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Mechanical Ablation of the Intervertebral Disc

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Abstract

Chronic lower back pain is a major public health problem, with direct and indirect economic costs comparable to those of heart disease, depression and diabetes. In many cases this pain derives from degeneration of the intervertebral disc (IVD), a fibrous, avascular tissue that sits between the vertebrae in the spinal column. A novel treatment approach for this 'discogenic' pain is the injection of a hydrogel that hybridises *in situ* and restores the normal biomechanical function of the disc. While a number of promising materials are currently under development, existing approaches to removing degenerate material from the disc prior to injection are invasive and compromise the structural integrity of the disc. Mechanical fractionation of the tissue using acoustic cavitation generated by high intensity focussed ultrasound (HIFU) has the potential to be non-invasive, and to enhance the effectiveness of the procedure by preserving the outer regions of the disc. The primary goal of this thesis is to investigate the feasibility of this approach.

The acoustic properties of the disc were first measured using a modified scanning acoustic microscope. The outer region of the disc, the annulus fibrosus (AF) was found to be highly attenuative compared to the central nucleus pulposus (NP). These measured properties were then used in a simplified two-dimensional model to simulate the shape of the acoustic pressure field within the disc. A configuration using two confocal spherically focussed 0.5 MHz single-element transducers was able to produce a tightly focused field suitable for use in the IVD.

As preliminary experiments suggested that high pressure amplitudes were required to initiate cavitation inside the disc, the use of exogenous nuclei to lower this threshold was investigated. A novel class of solid sonosensitive nanoparticles (SNPS) suitable for use in the IVD were developed and characterised. These SNPS comprise a layer of hydrophobic silica particles deposited onto a polystyrene core, and are thought to trap small gas pockets in surface crevices. Coated particles were found to reduce the cavitation threshold significantly in both water and blood, from some 2.0 - 2.5 MPa at 1.067 MHz to below 1.0 MPa. The particles were also found to provide repeatable initiation of cavitation activity during prolonged or repeated exposures, and to exhibit good storage stability, suggesting that they may be appropriate for use within the IVD.

Finally, a combined therapy and monitoring system was designed, built and validated. The system comprised two confocal 0.5 MHz spherically focussed HIFU transducers with central openings, each co-axially aligned with either a single element passive cavitation detector or a 64-element array that could be used for both active and passive imaging. The system was found to be capable of initiating inertial cavitation in the disc at pressures as low as 2.5 MPa in the presence of sonosensitive nanoparticles. Use of the array in active mode enables creation of a B-mode image that provides anatomical information on the boundaries of the IVD, whilst the same array could be used for passive mapping of acoustic emissions arising from the HIFU focus during therapy. Two different exposure regimes were found to be capable of producing sizeable perforations within the NP without significantly damaging the AF, and preliminary investigations were carried out into the mechanism of damage. The location and extent of cavitation as seen on passive maps acquired during treatment was found to coincide with the regions of NP fractionation. This confirms that passive acoustic mapping can provide the real-time treatment monitoring necessary to ensure both safety and efficacy of ultrasonic IVD fractionation.

Prior to clinical application, a significant amount of further development is required to further validate non-invasive disc fractionation by HIFU and the subsequent steps for minimally invasive disc replacement using injectable hydrogels. The present work has nonetheless demonstrated for the first time that minimally invasive removal of degenerate disc tissue is feasible through the combined use of sonosensitive nanoparticles and a relatively low-cost therapeutic ultrasound system that provides simultaneous anatomical imaging and real-time treatment monitoring by passive acoustic mapping.

Statement of Originality

I hereby declare that this submission is my own work and to the best of my knowledge it contains no materials previously published or written by another person, or substantial proportions of material which have been accepted for the award of any other degree or diploma at the University of Oxford or any other educational institution, except where due acknowledgement is made in the thesis.

Any contribution made to the research by others, with whom I have worked at the University of Oxford or elsewhere, is explicitly acknowledged in the thesis.

I also declare that the intellectual content of this thesis is the product of my own work, except to the extent that assistance from others in the project's design and conception or in style, presentation and linguistic expression is acknowledged.

Michael Molinari.
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Patent Applications

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CONTENTS

Contents	ix
List of Figures	xii
Acronyms	xv
1 Physiology, Current Treatments and Motivation	1
1.1 The Healthy Intervertebral Disc	2
1.1.1 The Annulus Fibrosus	3
1.1.2 The Nucleus Pulposus	5
1.1.3 The Cartilaginous End-Plate	6
1.2 The Degenerate Intervertebral Disc	7
1.2.1 Symptoms	7
1.2.2 Causes of Degeneration	10
1.2.3 Low Back Pain	12
1.3 Treatment of Discogenic Low Back Pain	12
1.3.1 Conservative Management	14
1.3.2 Spinal Fusion	15
1.3.3 Nucleotomy	16
1.3.4 Annuloplasty	17
1.3.5 Arthroplasty	19
1.4 HIFU for Treatment of Discogenic Low Back Pain	22
2 Biomedical Acoustics	24
2.1 Basics of Linear Sound Propagation	25
2.2 Non-Linear Sound Propagation	27
2.3 Acoustic Cavitation	28
2.3.1 Cavitation Inception	29
2.3.2 Cavitation Dynamics	40

2.3.3	Cavitation Detection and Monitoring	44
2.3.4	Effect on Surrounding Tissue	53
2.4	Therapeutic use of High Intensity Focussed Ultrasound	59
2.4.1	Lithotripsy	60
2.4.2	Thermal Ablation	61
2.4.3	Mechanical Fractionation	63
2.5	Implications for the Current Work	67
3	Ultrasound and the Intervertebral Disc	70
3.1	Measurement of the Acoustic Properties of the Intervertebral Disc . .	71
3.1.1	Experimental Setup	72
3.1.2	Measured Acoustic Properties of the Intervertebral Disc	89
3.2	Modelling the Acoustic Field in the Intervertebral Disc	99
3.2.1	Modelling Approach	99
3.2.2	Linear Simulation	105
3.2.3	Non-linear Simulation	108
3.2.4	Implications for the Present Work	110
3.3	Experimental Validation	111
3.3.1	Experimental Setup	111
3.3.2	Single Transducer Field	112
3.4	Summary	114
4	Sonosensitive Nanoparticles	116
4.1	Particle Production	119
4.1.1	Materials and Methods	119
4.1.2	Characterisation	124
4.1.3	Discussion	127
4.2	Effect on Cavitation Thresholds	128
4.2.1	Experimental Setup	129
4.2.2	Experimental Procedure	133
4.2.3	Data Analysis	139
4.2.4	Results and Discussion	144
4.3	Summary	153
5	Mechanical Fractionation of the IVD	158
5.1	The High Intensity Focussed Ultrasound System	159
5.1.1	Setup	159
5.1.2	Characterisation of the High Intensity Focussed Ultrasound Field	165

5.2	Inducing Cavitation in the Intervertebral Disc	170
5.2.1	Experimental Setup	170
5.2.2	Data Processing	173
5.2.3	Results and Discussion	173
5.3	Passive Cavitation Mapping Inside the Intervertebral Disc	178
5.3.1	Experimental Setup	178
5.3.2	Results and Discussion	180
5.4	Extended HIFU Exposures for IVD Fractionation	182
5.4.1	Materials and Methods	183
5.4.2	Results	186
5.4.3	Discussion	201
5.5	Summary	205
6	Conclusions and Future Work	209
6.1	Conclusions	209
6.2	Future Work	214
6.2.1	Improvement of Device	214
6.2.2	Treatment Protocol	216
6.2.3	Trials	218
6.2.4	Sonosensitive Nanoparticles	219
	References	221

LIST OF FIGURES

1.1	Schematic of IVD structure	3
1.2	Relationship between IVD structure and function	5
1.3	Detail of AF and NP structure	6
1.4	Image of healthy and degenerate human discs	8
1.5	Pressure distribution in healthy and degenerate discs	8
1.6	Grading system for disc degeneration	9
1.7	Comparison of MRI images of healthy and degenerate disc	10
1.8	Schematic and example of spinal fusion	15
1.9	Ultrasound-based devices for annuloplasty treatment	18
1.10	Current NP replacements	21
2.1	Shockwave formation due to non-linear behaviour	27
2.2	Nomenclature definitions of the crevice model	33
2.3	Theoretical explanation of crevice model	33
2.4	Size distribution of Sonovue [®] microbubbles	39
2.5	Comparison of stable and inertial cavitation	43
2.6	Cavitation threshold of pre-existing microbubbles in water	44
2.7	Comparison of signal from different cavitation events	47
2.8	Geometry for passive mapping	50
2.9	Example of cavitation mapping results	51
2.10	The effect of cavitation-enhanced heating	54
2.11	Fluid jetting at a rigid boundary	58
2.12	The mechanical effects of high-amplitude HIFU exposure on tissue	58
2.13	Idealized pressure-vs-time waveform	60
3.1	Schematic showing the setup of the SAM	74
3.2	Axial scans of the field produced by the 35MHz transducer used in the SAM	74
3.3	Custom-built device for cutting uniform thickness slices of IVD	78

3.4	Determination of the iso-osmotic concentration of PEG required for measurements	81
3.5	Example reflected signals from a glass slide and with tissue in place	84
3.6	Example of the curve-fitting to measure frequency dependence of attenuation	88
3.7	Variation of acoustic properties along a disc cross-section	90
3.8	Variation of acoustic properties along the transverse axis of the disc cut into strips	91
3.9	Comparison of field produced by transducers modelled in two and three dimensions	101
3.10	Transducer and disc geometries used in simulations of the acoustic field .	101
3.11	Geometry of idealised disc used for modelling	103
3.12	Linear modeling of the acoustic field for a single transducer	106
3.13	Linear modeling of the acoustic field for two perpendicular transducers .	107
3.14	Comparison between normalised pressure profiles from single and dual transducers	108
3.15	Effect of non-linearity on the acoustic field within the disc	109
3.16	Effect of non-linearity on the pressure profile within the disc	110
3.17	Measured pressure profile within a disc, single transducer	112
3.18	Comparison between experimental and modelled pressure profiles in the disc	113
4.1	Schematic of the process to produce the SNPs	120
4.2	Size distribution of LUDOX [®] particles	121
4.3	SEM images of the SNPs	126
4.4	Schematic showing the experimental setup for the SNPs	129
4.5	Images of the experimental setup	130
4.6	Spatial calibration of the H102 transducer	132
4.7	Absolute calibration of the HIFU transducers used	132
4.8	Effect of concentration on cavitation detection	135
4.9	The HIFU pulse sequence used for threshold measurement	138
4.10	Outline of cavitation detection algorithm	141
4.11	Variation of mean threshold with changing core and coating	145
4.12	Depletion of cavitation activity over exptended pulses - SNPs	148
4.13	Depletion of cavitation activity over exptended pulses - Sonovue	149
4.14	Variation of mean threshold with changing frequency across core sizes and coatings	151
4.15	Cavitation threshold of the SNPs in porcine blood	152

4.16 Depletion of both the threshold and the cavitation activity of SNPs and Sonovue over time	154
5.1 Experimental setup for the dual-transducer experiments	160
5.2 Schematic showing thermocouple placement	165
5.3 Absolute calibration of the dual-transducer therapy system	167
5.4 Spatial calibration of the dual-transducer therapy system	168
5.5 Effect of varying relative phase of the two HIFU transducers	170
5.6 A motion segment obtained from bovine tail	171
5.7 Effect of exogenous nuclei on cavitation in the IVD	174
5.8 Cavitation maps of discs injected with exogenous nuclei	176
5.9 Validation of the accuracy of the passive mapping	181
5.10 Results of HIFU exposures at moderate PRFP	188
5.11 Change in time-of-flight to the front of the cavitating bubble cloud	192
5.12 Varying results of discs with similar exposure conditions	193
5.13 Difference images showing the appearance of thermal damage on a B-mode scan	194
5.14 Example results of a high amplitude, long pulse exposure	195
5.15 Temperature rise and disc appearance from a high amplitude, long pulse exposure	196
5.16 Difference image showing a 'bright-up' for a long-pulse exposure	197
5.17 Summary of cavitation activity during high amplitude, short pulse exposures	199
5.18 Results showing cavity formation with high amplitude, short pulse exposures	200

ACRONYMS

AF	Annulus Fibrosus
ACD	Active Cavitation Detection
AVD	Acoustic Vaporising Droplet
CEM	Cumulative Equivalent Minutes
CEP	Cartilaginous End-Plate
DC	Duty Cycle
DLBP	Discogenic Low Back Pain
FFT	Fast Fourier Transform
FWHM	Full-Width Half-Maximum
HIFU	High Intensity Focused Ultrasound
IDET	IntraDiscal Electrothermal Therapy
IVD	Inter-Vertebral Disc
LbL	Layer-by-Layer
MRI	Magnetic Resonance Imaging
NP	Nucleus Pulposus
PCD	Passive Cavitation Detector
PEG	Polyethylene Glycol
PG	Proteoglycan
PRF	Pulse Repetition Frequency
PRFP	Peak Rarefactional Focal Pressure
RF	Radio-Frequency
SAM	Scanning Acoustic Microscope
SEM	Scanning Electron Microscope
SNP	Sonosensitive Nanoparticles
TEA	Time Exposure Acoustics
TMM	Tissue-Mimicking Material
UCA	Ultrasound Contrast Agent



PHYSIOLOGY, CURRENT TREATMENTS AND MOTIVATION

Low back pain is one of the most common health problems in the developed world, with over 80% of adults experiencing pain at some point in their life. In a high proportion of chronic cases this pain derives from the intervertebral disc (IVD), a small fibrous tissue that separates the vertebrae in the spinal column. While the aetiology of this discogenic pain is not well understood, degeneration of the disc tissue has been widely implicated. This chapter describes the basic anatomy of the disc and outlines the postulated causes of denegeration and its effects on both the IVD and the patient. Existing treatment options for discogenic low back pain are then reviewed, highlighting the need for a novel treatment approach and the potential role of High Intensity Focussed Ultrasound (HIFU) therapy.

Four out of five adults will, at some point in their life, suffer from low back pain. The statistics place it squarely among the major health problems in developed countries - in a 2006 study by the National Center for Healthcare Statistics in the US (NCHS, 2006) 27% of adults reported experiencing low back pain in the previous 3 months, with 28% of this group reporting that it limited their activity; low back pain was found to be the leading cause of disability in Americans under the age of 45. While estimates of costs vary widely depending on the methodology employed (Dagenais et al., 2008), there is general agreement that they are comparable to disorders

such as headache, heart disease, depression or diabetes (Maetzel and Li, 2002). As a ballpark figure, Maniadakis and Gray (2000) estimated the *direct* cost for treatment of low back pain in the UK in 1998 to be £1.6bn, dwarfed by *indirect* costs (such as informal care, productivity losses) of £10.7bn - a total economic burden of £12.3bn, or just over £200 per person per year if spread across the whole population.

For most patients this pain derives primarily from a soft tissue injury or strain and lasts for only a week or so, with the symptoms relieved by over-the-counter painkillers and the underlying factors resolved by rest and/or self-care. For an important group of patients, however, low back pain is a chronic and debilitating problem that can have a huge impact on their lifestyle. The underlying causes of pain in many of these situations are complex and poorly understood, which can make diagnosis and identification of the optimal treatment approach difficult. Studies have shown that in a substantial proportion of patients suffering from chronic pain, the degeneration of the intervertebral disc (IVD) is implicated (Luoma et al., 2000). To understand the basis for this, we first consider the structure and function of the healthy IVD, and how this is affected by degeneration.

1.1 THE HEALTHY IVD

The intervertebral disc is an avascular and anervous tissue that lies between the vertebral bodies of the spine and has a critical role in the biomechanics of the spine. It distributes and transmits the substantial loads that arise from body weight and muscle activity in the back, and provides the flexibility that allows the spine to undergo bending, flexion and torsion (Raj, 2008). The adult human lumbar disc typically measures 10-14mm in thickness, and 3-4cm in diameter (Urban and Roberts, 2003). Note that as this thesis is primarily concerned with lumbar, as opposed to cervical or thoracic, discs; the term ‘disc’ shall be taken throughout to mean ‘lum-

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Figure 1.1: Schematic diagram of the structure of the IVD, showing the three main regions and the two adjacent vertebral bodies (Adapted from Raj (2008))

bar disc’.

To meet the diverse biomechanical requirements placed upon it, the IVD has a complex structure. It is considered to be made up of three distinct regions (Figure 1.1): the annulus fibrosus (AF), the nucleus pulposus (NP) and the cartilaginous endplate (CEP).

1.1.1 The Annulus Fibrosus

The annulus fibrosus is the outer region of the IVD. It is highly organised on several scales, and its structure can be visualized by eye. At the smallest scale, the AF is comprised primarily of individual collagen fibres that are organised into bundles in a similar fashion to tendon (Marchand and Ahmed, 1990). At the next level of structure, these bundles of many fibres are arranged into 15-25 concentric circumferential rings, or ‘lamellae’. Within these lamellae, the bundles in each layer are aligned parallel to each other, and arranged at a 60° angle to the spinal axis. There are typically 15-20 of these lamellae making up the AF, with bundles in adjacent lamellae oriented in opposite directions to give a 120° angle between them (Figure 1.2). At their ends the collagen bundles are attached to the vertebral bodies and/or the cartilaginous endplates, thus securing the IVD to the adjacent structures of the spine. The individual lamellae are clearly distinguishable in a healthy disc and only loosely interconnected, allowing them to slip past each other as the IVD is compressed and extended during normal activity. Elastin is known to be present in the interlamel-

lar region and is thought to play an important role in controlling and limiting this slippage (Yu et al., 2002), however the interaction between this and other proteins present between layers is not well understood. The AF thus provides a crosswoven and reinforced structure (Figure 1.2) that provides strong resistance to tensile forces, yet at the same time is highly deformable - confining the material of the NP within it, and allowing torsion and compression of the spine.

The outer annulus of a young adult comprises around 60-70% water, decreasing closer to the disc boundary; collagen makes up 70-80% of the dry weight, with the remainder a complex mixture of structural proteins and proteoglycans (PG) (Cs-Szabo et al., 2002). The cells in the AF are present in very low density ($\sim 9,000/\text{mm}^3$ (Maroudas, 1975), compared with $\sim 15,000/\text{mm}^3$ in articular cartilage and much more in cellular soft tissue such as the liver) and are largely fibroblast-like, aligned along the axis of the collagen bundles. This low cell density means that each cell is 'responsible' for the maintenance of a relatively large volume of extracellular matrix, resulting in a slow response to any disruption or injury to the structure of the AF. Nutrient supply to the cells of the AF is believed to be a combination of diffusion from vascular buds within the vertebral body and diffusion from blood vessels close to the external surface of the disc (Brodin, 1955).

In addition to the primary population of fibroblast-like cells, the outer third of the AF is partially enervated to a depth of 1-3mm and vascularised in the young disc, with some of this lost at maturity (Bogduk et al., 1981). The nerves present in the posterior annulus are supplied by the sinuvertebral nerve, whereas the anterior and lateral regions are supplied by autonomic nerves.

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Figure 1.2: The role that IVD structure plays in function. **(a)** Compression along the spinal axis (C) generates a hydrostatic pressure (P) in the NP, and tensile stresses (T) in the AF. **(b)** Lamellae in the AF showing the orientation of collagen bundles in adjacent lamellae, together with typical values for the number of lamellae per disc, and bundles per lamella (Adapted from Adams and Roughley (2006))

1.1.2 The Nucleus Pulposus

The NP is a soft, pulpy tissue confined radially by the AF and vertically by the CEP. It is comprised of proteoglycan (PG) molecules (largely aggrecan) supported by a randomly organised matrix of collagen fibrils (Figure 1.3) (Inoue and Takeda, 1975). The aggrecan molecules are highly charged, exerting a substantial osmotic swelling pressure and drawing in water from surrounding tissue (Urban et al., 1979). The high proportion of PG (collagen makes up only 20-30% of the dry weight of the tissue, 70-80% is PG) means that the NP is highly hydrated, containing roughly 80% water in a young adult and taking on a gelatinous consistency. This gel-like material then acts as a hydraulic fluid, transmitting axial compressive load from the vertebral bodies into tensile stress on the collagen fibrils of the AF and ensuring that the lamellae do not buckle inward and separate (Figure 1.2) (Adams and Roughley, 2006). The hydrostatic pressure inside the NP can vary greatly with posture and physical activity, but has been found to range from 0.1-2.5MPa gauge during most activities (Wilke et al., 1999).

The adult NP contains chondrocyte-like cells that are responsible for maintaining the composition of the matrix through a complex and incompletely understood

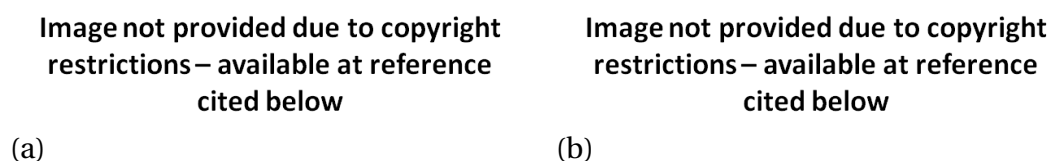


Figure 1.3: SEM images showing the detailed structure of **(a)** the AF, and **(b)** the NP. Note the organised lamellar structure of the AF and the seemingly random arrangement of the fibres in the NP. (Adapted from Inoue and Takeda (1975))

balance of anabolic and catabolic processes. These cells are present at even lower densities than in the AF ($\sim 5,000/\text{mm}^3$), meaning that there is an even lower ability for the NP to recover from any disturbance than the AF. To illustrate just how slow recovery is, the half-life of the collagen in the NP has been estimated to be in the vicinity of 100 years (Verzijl et al., 2000), meaning that most people will still have more than half of the collagen with which they were born when they die. Nutrient supply for these cells is thought to come primarily by diffusion from vascular buds in the vertebral bodies.

1.1.3 The Cartilaginous End-Plate

The CEP is a thin layer ($<1\text{mm}$) of hyaline cartilage that lies between the disc and the vertebral body. It is thought to play an important role in distributing load evenly over the NP and also in allowing nutrient supply to the NP in particular. In young discs the CEP is permeable, allowing for the transport of nutrients from the vascular bud close to the surface of the vertebral body. However, with age the CEP is known to calcify (Benneker et al., 2005) and the resulting decrease in permeability can limit nutrient supply. This loss of nutrient supply has been implicated in degeneration (Section §1.2).

1.2 THE DEGENERATE IVD

The IVD undergoes extensive destructive changes with age (Buckwalter, 1995; Boos et al., 2002), with an earlier onset than for any other musculoskeletal tissue. In most people this process of ‘degeneration’ begins between the ages of 11-16 and continues throughout life, although certain individuals can exhibit greatly accelerated degeneration at a young age. The precise definition of disc degeneration has been the subject of debate. Adams and Roughley proposing to define it as ‘an aberrant, cell-mediated response to progressive structural failure’ Adams and Roughley (2006) in order to differentiate from age-related ‘constitutional changes’ and relate it more closely to pain; other authors (Urban and Roberts, 2003; Boos et al., 2002) prefer to define it in terms of biochemical changes to the IVD. The debate is somewhat arcane, and for the purposes of this thesis it is sufficient to say that degeneration, however defined, is very common in the population and is closely linked to chronic back pain. This section begins by describing the changes that are seen in the degenerate disc, briefly discusses the hypothesised causes of these changes, and then outlines how these are thought to lead to pain.

1.2.1 Symptoms

As the disc degenerates, it begins to exhibit changes in its properties, behaviour and appearance (Urban and Roberts, 2003; Adams and Roughley, 2006). An example of a healthy disc compared to a degenerate disc can be seen in Figure 1.4; the differences are obvious to the naked eye.

Degeneration is generally believed to begin in the NP (Section §1.2.2 gives a detailed discussion of the causes and progression), with this region losing hydration, which leads to a drop in the intradiscal pressure. This drop in pressure means that the NP begins to lose some of its ability to hydraulically distribute axial loads, local

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Figure 1.4: Photographs of excised human discs: (a) healthy and (b) degenerate. Note the loss of hydration and internal structure in the degenerate disc. (Adapted from Urban and Roberts (2003))

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Figure 1.5: Stress profiles showing the distribution of compressive stress along the diameter of a lumbar disc under 2kN of compressive load. Solid and broken lines show vertical and horizontal stresses respectively. (a) A young 'grade 1' disc, (b) A middle-aged 'grade 2' disc, with dashed lines showing the extent of the NP, (c) A 'grade 3' disc with load borne by the AF and indicating the location of stress concentrations (arrow) (Adapted from Adams and Roughley (2006))

stress concentrations appear (Figure 1.5) as the AF supports more of the compressive load directly, and the AF can begin to buckle inwards. The clear delineation between the AF and the NP disappears, and the internal structure of the disc is lost. The lamellae of the AF can begin to separate circumferentially and split radially, which allows both NP material to leak outwards, and blood vessels and nerves to grow into the IVD from surrounding tissue. Osteophytes (bone ingrowths from the vertebral bodies or CEP) are increasingly seen, and the endplate begins to exhibit irregularities. Visually, the disc tissue begins to discolour, taking on a yellowed appearance.

At a biochemical/histological level, the NP begins to lose PG content, and it is thought that the length of the PG chains that are present decreases. The degree of

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Figure 1.6: Images showing examples of the degeneration grades as defined by Thompson's system, ranging from Grade 1 (normal juvenile disc) to Grade 5 (severe degeneration), and clearly showing the loss of internal structure and disc height with increasing degeneration (Adapted from Boos et al. (2002)). This grading system is commonly used to assess and classify the gross morphology of lumbar discs.

cross-linking in the collagen within the NP increases. The behaviour of the cells in the IVD begins to change, with increases in both proliferation and cell death, the formation of cell clusters in the AF and the CEP, increased levels of senescence (Roberts et al., 2006a), and a decrease in the cell density in the NP. Cell types from surrounding tissue are increasingly seen to infiltrate the IVD. A classification system (Boos et al., 2002) for age-related histologic disc alteration has been developed based on an extensive assessment of degenerate disc samples, which provides a detailed description of these changes and their histological signatures (see Figure 1.6).

It is important to note here that disc degeneration is not synonymous with a painful disc - patients present all of the above changes in disc appearance without experiencing pain (Boos et al., 2000; Borenstein et al., 2001; Videman et al., 2003), and vice versa: a small number of patients may experience back pain without presenting any symptoms of degeneration (Section §1.2.3). As might be expected, this lack of a direct relationship makes diagnosis of the causes of chronic back pain difficult.

Assessment of disc degeneration in patients is typically done using x-ray or MRI. X-ray images allow for an assessment of the decrease in disc height and the presence

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Figure 1.7: MRI images of spines with: **(a)** healthy discs, clearly showing signal from the highly hydrated NP region, and **(b)** degenerate discs, with no clear delineation between the NP and the AF and a decrease in height of the disc space

of any osteophytes. However, MRI is now more commonly used as the intensity of the T2 signal allows for a direct assessment of the hydration of the NP in a similar fashion to cartilage (Liess et al., 2002). However, it has been shown that there is little, if any, relationship between disc signal intensity on MRI and pain (Videman et al., 2003), which means that it is of limited use in screening and diagnosis.

In the absence of a suitable imaging technique, pain-generating discs are often identified using discography. In this procedure, the clinician seeks to replicate the patient's reported pain and locate fissures in the AF by injecting a radio-opaque material into the disc under fluoroscopic guidance. However, a recent study (Carragee et al., 2009) has raised concerns over whether this procedure may lead to increased degeneration over time, and anecdotal evidence (Dr Jill Urban, personal discussion) suggests that many clinicians are choosing to avoid the technique.

1.2.2 Causes of Degeneration

The previous section described the symptoms of degeneration; the underlying causes are less well-understood, but are essential in identifying therapies capable of preventing, arresting or reversing the process. At a high-level, disc degeneration can be thought to be caused by a combination of three different factors - underlying epidemiological risk, age-related changes, and specific mechanical effects.

The underlying epidemiology of degeneration is poorly understood. It is known

that the highest risk factor is genetic inheritance, which accounts for 50-70% of the variability between identical twins (Battie et al., 2004). However, the consistent finding that L4-S1 discs are more degenerated than L1-L4 discs suggests that lifestyle and environmental factors also play a role. Such environmental factors are known to include high and repetitive mechanical loading, and smoking.

Age-related changes are generally thought to begin in the second decade of life (Boos et al., 2002) with a decrease in the density of the blood vessels in the CEP that deliver nutrients to the NP, and an increased diffusion distance as the disc grows in size. This loss of nutrition triggers metabolic changes in the cell population of the NP, leading to a decrease in matrix turnover - both through a decrease in PG synthesis per cell and in the number of cells present. The number of crosslinks between the collagen fibrils in the NP increases over time. The net result of these changes is to reduce the mechanical strength of the disc tissue, and bring about a loss of hydration and hydraulic function of the NP, thus 'pre-disposing' (Adams and Roughley, 2006) the disc to structural disruption.

Structural disruption may occur as a result of a single mechanical event; most commonly either an annular tear or damage to the CEP. This disruption compromises the load-spreading function of the healthy disc, leading to altered stress distributions and progression of the damage (Adams et al., 2006). The ongoing changes in the mechanical environment in turn affect the behaviour of the cells of the disc, producing a degenerative cascade that ultimately results in the observed loss of structure and disc height in severely degenerate discs.

Each of these factors may be present to varying degrees in any individual, and it is the interplay between them that creates such a complex environment for both diagnosis and treatment.

1.2.3 Low Back Pain

Although the pathological cause of most low back pain is undiagnosed, there are thought to be several mechanisms by which degeneration results in low back pain. As a result of the structural changes associated with degeneration, the disc can become herniated or prolapsed, meaning that radial fissures in the AF have allowed gross migration and bulging of the NP into surrounding tissue. In doing so, the herniated disc impinges on surrounding nerve structures and can stimulate them directly to produce the sensation of pain. The NP material is also known to have an inflammatory effect, and can sensitise nerves in the outer annulus and surrounding tissue to trigger a pain response under otherwise normal physiological loading (Cavanaugh et al., 1997). Further, it is known that the loss of structure in the AF can allow the intradiscal pressure to drop sufficiently to allow nerves and their attendant blood supply to grow into the disc (Boos et al., 2002), generating a pain response.

This suggests that prevention or minimisation of the degeneration-related structural changes to the disc may be able to reduce the impact of chronic low back pain. The following section discusses the treatment options available, and a brief review of their effectiveness.

1.3 TREATMENT OF DISCOGENIC LOW BACK PAIN

In deciding on the appropriate treatment for a given patient experiencing low back pain, the clinician, and to an even greater extent the patient, is faced with an array of options (Fairbank et al., 2011). The decision is complicated by a lack of clear indications for one treatment over another, and the often contradictory results of clinical trials. This section provides a brief overview of the five main treatment approaches

¹, summarised in Table 1.1. For a detailed description and comparison of treatments the interested reader is directed to one of the many reviews and good basic studies on the various aspects of the topic (Freeman, 2006; Freeman and Davenport, 2006; Freeman and Mehdiian, 2008; Gibson and Waddell, 2007; Fairbank, 2011; Fairbank et al., 2005).

Approach	Basis	Available Devices	Clinical Trials
Conservative Management	Rest, stretching, pain relief medication and rehabilitation	-	(Fairbank et al., 2005)
Fusion	Elimination of motion between adjacent vertebrae by fusing together, typically used where pathology is unclear	Many products are on the market	(Fairbank et al., 2005)
Annuloplasty	Thermal treatment of the AF to necrose nociceptors and/or coagulate collagen to close fissures	IDET DiscTrode Ultrasound devices	(Pauza et al., 2004)* (Freeman, 2006)* (Saal and Saal, 2000a,c, 2002)
Nucleotomy/ Discectomy	Removal of tissue from the NP in order to reduce the intradiscal pressure and reduce nerve impingement	APLD PLDD Coblation® Various manual procedures	(Revel et al., 1993)* (Chatterjee et al., 1995)* (Nerubay et al., 1997)*
Chemonucleolysis	Enzymatic degradation of NP material in order to reduce intradiscal pressure	None (chymopapain withdrawn from use)	(Gibson and Waddell, 2007)
Arthroplasty	Replacement of all or part of the disc in order to restore normal biomechanical and/or biological function	LINK-Charite ProDisc PDN NewCleus Aquarelle DASCOR BioDisc	(Griffith et al., 1994) (Freeman and Davenport, 2006)

Table 1.1: Surgical options for the treatment of chronic low back pain. References describing the available devices and selected clinical trials are indicated in brackets, randomised clinical trials are indicated with an asterisk. Chemonucleolysis is not elaborated on elsewhere in this thesis as it is no longer performed clinically.

¹Biological or drug-based treatments of the IVD aimed at restoring normal biological activity are not considered here as they remain a long way from clinical use (Alini et al., 2002).

1.3.1 Conservative Management

Most low back pain is short in duration, and the first stage in treatment upon presentation is typically a combination of rest, stretching and pain relief medication, collectively termed ‘conservative management.’ The National Institute for Health and Clinical Excellence (NICE) publishes guidelines for recommended treatment of early-stage non-specific low back pain in the UK (accessible at <http://www.nice.org.uk/CG88fullguideline>). These guidelines recommend that presenting patients should in the first instance be offered a choice between an exercise program of up to 12 weeks, a course of spinal manipulation over up to 12 weeks or a course of acupuncture needling over 12 weeks. In the event of continuing pain, patients may be referred to a combined physical and psychological program of around 100 hours over 8 weeks. If pain continues, spinal fusion (Section §1.3.2) is suggested as a surgical option.

In the Spine Stabilisation Trial conducted in the UK (Fairbank et al., 2005) to compare the economic effectiveness of conservative rehabilitation against fusion, the rehabilitation program consisted of about 75 hours of an exercise and education programme over a 2 year period that included hydrotherapy and gym activities. Interestingly, this study found that conservative rehabilitation showed no significant difference in outcome compared to fusion over a 2-year followup period, and came at a significantly lower cost.

However, there will always be a group of patients for whom conservative management does not relieve their symptoms, and who require interventional therapy.

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Figure 1.8: Spinal fusion. (a) Schematic of spinal fusion, with a rod fixing the vertebrae in place, and a cage holding them apart (adapted from <http://www.umm.edu/spinecenter/education>, accessed 11 Jan 2012), (b) The BAK cage from Zimmer Spine (adapted from Williams et al. (2005))

1.3.2 Spinal Fusion

Spinal fusion is the current gold standard for surgical treatment of chronic low back pain. The procedure works on the principle that it is changes in the stress distribution in the IVD (Figure 1.5) that causes pain, and seeks to fuse the adjacent vertebrae together to prevent movement of the pain-producing disc. Fusion usually involves the removal of the disc, followed by the insertion of pedicle screws into the vertebrae to lock them together with either a plate or a cage positioned in the cavity where the IVD has been removed (Figure 1.8). Fusion is regarded as a relatively successful procedure, with success rates (variously defined) in the range of 70-90% (Andersson et al., 2006).

It is, however, a highly invasive procedure that requires a patient to be placed under general anaesthetic, and requires significant hospital recovery time. Fusing adjacent vertebrae together inherently alters the natural biomechanical function of the spine, reducing the range of flexion and tension and altering the stress distribution within the spine. This altered stress environment has been reported to contribute to increased prevalence of degenerative symptoms in adjacent segments (Lee, 1988; Hilibrand and Robbins, 2004), which makes it much less suitable for younger patients who are likely to be more active and to live long enough to require

revision.

1.3.3 Nucleotomy

Nucleotomy, or discectomy, is a procedure designed to remove a small amount of tissue from a pain-generating disc to reduce the pressure on the nerves producing pain. In many cases it is uncontained herniated tissue impinging directly on adjacent nerves that is removed, however material from inside the disc boundary is also commonly extracted (Manchikanti et al., 2009). This approach is also widely used for the treatment of sciatica or radicular pain, where the herniation of the disc leads to the experience of pain elsewhere in the body (Hout et al., 2008).

The traditional open nucleotomy using manual cutting tools has been largely replaced by minimally invasive procedures that are designed to minimise the impact on the surrounding tissue, and reduce post-operative recovery time. These minimally invasive procedures include automated percutaneous lumbar discectomy (APLD) (Onik et al., 1985); percutaneous laser disc decompression (PLDD) (Choy, 1998, 2004); and Coblation[®], a procedure that uses an RF probe to generate a low-temperature plasma inside the nucleus that ablates small regions of the NP (Singh et al., 2003, 2004; Chen et al., 2003; Lee et al., 2003; Zhu et al., 2011). The injection of enzymes to degrade disc tissue ('chemonucleolysis') has been widely used in the past, but has been largely discontinued due to complications associated with its use (Gibson and Waddell, 2007).

A 2007 Cochrane review (Gibson and Waddell, 2007) found that the results of open and percutaneous nucleotomy were broadly comparable, and superior to those of chemonucleolysis. They reported that there was insufficient evidence of the effectiveness of other treatments - three small randomised controlled trials (RCTs) of PLDD gave inconclusive results, and there were no RCTs available on the effect of

Coblation[®]. A more recent review (Manchikanti et al., 2009) using a similar methodology found evidence to suggest that Coblation[®] at least had no negative effects, and may provide relief in a subset of properly selected patients.

While less invasive than fusion, nucleotomy is explicitly aimed at relief of symptoms rather than addressing the underlying causes of degeneration and pain. The removal of tissue from the disc changes the biomechanics of the spine, which can lead to the need for revision, and potentially effects in adjacent discs.

1.3.4 Annuloplasty

The underlying goal of annuloplasty is to thermally treat the AF of a degenerate disc. There is some disagreement over the mechanism by which this relieves discogenic pain: some research groups (most notably Saal and Saal (2000b)) claim that it brings about a contraction of the collagen fibres due to the loss of organised structure and coagulation (Hayashi et al., 1996), which then seals any microscopic fissures that are present; other groups (Nau et al., 2005) postulate that the effect is simply due to thermal necrosis (Dewey et al., 1977) of any nociceptors present in the disc.

Intradiscal electrothermal therapy (IDET, Smith and Nephew, London, UK) is the most prominent of the devices used for annuloplasty. The process consists of placing a resistive thermal element inside the disc against the posterior wall of the nucleus, and heating it to 90°C for a 16 minute treatment period. An RCT by Pauza et al. (2004) found that it could achieve pain relief for a very tightly indicated cohort of patients, however Freeman (2006) reported no significant benefit over placebo for another patient cohort. In spite of the limited clinical evidence in support of the procedure it has reached widespread clinical usage.

Two separate groups have also proposed devices that use ultrasound to produce

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Figure 1.9: The ultrasound-based annuloplasty probe developed by the group from UCSF. **(a)** A direct-coupled transducer (top) and catheter-cooled transducer (bottom) of 1.5mm OD and 10mm long, **(b)** A fluoroscopic image showing the position of the probe (red arrow) inside the NP, with the thermocouples for measuring the temperature distribution (adapted from Nau et al. (2007))

heating in the IVD. As a treatment modality, ultrasound has the potential to heat a larger volume of tissue more uniformly and in a more controlled fashion than the resistive element used in IDET. Chapter 2 gives a detailed discussion of the uses of ultrasound for therapy.

A Swedish group at the University of Lund published on their development of a 4.1MHz ultrasound transducer that is designed to be inserted into the body and placed against the surface of the IVD, and used to heat the tissue inside the AF. They demonstrated that this device was capable of heating the AF to the temperatures required for coagulation, with the peak temperature rise recorded 4mm into the disc (Forslund et al., 2006). The main issue reported was that the surface of the transducer heated up substantially, and raised the temperature of the outer AF through direct heat conduction. The same group had earlier proposed the use of an extracorporeal transducer array to target the IVD in a completely non-invasive fashion (Persson et al., 2002, 2005), however they reported difficulty with targeting and alignment.

A group at the University of California San Francisco have also published on the development of an ultrasound device (7-8MHz) to heat the IVD for annuloplasty. In

their approach, a side-firing transducer capable of producing a directional field was implanted directly into the NP and aimed at the internal wall of the AF to thermally necrose any nociceptors present Figure 1.9. They were able to achieve a sufficient temperature rise, in a more tightly controlled region and at greater depth than the Swedish device, in both *ex vivo* human discs (Nau et al., 2005) and *in vivo* ovine cervical discs (Nau et al., 2007). They also demonstrated the use of a simple control system for treatment based on the insertion of a small thermocouple into the disc.

Both of these ultrasound devices demonstrate the feasibility of the approach, but to the best of our knowledge they have not been progressed to clinical use. Annuloplasty in general has the advantage of being a straightforward, minimally invasive procedure that is easily revised. However, similar to other surgical treatments it is aimed at symptom relief rather than restoration of normal disc function, and has the danger that excessive heating may result in the death of cells in the CEP with uncertain long-term effects.

1.3.5 Arthroplasty

Disc arthroplasty, or replacement, follows one of two approaches; replacement of the whole disc, or nucleus replacement only. Both of these approaches have the potential to restore normal biomechanical function of the disc.

Several devices for total disc replacement have been developed, and four appear to have been approved for clinical use: the LINK-Charite device (Depuy Spine, Raynham, MA, USA) (Figure 1.10), the ProDisc (Synthes, West Chester, PA, USA), Maverick (Medtronic, Minneapolis, MN, USA - since withdrawn from the market) and the AcroFlex (DePuy Acromed, Raynham, MA, USA - since withdrawn from market). All three of these devices use a multi-component system comprising a bearing material sandwiched between endplates that allows for flexion and torsion.

The main challenge in design of an implant is to identify a suitable material for the bearing component - if it is too stiff or too flexible it can lead to significant remodelling and/or subsidence in the vertebrae. Systematic reviews by Freeman and Davenport (2006) and Resnick and Watters (2007) found that, while the LINK-Charite can be considered a safe and effective treatment for single-level disc degeneration, the clinical outcomes are only equivalent to (i.e. no better than) those of fusion. Implanting the replacement is an invasive, time-consuming and expensive procedure, and as a result none of the devices have achieved widespread clinical usage.

In nucleus replacement, the degenerate NP is broken down and removed, and a replacement inserted. There are several advantages to NP replacement over total disc replacement: it goes closest to retaining normal physiological function of the disc as the AF and surrounding tissue is retained; it can be minimally invasive; it is potentially much cheaper in terms of both direct and hospital costs; and the treatment can be progressed to fusion or total disc replacement in the event of continued degeneration. As with total disc replacement, the nature of the replacement material is critical: if its properties are too different from healthy NP it can accelerate degeneration and/or lead to remodelling of the intervertebral bodies. Additionally, the AF has to be sufficiently intact to retain the nucleus replacement in place and prevent it from excessive lateral spreading.

Among pre-formed NP replacement devices, the most successful are the Prosthetic Disc Nucleus (PDN, Raymedica, Minneapolis, MN, USA) (Figure 1.10), the NewCleus (Zimmer Spine, Warsaw, IN, USA), and the Aquarelle (Stryker, Kalamazoo, MI, USA) (Figure 1.10). These replacements are all formed into a complete 'nucleus' prior to insertion into the disc. The PDN and the Aquarelle are based on the insertion of a pellet containing a hydrogel that absorbs water once in place and seeks to replicate the mechanical properties of a healthy nucleus; the NewCleus uses a spi-

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Figure 1.10: Examples of devices currently being used for disc arthroplasty. **(a)** The LINK-Charite total disc replacement showing the endplates and bearing material (adapted from Link (2002)), **(b)** The Aquarelle hydrogel implant shown *in situ* inside a disc (adapted from Allen et al. (2004b)), **(c)** The Prosthetic Disc Nucleus, with hydrogel confined by a sturdy woven material (adapted from Ray (2002))

ral implant that expands following insertion Figure 1.10. All these devices require a relatively large incision in the AF in order for degenerate material to be removed and the device inserted. This large incision has been implicated in migration and extrusion of the implant following surgery in 20-33% of patients, leading to the need for revision or progression to fusion (Martino et al., 2005; Allen et al., 2004a).

The development of injectable gels that can polymerise *in situ* overcomes this difficulty, as the large incision can be replaced by a hole the size of a small-gauge needle. Such a gel must be able to restore the swelling pressure of the nucleus (and hence disc height), be non-toxic, set quickly, and (importantly) should not give off excessive heat during polymerisation (Martino et al., 2005; Goins et al., 2005). Several gels have been developed and are reported to be in trials, including the DASCOR device (Disk Dynamics, Eden Prairie, MN, USA) and the BioDisc (Cryolife, Atlanta, GA, USA). The major problem with these products to date has been the long time required for the polymerisation after injection, and the heat evolved by this process causing damage to the endplates.

A key issue in nucleus replacement that has not received sufficient attention to date is the procedure for removing degenerate material from the NP without dam-

aging or excessively weakening the AF. The current means of achieving this is to use standard manual surgical equipment and techniques (trochar and rongeurs) to perforate the AF and to mechanically break down the NP before extracting the broken down material under suction. While this can be achieved through a minimally-invasive approach, it does require the creation of a relatively large incision in the AF. This incision can result in migration and extrusion of preformed implants, and complete extrusion of in situ polymers. It has also been shown that the approach often leaves a substantial amount of material inside the disc (Dang et al., 2007). The development of a technique to address this would greatly assist in the ongoing clinical development of injectable disc replacements.

1.4 HIFU FOR TREATMENT OF DISCOGENIC LOW BACK PAIN

In addition to the thermal effects of ultrasound described above (Section §1.3.4), high-intensity ultrasound is capable of producing mechanical effects that may be relevant for the treatment of the IVD. In particular, ultrasound can trigger the formation, oscillation and collapse of microbubbles, an effect known as ‘acoustic cavitation’, which is capable of delivering a wide range of bioeffects, including the mechanical (i.e. non-thermal) fractionation of tissue into a smooth emulsion.

The following chapter presents a detailed discussion of the use of ultrasound and acoustic cavitation in therapy, and the basis for the bioeffects. In subsequent chapters, original work aimed at using the effects of acoustic cavitation to break down and remove the tissue of the degenerate NP is described. The aim is to create a void within the NP without surgical incision that will then enable the injection of a replacement material to restore normal physiological disc function and provide

relief of chronic discogenic low back pain for the patient.



BIOMEDICAL ACOUSTICS

The use of ultrasound in a clinical context is underpinned by an understanding of the basic physics of sound propagation. For therapeutic ultrasound in particular, cavitation is a critical element with bio-effects that can variously enhance or reduce treatment efficacy, effectiveness and safety. This chapter describes the relationships that define the propagation of soundwaves through the body, the conditions under which cavitation can occur and how it can be detected, enhanced and controlled. The use of HIFU-induced cavitation to fractionate tissue mechanically is explored, with the aim of exploiting this mechanism for degenerate intervertebral disc removal.

Historically, ultrasound has been predominantly used as an imaging modality for diagnosis and monitoring; recently it has begun to be considered as a therapeutic modality of considerable potential, with extensive research being undertaken to understand and control ultrasound-induced bioeffects. These bioeffects can vary widely depending on the nature and duration of the exposure. At one end of the scale, ultrasound is capable of reversibly opening the blood-brain barrier to allow the delivery of large drug molecules, or of enhancing the delivery of thrombolytic agents to the site of clotting in a stroke. At the other end, very short, high-intensity pulses of ultrasound are capable of breaking up a kidney stone, or ‘mechanically fractionating’ (effectively liquefying) the soft tissue in the kidney itself. In order to

understand the relationship between exposure parameters and clinical effects, and how these are relevant to the treatment of a degenerate IVD, it is important to first understand the physics of ultrasound propagation through tissue.

2.1 BASICS OF LINEAR SOUND PROPAGATION

Sound is a waveform consisting of density variations in a fluid medium, propagating away from a source (Leighton, 1994). The molecules comprising the medium oscillate about their equilibrium position parallel to the direction of propagation.

Ultrasound is simply defined as a sound wave oscillating at a frequency above the range of human hearing (generally taken to be 20kHz). The simplest way to model the propagation of ultrasound is by assuming *linear*, lossless behaviour. By ignoring non-linear terms in the equations of continuity, momentum conservation and state, the linear wave equation for propagation through a lossless, homogeneous medium can be derived (in one-dimensional form):

$$\frac{\partial^2 p(x, t)}{\partial x^2} - \frac{1}{c^2} \frac{\partial^2 p(x, t)}{\partial t^2} = 0 \quad (2.1)$$

where $p(x, t)$ is the pressure disturbance, x is the spatial dimension, t is time, and c is the wave speed.

This assumption of linearity is only valid as long as particle velocities remain small relative to the speed of sound in the medium, and density variations are small relative to the equilibrium density - criteria which are met only for small-amplitude disturbances.

In a lossy inhomogeneous medium, such as tissue, the ultrasound wave loses energy through a combination of viscous damping, thermal conduction and scattering from tissue inhomogeneities, effects which are collectively referred to as ‘attenua-

tion'. Modifying the solution of Equation (2.1) to include the effect of attenuation gives a general solution of the form:

$$p(x, t) = \Re\{P_o e^{i(\omega t - kx)} e^{-\alpha x}\} \quad (2.2)$$

where $c = \omega/k$, ω is the angular frequency of the wave, k is the wavenumber, and α is the amplitude attenuation coefficient. Attenuation is frequency-dependent, with the frequency dependence of α generally taking the form $\alpha = \alpha_o (f/f_o)^\eta$, where α_o is the attenuation coefficient at f_o , $\eta = 2$ for water and η is in the range 1.1 – 1.5 for most tissues (Liu et al., 2006).

Higher frequencies are thus more readily attenuated than low frequencies. This requires a compromise for imaging applications, as the use of higher frequencies yields greater spatial resolution but smaller achievable imaging depths due to attenuation. Additionally, since much of the acoustic energy lost is converted into thermal energy, a higher frequency can result in heat deposition and elevated temperatures along the path of propagation, with potentially undesirable bioeffects.

In therapy, heat deposition by ultrasound can be exploited, for example in the non-invasive thermal ablation of cancerous tumors (Coussios et al., 2007). A similar tradeoff between frequency and penetration depth exists in this context, with the potential for heat deposition to result in tissue damage occurring outside the desired region.

The situation in most ultrasound therapy is not so simple, however, as it employs pressures at a level where the assumption of small disturbances is no longer valid, and *non-linear* effects become significant. The most significant of these effects are distortion of the propagating wave, and cavitation.

