

Design of a New 3D Printed Joint Plug

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Abstract

This article introduces a kit of parts as a novel three dimensional (3D) printed joint plug, in which each of the parts function cooperatively to treat cartilage damage in joints of the human body (e.g. hips, wrists, elbow, knee and ankle). Two 3D printed scaffolds are involved in this plug: one of which is a hard scaffold (Bone Portion) to accommodate bone cells, while the other is a 3D printed soft scaffold (Cartilage Portion) overlying the Bone Portion to accommodate chondrocytes. Both scaffolds can have gradient configurations. The third part of joint plug is a permeable membrane, termed Film, to cover the entire plug to provide coordinated sliding of the joint during the regeneration of the cartilage. Retention of the chondrocytes in their proper location is a major challenge in cell therapy of joints; in our plug, the Film is responsible for both this and allowing nutrients to diffuse through the membrane. The kit of the joint plug may further include a fourth part, called the Barrier, which is a membrane with small pore size to assist the Bone Portion in avoiding the loss of chondrocytes from the Cartilage Portion beyond the Barrier. Various engagement means among the parts of the plug are assumed, which are discussed in this paper. Moreover, the detailed design criteria and selection of suitable materials for different parts of the joint plug are elaborated. The 3D printing practice allows the plug to be personalized and fabricated to fit the shape and size of the target joint and the injured section. Also discussed are the different configuration options of the plug to be implanted in a joint, through consideration of seeding the relevant cells in different parts. Although the focus of this paper is on the design, a brief overview of a prototype using specific material and engagement means are presented.

Keywords: Tissue Engineering, 3D Printing, Joint Plug, Soft Scaffold, Hard Scaffold

1. Introduction

Bones of joints are protected by a thin tissue of cartilage (articular cartilage), which permits the easy slide of the joint tissues [1]. Cartilage lacks neural networks; when it is damaged, the joint exhibits pain, as the nerve system of the bony part underlying the cartilage becomes unprotected and exposed [1]. Damaged cartilage is a widespread occurrence among many people, particularly the elderly. In United States alone, there are reports of over five hundred thousand procedures per year associated with such cartilage disorders [2]. Osteochondral deficiencies disturb both articular cartilage and the underlying subchondral bone, with

the possibility of prompting osteoarthritic degenerative alteration by influencing the mechanical instability of the joint, which indicate the importance of its treatment [3-5].

Due to the limited capacity of cartilage self-restoration, several procedures are applied by surgeons for its treatment [6-8]. In an effective method, surgeons remove the damaged cartilage and sections of the bone underneath, followed by an attempt to stimulate the affected area to produce new tissues by cell therapy [6].

For this purpose, the approach of using basic synthetic implants and tissue grafts is changing toward employing degradable tissue engineering scaffolds with appropriate pore size and configuration, which can be seeded by relevant cells or regenerative agents [9-11]. For example, to regenerate cartilaginous tissues, some investigators studied the use of degradable scaffolds made of polyglycolic acid seeded by mammalian chondrocytes [12, 13], and to regenerate bone tissues, studies indicate the use of bioceramics scaffolds seeded by mesenchymal stem cells (MSCs) [14], or poly(lactic-co-glycolic acid) scaffolds seeded by osteoblasts [15].

Although there are various reports of using bone and cartilage scaffolds, osteochondral scaffolds that aid regeneration of both cartilage and bone together are more appropriate for repair of such articular cartilage (and the underlying bone) [16]. For example, it was shown that the repair of osteoarthritis is more effective using tissue engineered osteochondral constructs than cartilage scaffolds [17, 18].

To design appropriate osteochondral scaffolds, the use of biphasic, triphasic and gradient constructs are popular [5, 17, 19-21]. More specifically, two or more layers of different materials and configurations are assumed for a good osteochondral construct to accommodate both chondrocytes and bone cells. The transitional area from the cartilage to the bony area is usually designed in a way to prevent construct delamination [22, 23]. While usually two or three different compositions and configurations are employed for different layers in biphasic and triphasic osteochondral scaffolds, such constructs can also be made by a single composition, in which the physical properties of different layers are considerably different [20].

