Biocompatibility of implantable materials: an oxidative stress viewpoint

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Abstract:

Oxidative stress occurs when the production of oxidants surpasses the antioxidant capacity in living cells. Oxidative stress is implicated in a number of pathological conditions such as cardiovascular and neurodegenerative diseases but it also has crucial roles in the regulation of cellular activities. Over the last few decades, many studies have identified significant connections between oxidative stress, inflammation and healing. In particular, increasing evidence indicates that the production of oxidants and the cellular response to oxidative stress are intricately connected to the fate of implanted biomaterials. This review article provides an overview of the major mechanisms underlying the link between oxidative stress and the biocompatibility of biomaterials. ROS, RNS and lipid peroxidation products act as chemo-attractants, signalling molecules and agents of degradation during the inflammation and healing phases. As chemo-attractants and signalling molecules, they contribute to the recruitment and activation of inflammatory and healing cells, which in turn produce more oxidants. As agents of degradation, they contribute to the maturation of the extracellular matrix at the healing site and to the degradation of the implanted material. Oxidative stress is itself influenced by the material properties, such as by their composition, their surface properties and their degradation products. Because both cells and materials produce and react with oxidants, oxidative stress may be the most direct route mediating the communication between cells and materials. Improved understanding of the oxidative stress mechanisms following biomaterial implantation may therefore help the development of new biomaterials with enhanced biocompatibility.

1. Introduction

Implanting materials to replace or to repair damaged tissues in our body, to restore the function of a deteriorating organ, or simply to improve our aesthetics, is a routine procedure in medicine. The medical device industry represented a global market of $360 billion in 2014 [1]. Implantable devices make use of all types of materials, i.e. metals, ceramics (comprising glasses) and polymers. Metals and ceramics find mostly applications in orthopaedics for procedures including total hip replacements, and in dentistry for fillings and tooth implants. Polymers are more appropriate for soft tissues repairs, for example as sutures, patches and cardiac valves.

Improving the biocompatibility of biomaterials, i.e. their "ability to perform with an appropriate host response in a specific application"[2], has been a major driver in their development. For example, researchers have put much effort into improving the surface properties of materials to stimulate cell adhesion, proliferation and differentiation. However recent findings show that the pre-existing tissue reactivity at the site of implantation can affect the healing response, suggesting that biocompatibility assessment may require a case-by-case approach [3]. Moreover, several degradable materials previously described as 'biocompatible' can induce significant oxidative stress and inflammation in the surrounding tissue during their degradation [4-7]. Thus the current understanding of the biocompatibility of implants requires further investigation.

Oxidative stress occurs when the production of oxidants, which mainly include reactive oxygen species (ROS), nitrogen species (RNS) and consequently formed lipid peroxidation (LPO) products, surpasses the antioxidant capacity of cells or tissues. It is implicated in a number of pathological conditions such as cardiovascular and neurodegenerative diseases, cancer and aging. In recent years, evidence showed that it plays critical roles in inflammation, fibrosis and healing, the major events occurring during the implantation of biomaterials. In particular, ROS contribute to the recruitment and the function of leukocytes and macrophages, suggesting the importance of oxidative stress in the orchestration of the inflammation and healing phases [8, 9]. Interestingly, the events stimulated by oxidants often lead to further oxidant production, propagating the inflammatory response [10].

Implanted materials can also stimulate oxidant formation, through the constant oxidative attack by immune cells and through their degradation products [11-14]. This may result in excessive or prolonged oxidant exposure, which can then lead to chronic inflammation and loss of the biomaterial's biocompatibility and function [15]. Successful biomaterial implantation thus requires the balanced expression of both oxidant production and elimination. Designing biomaterials that modulate oxidants is therefore a promising strategy to improve their outcomes *in vivo*.

The aim of this review is to provide an overview of the major mechanisms underlying the link between oxidative stress and fate of implanted materials. Little is known about how implanted biomaterials affect and are affected by ROS, RNS or lipid peroxidation products. It is also challenging to find a diagram of signal transduction pathways induced by implanted materials that takes oxidants into account. A recent book published on the topic of oxidative stress and biomaterials supports the need for a better understanding of the cell-material interactions from an oxidative stress viewpoint [16]. Substantial advances in this area could lead to novel biomaterials with improved biocompatibility and better patient outcomes.

2. Oxidative stress: physiological and pathophysiological roles

Although oxidative stress was initially described as a pathological condition leading to degenerative diseases, extensive research also shows that it plays an important role in the subtle modulation of cell signalling pathways. Reduction-oxidation (redox) reactions are key for the regulation of biological activities such as inflammation, wound healing, immune function, stem cell self-renewal, carcinogenesis and ageing [17, 18].

Oxidants generated by the body that participate to oxidative stress include ROS, RNS and the consequently formed LPO products. ROS include both free radical and non-radical species such as superoxide, hydrogen peroxide, singlet oxygen, hydroxyl radical, ozone, hypochlorous acid, alkoxyl and peroxyl radicals. ROS have either unpaired valence electrons or unstable bonds that makes them reactive and they easily transit from one species to another through reaction cascades [19]. Radicals such as hydroxyl radical are particularly reactive and thus extremely short-lived. ROS are the result of normal cellular metabolism (mostly in mitochondria, peroxisomes and endoplasmic reticulum) but are also generated from xenobiotic exposure, as shown in Figure 1a. Xenobiotic sources of ROS include implanted materials. RNS designate mostly nitric oxide, which is generated from L-arginine substrate by NO synthases in cells such as neutrophils and macrophages. These cells exploit oxidants as our first line of defence against pathogens [20]. Nitric oxide can react with ROS to produce stronger oxidants, such as peroxynitrite or dinitrogen trioxide [21, 22]. Because of their reactivity, ROS and RNS easily react with biomolecules such as protein, DNA, carbohydrates and lipids. Lipid peroxidation is autocatalytic process yielding different products some of which are well-known markers of oxidative stress. When attacked, lipids undergo a chain reaction involving peroxyl radical that lead to the formation of aldehydes such as 4-hydroxyl-2-nonenal (HNE) or malondiadehyde (MDA). HNE is often referred to as the secondary messenger of oxidative stress and itself can easily react further with other macromolecules [23, 24].

All organisms, including simple life forms such as yeasts and bacteria, possess a complex system of antioxidants that counterbalance the oxidants constantly generated in the body. This antioxidant system, both endogenous and exogenously obtained, includes small water

and lipid soluble molecules (e.g. glutathione, vitamin C, vitamin E) and enzymes (e.g. superoxide dismutases, catalase, peroxiredoxins, sulfiredoxins, glutathione peroxidases). Glutathione (GSH), γ-L-glutamyl-L-cysteinylglycine, is one of the most ubiquitary small molecules in antioxidant defence. GSH reacts with its cysteine residue with oxidants thereby forming oxidised glutathione. Other well-known small antioxidant molecules are vitamins, such as vitamin C (ascorbic acid), which protects the aqueous compartments of cells, and Vitamin E (name given to a group of tocopherols and tocotrienols), which protects the lipid compartments. In brief, vitamin C quenches radicals and forms in turn an ascorbyl radical, which is more stable and causes little oxidative damage. Vitamin E acts by terminating the lipid peroxidation chain reaction or by inactivating ROS. Oxidised molecules of vitamin E are regenerated by vitamin C molecules, themselves regenerated through the redox cycle [25]. Antioxidant enzymes, which are more specific than small antioxidant molecules, work by enzymatic cascades leading to complete detoxification of oxidants. For instance, superoxide dismutases catalyses the dismutation of the superoxide radical into hydrogen peroxide and molecular oxygen. Hydrogen peroxide, which still represents a danger for the cell, and is then becoming the substrate for the enzyme catalase. Catalase decomposes hydrogen peroxide into water and oxygen.