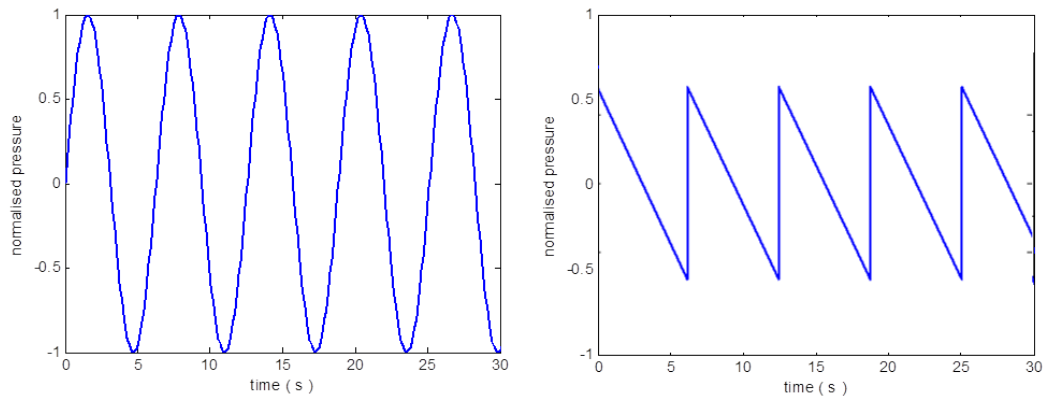


Figure 2.1: Graphical representation of a sinusoidal pressure wave (left) and a shock wave resulting from non-linear behaviour (right). Note that the x-axis is time - the corresponding plot for position would be inverted

2.2 NON-LINEAR SOUND PROPAGATION

When the assumption of small disturbances is no longer valid, two effects become important. The particle velocity becomes non-negligible relative to the bulk speed of propagation, meaning that in the compressive part of the sound wave the particles travel at $c + |\nu|$, while particles in the rarefactional region travel at $c - |\nu|$. Additionally, the pressure in the compressive region is much higher than at equilibrium, resulting in a higher bulk modulus and stiffness and thus a higher speed of sound; the opposite applies in the rarefactional region. Thus the peaks travel faster than the troughs, resulting in a distortion of the wave into a sawtooth shape (Figure 2.1).

The degree of non-linearity in a given medium depends on the material properties, and is characterised by the parameter of non-linearity (B/A) (Hamilton and Blackstock, 1998). For a given medium with coefficient B/A , the distortion of the wave is increased at higher amplitudes and higher frequencies, and becomes more pronounced over long propagation distances.

Distortion in the time/displacement domain corresponds in the frequency domain to an increase in high-frequency content of the waveform, transferring energy

from the fundamental frequency to its harmonics. Since higher-frequency components are more highly attenuated than low frequencies, non-linearity results in more rapid attenuation of a propagating wave, corresponding to more rapid heat deposition into the medium.

This has several implications for the use of ultrasound in therapy. At one level, it can be harmful. Fat is the most highly non-linear tissue in the body, which can mean that much of the ultrasound energy is attenuated in fat layers in the propagation path between the ultrasound transducer and the targeted region at the focus, particularly in obese patients or when targeting the deep abdomen. This can create burns in the fat, and also prevent sufficient energy from reaching the focus to achieve the desired therapeutic effect.

At another level, non-linearity can be exploited in therapy. By using a focused transducer or array of transducers, it is possible to produce an acoustic field that is sufficiently low-amplitude close to the skin, and in surface fat layers, that it is accurately described by Equation (2.2), but that interferes constructively closer to the focus to produce much higher pressures, resulting in greater non-linearity and higher rates of attenuation and heat deposition than for a single-element unfocused transducer.

2.3 ACOUSTIC CAVITATION

Although definitions vary depending on context, in this work the term *acoustic cavitation* is used to describe the nucleation (formation), oscillation and collapse of a bubble subject to an external ultrasound field. Here we treat the nucleation process separately from oscillation and collapse, whilst acknowledging their strong interdependence (Brennen, 1995).

Cavitation for many years was viewed as undesirable and something to be avoided clinically, as it was known to be responsible for tissue damage in diagnostic imaging (Holland et al., 1996). It is a highly non-linear and stochastic process, making it difficult to predict and to control. However, as ultrasound has increasingly come to be seen as a therapeutic modality, there has been a renewed focus on harnessing the physical effects of cavitation to enable and enhance desirable bioeffects in treatment (Coussios and Roy, 2008). It is currently being explored for use in thermal necrosis, mechanical fractionation, reversible opening of the blood-brain barrier, sonoporation for gene therapy, thrombolysis, and enhanced drug delivery; these therapeutic applications are discussed further in Section §2.4.

2.3.1 Cavitation Inception

Acoustic cavitation requires a bubble or vapour cavity to be present in the transmission medium. Such a bubble can be produced spontaneously within the medium ('homogenous' nucleation), as a result of discontinuities in the medium ('heterogeneous' nucleation), or introduced from outside the medium ('exogenous' nucleation). This section describes the physical basis for each of these mechanisms, with a particular focus on the clinical environment.

2.3.1.1 Homogeneous Nucleation

In a homogeneous material there are (by definition) no predetermined nucleation sites. In this situation, it is the random thermal fluctuations of kinetic theory that are responsible for the formation of temporary, microscopic voids within the medium that can act as cavitation nuclei (Brennen, 1995). In order for these voids to form, the molecules within a small region must possess sufficient thermal/kinetic energy to overcome the intermolecular forces that are holding them together (in a pure liquid

this is the surface tension). This gives rise to an expression for the net energy, W_{CR} , that must be deposited in order to form a void of radius R_C :

$$W_{CR} = \frac{4}{3}\pi R_C^2 \sigma \quad (2.3)$$

where σ is the surface tension.

Since kinetic theory dictates that the energy of the molecules in the medium follows a Boltzmann distribution, the formation of these voids is probabilistic, and they have only a short lifetime before ongoing thermal fluctuations cause them to disappear. In order for cavitation to occur, in this narrow window of existence the void must be exposed to an external pressure sufficient to cause it to grow. This critical external pressure (Δp_C , known as the Laplace pressure) is given by the expression $\Delta p_C = 2\sigma/R$, which is essentially the tensile strength of the liquid. In a typical fluid the intermolecular separation distance is of the order of angstroms (10^{-10} m), which suggests that rarefactional pressures in excess of 100MPa are required to nucleate cavitation. As void formation is probabilistic, it follows that homogeneous nucleation is unpredictable in nature, and difficult to control.

2.3.1.2 Endogenous Nucleation

Empirically, with the exception of certain experiments under highly controlled conditions that aim to measure the tensile strength of water (Herbert et al., 2006; Crum, 1979), cavitation is observed at pressures below those predicted by the homogeneous theory (Holland et al., 1996). This implies that there is something else present that gives rise to a localised weakness in the medium, for which a number of different mechanisms have been proposed.

The two most commonly cited models are the crevice model of Harvey et al. (1944), and the organic surface skin model of Yount (1979); other proposed models

include nucleation due to spontaneous nuclear fission (Walder and Evans, 1974), or due to cosmic radiation (Sette and Wanderlingh, 1962). None of these models are mutually exclusive, and it is likely that they are all sources of endogenous nucleation to varying degrees in different contexts.

The theory underlying the formation of stable gas cavities in a crevice, and their behaviour when exposed to ultrasound, is summarised briefly here, as it is particularly relevant to the work in Chapter 4 describing the use of solid nanoparticles to facilitate initiation of cavitation in the nucleus pulposus. It was first considered in detail by Apfel (1970). In his analysis, Apfel considered an idealised conical crevice on the surface of a hydrophobic mote with a gas cavity located at the apex, as depicted in Figure 2.2.

The curvature of the interface (R) is governed by the following relationship:

$$P_{\text{hyd}} = (P_{\text{vap}} + P_{\text{aq}}) + \frac{2\sigma}{R} \quad (2.4)$$

where P_{hyd} is the hydrostatic pressure in the liquid, P_{vap} is the vapor pressure of the liquid, P_{aq} is the equilibrium pressure of the gas dissolved in the liquid, and σ the surface tension. This is essentially a restatement of the relation stated in Section §2.3.1.1; note, however, that the sign of the surface tension term has reversed, implying that the surface tension acts to stabilise the inward-curving interface in the crevice, rather than encouraging dissolution as in an outward-curving free bubble.

The interface curvature determines the contact angle between the interface and the solid surface (α in Figure 2.2), which is limited by the surface energetics to values within a certain range. These two factors (interface curvature and contact angle) govern the behaviour of the gas cavity in response to a changing hydrostatic pressure.

To illustrate this effect, consider the case of a cavity during the compression phase of an ultrasound wave. The increased hydrostatic pressure causes the radius of curvature to decrease (in accordance with Equation (2.4)), pushing the interface towards the apex of the crevice and increasing the contact angle. At a certain point, this contact angle hits a limit (the ‘advancing contact angle’), and the only way in which the radius of curvature can continue to decrease is for the gas-solid-liquid contact line to advance further into the crevice.

Conversely, in the rarefactional phase, the radius of curvature increases, often changing sign as it curves outwards until the contact angle reaches a lower limit (the ‘receding contact angle’), and the contact line begins to move toward the mouth of the crevice. When it reaches this point the gas cavity continues to expand until it leaves the crevice and the interface begins to move outwards on the surface of the mote. If the rarefactional pressure is high enough for a sufficient period of time to allow cavity growth to continue, the gas cavity can nucleate cavitation, as depicted and described in Figure 2.3. The subsequent cavitation dynamics differ from those of a free bubble due to the presence of the attached particle, however if the cavity is sufficiently large relative to the particle size the effect is minimal; the bubble can also escape from the particle.

The limiting values of the contact angle are determined by the surface energetics of the interface, which are dependent upon the composition of the solid, liquid and gas phases. A solid is deemed to be hydrophobic if the contact angle (with air and water) is greater than 90° , and hydrophilic if it is less than 90° . On this basis, gas cavities will only be stabilised in crevices on the surface of hydrophobic particles, with crevices in hydrophilic particles rapidly wetting due to dissolution.

Apfel’s analysis, while providing insight into the basis of observed phenomena, was limited in that it considered only an idealised geometry, and a static tensile

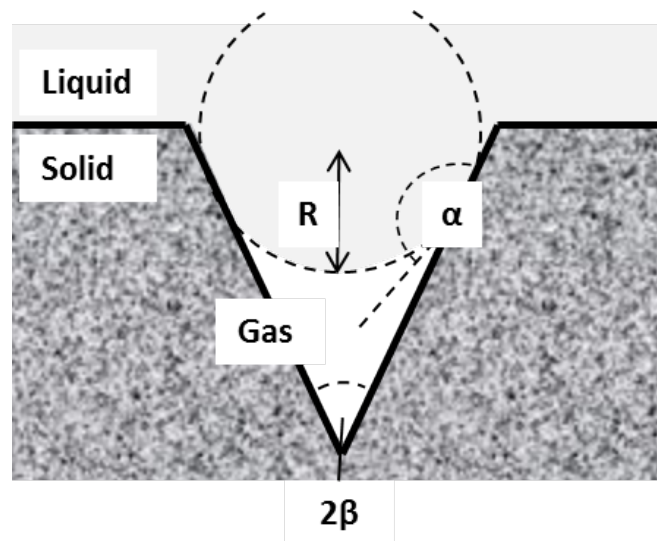


Figure 2.2: An imperfectly wetted conical crevice in a mote, showing the contact angle between the gas-liquid interface and the crevice wall (α) and the crevice angle (2β)

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Figure 2.3: Diagram showing stages in the production of a cavitation nucleus from a theoretical conical crevice (a) an imperfectly wetted crevice with a residual gas pocket; (b) as the hydrostatic pressure is reduced by the ultrasonic field, the contact angle decreases as the gas pocket increases in volume; (c) once the contact angle reaches the receding limit, the gas cavity begins to move out of the crevice; (d) the gas cavity expands out of the crevice and acts as a nucleus for cavitation (adapted from Atchley and Prosperetti (1989))

stress. In his work, the cavitation threshold was taken to be the hydrostatic pressure at which the interface reached the receding contact angle and the contact line began to move. However, Atchley and Prosperetti (1989) point out that this can only be the case if diffusion across the cavity boundary occurs sufficiently rapidly to ensure that the decline in gas pressure inside the cavity is limited. At high excitation frequencies (kHz-MHz) used in medical ultrasound this is less likely to be the case (diffusion takes place over a longer timescale than a single acoustic cycle), and the appropriate limit for cavitation to occur is that there be *unstable* growth of the gas cavity, which can differ from Apfel's criterion. Atchley and Prosperetti go on to identify four distinct nucleation thresholds based on different limiting mechanisms - the unstable movement of the contact line within the crevice; the reaching of a 'critical radius' at which expansion becomes unstable within the crevice; unstable movement of the contact line *outside* the crevice (on the particle surface); and the reaching of a critical radius *outside* the crevice. All four of these conditions must be met before cavitation is observed macroscopically.

Atchley and Prosperetti also consider the effect of changing the angle of the crevice apex, and the angle between the crevice walls and the particle surface, and show that this can affect which of their four mechanisms determines the macroscopic threshold. The effect of shape on threshold was developed further by Chappell and Payne (2007), who consider the effect of going from a conical crevice to alternative geometries. While the results are reflective of the specific geometries selected, the generalisable conclusions of their results are that relatively small changes in crevice geometry can have a large impact on the pressure required to nucleate cavitation.

In any practical situation, where a given volume of liquid will contain a substantial population of motes with diverse crevice sizes, geometries and histories,

cavitation will occur over a range of pressures. Theoretical modelling is not able to give any quantitative predictions on the conditions required to induce nucleation, meaning that any such predictions must be based on empirical observations.

Clinically, the pressures at which endogenous nucleation occurs is known to vary depending on the nature of the tissue and its history. Some tissues such as oxygenated blood or liver produce cavitation at a relatively low pressure (2-4MPa at 1MHz (S. Labouret, personal communication)), while others require a substantially higher pressure amplitude. The underlying causes of this variation are not completely understood. At a macroscopic level the material composition of the tissue is known to be a factor (eg the concentration of collagen (Goss and Dunn, 1980)), as are the dissolved gas concentration, the concentration of hydrophobic chemical species, the structure of the cellular network (Miller et al., 1996), the tissue viscosity, and the temperature. The link between these observations and the microscopic mechanism of action is not well defined. Such a complex mix of factors means that it is not possible to accurately predict *ab initio* the pressure required to induce cavitation in a given tissue in a given patient.

This inherent unpredictability, and the potential for cavitation to cause damage to tissue if not carefully controlled (Coussios and Roy, 2008), means that endogenous nucleation is generally viewed as undesirable in both diagnostic and therapeutic applications of ultrasound. To exploit cavitation under these conditions it is necessary either to operate under extreme conditions where endogenous nucleation is certain to occur (see Section §2.3.2), to develop feedback systems (Hockham et al., 2010) to detect and adjust system performance, or take some other step to make nucleation more predictable.

2.3.1.3 Exogenous Nucleation

An alternative to relying on homogeneous or endogenous nucleation to produce cavitation is to introduce nuclei into the region of interest directly, producing a known population density of these ‘exogenous’ nuclei in the target volume of tissue. If the conditions required to induce these nuclei to cavitate are known, the design of therapies exploiting the potentially beneficial bioeffects of cavitation (described in Section §2.4) can be facilitated.

The simplest approach, that of introducing bubbles of air or other gas into the liquid by releasing bubbles is suitable for inducing cavitation in a sample medium in the laboratory, but the introduction of such ‘free’ gas bubbles into the body (by intravenous injection) is not practical. If the volume of the bubbles is relatively large, such as for diameters in the range 40-50 μm (Kripfgans et al., 2000), they are bouyant and will occlude blood vessels with potentially fatal effects (the injection of air has been a favoured method of murder by villains in literature from Agatha Christie to Kill Bill). Free gas bubbles small enough to avoid these effects (micron-scaled bubbles or ‘microbubbles’) are readily dissolved in blood by the excess internal pressure required to balance the surface tension at the bubble surface, typically in less than a second.

To overcome this, strategies have been developed to stabilise microbubbles against dissolution. The initial impetus was the desire to use the increased reflections from the gas-medium interface at the surface of the stabilised gas pockets to enhance the contrast between blood vessels and bulk tissue, and this has now expanded to include a range of both therapeutic and diagnostic applications, and even so-called ‘theranostic’ particles that combine both functions (Sumer and Gao, 2008). The major classes of exogenous nuclei are described below, together with a brief discussion of the advantages and limitations.

Solid-Shelled Microbubbles The first generation of stabilised microbubbles to become available were based on a similar approach to the ‘surface skin’ model for endogenous nucleation proposed by Yount (see Section §2.3.1.2). Albunex[®], which became available in 1994, was the first product to receive FDA marketing approval. Albunex[®] is an air-filled bubble with a mean diameter of $4.3\mu\text{m}$, surrounded by a shell of aggregated albumin that is approximately 15nm thick (Christiansen et al., 1994).

The albumin shell acts to stabilise the air cavity in two ways. Firstly it reduces the surface tension at the gas-liquid interface - which lowers the pressure inside the bubble (Section §2.3.1.1) and the concentration gradient across the interface, and thus the rate of dissolution of the gas into the liquid. Secondly, the albumin provides a physical barrier to mass transport. The combination of these mechanisms increases the lifetime of these microbubbles from seconds up to 2 years (when refrigerated (Christiansen et al., 1994)), enabling them to be used clinically in a range of applications - most notably contrast enhancement in diagnostic imaging.

However, the albumin shells tend to be rigid (Dayton et al., 1999), which inhibits oscillation in response to stimulation by ultrasound, and particularly their non-linear response. This non-linearity in particular is important to several modern imaging algorithms (such as harmonic imaging), and a number of therapeutic applications. In addition, the use of xenogenic albumin can present immunological issues.

Improvements on the original Albunex[®] microbubbles have included the development of polymeric shell materials (Cavaliere et al., 2008) which can be tailored to control surface, localisation and dissolution behaviours; and the use of gases other than air. Perfluorocarbons are widely used for this purpose, as they have far lower solubility and diffusivity in water than air, and therefore result in more stable mi-

crobbles.

Lipid-Shelled Microbubbles Lipids are a family of large organic molecules that comprise a hydrophobic hydrocarbon chain attached to a hydrophilic ‘head’ group. Due to this ‘amphiphilic’ nature, lipids will tend to spontaneously self-assemble at a liquid-gas interface and reduce the surface energy, stabilising a gas cavity against dissolution.

Since the lipids on the surface of the gas cavity are only loosely associated, such lipid-shelled microbubbles are less stable than the albumin- or polymer-shelled microbubbles described above, and have a shorter lifetime in solution. However, they are easier to produce given that they self-assemble, and are also easier to resuspend. More important from a functional perspective, the thin, loosely associated lipid layer provides much less resistance to oscillation of the gas cavity when stimulated by ultrasound, which gives rise to greatly improved acoustic properties. Such microbubbles are more echogenic than solid-shelled equivalents, and more readily give rise to non-linear effects (Dayton et al., 1999).

Sonovue[®] is a commercially available lipid-shelled microbubble, comprising a phospholipid outer shell surrounding a sulfur hexafluoride (SF₆) cavity, and with a mean diameter of 2.5 μm (Figure 2.4). While it is primarily used as a contrast agent in imaging applications, it has also been used to provide exogenous nuclei for cavitation in therapeutic applications (see Chapter 5).

In a similar fashion to polymer shells, the surface of the lipid shell can be altered to present various functional ligands, either through direct covalent bonding or through the use of various bonding intermediates (e.g. biotin - streptavidin). These ligands can be used to control the fate of the microbubbles within the body, for example using a poly-ethylene glycol coating to enhance circulation

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Figure 2.4: Size distribution of Sonovue microbubbles[®] (Gorce et al., 2000)

time (Langer, 1998), or attaching antibodies specific to surface receptors expressed by cancer cells.

Acoustic Vaporising Droplets Acoustic vaporising droplets, first reported in 2000 (Kripfgans et al., 2000), represent an interesting sub-class of shell-stabilised ‘microbubbles’. Rather than enclosing a gas body, the shell of these droplets (which can be either solid or lipid) encloses a perfluorocarbon emulsion with a boiling point similar to body temperature. When present in the bloodstream, these droplets remain in liquid form due to the surface tension of the droplet and the physical effect of the shell. When exposed to ultrasound, the droplet is perturbed and the liquid vaporises, expanding significantly and creating a microbubble *in situ*.

By expanding only upon the application of ultrasound, these droplets overcome many of the limitations placed upon gas-containing microbubbles by virtue of their size. In particular, this enables them to penetrate into the smallest capillaries, and to naturally extravasate and concentrate in tumours.

The combination of smaller size in non-active form, and the ability to generate nuclei on demand has a lot of potential, and can be exploited to enable localised occlusion of blood vessels (embolotherapy), localised delivery of therapeutic agents encapsulated in the droplets (Rapoport et al., 2009), localised enhanced contrast in imaging applications, and for localised production of cavitation nuclei (Giesecke

and Hynynen, 2003) in therapeutic applications.

Solid Hydrophobic Particles Whilst all of the above sources of nuclei are based on variants of the premise of the surface skin model of endogenous nucleation, an alternative approach is to exploit the crevice model proposed by Harvey et al. (1944) and described in some detail in Section §2.3.1.2. This approach uses solid particles with a combination of a hydrophobic surface and a high surface roughness, which provide a large number of surface crevices in which gas pockets can be trapped. When exposed to ultrasound of high-enough amplitude, these gas pockets can be ‘activated’ to act as nuclei for cavitation.

The advantages of these particles over microbubbles are their size (nm- μ m in diameter), their scattering behaviour (they are weakly scattering until bubbles are nucleated), and the possibility to ‘tune’ their acoustic response by controlling crevice size during the production process.

While they have substantial potential these particles have not been approved for clinical use, and this is an ongoing area of research.

The work described in this thesis uses a novel class of solid hydrophobic particles, which use layer-by-layer techniques (Caruso, 2001, 2004) to control the size and surface properties, and which have never been previously used as cavitation nucleation agents. The production and characterisation of these solid nanoparticles is described in detail in Chapter 4. Their use in providing nuclei to enable cavitation-based therapy is described in Chapter 5.

2.3.2 Cavitation Dynamics

Once it is nucleated, the effect of the cavitating bubble on the surrounding tissue is determined by its response to ultrasonic excitation - that is to say, the *cavitation*

dynamics. It is worth noting that the behaviour of a bubble is not independent of the mechanism of nucleation - a homogeneously nucleated bubble will behave differently to one with a solid or lipid shell. A bubble nucleated from the crevice of a solid particle is expected to behave similarly to a free bubble, since in most cases the size of the solid particle is insignificant compared to the size of the bubble and has only a small effect on bubble oscillation. Since the experimental work in this thesis is primarily concerned with the use of solid particles, the discussion of cavitation dynamics is limited to free bubbles.

In the simplest case of a free spherical bubble in an infinite fluid medium, assuming an incompressible fluid and a bubble radius much smaller than the wavelength of the driving sound, the radial oscillations of the bubble are described by the Rayleigh-Plesset equation (Plesset and Prosperetti, 1977):

$$R\ddot{R} + \frac{3}{2}\dot{R}^2 = \frac{p_g + p_v - p_\infty - p_{ac}}{\rho_L} - \frac{2\sigma}{\rho_L R} - \frac{4\nu_L\dot{R}}{R} \quad (2.5)$$

where p_g is the bubble gas pressure (taken to be $(\frac{R}{R_0})^\gamma$ under adiabatic conditions, where γ is the heat capacity ratio), p_v is the bubble vapour pressure, p_∞ is the ambient pressure, p_{ac} is the acoustic driving pressure, σ is the surface tension coefficient, ν_L is the kinematic viscosity of the liquid, and ρ_L is the density of the liquid.

Both this equation and the widely-used Keller-Miksis equation, which incorporates a compressibility term (Keller and Miksis, 1980), are simplified representations. While this limits their ability to accurately model bubble behaviour in the complex physiological environment, they can be used to identify characteristics of a variety of bubble behaviours that can then be recognised and used clinically. Most notably, as the acoustic driving pressure is varied (for a given frequency), the response of the bubble changes dramatically. This can be used to divide the discus-

sion of cavitation dynamics into two broad categories: *stable* and *inertial* cavitation (Figure 2.5).

Stable Cavitation: At low pressure amplitudes, equation (2.5) is dominated by the pressure terms, and the bubble oscillates periodically about an equilibrium radius (light shading in Figure 2.5). However, the non-linear nature of equation (2.5) means that the oscillation is not necessarily sinusoidal even if the driving wave is. The magnitude of the oscillation is determined primarily by the compressibility of the gas within the bubble (and of course the acoustic pressure), and can create *microstreaming* patterns in the surrounding medium (Tho et al., 2007). The equilibrium radius is determined primarily by the mass of gas present in the bubble, which can decrease over time due to dissolution, or increase by the process known as rectified diffusion (see Brennen (1995)).

Inertial Cavitation: At higher pressure amplitudes, the inertial ($\dot{R}$) terms of equation (2.5) dominate, and the bubble undergoes rapid expansion during the rarefactional phase, followed by a rapid collapse during the compression phase (dark shading in Figure 2.5). This collapse is driven by the inertia of the surrounding fluid, hence the term ‘inertial’ cavitation. The adiabatic collapse can result in extreme pressures and temperatures being reached inside the bubble, giving rise to the unique effects forming the basis for the study of sonochemistry and sonoluminescence. Inertial cavitation is also associated with fluid jetting, shockwave formation, and enhanced heat deposition (§2.3.4.1).

The transition between stable and inertial cavitation is not abrupt, and rather occurs along a continuum (as illustrated in Figure 2.5). For a given excitation frequency, a bubble will initially oscillate linearly at low amplitudes, with non-linear oscillations becoming more prominent as the amplitude is increased. Beyond a certain point the system loses periodicity and becomes more chaotic, eventually

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Figure 2.5: A series of radius-time curves for a $7\mu\text{m}$ bubbles excited at 25kHz for a range of pressure amplitudes (p_a). At low pressures (light shading) the bubble oscillates smoothly with the ultrasound wave (shown at bottom). As the pressure is increased, it begins to exhibit the expansion and collapse behaviours characteristic of inertial cavitation (adapted from Lauterborn and Kurz (2010)).

reaching a stochastic zone where oscillations are completely devoid of periodicity (Ilyichev et al., 1989).

2.3.2.1 Cavitation Thresholds

Even though the transition from stable to inertial cavitation is not sharply defined, the two regimes generate very different effects in the surrounding tissue (§2.3.4), and it is useful to have a means of distinguishing between them. The theoretical definitions that have been proposed for this transition point, known as the ‘inertial cavitation threshold’ or simply the ‘cavitation threshold’, are:

- **R_{max} threshold:** Maximum bubble radius equal to twice the initial radius (Flynn, 1975; Church, 2005)
- **T_{max} threshold:** Maximum temperature within the bubble during collapse equal to 5000 K (Flynn, 1975; Apfel and Holland, 1991)
- **$\dot{R}$ threshold:** Maximum bubble wall velocity greater than the speed of sound in air (Church, 2002)

These criteria have been selected to approximate phenomena known to be correlated with inertial cavitation, and the appropriate criteria for use depends on the

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Figure 2.6: Cavitation threshold in water as a function of initial nucleus radius for three frequencies of sonication in response to a single cycle of ultrasound, assuming that all nuclei sizes exist a priori. At pressures above the threshold a bubble will cavitate inertially, below the threshold stable cavitation will be observed. The shaded region indicates the range of bubble sizes that will inertially cavitate when driven at 5 MHz with amplitude P' (adapted from Apfel and Holland (1991)).

intended application. Using the $T_{\max}$ criterion, Apfel and Holland derived a family of curves that relate the cavitation threshold to the initial bubble size and the excitation frequency (Figure 2.6) for a single excitation pulse. As this illustrates, the cavitation threshold increases with frequency, and there is an optimal bubble size for which the threshold is a minimum at any given frequency.

These calculations require that the properties of both the tissue and bubbles are known to a high degree of accuracy, which is rarely the case. The physical properties of tissue are known to vary substantially between patients and even within a single patient over time, and the size and density of the bubble population in tissue is not known. As a result, the cavitation threshold in biological media is typically measured empirically.

2.3.3 Cavitation Detection and Monitoring

To validate the theories described in the previous section, empirical data on bubble formation, size and oscillation was required. This is collected most readily by using high-speed cameras and other optical systems to directly capture images of bubbles formed in optically transparent materials, which can then be analysed computationally to extract the desired parameters. Such systems need to operate at ex-

tremely high shutter speeds (>4MHz) to be able to capture multiple images within a single excitation pulse, since medical ultrasound systems typically operate at frequencies >1MHz.

Optical systems are very useful in controlled laboratory experiments, but the opacity of biological tissue requires different techniques. Monitoring the acoustic signals produced when tissue is sonicated provides an alternative approach, and insight into cavitation onset and dynamics can be gained from the analysis of these signals. This section first discusses the basis for such emissions, then describes the techniques used for the detection and processing of acoustic signals, and finally considers their use in clinical monitoring.

2.3.3.1 Acoustic Signals from Cavitation

The acoustic signal emanating from a volume of cavitating tissue is typically complex, and interpreting it to gain insight into cavitation behaviour is a challenge. The two main sources of this signal are *secondary acoustic emissions* deriving from the bubble oscillation, and *scattering* of the incident pressure wave from tissue inhomogeneities.

In the absence of attenuation, the oscillation of a bubble will produce a radiated pressure field (P_{rad}) given by:

$$P_{\text{rad}} = \frac{\rho_L R}{d} (2\dot{R}^2 + R\ddot{R}) + O\left(\frac{1}{d^4}\right), \quad (2.6)$$

where ρ_L is the liquid density and d is the distance from the bubble center (Ilinskii and Zabolotskaya, 1992). Since detection is typically done at a distance $d \gg R$, the near-field components (grouped as $O\left(\frac{1}{d^4}\right)$) are often ignored, giving a simplified expression where the radiated pressure at a given distance depends entirely on the radial oscillations of the bubble.

Analysis of radiated signals is typically done in the frequency domain. At low pressure amplitudes, a stably cavitating bubble driven at a frequency f_0 (the *fundamental* frequency) will oscillate linearly, while at higher insonation amplitudes it will undergo both non-linear radial oscillations and non-radial shape oscillations. These oscillations produce acoustic emissions at fractional multiples of the fundamental $n f_0 / m$ ($n = 1, 2, 3, \dots; m = 1, 2, 3, \dots$), where f_0 is the fundamental frequency. The nomenclature applied to these components is as follows: fundamental (or principal) ($n=1, m=1$), harmonic ($n = 2, 2, 3, \dots; m = 1$), subharmonic ($n = 1; m = 1, 2, 3, \dots$), and ultraharmonic ($n=2, 3, 4, \dots; m = 2, 3, 4, \dots$) (Ilyichev et al., 1989). The presence of spikes at these points in the frequency domain of the acoustic signal can be indicative of stable cavitation.

As the pressure amplitude is increased further, spherical bubbles begin to exhibit more characteristics of inertial collapse (Figure 2.5). When this collapse occurs, the velocity of the vessel wall can approach the speed of sound and result in the formation of a shockwave. The collapse, driven by the inertia of the surrounding fluid, happens over a very short time duration, giving rise to a signal that is correspondingly broad in frequency content. This gives rise to an increase in the ‘noise floor’ of the acoustic signal that is uniquely associated with the presence of inertial cavitation (Figure 2.7).

As discussed in Section §2.2, as a wave propagates through tissue energy is redistributed from the fundamental into the harmonics. Any inhomogeneities present in the tissue will act as scatterers, producing a signal dominated by the fundamental, but with similarities to the harmonic content produced by a stably cavitating bubble. Possible scatterers in a clinical situation include, for example, tissue-tissue interfaces, the walls of blood vessels, cells in the bloodstream, and inhomogeneities in the extracellular matrix. Bubbles are also very strong scatterers, and this can result

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Figure 2.7: Comparison of the radiated acoustic signal for increasing sonication intensity (proportional to the square of pressure amplitude) at 1.075MHz: **(a)** 12mW/cm² **(b)** 24mW/cm² **(c)** 60mW/cm². The spectrum in (a) contains primarily harmonics of the fundamental, (b) begins to illustrate increasing ultraharmonic content as the insonating pressure is increased, while (c) shows the increase in the broadband noise floor that is characteristic of inertial cavitation. Note that (c) still contains strong harmonic elements of the signal, which may be due to scattering of the incident wave, or acoustic emissions arising from the bubble oscillations (adapted from Frohly et al. (2000)).

in a single bubble acting as a source of both scattering and secondary emissions, making unique interpretation of the signal difficult. Alternatively, a jump in scattered signal can provide a clear indication of the presence of bubbles, which can be of use in determining either the onset of cavitation or of boiling in thermal ablation (Section §2.3.4.1). This is also exploited in active cavitation detection, discussed in the next section.

2.3.3.2 Acoustic Detection of Cavitation

Acoustic detection of cavitation activity can be done actively or passively.

In Active Cavitation Detection (ACD) a short burst of ultrasound is sent into the tissue, and the returns from bubbles and other scatterers are detected and processed in a manner similar to conventional B-mode imaging. While the use of a transducer array can enable spatial information to be obtained, the applicability of the technique is limited by the need to switch off the primary insonating beam during ACD (Owen et al., 2006) to prevent oversaturation of the detector. ACD thus

provides only a snapshot of the existence of a bubble and its location, and is not able to give any insight into cavitation dynamics and bubble behaviour.

In Passive Cavitation Detection (PCD, note that this abbreviation is used interchangeably throughout this thesis to describe both the technique and the detector itself), a transducer is used to passively receive the acoustic signals produced from within the tissue being insonated. Since this approach is not reliant on the transmission of a separate, short-duration excitation pulse, the signal can be acquired *during* insonication, and therefore provide real-time information on cavitation dynamics and bubble behaviour over time. The receiving transducer is typically selected to have a wide bandwidth, with a centre frequency much higher than the fundamental to reduce saturation from scattering. The signal is usually high-pass filtered prior to digitisation in order to further reduce this effect.

Focussed single-element transducers have typically been used as passive cavitation detectors (PCD), since these can provide information on cavitation activity within a known region, usually selected to be the focal region of the primary insonating transducer. Since the signal received on the PCD is an aggregate signal from all cavitation activity within this focal region, it is not able to resolve signals from distinct bubbles or sources, nor is it able to provide information on cavitation activity occurring outside the focal region.

Passive Acoustic Mapping By using a transducer array rather than a single-element PCD, it has been shown that it is possible to map cavitation activity in two dimensions (Gyongy and Coussios, 2010a,b), and theoretically in three-dimensional space as well. This ‘passive acoustic mapping’ can provide information on the extent of the cavitating region, both inside and outside the focal region of the therapeutic transducer, and resolve separate sources of cavitation noise, all of which is of interest clinically (Section §2.3.3.3). As this technique is central to the experimental

work presented in Chapter 5, the underlying theory is described briefly here.

In mapping, signals from a cavitating source manifest themselves as spatially correlated signals over a receiving array. While these signals can be beamformed to produce an image using conventional B-mode imaging techniques (usually *dynamic receive beamforming*), the resulting images have a low signal-to-noise ratio and a low axial resolution (Farny et al., 2009; Salgaonkar et al., 2009). By using an explicitly broadband and near-field variant of beamforming, so-called *time exposure acoustics* (TEA) (Norton and Won, 2000), it is possible to produce a map of cavitation intensity with much higher signal-to-noise ratio and improved axial resolution (Gyongy and Coussios, 2010b). The intensity calculated for these maps provides an estimate of the cavitation energy deposited into a region of tissue, which can be correlated with various bio-effects.

Mathematically, the algorithm functions as follows. If $u(\mathbf{r}_n, t)$ is the voltage signal (assumed proportional to pressure) recorded at the n^{th} element of the array at position $\mathbf{r}_n$, then an estimate of the signal emitted by a control volume or pixel ($S(\mathbf{r})$) is given by:

$$S(\mathbf{r}) = \sum_{n=1}^N u(\mathbf{r}_n, \frac{|\mathbf{r} - \mathbf{r}_0|}{c} + t) |\mathbf{r} - \mathbf{r}_n| \quad (2.7)$$

where $\mathbf{r}$ is the vector position of the control volume or pixel as described in Figure 2.8, and the $|\mathbf{r} - \mathbf{r}_n|$ term is included to compensate for loss due to spherical spreading. In this implementation there is no allowance for differential attenuation between the paths to different receiving elements, but if this information is available it can be readily incorporated into a (now frequency-dependent) correction factor. If the expression for the delayed time ($\frac{|\mathbf{r} - \mathbf{r}_0|}{c} + t$) is represented by t' , then Equation (2.7) can be written as:

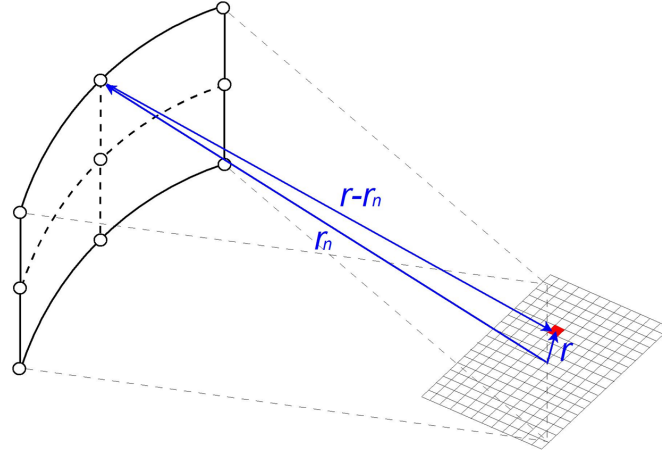


Figure 2.8: Model geometry for the implementation of the Time Exposure Acoustics algorithm, where $\mathbf{r}$ is the vector position of the volume element or pixel, and $\mathbf{r}_n$ is the location of the n th receiving element

$$S(\mathbf{r}) = \sum_{n=1}^N u(\mathbf{r}_n, t') |\mathbf{r} - \mathbf{r}_n| \quad (2.8)$$

The signal is assumed to be a random variable with zero mean, which means that the images cannot be averaged on an amplitude basis, and are instead averaged on an intensity basis (i.e. by averaging $I(\mathbf{r}) = |S(\mathbf{r})|^2$, where $I(\mathbf{r})$ is the intensity). Thus the expression for a source intensity map at $\mathbf{r}$ for the time interval $[t_0, t_0 + T]$ is given by:

$$I(\mathbf{r}) = \frac{1}{T} \int_{t_0}^{t_0+T} [S(\mathbf{r})]^2 dt \quad (2.9)$$

Note that the above expressions are valid for the three-dimensional case - they can equally well be implemented in two dimensions for a linear array, as is the case in the experimental work in this thesis.

This technique has been shown to accurately resolve both continuous and disjoint cavitation regions in agar, as depicted in Figure 2.9 (Gyongy and Coussios,

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Figure 2.9: Example of passive acoustic maps produced of disjoint (left) and a single cavitation region (right). The figure shows a map of acoustic source intensity in the imaging plane of a passive array located at the top of the map. Both maps were produced by insonating with a single focused 1.06MHz transducer; the single region was produced at 1.67MPa PRFP in an agar gel, the disjoint regions at 0.80MPa in channels in an agar gel through which a talc solution was flowing. Note the tight transverse resolution and the axial ‘smearing’ (adapted from Gyongy and Coussios (2010b)).

2010b). This work, which was done with a point source and an adapted linear diagnostic array, demonstrates the limitations of the spatial resolution of the technique. For the configuration used in these experiments the transverse resolution can be sub-millimetric, but the axial resolution, which is determined by the aperture of the receiving array, can be up to 5mm. Work using three-dimensional sparse and random arrays to provide a higher-resolution image without the axial smearing is ongoing (Faragher et al., 2011).

2.3.3.3 Use in Therapy

Detection and monitoring of cavitation is of primary interest in this thesis for its application to therapy in the clinic, and the way it can enable cavitation activity to be either avoided or controlled.

Most current HIFU treatments seek to avoid cavitation due to its unpredictability and potentially damaging bioeffects. In thermal ablation the presence of bubbles can lead to rapid, uncontrolled lesion growth extending pre-focally and damaging tissue outside the target region, or alternatively, it can be an indicator of overtreatment in the case of large boiling bubbles. ACD, with its ability to detect the presence

and location of a bubble region, is widely used for this purpose in preference to passive monitoring since it requires no specialised equipment beyond a conventional diagnostic imaging device, and clinicians are familiar with the presentation and interpretation of images.

However, the limitations of ACD mean that it is much less suited to monitoring therapies that exploit cavitation bioeffects, where more information is required. In such therapies, the two most important requirements are to know the nature of the cavitation activity, and to be able to monitor treatment progress and extent in real time.

Qualitative insight into the nature of cavitation behaviour may be gained from analysis of the signal acquired on a PCD, in line with the theoretical considerations outlined in Section §2.3.3.1. For example, a spike in the broadband components of a signal might suggest the onset of inertial cavitation, whereas a signal dominated by harmonic components may suggest a stably cavitating bubble. Such analysis can only be indicative, because the wide range of bubble sizes, populations and behaviours make it impossible to completely reconstruct the source field, giving rise to multiple possible causes for any observed signal. Any clinical use of such techniques would therefore require a large degree of interpretation on the part of the clinician, and introduce the potential for human error.

In monitoring treatment progression and extent, the goal is to identify a metric based on acoustic emissions that is correlated with treatment success; i.e. a ‘cavitation dose’. Various metrics can be used, the choice between them depending largely on the treatment effect being monitored. For example, it has been demonstrated that in a putative thrombolysis treatment there is a strong correlation between mass loss in the clot and the presence of ultraharmonics in the acoustic signal (Datta et al., 2008), and in thermal ablation that there is a correlation between

the total broadband energy detected, and the size of the resulting lesion (Coussios et al., 2007). These examples are based on the use of a single-element PCD; a passive array has also been used to demonstrate a relationship between total cavitation energy and lesion formation in *ex vivo* liver (Jensen et al., 2012).

It is important to note, however, that these studies have all been performed under controlled experimental conditions and the results are very much a suggestion that such an approach *may* work in a clinical situation, rather than allowing for a definitive statement that it *will* work. The quantitative threshold values or dose-effect parameters are empirical and specific to a particular experimental setup, and can't be directly transferred from one experiment to another, or from an experiment to a patient. Taking this next step to clinical usage requires the development of an approach to calculate the required thresholds pre-operatively on a patient-specific basis, which is far from straightforward; work in this area is continuing.

2.3.4 Effect on Surrounding Tissue

Cavitation is of primary interest to this work because of the range of effects that it can have on tissue, and the potential to exploit these in therapy. This section discusses the physical basis and effect of two of these effects that are most relevant to the experimental work in later chapters.

2.3.4.1 Cavitation-Enhanced Heating

As discussed in Section §2.2, a propagating acoustic wave loses energy to the surrounding medium by attenuation, much of which is converted into heat. The rate of this attenuation, and thus the rate of temperature rise of the medium, is dependent on the nature of the propagating medium, and on the frequency of the acoustic wave. It is this phenomenon that is primarily exploited in the thermal ablation of

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Figure 2.10: An example of the effect of cavitation-enhanced heating. Figures show the temperature rise measured by a thermocouple embedded in an agar-graphite tissue-mimicking material exposed to 1-MHz high-intensity focused ultrasound for 1 s. **(a)** Measured (blue) and predicted (orange) peak temperature rise versus the acoustic peak-rarefaction pressure amplitude. Note the deviation beginning at a pressure of 1.2MPa, which is thought to be due to the onset of cavitation activity. **(b)** Measured temperature versus time for increasing peak-rarefaction pressure amplitudes; each curve corresponds to an increment of approximately 0.1 MPa (adapted from Holt and Roy (2001)).

tissue, also referred to as ‘lesioning’, discussed in Section §2.4.2.

Acoustic cavitation is known to greatly increase the rate of heat deposition in tissue above that predicted by tissue non-linearity and attenuation (Figure 2.10). The three primary mechanisms by which this occurs are multiple scattering, viscous absorption at the boundary layer, and absorption of secondary acoustic emissions (Coussios et al., 2007).

As discussed previously, bubbles act as strong scatterers of an incident sound field due to the large impedance mismatch. When multiple scatterers are present, as in a cavitating bubble cloud at the focus of a HIFU transducer, sound will be bounced back and forth between the scatterers numerous times. This increases the path length over which attenuation can occur, resulting in increased heat transfer to the medium and an increased temperature rise.

Viscous absorption occurs as the bubble oscillates in response to the sound wave, resulting in increased shear within the bubble boundary layers and a higher rate of viscous losses in this region. This mechanism is most prominent when a

bubble is cavitating stably, and as might be expected the rate of heat deposition is highest for larger bubbles (with larger volume boundary layers) and high-viscosity media (Holt and Roy, 2001).

The third mechanism of heat transfer derives from the increase in attenuation with frequency. As discussed previously, a bubble oscillating non-linearly will emit an acoustic signal (Figure 2.7) with significantly more energy at high frequencies than the incident acoustic wave. Since these higher frequencies are attenuated much more rapidly, this results in increased heat deposition and a more rapid temperature rise. From equation (2.6) it can be seen that the level of radiated pressure (and therefore intensity) is dependent upon wall velocity and acceleration; this mechanism is therefore most prominent for an inertially cavitating bubble where the values of these parameters are highest.

The extent of temperature rise in the tissue is governed by the balance between the amount of energy deposited by ultrasound and metabolism, and the amount of energy dissipated by conduction, metabolism and perfusion. This is represented mathematically in Pennes Bio-Heat Transfer Equation (Pennes, 1998):

$$\rho_t C_t \frac{\partial T_t}{\partial t} = K_t \nabla^2 T_t - \omega_b C_b (T - T_\infty) + q_{ac} + q_{met} \quad (2.10)$$

where $T_t(r, t)$, ρ , C and K are the temperature, density, specific heat, and thermal conductivity of either the tissue (subscript t) or flowing blood (subscript b), q_{met} is the metabolic heating rate, and q_{ac} is the rate of heat deposition by ultrasound. The contribution of ultrasound heating is shown here as a single parameter, but will be comprised of terms describing viscous attenuation as well as cavitation-enhanced mechanisms. Full modelling of the system also requires a consideration of convective heat transport in the blood phase (Coussios and Roy, 2008), which is omitted

here for the sake of simplicity.

Cavitation-based effects are capable of enhancing heat deposition at least five-fold (Coussios and Roy, 2008), which can have significant clinical effects. The increased rate of heat deposition reduces the time required for ablative treatments and increases the volume ablated for a given exposure, enabling shorter operations with fewer side-effects. An enhanced rate of heating can also result in the formation of boiling bubbles, which have been associated with the formation of irregular tadpole-shaped lesions and excessive heat deposition in the prefocal region. This further highlights the need for careful monitoring and control of cavitation in a therapeutic context.

2.3.4.2 Mechanical Damage

The creation and subsequent oscillation of a bubble exerts mechanical forces on the surrounding medium, with a number of different physical effects depending on the nature of the bubble and its excitation. The forces involved can be substantial, and the early work on cavitation was largely motivated by attempts to minimise the damage to ship propellers caused by hydrodynamic cavitation. This section focuses predominantly on the analogous ability of acoustic cavitation to mechanically erode biological tissue; more subtle mechanical effects such as microstreaming (Datta et al., 2006) and physical stimulation of cardiac and neural tissue (Dalecki, 2004) are not considered in any detail here.

The two phenomena believed to be responsible for tissue erosion by cavitation are shock wave formation and jetting; a third mechanism of tissue erosion caused by the rapid formation of boiling rather than cavitation bubbles is also described here as it can, somewhat paradoxically, cause predominantly mechanical rather than thermal damage.

Shock wave formation: As a bubble undergoes inertial collapse in a symmetric fashion, for example in a bulk medium with uniform properties, it can emit a shockwave with an extremely high pressure amplitude. This shockwave is very short in duration and can therefore set up large pressure gradients in the tissue around the bubble capable of breaking down the physical structure of the tissue - in effect tearing it apart. However, since this shockwave is attenuated very rapidly, effects are limited to the immediate vicinity of the bubble (μm scale).

Jetting: At a rigid boundary, bubbles collapse in a non-symmetric fashion, which can cause the formation of a jet directed towards the rigid surface (Figure 2.11). The strength of this jet is strongly dependent on the ratio of the distance of the bubble from the wall to the maximum bubble radius (Vogel et al., 1989), and speeds can reach up to 150m/s. When this jet impacts on the surface it can crack the surface or cause pitting, resulting in a pockmarked tissue surface.

Since the individual bubbles responsible for these two mechanisms are small, and the effects are local (μm scale), causing damage at a macroscopic scale requires many of these events, meaning that it requires either an extended exposure time, a large number of individual bubbles (a bubble cloud) or both. This is the case in the technique referred to as *histotripsy* (Section §2.4.3), which has been applied to erode tissue both at tissue boundaries and in bulk tissue.

Clinically, the mechanical effects of cavitation are capable of *fractionating* (equivalently referred to as *emulsifying*, *mechanically ablating* or *homogenising*) tissue, producing lesions with no cellular or matrix structures within them. Fractionation appears to occur at differing rates for different tissue types, and of particular interest to this work it appears that collagenous tissue is more resistant to erosion than bulk cellular tissue (Lake et al., 2008b). Following fractionation, the remnant material within the focal volume has been shown to be micron-sized (Xu

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Figure 2.11: Fluid jetting during the collapse of a 2mm bubble subject to 60Hz ultrasound at 4-5kPa (adapted from Prosperetti (1984)). While the experimental conditions are very different from those used in ultrasound therapy, the same physical phenomenon is exhibited.

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Figure 2.12: Damage to porcine myocardial tissue from a cavitating bubble cloud. (a) shows a region of homogenized tissue, that has been removed by irrigation in (b). (c) shows the histological results at the tissue boundary, indicating a very sharp edge to the treatment region that cuts cells in half (adapted from Parsons et al. (2006)).

et al., 2009), with some sensitivity to the acoustic parameters used. The boundaries of these ablated regions can be very sharp, even bisecting cells (Figure 2.12).

Millisecond boiling: At the high pressure amplitudes that are produced at the focus of a therapeutic transducer, the nonlinear conversion of energy from the fundamental to the harmonics is greatly increased. This can result in the rapid formation of a shockwave in the tissue, greatly enhancing the rate of heat deposition and leading to boiling over a millisecond timescale (Canney et al., 2010). The formation of these shockwaves and the corresponding enhancement in heating occurs in a region only several hundred microns or less in width (due to the shorter wavelength of higher-frequency harmonics), and thus the heated region is substantially smaller than the millimeter-sized boiling bubbles that are created (Khokhlova et al., 2011).

The creation of these bubbles results in damage to the surrounding tissue that is significantly larger than the region of thermal damage, thus using a thermal effect to create mechanical damage.

Two mechanisms have been proposed for the damage that is caused to tissue (Khokhlova et al., 2011). The first proposed mechanism suggests that the rapid expansion of the boiling bubble exerts a shear stress on the tissue that ruptures connective tissue and cell membranes, leading to the emulsification of tissue over multiple cycles. The second mechanism is based upon the experimental observation of cavitating bubbles located pre-focally to the main boiling bubble. It is thought that these bubbles may lead to the weakening of the layer of tissue directly in front of the bubble, allowing the radiation force associated with the shockwave to tear off small fragments and push them into the lesion. This has been characterised as a miniature ‘acoustic fountain’ (Simon et al., 2011).

The tissue fragments resulting from damage by millisecond boiling have been shown to be in the micron-scale, macroscopically similar to those produced by cavitation damage (Khokhlova et al., 2011), and producing a very similar fractionated region of tissue.