During the last decades, many different designs have been suggested for use on osteochondral defects, including monophasic and multiphasic scaffolds [20, 24-28]. Some recent review articles have extensively elaborated the capabilities of different multicomponent constructs to be used for tissue engineering of osteochondral defects and discussed their challenges for clinical applications [24-27]. Fundamentally, a good design must be able to provide a suitable environment for attachment and growth of the relevant cells, bearing in mind that the porosity, pore size, interconnectivity and geometry are extremely important in such an environment [29].

One of the most recent models—which is one of the most similar to the design we are introducing in this paper—is a tri-layered plug in a shape of a simple cylinder with a diameter and height of a few millimeters [30]. While the top layer of the cylinder is made of a nonwoven poly(glycolic acid) (PGA) felt, the mid layer is composed of poly(l-lactic acid) (PLLA) hybrid with a colorant, and the bottom-most layer is made of a porous poly(l-lactic acid)/poly(ϵ -caprolactone) (PLLA/PCL) structure coated with collagen Type I and calcium phosphate microparticles [30]. This tri-layered plug is produced by molding/salt-leaching technique and implanted *in vivo* without any cell seeding [30]. However, it has been demonstrated that molding/salt-leaching method cannot provide a good control over the pore size, porosity and interconnectivity of the produced constructs [31], which are important factors in osteochondral scaffolds [29]. Therefore, we introduce a design of a joint plug using 3D printing method, which is an advanced

technique that can create fully customized constructs with detailed control over the pore size, porosity, geometry, gradient configurations and interconnectivity of pores [32].

More specifically, in this study, we have designed a new 3D printed joint plug as a multi-part system with cooperative function to provide suitable support for accommodating the relevant cells in the area of the damaged joint during the cell therapy. The parts, as shown in Figure 1, include two 3D printed scaffolds and one permeable membrane, which are called Bone Portion, Cartilage Portion and Film, respectively. The fourth optional part, the Barrier, may also be included in the plug to prevent the fall of cells from Cartilage Portion deep into the Bone Portion, while keeping the cells of two Portions in contact (Figure 4). This paper will describe the design criteria and materials that can be used for each part. Although the paper is mostly focused on the design and its capabilities and possible configurations as a joint plug, a brief outline of a prototype made with specific materials is presented to show the function of the plug in accommodating chondrocytes and osteoblasts.

2. Materials and Methods

Sodium Hyaluronate (Research Grade, 500-749 KDa) was purchased from Lifecore Biomedical. Alfa Aesar (USA) was the vendor of carboxymethyl cellulose (CMC), sodium tripolyphosphate (TPP), 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-Hydroxysuccinimide (NHS). Polycaprolactone (PCL) (Mn 80000), β -tricalcium phosphate (TCP), polyethylene glycol (PEG, Mn 2000), gelatin (Type A, from porcine skin) and hydroxyapatite (HA) were obtained from Sigma (USA). Elastin Products Company, Inc. provided the elastin (Elastin-Soluble, No. ES12) with the molecular weight of 60 KDa.

To prepare the paste for 3D printing of the Bone Portion, 12 g TCP, 3 g HA, 0.5 g TPP, 0.075 g CMC and 5.75 ml water were homogenized in a centrifugal mixer (Thinky, USA) at 2000 rpm for 2 min. A solution of 8% w/v gelatin, 2% w/v elastin and 0.5% w/v sodium-hyaluronate was used as the ink to 3D print the Cartilage Portion.

Both scaffolds were 3D printed using a 3D-Bioplotter® system (EnvisionTEC, Germany). The optimized parameters for printing the bone scaffold were pressure of 2 bar, nozzle speed of 4 mm/s, and cartridge and platform temperature of 22 °C. These parameters for printing the cartilage scaffold were pressure of 2 bar, nozzle speed of 15 mm/s, cartridge temperature of 31 °C and platform temperature of 12 °C. The bone scaffold was sintered by keeping it at 600°C for 2 hours (h) to remove the organic additives, and then increasing the temperature to 1100 °C for 4 h.

To make the Film, 1 g PCL, 15 ml 2,2,2-Trifluoroethanol and 1.5 g PEG 2000g/mol were dissolved and then casted. After overnight evaporation of the solvent, the membrane was then waterlogged to eliminate/sacrifice the PEG component. For surface modification of the Film, one side of the membrane was floated on 10% w/v NaOH for at least 8 h. Residual NaOH was washed away using water.