Inadequate removal of ROS or RNS, such as when the antioxidant capacity is not sufficiently high or when enzyme activity is inhibited, may cause disturbance of the redox balance and be harmful to tissue and organs. However, whether oxidants are biologically beneficial or harmful depends on their concentrations, chemical nature, origin (physiological or non-physiological), location (intra- or extracellular), time of exposure and stability. As illustrated in Figure 1b, high concentrations of oxidants lead to cell damage which may cause in turn a number of pathologies, ranging from brain disorder, like Alzheimer's and Parkinson's disease, to various forms of cancer (skin melanoma) and eye pathologies (cataract, macular disease) and aging. In the presence of implants, it can lead to the loss of function of the material and its rejection. At low/moderate concentrations, oxidants induce subtle changes in signalling intracellular pathways in response to changes of intra- and extracellular environmental conditions, ensuring physiological function of the cell [26]. In wound healing involving biomaterials, they orchestrate the different phases of inflammation and healing that lead to its successful integration. Too low concentrations of oxidative species may lead

to a defence system which is deficient against pathogens and thus may be harmful to the organism. In presence of a biomaterial, complications such as loosening of the material may arise if pathogens are not adequately eliminated.

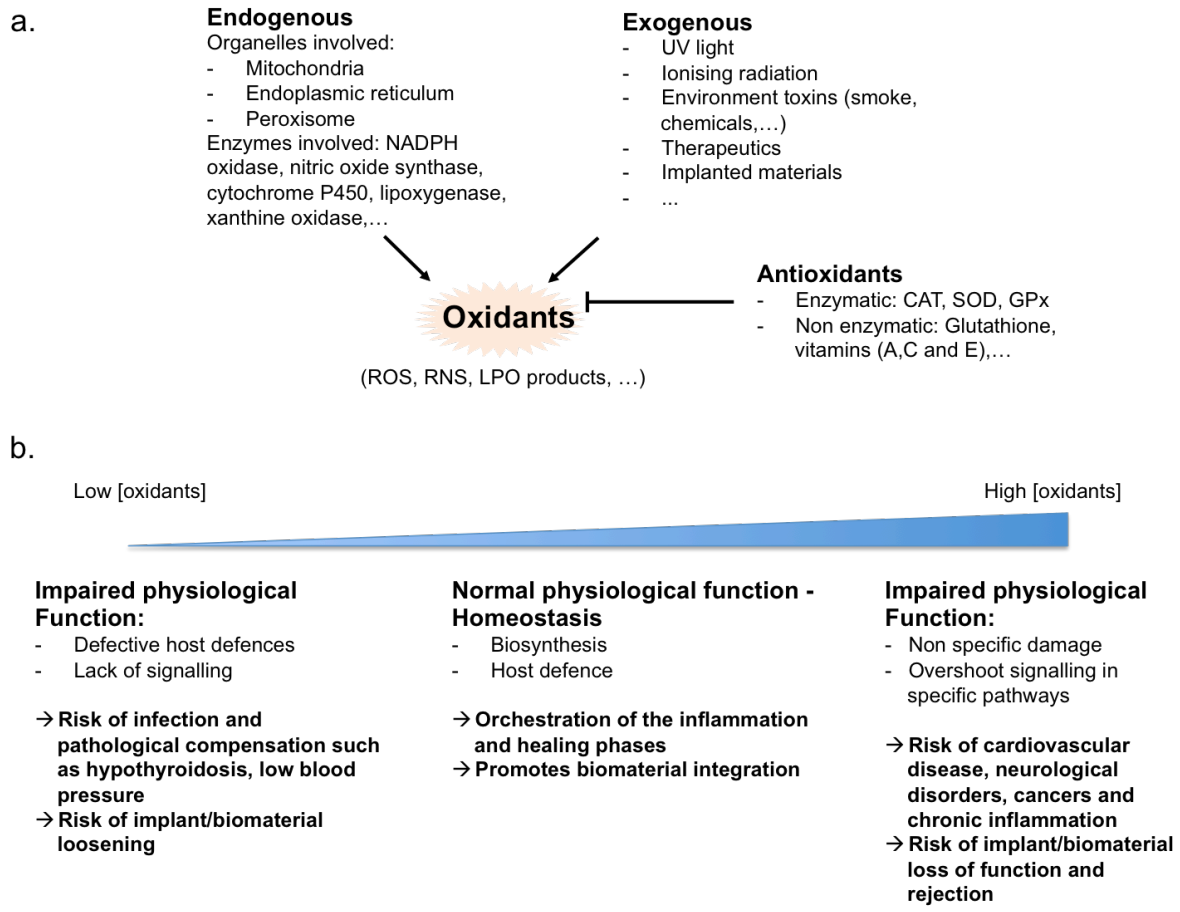

Figure 1: Origins and consequences of oxidative stress. a. Endogenous and exogenous sources of oxidants. b. At moderate levels, oxidants (net concentration) play crucial roles in normal cell function through signalling pathways and host defence. Levels of oxidants that are too low may lead to decreased antimicrobial defence and pathological conditions such as hypothyroidosis or low blood pressure. When levels are too high, the condition might result in cardiovascular disease, neurological disorders, cancers, and chronic inflammation (modified from [18, 19, 27]).

3. Oxidative stress and cell signalling pathways during wound healing

Extensive studies have identified that oxidative stress plays a critical role in the orchestration of inflammation, fibrosis and wound healing [10]. Phases of inflammation and healing are linked with significant alterations of redox equilibrium. Both acute and chronic inflammatory states *in vivo* are associated with enhanced oxidant generation [17, 28, 29] and oxidative stress is considered a major factor amplifying the inflammatory state of non-healing wound [30]. In fresh wounds, ROS release by platelets, mast cells and macrophages plays an important role in the recruitment and function of immune cells as well as resident stromal cells.

ROS and RNS can regulate cell behaviour and consequent response to a wound through both direct and indirect mechanisms. These include:

1) Regulation of redox-sensitive transcription factor activity (e.g. NFkB, Nrf2, AP-1, p53, HIF-1α, PPAR-γ and β-catenin) via direct thiol group oxidation; altering accessibility of DNA for transcription (e.g. HDAC activity modulation); or via regulation of intracellular signalling pathways (e.g. p38 MAPK, JNK, PI3K/Akt, PKC, Smad2/3) upstream of these transcription factors [31-35]. These changes in transcription factor activation leads to altered expression of target genes including those for inflammatory mediators and for oxidant generation (e.g. NAPDH oxidases, NOX) which both regulate generation of ROS/RNS via activation of cell signalling and consequent alterations in gene expression (Figure 2).

2) Lipid peroxidation damage of cell membrane phospholipids leading to direct activation of apoptosis due to severe cell damage or through altered cellular PKC, JNK and NFkB pathway activity by lipid peroxidation products [36, 37].

3) Oxidation of cellular proteins, generating protein carbonyls and proteins modified by reactive aldehydes, leading to aggregation and proteasome-mediated cell death or altered protein activity (e.g. matrix metalloproteinases, apoptosis-mediating caspases and intracellular signalling proteins) [38-40].