2.4 THERAPEUTIC USE OF HIGH INTENSITY FOCUSSED ULTRASOUND

Having explored the nature of cavitation and the effects of a cavitating bubble on tissue, the uses to which HIFU has been put clinically are described in some detail. Of particular interest are the acoustic parameters typically employed in different treatments; the most relevant parameters to therapy are shown in Figure 2.13, and include acoustic frequency (f_{ac}), peak rarefactional focal pressure (PRFP), duty

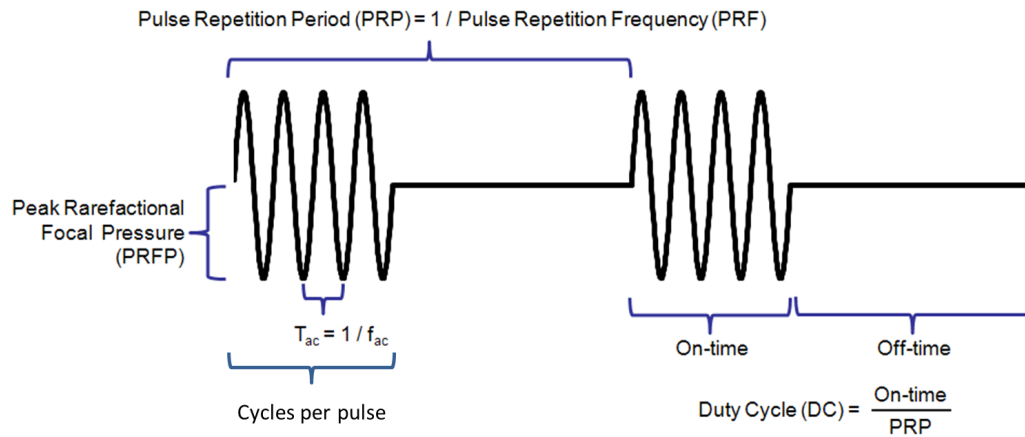


Figure 2.13: Idealized pressure-vs-time waveform elucidating various commonly cited acoustic parameters.

cycle (DC), cycles per pulse, pulse repetition frequency (PRF), and total treatment duration. This section focuses on the therapeutic use of ultrasound at high pressure amplitudes - ultrasound at lower pressure amplitudes is capable of producing a range of other therapeutic effects ranging from bone healing to enhanced gene delivery that are not discussed here.

In each of the treatments outlined below, the ability of ultrasound to treat tissue deep within the body in a *non-invasive* and *non-ionising* fashion is one of the key advantages of the approach relative to alternative therapies.

2.4.1 Lithotripsy

Extracorporeal shockwave lithotripsy was the first clinically applied ultrasound therapy, used for the treatment of gallstones or kidney stones. It is in widespread use, with estimates that over a million patients are treated each year in the US alone. In lithotripsy, very short pulses (μs duration) of a very high amplitude (10s of MPa) are delivered to the body using a focused transducer, creating shockwaves in the target tissue. To eliminate the potential for bubbles at the focus to produce a shielding

effect, an extremely low PRF in the vicinity of 2Hz is typically used.

Typical treatments may take hundreds or even thousands of separate shots to break down the stone, with the progression of treatment directly observed using either ultrasound or fluoroscopic imaging. Five distinct mechanisms of stone destruction ('comminution') have been identified (Bailey et al., 2003): erosion, spallation, dynamic fatigue, shear, and circumferential compression. These mechanisms may be activated to varying degrees by either the lithotripter shock pulse directly, or the subsequent collapse of cavitation bubbles excited by the pulse in the vicinity of the stone.

2.4.2 Thermal Ablation

As was outlined in Sections §2.2 and §2.3.4.1, ultrasound can deposit energy locally in tissue, with the rates of deposition enhanced by the presence of cavitation. Clinically, this can be exploited to thermally ablate tumours and other malignant tissue within the body (Coussios et al., 2007).

In conventional thermal ablation therapy (i.e. without cavitation-enhanced heating), energy is rapidly deposited into a small region at the focal point of the transducer, the size and shape of which is dictated by the spatial distribution of the acoustic field. For a typical single-element 1MHz HIFU transducer, the focus is an oblate ellipsoid with a major diameter of approximately 2mm and a length of approximately 10mm.

The delivery of sufficient thermal energy to tissue can result in *thermal necrosis* or *coagulation*, leading to cell death and ultimately resorption of the dead tissue by the body's natural degradative processes. This is captured in the definition of the *thermal dose*, which is a temperature-time metric referred to as 'Cumulative Equiv-

alent Minutes' (CEM) (Sapareto and Dewey, 1984):

$$CEM_{43} = \int R_T^{(T_{\text{ref}} - T(t))} dt \quad (2.11)$$

where R_T is a temperature dependent parameter typically approximated to 0.25 below T_{ref} and 0.5 above, with T_{ref} usually taken to be 43°C. An empirical threshold of 240 CEM is usually accepted as the level at which thermal necrosis has occurred, although this is now increasingly believed to vary depending on the nature of the tissue and on the rate of heating to which it has been exposed. For example, some tumour cells express particularly high levels of heat-shock protein which can protect them against thermal death, and may require an increased thermal dose to be sure that ablation has taken place.

A second, related process in thermal ablation is the effect of elevated temperature on the extracellular matrix. While this is not an issue for highly cellular tissue such as the liver, in connective tissue with a high proportion of collagen such as ligament, tendon or the IVD it becomes an issue. Collagen is known to coagulate at temperatures above 63°C, losing its structural configuration and forming a shapeless mass, which can result in a loss of tissue function even if the exposure is sufficiently short so as not to reach the CEM threshold.

In a typical protocol for thermal ablation, continuous wave excitation (duty cycle of 1) is used at intensities of several thousand watts per square centimetre - giving pressures in the range of 2-5MPa PRFP - for several seconds to reach the necrosis threshold. Treating a larger region such as a tumour is achieved by moving the transducer (or the patient) to cover the entire tissue volume. The two major drawbacks associated with the technique at present are the time required to create such a large number of targeted lesions, and the difficulty associated with the monitoring of treatment progress and extent in a non-invasive procedure.

Cavitation-enhanced therapy has the potential to greatly increase the rate of focal heating §2.3.4.1, but has not achieved widespread clinical usage due to the unpredictability of endogenously-nucleated cavitation. Scattering of the incident wave from a cavitating bubble cloud is also known to lead to pre-focal heating and a distortion of the lesion back towards the transducer (Coussios and Roy, 2008). One potential means of achieving greater control over cavitational heating is by implementing a feedback-control system (Hockham et al., 2010), and work is ongoing in this area.

2.4.3 Mechanical Fractionation

The mechanisms of mechanical damage described in Section §2.3.4.2 give rise to two distinct treatment regimes for the mechanical fractionation of tissue: histotripsy and millisecond boiling. The majority of this section is focused on histotripsy as there has been a substantial amount of work into the clinical applications of the treatment; the discussion of millisecond boiling is limited to describing the ultrasound parameters used in treatment.

Histotripsy uses an extended series of very short ($<50\mu\text{s}$), very high-amplitude ($>10\text{MPa}$ PRFP) pulses of focused ultrasound to generate bubble clouds within a well-defined treatment region, and of using these bubble clouds to gradually both ‘erode’ material from tissue boundaries, and ‘fractionate’ regions inside bulk tissue. Since the mechanisms responsible for the damage have only a short range, there is the potential to create very tightly-defined treatment boundaries (Figure 2.12), which can make it suitable for use close to sensitive anatomical areas. Thermal treatments, in contrast, will always have a diffuse boundary due to the conduction of heat into surrounding tissue.

The typical treatment parameters, and the effect of varying them are described

in Table 2.1. Thermal effects are avoided by the combination of the short pulse length and low duty cycle, meaning that despite the extremely high instantaneous energy intensity at the focus only a small amount of thermal energy is deposited, and this is allowed to dissipate through conduction and perfusion before the next pulse arrives.

The presence of exogenous nuclei in the tissue has been shown to both enhance the rate of tissue erosion, and decrease the pressure required to bring about cavitation damage (Tran et al., 2003). This is of particular interest to the experimental work presented in Chapter 5.

A versatile procedure, histotripsy is particularly well-suited to debulking of benign tissue, removal or puncturing of membranes, and the fractionation of cancerous tumours. Pre-clinical work to date has been predominantly focused on the treatment of benign prostate hyperplasia (BPH) (Hall et al., 2008; Roberts et al., 2009; Lake et al., 2008a), renal tumours (Hall et al., 2007; Kieran et al., 2007; Lake et al., 2008c), hyperplastic left heart syndrome (Xu et al., 2004, 2007), and thrombolysis (Xu et al., 2009; Maxwell et al., 2009).

Monitoring of the large cavitating bubble cloud created by histotripsy treatment can be readily achieved using conventional active ultrasound imaging techniques, and treatment extent is confirmed by the presence of an anechoic region on a diagnostic image once fractionation is complete.

The proposed use of millisecond boiling for mechanical tissue fractionation differs from histotripsy primarily in two respects. The acoustic pulse that is sent into tissue is three or four orders of magnitude longer (10-40ms) (Khokhlova et al., 2011; Canney et al., 2010) than the μ s pulses sent in histotripsy; and the PRFP is approximately two times lower. Interestingly, and importantly for therapy, the presence

Treatment Parameter	Conclusion	Reference
Number of cycles	Increased extent and homogeneity of damage in focal region with increased number of cycles ; complete homogenization was achieved for 1,000+ pulses	(Roberts et al., 2006b)
Duty Cycle	Thermal effects are negligible below a duty cycle of 4% ; most histotripsy treatments reported to date appear to use duty cycles of 0.1% to 0.4%	(Parsons et al., 2006) (Kieran et al., 2007) (Lake et al., 2008a)
Cycles per Pulse	The rate of tissue erosion is greatly increased when using a lower number of cycles per pulse for a constant duty cycle; maximum effect is seen for 3 pulses per cycle	(Xu et al., 2004)
Pulse Repetition Frequency (PRF)	There is an optimal range of PRF for causing tissue damage. This is proposed to be due to need for a 'just right' bubble population – too low a PRF allows bubbles to dissolve or migrate, too high leads to shielding	(Xu et al., 2004) (Parsons et al., 2006)
Microbubbles	Introducing UCAs into the treatment region can reduce the intensity and duration of insonication required to cause tissue damage	(Tran et al., 2003)
Acoustic Pressures	Studies reported to date have used a PRF in the vicinity of 20MPa or greater	See all of the above
Ultrasound Frequency	Studies to date have used transducers with frequencies in the range of 0.783-1.5MHz	See all of the above

Table 2.1: Overview of studies investigating the impact of various ultrasound treatment parameters on the nature, extent and efficiency of tissue damage caused by histotripsy

and nature of tissue damage can be varied by varying the length of the excitation pulse. If each pulse is shorter than the boiling time for the tissue, no macroscopic tissue damage is observed, although there may be microscopic damage caused by the presence of individual cavitating bubbles. If the pulse length used is close to the minimum required to achieve boiling, a region of non-thermal damage is observed, while if the pulse length is increased beyond the minimum required to induce boiling, it was found to be possible to induce a certain amount of thermal damage in the tissue both within the lesion and surrounding the lesion. Successful clinical use of the technique therefore requires either the accurate knowledge of the time to boiling for the tissue at the focus, or the development of a heuristic procedure for this to be measured at the start of the procedure.

Whilst it has not yet been used in animal or clinical trials, millisecond boiling has been demonstrated to be capable of creating lesions in *ex vivo* bovine liver and heart.

Assuming that millisecond boiling is shown to be viable *in vivo* (and this will require detailed histological work on the residual material inside the lesion that has not been reported), the choice between employing a strategy based on histotripsy and one based on millisecond boiling for an application requiring non-thermal mechanical fractionation of tissue is not clear-cut. It may be that histotripsy is recommended for therapies where thermal damage would have dire consequences, and millisecond boiling where the use of higher pressures could encourage non-focal cavitation damage, but this remains to be seen as both techniques continue to develop.

2.5 IMPLICATIONS FOR THE CURRENT WORK

The objective of the current work is to use HIFU to improve on the treatments available for the treatment of chronic lower back pain. As was highlighted in Chapter 1, NP replacement is a promising approach with the potential to restore normal biomechanical function to the IVD at an early stage of degeneration. Doing so requires the existing degenerate tissue to be removed, and there is an outstanding need for a technique capable of doing this whilst retaining the undamaged external structure of the disc.

Given the discussion of the biomedical applications of ultrasound in this chapter, an obvious candidate approach is to mechanically fractionate this tissue using HIFU reducing it to a liquid consistency and enabling it to be removed through a small-gauge needle. This *minimally invasive* procedure would leave in place the existing tissue of the AF and retain its essential structural integrity, increasing the chance of an injected replacement both remaining in place and restoring normal spinal function to the patient.

Whilst enabling NP replacement is the primary goal of the work presented in this thesis, the results may also be applicable to other treatment strategies for the disc. The mechanical fractionation of NP tissue by HIFU would be expected to have a similar effect to the plasma ablation used in Coblation[®], and may also achieve a similar result to the mechanical removal of tissue in a nucleotomy. These are mentioned here as potential options for future development, and are not expanded upon further in the experimental sections of the thesis.

However, fractionating the IVD with HIFU it is not as simple as taking the published exposure parameters for histotripsy or millisecond boiling and applying them to new tissues. The IVD in particular presents four major challenges that make treat-

ing the region more difficult than bulk liver, kidney or prostate tissue:

- **Acellular tissue:** The NP is primarily composed of PG supported by an amorphous network of collagen fibrils (Section §1.1), with a very low cell density. Given that connective tissue is known to be more resistant to mechanical damage from cavitation than cells, mechanically fractionating the disc tissue is likely to require either more erosive conditions, longer exposures, or both.
- **Hydrostatic pressure:** The high hydrostatic pressure within the NP, even when unloaded, will require correspondingly higher acoustic pressures to produce the same cavitation dynamics. In addition, exogenous nuclei based on stabilised microbubbles are prone to collapse when subjected to an elevated external pressure (Chitnis et al., 2011), making them unsuitable for use as damage-enhancing or pressure-reducing agents (Tran et al., 2003).
- **Sensitive anatomy:** The IVD is surrounded by numerous nervous structures and the spinal column, making the consequences of any damage outside the intended treatment region potentially devastating.
- **Constrained geometry:** The IVD is relatively narrow, and presents a limited acoustic window between the vertebrae through which an acoustic wave can enter the disc (bone is highly reflective). This is further complicated by the presence of the facet joints limiting extracorporeal access to the disc surface posteriorly, and the intestine (containing gas bodies and numerous potential nuclei (Dalecki, 2004)) limiting anterior access.

At the same time, since there are a range of accepted treatments for degenerative discs based on heating and coagulation of the material within the NP (Section §1.3.4), minimising the level of temperature rise and thermal damage surrounding

the lesion is perhaps less critical than in other applications. As long as the heating in the outer regions of the AF is sufficiently low to prevent necrosis of the cell population there, some heating may even necrose intruding nerve tissue and benefit the patient.

Additionally, despite the fact that microbubble-based nuclei are likely to be unsuitable for use under high hydrostatic pressures, the avascular nature of the IVD presents an opportunity to use solid hydrophobic particles (Section §2.3.1.3) as nuclei to enhance the rate of tissue damage. Whereas in vascular tissue these nuclei would rapidly be lost into the systemic circulation and increase the possibility for damage outside the focus, in the IVD they will be retained at the point of injection.

The following three chapters describe the experimental work that has been undertaken to support the central aim of this thesis - demonstrating that HIFU can be used to mechanically fractionate the tissue of the NP whilst leaving the AF intact. Chapter 3 details an investigation of the acoustic properties of the IVD and the implications of this for the design of the treatment array, while Chapter 4 describes the preparation and characterisation of a novel class of solid hydrophobic particles capable of acting as exogenous cavitation nuclei within the disc.

Based on the results of this work, Chapter 5 then describes the design and characterisation of an ultrasound system capable of producing the necessary acoustic pressures inside the IVD to mechanically fractionate tissue, and simultaneously reducing the potential for damage by monitoring treatment inception and location using passive cavitation mapping techniques. This chapter also presents the results of experiments on *ex vivo* bovine intervertebral discs to demonstrate the feasibility of HIFU-based mechanical fractionation.



ULTRASOUND AND THE INTERVERTEBRAL DISC

In developing a technique for disc tissue fractionation by ultrasound, it is essential to first understand the interaction between ultrasound and the IVD. Despite several reports of ultrasound being used to either treat or study the IVD, there has been no detailed investigation of the acoustic properties of the tissue. This chapter presents the results of experiments using a scanning acoustic microscope to measure the speed of sound and attenuation of disc tissue, and how these two properties vary across the profile of the IVD. These results are then used in a simplified model to investigate the acoustic field produced inside the IVD by different configurations of transducer. The highly attenuative nature of disc tissue and the large variation between regions of the disc both present challenges for the design of a therapeutic device.

This chapter has been submitted for publication in an alternative form in *Ultrasound in Medicine and Biology*.

In Chapter 1 the pathophysiology of the degenerate disc, and the role that it plays in chronic lower back pain, was discussed. Current treatments were outlined, and in particular the potential for NP replacement during early-stage degeneration to restore ‘normal’ function to the spine. To enable this procedure, there is an outstanding need for a non-invasive procedure capable of removing the degenerate tissue. Chapter 2 discussed the ability of high-intensity focused ultrasound (HIFU) to mechanically ablate tissue in a tightly controlled region, and highlighted this as a

potential means of ablating degenerate tissue in the disc.

In order to determine whether this technique can be adapted from soft tissue (in which it has been applied to date) to the acellular tissue of the IVD, the first step is to understand how ultrasound propagates through the disc, and the pressure amplitudes and field shape that can be achieved. A modified scanning acoustic microscope (SAM) was used to measure the acoustic properties of the disc, and their variation across the different regions of the IVD. Using these values, the acoustic field produced within the disc by several different transducer configurations was then modelled using a commercially available modelling software package. The outputs of this modelling were compared against empirical measurements to validate the results, and used to design the setup used in the experimental work described in later chapters.

This chapter is structured in three parts: the first describes the measurement of the acoustic properties of the disc, the second the modelling work, and the final section the experimental validation and implications for the design of a therapeutic setup.

3.1 MEASUREMENT OF THE ACOUSTIC PROPERTIES OF THE IVD

The measurement of the acoustic properties of tissue is important for both diagnostic and therapeutic applications of ultrasound. The accurate modelling of acoustic fields for the design of transducers and arrays requires a detailed knowledge of the acoustic properties of tissue, as does the creation and interpretation of accurate images from signals reflected or produced from within the tissue. Additionally, since the acoustic properties of tissue are determined by the physical properties of the

tissue, the measurement of these properties can be diagnostic or at least suggestive of a range of pathologies (Kuc and Schwartz, 1979).

The primary parameters of interest are typically the speed of sound (c), attenuation coefficient (α) and its frequency dependence, and the tissue non-linearity (B/A). A number of techniques have been developed to measure them, ranging from destructive *ex vivo* techniques to approaches capable of measurement *in situ* and *in vivo*.

The IVD is a challenging tissue to measure the acoustic properties of, as it is relatively small and with a large variation in material properties over a short distance. This is further complicated by the gelatinous nature of the NP making it difficult to handle physically, and the high swelling pressure limiting the range of transmission media that can be used to couple the transducer to the tissue.

In these experiments, a modified SAM was used to measure the speed of sound and attenuation for a cross-section of disc at a sufficiently high resolution to capture the variation in properties between the regions of the disc. In the measurements, the tissue is placed onto a highly (acoustically) reflective surface and placed under a high-frequency ultrasound transducer. A short pulse is sent from the transducer, and the reflection from the glass plate with the tissue in place is compared with that from just the glass to determine the acoustic properties (Section §3.1.1.7).

3.1.1 Experimental Setup

3.1.1.1 Scanning Acoustic Microscope

A schematic of the experimental setup up is shown in Figure 3.1. All measurements were carried out in either an acrylic tank or a glass beaker filled with iso-osmotic medium (see Section §3.1.1.5), with an ultrasonic absorber placed placed

in the base of the vessel to minimise stray reflections. The tissue was mounted on a glass microscopy slide resting on a flat acrylic plate. The acrylic plate was mounted on a goniometer stage (GN1/M, Thorlabs, Newton, NJ, USA) attached to a one-dimensional translation stage allowing the tissue sample to be tilted in two dimensions and shifted vertically.

A spherically-focused broadband PVDF transducer with a centre frequency of 35MHz (Olympus NDT, Essex, UK) was mounted above the sample holder on a software-controlled, three-dimensional motorised positioning system of sub-micron precision (Z825 and TST001, Thorlabs, Newton, NJ, USA) controlled by a PC using activeX controls in a Matlab user-interface. The transducer was mounted with its axis perpendicular to the tissue surface, and driven in pulse-echo mode using a high-frequency pulser-receiver (DPR500, JSR Ultrasonics, NY, USA). The waveform received by the pulser-receiver was recorded by a digital oscilloscope (1GHz bandwidth, 8-bit, Wavesurfer 104Mxi, LeCroy, NY, USA), and transferred to a PC (XPS, Dell, Round Rock, TX, USA) via TCP/IP for storage and further processing.

The scanning and acquisition process was controlled using a custom GUI created in Matlab (Mathworks, USA).

3.1.1.2 Transducer Characterisation

The size of the focal region of the transducer was measured by using the tip of a 100 μ m needle (Hamilton Company, Bonaduz, Switzerland) aligned along the transducer axis as a scatterer, and scanning this throughout the focal region. The results are shown in Figure 3.2, which shows a FWHM in the transverse (x and y) dimensions of approximately 240 μ m, and in the axial (z) dimension of approximately 7mm.

The implications of this result are twofold. Axially, the depth of the focal region is such that it can be assumed that the ultrasound wave incident upon the

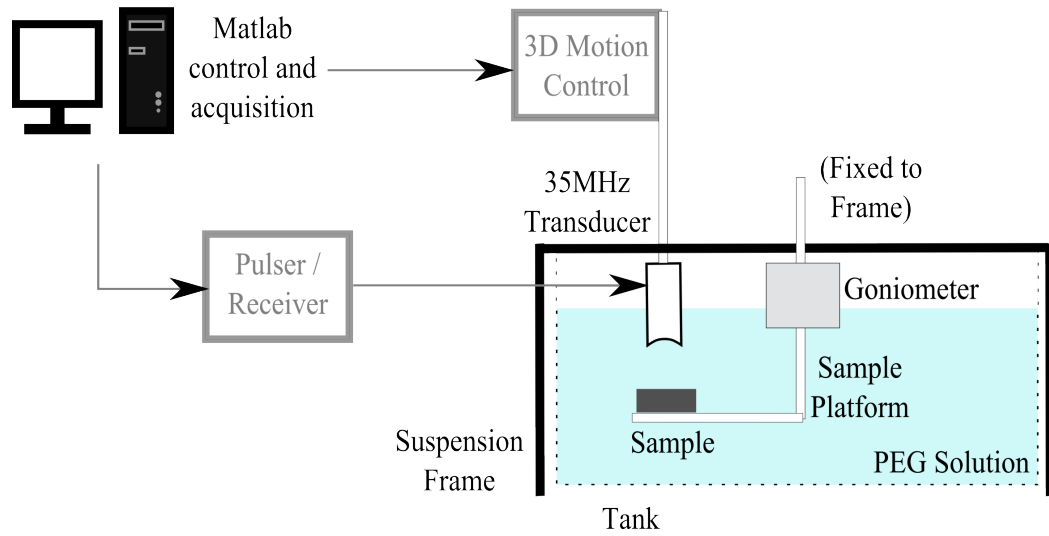


Figure 3.1: Schematic diagram showing the experimental setup of the SAM used in measuring the acoustic properties of disc tissue.

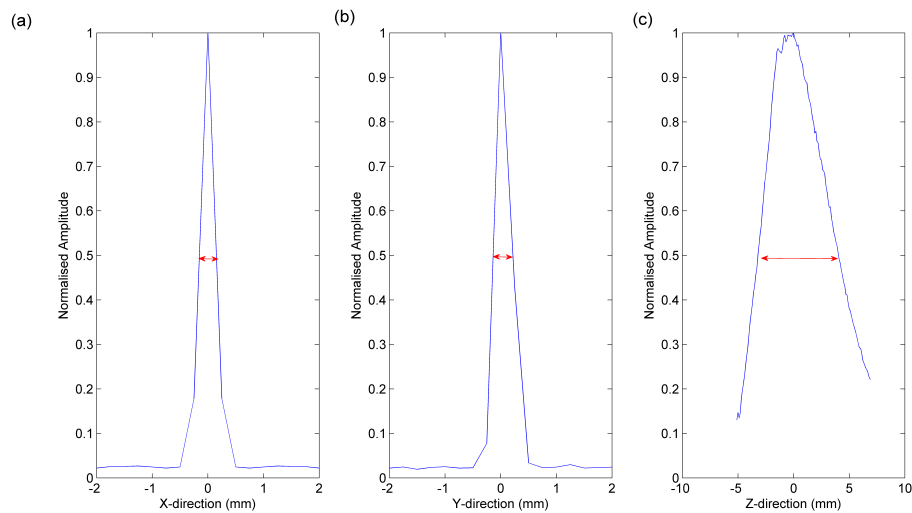


Figure 3.2: Normalised pressure fields along the three dimensions of the acoustic field produced by the 35MHz transducer used with the SAM, as measured by scattering from the point of a $100\mu\text{m}$ needle. (a) and (b) show the narrow transverse extent of the field; (c) the longer axial extent of the field

tissue is effectively constant over a distance of approximately 3mm (the thickness of the tissue), meaning that it can be assumed to behave as a plane wave in this region. Further, the transverse beam dimensions determine the spatial resolution of the measurement, and the number of steps required to completely characterise the sample.

3.1.1.3 Choice of Tissue Model

As complete human discs were not available, it was necessary to identify a suitable substitute. While agar and polyacrylamide are commonly used in acoustic experiments as mimics for soft tissue, there is no equivalent artificial mimicking material for the IVD with its complex mechanical properties and non-uniform structure. As such, most experimental investigations of the biology and mechanical properties of the IVD have been performed using animal models. The characteristics of animal models commonly used in IVD research have been well reviewed previously (Alini et al., 2008).

The major limitation of using animal models of the disc is that very few are known to exhibit degeneration, and even where it does occur it is not clear that it has the same causes or progression as in humans. In many investigations this is addressed by treating an otherwise healthy animal disc to mimic one or more of the changes in the disc associated with degeneration, but even this has its limitations. For example, partial digestion with proteolytic enzymes (such as chymopapain) to break down PG molecules may replicate the reduced PG content of a degenerate disc, but not any of the conditions of reduced nutrient supply or changes in cell phenotype. Another commonly used approach is the so-called ‘injury’ model, often used with rabbits (Masuda et al., 2005), where the disc is stabbed with a scalpel. Penetration all the way through the AF results in the relatively rapid degeneration of the

disc (within 2-8 weeks) which can then be used as a pre-clinical testing platform for regeneration therapies.

However, as Alini points out Alini et al. (2008), ‘no one model results in an identical situation to that found in the human disorder... a compromise is the best that can be achieved and the choice of the optimum model will depend on the questions being asked’.

In this work, the two major questions relating to the disc model are whether it is possible to induce inertial cavitation inside the disc without damaging the surrounding tissue, and whether this cavitation can produce fractionation of the NP. To answer these, it is important that the model have similar dimensions and disc geometry, and similar mechanical properties and biochemical composition to the degenerate human disc. After consideration of the possible alternatives, healthy bovine coccygeal discs were selected as a model.

The diameter of bovine coccygeal discs (25-30mm) is comparable, and although they are slightly thinner than healthy human discs (8-10mm compared to 12-14mm), the aspect ratio is more similar to humans than for other large animals (Alini et al., 2008). The aspect ratio is of particular importance in the experimental work detailed later in this thesis, as the target ‘window’ between the vertebrae is a factor in determining the proportion of acoustic energy that can be delivered to the focus. Since the narrowing of the disc space is one of the major symptoms of disc degeneration, the lower aspect ratio of bovine discs makes them somewhat representative of a degenerate disc.

Biochemically, bovine coccygeal discs have been shown to be quite similar to human discs in the type and distribution of aggrecan and collagen, in proteoglycan synthesis rates, and in matrix synthesis responses to hydrostatic pressure (Demers

et al., 2004). This suggests a similar cell population, which is supported by the finding that bovine IVD cells have a phenotype more similar to human than other animal models (Horner et al., 2002).

Mechanically, the bovine IVD is believed to have a similar internal hydrostatic pressure to healthy human discs (0.1-2.5MPa). In contrast to the human disc, where much of this pressure comes from the load-bearing nature of the spine as it supports the upper body, this pressure is produced by the muscles and ligaments that compress the discs of the tail. This hydrostatic pressure is important, as it affects the acoustic pressure required to produce the necessary tension in the tissue for cavitation nucleation.

The major difference between the healthy bovine coccygeal disc and the degenerate human disc is in the hydration of the NP: the healthy IVD is substantially more hydrated than the degenerate one. This will have an impact on both the cavitation behaviour and the response of the tissue to cavitation, and it is addressed throughout this thesis in the discussion of the experimental results and the extent to which they can be generalised.

3.1.1.4 Tissue Preparation

Bovine tails were obtained from the abattoir (Mutch Meats, Witney, UK) within 4 hours of slaughter. Overlying fat, muscle and ligament tissue was removed, and the tail separated into individual motion segments by cutting through the centre of vertebrae using a hacksaw.

Discs to be used for cross-sectional measurements were wrapped in a moist paper towel and clingfilm before being placed at -20°C for up to 7 days. Immediately before measurement discs were removed from the freezer, and uniform 2-4mm sections were cut by hand using a sharpened knife before the NP became fully defrosted

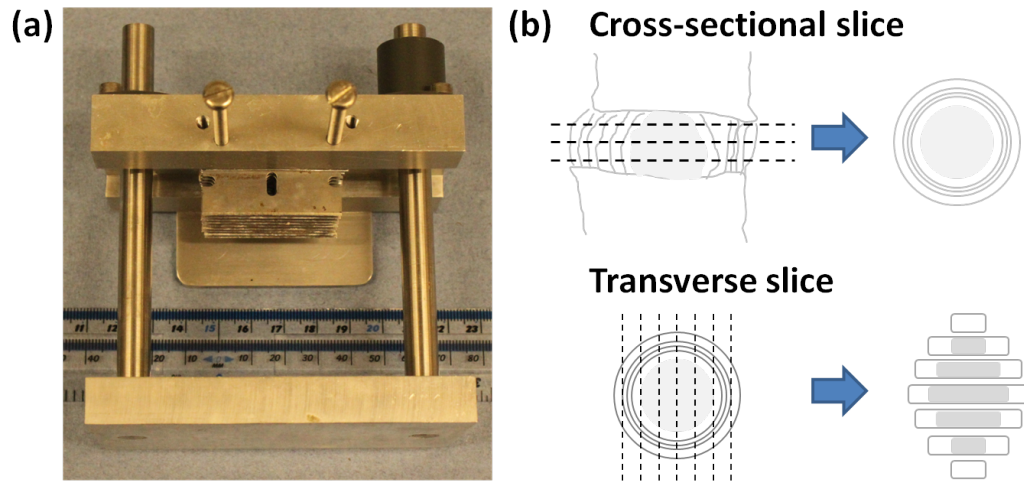


Figure 3.3: **(a)** Custom-built device for cutting uniform thickness slices of IVD for transverse measurement. The razor blades are removable, and are separated by metal dividers of a known thickness to vary the slice thickness. To prevent the disc defrosting and deforming when removed, the device was placed at -20°C for 30 minutes prior to use; **(b)** Illustration showing how cross-sectional and transverse slices were taken from discs. The dotted lines on the left show the lines along which slices were taken; the resulting slices are shown on the right.

and deformable. During cutting care was taken to ensure that the surfaces of the disc cross-section were parallel.

Discs used for transverse measurement were removed from the vertebrae and wrapped in a moist towel and clingfilm prior to freezing at -20°C . Immediately prior to measurement the discs were partially defrosted and cut into slices of controlled, uniform thickness (2-4mm) using a custom-built device (Figure 3.3(a)). The device was cooled to -20°C prior to cutting to prevent the disc sections from defrosting and then deforming when removed from between the blades.

Figure 3.3(b) provides an illustration of how the transverse and cross-sectional slices were obtained.

The tissue thickness was chosen so as to be sufficient to allow for resolution between the reflections from the front and back faces of the tissue in the time domain

(dictated by the pulse-length from the transducer), but thin enough not to completely attenuate the signal reflected from the back surface of the disc.

Cross-sectional slices from a total of 16 discs from 5 different animals were prepared; transverse slices were prepared from a total of 11 discs from four different animals.

3.1.1.5 Iso-Osmotic Medium

The high concentration of PG in the IVD (the NP in particular) gives rise to a high charge density and therefore a high osmotic swelling pressure (Urban et al., 1979), seeking to draw in water from surrounding tissue. In the static disc, this swelling pressure is balanced by the external load placed upon the disc by the muscles and ligaments that hold the vertebrae together, and the weight of the upper body. Interestingly, when the weight of the upper body is taken off the spine the IVD takes in water and expands. Thus it is regularly observed that people will gain several centimetres in height while sleeping, and the Skylab astronauts were anecdotally reported to gain 5cm in height over their 85 days in a weightless environment (Urban and Maroudas, 1981).

This presents an issue for the current work, as placing unconstrained IVD in either water or physiological saline (the most commonly used coupling/transmission media) will result in substantial and rapid swelling, changing the acoustic properties. In previously documented work with discs where swelling has been considered, it has been prevented by the application of an axial mechanical load (Urban and Maroudas, 1981) or osmotically by placing the IVD inside a dialysis sac placed in a solution with a known concentration of polyethylene glycol (PEG) and sodium ion (Urban and McMullin, 1988).

Neither of these approaches are suitable for measuring tissue properties using

the SAM: an axial mechanical load does not allow a beam path for the ultrasound to pass through the disc, while the dialysis sac introduces additional interfaces that interfere with the interpretation of reflections. As a proxy, the disc was placed directly into a solution of iso-osmotic PEG, without a surrounding dialysis sac. This approach does not prevent the loss of PG from the disc that has been reported under such conditions; to mitigate this effect measurements were taken as quickly as possible after the disc was placed into the solution, since leaching is known to take place over a period of several hours (Urban and Maroudas, 1981).

To determine the concentration of PEG required to balance the osmotic pressure of the IVD, fragments (roughly one quarter of a disc) of bovine coccygeal discs acquired and prepared as outlined above were first weighed and then each fragment placed into a vial with a known concentration of PEG (MW 20k, Fluka, Sigma Aldrich, St Louis, MO, USA). The fragments were removed at periodic intervals, blotted dry, and weighed before being placed back into the vial. A hypotonic (ie too high) concentration of PEG will cause a disc to shrink and decrease in weight as it loses water, while a hypertonic concentration will result in a weight increase. The results of the experiment are shown in Figure 3.4.

As a result of these experiments it was decided to use a PEG concentration of 0.167g PEG / g of solute. Sodium chloride at a concentration of 0.15M was used to minimise the loss of sodium ion from within the disc tissue.

The speed of sound and the attenuation of the PEG solution were measured using a substitution method. In short, an unfocused ultrasound transducer with a 5MHz centre frequency was placed in a tank of distilled, degassed water, and aligned with a 200 μ m needle hydrophone (S/N 1360, Precision Acoustics, UK). The transducer was driven with a broadband pulser-receiver (DPR300, JSR Ultrasonics, NY, USA), and the hydrophone connected to a preamplifier and the received signal dis-

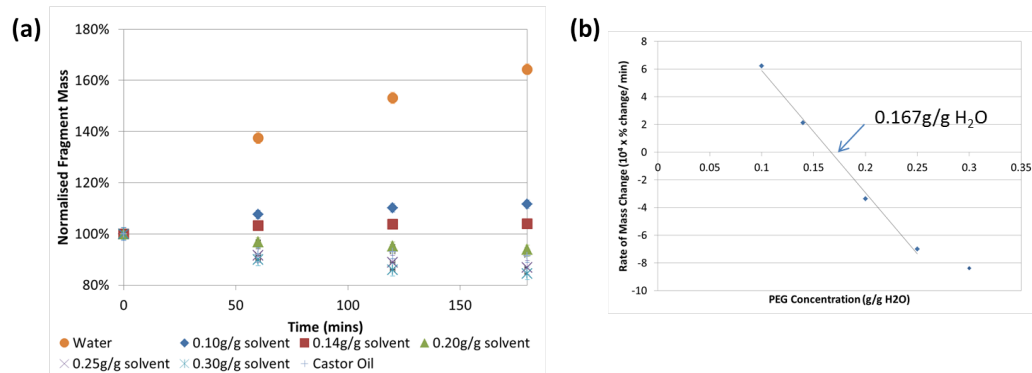


Figure 3.4: Determination of the iso-osmotic concentration of PEG required for measurements. (a) shows the change in normalised fragment mass over time ($n=2$) for different concentrations of PEG, while (b) shows the rate of change of normalised mass plotted against PEG concentration. The zero-crossing of a linear fit to the data in (b) is at $0.167\text{g}_{\text{PEG}}/\text{g H}_2\text{O}$.

played on an oscilloscope (Waverunner, LeCroy, NY, USA) triggered by the pulser-receiver.

Two different holders with acoustically transparent mylar faces a known distance apart (20mm and 35mm) were separately placed between the transducer and the hydrophone, and alternately filled with distilled, degassed water and the PEG solution. For each solution, the trace from the pulser-receiver and the hydrophone were recorded at 3-6 different points within the holder to minimise the effect of any imperfections in the mylar sheet. The speed of sound was determined by comparison of the time-of-flight for water and the PEG solution, and averaged between the measurements for the different holders. For the attenuation measurements the hydrophone was moved to give the same time-of-flight as for the water to minimise diffraction effects. The difference between the hydrophone signal for the two thicknesses of PEG solution was calculated to eliminate the effect of surface reflections, then converted into the frequency domain and the power spectrum normalised by the water spectrum to obtain a measure of the frequency dependence of attenuation. This relationship was then used to calculate the attenuation of the PEG solu-

tion at frequencies outside the range directly measured here, assuming consistent behaviour over the frequency range of interest.

One alternative to PEG that was considered was to use a non-aqueous oil to couple the transducer to the IVD, on the premise that this would not affect tissue hydration levels. Both castor and cottonseed oil were considered and their properties measured, however they were found to be too highly attenuating to allow for measurement on the SAM system that was available.

The results for the PEG solution compared to water are shown in Table 3.1. The measured values for cottonseed and castor oil are also shown for the purpose of comparison. The values for castor oil compare reasonably well with the values quoted in the literature (Kaye&Laby, 1995; Shore et al., 1986); no literature values for cottonseed oil at 1MHz could be found.

Medium	Speed of Sound (m/s @ 20°C)	Attenuation (Np/cm @ 1MHz)	Frequency Dependence
Water	1484	2.53×10^{-4}	2
PEG	1581.6	6.9×10^{-4}	1.95
Castor oil	1527	6.25×10^{-2}	1.80*
Castor oil (literature)	1494	9.6×10^{-2}	1.75
Cottonseed oil	1479	7.4×10^{-3}	2.07*

Table 3.1: Comparison between the speed of sound, attenuation and the frequency dependence of attenuation for potential media for the IVD. (*) signifies that the ability to measure these parameters was limited by high attenuation

3.1.1.6 Experimental Protocol

For the measurement of the properties of each sample of disc, the tank or beaker was filled with isotonic PEG, and an uncoated crown glass microscope slide (VWR, Radnor, PA, USA) was placed on the acrylic plate. The plate was positioned close to the acoustic focus of the transducer, and the goniometer adjusted to ensure that

the microscope slide was perpendicular to the transducer axis in both transverse dimensions. This was achieved by monitoring the time-of-flight of the reflection from the glass surface as the SAM was scanned through the full 25mm range of the motorised stages. The transducer was then scanned 5mm each way in the axial direction about the focus in 100 μ m increments, and the reflected ('reference') signal from the glass slide recorded.

The partially-defrosted disc section prepared as per Section §3.1.1.4 was placed onto the microscope slide. Any surface bubbles adhering to the tissue were gently removed using a pipette. In the 5-10 minutes required for the tissue to defrost and reach thermal equilibrium with the PEG solution, the slide was positioned so as to maximise the surface of the disc within the range of motion of the motorised stages. The position of the slide on the z axis was selected so as to place the focus of the transducer in the centre of the disc thickness.

Scan parameters were set using the user interface, and the scan run to completion (Figure 3.1). The typical scan ran for 25mm in each of the x and y dimensions, with a step size of 0.5mm giving approximately 2500 measurement points.

3.1.1.7 Signal Processing

The signal processing is based on a substitution approach, comparing the time and frequency domain data obtained with the disc tissue in place to the reference data taken from the glass slide. The time domain data is used to determine the tissue thickness, the speed of sound and the 'bulk' attenuation coefficient in a similar fashion to the time-domain reflectometry approach outlined by Daft et al. (1989), with the frequency dependence of the attenuation coefficient determined from frequency domain data (D'Astous and Foster, 1986). The calculation approach is described briefly below - Figure 3.5 gives an example of the signals received from the

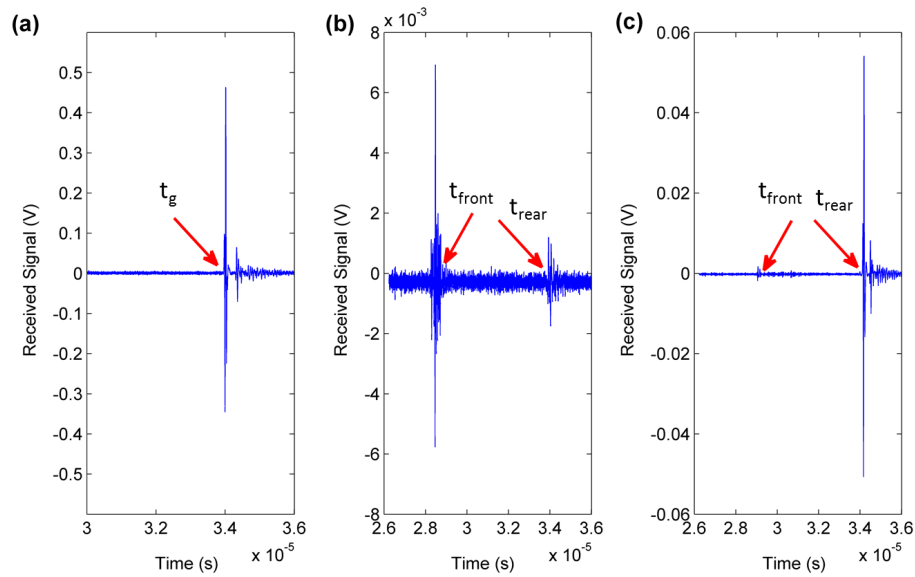


Figure 3.5: Signals reflected from (a) a glass microscope slide; (b) a slide supporting AF tissue, and (c) a slide supporting NP tissue. Note the clear separation in the time domain between the signal reflected from the PEG-tissue interface (first peak) and the tissue-glass interface (second peak) in both (b) and (c), and the difference in the amplitude of the surface reflections between the two tissue samples

SAM and the terminology used in processing.

It is worth noting here that all of these calculations are based on three key assumptions: that the wave in the region of the tissue can be considered a plane wave and that therefore the effects of diffraction can be ignored (Cloostermans and Thijssen, 1983); that the effect of dispersion is minimal (Verhoef et al., 1985); and that the tissue and medium behave linearly.

Filtering, Windowing and Peak Estimation The signal received from the pulser-receiver was passed through a bandpass filter implemented in software (Matlab, Mathworks, MA, USA). A Kaiser window filter was used in zero-phase implementation, with the pass band set at 5-50MHz with 60dB dropoffs over a 3dB range at the edges of the band.

The filtered signal was then windowed to define the regions where reflections

from the front and back surface of the disc are expected. The boundaries of these windows were defined manually based on visual examination of a trace where both signals are clearly visible; the same windows were applied to all traces for a given tissue sample.

The timing of the reflected signal from the front (PEG/disc) and rear (disc/glass) surfaces was extracted by first subtracting any dc bias from the signal and then finding the local maximum value of the signal. If this value was found to be below a threshold value related to the level of background noise it was ignored, and subsequent calculations were not performed for that point.

Parameter Estimation Each of the ultrasonic parameters was calculated for each of the points (i, j) in the two-dimensional scan over the surface of the disc.

Disc thickness ($d_{\text{disc}}(i, j)$) was estimated by comparing the timing of the reflection from the surface of the disc to the reflection from the glass slide without the disc present (Daft et al., 1989):

$$d_{\text{disc}}(i, j) = c_{\text{PEG}} \left(\frac{t_{\text{g,mean}} - t_{\text{front}}(i, j)}{2} \right) \quad (3.1)$$

where c_{PEG} is the speed of sound in PEG, t_{front} is the time of the peak reflected from the surface of the disc, and $t_{\text{g,mean}}$ is the time of the peak reflected from the glass slide without disc present.

The speed of sound in the disc ($c_{\text{disc}}(i, j)$) at each point in the scan was estimated from the difference between the timing of the reflection from the glass plate with and without the disc present, by the following relationship:

$$c_{\text{disc}}(i, j) = c_{\text{PEG}} \left(\frac{t_{\text{g,mean}} - t_{\text{front}}(i, j)}{t_{\text{rear}}(i, j) - t_{\text{front}}(i, j)} \right) \quad (3.2)$$

where definitions are as previously, and t_{rear} is the time of the reflection from the disc / glass interface.

The specific acoustic impedance at each point on the disc was calculated from the amplitude of the pulse reflected from the disc surface. Assuming a plane wave normally incident on a surface, the reflection coefficient (ratio of reflected pressure amplitude to incident pressure amplitude) is given by:

$$R = \frac{A_{\text{reflected}}}{A_{\text{incident}}} = \frac{z_{\text{reflecting}} - z_{\text{trans}}}{z_{\text{reflecting}} + z_{\text{trans}}} \quad (3.3)$$

where $A_{\text{reflected}}$ and A_{incident} are the reflected and incident pressure amplitudes, and z_{trans} and $z_{\text{reflecting}}$ are the specific acoustic impedance of the transmission medium and the reflecting medium respectively. The impedance for an unknown material (in this case the disc) can be determined by comparison of the reflected pressure amplitude with that of a known material at the same time-of-flight from the transducer. The ratio between the two reflected amplitudes is given by:

$$\frac{A_{\text{reflected,ref}}}{A_{\text{reflected,unk}}(i, j)} = \frac{(z_{\text{ref}} - z_{\text{trans}})(z_{\text{unk}}(i, j) + z_{\text{trans}})}{(z_{\text{ref}} + z_{\text{trans}})(z_{\text{unk}}(i, j) - z_{\text{trans}})} \quad (3.4)$$

where the ref, trans and unk subscripts refer to the reference material, transmission medium and the unknown material respectively. Algebraic rearrangement gives the following expression for the impedance of the unknown material:

$$z_{\text{unk}}(i, j) = z_{\text{trans}} \left(\frac{\alpha + \beta}{\alpha - \beta} \right) \quad (3.5)$$

where $\alpha = A_{\text{reflected,ref}} \times (z_{\text{ref}} + z_{\text{trans}})$ and $\beta = A_{\text{reflected,unk}}(i, j) \times (z_{\text{ref}} - z_{\text{trans}})$.

Two attenuation coefficients were calculated - a *bulk* coefficient based on the overall reduction in signal amplitude in the time domain and which is specific to the experimental setup, and a *frequency dependent* coefficient calculated from the changes in the power spectrum between the signals with and without tissue in place.

The aggregate attenuation coefficient (α) for each point is given by:

$$\alpha_{\text{unk}}(i, j) = \alpha_{\text{trans}} - \left(\frac{0.5 \ln \frac{A_{\text{disc}}(i, j)}{\gamma_{\text{corr}}(i, j) A_{\text{ref}}}}{d_{\text{disc}}} \right) \quad (3.6)$$

where α_{trans} is the attenuation coefficient of the transmitting medium, A_{disc} is the reflected pressure amplitude from the glass plate with the disc in place, A_{ref} is the reference reflected pressure amplitude from the glass plate at the same time-of-flight, and γ_{corr} is a factor to account for the proportion of acoustic energy reflected from the disc surface:

$$\gamma_{\text{corr}}(i, j) = \frac{4z_t z_d(i, j)(z_g + z_t)}{(z_d(i, j) + z_t)^2 (z_g + z_d(i, j))(z_g - z_t)} \quad (3.7)$$

where the t , d and g subscripts refer to the transmission medium (PEG), disc and glass plate respectively.

The frequency-dependent attenuation coefficient was determined by following a similar approach to that described by D'Astous and Foster (1986), modified slightly to include a term to account for the attenuation by the PEG in the reference sample. In short, the magnitude spectrum of the reference pulse ($S_{\text{ref}}(f)$) reflected from a glass slide is compared to the same pulse reflected from the same glass slide with the tissue in place ($S_{\text{sample}}(f)$), which upon re-arrangement gives the following expression for the attenuation coefficient:

$$\alpha(f)_{\text{sample}} = \alpha(f)_{\text{trans}} - \frac{1}{2d_{\text{disc}}} \ln \left(\frac{S_{\text{sample}}(f)}{S_{\text{ref}}(f)} \right) \quad (3.8)$$

where f is frequency, $\alpha(f)$ is the frequency-dependent attenuation coefficient, and d_{disc} the tissue thickness. $S_{\text{sample}}(f)$ and $S_{\text{ref}}(f)$ are calculated by performing a fast Fourier transform (FFT) on a signal trace 600ns long centred on the reflected peak. To account for the effect of the observed reflections of ultrasound from the disc surface, the signal with the tissue in place was first corrected using $\gamma_{\text{corr}}(i, j)$ (as defined previously) before calculating the magnitude spectrum.