Aminolysis treatment can be used to adhere the PCL Film to the Cartilage Portion. To do the aminolysis treatment of the PCL Film, the Film must be washed with isopropyl alcohol and immersed in 10% (w/w) 1,6-hexanediamine in isopropyl alcohol at 35 °C for 3 h, rinsed with copious amounts of deionized (DI) water followed by drying in a vacuum oven at 30 °C overnight, and then immersed in 2.5 wt% of glutaraldehyde/PBS solution for 24 h at room temperature. Finally, the free glutaraldehyde must be removed by rising with water [33]. Scanning electron microscopy (SEM) of the Film was performed using a JEOL JSM6510 system (Japan) at the accelerating voltage of 3kV after sputter-coating with gold.

Human osteoblasts (HOB, Cell Applications, USA) and normal human chondrocytes (Cell Applications Inc, USA) were cultured under standard aseptic conditions. 2500 cells/mm² and 750 cells/mm² were used to seed scaffolds of Bone and Cartilage Portions, respectively.

At desired time intervals, the cell culture media was substituted with 10% v/v prestobblue reagent (Life Technologies, USA) in phenol red free DMEM following by 1.5 h incubation at 37°C and 5% CO₂.

3. Result and Discussion

As shown in Figure 1, the design is a kit of three parts that form a joint plug as an implant for insertion within an opening of a bone layer in a joint and through a cartilage layer. Part 1 is the Bone Portion and composed of a 3D printed bone scaffold. Part 2, the Cartilage Portion, is a 3D printed soft thick membrane (1-3 mm thick), and part 3 is a thin permeable porous membrane, termed Film (0.3-0.7 mm thick).

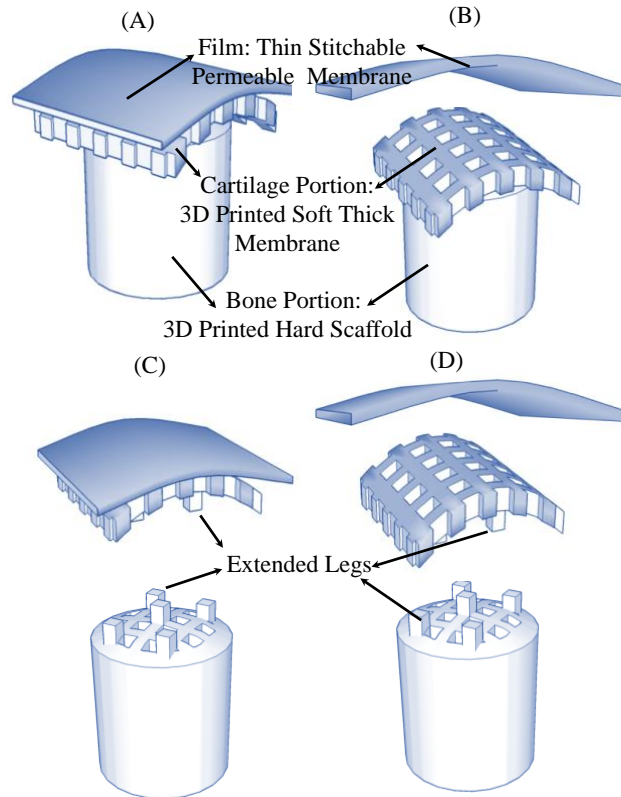


Figure 1: Different designs of the plug that can be used based on the surgery needs: (A) Fully assembled, (B) Adhered Cartilage and Bone Portions, (C) Adhered Film and Cartilage Portion, (D) Unattached form.

The joint plug can be implanted in the body as a fully assembled joint plug in order to simplify the surgery (Figure 1 A). In this case, the desired cells should be seeded into the plug prior to implantation in the body. Other options allow some portions of the plug to be engaged separately. For example, the plug can be implanted in the body while Bone and Cartilage Portions (Figure 1B) or the Film and the Cartilage Portion (Figure 1C) are already attached. In this case, the third part can be engaged to the plug during the

surgery. In another scenario, the components of the kit can be separately implanted, then engaged with each other inside the body (Figure 1D). In such cases, the surgeon has the option of seeding some cells to the accessible parts of the plug during the surgery.