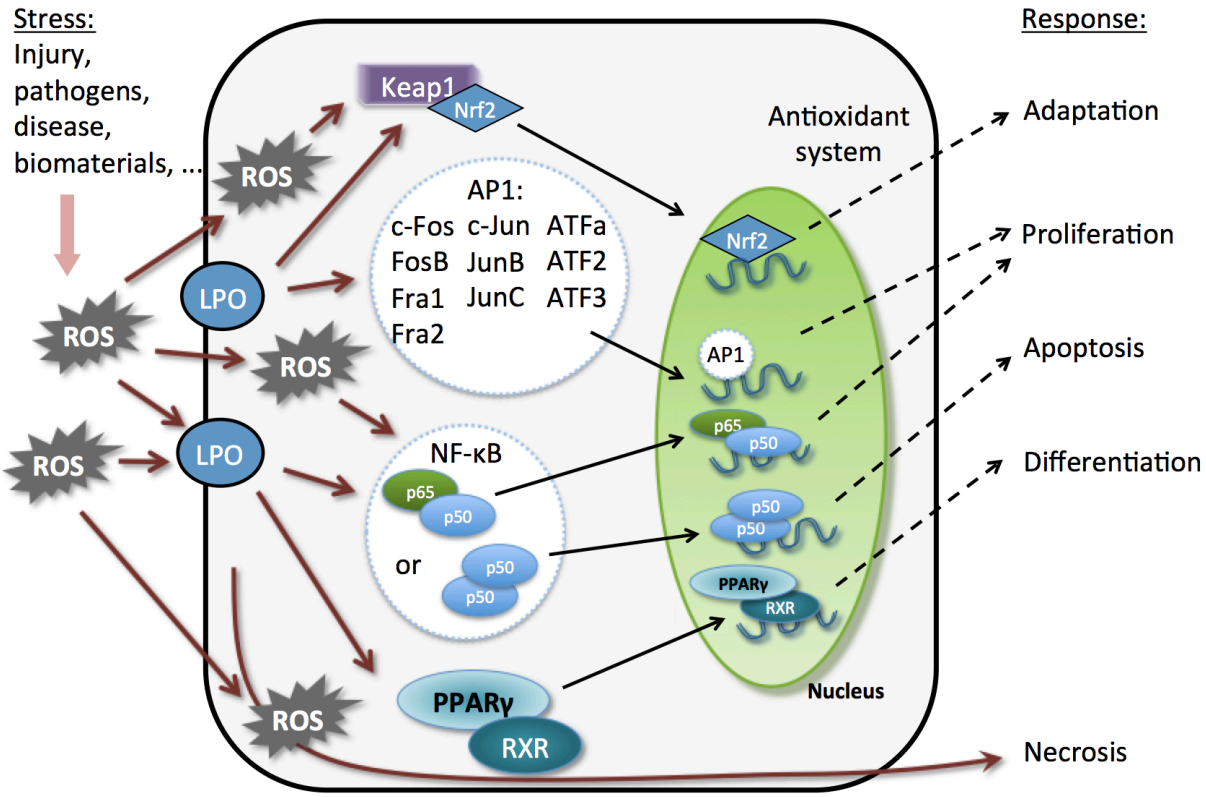

Figure 2: Examples of transcription factors involved in the cellular response to oxidative stress. ROS and LPO products react with redox sensitive parts of transcription factors and/or their regulatory molecules causing their activation directly or indirectly by releasing the inhibition.

4. Oxidative stress, inflammation and healing following material implantation

For improved understanding of the origins, roles and consequences of oxidative stress following biomaterial implantation, it is worth highlighting some of its involvements in the major steps of the wound healing process, in particular during the inflammatory and healing responses.

The inflammatory response to material implantation is classically described as a timely sequence of events, including protein absorption (within seconds), neutrophil invasion (1 day), monocyte/macrophage infiltration (3 days), foreign body giant cell (FBGC) formation (1-2 weeks) and collagenous encapsulation (3-4 weeks) [41, 42]. Inflammation is an integral

component of successful tissue healing. The healing response itself usually starts with a proliferative phase a few days after the implantation, and includes fibroblast infiltration, angiogenesis and granulation tissue formation. This phase is then followed by maturation and tissue remodelling within weeks and generally lasts for months or years [42, 43].

The boundaries between these different events and phases are often unclear and mainly depend on the material implanted and the nature of the injury. Moreover, their order and duration might be influenced by the state of the tissue prior material exposure. Since damage to tissues by trauma or disease often takes place prior biomaterial implantation, the extent and nature of inflammation in the diseased environment may influence the inflammatory response to materials [3].

Oxidative stress can occur at all stages of the biological response to biomaterial implantation and the consequent changes in cell behaviour mediated by cell signalling are the mechanism by which this stress is communicated to the cells present and to those recruited to the site of implantation (see Section 3). A representation of the changes in oxidative stress levels during the process of trauma and healing with a biodegradable biomaterial is shown in Figure 3.

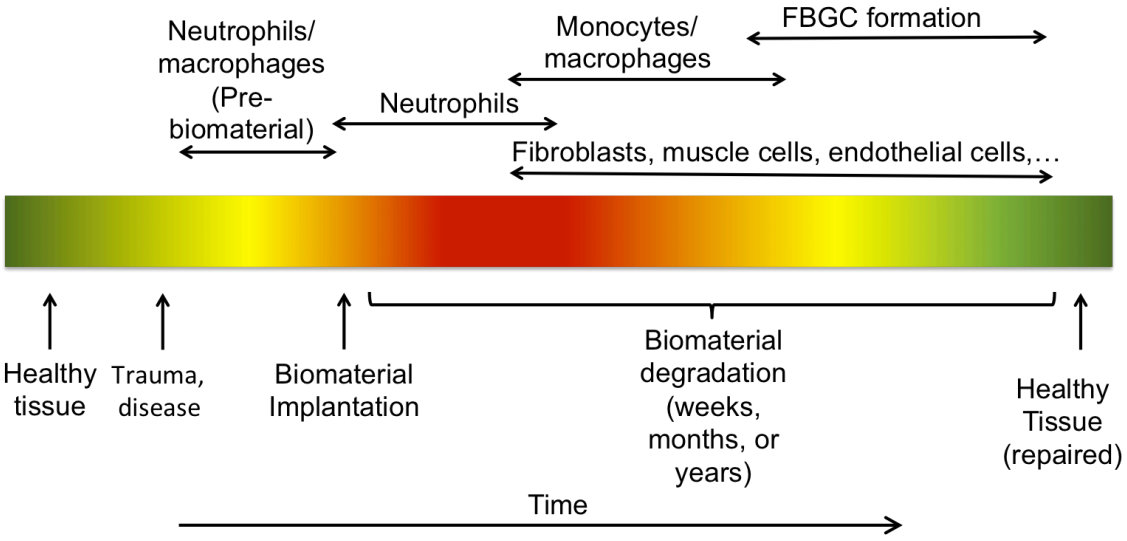

Figure 3: Expected oxidative stress dynamics in tissue during the process of trauma and healing with a biodegradable biomaterial (green: physiological levels of ROS, RNS and/or LPO products, yellow: slightly elevated levels, red: high levels that may lead to pathological processes if present for prolonged time).

4.1 Sources of oxidative stress prior to material-tissue contacts

Two factors define the redox state or reactivity of the site of implantation before any contact with the biomaterial itself: the degree of pre-existing inflammation in the host tissue and the immediate stress resulting from the surgical wound. Although they are often underestimated, these factors might influence the success of a biomaterial and therefore biocompatibility.

- Pre-existing oxidative stress

There is often some level of inflammation existing prior to material implantation as the damaged (by trauma or disease) tissue attempts to heal itself or due to the inherent tissue damage related to the condition. It is thus likely that the oxidative stress is already present.

During healing, H_2O_2 can stimulate NFkB activity and thus expression of inflammatory genes and NOX enzymes [44, 45]. Furthermore generated inflammatory cytokines such as IL6, IL1b and IFNγ not only induce further inflammatory gene expression but also induce NOX enzymes via altered NFkB and PKC pathway activity leading to a further increase in ROS generation [46-48].

Pathological conditions linked to oxidative stress can also display elevated levels of oxidants. For example, cardiovascular diseases are characterised by elevated inflammation and oxidation of lipids. Oxidation of lipids, especially in lipoproteins, creates a variety of oxidative stress markers, which have been reviewed by Frijjhof et al. [23]. Almost all cancers also show elevated levels of ROS. They promote many aspects of tumour initiation and progression such as by inducing mutations in genes regulating cell cycle. In addition, metabolic and signalling pathways such as Nrf2 (anti-oxidative) and NFkB (inflammation) are also affected in tumours, leading to destabilisation of the normal redox balance [49, 50].

Pre-existing oxidative stress might have a significant effect on biomaterials, and in particular degradable materials as they may degrade faster in environment with higher concentrations of oxidants [51]. These conditions may also affect the surrounding tissue surface chemistry and the biological microenvironment, which may in turn alter the performance of biomaterials [3]. Selection of a biomaterial should therefore be done in full knowledge of the state of the extent and nature of inflammation in the diseased or damaged environment [3, 52].

- The surgical wound and the resulting oxidative stress

The surgical procedure to implant materials into the body inevitably creates a wound and tissue damage. This cellular and tissue damage results in the release of the intra- and extracellular components in the wound environment, which contribute to increasing the levels of oxidative stress. This partially results from the direct release of existing ROS from damaged cells. The wound also triggers an instantaneous calcium flux, which travels as a wave via gap junctions several cell rows back from the wound edge. This calcium flash activates the DUOX/lactoperoxidase system, responsible for hydrogen peroxide (H_2O_2) production [8, 53]. At the wound margin, H_2O_2, besides the killing of invading bacteria, plays a role in the rapid recruitment of phagocytic leukocytes from distant sites [8].