In much of the work investigating the use of the frequency dependence of the attenuation coefficient to detect pathology, it has been assumed that there is a linear relationship between the two parameters (Kuc and Schwartz, 1979). However, a

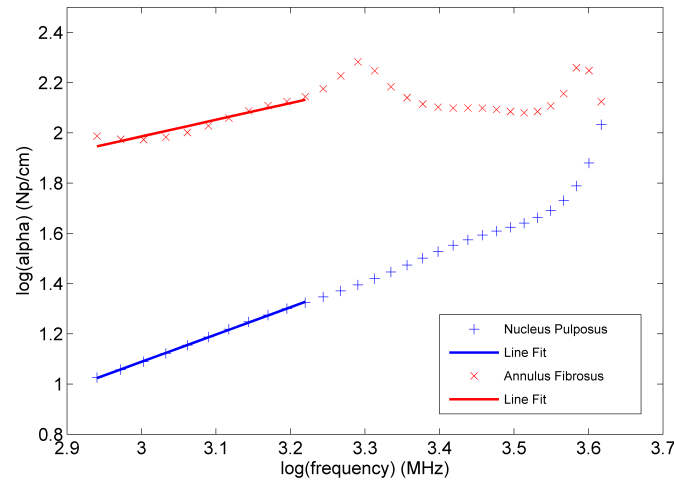


Figure 3.6: Example of the curve-fitting to measure the frequency dependence of attenuation. The relationship is linear over a shorter range for the AF due to increased attenuation at higher frequencies. The region over which the line is fitted corresponds to the range 19-25MHz

power-law relationship often provides a better fit to experimental data. The power-law dependence (n) of the disc tissue was calculated from the gradient of a least squares fit to a plot of $\log(\text{frequency})$ against $\log(\alpha)$, since:

$$\alpha = \alpha_0 \cdot \left(\frac{f}{f_0} \right)^n \quad (3.9)$$

The high attenuation in the AF in particular meant that the signal-to-noise was very low at the upper end of the transducer bandwidth, so the region over which the frequency-dependence of the attenuation was calculated was limited to the range 19-25MHz which was found to follow a power-law relationship, as illustrated in Figure 3.6.

3.1.2 Measured Acoustic Properties of the Intervertebral Disc

As discussed in Section §3.1.1.4, a series of intervertebral discs were prepared and scanned in both cross-sectional ($n=16$) and transverse ($n=11$) directions. Sample results for a cross-sectional scan of a disc are shown in Figure 3.7, with results for strips cut from a single disc shown in Figure 3.8. These particular scans were selected as they gave a nearly complete image of the disc (i.e. reflections from both surfaces could be detected for nearly all points). The limited regions where values could not be calculated are given the same value as the background.

From the sample images, the substantial variation in acoustic properties across the different regions of the disc can be seen, with the transition between regions taking place over a short distance. The speed of sound, specific acoustic impedance and the attenuation coefficient are higher in the annulus fibrosus than in the NP for both transmission directions, while the frequency-dependence of the attenuation coefficient is noticeably lower in the AF than the NP. There is variation in the measured tissue properties within each of the regions which is attributed primarily to local tissue variations and methodological artefacts; the irregular values at the edge of the tissue in both sets of scans are attributed to the finite size of the transducer focus lying across the tissue boundary.

Interestingly, the region of high attenuation on the outer boundary of the disc appears to be wider than the corresponding region of high transmission speed - a finding consistent with qualitative results reported elsewhere (Tervonen et al., 1988). This high-attenuation, low-sound speed region corresponds to the part of the inner annulus where the highly organised collagenous structure of the outer AF transitions to the less organised and proteoglycan-heavy NP, which suggests a role for both tissue structure and composition in determining the acoustic properties.

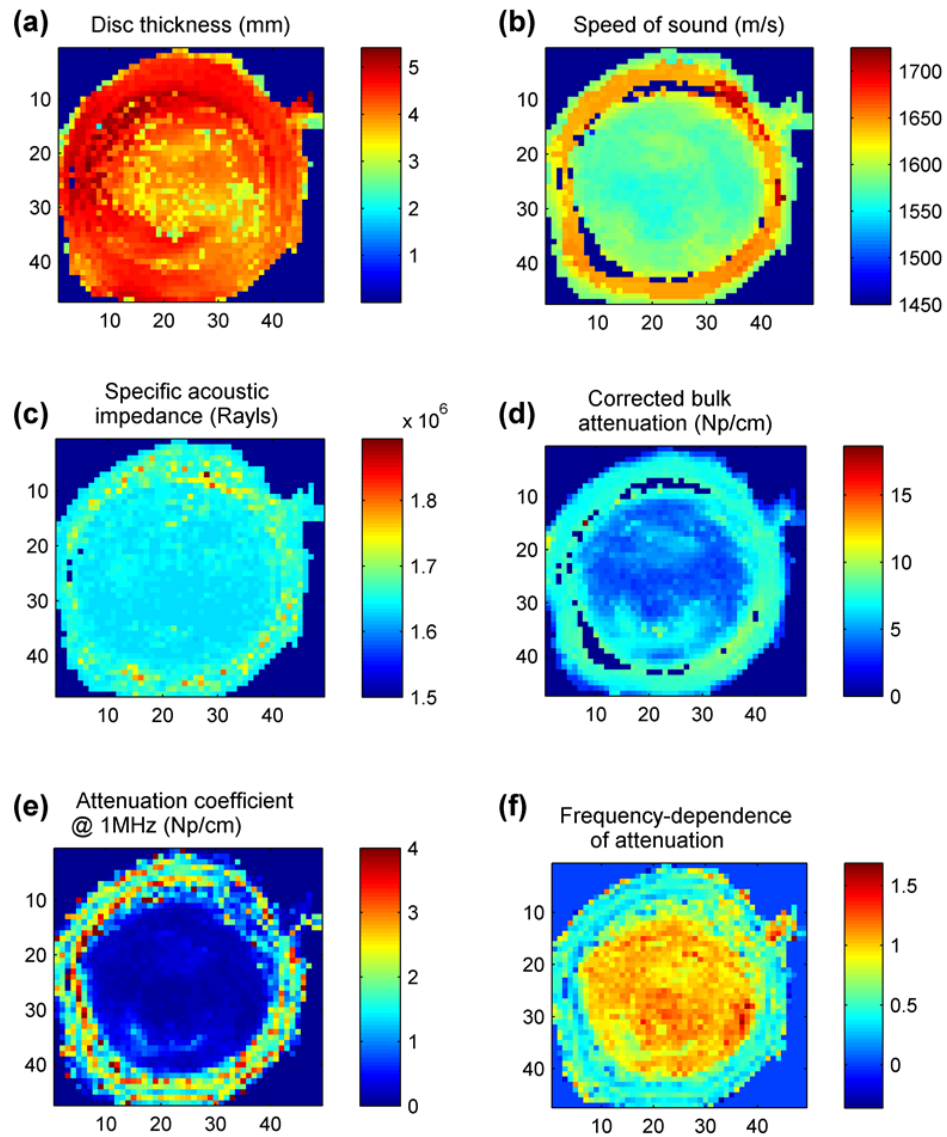


Figure 3.7: Results from an example disc showing how the acoustic properties vary along the cross-section of the disc. The large difference in the attenuation and speed of sound between the AF and NP can be clearly seen; other discs examined show a very similar effect. Axis units are measurement number; measurements were taken at a 0.5mm spacing

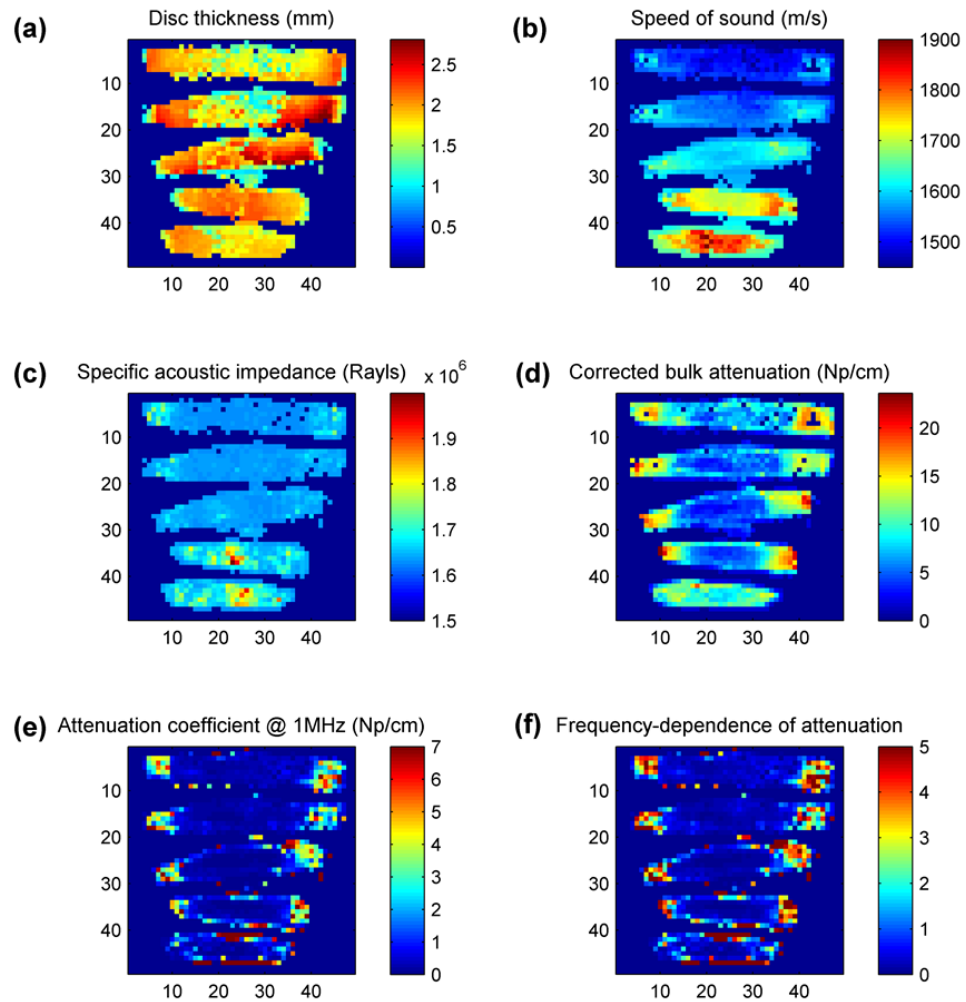


Figure 3.8: Results from an example disc showing how the acoustic properties vary along strips cut from the disc (acoustic propagation in the transverse plane of the disc). The large difference in the attenuation and speed of sound between the AF and NP can be clearly seen. Note that the high values for the frequency dependence in (f) (i.e. >1) are due to errors associated with reflections from the edges of the strips

To obtain quantitative estimates of tissue properties in the two primary regions of the disc, the values obtained from the two sets of scans (cross-sectional and transverse) were aggregated and averaged.

Before processing, the results for each disc slice were subjected to two qualitative screening tests. Where reflections from both disc surfaces could not be detected (due to excessive attenuation or a non-normal incidence) for more than approximately 30% of the scan points, the results from the slice were excluded from further analysis. Likewise, where the disc slices were seen to have curled and distorted in the PEG solution (giving rise to a substantial variation in measured tissue thickness) the results were not included in further analysis. In total, cross-section measurements from 7 discs (of 16 in total) were included, with transverse measurements from 6 discs (of 11 in total).

Following this initial screening, the first step in the process was to define the disc regions on the scans. For the cross-sectional scans, an algorithm was devised where the centre of the disc and six points on the boundary were manually indicated, and used to calculate the disc radius. Radial regions were then defined to correspond to each tissue type - the AF from 0.84-0.96 times the radius, and the NP from 0-0.30 times. This deliberately excluded the transition between the two regions and the edge of the disc, concentrating only on the regions that could be clearly defined. For the transverse scans, regions of AF and NP were manually selected from the image in order to exclude any of the points on the tissue boundary. The regions of AF were limited to those where the acoustic transmission axis was *along* the lamellae, rather than directly across the lamellae (in the smallest slice of disc scanned).

The values from each of these regions were then sorted, and the upper and lower quartiles excluded to eliminate any outliers or methodological artefacts. The mean of the middle quartiles was taken as the measured value for each region of each

disc, with the uncertainty in that value taken as the range between the limits of the middle quartiles. The values reported in Table 3.2 are the mean of the values across the different discs, with uncertainties derived from standard error propagation. It is worth noting that the uncertainties thus obtained are a measure of the internal consistency of the experimental approach, and do not take into account any systematic uncertainty (discussed later in this section).

Property	Unit	Annulus Fibrosus		Nucleus Pulposus	
		Transverse	Cross-Section	Transverse	Cross-Section
Speed of sound	(m/s)	1625 ± 12	1626 ± 13	1582 ± 9	1574 ± 3
Impedance	kRayls	1648 ± 8	1655 ± 7	1635 ± 2	1632 ± 1
Bulk attenuation	Np/cm	11.8 ± 0.8	6.6 ± 0.6	4.4 ± 0.5	4.4 ± 0.5
Attenuation (1MHz)	Np/cm	2.5 ± 1.8	1.24 ± 0.3	0.17 ± 0.05	0.21 ± 0.05
Frequency-Dependence	-	0.63 ± 0.12	0.64 ± 0.06	1.07 ± 0.06	0.98 ± 0.06

Table 3.2: Values for the acoustic properties within the two main regions of the IVD as measured both transversely ('slice', n=6) and sagittally ('cross-section', n=7)

The quantitative results support the qualitative impressions from the raw scans. The speed of sound is approximately 45m/s (3%) higher in the AF than the NP, and both the bulk attenuation and attenuation at 1MHz are significantly higher in the AF. However, while the results for cross-sectional and transverse measurements are similar for the NP, the attenuation coefficient in the AF is much higher in the transverse scan than the longitudinal scan.

The anisotropy in the AF is as a result of the highly organised nature of the tissue. As discussed in Chapter 1, the AF predominantly comprises bundles of collagen fibrils which are arranged at 60° to the spinal axis, alternating in adjacent layers. Thus in the cross-sectional measurements the sound is propagating at an angle of 60° to the fibrillar axis, and in the transverse measurements sound is propagating at 30° to the fibrillar axis. It has been shown in tendon (Miles, 1996) and skeletal muscle (Shore et al., 1986) (see Table 3.3), which both also have a fibrillar structure, that the

attenuation is higher along the fibre axis than across it. Thus the transverse scan, which is closest to propagating along the fibrillar axis, would be expected to give rise to a higher attenuation value, as observed.

The similar values measured for the two propagation directions in the NP are expected from the disorganised tissue structure.

Table 3.3 provides data comparing the measured properties for disc tissue against the reported acoustic properties for other biological tissue.

There is very limited data available in the literature on the acoustic properties of the IVD, with a single paper reporting speed of sound data and providing a qualitative assessment of attenuation (Tervonen et al., 1988). The reported values (see Table 3.3) are lower than those measured in this work, although the difference in speed between regions is similar. Tervonen also looked at different directions of propagation through the AF, and found minimal variation in speed of sound between the two directions considered in this work (1,597m/s for cross-section vs 1,601m/s transverse) which was similar to the current work. However the speed of sound *across* the lamellae, which was not measured in this work, was found to be significantly lower (1,556m/s). As there is no information provided on the methodology employed by Tervonen it is not possible to postulate reasons for the discrepancies in values.

When compared to the attenuation of other tissues, the NP appears to behave most similarly to fat, with a higher attenuation coefficient than both muscle and liver. The frequency dependence of attenuation is roughly linear, which is in line with most soft tissue (Hill et al., 2004) values reported.

The attenuation coefficient of the AF is very high relative to other tissues. This is likely due to the high collagen content of the AF, as collagen has been implicated

in the attenuation of ultrasound by tissue (Tervonen et al., 1988; Goss and Dunn, 1980), although the precise mechanism has not been elucidated. The only soft tissue known to have an attenuation coefficient higher than that measured for the AF is the tendon. This effect, however, may be due to the parallel alignment of the collagen fibrils within the tendon structure allowing for a direct measurement, whereas in the AF it is only possible to measure properties at a 30° angle to the fibrillar axis.

The frequency-dependence of the AF is lower than that seen in other tissue, though similar values have been reported. Tendon has been reported to have a dependence of 0.8 (Hill et al., 2004) and pancreas 0.78 (Kaye&Laby, 1995), which is only just outside the range of values seen in this work. The measured value may also be skewed downward by excessive attenuation at the higher end of the range of frequencies over which it is measured, as discussed in Section §3.1.1.7.

Tissue	Speed of Sound (m/s @ 20°C)	Attenuation (Np/cm 1MHz)	Frequency De- pendence @	Source
NP (measured)	1574	0.21	0.98	current work
AF (measured)	1626	1.24	0.64	current work
NP (reported)	1553	-	-	(Tervonen et al., 1988)
AF (reported)	1557 – 1597	-	-	(Tervonen et al., 1988)
Tendon, along	1713	6.77 @ 7MHz	0.8-1	(Miles, 1996; Hill et al., 2004)
Tendon, across	1650	1.3 @ 7MHz	0.8-1	(Miles, 1996; Hill et al., 2004)
Cartilage	1665	0.58	-	(Goss et al., 1978)
Muscle	1529 – 1629	0.13	1 – 1.2	(Kaye&Laby, 1995; Shore et al., 1986)
Liver	1578 – 1640	0.07	0.9 – 1.3	(Kaye&Laby, 1995)
Fat	1412 – 1487	0.21	-	(Kaye&Laby, 1995; Goss et al., 1978)
Bone	2190 – 3289	1.5 – 2.15	approx. 1	(Kaye&Laby, 1995; Sasso et al., 2007)

Table 3.3: Comparison between the measured values for acoustic properties of the IVD and values for other tissue quoted in the literature (blanks shown where no values are available, IVD values are measured in cross-section)

These results have two main implications for the development of a procedure to achieve sufficiently high pressures in the centre of the IVD to mechanically disrupt

degenerative disc tissue.

Firstly, the high attenuation coefficient means that a high proportion of the acoustic energy delivered to the disc will be attenuated in the AF. This will have the dual effect of reducing the pressure amplitude that can be achieved inside the NP, and potentially heating the AF to levels that will damage both cells and the collagen matrix. One way to reduce the potential for this damage to the AF is to design a transducer, or array of transducers, that increases the focusing gain so that the acoustic intensity inside the AF is reduced for a given focal pressure inside the NP.

The second major implication of these results is that the variation of tissue properties over short distances within tissue makes the modelling of such a transducer array much more complex than the simple models that have been reported previously (Persson et al., 2005) which were based on a uniform disc composition.

As noted earlier, the error estimates presented in this work are based on the internal consistency of the results, rather than on the accuracy of the method itself. The methodology has several areas where error may be introduced, and while every attempt has been made to minimise them it is likely that some will be present in the measured values, driven largely by the restrictions imposed by the difficulty of working with IVD tissue. These areas are:

- The manually-cut surfaces of the disc are not perfectly parallel, which affects the accuracy of the impedance values and the reflection-correction factors applied. One possibility to address this is to place the sample between solid buffer rods, though the applicability of approach is limited by the need to use a focussed transducer to measure property changes over small distances; a second approach is to use samples of varying thickness to eliminate the need for explicit reflection correction. This approach is rendered impractical by the

limited height of a bovine coccygeal disc, the need for a sufficient thickness to resolve reflections in the time domain, and the difficulty in obtaining more than one slice from a single disc;

- The centre frequency of the transducer used for measurements is 35MHz, with the centre frequency of the returned signal in the range 20-25MHz. This is substantially higher than the planned therapeutic frequency (500kHz), and an extrapolation of the attenuation results down into this frequency range for modelling work may be a source of error. The choice of frequency in these experiments was driven by the desire to measure properties with high spatial resolution;
- It is assumed in the analysis that both the transmission medium and the tissue itself behave linearly. While the filtering applied to the raw signal will prevent most harmonics from affecting the measured signal amplitudes, the harmonics will be more readily attenuated and may result in an overestimate of the frequency-dependent coefficient;
- Freezing and then thawing the disc prior to measurement may have some effect on the acoustic properties of tissue. This was reported to be an issue in early measurements of the acoustic properties of fish muscle, but subsequent measurements in liver have shown no such effect (Miles, 1996);
- IVD placed directly in PEG solution is known to lose proteoglycan into solution over time, as mentioned in §3.1.1.5. Since a complete scan over a disc cross-section takes 2-3 hours, it is likely that there will be a significant difference in the PG content of tissue measured at the start and at the end of a scan. However, this effect was not obvious in our work, and it may be that the effect of minor PG loss on the acoustic properties of tissue is minimal.

A final consideration is that these measurements are carried out on healthy bovine coccygeal discs, which do not show any effects of degeneration. The effect of degeneration on the acoustic properties has not been systematically investigated, however it is likely that it will lead to some changes as a result of the compositional changes to the disc. It has been reported that the speed of sound in the NP increases with age (Tervonen et al., 1988), and it is reasonable to assume that this is related in some way to the loss of hydration and consequent increase in PG and collagen concentration that occurs in age-related degeneration. Such a systematic study is suggested as further work in the event that suitable samples of human tissue are made available.

3.1.2.1 Implications for the Present Work

This work presented the first quantitative measurement of the acoustic properties of the intervertebral disc, and their quantitative variation across the disc profile. The NP was found to have a substantially lower speed of sound and attenuation coefficient than the AF, to have an approximately linear relationship between frequency and attenuation, and to be isotropic (i.e. properties are independent of propagation direction). The AF, on the other hand, has an attenuation coefficient among the highest reported in soft tissue, and to be highly anisotropic.

The primary implication of this for the procedure proposed in this thesis is that there is the potential to damage cells and matrix in the AF due to excessive heating due to attenuation, and that this energy loss may present difficulties in achieving sufficiently high pressures in the NP to mechanically damage the tissue.

The next section presents the results of modelling the acoustic field inside the disc for two different transducer geometries (using the measured acoustic properties) in order to understand both the field shape and amplitude that can be

achieved.

3.2 MODELLING THE ACOUSTIC FIELD IN THE IVD

The measured acoustic properties of the disc tissue were used to model the acoustic field produced inside the disc. The modelling was performed using a commercial software package (PZFlex, Wiedlinger Associates, CA, USA), for which the underlying equations and calculation approach are not disclosed. In order to reduce the complexity of the modelling to be computationally tractable, the transducer-disc system was approximated by a two-dimensional system. This necessarily introduces a number of distortions in behaviour from the three-dimensional geometry, but it does allow for important general conclusions to be drawn.

Two transducer configurations were modelled to investigate the effect on the location and extent of the focal region - a single transducer, and two transducers with their axes aligned at right angles. The acoustic field was first simulated assuming linear propagation through the disc tissue; the effect of tissue non-linearity on the pressure waveform and acoustic field was subsequently investigated by assuming a value of B/A and varying this through a range of possible values.

3.2.1 Modelling Approach

Modelling the acoustic field within the disc can be considered in three primary stages - definition of the model geometry, specification of the acoustic properties at each point in the geometry, and then simulating the propagation of an acoustic waveform through the geometry.

3.2.1.1 Model Geometry

For the purposes of this work, the HIFU transducers and disc were modelled as elements on a two-dimensional plane. Two different transducer configurations were modelled - a single transducer and a two transducer configuration where the axes of the two transducers are aligned at 90° to each other. The dimensions of the HIFU transducers were specified to be the same as those that were available for experimental work in our laboratory - 60mm element diameter, spherically focussed with a radius of curvature of 62.64mm.

The transducers were approximated as solid surfaces, without a central cutout. Initial simulations comparing the results of two-dimensional transducers both with and without a cutout (for a PCD) against an axisymmetric three-dimensional transducer with a cutout (Figure 3.9) suggested that although both approximations introduced distortions to the field (noticably a lower focusing gain) the cutout introduced more significant distortions in the acoustic field than the solid model.

The disc was assumed to be a circle, with the centre located at the geometric focus of the transducer. While human lumbar discs are not circular (with a shape more similar to a cardioid), the bovine coccygeal discs that are used as an experimental model are roughly circular (Oshima et al., 1993). The diameter was assumed to be 27mm, which is consistent with the sizes of discs measured experimentally.

The two geometries modelled are shown in Figure 3.10.

As described above, modelling the transducers as two-dimensional objects distorts the acoustic field and reduces the focusing gain. Modelling the disc as a flat planar object ignores the effect of the vertebral bodies, which are highly reflective of acoustic energy and will both act to reduce the amount of acoustic energy able to enter the disc, and to affect the shape of the field within the disc.

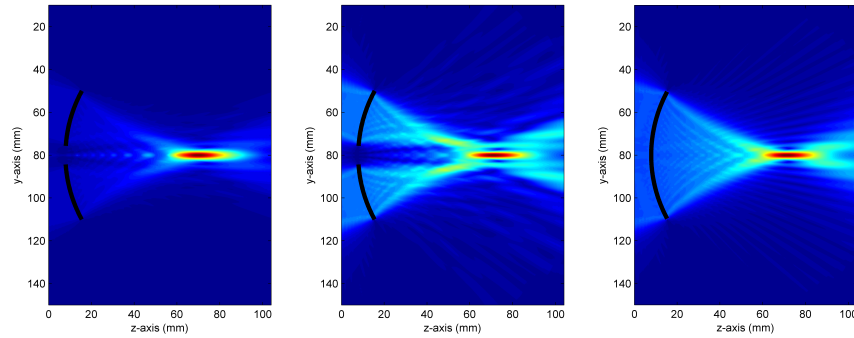


Figure 3.9: Comparison between the field produced by a transducer modelled as **(a)** a three-dimensional axisymmetric transducer with a central cutout, **(b)** a two-dimensional transducer with a central cutout, and **(c)** a solid two-dimensional transducer. The transducer dimensions and frequency (500kHz) correspond to those of the H107D used in experimental work in subsequent chapters

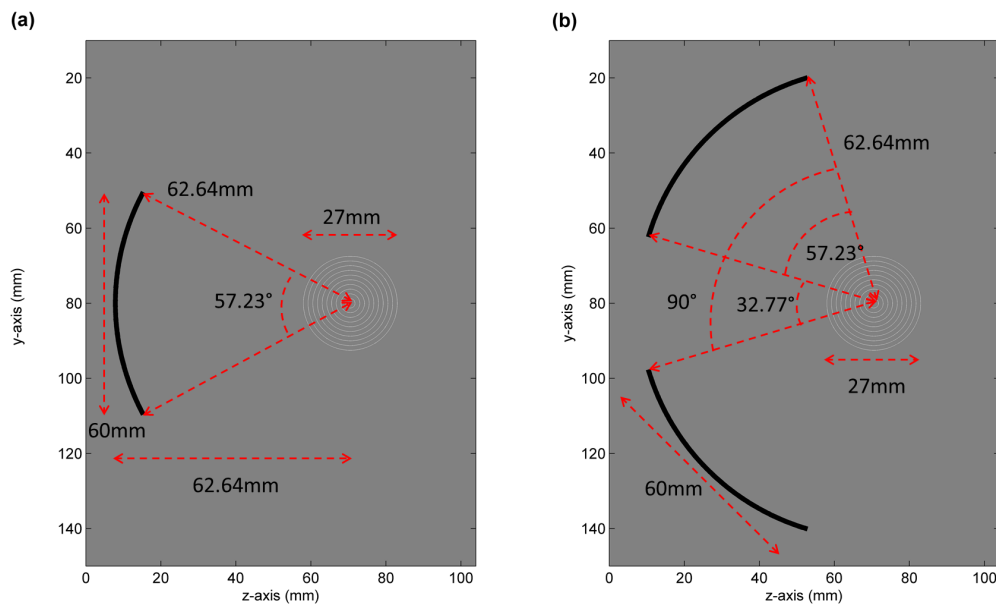


Figure 3.10: Transducer and disc geometries used in simulations of the acoustic field. **(a)** shows a single transducer, **(b)** the configuration with two transducers. Note that both geometries have reflection symmetry about the x-axis

Whilst modelling both the transducers and the disc-vertebrae in three dimensions would provide a more realistic simulation, this comes at the cost of computational complexity. Running a simulation of sufficient resolution to capture the variation of acoustic properties across the disc in three dimensions would require several days, as opposed to less than an hour for the two-dimensional case.

The results obtained in the simulations should be interpreted with these limitations in mind, focusing on directional conclusions rather than absolute values. The impact of shifting to three dimensions is considered in the discussion of the results.

3.2.1.2 Parameter Estimation

As described in Section §3.1.2, the measured values for the acoustic properties are observed to vary significantly along the cross-section of the disc. The variation is primarily in the radial direction (i.e. properties are relatively consistent at a given distance from the centre of the disc) as expected given the disc structure and composition. However, the values at a given distance from the centre of the disc do vary slightly, and there are a number of outliers as an artefact of the measurement approach.

Rather than specifying a unique set of properties at each of the approximately 2000 points measured on the cross-section of the disc, or reducing the disc to a simple two-component geometry comprising only AF and NP, the idealised disc was divided into radial regions, and the measured acoustic properties within these regions averaged to provide an input to the model. This averaging was done using a similar approach to that described in Section §3.1.2 - by selecting a representative disc, manually indicating both the centre and outer boundary, and then dividing the radius into 10 regions of equal width as shown in Figure 3.11. The measured values within each of these regions were grouped together, the top quartile and bottom

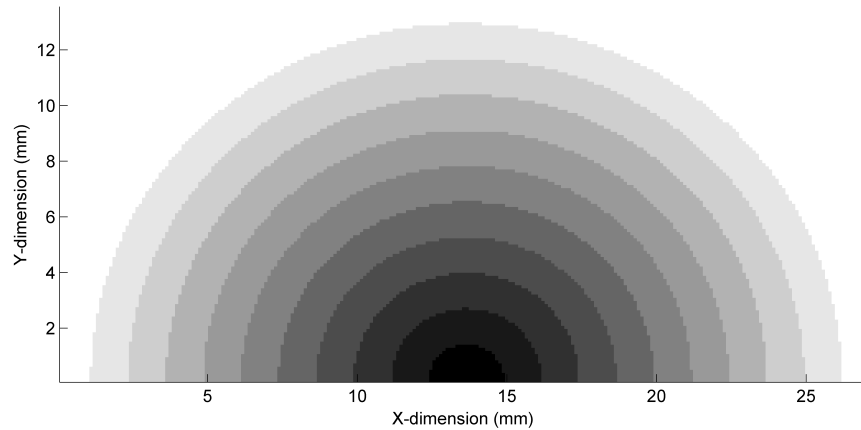


Figure 3.11: Geometry of idealised disc for specifying the acoustic properties used in modelling the acoustic field; only a half-disc was specified as the model used reflection symmetry about the central axis

quartile discarded as potential outliers, and the mean of the middle quartiles taken as the acoustic property for the whole of that region. To prevent issues associated with differing AF widths between different discs, the values were calculated from a single disc. The values thus determined are shown in Table 3.4.

While these values do not agree perfectly with the average values reported in the previous section, they are broadly similar and any variation from the average values is likely to fall within the natural variation in properties between discs.

For models investigating the effect of tissue non-linearity, there was no empirical data available as the measurements assumed linear behaviour, and as there are no reported values in the literature for the IVD or other connective tissue it was necessary to assume a value for B/A . A value was therefore assumed, and varied between simulations to investigate the effect on the field shape and amplitude. Rather than assume a uniform value for the whole disc, a value was set for the outermost region of the disc and this was then used as a baseline for the other regions.

The baseline value of B/A was varied from 4 to 12 in five equal steps in order to

Region	Sound Speed (m/s)	Density (kg / m ³)	Attenuation (1MHz) dB/cm	Freq Dependence
1	1578	1037	2.28	0.91
2	1576	1040	1.85	0.98
3	1573	1040	1.39	1.05
4	1573	1041	1.40	1.06
5	1575	1041	1.51	1.04
6	1578	1037	2.26	1.00
7	1588	1034	4.23	0.89
8	1608	1028	7.77	0.75
9	1608	1030	12.0	0.58
10	1619	1025	16.2	0.58

Table 3.4: Acoustic parameters used in modelling the acoustic field inside the IVD. Regions are as defined in Figure 3.11 from the centre outwards

cover the range of values reported for biological tissue (Kaye&Laby, 1995). To estimate the variation across the disc profile, the empirical results reported by Mast (Mast, 2000) indicating relationships between B/A and both speed of sound and density were used. Since density variation across the disc was observed to be minimal, the variation from the baseline value was determined from the speed of sound by the formula $\Delta \frac{B}{A} = -16.58 \Delta c$ where c is measured in mm/ μ s.

3.2.1.3 Simulation

Simulation was performed using PZFlex, a commercially available finite element modelling software package designed to specialise in piezoelectric transduction and ultrasonic wave propagation. The package uses an explicit time-domain approach, and is capable of modelling non-linear wave propagation in one-, two- or three-dimensions.

In this study, rather than specifying a voltage load and simulating the response of the piezoelectric elements of the transducer, the pressure waveform at the surface

of the transducer was simply specified as a continuous wave sinusoid. This resulted in both forward and backward propagation as no backing layer was specified - the backward propagating wave was absorbed at the model boundary and ignored. In each simulation the transducer produced continuous wave excitation until steady state was reached, when the simulation was halted.

The model boundaries were set as perfect absorbers (an inbuilt boundary condition in PZFlex), and the reflection symmetry of the model about the x-axis was exploited to reduce processing time. Element size was specified to contain 30 elements per wavelength of the fundamental frequency of the pressure waveform. Since pressure amplitude does not affect the form of the acoustic field in a linear model, the pressure amplitude at the transducer surface was set to an arbitrary value of 100Pa; in the non-linear model the pressure amplitude was adjusted to produce a freefield PRFP of approximately 10MPa at the focus.

3.2.2 Linear Simulation

The first stage of modelling assumed linear acoustic behaviour in both the transmission medium (water) and the disc. Figure 3.12 shows the shape of the acoustic field simulated for a single transducer both in water and with a disc present, and compares the profile of the PRFP along the transducer axis for both situations. From the field profiles it can be seen that the disc does not significantly distort the acoustic field, and that the axial extent of the focal region is similar to the disc diameter. The axial profile shows two main effects - the drop in pressure amplitude within the disc due to attenuation in the AF, and the slight shift of the focus towards the transducer that this causes (approx. 2mm).

These effects present a challenge to using cavitation to mechanically ablate the NP while preserving surrounding tissue. While the highest pressures produced by

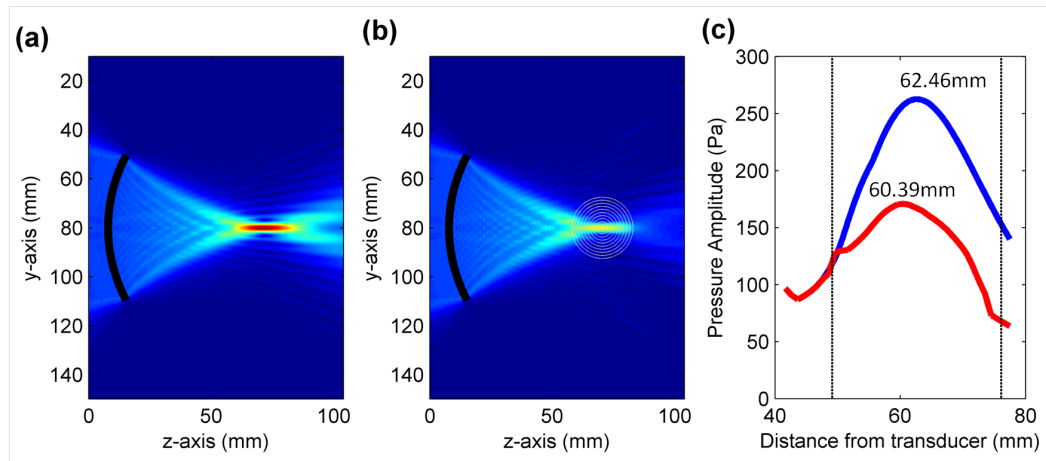


Figure 3.12: Linear modeling of the acoustic field for a single transducer. **(a)** Field with no disc present, **(b)** Field with the disc present, **(c)** Profile of the peak PRFP along the transducer axis with no disc (blue) and disc (red) present. The dashed lines show the location of the disc boundaries, and the location of the peak pressure is annotated.

the single transducer are inside the NP, the probability of inducing cavitation is a function not only of the PRFP, but also the local tissue environment. Although the cavitation threshold of disc tissue is not known, it is reasonable to believe that there will be a greater concentration of nuclei present at the (vascularised) disc surface than inside the (avascular) disc, and thus that the threshold in the NP will be substantially higher than at the disc surface. Since the focussing gain from the disc surface to the NP is only approx. 50%, the single transducer is likely to be unsuitable for therapy due to the high possibility for damaging sensitive tissue surrounding the disc.

In order to reduce the axial extent of the focal region, simulations were performed for two confocal transducers oriented at 90° - the results are shown in Figure 3.13. In the field profiles it is immediately obvious that the focal region has a greatly reduced axial extent. Attenuation means that the pressure inside the disc is again substantially reduced from the freefield case, but the position of the focus does not have the same shift seen with the single transducer.

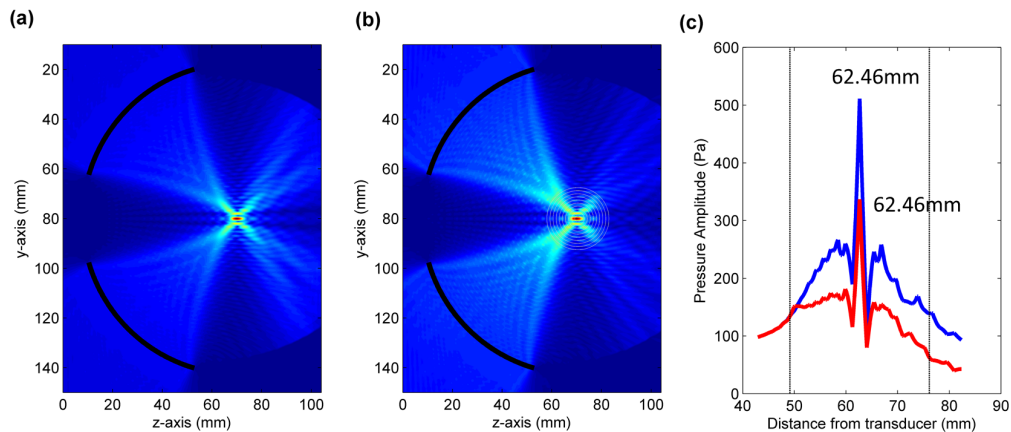


Figure 3.13: Linear modeling of the acoustic field for two perpendicular transducers. **(a)** Field with no disc present, **(b)** Field with the disc present, **(c)** Profile of the peak PRFP along the axis of one of the transducers with no disc (blue) and disc (red) present. The dashed lines show the location of the disc boundaries, and the location of the peak pressure is annotated. Note that **(a)** and **(b)** do not have the same scale as in Figure 3.12 above.

The reduced size of the focal region means that this configuration is much less likely to produce damage in surrounding tissue. The relationship between focal pressure and the pressure at the disc surface is best illustrated by a plot of normalised PRFP along the transducer axis. Figure 3.14 shows that for the single transducer the pressure amplitude at the disc surface is roughly 70% of that at the focus, whereas for the dual-transducer configuration it is only 45%. The more sharply-defined focus means that it will be possible to target the treatment within the NP accurately.

Figure 3.14 also shows the shape of the curve for a single transducer modelled in 3 dimensions ('axisymmetric'), which is broadly similar to that of the single two-dimensional transducer. This similarity suggests that the results obtained with the simplified model geometry will provide at least directional guidance on experimental outcomes.

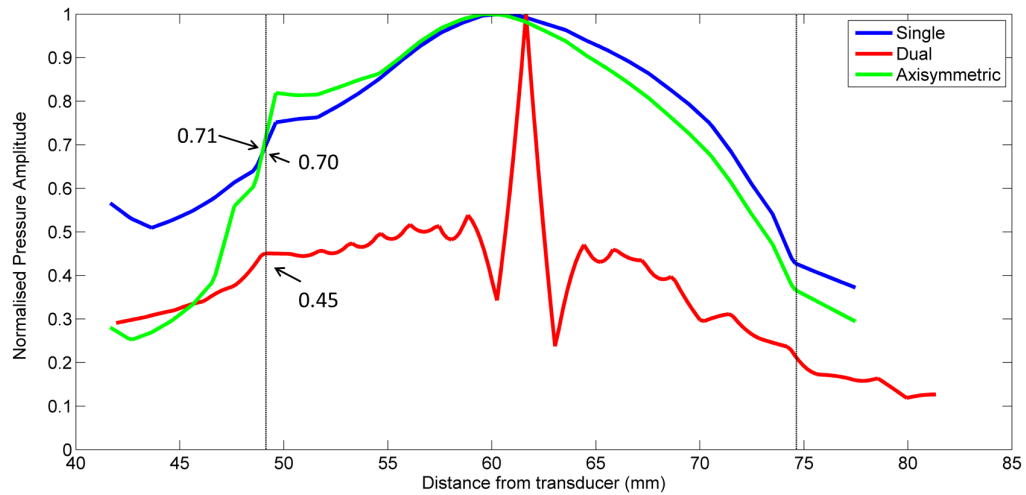


Figure 3.14: Comparison between the normalised pressure profiles through the disc for a single 2D transducer, a single axisymmetric transducer, and dual 2D transducers. The borders of the disc are shown by the vertical dotted lines.

Thus the linear modelling work suggests that using a dual-transducer configuration is likely to produce better outcomes than a single transducer. In a similar fashion, more complex transducer geometries and arrays are likely to be capable of producing a more tightly defined focus with a higher gain over the pressure at the disc surface. These have not been considered in the current work as it was impractical to reduce them to experimental practice, but the optimisation of transducer design is suggested as a topic for future work.

3.2.3 Non-linear Simulation

Despite the assumption of linearity to this point in both the measurement of tissue properties and modelling of the acoustic field, tissue is non-linear and the IVD is no different. To model the effect of non-linearity on the acoustic field in the disc, simulations were performed for a range of assumed values of the non-linearity parameter B/A (with the driving pressure set such that the PRFP was 10MPa). Figure 3.15 compares the field profile for the linear model to the field for the most highly non-linear

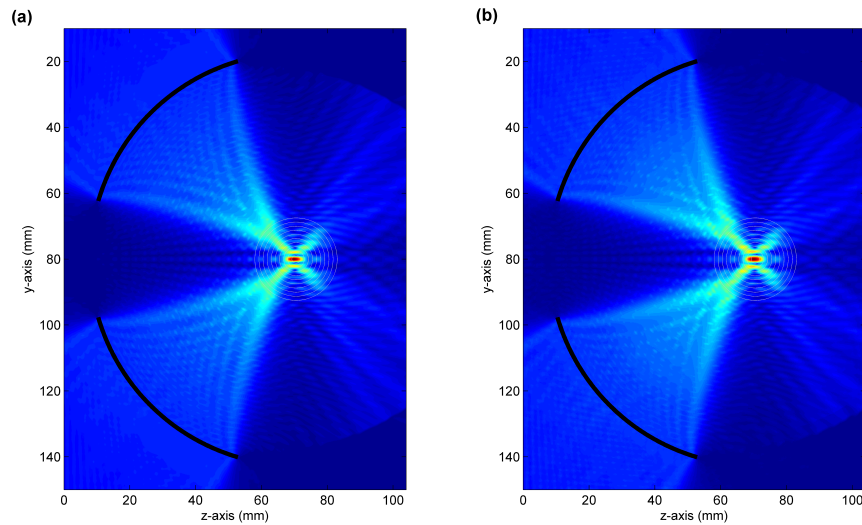


Figure 3.15: Effect of non-linearity on the acoustic field produced by two transducers within the disc. **(a)** shows the acoustic field for a linear simulation; **(b)** shows the profile for a baseline non-linearity coefficient (B/A) of 12

model (baseline $B/A=12$), and it is evident that the non-linearity has had minimal effect on the field shape. This is reinforced by the plot of the PRFP profile along the transducer axis (Figure 3.16), which shows that the profile changes only slightly. This figure also shows the waveform at the focus for B/A equal to 4, 8 and 12, which does not exhibit any obvious sharpening or asymmetry as a result of non-linearity.

This is not entirely unexpected, as the propagation distance through the disc is too short to produce shocks at the pressure amplitudes considered in these simulations (a rough calculation of the shock formation distance for a sinusoid at 7MPa PRFP gives 75mm, much greater than the disc diameter). Thus, for the purposes of the experiments with excised discs detailed in subsequent chapters, non-linearity will not affect the field profile even if the B/A is at the higher end of the range considered. The main issue in therapy is likely to come from the non-linearity of the tissue overlying the disc, and in particular any extensive fat layers. As with the optimisation of the transducer design, assessing the impact of overlying tissue on the

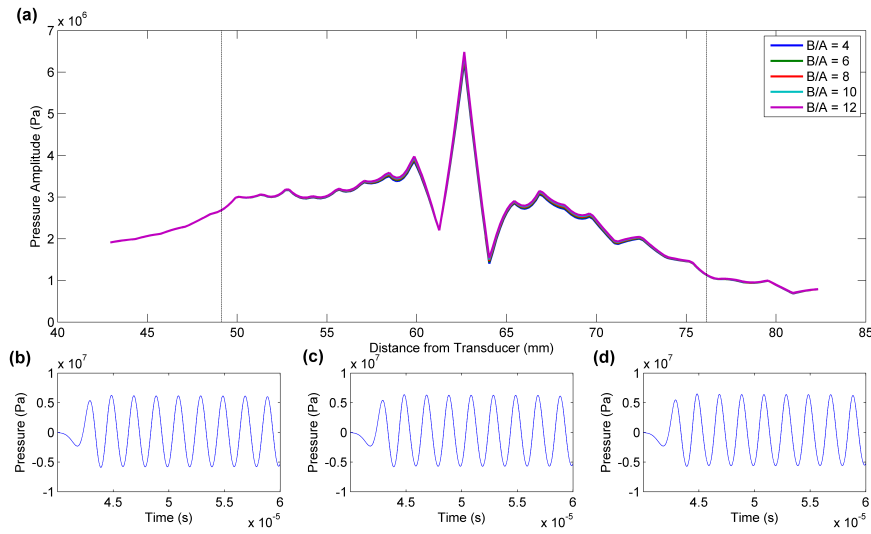


Figure 3.16: Effect of non-linearity on the pressure profile produced by two transducers within the disc. (a) shows the pressure profile along the transducer axis for a range of baseline non-linearity values; (b), (c) and (d) show the pressure signal at the focus for baseline B/A equal to 4, 8 and 12 respectively. Note that the ‘jagged’ shape of the plot is due to coarse sampling of result points rather than model meshing

field within the disc is suggested as a topic for further work.

3.2.4 Implications for the Present Work

Using the acoustic properties of the disc measured in Section §3.1, the acoustic field produced within the disc for two different transducer configurations was modelled in PZFlex. These simulations showed that using a single HIFU transducer produces a field with a focus that extends axially, resulting in minimal gain from the disc surface to the NP, and creating the potential for cavitation and tissue damage outside of the desired region. Moving to a two-transducer configuration produces a much tighter focus located within the NP, and with a much higher focal gain from the surface of the disc. While the results obtained with the simplified two-dimensional geometry will differ from the full three-dimensional environment, the underlying trend indicates the advantages in using a dual-transducer configuration for the experimental

work described in the following chapters.

Further simulation work to optimise the transducer design, and to investigate the effect of overlying tissue on the acoustic field within the disc is recommended.

3.3 EXPERIMENTAL VALIDATION

To validate the results of the modelling, experiments were conducted to measure the pressure field within the IVD. A needle hydrophone was inserted into a disc and moved through a range of positions. The pressure measurements obtained were compared to the free-field pressures, and to the modelling results.

3.3.1 Experimental Setup

Fresh bovine tails were obtained from the abattoir (Mutch Meats, Witney, UK), and overlying muscle and ligament were removed before cutting into individual motion segments. The motion segments were placed into a custom holder, and a needle hydrophone (HNA, 500 μ m tip, S/N 1056, Onda, Sunnyvale, CA, USA) manually inserted into the disc horizontally. The disc was then placed so that the NP was at the focus of a 500kHz HIFU transducer (H107D, Sonic Concepts, Bothell, WA, USA), with the hydrophone aligned along the transducer axis. The position of the disc was adjusted laterally so that the hydrophone was at a pressure maximum.

The transducer was driven by a signal generator (33250A, Agilent, Santa Clara, CA), and the hydrophone signal recorded on an oscilloscope (LeCroy WaveRunner, LeCroy, NY, USA). The signal from a 15MHz PCD coaxially and confocally aligned with the HIFU transducer was also recorded. These two signals were used to locate the position of the front of the disc, the distance of the hydrophone from the front of the disc, and the pressure waveform at the hydrophone tip. The hydrophone was

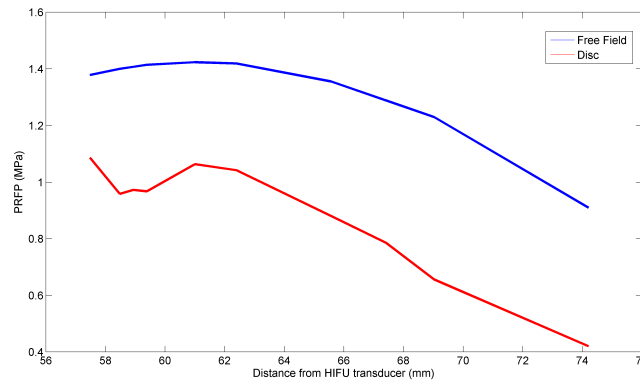


Figure 3.17: Comparison between the measured freefield pressure profile, and the pressure profile within a disc generated by a single transducer driven at 500kHz. The front of the disc in these measurements is positioned roughly 50mm from the transducer

then manually moved a small distance within the disc (away from the transducer), and the readings repeated at this new position; this process was repeated until the hydrophone was completely removed from the disc.

Freefield pressures were acquired using a similar process in the absence of a disc in the holder.

3.3.2 Single Transducer Field

The comparison of the free-field pressure profile with the profile measured inside the disc (Figure 3.17) shows that the presence of the disc results in a substantial reduction in pressure amplitude, ranging from a 20% reduction at the front of the measured region to 50% at the back.

When these results are normalised against the free-field profile and compared against the results obtained from the simulation work (Figure 3.18), it can be seen that there is similarity between the pressure profiles. This suggests that the results of the modelling work are at least broadly representative of the physical situation.

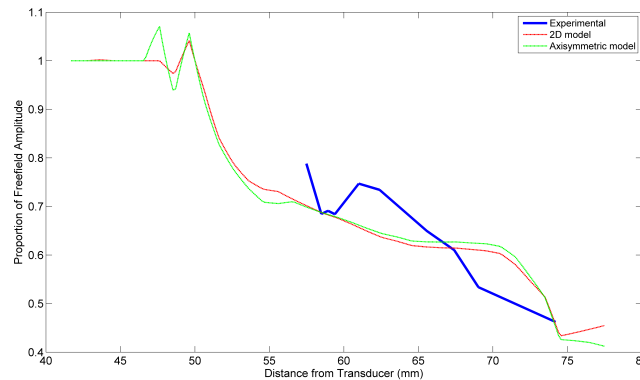


Figure 3.18: Comparison between the experimental and modelled pressure profiles in the disc, showing pressure as a proportion of the freefield pressure (i.e. without a disc present). The disc is positioned similarly in both the experimental and modelled data, with the front face at approximately 49-50mm from the transducer

A more precise and conclusive validation of the modelling work was not possible due to both the simplified two-dimensional geometry employed in the simulations (discussed in previous sections), and the difficulty of obtaining pressure measurements inside the disc. The presence of the vertebral bodies in the empirical measurements is the major difference between the two geometries, and may have led both to the reflection of part of the HIFU beam prior to entering the disc, and also to the reflection of sound within in the disc, creating local interference effects.