3.1. Part 1: Bone Portion

The Bone Portion of our design is a 3D printed hard scaffold made of an appropriate biodegradable material with the size of the removed bone from the patient's specific joint. During the treatment of a joint, a zone of the joint is predictably removed by a surgeon to make an opening in the bone section. 3D printing technique enables manufacturing of controlled and customizable structures to fit the desired shape and size of the opening in the bone section of an individual joint in which the plug is going to be inserted. Thus, manufacturing of the Bone Portion of the joint plug can be personalized, which allows for easier handling and more efficient treatment of the injured joint.

The materials selected for the scaffold of the Bone Portion must be able to accommodate osteoblasts. Ceramic or composite materials are good options for this purpose. Some examples include bioactive glasses, various calcium phosphates, aluminum oxide, calcium oxide, zirconium oxide, HA, composites of polymers (such as PCL) and calcium phosphates. We have suggested that a combination of TCP and HA (TCP/HA 80:20 (w/w)) is the most suitable material to fabricate the Bone Portion (Figure 2A,B). Figure 2C shows the attachment of osteoblasts in our bone scaffold.

3.2. Part 2: Cartilage Portion

The plug further includes a 3D printed soft scaffold for accommodating chondrocytes. This middle part, the Cartilage Portion, is configured in a way to be positioned over the Bone Portion. The materials of this part must be a biodegradable biocompatible material, preferably hydrogels, which can accommodate chondrocytes. Examples of the materials that can be used for this purpose include collagen, hyaluronic acid, gelatin, alginate, elastin, poly(amino acids), elastin-like peptides, albumin and fibrin. An example of the Cartilage Portion is demonstrated by the use of a composition of gelatin/elastin/sodium-hyaluronate (8:2:0.5) (Figure 2A,B). As seen in Figure 2D, chondrocytes can robustly attach to this composition. The soft porous scaffold of gelatin/elastin/sodium-hyaluronate not only facilitates the cartilage growth within the cartilage layer but also retains the chondrocytes within the layer by their attachments, enhancing cell differentiation between the bone and cartilage portions.

3.3. Engagement Means Between the Bone and Cartilage Portions

There are engagement means to mount the Cartilage Portion on the Bone Portion. The engagement means can be in the form of several protrusions or legs/knobs extending from either the bone or cartilage 3D printed scaffolds, or from both of them (Figure 1A-D). This can confine the location of the Bone Portion relative to the Cartilage Portion. Engagement between the Cartilage and Bone Portions can also allow the two portions to be coupled prior to implantation into the joint to simplify the surgical process (Figure 1A, B). In the schematic view of the design shown in Figure 1A-D, legs are fabricated in the Cartilage and Bone Portions to mechanically interlock them together. Another means of engagements is suturing/stitching between the two portions.

3.4. Part 3: Film

The third component of the plug is a permeable membrane (Film) configured to be placed over the Cartilage Portion. It is located on top of the plug to retain chondrocytes within the Cartilage Portion, while

allowing nutrients to diffuse through the Film into the Cartilage Portion to facilitate cell propagation and growth. The Film also eases sliding of the joint during the regeneration and repair procedure of the cartilage tissue. While covering the cartilage portion, the Film may also cover an area of the surrounding joint tissue.

The Film can be composed of hydrogels, biodegradable polymers or a composite material. The material should be selected such that the Film can be attached to the surrounding joint tissue and the Cartilage Portion by suturing/stitching. The pore size of the Film should be less than $15\text{ }\mu\text{m}$, so the route of cells from the Cartilage Portion out of the joint plug through the Film can be considerably reduced or prevented. In our prototype, we created the Film using PCL with the pore of size of $10\text{ }\mu\text{m}$ using a combination of film casting and sacrificial material leaching methods [34]. The sacrificial material in our case was PEG and the PCL/PEG w/w ratio was 3:2 (Figure 2A,B,E).