Other components released after tissue injury from either dying cells or from the breakdown of the extracellular matrix components are the numerous damage-associated signals (DAMPs, also called “alarmins”). Examples of intracellular DAMPs include heat shock proteins, S-100 proteins, high mobility group box-1 (HMGB1), ATP, and DNA [54]. Extracellular DAMPs comprise fibronectin, hyaluronic acid, as well as peptides. DAMPs activate the innate immune defence mechanisms. Oxidative stress represents both a cause and a consequence of release of DAMPs in multiple situations. For instance, HMGB1 itself may induce significant redox modifications by fostering the cellular generation of ROS and RNS [10, 55]. DAMPs are sensed by a complex set-up of pattern recognition receptors (PRRs), which include primarily the Toll-like receptors (TLRs). Activation of TLRs upregulates NFkB, p38 and JNK pathways leading to upregulation of redox enzymes including iNOS and inflammatory cytokines such as TNFa, IL6 and CCL2 [56, 57]. Cells localised in the connective

tissue surrounding the wound, such as mast cells, macrophages and resident stromal cells, are able to respond to DAMPs early on following tissue injury (Figure 4A).

The mechanical stress caused to the microvasculature and tissue by the surgery might also cause the lysis of red blood cells that can result in the release of large amount of pro-oxidant molecules such as methemoglobin (Fe^{3+}) derived from haemoglobin (Fe^{2+}) auto-oxidation. Methemoglobin stimulates ROS production that potentiates platelet and leukocyte activation, enhances thrombus formation and produces cellular damage. Methemoglobin reacts with H_20_2 and other organic peroxides to form Ferryl-hemoglobin (Fe^{4+}) and protein radicals, which are highly reactive and can initiate lipid peroxidation and oxidant damage to proteins. Moreover, if traces of iron are freed from heme during hemoglobin autoxidation and degradation, superoxide and H_20_2 can lead to the production of the harmful hydroxyl radical by the metal-catalyzed Haber-Weiss and Fenton reactions [58, 59].

Temporary hypoxia may be caused after injury, as restriction in blood supply to tissues can cause a shortage in oxygen. During hypoxia, lack of oxygen triggers series of metabolic events leading to increased ROS production once the blood flow is established and oxygen supply is normalised [60]. Hypoxia can also be the result of a disease, in which case it might contribute to the oxidative stress levels existing prior the surgery. On the other hand, hyperoxia can also occur in the normally less oxygenated tissues due to air exposure during surgery. Air exposure might create gradients of oxygen in the opened wound, which could contribute to the production of oxidants and to tissue inflammation [61]. This suggests that minimising air exposure should be encouraged in clinical practice [62].

4.2 Oxidative stress following protein adsorption

Immediately after implantation in the host tissue, biomolecules from blood and interstitial fluid competitively adsorb onto the surface of the material (Vroman effect). These include serum proteins, such as albumin, fibrinogen, complement proteins, globulins and other immunomodulatory proteins. Depending on the environment at the implant site and the properties of the implant itself, these biomolecules will adsorb in different quantities, proportions, distributions, orientations and conformations. The resulting provisional matrix

leads to an amalgamation of molecular binding events, triggering oxidant formation. This further contributes to the existing oxidative stress at the implanted site (Figure 4B). For instance, complement activation is an early event happening at the surface of implanted materials resulting in pro-inflammatory signal transduction involving anaphylatoxins and cumulating in phagocytosis, NADPH oxidase activation, ROS generation [41, 55]. Oxidative stress is not only a cause but also a consequence of complement activation [63]. Immunoglobulins and DAMPs attached to the biomaterial can bind the F_C Receptors present on the surface of inflammatory cells further regulating ROS and inflammatory mediator production via complement activation and TLR signalling respectively [55].

4.3 Oxidative stress induced by immune cells during the acute and chronic inflammation

- Mast cells

Mast cells are a heterogeneous class of cells that are localised in connective tissues surrounding the implantation site and that can be stimulated by DAMPs. Upon activation, they produce a variety of substances, including histamine, prostaglandines, cytokines and ROS. These trigger the rapid migration of inflammatory cells, such as neutrophils and macrophages, towards the implanted material [64, 65].

- Polymorphonuclear leukocytes (neutrophils)

Following the signalling cascades triggered by the wound creation and the formation of a provisional matrix at the surface of the material, polymorphonuclear leukocytes such as neutrophils are quickly recruited to the implantation site. This stage is often denoted as the acute inflammation phase. After adhesion onto the provisional matrix (via integrins and PRRs), leukocytes immediately begin to release destructive agents such as proteolytic enzyme and ROS as an attempt to degrade and phagocytose the material. This is often referred to as the oxidative burst and is generated by NOX enzymes. Even if materials are often too large for phagocytosis, the damage done to the surface (corrosion or cracks formation) can compromise the function of the device. Leukocytes also remove pathogens that enter the wound (during the surgery or from the material itself) but due to the metabolic exhaustion and depletion of oxidants to destroy the material, the microbial killing capacity of leukocytes is significantly reduced, which can cause severe infections [55].

Leukocytes also release chemokines which attract and activate other monocytes, macrophages, immature dendritic cells and lymphocytes. At the same time increased release of these chemokines suppresses their own infiltration in favour of mononuclear cell. Due to a lack of further activation signals leukocytes then undergo apoptosis and are engulfed by macrophages. Leukocytes should disappear within a few days after implantation as their prolonged presence usually indicates an infection [41]. However they can also persist at sites of chronic inflammation [66].

- Macrophages

Macrophages usually follow leukocytes and their continued presence at the implant site indicates chronic inflammation, a crucial step of biomaterial-related inflammation to achieve effective healing [67]. Macrophages are key cells that orchestrate inflammation and tissue repair. They have numerous roles including the clearance of pathogens, xenobiotic material and apoptotic cells, the regulation of both innate and acquired immune responses through antigen presentation to secretion of a repertoire of cytokines and chemokines [68]. Remodelling of the extracellular matrix, proliferation of epithelial cells and the development of vasculature are vital processes for successful tissue repair.

Macrophages become activated in response to signals such as DAMPs and cytokines. The macrophage activation paradigm has recently been revised to more accurately reflect the key signalling mediators in common and distinct pathways. These include pro-inflammatory pathways containing interferon and NFκB (superseding M1 activation), pro-fibrotic pathways containing signal transducer of activator of transcription 6 (STAT-6) and inflammation resolving IL-10 and glucocorticoid receptor activation pathways (superseding M2 activation) [69]. The early phase of inflammation and wound healing is characterized by a pro-inflammatory macrophage phenotype. During the later phase of tissue repair and remodelling, alternative activation predominates. The type of macrophage activation status and abundance of these relative proportions appear to be a crucial event in the tissue remodelling process [70]. In aseptic wounds without material implantation, M2 macrophages usually rapidly downregulate the inflammatory response to promote tissue repair. The persistence of chronic inflammation at the site of material implantation may be attributable to failure of inflammation to adequately resolve [71].

Oxidative stress has been shown to participate to macrophage polarisation. In particular, ROS were shown to be critical for the activation and functions of M1 macrophages and necessary for the differentiation of M2 macrophages [72]. This suggests that designing biomaterials capable of modulating the oxidative stress at the implantation site could become a useful strategy to control the macrophage phenotype and, as a result, improve outcomes such as tissue healing. However oxidative stress is both a cause and consequence of macrophage activation. In activated macrophages, ROS are generated during the respiratory burst by the enzyme NADPH oxidase which catalyses the generation of superoxide (O_2^-) and hydrogen peroxide (H_2O_2). RNS are also produced by the enzyme NOS to produce nitric oxide. While important for host defence and direction of the adaptive immune system, ROS/RNS can also cause significant collateral damage on the microenvironment and therefore the implant itself.