In terms of the practical experimental considerations, it was difficult to ensure that the hydrophone was inserted horizontally and coaxially with the transducer, and to ensure that the disc remained static as the hydrophone was moved between measurements (this was checked before each measurement, but some slight movement was inevitable). The calibration results used to convert the hydrophone voltage signal to *in situ* pressure were based on measurements taken in water; the hydrophone will not necessarily have the same response characteristics when embedded in tissue, which would result in a distortion of the pressure readings. The presence of the hydrophone itself, but perhaps more importantly the track that it created

in the disc tissue when removed, is also likely to have affected the measured pressure inside the disc.

Thus the empirical measurements are taken to provide a limited validation of the results of the modelling work, but with an acknowledgement of the limited accuracy of both the simulations and the measurement. If the detailed three-dimensional modelling and transducer design work proposed in the previous section is undertaken, it is suggested that a more rigorous validation procedure be developed.

3.4 SUMMARY

This chapter set out to investigate the interaction between ultrasound and the IVD, first by measuring the acoustic properties of disc tissue and then using this information to model the acoustic field produced inside the disc by two different transducer configurations.

The acoustic properties of disc tissue were measured using bovine coccygeal discs and a modified scanning acoustic microscope setup. The speed of sound and attenuation of tissue were found to vary between the regions of the disc, with both substantially higher in the AF than in the NP. The AF was also found to exhibit a large degree of anisotropy, which is consistent with the behaviour of other fibrous tissue with a high collagen content. This combination of properties presents a challenge for designing a therapy aimed at inducing cavitation inside the NP, as the highly attenuating AF will make it difficult to achieve sufficiently high pressures, and also raise the potential for excessive heating of the AF causing damage to cells.

These results were then used in modelling work to investigate the shape of the acoustic field inside the disc. To reduce computational complexity, the geometry

was approximated by a simplified two-dimensional setup. A single HIFU transducer was found to have a focal region that was of similar axial extent to the disc diameter, which produced similar pressures at the disc surface and the centre of the NP. Given that the cavitation threshold inside the disc is expected to be substantially higher than at the surface of the disc, this creates the potential for damage to the sensitive tissue surrounding the disc and was therefore decided to be unsuitable for experimental work. A two-transducer configuration, with their axes at 90° to each other, was found to give a much smaller focal region at the centre of the disc, with much greater gain between the disc surface and the focus.

The results of the modelling for the single-transducer case were broadly supported by empirical measurements of the pressure inside the disc, however the accuracy of these measurements was limited by the difficulty of working with a hydrophone embedded into the IVD, and both more detailed modelling and validation are suggested for future work.

The major implication of the acoustic properties and modelling work for the design of a procedure for the non-invasive removal of degenerate material from the NP are that a single HIFU transducer is likely to cause damage to sensitive surrounding tissue and is therefore unsuitable. A two-transducer configuration produces a more tightly focused acoustic field that can be completely contained within the NP and will therefore result in a safer therapy.

The next chapter details a second approach to further reduce the possibility of damage to tissue outside the IVD, by developing solid sonosensitive nanoparticles that are capable of reducing the local cavitation threshold when injected into tissue. Chapter 5 then details the design and initial validation of a two-transducer setup for simultaneous therapy and monitoring, and the results of using this setup to mechanically ablate NP tissue.



SONOSENSITIVE NANOPARTICLES

In order to address the difficulty in initiating cavitation that is presented by the acoustic properties of disc tissue, the introduction of artificial cavitation nuclei prior to ultrasound exposure that enable the generation of cavitation activity at lower pressure amplitudes is considered. This chapter presents a novel formulation of solid, sonosensitive nanoparticles (SNPs) designed to lower the cavitation threshold in a consistent and reproducible manner. The design and manufacturing protocol for the SNPs is presented first, together with the results of initial characterisation of their coating thickness and regularity. Investigations of the effect of the SNPs on the cavitation behaviour of water show that they are capable of substantially reducing the cavitation threshold, maintaining their activity over repeated insonications, and remaining stable in suspension for an extended period - suggesting that these particles may be useful not only for lowering the cavitation threshold in the disc, but also for broader use in therapeutic ultrasound applications including enhanced drug delivery.

This chapter has been submitted for publication in an alternative form in *Ultrasound in Medicine and Biology*.

The previous chapter outlined experimental work to determine the acoustic properties of the bovine IVD, and presented the results of modelling of the acoustic field that can be achieved within the disc by different transducer configurations. The modelling work suggests that the highly attenuative nature of the tissue of the AF will make it difficult to achieve the high acoustic pressures required to achieve mechanical tissue fractionation (Section §2.4.3) inside the NP, without increased risk of damage to adjacent tissue structures.

It has been reported previously (Tran et al., 2003; Poliachik et al., 1999) that the presence of artificial or exogenous cavitation nuclei (the two terms are used interchangeably in this chapter) in the targeted region can lower both the intensity and the duration of ultrasound exposure required to mechanically damage tissue. The IVD is a particularly attractive target for this approach; as a largely avascular tissue with minimal fluid exchange with surrounding tissue, it may be possible to inject artificial nuclei directly into the IVD and not only use them to reduce the acoustic pressure required to mechanically fractionate degenerate tissue, but also to localise this damage to the injected region.

The most commonly used artificial cavitation nuclei to date have been stabilised microbubbles developed as ultrasound contrast agents (UCAs) for the enhancement of diagnostic imaging (see Section §2.3.1.3 for a more detailed discussion). However, these stabilised microbubbles have several limitations in their use as cavitation nuclei to enhance therapy. Their size, typically in the range of 1 - 5 μm (Gorce et al., 2000), limits transport into the smallest capillaries and cavities. As a result, they cannot passively accumulate in tumours, which is desirable for cancer therapy. Of particular interest in considering their use in the IVD, they are known to collapse, rupture and dissolve when subjected to static overpressure at the levels experienced in the IVD (Chitnis et al., 2011). Additionally, the gas diffuses and dissolves in solution over time; their ability to nucleate cavitation is lost following disruption of the shell; and strong non-linear scattering makes it difficult to detect the onset of inertial cavitation.

Arora et al. (2008) first investigated the use of rough-surfaced solid microparticles to overcome some of these limitations, finding that these particles lowered the cavitation threshold significantly, and that reproducible cavitation at the same location could be achieved. It is hypothesized that any solid, rough surfaced nanopar-

ticles will contain minute amounts of gas entrapped on the particle surface after drying and resuspension in solution, which will facilitate cavitation inception when exposed to negative pressures typically achievable by therapeutic ultrasound transducers.

This chapter describes the development, preparation and characterisation of novel sonosensitive nanoparticles (SNPs) produced by layer-by-layer (LbL) surface deposition, that decrease the cavitation threshold when present in solution. The techniques used to prepare the particles are briefly outlined together with the methods used to assess the results of the preparation procedure. The main focus of this chapter is then the testing of the effect of the SNPs on the acoustic cavitation threshold. A range of particles with varying core size and coating particle size were tested at different frequencies to investigate the effect of these parameters on the threshold, and whether this might represent a method of tailoring particles to specific therapeutic applications. Other clinically relevant behaviours (depletion with subsequent exposures, effect of storage time) of the particles are also investigated and compared to currently available artificial nuclei that have been used in previous work. Finally, the effect of the SNPs on the cavitation threshold of porcine blood is presented to demonstrate the potential for use *in vivo*. In light of these results, the potential use of these particles to enhance mechanical fractionation of the IVD is discussed.

The work described in this section was carried out in collaboration with a highly skilled and valued chemist, Dr Sarah Wagstaffe, who was responsible for the design, manufacture and physical characterisation of the SNPs and assisted in the cavitation threshold measurements.

4.1 PARTICLE PRODUCTION

Layer-by-layer surface deposition is widely used for the preparation of tailored nanoparticles and nanocapsules. In brief, a solid core of known size and monodisperse size distribution is treated to create an active surface, upon which a number of layers can be deposited to create a shell. Depending on the application, the core may then be removed either by calcination or dissolution to create hollow nanocapsules for drug delivery, or left in place to give a solid particle. The properties of the shell can be tightly controlled by altering the nature of the deposited layers: fluorescent to enable visualisation, immuno-invisible to extend circulation time, functionalised with proteins to enable targeting in the body, pH-dependent porosity to enable release of encapsulated drugs. The general approach and potential applications are well described in many review papers (Antipov and Sukhorukov, 2004; Caruso, 2004, 2001; Gil et al., 2008; Gittins and Caruso, 2000; Johnston et al., 2006; Shchukin and Sukhorukov, 2004), and they are not further elaborated upon here.

This work uses a hydrophobic colloidal silica solution to coat a polystyrene (PS) core. These materials were selected because of the monodispersity of both the core and the coating particles, the high contact angle and thus hydrophobicity of the silica, and the opportunity to ‘tune’ the surface roughness by altering the size of the silica particles. A single-layer coating is used for the purposes of demonstrating the effect on cavitation threshold; it is suggested as future work to investigate the potential for adding layers to confer additional functionality for therapeutic applications.

4.1.1 Materials and Methods

4.1.1.1 Preparation of SNPs

SNPs were prepared by deposition of poly(diallyldimethylammonium chloride)

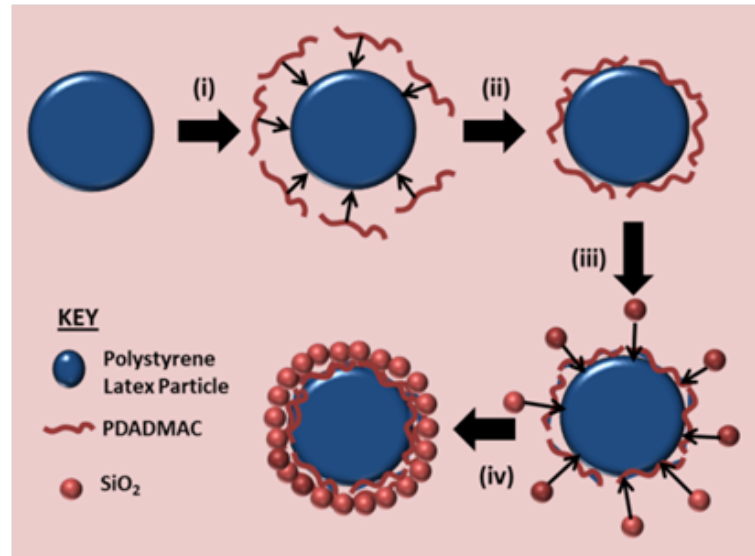


Figure 4.1: Schematic showing process of sonosensitive nanoparticle preparation. Construction of a single composite multilayer of and silica particles (SiO_2) on polystyrene latex particles exploiting electrostatic interactions. (i) Negatively charged core polystyrene nanoparticles (300 - 800 nm) were suspended in dilute poly (diallyldimethylammonium chloride) (PDADMAC) solutions; (ii) Following deposition of the cationic polymer layer, excess polyelectrolyte was removed by centrifugation, with intermediate water washings; (iii) PDADMAC coated latex suspended in SiO_2 suspension in NaCl; (iv) Excess SiO_2 removed by centrifugation, with intermediate water washings. Particles resuspended in deionized H_2O with sonication prior to characterization by Dynamic Light Scattering and Scanning Electron Microscopy analysis.

(PDADMAC) and colloidal SiO_2 on to PS core particles of varying diameters, and a range of surface morphologies were created by the deposition of various colloidal SiO_2 suspensions onto the surface. The deposition process is depicted in Figure 4.1. Core PS diameters were fixed at 300 nm, 600 nm and 800 nm. The range of colloidal SiO_2 suspensions used to coat the PS cores were: LUDOX[®] SM-30, LUDOX[®] TMA and LUDOX[®] TM-40.

Figure 4.2 shows the size distributions of each LUDOX[®] coating suspension and clearly shows the variation in the size distribution of SiO_2 particles. LUDOX[®] SM-30 showed an average particle size of 6.6 nm and LUDOX[®] TMA an average of 22nm;

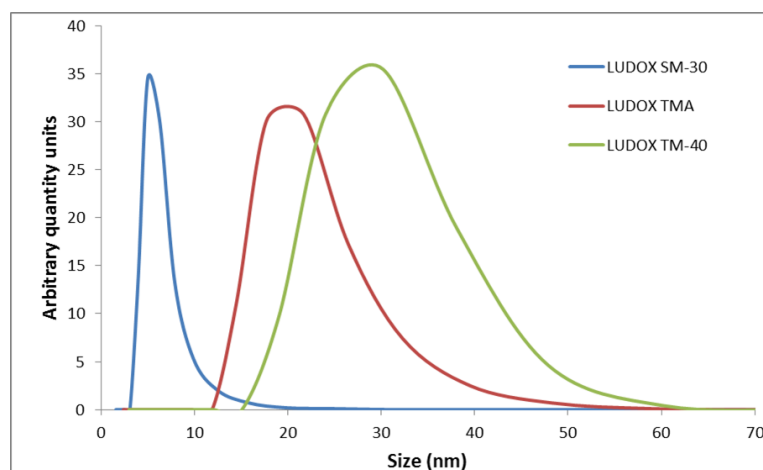


Figure 4.2: Size distribution of the LUDOX[®] colloidal silica particles used as coatings for the variously-sized polystyrene cores. Note the broad size distribution for the TMA and TM-40 particles (data provided by manufacturer, Sigma-Aldrich, St Louis, MO, USA)

the span in particle size for both suspensions were very narrow. However LUDOX[®] TM-40 had a much larger average size at 36.7nm but also showed a much broader range of particle sizes present in the suspension.

The following protocol was used for coating 600nm PS core particles with a single PDADMAC-SiO₂ shell, adapted from techniques reported previously (Caruso, 2001). Concentrations of core PS particles and coating material solutions were kept constant, centrifugation speeds were adjusted accordingly for 300 nm and 800 nm particles to ensure pellets were formed with minimal centrifugal force, preventing excess agglomeration.

Briefly, polystyrene nanoparticles (Sigma-Aldrich, St Louis, MO, USA) at a concentration of 1×10^{10} particles / mL were suspended in a solution of poly(diallyldimethylammonium chloride) (PDADMAC, 1 mg / mL, 0.5 M NaCl, Sigma-Aldrich, Mw < 200,000). Adsorption was allowed to proceed for 30 minutes, excess polyelectrolyte was then removed by three repeated centrifugation-wash-resuspension cycles (13,500g). Particles were suspended into colloidal silica

(LUDOX[®] 2 wt %, Sigma-Aldrich, SM-30, TMA and TM-40 dependent on depositing surface nanoparticle size) in 0.1 M NaCl solution. Following a 20 minute adsorption time, excess SiO₂ particles were removed by three repeated centrifugation-wash-redispersion cycles (13,500g). Final PS-PDADMAC-SiO₂ core-shell nanoparticles were resuspended in deionized water with sonication prior to characterization by dynamic light scattering and scanning electron microscopy (next section).

In total, twelve particle types (including uncoated particles) were generated using this methodology with varying diameters and surface morphologies. Four batches of each particle type were produced to allow method reproducibility to be estimated, both in particle preparation and cavitation threshold analysis.

4.1.1.2 Size Measurement

Particle sizes were measured using dynamic light scattering (DLS). Particle size distributions of each particle type (100 μ L of final suspension in 4 mL deionized water with sonication) were measured using a Zetasizer 3000 series instrument (Malvern Instruments, Malvern, UK) following PDADMAC-SiO₂ shell formation. Number-averaged particle size distributions were ascertained and the polydispersity index (PI) associated with the particle distribution was noted.

4.1.1.3 Scanning Electron Microscopy

Particle surface morphology was examined using a JSM-6500F scanning electron microscope (JEOL, Tokyo, Japan) at 10 kV for each particle type. Particle suspensions (20 μ L) were evaporated onto aluminium analysis stubs and coated with 3 nm platinum prior to analysis using a sputter-coater.

4.1.1.4 Freeze-drying

PS-PDADMAC-SiO₂ final particle suspensions were freeze dried in 10% (w/v) Trehalose (Sigma-Aldrich, D-(+)-Trehalose dehydrate) using a Virtis Advantage Plus freeze drying system. The protocol for freezing and drying is indicated in Table 4.1. The dried particles were resuspended with vortex mixing before DLS analysis as described above. Particles were only used for cavitation threshold measurements if a polydispersity index of below 0.25 was obtained. Smooth (uncoated) PS particles were freeze dried at comparable concentrations in 10% w/v Trehalose for controls in cavitation threshold experiments.

Thermal Treatment			Primary Drying			
Step	Temp (°C)	Time (mins)	Step	Temp (°C)	Time (mins)	Vac (mTorr)
1	20	5	1	-40	5	100
2	5	15	2	-20	40	100
3	5	10	3	-20	720	100
4	-5	10	4	-20	720	100
5	-5	10	5	-20	720	100
6	-40	35	6	20	160	100
7	-40	60	7	20	720	100
Freeze -40°C			8	20	720	100
Condenser -40°C			9	20	720	100
Vacuum 100mTorr						

Table 4.1: Freeze-drying recipe, thermal treatment and primary drying protocols

4.1.1.5 Particle Concentration Measurement

The concentrations of particles in the resuspended freeze-dried solutions were estimated using a qNano particle sizing and counting system (IZON, Cambridge, MA, USA). Briefly, particles were diluted in a standard electrolyte buffer and sonicated for 30 minutes prior to analysis. The concentration of particles in each suspension was calculated using a reference solution provided by the manufacturer. This con-

centration was used to prepare samples for cavitation threshold measurement with a concentration of 8.3×10^5 particles/mL.

4.1.2 Characterisation

The details for the 12 particle types produced are detailed in Table 4.2, which shows the core PS particle size, the colloidal silica (LUDOX[®]) coating suspension, the number-averaged particle size of the PS-PDADMAC-SiO₂ SNPs and the polydispersity index (Pdl) associated with the post freeze dried resuspended particle suspensions prior to cavitation threshold analysis. Each particle type was produced on multiple occasions (n=4), these ‘batches’ of particles were used to calculate the average sizes quoted in Table 4.2; the standard deviation between batches is also displayed.

PS Diameter (nm)	Colloidal Silica	Average size SiO ₂ (nm)	PS-PDADMAC-SiO ₂ size (nm)	Pdl
300	None		336.4±16.23	0.089
300	LUDOX [®] SM-30	6.1	351.98±9.38	0.171
300	LUDOX [®] TMA	22	388.7±9.86	0.182
300	LUDOX [®] TM-40	36.7	353.3±9.40	0.002
600	None		593.4±12.18	0.130
600	LUDOX [®] SM-30	6.1	684.4±13.1	0.190
600	LUDOX [®] TMA	22	716.25±13.38	0.268
600	LUDOX [®] TM-40	36.7	633.5±12.6	0.233
800	None		812.15±14.25	0.071
800	LUDOX [®] SM-30	6.1	863.6±14.7	0.016
800	LUDOX [®] TMA	22	851.3±14.6	0.030
800	LUDOX [®] TM-40	36.7	861.6±14.7	0.162

Table 4.2: Showing the twelve different particle types created with varying PS core size and coating colloidal silica solution. The number-averaged particle diameters of each colloidal silica solution as determined by dynamic light scattering is shown. The number averaged particle diameters of the PS-PDADMAC-SiO₂ SNPs are shown, together with the standard deviation of particle sizes between the four batches created. The polydispersity index associated with the SNP suspension following freeze drying is shown.

Table 4.2 shows that as expected, a general increase in particle diameter is ob-

served between uncoated, smooth PS core nanoparticles and PDADMAC-SiO₂ coated rough surfaced nanoparticles. However, no statistically significant difference was observed between coating particle size distribution and the thickness of the shell created, determined by the increase in particle diameter observed as a result of each coating type when compared to uncoated PS cores.

The surface morphology of the PS-PDADMAC-SiO₂ SNPs was examined by scanning electron microscopy prior to freeze drying. Figure 4.3 shows the images obtained for each uncoated core PS particle size and demonstrates the increase in surface roughness which is observed when particles are coated with the PDADMAC-SiO₂ shell. The variation in the surface morphology associated with the varying LUDOX[®] coating suspensions is clearly observed.

A uniform distribution is observed between core particle sizes for each LUDOX[®] coating suspension. LUDOX[®] SM-30 offers a homogeneous coating, producing an almost film-like surface but with an increased surface roughness over that associated with uncoated particles. LUDOX[®] TMA shows a highly rough surface with a highly close packed structure of the 22nm SiO₂ nanoparticles. The particles do not appear to have an ordered packing structure, this element of random packing of the SiO₂ particles at the surface offering an explanation as to the increase in polydispersity observed when coating 300nm and 600nm PS cores with LUDOX[®] TMA (Table 4.2). LUDOX[®] TM-40 coatings also show a high degree of surface roughness, the larger SiO₂ particles present in the colloidal suspension as indicated by DLS are clearly visible at the particle surface, offering a less uniform surface coating than is observed with the other colloidal suspensions.

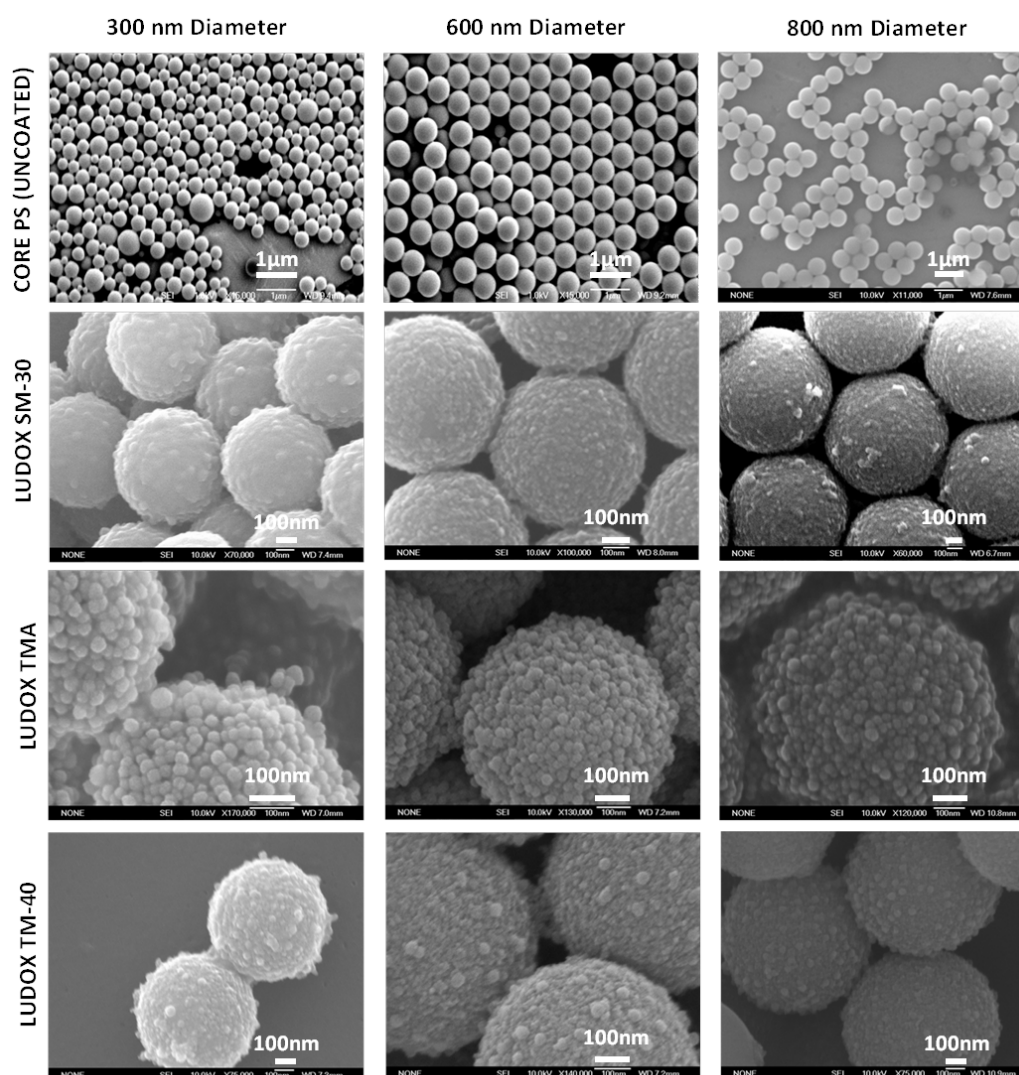


Figure 4.3: Scanning electron micrographs showing varying core particle diameters of generated PS-PDADMAC-SiO₂ nanoparticles (left to right) and the various surface morphologies obtained when LUDOX[®] coating solution is altered (top to bottom). Samples were evaporated onto aluminium sample stubs, coated with 3nm platinum and analyzed using a JEOL JSM-6500F FEGSEM at 10kV. Photographs show magnifications used and resulting scale bars show varying size particles.

4.1.3 Discussion

The results presented above clearly indicate that the protocol used is capable of producing a coating of LUDOX[®] particles on the surface of the PS cores - particles suspended in LUDOX[®] are larger than the uncoated particles, and a surface coating can be clearly seen in the SEM images. However, the observed particle size does not increase with the increasing size of the LUDOX[®] coating, as might be expected; there is no obvious pattern to the relative sizes for the three coatings.

Part of the explanation for this lies with the dispersity of the size of the LUDOX[®] particles themselves (Figure 4.2), and part of the explanation with the formation of multiple layers of LUDOX[®] particles on the surface. This second effect can be seen most clearly in the SEM image of the LUDOX[®] TMA on the 300nm PS core in Figure 4.3. This ‘aggregation’ of multiple layers should be inhibited by the electrostatic nature of the interaction (i.e. the like charge on all the LUDOX[®] particles should mean that they repel each other), but it may be either that the LUDOX[®] in suspension is aggregated to begin with, or that residual PDADMAC may be present to balance the charge and form aggregates of silica on the particle surface.

While there is some aggregation of the LUDOX[®] on the surface of the particles, the values of the polydispersity index suggests that there is minimal aggregation of the core-shell particles themselves. This issue was regularly experienced with early batches of particles, which is problematic as the larger aggregates formed are expected to have different circulation dynamics in the body.

It is also worth commenting on the nature of the surface coating as seen in the SEM images. The coating of LUDOX[®] particles is clearly not regular or close packed. From the perspective of cavitation nucleation, this is expected to result in a population of crevices on the surface of the particles with a broad distribution of both size

and geometry, each with their own cavitation threshold (Section §2.3.1.1). The impact of this on the results of the experimental work described in the next section are discussed later (Section §4.2.4).

The observed difference in surface morphology between particles with different coating sizes, and to a lesser extent core size, will also change cavitation behaviour. This variation may be an artefact of the particles selected for imaging, but is more likely an indication of the effect of the size of the colloidal silica on its behaviour, and the sensitivity of the production process to minor variations in experimental conditions.

Despite the lack of a uniform monolayer coating, the particles described here are expected to provide a strong indication of the effect of a LUDOX[®] coating on cavitation threshold. This work is described in the following section.

4.2 EFFECT ON CAVITATION THRESHOLDS

While the results presented above indicate that the preparation protocol has been successful in attaching silica particles to a PS core, they do not give any indication of whether the particles will succeed in their intended purpose of reducing the local cavitation threshold in solution, and ultimately *in vivo*. To test this, a simplified model system has been developed, with the particles suspended in a stirred, optically-transparent chamber and exposed to HIFU. As the amplitude of the short pulses was increased stepwise, the presence of cavitation was assessed by passively monitoring acoustic emissions which were then analysed using numerical implementations of the concepts discussed in section §2.3.3. The experimental setup, execution, results processing and interpretation are detailed below, together with a discussion of the potential use of the particles in a clinical setting.

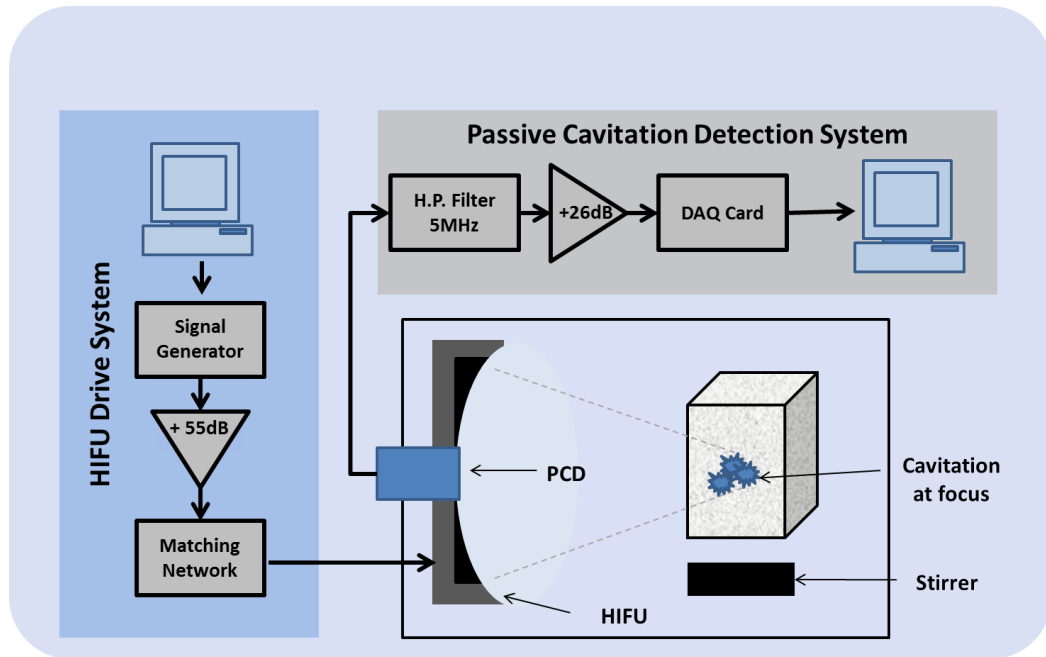


Figure 4.4: Schematic illustration of the experimental setup used for the characterisation of the acoustic cavitation threshold of the SNPs in aqueous suspension. A HIFU transducer was driven by a signal generator controlled from a PC, amplified 55 dB and passed through an impedance-matching network. The focus of the transducer was positioned inside the centre of a small chamber containing the SNP suspension. A confocally-aligned 15 MHz PCD acquired acoustic traces from the focus, which were filtered (5 MHz high-pass) and pre-amplified (26 dB) before digitization and storage in the memory of a PC for post-processing.

4.2.1 Experimental Setup

A schematic of the experimental setup used is shown in Figure 4.4. The components of the system can be divided into three groups: the particle holder, the sonication equipment, and the cavitation detection equipment.

4.2.1.1 Particle Holder

The primary concern in identifying a means of presenting the particles in front of the HIFU transducer was that the particles in the focal region should be renewed between exposures, thus avoiding any depletion effects associated with prior exposure of the particles to HIFU. Several different setups, including flow through a channel

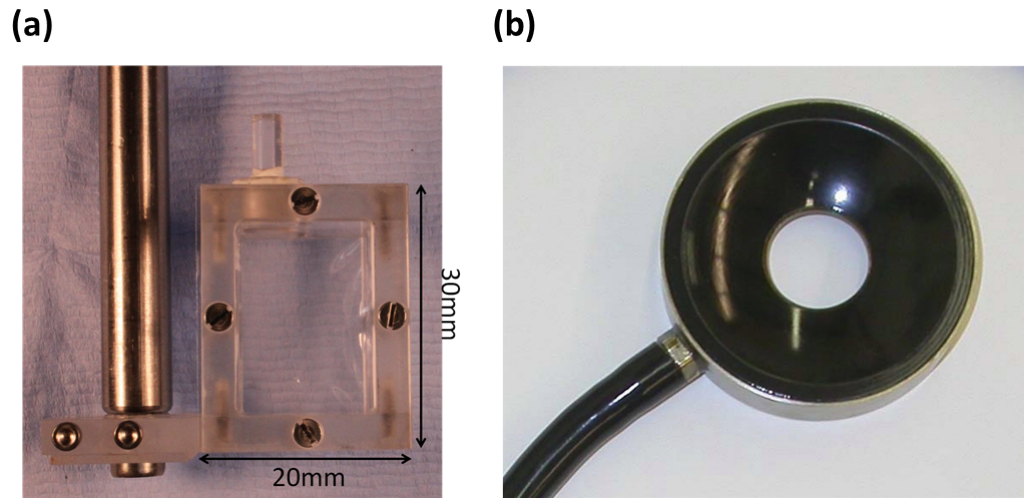


Figure 4.5: **(a)** Photograph of the chamber used for the cavitation threshold testing of the SNPs **(b)** Sonic Concepts H102 focused HIFU transducer, showing the circular opening for placement of a coaxial PCD

inside a tissue-mimicking phantom and injection of particles directly into multiple points within a phantom were considered and rejected due to difficulties in ensuring reproducibility, and the time required for data acquisition. In the end, it was decided to suspend the particles in degassed, distilled water inside a simple chamber and stir them during HIFU exposure with a magnetic stirrer bar. This simple setup had the benefit of being simple and reproducible, and allowed the relatively rapid processing of samples.

The stirred chamber (Figure 4.5) measured 30mm x 20mm x 20mm (total volume of 12cm³) and was made largely of perspex. The front and back walls were acoustically transparent mylar, fixed in place around their outer boundary by a polycarbonate plate. It was mounted on a three-dimensional positioning system (Thorlabs, Newton, NJ, USA) that could be controlled through software on the attached PC.

4.2.1.2 Sonication Equipment

Custom LabView software (National Instruments, TX, USA) was developed to control and synchronise the equipment used in the experiments. The software triggered a signal generator (33250A, Agilent, Santa Clara, CA, USA) to output a pulsed sinusoid, which was transmitted to a 55dB power amplifier (A300, E&I, Rochester, NY, USA), then through an impedance-matching network to the HIFU transducer. Several different HIFU transducers were used in these experiments to allow for testing at different frequencies - an H102 (Sonic Concepts, Bothell, WA, USA) with a narrow-band resonant frequency of 1.067MHz that could also be driven at 3.46MHz (third harmonic), and an H107 (Sonic Concepts, Bothell, WA, USA) with a resonant frequency of 508kHz and a third harmonic resonance at 1.682MHz. Both transducers had a circular opening in the centre to allow for the coaxial placement of a PCD (Figure 4.5).

Both spatial and absolute calibrations of both HIFU transducers were carried out using a 75 μ m needle hydrophone (Precision Acoustics, Dorset, UK). The absolute calibrations relating peak-to-peak voltage into the impedance matching network to the peak focal pressure were used to determine the focal pressures in later experiments with the SNPs. The spatial calibration was done by means of linescans along the three perpendicular axes of the HIFU transducer: results for the H102 driven at 1.067MHz are shown in Figure 4.6. For all of the transducers at all of the frequencies the transverse cross-section of the full-width half-maximum focal region was smaller than the cross-section of the particle holder. The axial extent of the focal region was of the same order as the depth of the chamber (~20mm), an issue which was addressed by windowing the monitoring signal prior to processing to ensure that any cavitation activity outside the chamber was excluded (see Section §4.2.3.1).

The results of the absolute amplitude calibration of the transducers used are

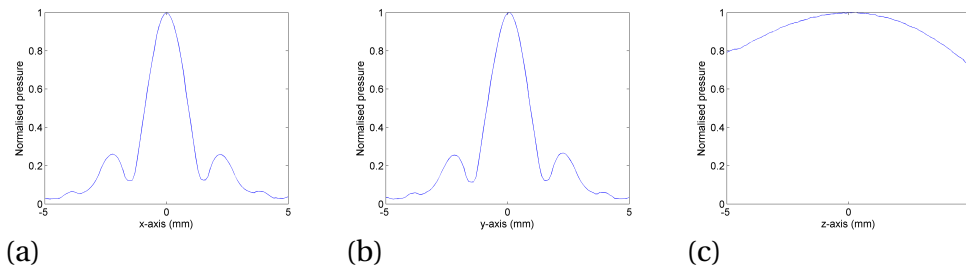


Figure 4.6: Spatial calibration of the H102 transducer at 1.067MHz. **(a)** x-axis, **(b)** y-axis, **(c)** z-axis. Note the similarity between (a) and (b) indicating the symmetry of the focus in the transverse plane. The low ratio of transducer diameter to focal length gives rise to the long axial focus, demonstrated by the small drop in pressure in (c)

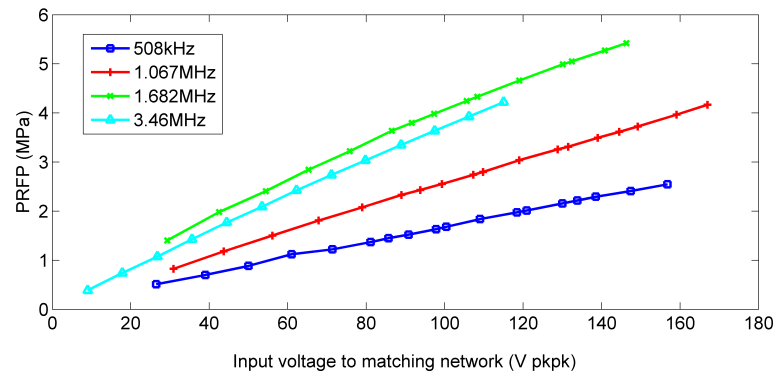


Figure 4.7: Absolute calibration curves for the HIFU transducers at the four frequencies used in the experiments to characterise the cavitation behaviour of the SNPs. Note the increased focusing gain of the higher frequencies

shown in Figure 4.7.

4.2.1.3 Cavitation Detection

Acoustic emissions were recorded by a broadband, spherically-focused PCD (Panametrics V319, Olympus, Essex, UK) with a 15MHz centre frequency and 75mm focal length, mounted coaxially and aligned confocally to the HIFU transducer. The received signal was high-pass filtered at 5MHz (Allen Avionics, Mineola, NY, USA) to suppress reflections at the driving frequency, and amplified 25-fold (SR445A, Stan-

ford Research Systems, CA, USA) to improve the detection sensitivity. The signal was digitised at a sample rate of 100MHz using a 14-bit DAQ card (PCI-5122, National Instruments, TX, USA), and passed to the custom LabView software to perform initial data processing, visualisation and storage (Section §4.2.3). For each shot, 250 μ s of signal was acquired, triggered by the synchronisation signal (hardware) from the HIFU signal generator.

4.2.2 Experimental Procedure

4.2.2.1 Alignment

Correct alignment of the transducer at the centre of the mixed chamber was essential for both reproducibility between samples, and to avoid any effects of the chamber walls. The first alignment step was to confocally align the HIFU transducer and PCD. Briefly, a pulser-receiver (DPR300, JSR Ultrasonics, NY, USA) was used to drive the HIFU in pulse-echo mode, with a 6 mm brass sphere approximately at the focus. Based on the water temperature, the speed of sound and the time delay corresponding to the pulse-echo path length was calculated (the focal length was determined during calibration). The pulse-echo received signal was displayed on an oscilloscope (WaveSurfer 434, LeCroy, NY, USA). The brass sphere was attached to a three-dimensional positioning system, and was aligned depthwise using the appropriate time delay, and transversely by maximizing the reflected signal. The procedure was repeated for the PCD (with the HIFU fixed in place) before it too was fixed in place. The signal received on each transducer when driven in pulse-echo mode was checked once again to ensure confocal alignment before beginning experiments.

The second alignment step was to ensure that the transducer is positioned with the focus at the centre of the stirred chamber containing the particle suspension.

The alignment was performed at the beginning of each day of experiments using the same pulser-receiver as above to drive the PCD as the smaller focal region of this transducer gives greater spatial resolution than the HIFU. The chamber was manually positioned approximately at the transducer focus, and moved axially so that the reflections from the front and back wall of the chamber were evenly spaced about the time-of-flight to the focus. The chamber was then moved transversely in both directions until reflections were seen from the perspex casing (much higher amplitude than the mylar), and positioned at the midpoint between the two sides. This position was clearly marked on the positioning system to enable the chamber to be returned to the same position each time the particle suspension was replaced.

4.2.2.2 Particles

Particles produced as described previously (Section §4.1) were resuspended in a minimal volume of distilled, degassed water, and allocated into aliquots of 1×10^7 particles per experiment (8.3×10^5 particles/mL). Particle counts for each vial of freeze-dried SNPs were obtained from dynamic light scattering measurements. These aliquots were then made up to ~10mL total volume (filling their vial completely) and vortexed to ensure a homogenous suspension with complete dissolution of the trehalose. The suspension was transferred to the stirred chamber together with a small magnetic stirrer bar (~12mm x 3mm), and completely filled with water. Care was taken to avoid the formation of any bubbles on the chamber walls, and any visible bubbles were removed before sealing the chamber and placing it in the tank.

Several preliminary experiments were carried out to determine the optimal particle concentration in the chamber. It was desirable to minimise the number of particles per experiment in order to maximise the number of samples per particle type,

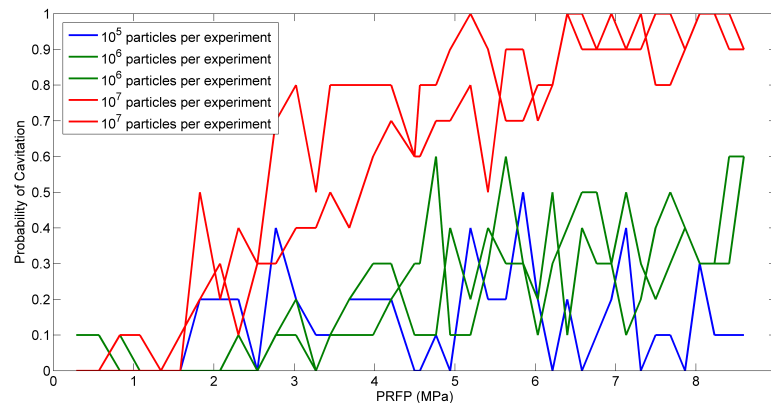


Figure 4.8: The effect of particle concentration on the detection of cavitation. For the lower concentrations it can be seen that the probability of cavitation remains low (0.5) as the PRFP is increased; with 10^7 particles per experiment (8.3×10^5 particles/mL) the probability increases to 1. Experiments were conducted using particles created with a 600nm PS core and LUDOX[®] TM-40 coating.

as particle production is a time-consuming process and supply was limited. Beyond a certain level, however, cavitation detection can become erratic as the number of cavitation events at the focus becomes too low to be readily detected by the PCD. A series of particle concentrations was tested, and it was found that 8.3×10^5 particles/mL was the minimum for which cavitation could be reliably and reproducibly detected - the results are shown in Figure 4.8. Note that higher concentrations were not tested due to a shortage of particles.

4.2.2.3 Blood

For the experiments to examine the behaviour of the SNPs in blood, the preparation protocol was similar to that outlined above for the particles in water. Fresh porcine blood was obtained from a heparinised animal, volume maintained with isotonic gelofusine (Product 6060326, B Braun, Melsungen, Germany), into standard clinical collection bags, and maintained at 4°C overnight. The next day, the

blood bag was gently palpated to resuspend the erythrocytes, and dispensed into a small beaker using sterile giving set. The bulk blood bag was then returned to storage at 4°C, while the dispensed blood was warmed to 37°C in a waterbath. SNPs were suspended in blood to the same concentration as in the water experiments. Cavitation threshold measurements were carried out in the tank with the water heated to 37°C.

It was found that the process of dispensing the blood and transferring it to the chamber resulted in the presence of numerous small bubbles in solution, and that these took >20 minutes to rise to the surface. To eliminate the effect of this on the cavitation threshold measurements, the blood was allowed to sit in the chamber for 30 minutes before measurements were taken, and was not stirred to minimise the likelihood of bubbles being recirculated through the bulk of the solution.

4.2.2.4 Sonovue

Sonovue (Bracco, Italy) is a commercially-available ultrasound contrast agent (UCA) that has been used in several studies as a source of artificial cavitation nuclei (Gorce et al., 2000; Arvanitis et al., 2011). For these experiments, Sonovue was made up into a 5mL volume as per manufacturers instructions. The concentration of particles is reported to be $1 - 5 \times 10^8$ /mL (manufacturer documentation). Assuming the midpoint of this range (3×10^8 /mL), serial dilutions were performed with distilled, degassed water in order to achieve a final concentration of 1×10^7 particles per experiment (8.3×10^5 particles/mL, equivalent to the SNPs). The diluted Sonovue solutions were mixed with a magnetic stirrer throughout, and used as quickly as possible after preparation, with the exception of the storage stability experiments.

4.2.2.5 Experimental Run

Cavitation Threshold The HIFU transducer and confocally-aligned PCD were placed in a tank of distilled water that had been passed through a $0.22\mu\text{m}$ filter, and degassed at -0.8 to 0.95 bar for at least 6 hours (Liqui-cel 2.5x8 membrane contactor, -0.9 bar vacuum). A waterproof magnetic stirrer was positioned inside the tank such that it sat just in front of and below the HIFU transducer. Prior to each test, the stirred chamber was carefully cleaned and rinsed several times with degassed, distilled water to ensure that no residual particles from previous tests were present. It was then filled as described above, and placed at the focus of the transducers. Any visible bubbles on the surface of the holder were removed by pipette.

The chamber was then exposed to a series of HIFU pulses (controlled by the Lab-View software) to investigate the incidence of cavitation at different acoustic pressure amplitudes. The suspension was subjected to 10 excitation ‘shots’ at each pressure amplitude, before the amplitude was stepped up and the process repeated. Each shot consisted of 10 cycles at either the fundamental or the third harmonic of the HIFU transducer, with each shot separated by 1.5s to allow for the mixing to replenish the particle population at the focus (Figure 4.9). The pressure amplitude was varied by the output voltage of the signal generator, and this was stepped up from 12.5mV to 500mV in 40 steps. The corresponding focal pressure range varied with the HIFU frequency due to variable acoustic efficiency of the transducers, and the variation in focal gain with frequency (Figure 4.7).

At the beginning of each run of tests, the water in the tank (ie without the chamber present) was subjected to the same threshold-testing protocol to check for any baseline cavitation activity, and additionally the chamber was filled with water and subjected to the exposure protocol to check for activity resulting from the presence of the chamber. Where a large number of samples were tested in a single session,

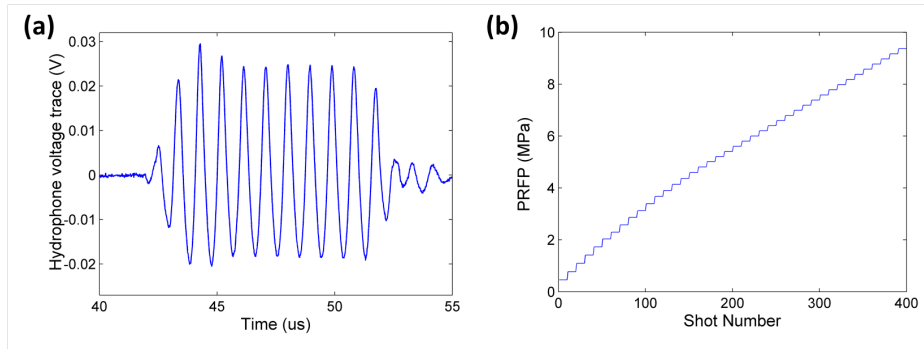


Figure 4.9: Representation of the HIFU pulse sequence used for the cavitation threshold measurements. **a** shows an example single shot with 10 acoustic cycles, while **b** shows how the pressure amplitude is maintained for 10 shots before it is increased stepwise

measurement of a water-filled chamber was repeated at several points throughout the run to test for any presence of residual particles within the chamber. In none of these control runs was a level of cavitation activity observed that would interfere with measurements of thresholds or cavitation signal levels.

Storage Stability SNPs were suspended as described above, and maintained in a sealed vial at room temperature between suspension and testing. Sonovue (Bracco, Italy) microbubbles were suspended as per the manufacturer's instructions and maintained in the buffer solution in a sealed vial between suspension and testing. Immediately prior to testing, the Sonovue was diluted to the appropriate concentration (8.3×10^5 particles/mL) using distilled, degassed water. All suspensions were vortexed immediately prior to measurement to ensure homogeneity.

The procedure for the cavitation threshold measurement experiments was identical to that described above for the SNPs.

Depletion Behaviour The same insonation setup was used. Rather than stepping up to 500mV output from the signal generator, the pressure amplitude was increased stepwise (10 shots at each pressure) until cavitation was observed in at

least 8 of 10 shots. This amplitude was then maintained for successive shots until the probability of observing cavitation in a rolling 10-shot window dropped to approximately 0.1. This same process was repeated three times on the same sample.

4.2.3 Data Analysis

Following experimental data acquisition, the raw PCD traces were processed and analysed to determine the probability and extent of cavitation, and the cavitation threshold for each sample. Statistical analysis was carried out to identify significant differences in the behaviour of different particle types. This section describes the approach to data analysis and the algorithms employed.

4.2.3.1 Windowing and Filtering

The significant axial length of the HIFU focus relative to the depth of the test chamber meant that the water close to the surface of the mylar may have experienced pressures sufficient to cavitate, potentially confounding results. To avoid this, the raw PCD trace was windowed to include only the section from 90-100 μ s (red lines in Figure 4.10(a)), which corresponds spatially to a small region about the HIFU focus (positioned at the centre of the test chamber). This window excluded the reflection of any part of the primary signal (10 μ s in duration at 1.067MHz) from the front face of the chamber, which was seen to begin at 78-79 μ s.

The raw PCD voltage trace potentially contains reflections of the fundamental HIFU signal in addition to the secondary acoustic emissions from cavitation at the focus. The direct effect of the fundamental is limited by both the finite bandwidth of the PCD and the analog 5MHz high-pass filter, but the non-linearity of water (Section §2.2) means that higher harmonics may be present and be reflected back to the PCD. Potential sources of reflection are the mylar faces of the test chamber, the

polycarbonate walls of the test chamber, and the (distant) walls of the tank itself. The SNPs are not expected to generate such non-linear reflections: by virtue of their small size, they are non-echogenic until inertial cavitation occurs.

To remove these components from the signal, a software filter was implemented to remove the harmonic components from the raw PCD trace. A custom comb-filter was implemented in the frequency domain, with notches (50kHz half-width unless otherwise specified) centred on multiples of the fundamental HIFU frequency to remove harmonic components. The mean value of the signal spectrum in both the 'broadband' and 'harmonic' regions of the signal was calculated, and the ratio between them taken as a rough measure of the character of the cavitation - a ratio of unity indicating a largely broadband signal characteristic of inertial cavitation. The reconstructed 'broadband' signal was further processed to detect cavitation events and calculate a cavitation threshold.

4.2.3.2 Cavitation Detection

Each trace of data acquired from the PCD was analysed for the presence of cavitation using an algorithm previously described (Kyriakou et al., 2011). In short, the level of background noise in the filtered PCD signal was calculated from an early section of the trace (10-40 μ s) that is assumed not to contain any reflection or direct transmission from the HIFU signal. Cavitation was deemed to have occurred if the amplitude of the PCD voltage trace in the windowed region (90-100 μ s, red lines in Figure 4.10(a)) exceeded a threshold set at six times the standard deviation of the background noise signal (marked by green lines in Figure 4.10(a)).