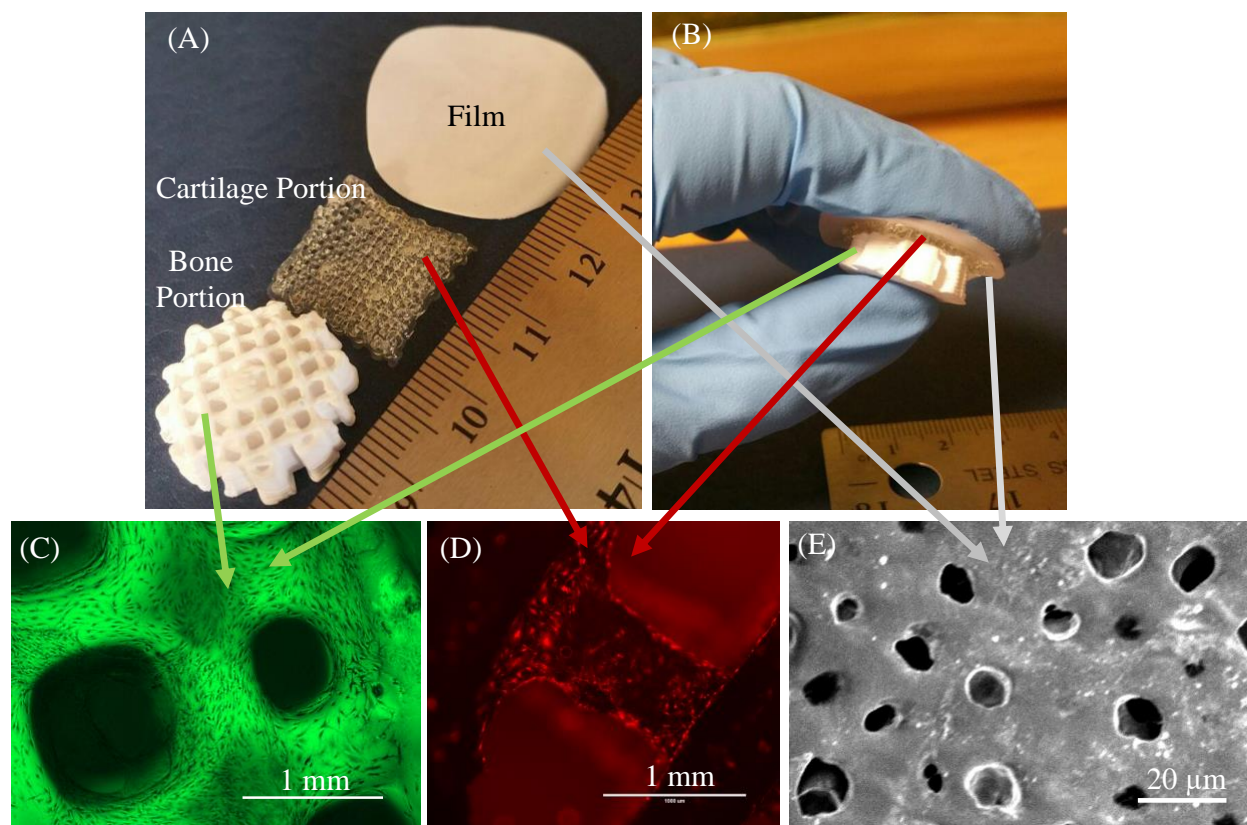


Figure 2: (A) Photographs of the Bone Portion made of TCP/HA (80:20), Cartilage Portion made of gelatin/elastin/sodium-hyaluronate (8:2:0.5) and Film made of PCL and sacrificial material of PEG (PCL/PEG 3:2), (B) Assembled plug with three parts of Film, Cartilage and Bone Portions (C) Fluorescence image of Bone Portion of the plug with attached human osteoblasts, (D) Fluorescent image of the Cartilage Portion with attached normal human chondrocytes, (E) SEM image of the PCL Film.

3.5. Engagement Means Between Film and Cartilage Portion

Mounting the Film on the Cartilage Portion secures it in place and prevents the migration of the chondrocytes. Retention of the chondrocyte within the Cartilage Portion is an important function of the

Film. Such engagement may occur before implantation of the plug into the body (Figure 1C or 1A), as coupling of Cartilage Portion and Film can simplify the surgery. Alternatively, it can occur after insertion of the components in the joint (Figure 1B or 1D).