- Foreign Body Giant cells

Due to the large size of the materials implanted, macrophages typically fail to digest and phagocyte them, and as a result they fuse into multinucleate giant cells, or foreign body giant cells (FBGCs), which is characteristic of the foreign body reaction. ROS have been shown to regulate interleukin-4, which is essential for the fusion process of macrophages [73, 74]. FBGCs further attack the material surface by secreting further ROS and MMPs in order to eradicate the foreign body and recruiting other inflammatory cells (Figure 4C). Both macrophages and FBGCs can still be found at the surface of the material years after implantation of non-degradable materials [70].

- DCs and lymphocytes

Dentritic cells (DCs) and lymphocytes also play an important role in the response to implanted materials, in particular those that contain immunogenic biological components [41, 75, 76]. DCs mark the foreign material with antigens that are recognised by lymphocytes for clearage. Both are sensitive and participate to the oxidative stress in the environment. DC's activity has been shown to be up-regulated by oxidative stress [77] while their endogenous ROS act as second messengers in altering their functions during antigen

presentation [78, 79]. Oxidative stress has also been shown to play a role in T cell activation and trafficking [77].

4.4 Oxidative stress during healing and tissue remodelling

As neutrophils, macrophages and other immune cells work on cleaning the implant site of cellular debris and potential sources of infection, the healing phase begins. This phase is characterised by the granulation tissue formed mostly by fibroblasts, which proliferate and produce provisional extracellular matrix (ECM), and endothelial cells, which form blood vessels. Other mesenchymal cells, including stem cells, are involved in the process. As for immune cells, cells that participate in the wound healing process are also influenced by oxidative stress (Figure 4D). Many *in vitro* studies have shown the influence of ROS and LPO on cell attachment, spreading, proliferation, differentiation [36, 80-87]. Redox variations also influence the viability, plasticity and lineage commitment of adult stem cells [55]. Interestingly, several studies have demonstrated that cells such as fibroblasts also actively contribute to the production of ROS [88-90].

Healing of the wound is characterised by a maturation and tissue remodelling phase. During tissue remodelling, mature ECM replaces the provisional ECM while the cell density decreases [91]. Degradation of the ECM by enzymes is an essential step in tissue repair as it enables cell migration and infiltration. Tissue remodelling also involves oxidative stress. Oxidative stress for instance regulates matrix metalloproteinases (MMP) [92]. The breakdown of the ECM by MMPs increases the presence of DAMPs in the microenvironment, which may lead to further macrophage activation and oxidative attack of the implant. Furthermore ROS have been shown to regulate activation of latent TGFβ and to alter activity of Smad protein activity. TGFβ is a pleiotropic fibrotic growth factor that exists in a latent and activated form and is produced by a range of cell types including fibroblasts, mast cells, macrophages and by platelets. It signals through the Smad2/3 signalling pathway and induces expression of a range of genes including those for ECM proteins. It is thus particularly interesting that NOX4 has been found to be necessary for TGFβ-mediated effects on fibrotic gene expression in cardiac and renal fibroblasts [93, 94] [95].

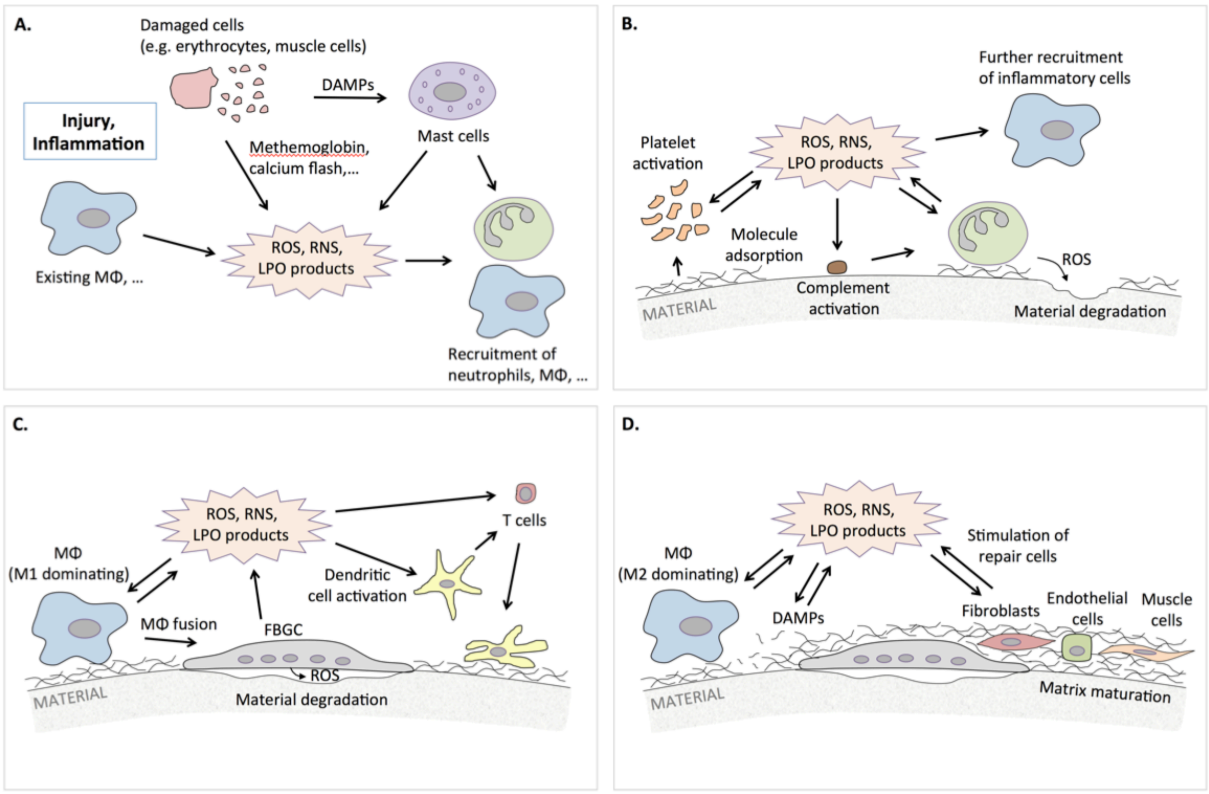

Figure 4: Oxidative stress involved during the inflammation and healing phase in presence of a biomaterial. A. prior material-tissue contacts, B. directly following material implantation, C. during the acute and chronic inflammation, D. during healing and tissue remodelling.

5. Biomaterial design considerations affecting oxidative stress

So far, we have discussed cellular events occurring around a material without discussion of the material properties that may induce and be regulated by oxidative stress. In reality, the material properties both influence and are influenced by oxidative stress, as shown in Figure 5. In particular, the degradation of materials (polymers, metals or ceramics) leads to the formation of products including ions, small molecules and particulate debris of various sizes. These products contribute to the local oxidative stress levels, as summarized in Figure 6. The extent of this contribution depends on their composition, their size, their release profile and their toxicity. Other properties, such as the size and shape of the material, the topography, the wettability and the mechanical properties, also influence the oxidative stress levels significantly. Moreover, each of these properties can be affected in turn by the oxidative stress. This section attempts to describe the main interactions between the material properties and oxidative stress.