The sensitivity of the algorithm is limited by the sensitivity of the detection system, and may therefore systematically over-estimate the cavitation threshold; it is also sensitive to electrical noise which may result in some false positives. However, it

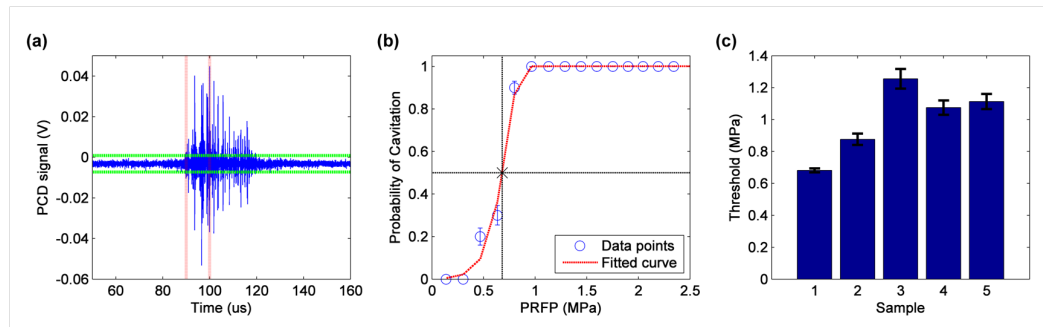


Figure 4.10: Illustration of the stages in the data processing, in this case for 300 nm particles with a LUDOX[®] TMA (22 nm) coating measured at 1.067 MHz. (a) A section of a PCD voltage signal that has been filtered to remove harmonic components. The red lines indicate the signal 'window' that is examined to determine if cavitation has occurred, while the green lines indicate the threshold (6 standard deviations of the signal background) above which cavitation can be said to have been induced by the HIFU signal. (b) Data points showing the probability of cavitation for one sample over the range 1 - 2.5 MPa peak rarefactional, or negative, pressure (PRFP). The red line is the sigmoidal curve fitted, with the 50% probability of cavitation threshold (0.68 MPa) indicated by the intersection of the black lines [errors are the binomial error]. (c) The measured cavitation threshold for 5 samples of the same particle, showing some sample-to-sample variation [errors are 95% confidence intervals of the fitting algorithm]

has the advantages of being consistent, computationally cheap and therefore able to provide realtime feedback, and simple to implement experimentally, and was therefore deemed to be an appropriate choice for this work.

4.2.3.3 Threshold Estimation

For a given sample at a given pressure amplitude, the probability of cavitation was calculated as the proportion of shots in which cavitation was observed, in line with that reported in the literature (Herbert et al., 2006). The probability was seen to increase with increasing pressure amplitude, as would be expected. The uncertainty in the probability data (error bars on Figure 4.10(b)) was estimated using a binomial distribution to calculate a standard error, using equation (4.1):

$$SE = \sqrt{\frac{P_r * (1 - P_r)}{n}} \quad (4.1)$$

where P_r is the probability of cavitation calculated as described previously.

This probability of cavitation takes a sigmoidal form when plotted against increasing PRFP (Herbert et al., 2006), and several forms of sigmoidal curve can be used in fitting the data. In this work, the form of equation used by Herbert et al (Herbert et al., 2006) was modified slightly (through the inclusion of the extra scaling factor γ in equation (4.2)) to enable it to give an improved fit for data that had not reached the upper asymptote at the highest pressures achieved in the experiments. The fitting equation used was:

$$\text{Probability of Cavitation} = \gamma \left\{ 1 - \exp^{-\ln(2) \exp[\alpha(P_{ac} - \beta)]} \right\} \quad (4.2)$$

where P_{ac} is the PRFP at the HIFU focus, and α , β and γ are fitting factors. The equation was fitted to the data using the Levenberg-Marquardt algorithm for non-linear least squares (built-in implementation in Matlab), and the initial conditions varied manually until a stable solution was found. Where γ fell outside the range of $0.5 < \gamma < 3.0$ the results were excluded as this was taken to be indicative of either data that could not be fitted to the curve form ($\gamma > 3$), or a cavitation threshold above the maximum pressures reached in the experiment ($\gamma < 0.5$). As a final check, the curve fit was checked visually against the raw data. An example of a fitted curve is shown in Figure 4.10(b).

From the fitting parameters, the cavitation threshold (P_{cav} - probability of cavitation equal to 0.5) was calculated by rearranging to give equation (4.3):

$$P_{cav} = \beta + \frac{1}{\alpha} \ln \left\{ \frac{\ln \left(1 - \frac{1}{2\gamma} \right)}{-\ln 2} \right\} \quad (4.3)$$

where α , β and γ are the fitting parameters as determined from the curve-fitting procedure.

The 95% confidence intervals for the fitting parameters in equation (4.2) were calculated using a method based on an asymptotic normal distribution for the parameter estimate described in detail elsewhere (Bates and Watts, 1988; 2008), and implemented in the standard form in Matlab. From the confidence intervals in the fitting parameters, the 95% confidence interval for the cavitation threshold was calculated by equation (4.4):

$$CI(P_{\text{cav}}) = P_{\text{cav}} * \left[\frac{\beta - CI(\beta)}{\beta} \right] \quad (4.4)$$

which assumes that γ is equal to unity - a valid assumption given that nearly all of the samples asymptote to a probability of 1 within the range of pressures tested. An example of the measured cavitation threshold of five samples of 300nm-core particles with a LUDOX[®] TMA coating, with uncertainties calculated as above, is shown in Figure 4.10(c).

4.2.3.4 Statistical Treatment

Comparisons of the cavitation threshold values of different particle formulations were carried out in order to investigate the effect of varying the size of either the PS or the LUDOX[®] coating. In these comparisons, the cavitation threshold results for the subset of particle types under analysis were grouped, and one-way analysis of variance (ANOVA) used to test the hypothesis that all particles belonged to the same population. If the hypothesis was rejected at a 95% confidence level ($p < 0.05$), a multiple comparison test was performed to identify which pair-wise relationships show a significant difference (ie $p < 0.05$), using a Tukey-Kramer correction to account for the multiple pairs being compared. All data analysis and statistical testing was done using built-in functions in Matlab (Mathworks, MA, USA).

4.2.4 Results and Discussion

4.2.4.1 Effect of Core and Coating

The 50% probability of cavitation threshold at 1.067MHz associated with all twelve particle types are shown in Figure 4.11. Figure 4.11(a) compares the effects of different LUDOX[®] coatings for a given core size, while (b) compares the effect of core size for a given LUDOX[®] coating.

It is clear from (a) that a LUDOX[®] coating on the polystyrene core significantly ($p < 0.05$) reduces the cavitation threshold when compared to an uncoated PS particle at the test frequencies, which strongly suggests that the colloidal silica is introducing a population of nuclei in some way. However, despite clear differences in microscopic surface morphology resulting from coating with the various LUDOX[®] suspensions, there is no significant change in threshold between the different LUDOX[®] coatings for any of the core sizes tested.

Figure 4.11(b) shows that for a given LUDOX[®] coating, the core size in general has no significant ($p < 0.05$) effect on the cavitation threshold. However, the 600nm core particles appeared to have a slightly lower threshold when compared to the other core particles with the same LUDOX[®] TMA coating at the surface. These particles had the highest polydispersity among the particle types examined across all four batches used (Table 4.2), which may indicate higher levels of agglomeration. We hypothesise that particle agglomerates trap air between particles as well as on coated surfaces, which may account for the lower threshold observed for these particular particles.

The ability of small particles (300nm core) to lower cavitation threshold as effectively as larger particles (800nm core) has relevance clinically, as these smaller particles are more readily capable of extravasation and natural accumulation in tu-

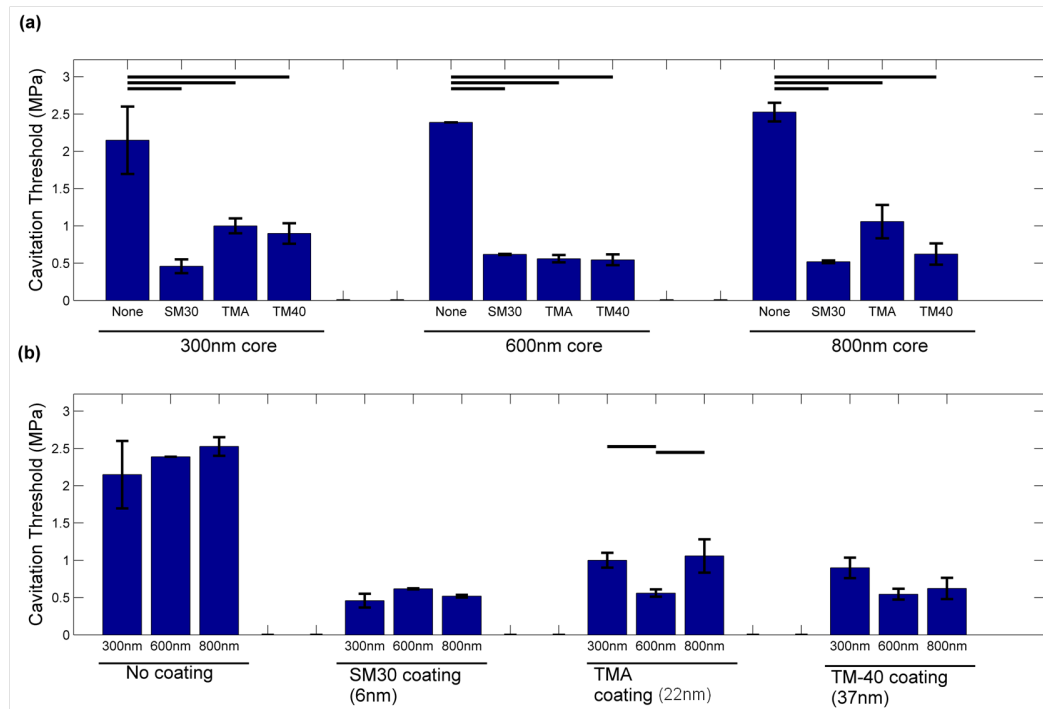


Figure 4.11: Comparison series showing the difference in cavitation threshold at 1.067MHz as **(a)** the size of the particles used in the coating, and **(b)** the size of the solid polystyrene core changes. Blue bars (errors are standard errors calculated from the 95% CI in the fitting algorithm) show the mean threshold from several samples ($n=3-5$), with the lines over the bars highlighting statistically significant differences ($p < 0.05$, one-way ANOVA with tukey-kramer correction in multiple pairwise comparisons). The only statistically significant differences in (a) are between uncoated particles and coated particles, which suggests that the size of coating particles has a relatively minor effect on cavitation nucleation. The low threshold of the 600nm core, LUDOX[®] TMA-coated particles in (b) may simply be the result of natural variation between samples.

mours, and may therefore be useful in enhancing cavitation-based treatments. Currently available microbubbles (e.g. Sonovue) are too large to achieve such extravasation in tumours by the enhanced permeability and retention (EPR) effect (Maeda et al., 2000).

The cavitation threshold measured for coated particles was in the range 0.5 - 1.0 MPa, while for uncoated particles it was 2.0 - 2.5 MPa (both at 1.067 MHz). This is much lower than the values reported for tissue in vivo, which range from 3 - 4

MPa for blood (Deng et al., 1996) and liver (S. Labouret, personal communication) at similar frequencies, and may therefore enable selective lowering of the cavitation threshold to enhance therapeutic effects of HIFU in the body. The diagnostic ultrasound used for imaging purposes is typically at frequencies from 2 - 10 MHz and lower pressure amplitudes than HIFU, and can therefore be used in conjunction with the SNPs without the risk of unintended cavitation events.

The exact mechanism of cavitation nucleation offered by the LUDOX[®] coating is not clear. It may be that gas pockets are trapped in the nanometer-scale crevices between individual silica particles on the surface of the PS or that larger gas bubbles are stabilized on the hydrophobic surface of the silica layer as a whole. Distinguishing between these two potential mechanisms conclusively is difficult given the limited resolution of current characterisation methodologies.

Theoretical models of cavitation nucleation provide only limited insight: models based on single, spherical gas bubbles (Apfel and Holland, 1991) do not consider the effect of either a relatively-larger solid surface that the bubble may be adhered to (if the gas pocket is on the surface of the SNP); models based on nucleation from a crevice are limited in the geometries considered (Section §2.3.1.2) and assume a static external pressure which is not the case for HIFU where the pressure varies on a sub-microsecond timeframe. Chappell and Payne (2007) consider several well-defined axisymmetric geometries, and show that crevice geometry and initial gas volume both have a large impact on the pressure required to initiate nucleation. The relatively random assembly of LUDOX[®] particles on the core PS surface (Figure 4.3) would be expected to give rise to a diverse population of crevices on the surface of the SNP, with a broad distribution of sizes and geometries, and therefore cavitation nucleation thresholds over a similarly wide range of pressures. Variation in the size of either the core or the surface coating would change the particle distri-

bution slightly, but if this change was small relative to the overall spread, then the measured cavitation threshold would not be affected, consistent with the results in Figure 4.11.

4.2.4.2 Depletion of Cavitation Activity

Figure 4.12 shows the effect of an extended series of shots at a single amplitude on the probability of cavitation for a single sample of SNPs. The pressure amplitude was ramped up until it was above the cavitation threshold, then maintained until cavitation activity was exhausted; this process was then repeated twice more for the same sample. As expected, a progressively higher pressure amplitude was required to initiate cavitation after each exhaustion sequence, increasing from $< 1\text{MPa}$ to $\sim 2\text{MPa}$, and then to $\sim 3.5\text{MPa}$ for the final run.

For the first exhaustion sequence at 1.4MPa PRFP, cavitation activity decreased gradually over 2500 shots, to a probability of ~ 0.25 . In the second sequence at 2.8MPa PRFP, the probability of observing cavitation decreased even more slowly, stabilising at ~ 0.4 after 3000 shots, while in the third sequence at 4MPa PRFP, cavitation continues to be seen out to 5000 shots.

By comparison, Sonovue microbubbles subjected to the same exposure protocol were shown to deplete in activity much more completely and rapidly (Figure 4.13). Cavitation was observed at much lower pressure amplitudes than for the SNPs (0.1MPa vs 1.4MPa), and initially it appeared as though cavitation was sustained for an extended period (Figure 4.13(b)). However, upon spectral analysis the first 750 pulses were found to be predominantly harmonic components of the fundamental, suggesting that the bubbles were not cavitating inertially, but rather cavitating stably or reflecting incident radiation. The pressure amplitude was then increased (at the point indicated with a red arrow) to 0.2MPa , at which point an el-

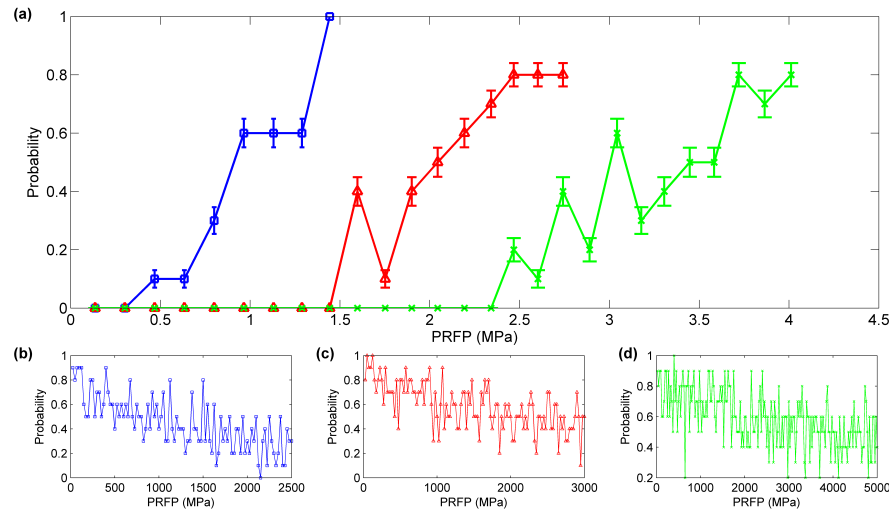


Figure 4.12: Behaviour of the SNPs (600nm core, LUDOX[®] SM-30 (6nm) coating) upon exposure to an extended series of HIFU pulses at 1.067MHz. In these experiments, the HIFU amplitude was increased (10 shots at each amplitude) until a probability of > 0.8 was observed (results shown in (a), blue line), and then pulses continued at this amplitude until the probability decreased to approximately 0.2 (results in (b)). The process (ramp to $Pr > 0.8$, extended series of pulses at this amplitude) was repeated three times, with the second run shown in red ((a) and (c)) and the third run in green ((a) and (d)). The results show that the ability of the SNPs to nucleate cavitation is sustained under successive excitation pulses (> 2500 for the chamber configuration used), and suggests that there is a broad population of nuclei present with different thresholds. Error bars in (a) are binomial uncertainty, the probability shown in (b), (c) and (d) is calculated as the proportion of pulses with cavitation events in successive 25-pulse windows.

evation of the noise floor suggestive of inertial cavitation was taking place, and the probability of cavitation decreased sharply over 800 shots. After a second sequence of 400 pulses at 0.24MPa, cavitation activity was effectively depleted, even for subsequent exposures at up to 6MPa PRFP.

The comparison between the depletion of the two types of exogenous nuclei is instructive. Sonovue microbubbles in suspension have a low threshold, but inertial cavitation causes them to rupture and their ability to nucleate cavitation in future is lost even at higher amplitudes, implying a relatively uniform population of nu-

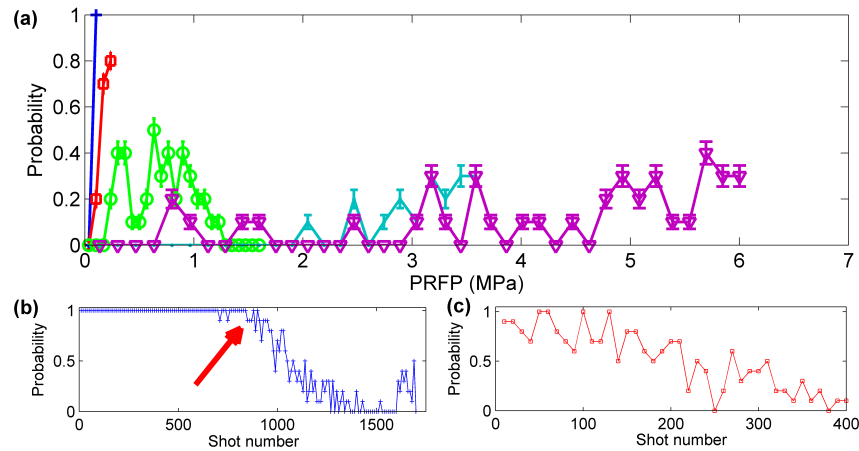


Figure 4.13: Behaviour of Sonovue microbubbles upon exposure to an extended series of HIFU pulses at 1.067MHz, following the same protocol (5 runs instead of 3) and display convention as described for the SNPs in Figure 4.12. Only two extended runs are shown, as the probability of cavitation never reached 0.8 for subsequent thresholding runs. The arrow in (b) indicates where the amplitude was increased from 0.1MPa to 0.2MPa. The results show the depletion of the cavitation nucleation of the Sonovue bubbles in an extended sequence of exposures. Error bars in (a) are binomial uncertainty, the probability shown in (b) and (c) is calculated as the proportion of pulses with cavitation events in successive 10-pulse windows.

clei. SNPs, on the other hand, have a higher initial threshold but are able to sustain cavitation activity over many more shots, and exhibit cavitation at high amplitudes even after depleting activity at lower amplitudes, implying a much larger and more diverse population of nuclei. Additionally, since the Sonovue and SNPs are present in the same concentrations, it would suggest that either there is more than one cavitation nucleus for each SNP particle in suspension, or that bubble fragments are stabilised on the surface of particles following collapse.

These observations support the hypothesis that nucleation in the SNPs occurs from small crevices in the surface of the particles. If nucleation for the SNPs were based on larger gas bubbles stabilised on their surface, they would be expected to be present in smaller numbers, behave similarly to the Sonovue and deplete rapidly. The storage stability results in Figure 4.16 are also consistent with this conclusion.

Thus, while not definitive, it does appear that cavitation is nucleated from crevices on the surface of the SNPs. This is of potential therapeutic importance; since the nuclei are contained within crevices it may be possible to attach additional layers on top of the LUDOX[®] to confer desirable properties on the SNPs, and/or regulate the nature of the surface to control the number and geometry of the nuclei, and therefore the cavitation threshold more tightly. This would not be possible if the cavitation were deriving from surface-attached bubbles.

4.2.4.3 Effect of Frequency

The effect of frequency on the cavitation threshold is shown in Figure 4.14. The cavitation threshold for a subset of particles (each core size coated with LUDOX[®] TMA (22 nm)) was measured at 508 kHz, 1.067 MHz, 1.682 MHz and 3.46 MHz, covering the range of frequencies relevant to therapeutic HIFU. As expected, the threshold increases with increasing frequency, although this reached statistical significance ($p < 0.05$) only for the highest frequency tested (3.46 MHz) due to high inter-sample variation and limited statistical power of the testing. This highlights the importance of considering the transducer frequency in the design of any therapeutic system based on the SNPs. Additionally, since higher frequencies have higher thresholds, diagnostic ultrasound is unlikely to trigger a cavitation event.

4.2.4.4 SNPs in Porcine Blood

An understanding of the behavior of the SNPs in distilled, degassed water would not necessarily be transferrable to an *in vivo* environment; particular consideration should be given to plasma proteins and their potential to adhere to the particle surface and change the wetting behaviour, which may result in the dissipation of gas cavities. To investigate these effects, SNP suspensions (600 nm core, LUDOX[®] TM-40 (37 nm) coating) were prepared in fresh porcine blood at 37°C and tested at 1.067

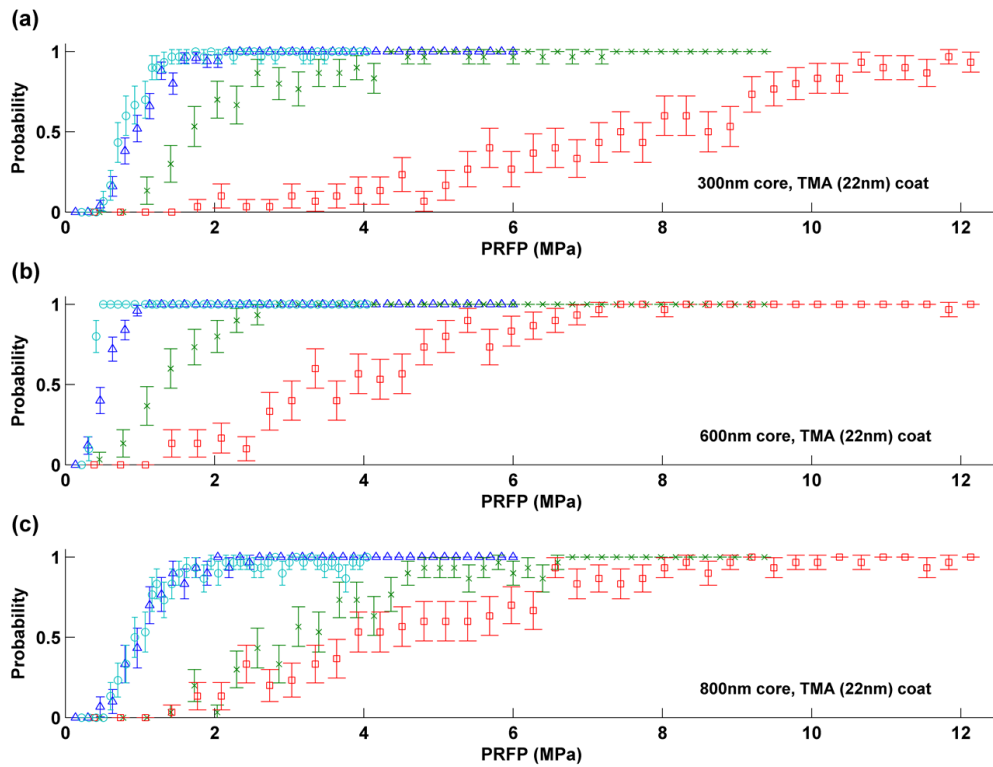


Figure 4.14: Comparison of the threshold for three particle types at different frequencies: 508kHz (light blue circles), 1.067MHz (dark blue triangles), 1.682MHz (green crosses) and 3.46MHz (red squares). The particle types in each plot are (a) 300nm core, LUDOX[®] -TMA (22 nm); (b) 600nm core, LUDOX[®] -TMA (22 nm) coating; (c) 800nm core, LUDOX[®] -TMA (22 nm) coating. As would be expected, the cavitation threshold increases (curve shifts to the right) with increasing frequency. The variation is not uniform, with some coating-core combinations appearing to exhibit lower thresholds at higher frequencies, although this effect is not statistically significant with the power of this experiment (data not shown) [errors shown are binomial errors].

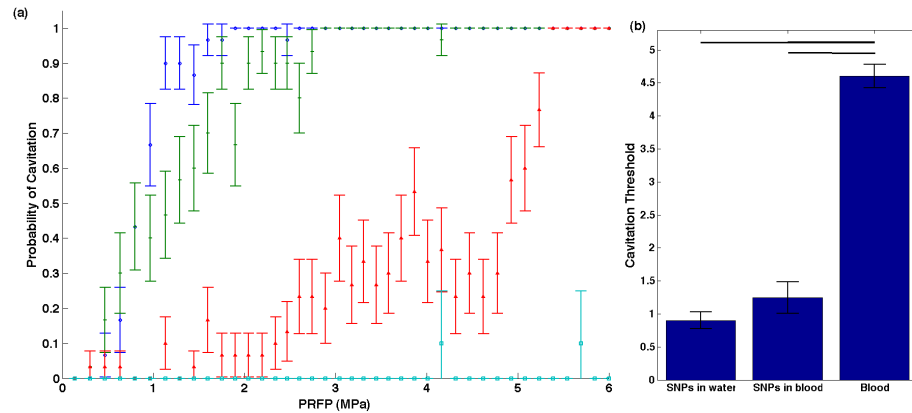


Figure 4.15: Characterisation of 600nm, LUDOX[®] -TM-40 (37nm) coated SNPs in blood at 1.067MHz. **(a)** Probability threshold charts showing SNPs in water (blue circles), SNPs in porcine blood (green + signs), untreated porcine blood (red triangles) and distilled, degassed water (blue squares). **(b)** Comparison between the mean thresholds of SNPs in blood, water, and untreated blood. There is no statistically significant difference between SNPs in blood and SNPs in water (one-way ANOVA, $p < 0.05$), but both are significantly lower than untreated blood [errors shown in (a) are binomial errors, in (b) standard errors from the fitting algorithm].

MHz in an otherwise identical manner to the distilled water. The results in Figure 4.15 show that native blood has a 50% probability of cavitation threshold of ~4.5 MPa (consistent with previously reported results (Deng et al., 1996), while adding SNPs significantly ($p < 0.05$) reduces the threshold to ~1.1 MPa, a similar threshold to that of SNPs in water. A number of other toxicity and biocompatibility tests are necessary before the biocompatibility of the SNPs can be understood. However, these results do suggest that their cavitation activity can be sustained *in vivo*.

4.2.4.5 Storage Stability

The storage stability of the SNPs in solution was examined and compared with Sonovue as shown in Figure 4.16. Over the 28 hours that the cavitation nuclei were left to sit after being resuspended, the cavitation threshold of the SNPs increased

from ~0.45 MPa to ~0.75 MPa (1.7x), and the Sonovue from ~0.1 MPa to ~0.35 MPa (3.5x). It is suggested that this is attributable to the gradual dissolution of gas from the surface of the SNPs or core of the microbubble.

An estimate of the amount of cavitation energy produced at the focus is obtained by calculating the variance of the filtered, windowed passive signal (illustrated by red and blue lines in Figure 4.16). It is evident that the cavitation activity of the SNPs is not affected by storage in solution, remaining effectively constant up to 28 hrs, while the activity of Sonovue decreases substantially.

These findings demonstrate significant advantages for the preferential clinical use of SNPs as artificial cavitation nuclei in clinical medicine. Firstly, they may be suspended and used over a significantly longer period of time, eliminating the requirement with microbubbles for a complicated suspension protocol at the point of administration. Additionally, if the surface of the SNPs can be modified or functionalized to target particular tissue or extend their circulation time in the body, they will remain active, permitting extended treatment protocols without requiring either continuous or periodic introduction of new nuclei. Finally, the level of cavitation activity is important in therapy, particularly for microstreaming applications, and the ability of the SNPs to maintain this over an extended period of time may enable novel therapies.

4.3 SUMMARY

This work has described the production, characterisation and testing of a novel class of exogenous cavitation nuclei - the (solid) sonosensitive nanoparticle.

The underlying premise of the SNP is that particles with a rough, hydrophobic surface will retain small pockets of gas in crevices on the surface, and that at a suf-

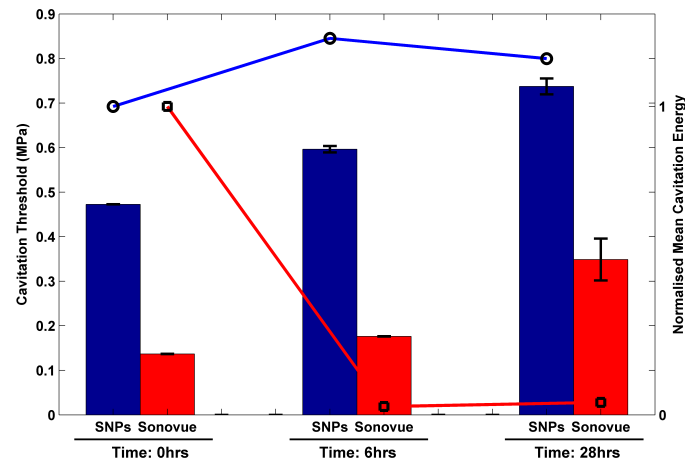


Figure 4.16: Comparison between the storage stability of the SNPs (in this case 300nm core with a LUDOX[®]-TM40 (37nm) outer coating) and Sonovue, a commercially available ultrasound contrast agent. Stock samples were prepared at $t=0$ and left to sit at room temperature, with aliquots taken at 6hrs and 28hrs for testing. The bars show that both the SNPs (blue) and Sonovue (red) exhibited a slight increase in threshold over the experimental timeframe, implying some loss of nucleation ability. The lines (RH axis) show a measure of the cavitation activity detected at each of the sample times, normalised to the signal at $t=0$. The magnitude of cavitation activity of the SNPs is maintained for at least 28hrs, while the level of cavitation activity nucleated by Sonovue drops off by >90%.

ficiently high negative pressure these cavities act as cavitation nuclei. To this end, layer-by-layer self-assembly techniques were used to deposit a layer of nanometer-sized LUDOX[®] particles on the surface of PS core particles. Examination of both particle sizing data and scanning electron microscope images of the particles suggest that the LUDOX[®] is successfully attached to the PS core, although there is variability in the surface morphology between different sizes of core-coating combinations.

It was subsequently demonstrated that the SNPs are able to significantly lower the cavitation threshold when suspended in water, against control samples of distilled water and uncoated PS cores. The threshold is higher than that reported for stabilised microbubbles (approx 1-1.5MPa PRFP against 100-200kPa), but sufficiently below the threshold for biological tissue to suggest that they are suitable

for biological use.

Interestingly, there was no significant variation in threshold between either different coatings or different core sizes - the primary determinant of the cavitation threshold was the presence of a coating. This is of clinical significance, as the smallest SNPs tested (300nm core) are small enough to penetrate into the microvasculature and to naturally accumulate in tumours, thus overcoming one of the limitations placed upon microbubbles by their size (5-10 μ m). Smaller core particles were not investigated in this work, but are suggested for further investigation.

The ultrasound frequency was varied throughout the range used in therapeutic ultrasound (508kHz to 3.46MHz), and it was demonstrated that the SNPs significantly reduced the cavitation threshold at each frequency (relative to water and uncoated PS), with the threshold increasing at the higher frequencies. Thus the SNPs can potentially be used with most existing therapeutic systems. Interestingly, diagnostic imaging systems regularly operate towards the upper end of this range, which opens the possibility for using the SNPs in a combined therapy/imaging device with a single transducer.

To conduct a very preliminary assessment of their ability to maintain their function *in vivo* (with proteins, surfactants and other biomolecules and wetting agents present), particles were suspended in fresh porcine blood, and again found to reduce the cavitation threshold significantly. A significant amount of further work is required to demonstrate immunocompatibility prior to use in humans.

Based on this combination of experimental results, it is suggested that these SNPs may be used in an analogous way to stabilised microbubbles: lowering the cavitation threshold in therapeutic ultrasound treatments where cavitation has been found to play a significant role, such as those described in §2.4.

However, it is worth noting that the SNPs do not simply replicate the properties of microbubbles, and exhibit several unique properties that may enable unique clinical applications:

- The layer-by-layer self-assembly technique is relatively straightforward, and can be readily modified to provide additional functionality to the particles: two potential options are combining the SNP with a drug, vaccine or other therapeutic agent to also enable cavitation-mediated targeted drug release and delivery; or an antibody to provide patient-specific targeting to desired treatment regions. While similar possibilities exist for microbubbles (see Section §2.3.1.3), the LbL technique provides scope for greater control over the particle geometry and morphology;
- The threshold-lowering activity of the SNPs is sustained over a greater number of acoustic pulses than microbubbles. This may mean that extended cavitation-based treatments can be carried out without replenishment of the exogenous nuclei, which typically requires systemic injection;
- The SNPs maintain their activity when stored in suspension for at least 28 hours. Sonovue microbubbles, on the other hand, lose their ability to nucleate cavitation over the same period, and as a result need to be resuspended at the point of delivery with a complex mechanism to ensure that the solution remains sterile. Further investigation of stability over extended periods is required, but it may be possible to distribute SNPs suspended in bulk sterile solution, greatly simplifying the process at the point of delivery and reducing packaging requirements;
- The SNPs have a low scattering profile until cavitation is nucleated, minimising interference with imaging and facilitating cavitation detection. The dif-

difficulties presented by using microbubbles was seen in the results shown in Figure 4.13, where the signal received on the PCD is dominated by scattering of the HIFU signal from the Sonovue, making clear detection of cavitation difficult.

It is acknowledged that a significant amount of additional work is required to get the SNPs from the laboratory to the clinic, and to demonstrate that the applications proposed above are feasible. The production process needs to be optimised to produce more consistent surfaces and reduced polydispersity, and to investigate the effect of adding multiple functional layers on the ability of the particles to nucleate cavitation; this may be assisted by an improved understanding of the mechanism of action. The effect of the SNPs on the immune system needs to be investigated and established, together with their pharmacokinetics in the body.

The work presented in this chapter has demonstrated that SNPs are capable of significantly reducing the cavitation threshold at a range of acoustic frequencies in water, and that they retain this ability in blood. The following chapter describes the development and characterisation of an ultrasound system that is capable of producing elevated pressures inside the IVD, and demonstrates the use of SNPs to lower the threshold and enable cavitation activity inside the IVD at therapeutically reasonable pressures, thus enabling the mechanical fractionation and minimally-invasive removal of tissue from the degenerate IVD.



MECHANICAL FRACTIONATION OF THE IVD

In this chapter, the development and validation of an experimental HIFU system capable of initiating, sustaining and monitoring cavitation activity inside the IVD is presented. A dual-transducer configuration is demonstrated to provide a focal region of similar size to the NP, with cavitation behaviour monitored simultaneously using both a single-element PCD, and a two-dimensional passive array to provide spatial resolution. The system is shown to be capable of inducing cavitation inside the IVD when SNPs are injected into the disc, with the location of cavitation accurately detected using passive acoustic mapping. Finally, the ability of the system to mechanically fractionate disc material inside the IVD with extended HIFU exposures is demonstrated, together with some initial investigations of the mechanism of damage.

This chapter has been submitted for publication in an alternative form in Spine Journal.

In Chapter 1 it was identified that the use of an injectable replacement for the degenerate material of the IVD requires a means of removing the material from the disc without compromising the structural integrity of the surrounding AF. Subsequent chapters described the proposed use of HIFU for this purpose, initial investigations into achieving sufficiently high pressure amplitudes in the disc, and using exogenous nuclei to lower the local cavitation threshold.

This chapter can be considered in two sections. The first describes the design

and characterisation of an ultrasound therapy system to both induce cavitation activity inside the NP and simultaneously monitor both the onset and location of this cavitation activity; the second section presents the results of experiments using extended HIFU exposures to fractionate tissue and create a cavity in the IVD.

For the therapy system, two HIFU transducers are used to produce a tightly focused, high-amplitude pressure field, along with two passive transducers (one single-element, one array) to monitor cavitation activity, and thermocouples to measure the temperature distribution within the disc. In the initial validation of the system, three key elements were considered: the size and shape of the focal region of the HIFU; the ability of the system to initiate cavitation inside the disc both with and without exogenous nuclei present; and the accuracy of the passive array in localising cavitation activity within the IVD.

In the extended exposures that followed, a wide range of ultrasound exposure parameters were explored in order to identify conditions under which a cavity could be formed inside the IVD. Once it was shown that a cavity could be formed, the parameter space was explored in a more systematic fashion to gain an insight into the potential mechanisms of damage, and then to suggest how these mechanisms may be exploited to enhance the further development of the therapeutic approach.

5.1 THE HIFU SYSTEM

5.1.1 Setup

A schematic of the experimental setup used is shown in Figure 5.1. The components can be divided into three groups: HIFU generation, acoustic monitoring, and temperature measurement.

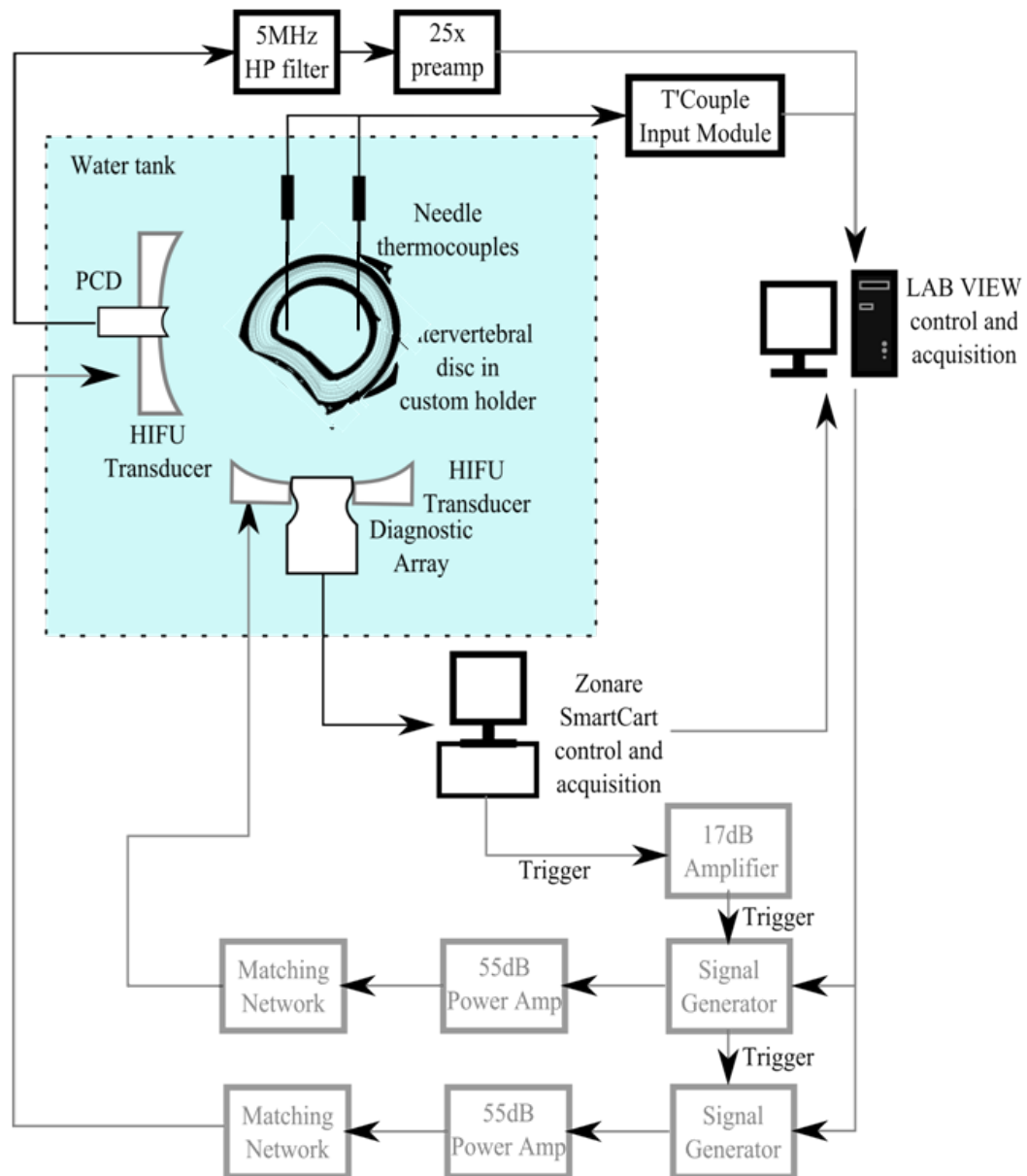


Figure 5.1: Schematic diagram of the experimental setup for HIFU exposure of the IVD. Two 500kHz transducers are confocally aligned together with a single-element PCD and multi-element passive array. A motion segment is placed at the focus and aligned using the array in active B-mode. In some experiments, thermocouples were also inserted into the disc

5.1.1.1 HIFU Generation

Custom LabView software (National Instruments, TX, USA) was developed to control the equipment used in the experiments.

Two HIFU transducers, each driven at their 500kHz centre frequency, were aligned at 90° to each other to produce the pressure field. The choice of 500kHz was made on a compromise between ease of inducing cavitation (the threshold is lower at lower frequencies - Figure 2.6) and the size of the focal region (larger at lower frequencies). This configuration also has the potential to be adapted (by using transducers with a longer focal length) to allow anatomical access to the IVD without impinging on the bony processes of the vertebrae.

One of the transducers (the ‘primary’ transducer) was an H107 (Sonic Concepts, Bothell, WA, USA) with a circular cutout at the centre to allow for a PCD to be inserted; the ‘secondary’ transducer (Sonic Concepts, Bothell, WA, USA) had the same dimensions (62.64mm geometric focal length, 60mm diameter), but was custom-built with a rectangular cutout at the centre to allow the insertion of a linear array conventionally used for B-mode imaging.

The transducers were driven by separate signal generators (primary transducer - 33250A, secondary transducer - 33220A, Agilent, Santa Clara, CA, USA), the output of each being amplified by a 55dB power amplifier (E&I A300, Rochester, NY, USA) and then passed through an impedance-matching network.

Synchronisation of the signals was achieved by means of a multi-step triggering cascade. The imaging scanhead (described in the following section) was modified to provide a TTL trigger ‘out’ signal at the commencement of each imaging frame (it was not possible to implement a trigger ‘in’ for this piece of hardware). This trigger signal was passed through a 17dB amplifier (DPR300, JSR Ultrasonics, NY, USA),

and used to trigger the output of a square wave from a signal generator (Agilent 33250A, not shown on Figure 5.1). This square wave was then used to trigger the signal generator of the primary transducer, and the trigger 'out' signal from this signal generator used to trigger the secondary transducer. Hardware triggers were used throughout the cascade to minimise system delays.

A delay was applied to both the square wave generator and the primary signal generator (i.e. the signal output did not occur immediately upon receipt of the trigger) to ensure that the last HIFU pulse ended 10-30 μ s before the acquisition of the subsequent frame on the passive array. This avoided any direct interference from the HIFU in the passive signal, and ensured that the signal from any cavitating sources at or near the focus would be captured in the voltage trace from the passive array (since the time of flight to the focus is $\sim$ 100 μ s).

The combination of the square wave parameters, imaging framerate, delays and cycles per pulse allowed the duty cycle and PRF of the HIFU pulse to be controlled.

5.1.1.2 Cavitation Detection and Monitoring

Both a single-element PCD and a passive array were used for cavitation detection and monitoring.

The spherically-focused single-element PCD was aligned coaxially and confocally (see next section) with the primary transducer. The PCD was typically a 7.5MHz centre frequency (Panametrics, Olympus, Essex, UK) with a focal length of 75mm, although occasionally a transducer with a centre frequency of 3.5MHz (Panametrics, Olympus, Essex, UK) and similar focal length was used. The centre frequency of the PCD was chosen to reduce the sensitivity to the scattered fundamental; the signal was also passed through an analog high-pass filter (5MHz for the 7.5MHz PCD, 2MHz for the 3.5MHz PCD, Allen Avionics, NY, USA) to further reduce the fundamen-

tal signal and enable detection of broadband acoustic emissions. After filtering, the PCD signal was amplified 25x/28dB (SR445A, Stanford Research Systems, CA, USA) before being digitized using a 14-bit DAQ card (PCI-5122, National Instruments, TX, USA) at a sampling frequency of 100MHz.

The rectangular cutout in the secondary transducer allowed a standard 128-element diagnostic scanhead (L10-5, Zonare Medical Systems, CA, USA) to be positioned coaxially in the horizontal plane. This transducer could be operated either in active imaging mode, or in passive acquisition mode to detect secondary acoustic emissions from cavitation. When in passive mode, 140 μ s of data sampled at 5MHz was acquired simultaneously for 64 elements at frames rates of up to 200Hz. After windowing to exclude any signal deriving from outside the IVD, this data was used to construct passive acoustic maps using previously described algorithms (Section §2.3.3.2). For each HIFU exposure a series of instantaneous passive acoustic maps were calculated. These were then summed over time to produce a cumulative acoustic map for a given exposure.

5.1.1.3 Alignment Protocol

An alignment protocol was devised to ensure alignment between the two HIFU transducers and the two passive detectors. The first stage in the protocol was to align each of the HIFU transducers with its coaxially mounted detector, and subsequently to align the two HIFU transducers.

To align the single element PCD with the H107 HIFU transducer, a procedure similar to that described in Section §4.2.2.1 was followed.

To align the custom HIFU transducer and the diagnostic scanhead, the brass sphere was similarly placed at the focus of the HIFU transducer. The scanhead was operated in pulse-echo mode (B-mode), and manually shifted relative to the trans-

ducer until the ball was seen in the centre of the image laterally. The imaging plane was then swept vertically to maximise the intensity of the image of the sphere, and the scanhead fixed in place in a custom holder.

Once aligned with their respective cavitation detector, the HIFU transducers were placed on separate three-dimensional positioning systems in a tank of deionised, degassed water. The faces of the two transducers were set at 90° to each other using a set square. A $75\mu\text{m}$ needle hydrophone (S/N 1360, Precision Acoustics, UK) was placed on a (third) three-dimensional positioning system at 45° to the axis of both transducers, and then positioned to maximise the peak-to-peak hydrophone voltage from the primary transducer, as measured on a digital oscilloscope (WaveRunner, LeCroy, NY, USA). The hydrophone and primary transducer were then fixed in place, and the secondary transducer moved so as to position its focus on the hydrophone tip (again maximising the hydrophone signal voltage). The phase delay on the signal generator driving the secondary transducer was then adjusted to ensure constructive superposition of their fields at the focus.

Once the HIFU transducers were aligned as described above, the diagnostic scanhead was operated in B-mode, and the position of the tip of the hydrophone (i.e. the transducer focus) marked on the screen of the imaging system. This mark was subsequently used in targeting to position the transducer focus inside the NP prior to insonication.

5.1.1.4 Temperature Monitoring

When temperature measurements were taken, they were obtained using a T-type embedded fine-needle thermocouple (HYP0, Omega Engineering Inc., CN, USA) of outer diameter $200\mu\text{m}$, connected to a multiplexer with embedded cold junction compensation (NI9211, National Instruments, TX, USA). A fine-tipped thermo-

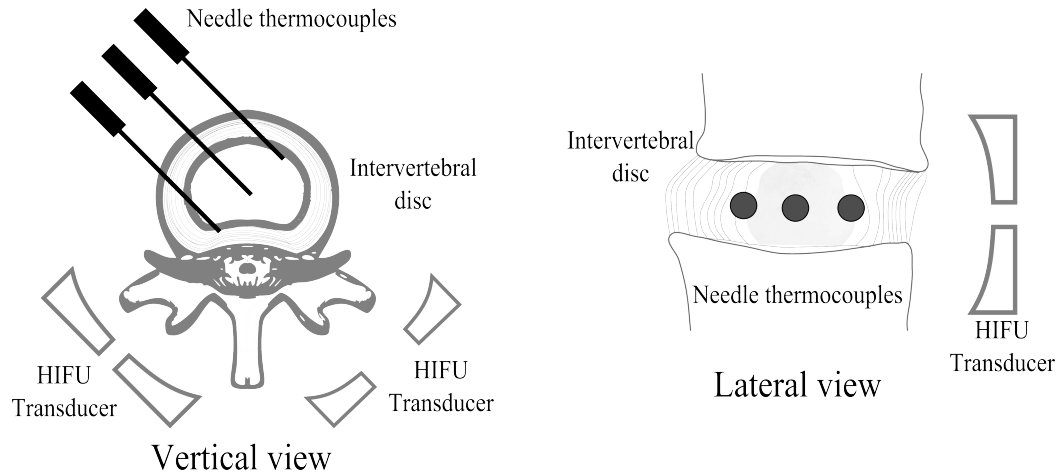


Figure 5.2: Schematic diagram showing the positioning of thermocouples within the IVD for measurements of the temperature rise profile associated with HIFU exposure

couple was selected to minimise viscous artefact and effect on the local cavitation threshold. Thermocouples were manually inserted at various locations in the disc, as indicated in Figure 5.2. Prior to inserting the thermocouple into the disc, a small track was created through the tough tissue of the AF using a 21G needle to enable penetration; care was taken to be as accurate as possible and to avoid leaving a larger track than necessary. During HIFU exposure, the temperature was sampled at 3Hz.

5.1.2 Characterisation of the HIFU field

The acoustic field produced by the dual-transducer configuration was measured experimentally to determine the size of the focal region, and measure the pressure amplitude.

5.1.2.1 Experimental Setup and Parameters

Calibration of the acoustic field was performed immediately following the alignment protocol (described above) using the same $75\mu\text{m}$ needle hydrophone

(SN1360, Precision Acoustics, UK). The voltage input to the primary HIFU transducer was recorded simultaneously with the voltage trace from the needle hydrophone using a two-channel DAQ card (PCI-5122, National Instruments, TX, USA). The hydrophone voltage trace was converted into a pressure reading using the calibrated sensitivity of the hydrophone, and the peak-to-peak pressure and the PRFP were calculated for a series of input voltages. The focal pressure amplitude was measured for each of the transducers independently, and then with both transducers in simultaneous operation. Measurements were carried out only up to 8MPa PRFP in order to avoid damage to the hydrophone from cavitation in the distilled, degassed water.