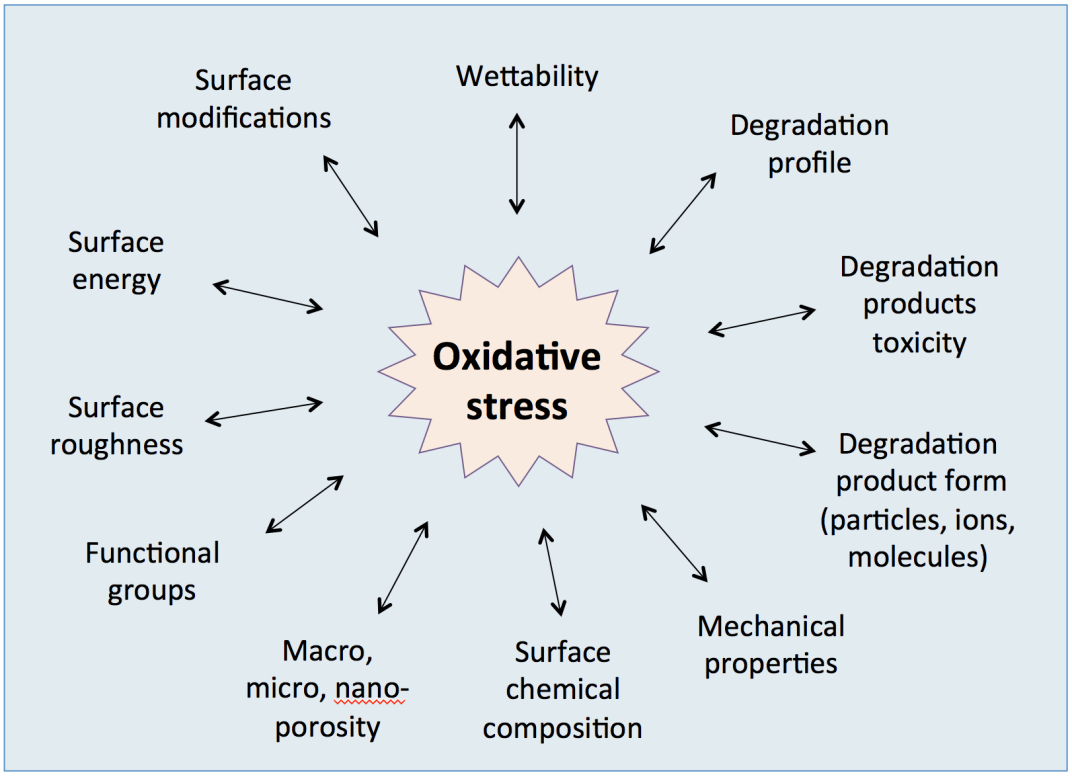

Figure 5: Relations between materials properties and oxidative stress. All materials properties may potentially affect the oxidative stress as well as be affected by it (modified from [65]).

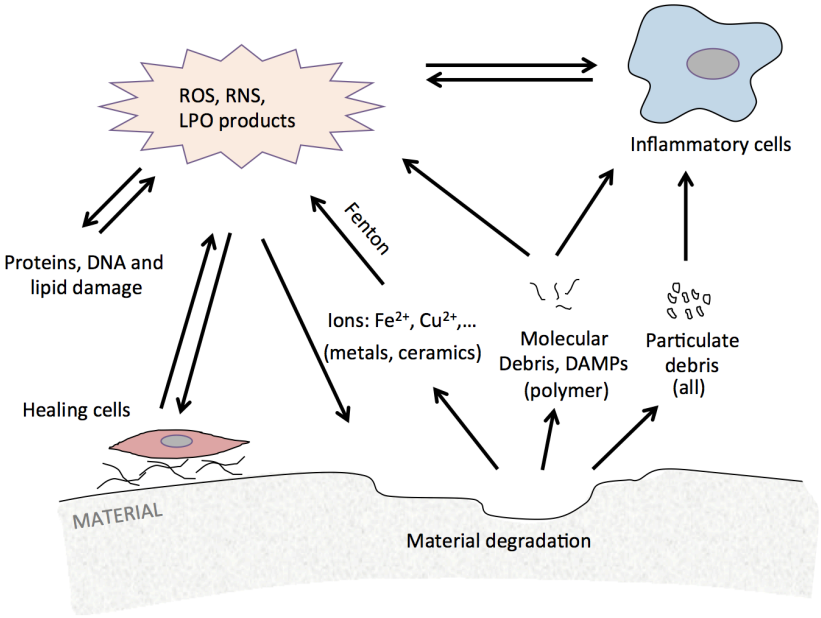

Figure 6: Interactions between the material's degradation products and oxidative stress. Oxidative stress may exacerbate the degradation of implanted materials. Products of degradation include ions (from metals and ceramics, and traces in polymers), molecular debris (from polymers) and particulate debris (from all types of materials). The size of particles typically ranges between 0.1 µm and above for polymers, from 10 to 50 nm for metals and from 0.1 to 10 µm for ceramics. Ions induce the formation of highly reactive hydroxyl radicals through the Fenton reaction with hydrogen peroxide. Molecular debris both directly and indirectly induces the formation of oxidants. Particulate debris is cleared by inflammatory cells such as macrophages and foreign body giant cells, which release oxidants in the process.

1. The effect of material degradation on oxidative stress

    a. Polymer

In the body, degradation of polymers can occur through chemical breakdown of the molecular chains and mechanical wear. Chemical breakdown is undergone by hydrolysis, by enzymatic reactions and by reactions with oxidants. Mechanical wear takes place mostly in load-bearing parts of implants under movement such as in joint implants.

- Synthetic polymers

Synthetic polymers are important and attractive biomaterials because of the ease to tailor their chemical, physical and biological properties for a specific application. The constant oxidative attack by inflammatory cells is one of the main causes of synthetic polymer degradation *in vivo* [51]. It can cause surface oxidation of polymers such as polyethylene (used in artificial joints) or polypropylene (suture material), which may compromise the function of the implant [64]. It can also induce polymer chain cleavage and generate radical species, leading to increased levels of oxidative stress. Radical species include alkyl radical, alkoxy radical, peroxy radical and hydroperoxide [96]. These can further degrade the material through radical propagation but also contribute to damage of surrounding biological tissues. Superoxide anion and hydroxyl radicals have been suggested as the main causes of degradation of biodegradable polyesters [97-99]. They have also been involved in the degradation of poly(ether urethane) materials in pacemakers [64]. As mentioned previously, hydroxyl radicals can be formed from hydrogen peroxide through the Fenton reaction in presence of iron, which might be found in traces in polymers. It has been suggested that polymers might also be oxidised by lipid peroxidation products [100].

The generation of ROS by polymer degradation products has been mainly demonstrated for dental resins such as triethylene glycol dimethacrylate (TEGDMA) and polyhydroxyethyl-methacrylate (HEMA). The release of monomers from the freshly polymerized resins has indeed been a concern for the safety of the materials. The presence of excessive monomers in the biological environment can induce ROS production, glutathione depletion and lipid peroxidation [11, 12]. The resulting oxidative stress has significant effects on signalling

pathways and has been shown to induce cytotoxicity, mutagenicity and apoptosis [101-103]. In the long term, “non degradable” polymers mostly degrade in the form of particles, with dimensions usually above 0.1 μm for polymer such as polyethylene. The generation of ROS by particles was observed for ultra-high-molecular-weight polyethylene (UHMWPE) used in joint implants [104].

Local accumulation of the degradation products is important to avoid given the dose-dependent effect on ROS generation [11]. This is particularly relevant for biodegradable polymers, such as poly(lactic acid), poly(lactide-co-glycide) and poly-ε-caprolactone, since high concentrations of their acidic degradation products can sometimes be found at the implantation site. Little evidence has shown that the pH influence oxidative stress [105], and its effect on the inflammatory response remains unclear [106]. However, a decrease of extracellular pH occurs during inflammation and increases the cellular influx of calcium, which might enhance the onset of oxidative stress [107]. On the other hand, both degradation molecules and fragments made of biodegradable polymers have been shown to stimulate ROS production after internalisation by phagocyte [106, 108]. Moreover, ROS stimulation by degradable materials depends on their degradation profile. A transitory but significant oxidative stress was shown in fibroblasts cultured on PCL films for short culture periods [109]. Nevertheless, after 7 days, cells returned to similar oxidant levels that those observed in the controls. Moreover, neither cell viability nor membrane integrity appeared significantly affected by PCL-induced oxidative stress with respect to control cells at any culture time. In a longer term study, no change has been observed in the levels of LPO or of glutathione at either 3 or 12 months after implantation of PCL materials in rats compared to polyglactin controls [110]. However, PCL materials usually degrade for periods longer than 18 months, meaning that the authors failed to investigate the effect of the final stage of degradation of the material, during which it fragments into small particles. Other studies have shown that PCL nanoparticles stimulate ROS formation after internalisation by phagocyte [108].

- Natural polymers

Natural polymers, such as collagen or elastin, generally show improved biological properties, such as adhesion and proliferation, compared to synthetic materials *in vitro*.