In a separate experiment, the HIFU transducers were excited at a constant voltage (corresponding to a PRFP of 2.2MPa) and the hydrophone scanned around the acoustic focus in the three planes of the motorised position system to measure the spatial distribution of the pressure field. Measurements were taken at a spacing of $500\mu\text{m}$ for 2.5mm in each direction about the focus, to cover a region similar in size to the smallest NP expected in bovine coccygeal discs (which are themselves smaller than human lumbar discs). Three scans were performed, one with each transducer operating independently, and a third with both transducers synchronised.

5.1.2.2 Results

The results of the absolute calibration of the therapy setup are shown in Figure 5.3. From this it is apparent that the dual transducer configuration produces a PRFP that is the sum of the field due to each of the transducers individually, at least as measured up to $\sim 7\text{MPa}$.

Importantly, this means that the dual-transducer setup is capable of producing similar pressure amplitudes to those reported to cause visible damage to liver im-

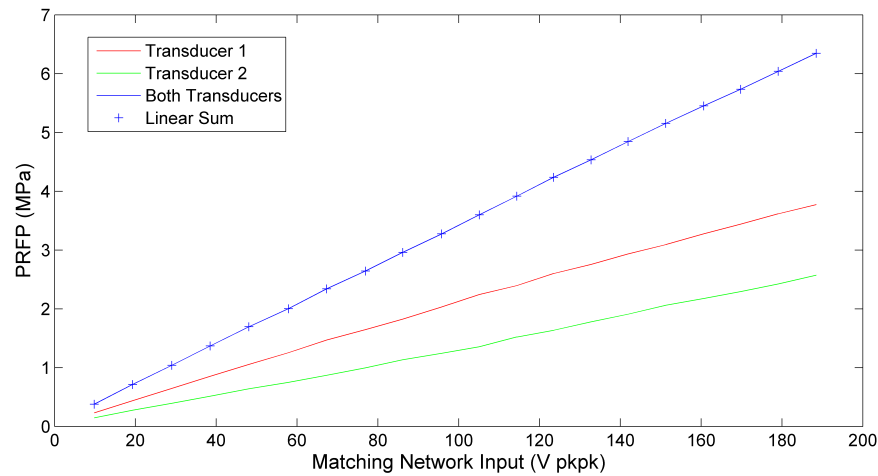


Figure 5.3: Absolute calibration of the dual-transducer therapy system (focal PRFP against voltage input to the matching network of one transducer), showing amplitude due to each of the transducers individually, the amplitude expected from a linear sum of the two transducers, and the measured field with both transducers

pregnated with exogenous cavitation nuclei (Tran et al., 2003), without needing to excite the individual transducers with a sufficiently high voltage/power load to risk thermal damage to the transducer electrodes.

The linear relationship between PRFP and the input voltage to the transducers seen in this data will be lost at higher pressures or in biological tissue due to the non-linearity of the medium. However, measurements could not be carried out using the needle hydrophone at these higher pressures due to the increased risk of cavitation activity damaging the hydrophone. Attempts were also made using a fibre-optic hydrophone, however cavitation in the surrounding water distorted measurements.

The spatial extent of the focal region for each transducer driven separately, and for the two transducers driven together, is shown in Figure 5.4. For the transducers driven individually, the stretching of the focus along the transducer axis predicted from the modelling in Chapter 3 can be clearly seen, with almost no pressure drop within 2.5mm of the focus, and an overall full-width half-maximum (FWHM) length

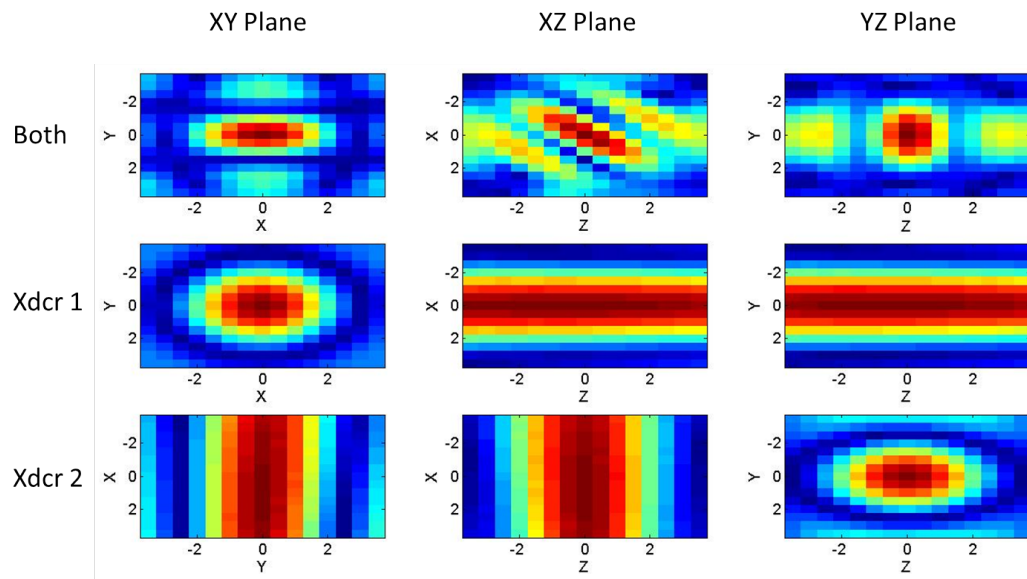


Figure 5.4: Spatial calibration of the dual-transducer therapy system, showing scans of three perpendicular planes through the transducer focus. The top row shows the field with both transducers on, the bottom two rows show the field due to each of the transducers individually (primary transducer in the second row, secondary transducer in the second row). All dimensions are given in mm, with the focus at the origin, the color scale is based on the pressure amplitude normalised to the PRFP.

of approximately 20mm (data not shown). In the transverse plane the FWHM is approximately 3mm, with a slight distortion for the secondary transducer due to the assymetrical rectangular cutout.

When the two transducers are driven together, the focal region was seen to be much smaller, as predicted in the modelling work. In the two transverse planes (XY and YZ), the primary lobe was reduced to assymetrical dimensions of approximately 2mm x 2.5mm, with a secondary lobe with a peak amplitude 45% of the PRFP located 2.5mm from the focus. In the XZ plane, the focus was seen to be elongated along in a direction at 45° to the transducer axes, approximately 4mm long and 1.5mm wide. Two side lobes were observed, with similar orientation and size, roughly 2mm from the primary lobe and with an amplitude approximately 50% of the PRFP.

As discussed in Chapter 3, this reduction in the size of the focal region has im-

portant implications for therapy. With a single transducer, the pressure amplitude inside the AF and even at the surface of the IVD is similar to that at the focus, especially once the effect of the attenuative disc tissue is considered. Thus, insonicating at a sufficiently high pressure amplitude to produce cavitation inside the NP at the geometric focus will also give a high probability both of cavitation occurring at the disc surface, and of a high level of heating along the beam path inside the AF. With the dual transducer configuration, the size of the focal region is reduced to be smaller than the NP (which in humans typically has a diameter of 10-12mm and a height of 8-13mm), allowing it to be positioned completely within this region during therapy and greatly reducing the potential for damage to surrounding tissue.

In the procedure used for these experiments, the alignment and phase matching was performed in distilled water, where the medium along the acoustic path for each transducer was the same. In a clinical environment it is likely that the acoustic path for each of the transducers would be different, depending on the specific transducer position and patient anatomy. Any difference in time-of-flight due to variation in propagation speed along the path will affect the phase relationship between the two signals, and potentially the size, location and amplitude of the acoustic field.

To assess the effect of phase difference, the relative phase between the two transducers in water was changed by 180° to give a minimum in pressure amplitude at the focus, and a second scan performed in the XZ plane. Figure 5.5 shows that the maximum pressure amplitude when out of phase is located in the minima for the in-phase scan. While this region was displaced from the nominal 'target' marked on the B-mode image during alignment that is used for subsequent targeting, it was still well within the confines of the spatial scan; thus suggesting that any phase difference resulting from path differences is unlikely to lead to tissue damage outside of the targeted region inside the NP.

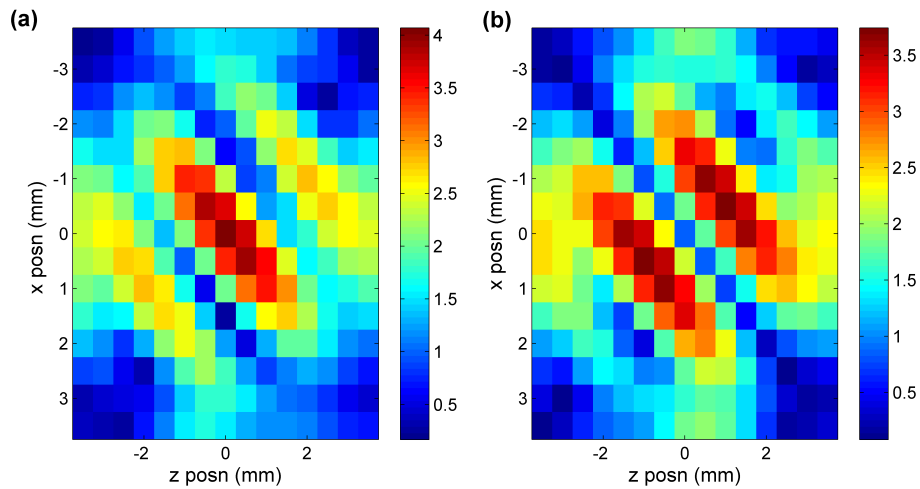


Figure 5.5: Scans in the transverse plane about the focus of the dual-transducer therapy setup with the relative phase between the two transducers set to **(a)** maximise PRFP and **(b)** 180 degrees out of phase with the maximum. Note that shifting the relative phase by 180 degrees provides two pressure maxima symmetric about the geometric focus, displaced by approximately 1mm, and with less than 10% decrease in absolute pressure

5.2 INDUCING CAVITATION IN THE IVD

The HIFU setup was then used to investigate the cavitation threshold inside the IVD, and the effect of injecting exogenous nuclei into the NP. Both Sonovue microbubbles and the novel SNPs described in Chapter 4 were used, and the cavitation threshold and location of cavitation measured.

5.2.1 Experimental Setup

5.2.1.1 Materials

The HIFU generation and monitoring system described above was used in these experiments. The SNPs used were prepared as described in Chapter 4. In the experiments described here, particles with a PS core 600nm in diameter with a LUDOX[®] TMA coating were used at a concentration of approximately 2.5×10^7 /mL. Sonovue microbubbles (Bracco, Italy) were prepared as per the manufacturer's instructions

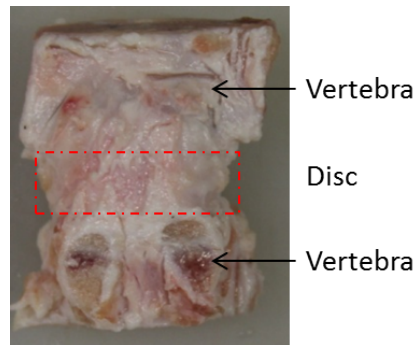


Figure 5.6: A motion segment (vertebrae still attached) obtained from bovine tail with bony processes removed. Image taken post-degassing and immediately prior to insonication

at a concentration of $2 - 5 \times 10^8$ /mL.

5.2.1.2 Intervertebral Disc Preparation

As discussed previously (Section §3.1.1.3), bovine coccygeal discs were selected as an experimental model. In these experiments, bovine tails were obtained from the abbatoir (Mutch Meats, Witney, UK) within 4 hours of slaughter. Overlying fat, muscle and ligament tissue was removed, and the tail separated into individual motion segments by cutting through the centre of vertebrae using a hacksaw (Figure 5.6). Motion segments were kept at 4°C for up to 48 hours before use. Prior to use, motion segments were degassed in deionised water for 2-4hrs at -0.9 bar before being mounted in a custom holder.

Four motion segments were prepared from two separate tails: the first control disc was left without injection and the second injected with degassed, distilled water; a third disc was injected with Sonovue microbubbles; and a fourth with SNPs. To inject the discs, a shortened (13mm in length) 25-gauge hypodermic needle mounted on a 1mL syringe was inserted into the centre of the disc at 45° to the transducer axes. The maximum possible volume was injected into the disc using manual force, and the approximate injected volume recorded. The injected volume

was found to vary within the range 140-200 μ L.

During injection, motion segments were kept submerged in water to reduce the potential for air bubbles to become attached to the tissue surface.

5.2.1.3 Experimental Procedure

Motion segments mounted in the custom holder were attached to a three-dimensional positioning system in a tank of distilled, degassed water, and care taken to remove any surface bubbles using a pasteur pipette. The diagnostic scanhead was operated in B-mode, and the motion segment moved until the transducer focus (as marked on the B-mode display screen during alignment) was located at the centre of the IVD. Vertical alignment was performed by moving the motion segment along this axis, and placing it midway between the points at which it became clear that the vertebral bodies were reflecting the incident ultrasound (i.e. the disc boundaries). The B-mode image of the motion segment at this point was then saved, and the diagnostic scanner placed in passive mode.

Each disc was then subjected to a series of 10s HIFU exposures, beginning at a PRFP of 1.2MPa and increasing stepwise up to 7.4MPa PRFP for the final exposure. Successive exposures were separated by 1-2 minutes to allow any residual bubble fragments to dissolve, and for initial processing of data between pulses. Pulsed HIFU at a low duty cycle was used, with 25 cycles per pulse at a duty cycle of 5% (PRF 1kHz). A 400 μ s trace was acquired from the PCD every 250ms during exposure, and ~100 frames acquired on the passive array at a frame rate of 10Hz.

Note that all pressures quoted hereafter are based on the values measured in degassed, distilled water, and have not been derated. Preliminary experiments conducted with a hydrophone embedded in the disc suggest losses of 15-30% at the focus (results not shown), however this figure would be expected to vary with disc

geometry and animal physiology. Where the input voltages exceed those measured in the calibration, the PRFP is extrapolated assuming a linear relationship.

5.2.2 Data Processing

The traces acquired on the single-element PCD were used to calculate the signal variance as a proxy for cavitation activity (Coussios et al., 2007). The variance of the PCD trace was calculated using only data from 60-200 μ s, corresponding to the time-of-flight for the position of the front and back of the disc for the start and end of the HIFU pulse respectively. The onset of cavitation was detected based on the algorithm described previously in Section §4.2.3.2.

A similar subset of the array data was used to construct passive acoustic maps using the algorithms described in Section §2.3.3.2. Maps for each individual frame were calculated ('instantaneous' maps), and a cumulative map for the entire exposure was also produced by simply summing the instantaneous maps. Passive acoustic maps were overlaid on the pre-exposure B-mode images prior to display.

All data processing was performed using the Matlab software package (Mathworks, MA, USA).

5.2.3 Results and Discussion

The results of experiments investigating the cavitation threshold of the IVD, and the effect of both SNPs and Sonovue microbubbles, are shown in Figure 5.7. Figure 5.7(a) and (b), for discs injected with nothing and degassed water respectively, show only a slight increase in PCD variance with pressure amplitude, suggesting that there is no focal cavitation activity. The results of applying the cavitation detection algorithm to the PCD data supports this; there is no visible jump in the individual traces where the signal from any activity in the disc is expected. There is a weak relation-

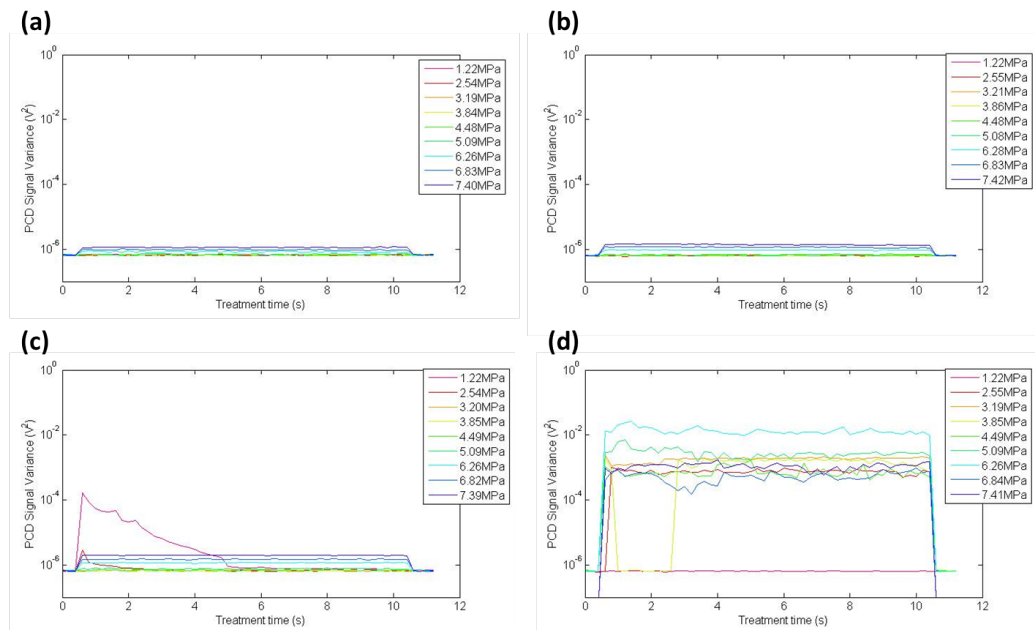


Figure 5.7: Plots showing the variance of the PCD trace during HIFU exposures for **(a)** native disc and discs injected variously with **(b)** degassed, deionised water, **(c)** Sonovue ultrasound contrast agents, and **(d)** sonosensitive nanoparticles. Each disc was subjected to nine 10s HIFU exposures (beginning at $t=0.5$ s on these plots) separated by 1-2 minutes, with the PRFP increased between each exposure. The 500kHz HIFU was pulsed, with 25 cycles per pulse and a duty cycle of 5% (PRF1kHz).

ship between variance and pressure amplitude which is explained when the traces are examined in the frequency domain; high HIFU pressure amplitudes are generating higher harmonics in the non-linear IVD tissue which are above the cutoff of the highpass filter, and therefore passed through into the recorded signal. The absence of any cavitation activity in the native tissue at up to 7.4MPa PRFP implies that the cavitation threshold of the native IVD is above this level.

The disc injected with Sonovue contrast agents (Figure 5.7(c)) shows an initial increase of three orders of magnitude in the PCD variance at 1.2MPa PRFP which drops off back to the level of noise over the first 5s of exposure. At 2.5MPa PRFP a similar effect is observed to a lesser extent, lasting only 2s; higher pressure amplitudes show no elevation in variance above background. The individual traces

acquired during periods of elevated variance (not shown) are consistent with cavitation activity and passive mapping results place the activity inside the NP (Figure 5.8(c)), however this activity is rapidly depleted.

The cavitation activity at the lowest pressure amplitudes, implying a cavitation threshold of $<1.2\text{MPa}$, is consistent with the behaviour of Sonovue observed in Chapter 4. There are two possible explanations for the rapid depletion observed in cavitation activity. The first is that the overpressure that is required to inject the microbubbles into the disc against the static internal pressure leads to the rupture of the stabilising shell in the vast majority of particles, leaving only a small residual population capable of nucleating cavitation. This explanation is consistent with the results reported by Chitnis et al. (2011) with different polymer-shelled microbubbles at over-pressures from 2-180kPa. The second possible explanation is consistent with the depletion behaviour of Sonovue reported in Section §4.2.4.2. At a PRF of 1kHz, the 5s of activity inside the IVD represents over 5000 individual ‘shots’ as compared to the <800 shots required to deplete activity in water solution; however the difference in PRF between the two experiments makes this comparison imperfect, as bubble fragments from a collapse in one shot may still be present to act as nuclei in the following shot at the higher PRF used in these experiments.

It is not possible to distinguish between these two mechanisms based on the current experiment, and it may be that both of them play a role. Regardless, these results clearly indicate that Sonovue microbubbles are not a suitable source of exogenous nuclei for sustaining cavitation inside the IVD over an extended HIFU exposure. Regular replenishment of the microbubble population in the disc may avoid depletion, but would require regular insertion of a needle which is inconsistent with a minimally-invasive procedure.

In contrast to the Sonovue, the disc injected with SNPs (Figure 5.7(d)) shows an

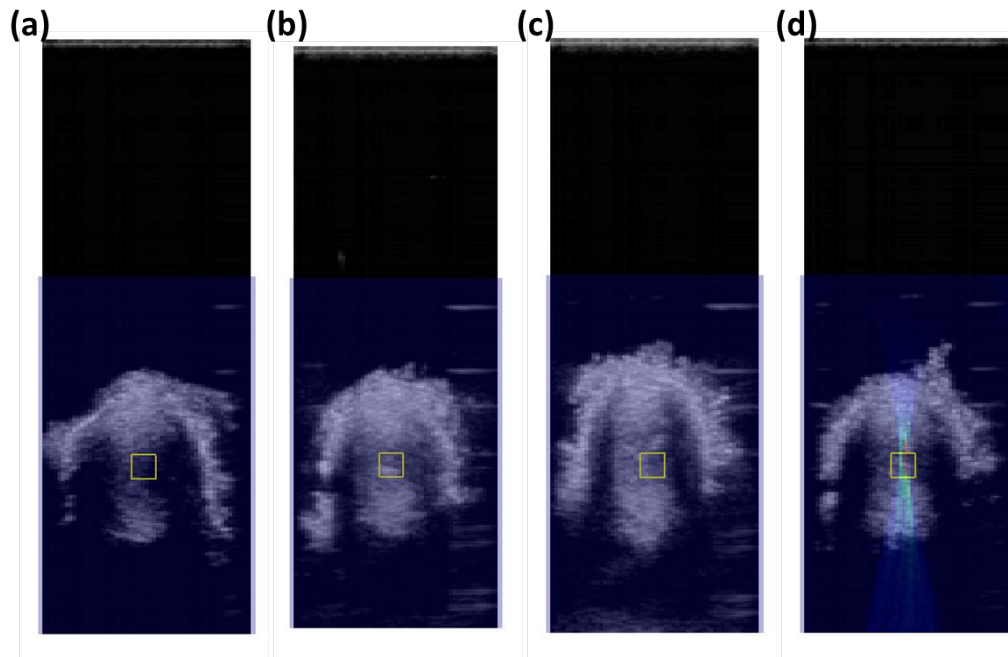


Figure 5.8: Cumulative passive maps acquired during HIFU exposures of (a) native disc and discs injected variously with (b) degassed, deionised water, (c) Sonovue ultrasound contrast agents, and (d) sonosensitive nanoparticles (results from PCD signals during the same exposure are shown in Figure 5.7). All exposures shown here were at 4.5MPa PRFP for 10s, 25 cycles per pulse at a 5% duty cycle. Passive maps are overlaid on B-mode images of the disc acquired prior to insonication.

increase in PCD variance of 4-5 orders of magnitude for all exposures with a PRFP of 2.5MPa or higher, and this is sustained throughout each of the exposures. The one exception is a short drop in activity from 1-2.5s for the exposure at 3.85MPa, which does not have an obvious explanation. The individual traces (not shown) are consistent with cavitation activity, and passive acoustic maps (Figure 5.8(d)) show activity inside the NP. A cavitation threshold for SNPs inside the disc of between 1.2-2.5MPa PRFP is implied.

The observed behaviour of the SNPs is generally consistent with the results presented in Chapter 4. The cavitation threshold is similar, as is the ability to continue to nucleate cavitation over an extended exposure. As discussed previously, this extended activity may be due to either the solid core of the nanoparticles protecting

them against the effect of overpressure, the presence of multiple nucleation sites on the rough surface of each particle, or a combination of the two. In either case, these results suggest that SNPs are a suitable source of cavitation nuclei to lower both the pressure and exposure time required to mechanically damage the tissue of the NP.

The generality of these results is somewhat limited as only a single disc was tested for each experimental condition. This raises the possibility that the results may be affected by exceptional events, especially since the result of each exposure is dependent to at least some extent on the prior exposures on that motion segment. Additional or different insights may come from the testing of more discs per condition, however since these results are consistent with the qualitative results of exploratory experiments conducted to investigate the feasibility of this protocol (results not shown) these were not pursued.

It is also worth noting two limitations of the passive mapping data presented here. Firstly, since the frame rate of the data used to construct the passive maps was only 10Hz against a PRF of 1kHz for the HIFU, passive mapping data was only acquired for 1% of all pulses. Interpretation of the passive cavitation maps therefore requires caution, as the cavitation location and amplitude may vary in the intermediate pulses. Since the total number of frames that can be stored by the passive mapping system is constrained by the memory constraints of the hardware, it was not possible to overcome this limitation. Secondly, the validity of overlaying the passive cavitation map onto a pre-exposure B-mode image, and then assuming that the location of a maximum in the passive map/B-mode image corresponds to the location of cavitation in the disc itself has not been tested. This assumption is tested in the following section.

5.3 PASSIVE CAVITATION MAPPING INSIDE THE IVD

As suggested above, passive maps can not necessarily be related directly to B-mode images of the disc to determine the anatomical location of cavitation activity. Regions of cavitation measured by passive acoustic mapping have been shown in several studies (Section §2.3.3.2) to correlate well with known cavitation regions using fixed-geometry phantoms, and in liver tissue (Jensen et al., 2012).

To test the assumption and the suitability of passive mapping to localise cavitation within the IVD, discs were injected with SNPs at a single point, the location of which was marked with dye. As demonstrated in the previous section, the presence of SNPs substantially lowers the cavitation threshold from native disc tissue, and it can be expected that cavitation at low pressures will only occur in areas where they are present. The discs were subsequently exposed to HIFU and passive maps produced, and the location of cavitation in the maps compared with the known location of the SNPs to give an indication of the accuracy of the passive mapping within the IVD.

5.3.1 Experimental Setup

The HIFU therapy system was prepared and aligned as described in §5.1.1 above.

The SNPs used were prepared as described in Chapter 4. In these experiments, particles with a PS core 600nm in diameter with a LUDOX[®] TMA coating were used at a concentration of approximately 2.5×10^7 /mL. Particles were suspended in a solution of Evans Blue at a sufficiently high concentration for the solution to strongly contrast with the white tissue of the IVD.

Bovine coccygeal motion segments were obtained and prepared as described above in Section §5.3, but injected with the suspension of SNPs in dilute Evans Blue

solution rather than water. The location of injection was deliberately varied in different discs by altering both the length of the needle used and the angle of the needle.

5.3.1.1 Experimental Procedure

Motion segments were mounted and aligned as described in Section §5.2.1.3.

Each disc were then subjected to four 2s exposures with pulsed HIFU, 25 cycles/pulse and 5% duty cycle (PRF 1kHz); the pressure amplitude was increased between each exposure from the lowest amplitude at 1.6MPa PRFP, up to 5.05MPa PRFP for the final exposure. PCD and passive array data were acquired and processed in the same way as for the previous experiments. Following the HIFU exposure the discs were marked to indicate their position relative to the two transducers, removed from the custom holder and cut in two along the transverse (anatomical) plane. The location of the injection point was clearly indicated by the blue-stained region inside the NP. Images of the resulting two sections of disc (superior and inferior) were acquired using a camera (EOS-400D, Canon, Surrey, UK) fixed to a stable mount.

5.3.1.2 Data Processing

In post-processing, the results of the lowest-amplitude exposure found to result in sustained cavitation activity in each disc was selected for analysis. The cumulative passive acoustic map and pre-exposure B-mode image for this exposure could be readily overlaid as the position of each relative to the (unmoved) diagnostic array was known. The photographic image of the inferior disc section was then rotated to share the alignment of the B-mode image, and overlaid with the B-mode image. Finally, the photograph was manually re-sized until the outer edge of the

disc was seen to align with the disc boundary on the B-mode image. The images were then layered with the B-mode image below the photographic image, and the semi-transparent passive map laid over the top, allowing simultaneous visualisation of the disc anatomy, the location of the region stained by the Evans Blue / SNP solution, and the passive cavitation map.

The addition and normalisation/scaling procedure used to construct the cumulative passive maps used for the above analysis results in a bias towards the strongest cavitation events, and is not necessarily representative of the distribution of cavitation activity. To address this, a separate analysis was performed whereby in each frame for which cavitation is observed, the image is thresholded at the -3dB level of the maximum pixel value. Frames are then summed and thresholded again to provide an image which is indicative of the region over which cavitation occurs, regardless of the strength of the individual events.

Processing of the B-mode images and passive maps was done in Matlab, while processing of the photograph and combined image was performed using Inkscape (v0.48, downloaded from inkscape.org).

5.3.2 Results and Discussion

The combined images resulting from the experiments to validate the accuracy of the passive acoustic mapping algorithms in the disc are shown in Figure 5.9. The location of the dyed region can be clearly seen in the photographic images, with the size of the region indicating the (minor) extent of spreading from the injection point. Given their relative size, the molecules of Evans Blue should diffuse through the disc more rapidly than the SNPs; the SNPs should therefore be contained completely within the dyed region.

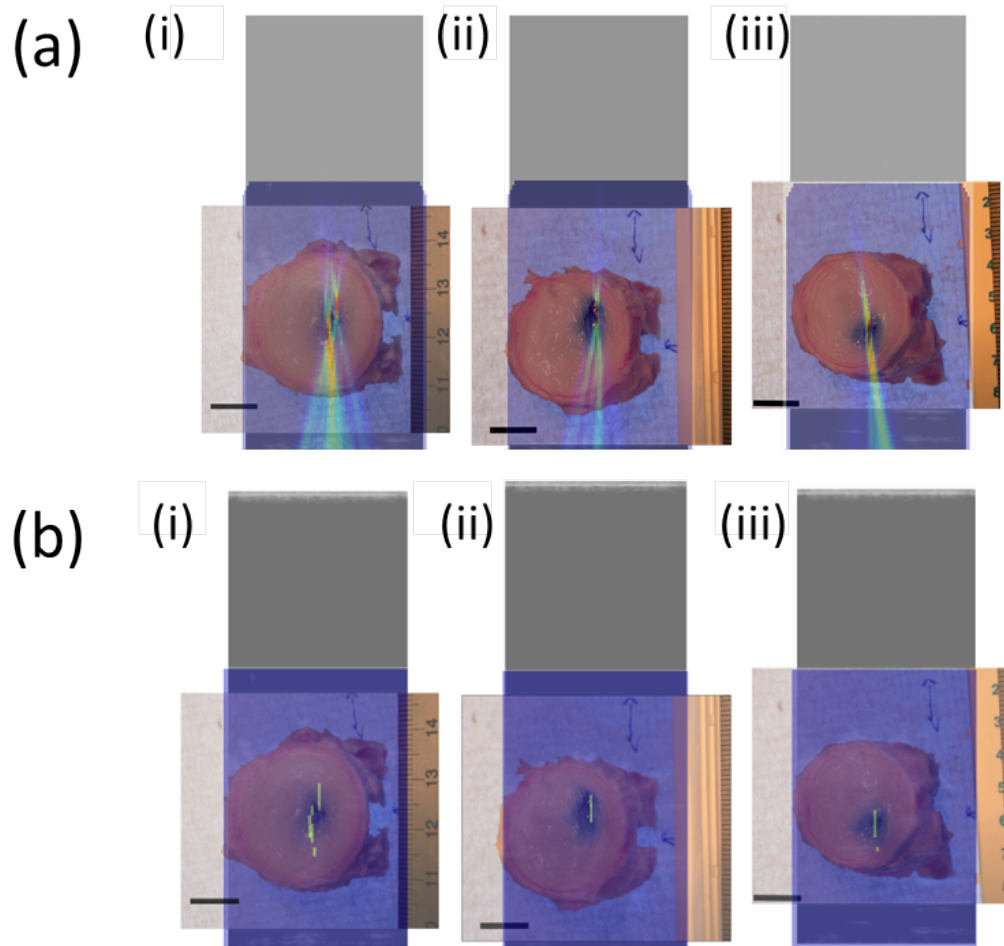


Figure 5.9: Merged images comprised of a pre-exposure B-mode image (black and white), the post-exposure photograph of a disc cross-sectionally sliced (color image) and the passive acoustic map (transparent overlay) for discs injected with a mixture of SNPs and dilute Evans Blue dye and subjected to a 2s HIFU exposure. Images in row **(a)** show cumulative maps; row **(b)** shows thresholded maps. The HIFU transducers are located to the top and to the right of the images as shown. The strong colocalisation of both the cumulative and thresholded maps with the blue dye in the disc demonstrates the accuracy of the passive mapping equipment and algorithm for the disc. HIFU was pulsed with 25 cycles per pulse, and a PRFP of (i) 3.2MPa (ii) 4.0MPa (iii) 3.2MPa. Solid black bars mark 10mm.

Both the cumulative and threshold passive maps clearly show a strong colocalisation between the regions with dye/SNPs and the maxima on the maps - the peaks on the thresholded passive maps are all completely contained within the regions containing SNPs. The axial 'stretching' that can be seen in the cumulative passive maps has been reported previously with the passive acoustic mapping algorithm used (Gyongy and Coussios, 2010b), and is primarily due to the limited aperture of the diagnostic scanhead used to acquire the data.

In two of the three discs shown ((ii) and (iii)) cavitation occurs at a single location, while in the disc shown in (i) cavitation occurs at multiple locations within the disc. This suggests that SNPs may 'pool' at certain points in the matrix of the NP which differ between discs, or an indication that the population of SNPs is not homogenous. It also shows that the passive acoustic mapping technique is capable of detecting and localisation cavitation activity at multiple sites within the disc.

These results clearly show that the level of resolution is sufficient to enable cavitation occurring at the desired location inside the NP to be distinguished from potentially damaging cavitation on the surface of the disc or in surrounding tissue. Thus passive mapping may be a suitable technique for monitoring the location and extent of cavitation inside the IVD during HIFU therapy.

5.4 EXTENDED HIFU EXPOSURES FOR IVD FRACTIONATION

While the results presented above show that the HIFU system is capable of inducing cavitation inside the NP in the presence of exogenous nuclei, it does not necessarily follow that this cavitation will produce the desired mechanical effects on the tissue. Indeed, collagenous tissue has been reported to be more resistant to mechanical

fractionation than cellular soft tissue, and may therefore require a different treatment approach to that reported in the literature for liver, kidney and myocardium.

However, existing treatment regimes at least provide a starting point for identifying parameters that can mechanically fractionate the IVD. As discussed in Chapter 2, three broad regimes have been shown to produce cavities in tissue. While histotripsy and millisecond-boiling both rely on extremely high focal pressures that are undesirable in a sensitive anatomical location such as the IVD, the strategy employed by Tran et al. (2003) used exogenous nuclei to lower the threshold significantly and produce damage at relatively moderate pressures.

This section presents the results of experiments aimed at testing several different HIFU regimes for their ability to produce macroscopic mechanical damage in the IVD following the injection of sonosensitive nanoparticles. The nature and location of cavitation activity during the exposure is monitored using the PCD and passive mapping, and the results are analysed to suggest areas of future development.

5.4.1 Materials and Methods

5.4.1.1 Hardware and Tissue Preparation

The HIFU system used in these experiments is the same as that described and characterised in detail in Section §5.1 above. For these experiments the signal generators and data acquisition system were controlled by custom-built Labview software (National Instruments, TX, USA), with the ability to trigger and synchronise the system either from software or the Zonare imaging system.

Bovine coccygeal discs were obtained and prepared as described in Section §5.2.

5.4.1.2 Experimental Procedure

Immediately prior to insonation, motion segments were injected with SNPs and had thermocouples inserted as described previously (Section §5.2.1.2), and were then placed at the focus of the HIFU system, using the imaging system in B-mode for targeting. A B-mode image of the disc prior to insonication was stored before placing the system in passive mode.

The exposure parameters were varied between exposures; the specific parameters used are outlined in the following section together with the results.

During each HIFU exposure, a $400\mu\text{s}$ trace was acquired from the PCD at a sampling rate of 100 MHz either every 250ms (for shorter exposures) or 500ms (to reduce memory requirements for longer exposures). Data were acquired on the diagnostic array operating in passive mode at a frame rate of 1Hz; as the buffer of the imaging engine is only capable of storing 325 frames, data could only be stored for the last 325s of each exposure.

In the long-pulse low duty cycle exposures, a longer PCD trace was captured. In order to capture the whole exposure within the capacity of the hardware buffer, the sampling rate for the trace was reduced to 30MHz. Since the diagnostic array is unable to store traces of this length, it was operated in active mode at a frame rate of 10 Hz in an attempt to identify ‘bright-ups’ indicating the presence of echogenic regions on bubbles inside the disc immediately after the HIFU pulse (these HIFU pulses were triggered by software rather than the scanner trigger ‘out’).

Temperature measurements for each of the thermocouples were taken at a sample rate of 3Hz.

Following insonation, a ‘post-exposure’ B-mode image was saved before motion segments were removed from the holder, the vertebral bodies removed, and pho-

tographs of the treated disc taken using a camera (EOS-400D, Canon, Surrey, UK) fixed to a stable mount.

In total, 81 discs from 25 different animals were subjected to extended HIFU exposures. Of these, roughly 50 were used in preliminary experiments to establish the experimental procedure and parameters. The remaining 33 discs were used for the more systematic examination of the parameter space outlined below.

5.4.1.3 Data Processing

The PCD traces were analysed as described previously (Section §5.2.2) to obtain a measure of cavitation activity within the disc. The timing of both the onset and end of the cavitation activity were also determined, and used to provide a rough one-dimensional indication of the location of front of the cavitating region (following the approach described in Hockham et al. (2010)). Both of these measures were calculated and displayed in real-time by the custom Labview software during insonation. After the exposure, a spectrogram was produced of the PCD traces to provide an indication of the spectral content of the signal, and a measure of the relative power in the broadband and harmonic regions of the signal was also calculated.

Passive data from the diagnostic array was used to construct maps of cavitation activity as described previously for the short pulse exposures.

Since the individual ‘long pulses’ were orders of magnitude longer than the maximum frame length of the passive mapping system ($140\mu\text{s}$), passive mapping could not be used during these exposures. To gain some information on cavitation location, the imaging system was driven in B-mode at a frame rate of 10Hz during the HIFU exposure. The lack of a ‘trigger-in’ function meant that the acquisition could not be synchronised, so frames were examined post-exposure to identify frames acquired when the HIFU was ‘on’ (substantial interference in the image). A difference

image was then produced from the frames immediately before and after the HIFU pulse to identify bright regions suggestive of bubble activity.

Similarly, a difference image was constructed by comparing the pre-exposure B-mode image to the post-exposure B-mode image for all discs in an attempt to relate any changes in this image to the macroscopic disc appearance.

Values for the spatial peak, temporal average intensity (I_{SPTA}) and total energy delivered in each exposure were calculated using an approach described elsewhere (Nandlall et al., 2011). Briefly, the I_{SPTA} calculation assumes a linear plane wave at the focus and tissue properties identical to water; the total energy delivered is equal to the intensity multiplied by the total ‘on time’ of the HIFU system and the focal area at FWHM (approximately 6.6mm^2). The low I_{SPTA} values are a result of the low duty cycles employed. As previously stated, the pressure values quoted in this thesis are the pressures measured in water for the same excitation parameters; they have not been derated to account for attenuation within the tissue.

5.4.2 Results

To investigate the effect of varying the parameters of the HIFU exposure on the IVD, a large number of individual motion segments were prepared and subjected to extended HIFU exposures. Three broad regimes were tested:

1. Moderate PRFP with short pulses and moderate duty cycle (1-20%) to limit the potential for damage to surrounding tissue, and minimise the total exposure time without excessive heating;
2. High PRFP with low duty cycle (1%) and extended individual pulses to produce thermal damage/boiling and mechanical damage within a limited region of the IVD;

3. High PRFP with short pulses and low duty cycle (0.1%) to increase the chance of tissue fractionation while preventing heating.

5.4.2.1 Moderate Amplitude, Short Pulses

The first regime tested used a relatively low pressure amplitude (above the cavitation threshold of the SNPs) to initiate cavitation inside the disc, and a pulsed HIFU sequence to maintain this cavitation activity over an extended period of time. In this way, it was hoped to exploit the mechanical forces associated with a cavitating bubble cloud in much the same way as histotripsy, whilst avoiding the need for extremely high pressures and the associated potential for damage to surrounding tissue. Tran et al. (2003) had previously shown that in the presence of Optison microbubbles it is possible to consistently produce visible lesions in kidney with a PRFP of 6.1 MPa; this regime used a slightly higher PRFP to allow for the increased attenuation of the disc tissue.

Given the difficulties reported with the fractionation of collagenous tissue and encountered in preliminary experiments, extended exposures were employed. The duty cycle was increased from the extremely low values typically employed in histotripsy in an attempt to minimise the total exposure time, with the temperatures inside the disc monitored to detect excessive heating of the disc tissue.

A series of discs were exposed to HIFU using the parameters described in Table 5.1.

A total of 22 discs were used for these experiments - 3 discs at 1% duty cycle, 7 discs at each of 5% duty cycle and 10% duty cycle, and 5 discs at 20% duty cycle. The difference in the number of discs exposed to each set of parameters was due to the high level of variation that was seen in both the presence and nature of cavitation activity between discs (see Figure 5.12 below); particularly at the interme-

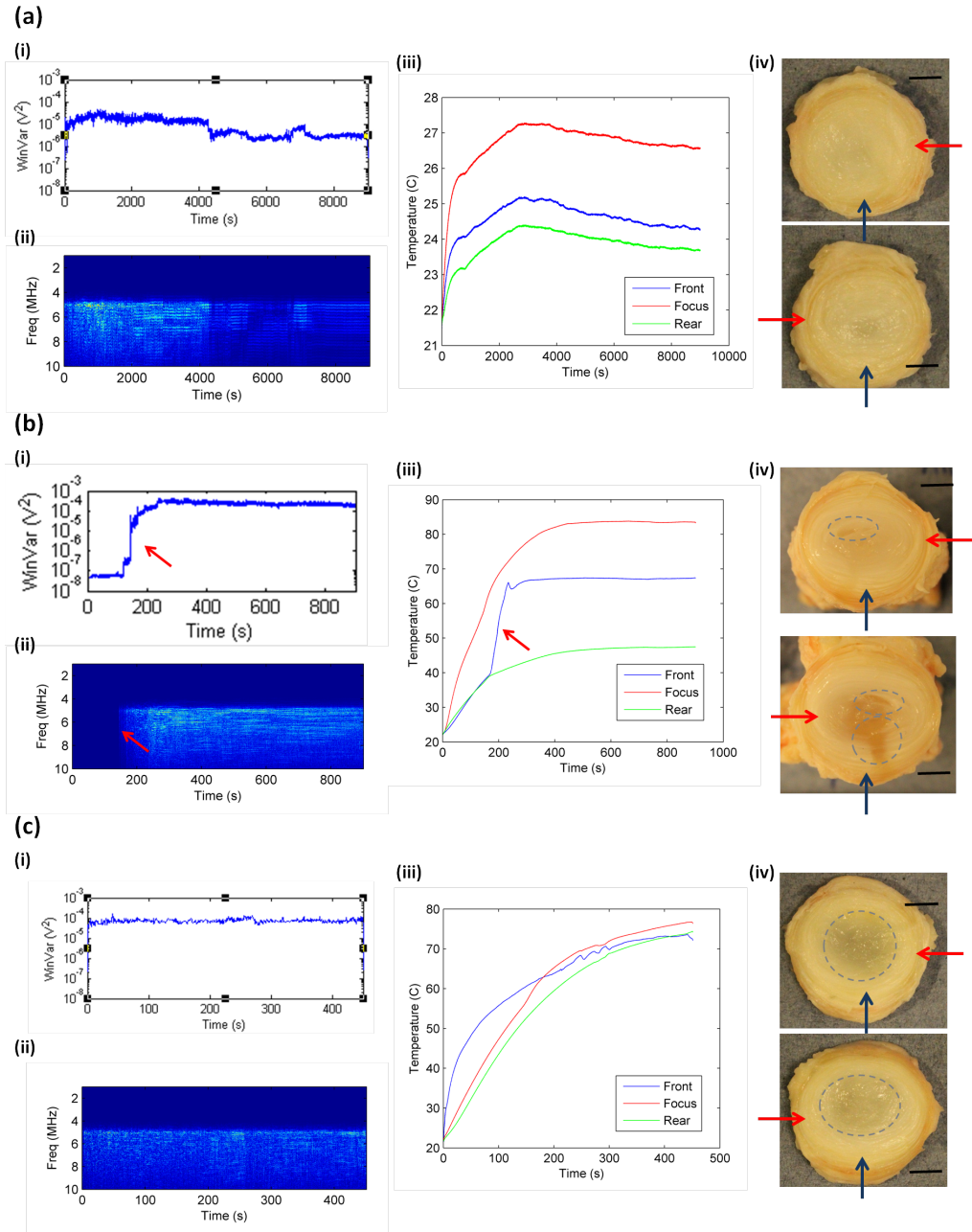


Figure 5.10: Results of HIFU exposures at moderate PRFP, using the parameters described in Table 5.1: **(a)** 1% duty cycle, 9000s exposure time; **(b)** 10% duty cycle, 900s exposure time; **(c)** 20% duty cycle, 450s exposure time. The results for the exposure at 5% duty cycle are not shown here as they are essentially similar to the 1% results. For each disc, (i) shows the squared variance of the PCD signal, a measure of cavitation ‘activity,’ (ii) a spectrogram of the PCD signals, (iii) the thermocouple measurements at three points in the disc, and (iv) photographs of the excised disc after exposure (black scale bars indicate 5mm).

PRFP (MPa)	Duration (s)	Duty Cycle	Cycles per Pulse	ISPTA (W/cm ²)	Total Energy (kJ)
6.45	9000	1%	5	14	8.3
6.45	1800	5%	5	70	8.3
6.45	900	10%	5	140	8.3
6.45	450	20%	5	280	8.3

Table 5.1: Parameters used for the first series of extended HIFU exposures of the IVD

diate duty cycles, cavitation was often not detected at all, or not sustained where it was detected. Sets of exposure parameters were repeated until cavitation was seen throughout the exposure for multiple discs. Potential reasons for this variability in behaviour, and approaches to controlling it, are discussed later in this chapter (see Section §5.4.3).

Figure 5.10 provides representative data showing the three different effects of the extended exposures that were observed on disc tissue with sustained cavitation activity; the 5% duty cycle results are not shown as they were substantively similar to those for the 1% duty cycle.

In the first exposure shown, at 1% duty cycle, the elevated variance in the PCD signal indicates that cavitation occurred and was sustained throughout the exposure. The spectrogram shows mostly broadband signal, which suggests inertial cavitation rather than either boiling or direct reflection of the HIFU. The temperature rise at all three points measured was less than 8°C, and as expected was highest close to the focus. The temperature rise at the AF-NP boundary closest to the transducer was higher than that at the distant boundary, as a result of attenuation and potentially shielding from a bubble cloud at the focus. Most importantly, there was no visual indication of damage to the disc following the exposure, with the NP conserving a gelatinous texture and no discoloration.

It is worth noting that the temperature values shown in Figure 5.10 are only suggestive of the peak temperatures reached and the profile within the disc, as it was not possible to align the thermocouples perfectly with either the transducer or the motion segment.

At a 10% duty cycle (Figure 5.10(b)), there is cavitation initially (low signal amplitude), and then between 150-200s there is a jump in the variance of the PCD signal. At around this time, there is also a jump in the rate of heating measured by the thermocouple at the AF-NP boundary closest to the transducer, while the rate of heating at the other thermocouples remains relatively constant. This is suggestive of a change in the cavitation activity, potentially marking the formation of a boiling or a cavitating bubble cloud that is either located toward the front of the disc or rapidly migrates there (Figure 5.11 has a further discussion of this). The spectrogram again shows the predominantly broadband nature of the PCD traces, which would conventionally be interpreted to indicate the presence of inertial cavitation rather than boiling, where harmonic oscillations and reflections of the fundamental would be expected to dominate.

The temperature in the NP reaches at least 85°C at the thermocouple, and potentially higher elsewhere, which may be consistent with boiling elsewhere in the NP. Both the NP and the front AF-NP boundary reach temperatures above 70°C, which is above the temperature required for the thermal coagulation of collagen (Section §1.3.4), and would therefore be expected to result in thermal damage to the tissue. This is seen in the post-exposure images of the disc - the NP and parts of the AF along the axis of the 'primary' transducer were palpably stiff to touch, and discoloration of the disc tissue was seen. However, along with this thermal damage, an elongated cavity is observed in the NP (indicated by the grey oval in the upper image), filled with a viscous fluid that leaked out upon dissection.

At a 20% duty cycle (Figure 5.10(c)) there is a relatively consistent (high) level of cavitation signal throughout the exposure, and the spectrogram does not show any clear harmonic banding. The temperature rise is very similar at each of the thermocouples, with all three showing a temperature rise to in excess of 70°C. The extensive region of thermally damaged tissue (as indicated by discoloration and palpable stiffness) is indicated by the grey ovals in the photographic images, and covers most of the disc cross-section. No cavity in the disc was observed.

The behaviour of the disc exposed to the 10% duty cycle was further examined by looking at the location of the front of the bubble activity relative to the PCD - shown in Figure 5.11. From this figure, it can be seen that the initial low level of activity is taking place just behind the focus of the transducers (which is at a time-of-flight of approximately 90 μ s). At the same time that the signal variance and temperature at the front of the disc jump, the front of the bubble cloud migrates towards the transducer, eventually stopping at a time-of-flight close to the front of the disc. This behaviour is highly suggestive of the formation of a cavity or boiling bubble at or near the focus that reflects acoustic energy back towards the transducer and results in excessive pre-focal heating and potentially boiling. The passive mapping data (not shown) contains only the last 325s of data, and therefore provides no information on this migration effect.

Inertial cavitation was observed for all of the exposure conditions shown in Figure 5.10. However, the amplitude of these secondary acoustic emissions varied substantially, and in a number of other exposures (not shown) it was observed to be sporadic. This variability was also observed in the appearance of discs after exposure - Figure 5.12 illustrates this for several exposures at 10% duty cycle. While cavities are formed in all three cases, the size and location of the cavity varies, as does the nature and extent of thermal damage to surrounding tissue.

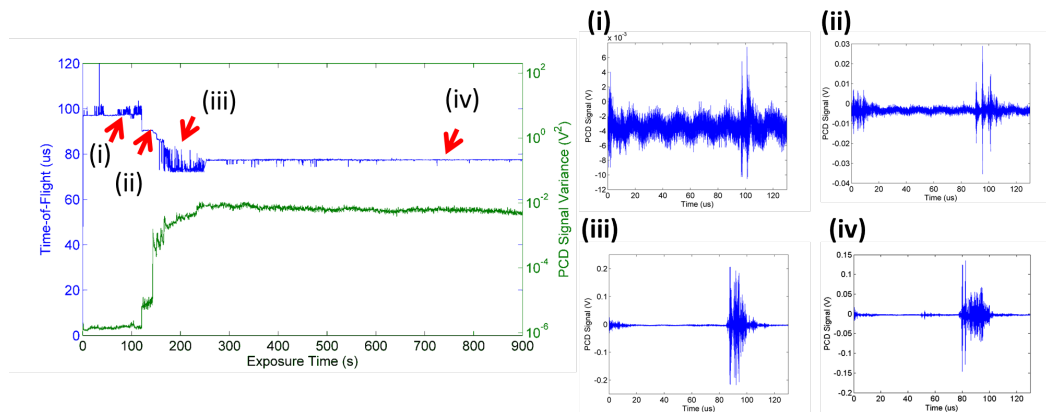


Figure 5.11: **Main Plot:** Time of flight (tof) to the front of the cavitation signal (blue line) for a HIFU exposure at 6.45 MPa PRFP, 10% duty cycle, 5 cycles per pulse. The green line shows the corresponding variance of the PCD signal. The tof to the focus is the red dashed line, the tof to the front of the disc the black dashed line. **(i)-(iv)** show the individual PCD traces at the timepoints indicated with red arrows in the main plot, showing the forward migration of the cavitation signal.