However, they often elicit a strong inflammatory response *in vivo* due to the immune response to the materials, which is likely to cause higher oxidative stress levels. Factors affecting this response include manufacturing processes, the rate of material degradation, and the presence of antigens [111]. ECM scaffolds are typically processed by methods of decellularization and chemical crosslinking to remove or mask antigenic epitopes, DNA, and DAMPs. Inappropriate removal of DAMPs from biological materials may lead to oxidative stress following their release *in vivo* (see Section 4.1). Moreover, the degradation of ECM might also induce the formation of oxidants indirectly as ECM fragments have been shown to promote immune cell recruitment [112, 113]. However, some natural polymers used in scaffolds can degrade into products that act as antioxidants. For example, glycosaminoglycans (such as chondroitin sulfate and hyaluronic acid) were identified as antioxidants capable of reducing free radicals, protecting both cells and materials from ROS damage [114].

b. Metal

The oxidative stress caused by metal materials is generally more accentuated than with polymers [13, 14]. All metallic materials undergo electrochemical corrosion, which releases products of degradation at the implanted site thus causing the formation of ROS and RNS. *In vivo,* corrosion is mainly caused by galvanic effects, which results from redox reactions. The tendency of a metal to corrode depends on it half reaction and on the composition of the synovial or organic fluids it is exposed to [115]. Degradation products can be found in various forms including free metallic ions, colloidal complexes, inorganic metal salts or oxides, organic forms (such as hemosiderin) and wear particles [115]. Because corrosion is continuous through the life of the implant, ROS and RNS continuously form at the implant surface. This can result into prolonged inflammation, unsuccessful healing of the surrounding tissues and aseptic loosening of the implant [116].

Metals commonly employed in implants include Fe, Co, Cr, Ti, Ni alloys. As a result of corrosion, high concentrations of metallic ions are often found at the implantation site, with their natures depending on the composition of the alloy material. Redox reactions create ions on the anodic side and reduction of oxygen on the cathodic side, with ROS (such H_2O_2)

being formed as intermediate products [116]. The released ions, such as Fe^{2+}, Cu^{2+}, Cr^{6+} and Co^{2+} undergo further redox-cycling reactions and may contribute to the formation of hydroxyl radicals through the Fenton reaction upon H_2O_2 exposure, also present in the wound area [36, 116]. For other metals such as Ni, the toxicity mainly happens through depletion of antioxidants such as glutathione and bonding to sulfhydryl groups of proteins. However, a common outcome for all metallic elements is the generation of ROS and RNS [117, 118]. In turn, ROS and RNS cause various modifications to DNA, proteins and enhance lipid peroxidation. Increase levels of lipid peroxidation and decrease in activity of antioxidant enzymes (catalase, SOD, GPX) were observed in the tissue surrounding metallic implants [14].

Metal wear particles, generated mostly by articulating implant interfaces, are smaller than polymeric particles and are typically in the range of 10–50 nm [115, 119]. They are the main cause of aseptic loosening of metal implants, which occurs through macrophage-induced inflammation and oxidative stress[120]. Many studies have shown increased levels of level ROS, RNS, and LPO products in response to metal wear particles [121, 122]. Moreover, wear particles can further contribute to corrosion, as the surface in contact with the surrounding fluids becomes larger. Through mechanical friction, those particles can also disrupt the protective oxide film present at the surface of the implant (passivation layer), further inducing corrosion and ROS. Small debris usually migrate in the tissues surrounding the material, and are phagocytosed by histiocytes [123].

It is important to note that, in the body, the natural corrosion and wear that occurs with metals is not only exacerbated by mechanical stresses but also by the continuous chemical attacks by cells (e.g. macrophages and FGBCs) and by the biological fluids. ROS, which result both from the oxidative burst and from wear and corrosion, are electrochemically active and therefore can induce corrosion themselves. This effect was observed at the surface of CoCrMo alloys and Ti alloys [124, 125]. Moreover, studies have shown that the presence of H_2O_2 can reduce the thickness of the protective oxide films and can raise the oxide potential of the solution to increase the driving force of corrosion [126].

Little is known about the effects of oxidants produced by metal degradation on the surrounding tissues. The mechanism underlying metal toxicity is still not fully understood.

However, because corrosion is continuously occurring, cells at the surface of metallic implants are thought to be constantly exposed to oxidative stress [14]. It has long been known that metals are involved in production of reactive ROS and RNS that may initiate damage DNA [117], eventually leading to carcinogenesis [122].

c. Glass and ceramic

Ceramics are generally used for hard tissue repair such as bone defects, dental fillings and teeth implants. They are also used for total hip replacements and in cements. Oxidative stress plays a key role in the inflammatory reactions caused by ceramic implants. In a study comparing ceramics to metals and polymers, ceramics have surprisingly been shown to induce the largest increase in lipid peroxidation and the largest decrease in antioxidant enzymes in the tissues surrounding the material *in vivo* [13]. However, the study was limited as only one time point was investigated.

The degradation of ceramic happens through wear and dissolution. Wear fragment have various size which can range from 10 nm to 1 mm but on average in the range from 0.1 to 10 µm. There are usually generated where mechanical stresses are important, such as at the articulating surfaces of ceramic-on-ceramic prostheses. Ceramic particles, once internalized, can generate reactive oxygen species (ROS) and increase the oxidative stress [119]. Dissolution products, in particular from bioglasses, might also influence oxidative stress levels. In some cases, it was shown to cause a rise in MDA levels and a reduction in SOD, CAT, GPx activities [127]. This could cause damage to healthy tissues. In other cases, the delivery by dissolution of ions such as Zn^{2+}, Sr^{2+}, Co^{2+} or Cu^{2+} has been used to accelerate bone regeneration [128, 129].

Oxidative stress has been involved in the pathogenesis of bone diseases, such as osteoporosis [130]. However, growing evidence shows that oxidative stress also mediates the process of bone remodelling and that excessive production and accumulation of ROS and LPO products in the bone tissue has a detrimental impact on bone metabolism [129, 131]. For instance, LPO products (in particular HNE) were shown to be involved in cell growth on Cu containing bioglasses [129]. Low concentrations of HNE stimulated cell growth while higher concentrations inhibited growth. The combined use of bioglass dissolution

products, ROS and HNE has recently been proposed as a bone regeneration strategy [131].

d. Composites and tissue engineered constructs

Composites are increasingly used in medical devices in order to improve their performance. Dental resins, for instance, usually consist of a polymer matrix filled with ceramic particles to achieve better mechanical properties. The contributions to oxidative stress from the different components of a composite material therefore depend on their composition, their proportion, their size and their exposure to the physiological environment.

Tissue engineered constructs (or cell-material hybrids) themselves undergo some level of oxidative stress, which results from the cell-material interactions occurring during the tissue growth *in vitro* [132]. Such constructs rarely involve immune cells pre-implantation, so the oxidative stress is likely to remain moderate if the material is relatively inert. Upon implantation however, the immune cells might elicit a host response to the cellular component, which might participate to the local oxidative stress.

2. Other material properties affecting oxidative stress

a. Size and shape of the biomaterial

Size and shape have an important impact on the inflammatory intensity, time duration and wound healing processes. In general, the secretion of oxidants increases with the amount of material and therefore the size of the device [67]. In general, increased surface areas are also leading to higher levels of oxidative stress [133].

The effect of size on oxidative stress becomes particularly evident with particles. It is reported that macrophages are usually capable of phagocytosing particles below 5 μm, while large particles (above 10 μm) induce the formation of FBGCs [134, 135]. Both macrophages and FBGCs produce ROS as an attempt to eliminate the foreign material. However, the most biologically active particles are sub-micrometre in size [104].