In an attempt to identify a non-invasive indicator of the condition of the disc, the pre- and post-exposure B-mode images were compared, and a difference image calculated (Figure 5.13). Discs where thermal damage was apparent showed an increase in the brightness of the NP, whereas discs with no visible damage showed no change in the B-mode image. The appearance of this brighter region appeared to result from the increased stiffness associated with coagulation of the collagen rather than the formation of a cavity, as it was also seen in discs without a cavity present. However, obtaining good quality B-mode images of the whole disc cross-section is difficult due to high attenuation and low scattering, and it may be that a characteristic marker becomes apparent with improvements in imaging techniques.

These results for extended exposures at a moderate amplitude show that it is possible to create a cavity in the centre of the IVD. However, the formation of the cavity appears to be correlated with the presence of thermal damage - no cavities were observed in the absence of a stiffer texture in the NP. While the migration of bubble activity and the temperatures measured suggest boiling inside the disc, the

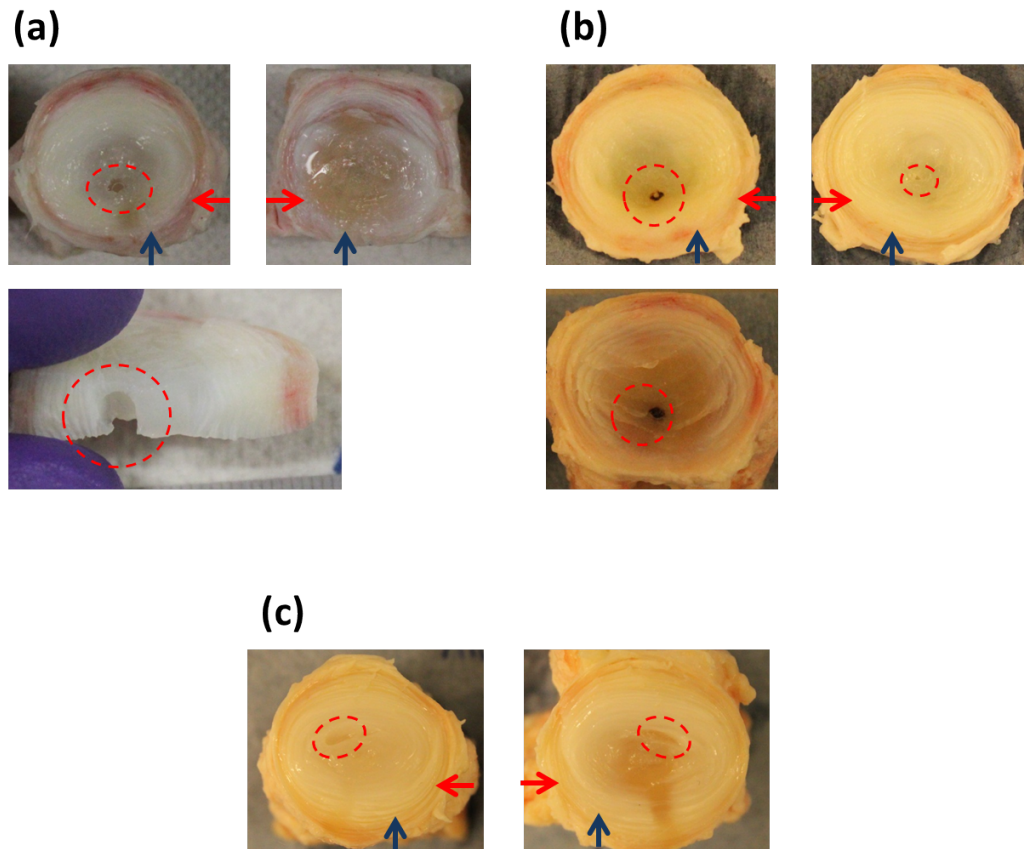


Figure 5.12: Images of post-exposure discs, showing the difference in outcome from similar conditions. All discs shown have been exposed to HIFU with PRFP 6.45 MPa, 5 cycles/pulse, 10% duty cycle; **(a)** and **(c)** for a total of 900s, **(b)** for approx 450s. Note that disc (c) is the same one shown in Figure 5.10

spectral emissions do not show a strong harmonic character. Interestingly, no cavities were observed at the 20% duty cycle exposure, where boiling would be most likely. The mechanism of damage therefore may well be due to the presence of boiling bubbles, but is dependent on the rate that heat is deposited in the disc.

Thus while these results suggest that mechanical fractionation of the IVD using HIFU at moderate amplitudes is possible, both the presence of thermal damage and the variability in cavitation activity have implications for how this would be exploited in the clinic. A detailed discussion of these implications and potential ways to address them is deferred to Section §5.4.3.

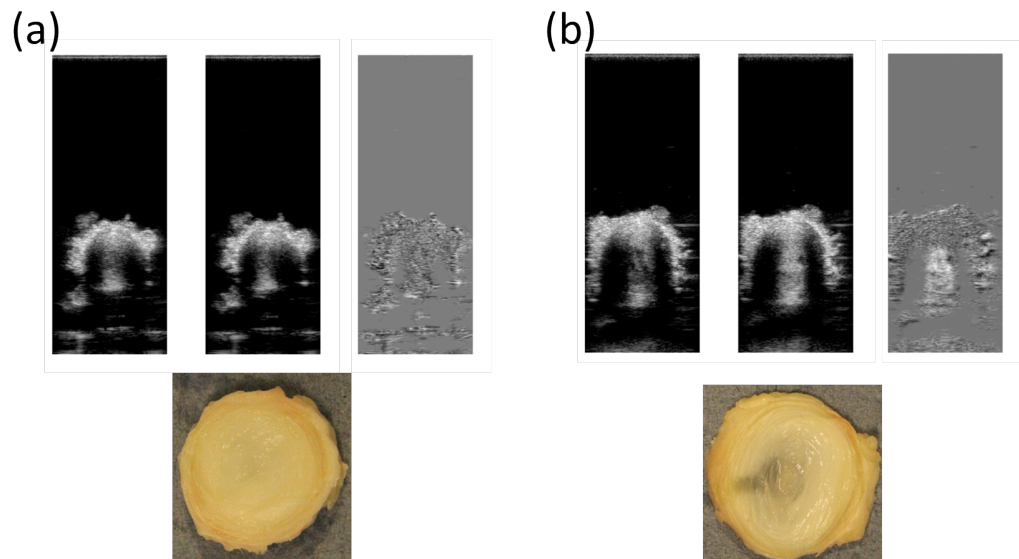


Figure 5.13: Comparison between the pre-exposure (left), post-exposure (centre) and difference (right) B-mode images for two discs. **(a)** shows a disc exposed to 6.45 MPa at a duty cycle of 1%; there is no observable damage to the disc and the B-mode image shows no appreciable difference after the exposure. **(b)** shows a disc exposed to 6.45 MPa at a duty cycle of 10%; there is a clearly visible thermally damaged region on the disc, and a brighter region in the difference B-mode image.

5.4.2.2 High Amplitude, Long Pulses

A second set of extended exposures used similar parameters to those used in millisecond-boiling, in an attempt to produce thermal damage/boiling and mechanical damage within a limited region of the IVD. These exposures used a PRFP of 10.7 - 12.8 MPa, at a duty cycle of 1%. Each pulse was either 50ms (at 12.8 MPa PRFP, $n=2$) or 100ms (at 10.7 MPa PRFP, $n=1$) long, and a total of 1000 pulses were delivered.

Given that the pressure amplitudes were (by necessity) substantially lower than those used in previously reported work on millisecond-boiling, shock formation was not expected; rather the intention was that the cavitating bubbles nucleated by the SNPs would result in the extremely rapid and local deposition of heat required to initiate boiling in a millisecond timeframe. To this end, the pulses were

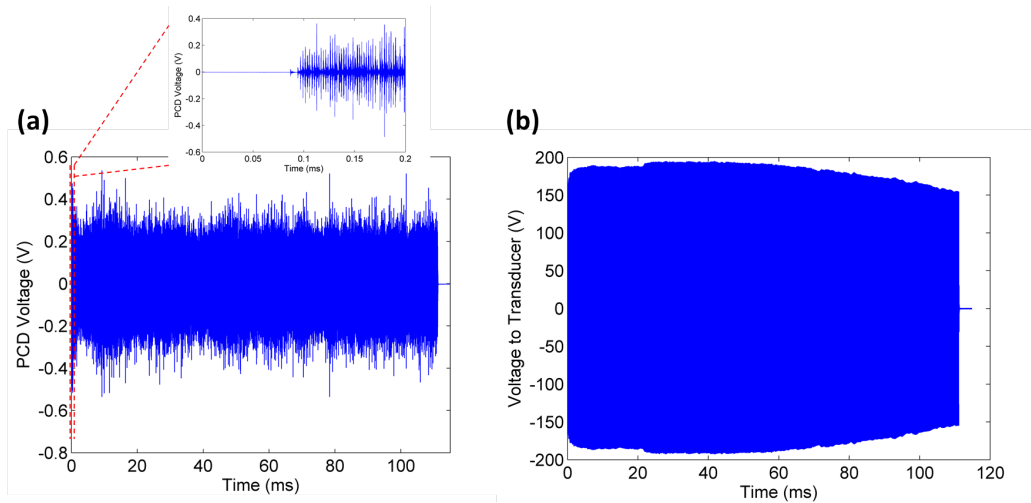


Figure 5.14: Example results of the PCD and voltage traces for an exposure with high amplitude (10.7 MPa PRFP) and long pulses (100ms). **(a)** shows the PCD trace, with the inset highlighting the initial 200 μ s, **(b)** shows the voltage trace measured at the input to the matching network.

slightly longer than those used to initiate millisecond boiling in liver (Khokhlova et al., 2011); rather they are comparable to the parameters that were reported for producing mechanical damage in kidney in the presence of Optison (Tran et al., 2003).

Results for a representative trace are shown in Figure 5.14. Rather than showing the summarised results from all of the PCD traces for the whole exposure, these plots show the PCD signal and the voltage to the transducer for a single, 100ms excitation pulse. The onset of boiling is expected to produce both a large jump in the PCD signal and fluctuations in the driving HIFU voltage (Canney et al., 2010); neither of these are seen in this trace or in any of the traces acquired.

The inset in Figure 5.14 shows a blown-up view of the first 200 μ s of the exposure. The appearance of the signal is broadly consistent with inertial cavitation activity, and the delay of approximately 90 μ s suggests that this activity is at or near the focus, at least initially.

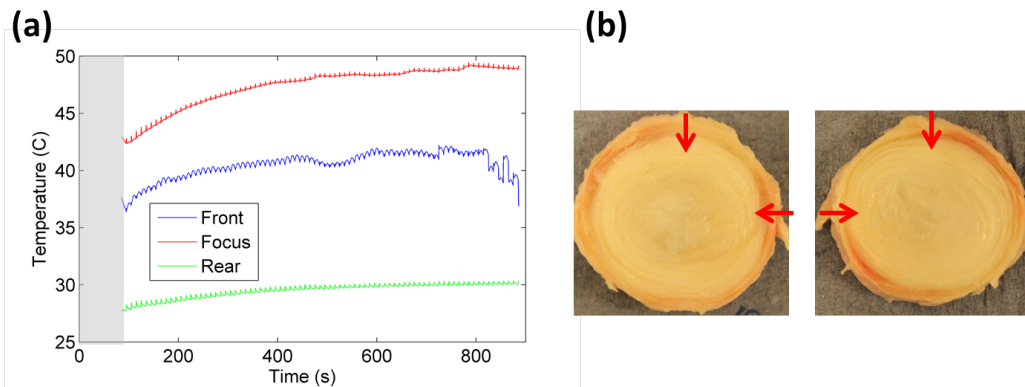


Figure 5.15: **(a)** Temperature rise in the disc during a an exposure at 10.7 MPa, with 100ms pulses at 1% duty cycle. Approximately the initial 85 seconds of the exposure are not included as these were lost due to an equipment malfunction (grey shaded region). **(b)** Appearance of the disc following the exposure (red arrows indicate the position of the transducers).

The absence of boiling is supported by the thermocouple readings for the same exposure (Figure 5.15), which show that while the temperature in the NP is slowly increasing throughout the exposure, it does not exceed 50°C at the thermocouple. While higher temperatures may be reached elsewhere in the NP, these would have to be extremely localised. Figure 5.15 also shows that there is no visible damage to the disc upon excision.

The B-mode difference images (Figure 5.16) are consistent with the presence of bubble activity inside the NP, as they indicate a brighter region immediately after the HIFU pulse. From these images it is not possible to distinguish between boiling and cavitation activity, however when taken in combination with the PCD results it is most likely that it is a region of cavitating bubbles that is seen.

Thus it appears that the set of parameters used in the ‘long pulse’ exposures was not able to produce the localised boiling effects that were intended. This is attributable to two effects: the pressure amplitudes that could be produced and sustained by the transducers used were not high enough to create shocks at the focus,

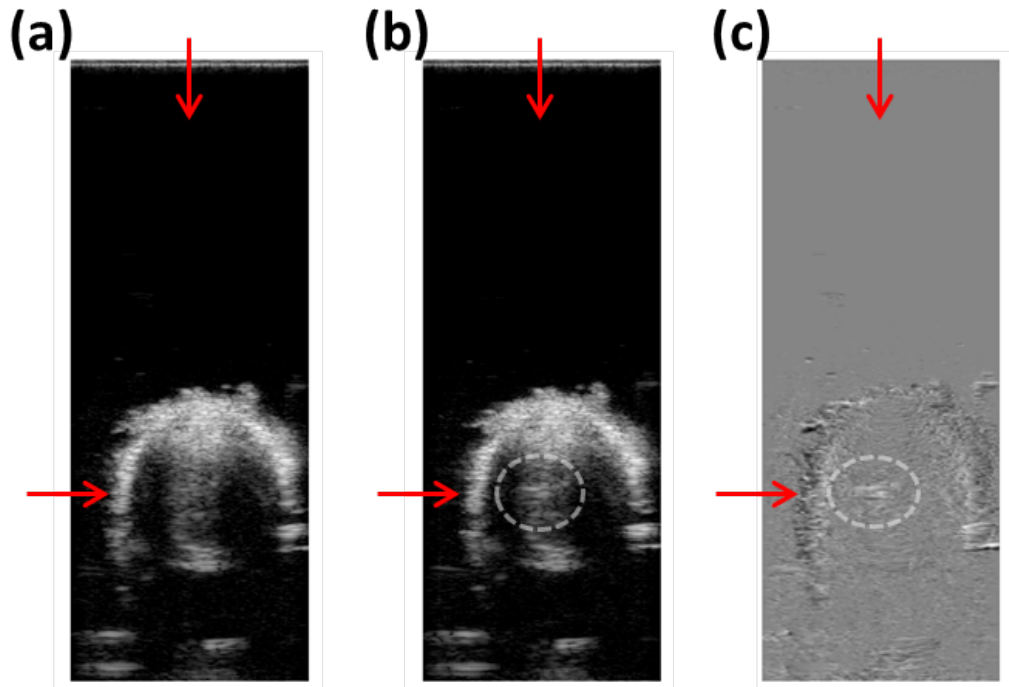


Figure 5.16: B-mode images showing the presence of a ‘bright-up’ during a high amplitude, long pulse exposure at a PRFP of 10.7 MPa. (a) shows an image before the HIFU pulse, (b) an image immediately afterwards, and (c) the difference image between the two. The ‘bright-up’ is indicated by the grey ovals, the red arrows show the positions of the transducers relative to the image.

and the pulses were too short for cavitation to produce high enough temperatures locally. Further work is suggested using a set of more robust transducers to examine the potential of this approach to create mechanical damage in the disc.

5.4.2.3 High Amplitude, Short Pulses

The third series of extended exposures of the discs used a very low duty cycle to avoid any thermal effects on the disc, and a higher PRFP in an attempt to produce a mechanical effect in the gelatinous NP. The limitations of the HIFU transducers meant that the maximum amplitude was restricted to a level below that typically used in histotripsy. Keeping the same energy as the moderate amplitude exposures would have made the exposures impractically long, both in terms of the experimen-

tal protocol and for clinical usage, so the length was limited to 900s which is similar to reported histotripsy procedures, and the IDET annuloplasty (Section §1.3.4) procedure. Table 5.2 gives the range of parameters used.

PRFP (MPa)	Duration (s)	Duty Cycle	Cycles per Pulse	I _{SPTA} (W/cm ²)	Total Energy (kJ)
8.6	900	0.1%	5	2.47	0.15
10.7*	900	0.1%	5	3.8	0.23
12.8*	900	0.1%	5	5.5	0.33
14.9*	900	0.1%	5	7.5	0.44

Table 5.2: Parameters used in high-amplitude, short pulse HIFU exposures

One disc was exposed to each of these sets of parameters. Four other discs were exposed to a similar set of parameters and demonstrated similar cavitation behaviour without exhibiting the formation of cavities within the NP.

Note that the pressures indicated with an asterisk in the above table have been linearly extrapolated from the values measured directly using the needle hydrophone at the transducer focus (which go only up to 8.5 MPa PRFP), due to the risk of damaging the hydrophone tip at higher pressures¹. As water is non-linear, these values are likely to be an over-statement, and further work should seek to measure these pressures by alternative methods.

In these exposures, the presence of cavitation was much more sporadic than for the moderate amplitude experiments (Figure 5.17), with cavitation occurring in short bursts separated by periods of inactivity. One potential explanation for this is that the lower duty cycle allows for the dissolution of nuclei between pulses, while at higher duty cycles some fragments of nuclei are still present when the following

¹It was also attempted to measure the focal pressure directly using a fibre-optic hydrophone (Precision Acoustics, UK), however the presence of cavitation in the degassed water above pressures of 8.6MPa PRFP affected the pressure at the hydrophone tip and made direct measurements impossible

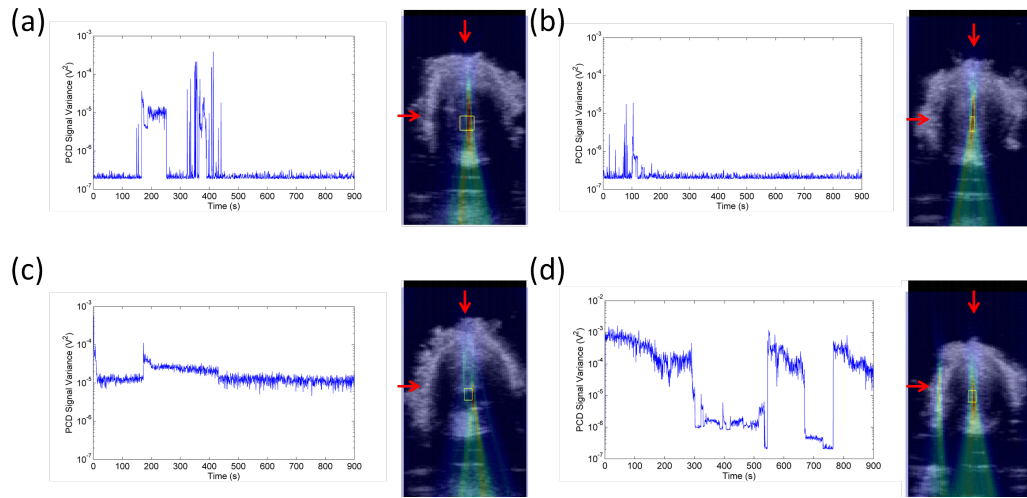


Figure 5.17: Cavitation activity during high amplitude, short pulse exposures using the parameters shown in Table 5.2. PRFP for each exposure was: **(a)** 8.6 MPa; **(b)** 10.7 MPa; **(c)** 12.8 MPa; **(d)** 14.9 MPa. The plots on the left for each disc show the variance of the PCD signal throughout the exposure; red arrows show the location of the transducers in the (cumulative) passive acoustic maps on the right.

pulse arrives. While this was not seen in the results of Chapter 4 where the SNPs were observed to sustain cavitation over thousands of pulses at an even lower duty cycle, the difference in behaviour may be due to the higher pressure amplitude leading to more rapid depletion or the increased overpressure in the disc, and/or be an effect of the lower absolute number of particles that could be injected into the IVD.

A set of results for a disc where cavitation was sustained at the focus throughout the exposure are shown in Figure 5.18. In this exposure there was cavitation at or near the focus throughout (see Figure 5.17 (c)), and the spectrogram indicates that the signal is largely broadband in nature. The temperature rise was kept to less than 3°C in the NP (and lower in the AF), and a clearly visible cavity is formed inside the NP. From an examination of the time-of-flight of the front of the bubble cloud (not shown), it was apparent that there was no migration of the bubble activity over time.

This suggests that, if cavitation activity can be sustained at the focus throughout

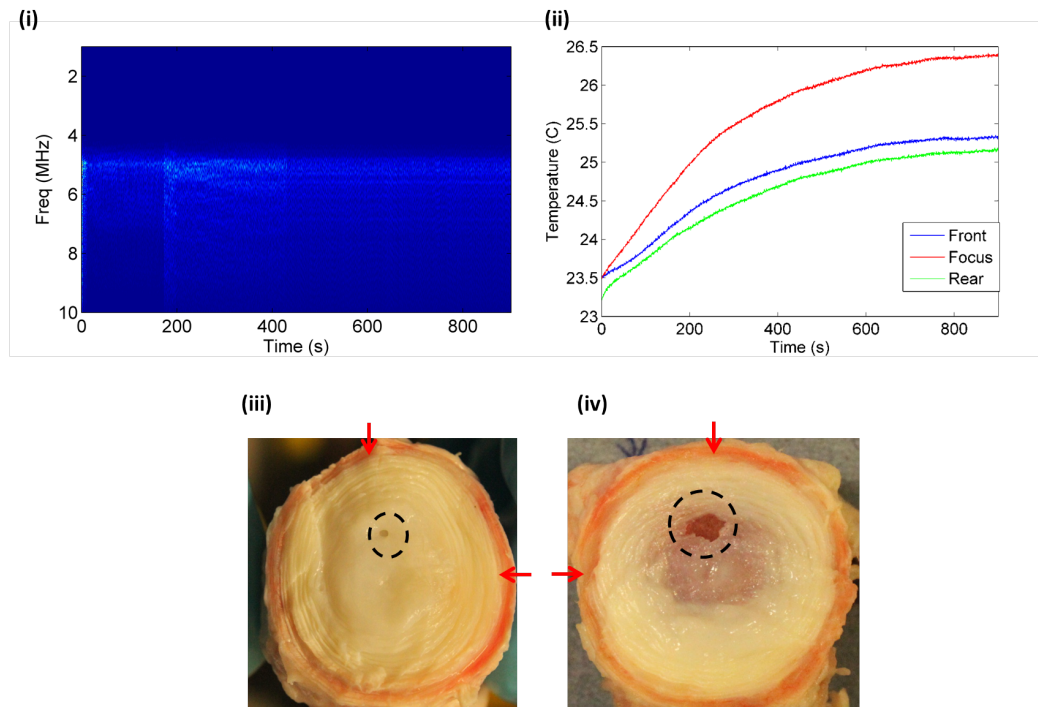


Figure 5.18: Combined results for a disc subjected to a HIFU exposure at 10.7 MPa PRFP (PCD variance and passive acoustic maps for this exposure are shown in Figure 5.17(c)). (i) shows the spectrogram of the PCD traces, (ii) the thermocouple readings, and (iii)-(iv) the post-exposure images of the IVD. The red arrows show the positions of the two transducers relative to the disc, the cavity is indicated by the dotted region.

the exposure, a high amplitude low duty cycle exposure is capable of mechanically fractionating the IVD without producing thermal damage. Of particular interest is that the presence of the SNPs appears to produce mechanical damage at a PRFP substantially below that typically employed in histotripsy, thus reducing the potential for damage to sensitive tissue anatomically adjacent to the disc.

While the cavity observed is substantially smaller than that produced by the moderate amplitude exposures, the low duty cycle means that far less energy has been delivered to the focus for the same exposure time. This low duty cycle was imposed by the limitations of the HIFU transducers available; investigating the use of a more robust set of transducers to increase the duty cycle and/or the pressure

amplitude may result in larger cavities and/or shorter exposures. Use of a higher duty cycle may also help to make cavitation activity more consistent throughout the exposure.

5.4.3 Discussion

The above results for extended exposures show that, by using the HIFU therapy system described in the first half of this chapter together with the SNPs described in Chapter 4, it is possible to mechanically fractionate the tissue of the IVD and create a cavity inside the NP using two distinct sets of exposure parameters.

The first regime involved the use of moderate HIFU amplitudes (6.5 MPa PRFP) and a relatively high duty cycle (10%) over a 900s exposure. The mechanism by which this was able to mechanically damage the disc appeared to be closely related to the appearance of thermal damage, and potentially boiling; it is hypothesised that this thermal damage is necessary to coagulate the material of the NP to become stiff enough to be broken down by bubble activity. A duty cycle of 20% produced significant thermal damage, but no mechanical damage, which suggests that more rapid heating prevents mechanical damage in some way - potentially by promoting the rapid migration of bubble activity towards the transducers and out of the focal region.

While the initial intention of the extended HIFU exposure was to avoid thermal damage, the heating associated with this exposure regime may not have a negative effect *in vivo*, and may even be beneficial. There are a number of existing annuloplasty treatments for chronic lower back pain (Section §1.3.4) that rely on heating the NP and AF to either necrose nociceptors or seal radial fissures by coagulating collagen. If the HIFU parameters can be tailored to produce mechanical damage inside the NP, an annuloplasty-like effect in the AF, and a minimal temperature rise at

the disc surface it may deliver an outcome that both restores normal biomechanical function and directly reduces discogenic pain. Based on the results seen in this work, a duty cycle of somewhere between 5-10% may be capable of delivering this with the *ex vivo* setup used.

Alternatively, it may also be possible to use the SNPs together with a lower duty cycle exposure to simply replicate the heating effects of annuloplasty in an improved, non-invasive treatment. Further work will be required to investigate these possibilities, and also to investigate the impact of either approach on the cells and matrix of the CEP.

The second regime capable of fractionating the disc involved the use of higher pressure amplitudes (up to 14.9 MPa PRFP) at low duty cycles (0.1%). While cavitation was seen to be sporadic in these exposures, when it was sustained it was shown to be capable of producing a cavity inside the NP without any visible thermal damage to the disc, and with minimal increase in the temperature ($<3^{\circ}\text{C}$) at the internal boundaries of the AF. The absence of thermal damage makes this potentially a more attractive approach, however the small size of the cavity that is formed means that further work is required to identify a set of parameters that can produce a cavity more rapidly. While in this case cavitation on the disc surface was not seen, the higher pressures do increase the possibility of this occurring, and the likelihood and effect of this *in vivo* needs to be investigated.

One option for making cavitation more consistent in this second regime is to increase the duty cycle or the pressure amplitude, making the parameters closer to those used in histotripsy. However, the ability to achieve this in the current work was limited by the capabilities of the hardware used, which also restricted the parameters that could be used in the investigation of millisecond-boiling behaviour. The initial hope was that the presence of the exogenous cavitation nuclei would allow

similar effects to be achieved at lower pressure amplitudes. While this appeared to have been the case for the ‘histotripsy’ regime tested, it was not the case in the parameters used in an attempt to produce millisecond boiling. The major restriction was on the power that could be transmitted to the HIFU transducers without causing damage. To fully investigate the range of exposure parameters, it is recommended that custom transducers (or a transducer array) capable of supporting these loads be developed and used for the next round of experiments.

The ability to carry out a systematic investigation of the parameter space for extended exposures was also limited by the high level of variability in the outcome for discs insonated with similar HIFU parameters. Some of this derives from the experimental approach, and may be reduced by improving the targeting method, particularly in the vertical direction/along the spinal axis, and by improving the consistency of the SNPs. However, much of this variability can also be attributed to the natural differences between discs, and the stochastic nature of cavitation.

In order for this approach to be developed into a viable clinical procedure, it is important that it is able to deliver a consistent clinical outcome despite this inherent variability. The most important aspects are to ensure that sustained cavitation is occurring, that it is occurring in the right place, and that the exposure is stopped at the right time. Achieving this will require some degree of feedback control. It may be possible to implement some of this automatically, using the information on cavitation activity derived from the PCD signal, and cavitation location from the passive array. (Hockham et al., 2010) describes an example of where a similar system has been implemented successfully in liver tissue, varying the driving voltage of the transducer to control the level of cavitation activity.

To detect the formation of a mechanically fractionated region inside the disc, these experiments attempted to use B-mode imaging. Thermally damaged regions

were found to become more highly scattering, but there was no obvious marker for a formation of a cavity. This was unexpected, as in histotripsy fractionated regions typically appear hypo-echogenic in B-mode, and this is used to determine the end of the exposure. It may be that the relatively wide vertical mixing of the imaging array masks the presence of a cavity, or more likely, that the IVD is highly attenuative, and the NP in particular is naturally hypoechogenic to start with. An improved B-mode imaging system may deliver improved results, or it may be necessary to identify an alternative method for determining the formation of a cavity inside the disc.

One limitation of the approach employed here is that the effect of the extended exposure on the disc tissue was monitored only from the visual appearance of the disc tissue after dissection. While this was able to provide an indication of the formation of a macroscopic cavity, which was the ultimate goal of this work, additional insight into the mechanism of damage may be gained from a histological analysis of the insonated tissue samples. In particular, it would be interesting to assess whether the discs that are visually undamaged in fact show signs of microscopic damage to the structure of the NP.

For the system to be used successfully in humans, it must also be shown to be able to fractionate tissue in degenerate discs. Human discs were not available for this work, and as discussed in Section §3.1.1.3 bovine coccygeal discs were used as an experimental model. It is worth briefly considering here how the changes that come with degeneration would be expected to affect the outcomes of an extended HIFU exposure.

Degeneration leads to a loss of hydration in the NP, and an associated increase in the stiffness of the tissue. This may make it easier for both of the regimes identified to mechanically fractionate the tissue, and in particular for the moderate amplitude

regime to operate at a lower duty cycle and reduce the extent of thermal damage necessary.

Degeneration also leads to a loss of structure and eventually fissuring within the IVD, which should make it easier to inject a large volume of the SNP suspension into the disc. However, at the same time, the fissuring may mean that the SNPs are more mobile, and may find their way out of the NP and into the AF and/or surrounding tissue. If this is seen, it may be necessary to restrict the procedure to early stage degeneration where fissuring is minimal, or to increase the mobility of the SNP suspension by using a solvent with a higher viscosity.

Ultimately, these questions can only be resolved by empirical work, initially on cadaverous discs and eventually *in vivo* in both animals and humans. The form that these trials might take is deferred to the following chapter.

5.5 SUMMARY

This chapter has presented the results of experiments aimed at using HIFU to achieve the selective mechanical fractionation of the NP within a bovine coccygeal disc, while simultaneously monitoring the location of cavitation activity using passive detection systems.

The early part of the chapter detailed the setup and characterisation of a custom HIFU system to produce cavitation within the IVD. In line with the results of the modelling described in Chapter 3, two spherically-focussed transducers were positioned at 90° to each other and confocally aligned; cavitation activity in the focal region was monitored by both a single-element spherically-focused PCD and a diagnostic scanhead. This system was found to have a focal region significantly reduced in axial extent from that produced by a single transducer, and to increase the PRFP

that could be achieved. Most importantly from a clinical perspective, the size of the focal region produced by this configuration was smaller than the size of the NP in both bovine coccygeal and human lumbar discs, greatly reducing the possibility of causing undesirable damage to either the AF or to tissue structures outside the disc.

It is worth noting that the choice of using two spherically-focused transducers in this system was largely dictated by the nature of the available equipment. It is expected that an array comprising multiple transducer elements would be capable of producing an acoustic field with an even higher focusing gain to further minimise the possibility of damage to surrounding tissue, and to provide greater control over the precise location of the pressure maximum within the IVD; this is suggested for further investigation and development in the following chapter.

This system was then used to investigate the cavitation threshold in the IVD. In native disc tissue no cavitation was observed up to a PRFP of 7.4 MPa, implying that the threshold is above this level. In order to reduce the threshold and produce cavitation at a pressure amplitude sufficiently low to minimise the potential for damage to surrounding tissue, both Sonovue microbubbles and SNPs were injected into discs as sources of exogenous cavitation nuclei. While Sonovue was found to have a lower threshold (<1.2 MPa) than the SNPs (1.2-2.4 MPa), the cavitation activity was rapidly depleted. The ability of the SNPs to continue to nucleate cavitation over a 10s HIFU exposure, on the other hand, made them a more appropriate choice to enable mechanical fractionation through extended exposures.

The ability of passive acoustic mapping to accurately localise cavitation within the disc was demonstrated by marking the location of the nuclei in the IVD (by injecting them together with a solution of a strong dye), and comparing this against the location of the peaks in the passive maps. These results strongly suggested that passive acoustic mapping can be used to accurately monitor the location of cavi-

tion during HIFU exposure of the disc, and thus to minimise the potential for dangerous tissue damage resulting from cavitation in the AF or on the disc surface.

The latter part of the chapter presented the results of extended HIFU exposures of the disc. The goal of this work was to identify a set of HIFU parameters that were capable of producing a cavity within the NP while leaving the AF intact.

After testing a wide range of exposure parameters, two regimes were identified that were capable of producing a cavity within the NP: a moderate PRFP regime with a duty cycle in the vicinity of 10%, and a high-PRFP regime with a much lower duty cycle (0.1%). For the moderate PRFP regime it appears as though the mechanism of damage is related to the onset of thermal coagulation of the collagen in the disc tissue and potentially to the presence of boiling, while in the high-PRFP regime the damage appears to be completely mechanical in nature. Histologic analysis would help to confirm the nature of the tissue damage and the impact on surrounding cells and tissue.

The ability to systematically determine the boundaries of these two regimes was limited by the high variability in the behaviour of discs when exposed to HIFU, as similar exposure parameters were regularly observed to give rise to different levels and durations of cavitation activity, and to have different effects on the appearance of the IVD. A number of potential causes for this variability were suggested, as were several techniques that may be applied in future to ensure consistency of outcome in a clinical setting.

Overall, these results from extended HIFU exposures suggest that while a significant amount of development remains, a procedure based on the use of HIFU to fractionate the material of the NP and enable the minimally-invasive injection of a replacement is feasible. The following chapter provides an overview of the further

work that is required before it can reach the level required for use in a clinical setting.

CONCLUSIONS AND FUTURE WORK

This thesis describes the development of a minimally-invasive procedure using ultrasound to remove degenerate material from an intervertebral disc, in preparation for the insertion of an injectable replacement. In this chapter, the results and implications of the work presented in previous chapters is reviewed and summarised. A number of suggestions are made for the further advancement of this approach, with the ultimate goal of achieving the use of the procedure in the clinic.

6.1 CONCLUSIONS

Degeneration of the IVD is strongly linked to the occurrence of chronic lower back pain, which has a significant impact both on the quality of life for individuals, and on the economy as a whole. Chapter 1 reviewed the pathophysiology of disc degeneration, and the treatments that are currently available. One promising minimally-invasive approach is to replace the degenerate NP with an injectable material in the early stages of degeneration in order to maintain 'normal' biomechanical function. The success of this approach is highly dependent upon leaving the AF largely intact, however at present the procedure requires the manual removal of material through a sizeable incision. This leads to the central aim of this thesis - the development of

a minimally-invasive procedure to remove degenerate material from the IVD based on the demonstrated ability of HIFU to mechanically fractionate tissue.

The mechanical effect of HIFU on tissue is related to the phenomenon known as acoustic cavitation, which is capable of exerting mechanical forces on the surrounding tissue. Chapter 2 provided a brief overview of biomedical acoustics, with a focus on the mechanisms by which cavitation can be initiated, and how the dynamics and bioeffects of cavitation can be controlled by varying the nature of the ultrasound used to excite it. The importance of duty cycle and amplitude were highlighted in particular - a high duty cycle gives rise to predominantly thermal effects, whereas mechanical fractionation typically requires a low duty cycle and extremely high amplitude pulse. The overview also discussed the potential for using the acoustic emissions from cavitation to detect and monitor cavitation initiation and behaviour.

In order to gain an understanding of the behaviour of ultrasound in the IVD, the acoustic properties of disc tissue were first measured (Chapter 3). For this work, and all of the experimental work in this thesis, bovine coccygeal discs were selected as a model for human discs. While they do not undergo degeneration, bovine discs are similar in key respects to healthy human discs and have the primary advantage of being readily available. By using a modified scanning acoustic microscope to obtain measurements for cross-sectional slices of disc tissue, the variation in acoustic properties between the different regions of the disc was clearly observed, with a significantly higher speed of sound and attenuation in the AF than in the NP, and a narrow transition region between the two. Interestingly, the attenuation coefficient in the AF exhibited a large degree of anisotropy, with higher values for horizontal transmission ('along' the collagen fibrils) than for vertical transmission, a finding that is in accordance with the behaviour observed in other fibrous materials such as tendon and muscle. The high attenuation coefficient in the AF suggested that it

may be difficult for the proposed treatment approach to produce the pressure amplitude required for mechanical damage inside the NP, and that it may also result in excessive prefocal heating and tissue damage within the AF and the surrounding tissue.

To investigate the effect of the acoustic properties of tissue on the acoustic field in the disc, computational modelling was performed in PZFlex, a commercially available software package. Using a simplified two-dimensional geometry, it was found that the extended axial focus of a single HIFU transducer created substantial potential for prefocal cavitation and tissue damage. Using two transducers aligned at 90° was found to produce a much smaller focal region with a higher focusing gain, and this configuration was therefore used for subsequent experiments. Despite the simplified geometry, the limited experimental validation that was possible suggested that the results of the modelling were at least directionally correct.

In order to further reduce the possibility of damaging the sensitive tissue surrounding the disc, the potential for artificial cavitation nuclei to reduce the pressure required to initiate cavitation was then investigated (Chapter 4). While such nuclei had previously been shown to both lower the cavitation threshold and enhance damage to tissue, they are not widely used clinically as their systemic administration and presence can result in pre-focal damage. The avascular nature of the disc means that it is almost uniquely suited to the use of such artificial nuclei, as they remain within the disc rather than entering circulation. As stabilised microbubbles were found to collapse and lose their activity under the overpressures experienced in the disc, a novel class of solid sonosensitive nanoparticles were developed. These SNPs have a rough silica coating layered onto a solid polystyrene core, with the roughness of the coating trapping small gas pockets that act as cavitation nuclei.

The presence of the silica coating was demonstrated to decrease the cavitation

threshold significantly compared to uncoated particles and a water control when the particles were suspended in water. This effect that was found to be independent of either the core size (down to 300nm) or the size of the particles in the coating layer (down to approx. 7nm). The particles were found to behave similarly in porcine blood, suggesting that they are capable of lowering the cavitation threshold *in vivo*, although further work on biocompatibility is required. The threshold was also found to increase with increasing HIFU frequency, as expected from previous results.

The behaviour of the SNPs was compared to Sonovue, a commercially available contrast agent based on stabilised microbubbles. While the threshold is slightly lower for Sonovue, the SNPs were shown to be more stable in storage, and much better able to maintain their activity when subjected to an extended series of ultrasound pulses. As a result, SNPs were used to lower the cavitation threshold in the disc for subsequent experiments. They also have a range of potential applications outside of the IVD, and some of these are discussed in the following section.

These particles were then used together with the two-transducer configuration to test if it was possible to initiate cavitation activity within the NP, without pre-focal activity within the AF or on the surface of the disc. Measurements of the acoustic field produced by the custom configuration showed that the focal region was substantially smaller than that with a single transducer, as predicted by the modelling work. This setup was found to be capable of producing cavitation in the disc in the presence of both Sonovue and SNPs, with Sonovue exhibiting a lower threshold. However, the activity of Sonovue was found to be rapidly depleted, which is consistent with the interpretation that the microbubbles collapse under the overpressure in the disc. The SNPs, on the other hand, maintained their activity over a 10s exposure and for subsequent exposures at increasing pressures.

Chapter 5 also describes the use of a 128-element diagnostic array for passive

acoustic mapping of cavitation activity during the HIFU exposure. While initial use of an established passive mapping approach suggested that cavitation was occurring inside the NP, the requirement of accuracy to ensure patient safety meant that validation was necessary. Discs were injected with a mixture of dye and SNPs and exposed to HIFU with data simultaneously acquired on the diagnostic array. The post-exposure discs were then sliced cross-sectionally, and the cavitation location as measured on the passive map was compared to the location of the dye. There was a strong agreement between the location of the two regions, validating the accuracy of the passive mapping to a level sufficient to distinguish between desirable and undesirable cavitation activity.

This experimental setup was then used for extended exposures of the IVD in an attempt to identify a set of ultrasound parameters that could produce mechanical fractionation of the NP tissue. After testing a wide range of exposure parameters, two regimes were identified that were capable of producing a cavity: a moderate PRFP regime with a duty cycle in the vicinity of 10%, and a high-PRFP regime with a much lower duty cycle (0.1%). For the moderate PRFP regime it was suggested that the mechanism of damage is related to the onset of thermal coagulation of the collagen in the disc tissue and potentially to the presence of boiling, while in the high-PRFP regime the damage appeared to be completely mechanical in nature.

The factors affecting the choice between these two regimes were discussed: the most important issues were the need to understand the impact of a high temperature on the tissue, cells and ultimately structural integrity of the AF and the endplates; and the limits placed on the focal pressure amplitude by the need to avoid damage to surrounding tissue (which is governed primarily by the unknown local cavitation threshold). A number of potential techniques were suggested for overcoming the natural variability between different patients and discs and achieving a

consistent outcome, as this is of critical importance in a clinical context and needs further development.

Overall, the results from extended HIFU exposures suggested that while a significant amount of development remains to be done, a procedure based on the use of HIFU to fractionate the material of the NP and enable the minimally-invasive injection of a replacement is feasible.

Suggestions for further development of this technique are outlined in the following section.

6.2 FUTURE WORK

While the experimental work detailed in this thesis demonstrates the feasibility of the proposed procedure, a significant amount of work remains before it can achieve widespread clinical usage. The four main areas that are suggested for further work are: improvements to the device itself; refinements to the exposure protocol; characterisation of *in vivo* bioeffects in better models, including the impact of degeneration; and development of additional applications for the SNPs.

6.2.1 Improvement of Device

Having demonstrated that the device described in Chapter 5 is capable of both inducing and monitoring cavitation, and producing mechanical lesions within the NP, further development work should be aimed at improving the design of the transducer, and developing the additional equipment needed for the clinical procedure.

The modelling work in Chapter 3 showed that a two-transducer configuration is capable of producing an acoustic field that is both smaller in area and higher in amplitude than a comparable single transducer. The experimental work was based

on the use of two spherically-focussed transducers predominantly because of their ready availability, but it is suggested that more sophisticated custom transducer designs may be capable of further improvements on the acoustic field.

Two potential approaches are suggested. Firstly, an array of transducers, with multiple independently-triggered elements may provide greater control over the size and location of the focal region. This is important in order to avoid excessive heating of the bone in situations where the patient has lost a substantial degree of disc height due to extensive degeneration, and also to be able to deal with the focussing issues produced by overlying tissue which will vary significantly between patients.

A second potential alternative is to use a lesser number (2-4) of shaped transducers to achieve a similar effect. Rather than using a spherical form, a truncated sphere or even a bi-curved form may allow the vertical extent of the focus to be greatly reduced and provide both a tighter focus and an increased focussing gain from the disc surface to the focus.

To assess the effect of different transducer configurations on the acoustic field, a three-dimensional model of both the disc and transducer geometry should be developed in PZFlex. A number of disc heights representative of different stages of degeneration should be modelled; the geometry for the various disc heights can be obtained from the segmentation of MRI scans of a series of human discs. The additional computational complexity involved in these simulations will require the use of a substantial amount of processing power, potentially more than can be achieved with a single desktop machine. The results of these simulations should also be carefully validated experimentally. A fibre-optic hydrophone may give greater control over the location of measurement within the disc; it may also be possible to validate the position of the hydrophone using either ultrasound imaging or fluoroscopy.

The work described in this thesis has been primarily concerned with demonstrating that HIFU can be used to create mechanical lesions inside the NP; the procedure and equipment required for the removal of fractionated tissue and insertion of an injectable replacement has not been considered. While the consistency of the fractionated disc tissue is appropriate for removal through a small-gauge needle, work is required to develop and validate the process for inserting the needle into the correct location. The potential for designing a single needle that can be used both for the removal of fractionated material and the injection of the replacement should also be examined, as this would reduce the complexity of the surgical procedure, and reduce the number of perforations of the AF.

6.2.2 Treatment Protocol

As highlighted in Chapter 5, there is a need to further investigate the optimal exposure parameters for producing a cavity in the NP, and additionally to develop techniques to ensure a consistent outcomes for patients and discs with very different characteristics.

The limitations of the transducers used in the extended exposures meant that it was not possible to test the effect on the IVD of extended exposures with the parameters that are commonly used in both histotripsy and ms-boiling applications. While the high pressure amplitudes involved are likely to make them unsuitable for clinical use, the effect of these regimes on the disc may provide insight into the mechanism by which cavities are formed, and enable optimisation of the exposure parameters at a more moderate pressure amplitude. With the new transducer designs proposed above, these regimes should be tested, and the resulting disc sections analysed to determine the nature of any damage observed.

Clinically, the level of variability in the outcome for discs subjected to the same

HIFU exposure that was seen in Chapter 5 is not acceptable. To ensure a more consistent outcome, several control measures are suggested for investigation.

A real-time adaptive controller has been shown to sustain cavitation for an extended period in an agar gel by adjusting the HIFU amplitude (Hockham et al., 2010); if this or a similar system can be used to maintain cavitation activity in the IVD at a constant level over the course of an extended exposure it should address the issue to some extent.

Along similar lines, while at present the passive acoustic maps are produced after the HIFU exposure has finished, hardware improvements should mean that in the near future maps can be produced in real time (i.e. during the exposure). This can be used to improve the inherent safety of the procedure by halting the exposure if cavitation is seen outside of a defined target region. It may also be possible to link these maps to the positioning system that controls the location of the HIFU transducers, and move the focus through the whole of the NP region during the exposure to ensure complete fractionation of degenerate material. Alternatively, it may be possible to develop a control mechanism based on detection of bright-ups in a conventional B-mode image that does not require passive acoustic mapping at all.

A final area of the treatment protocol that requires further work is to identify a means of determining the endpoint of the HIFU exposure - knowing when the lesion has been formed and the treatment can be stopped. Using the presence of hypo-echoic regions on B-mode images as an indicator of the presence of a lesion is suggested as the most obvious candidate. This will require the incorporation of new hardware; either a second imaging array, or an advanced dual-mode transducer capable of interleaving imaging and passive data acquisition at a high switching rate.

If this approach proves not to be possible due to either equipment constraints

or insufficient sensitivity, the potential for using empirical thresholds to determine endpoint should be investigated. It has recently been shown (Jensen et al., 2012) that aggregate secondary acoustic emissions can provide a measure of thermal lesion formation, and a similar threshold may exist for mechanical fractionation; an alternative may be to simply define a minimum time for which treatment must continue to achieve the desired effect (as in the existing IDET procedure for annuloplasty).

6.2.3 Trials

To take a finalised device design from the demonstration of feasibility in an *ex vivo* bovine coccygeal disc to a viable clinical procedure, there are a number of aspects of the procedure and its effects that must be demonstrated.

In the first instance, fractionated discs should be fixed and examined by histology to gain further insight into the mechanism of damage and the likely bioeffects *in vivo*. Two approaches are suggested initially. The first is a combination of H&E staining to examine the effects of the HIFU exposure on both the cells and extracellular matrix, and imaging of second-harmonic generation by the collagen matrix present in the disc. As this requires the discs to be fixed (to prevent elution of the fractionated material), it will provide information only on cellular morphology rather than viability. To investigate this, a second approach capable of distinguishing between live and dead cells (Poole et al., 1996) is suggested, with a particular interest in the cells in the pre-focal region of AF and the CEP. This should be done in conjunction with the work described above on identifying the optimal exposure parameters.

A second key area, and perhaps the most important, is the need to demonstrate that the approach will work with a degenerate disc. This is essential, as degenerate

discs differ from healthy discs in a number of important ways. As discussed at length in Chapter 3, very few animals exhibit degeneration, and it will be necessary to work with human discs as a model. If possible, a series of cadaverous human discs with varying degrees of degeneration should be obtained, and used to test the viability of the approach using the setup and parameters arrived at in the bovine disc. The key parameters to monitor are the location of cavitation and whether the degenerate disc material can be fractionated; the temperature in the pre-focal AF and at the cartilaginous endplates should also be monitored for excessive heating leading to cell death.

If and when *ex vivo* experiments show that the procedure can work in degenerate disc, *in vivo* trials will be required. The question of whether animal trials are required prior to human will be governed by the regulatory situation; since there are a number of injectable replacements already approved for use in humans, it may be possible to go straight to human trials. If animal trials are required, either sheep or baboons are commonly used for testing of disc replacement procedures and should be considered. The key question in these trials (outside of safety issues) will be whether the conservation of the AF in this procedure improves the performance of an injectable replacement, and whether the minimally invasive procedure provides a benefit in terms of both recovery time and patient comfort.

6.2.4 Sonosensitive Nanoparticles

The SNPs that are described in detail in Chapter 4 have potential applications outside of the focus of this thesis. When compared to existing sources of exogenous cavitation nuclei, their primary advantage is in their small size. This gives them the ability to more easily extravasate and naturally accumulate into tumours, potentially serving to enhance both thermal and non-thermal HIFU treatments. To

demonstrate that the SNPs are biologically compatible, the immune response of a suitable animal model needs to be tested; the procedures and protocols for this are relatively well-established.

The precise mechanism by which these particles are able to nucleate cavitation also needs to be investigated, and may yield insight into improvements in particle design and production. Direct imaging of the gas trapped in surface crevices is not feasible with currently available techniques, and so it is likely that this will need to be inferred from experimental testing of theoretical models - the development of such an approach is beyond the scope of this current work.

Finally, the potential for these particles to serve as a template for active targeting and drug delivery should be assessed. The use of the layer-by-layer approach has been documented in the literature for a range of applications. If it can be shown that the inclusion of a LUDOX[®] layer in the preparation of both particles and capsules confers the ability to nucleate cavitation, it has the potential to open up a whole new area of research into targeted therapy and drug delivery.

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