Nanoparticles can directly stimulate ROS formation or can otherwise trigger their production through activation or inhibition of enzymatic pathways. Both *in vivo* and *in vitro* studies showed that nanoparticles are closely associated with toxicity by increasing intracellular ROS levels [136]. This was mostly studied for ceramic [137] and metallic nanoparticles [138, 139] although similar effects have been observed with polymeric nanoparticles [108, 140, 141]. In general, nanoparticles with smaller diameter (and thus a larger surface area) produce higher amounts of ROS [142]. Although some aspects are still unclear, our understanding the mechanisms through which nanoparticles induce ROS generation has improved over the last few years and has been reviewed extensively [142-144]. Given their numerous applications in medicine, decreasing the cytotoxicity of nanoparticles has been an important focus in recent years. For instance, this resulted in the development of antioxidant polymer nanoparticles that are able to suppress local oxidative stress levels [145]. However, it is worth mentioning that nanoparticles can also cause harm to cells that is not related to oxidative stress, such as through non specific physical damage to cell membranes [141].

b. Topography

The texture and features at the surface of materials are well-known to influence the attachment of cells and this may affect the oxidative stress levels. In general, rougher surfaces attract more inflammatory cells than smooth surfaces and have a higher percentage of FGBCs [67]. This suggests that rougher surfaces induce higher levels of oxidative stress. This might also be due to the higher surface area in contact with the environment, which might increase degradation rates.

c. Wettability

Both the chemistry and the topography of materials dictate the wettability of materials. The wettability is known to influence the adsorption of biomolecules and cell attachment suggesting that it might have an effect on the local oxidative stress levels. NaOH treatment have for instance been used to induce the appearance of oxygen-containing functional groups at the surface of polymers and decrease ROS formation compared to untreated films

[109]. Moreover, macrophages on hydrophilic and anionic biomaterial surfaces were shown to undergo low integrin-mediated cell attachment and spreading. This may lead to macrophage apoptosis and, as a consequence, lower local oxidative stress levels.

d. Mechanical properties

The mechanical properties of a material might become a significant source of oxidative stress, in particular when these are not matched well with the properties of the host tissue [146]. A mismatch may result in physical damage of the host tissue that can lead to the decay of cells and matrix, which are known to induce oxidative stress (such as through DAMPs).

6. Managing oxidative stress to improve the biocompatibility of biomaterials: current and future directions

The evidence presented in this review suggests that oxidative stress may act as a common language between the material and the surrounding tissues, as they both interact with it. It also supports the idea of developing strategies to manage oxidative stress during biomaterial implantation in order to promote their integration.

Currently, the most common approach to manage oxidative stress is through the use of antioxidants. This is intuitive since antioxidants are released as a natural response to oxidative stress in the body. As mentioned earlier, the antioxidant defence mechanisms include small molecules antioxidants and antioxidant enzymes. Small molecules antioxidants are usually the preferred option, as they are less specific than enzymes and as they are less likely to lose their activity during incorporation in the material. It is relatively straightforward to incorporate these small antioxidant molecules (covalently or otherwise) into polymers for a therapeutic release by diffusion and/or degradation. Researchers have explored the possibility of using a wide range of antioxidant molecules, such as vitamin C, vitamin E, curcumin, trolox, etc. [64]. However, with this approach, it is difficult to provide antioxidant concentrations that are relevant and in particular that respond to variations of

oxidative stress levels. Recent studies have therefore been looking at the development of polymeric biomaterials that are sensitive to oxidative stress. Such materials, which can be used in various forms (e.g. as nanoparticles or as scaffolds), might undergo oxidative degradation and/or release bioactive molecules such as antioxidants in response to the oxidant concentrations [147-151].

Although less frequent, antioxidant enzymes have also been used to reduce oxidative stress during material implantation. For instance, researchers have attached superoxide dismutase mimics to the surface of polyethylene and polyetherurethane implants. The results showed a significant reduction of the fibrotic encapsulation compared to non-modified materials [152]. More recently, a research group demonstrated the potential of a nanocarrier loaded with superoxide dismutase and catalase enzymes to protect endothelial cells from killing by ROS [153].

Another approach to manage oxidative stress consists in modifying the expression of genes coding for antioxidant proteins or ROS-producing enzymes. For example, stimulating the expression of Nrf2, a major transcriptional activator of genes coding for enzymatic antioxidants, was an effective way to modulate the oxidative stress caused by dental resin monomers [154]. On the other hand, the use of degradable polyketal particles loaded with NOX2-siRNA showed a significant inhibition of the NADPH oxidases-2 (NOX2) expression *in vitro* and *in vivo* through RNAi-mediated gene silencing [155]. Some authors have suggested that the inhibition of NADPH oxidases (NOX family) is a better approach for combating oxidative stress compared to using conventional antioxidants [156].

The use of metal chelators is another possible way to decrease oxidative stress. A recent example is the development of nanogels for iron chelation that are able to degrade into small chelating fragments at rates proportional to the level of oxidative stress present [157]. Metal chelators have also been combined with antioxidants in some advanced polymeric biomaterials [158].

Metal compounds can also regulate levels of oxidative stress (ROS in particular) at the implant site. Platinum-ferritin substrates can act in a manner analogous to catalase and

peroxidase in ROS detoxification [159]. Moreover, it is thought that the biocompatibility of titanium is due to its ability to scavenge ROS on its surface during titanium oxide formation *in vivo* [159, 160]. Ceramic and bioglasses are the main biomaterials exploiting metallic elements to lower oxidative stress levels. Zinc for instance, may protect cells from oxidative protein and DNA damage as well as lipid peroxidation and improved the oxidative stress balance. It can directly inhibit H_2O_2 induced apoptosis by activation of the P13K/Akt and MAPK/ERK pathways. Strontium also has the ability to decrease MDA levels and increase the activities of antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase). The protective action against ROS was clearly observed in soft tissue surrounding bioglasses doped with strontium [127]. Other elements added to bioglasses, such as yttrium and cerium, may also have antioxidant effects and reduce the oxidative stress experienced after trauma [161, 162].

Pro-oxidant strategies may also be developed in the near future. Given the multiple roles of oxidants, which are not only pathological markers but also chemo-attractants and signalling molecules, pro-oxidant approaches could be used to regulate oxidative stress levels and stimulate the healing at the site of implantation. For instance, ROS and HNE have recently been proposed as a biomaterial supplement for bone regeneration strategy due they positive impact on cell proliferation and differentiation at low and moderate concentrations [131]. However, the potential of such approach remains to be demonstrated.

Because of the defensive role of oxidative stress against pathogen invasion, it is important to note that suppressing completely the oxidative stress might result in complications such as infections (see Section 2). The appropriate delivery of the therapeutic agents, in relevant concentrations that respond to oxidative stress variations, will therefore be one the main challenges for the future development of these strategies. Approaches must also take the risks of material degradation into better consideration. Finally, further understanding of the relation between the biocompatibility of implantable materials and oxidative stress should help to determine which strategy (or combination of strategies) is most appropriate for a specific application.

7. Conclusions

This review underlines the crucial role of oxidative stress in determining the biocompatibility and the fate of materials following their implantation. ROS, RNS and lipid peroxidation products act as chemo-attractants, signalling molecules and agents of degradation during the inflammation and healing phases. As chemo-attractants and signalling molecules, they contribute to the recruitment and activation of inflammatory and healing cells, which in turn produce more oxidant molecules. As agents of degradation, they contribute to the maturation of the extracellular matrix at the healing site and to the degradation of the implanted material. Interestingly, oxidative stress is itself influenced by the material properties, such as by their composition, their surface properties and their degradation products. Because both cells and materials produce and react with oxidants, oxidative stress may be the most direct route mediating the communication between cells and materials.

While high levels of oxidative stress may cause issues of implant failure and rejection, low levels might lead to infection due to the compromised defence system. Maintaining the oxidative stress to normal physiological levels is therefore crucial to prevent an implant failure and promote its integration. Studies establishing the oxidative stress profiles linked to biomaterials, in particular biodegradable ones, could become useful to guide their uses in clinics and to help regulators accepting new materials. Moreover, a better understanding of the cell-material interactions from an oxidative stress viewpoint may lead to novel biomaterials with improved biocompatibility. In particular for scaffold materials designed to influence surrounding endogenous cells such as for tissue engineering applications, a proper control of the redox balance may be crucial to achieve the desired effects and to prevent adverse events. For this purpose, the development of biomaterials with the ability to respond to oxidative stress variations at the implantation site and to maintain the oxidant concentrations within the physiological range represent a promising strategy for future biomaterial design.

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