The engagement means can be in the form of suturing/stitching between the Film and Cartilage Portion (Figure 3A), and/or can occur through surface modification of the film to provide specific affinity to the Cartilage Portion. Here, we explain two examples of such modifications:

1) *Aminolysis Treatment*: Aminolysis method is an effective method to create amine functional groups on the surface of polyesters where other molecules can be attached. For our case of PCL Film and the scaffold of gelatin/elastin/sodium-hyaluronate of Cartilage Portion, when PCL surface is undergoing the aminolysis treatment, the created anchored -NH_2 groups on PCL molecules can react with the -NH_2 groups of the gelatin/elastin/sodium-hyaluronate using aldehyde coupling to achieve the strong bonding between PCL Film and Cartilage Portion (Figure 3B) [35-37].

Since this method involves a chemical reaction between the Cartilage Portion and the Film, it must be used before seeding the chondrocytes in the Cartilage Portion, and is thus appropriate when the Film and Cartilage Portion are attached prior to the implantation in the joint (Figure 1A,C).

2) *Enhancing the Wettability of One Side of the Film*: Cell adhesion is highly influenced by wettability [38, 39]. Creating a Film with hydrophilic/hydrophobic sides can be an effective technique to engage the Film and Cartilage Portion (from the hydrophilic side), while also preventing the chondrocyte from leaving the plug (from the hydrophobic side) (Figure 3C).

For example, in the case of the PCL Film, treating one side of the Film with NaOH can significantly increase the wettability of the side facing the Cartilage Portion to facilitate the engagement between the Film and this portion, while keeping the hydrophobicity of the other side to help retain the cells within the plug. More specifically, in our method, treating one side of the film with NaOH decreases the contact angle from $76.7 \pm 3.3^\circ$ to $36.4 \pm 3.7^\circ$.

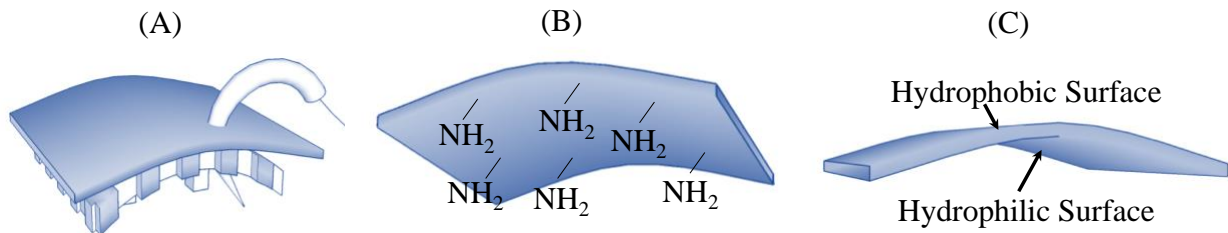


Figure 3: Various engagement means that can be used between the Film and Cartilage Portion: (A) Mechanical engagements, such as suturing/stitching of the two parts, which can be used when the materials of the Film and Cartilage Portion are suturable. (B) Aminolysis treatment of the Film, which usually can be used when the Film is made of some polymers (such as PCL) in which aminolysis can create amine functional groups on the surface of polyesters, which can react with the NH_2 groups of the Cartilage Portion (which is made of hydrogel materials) via aldehyde coupling. (C) Enhancing the wettability of one side of the Film in a way to have the hydrophilic surface facing the Cartilage Portion (to enhance the cell

attachment), while the hydrophobic surface faces outside of the plug (to prevent departure of cells). In our case (PCL Film), NaOH treatment could make such hydrophilic/hydrophobic surfaces.

3.5. Gradient Configuration

3D printing offers controllability of the pore sizes within the Bone and Cartilage Portions, which is important for the growth of tissues in different kinds of joints. A gradient in the pore sizes within the Bone and/or Cartilage Portion is also possible using the 3D printing approach. Generally, a gradient pore size in scaffolds can increase the cell seeding efficiency compared to homogenous scaffolds [40]. Pore size in the scaffolds plays direct role in cell binding, migration and ingrowth, ultimately affecting tissue regeneration. Large pores aid in effective nutrient delivery, gas diffusion and metabolic waste removal, while also offering low cell attachment and intercellular signalling [41-43]. Small pores, on the other hand, allow neither adequate nutrient supply delivery/gas diffusion, nor metabolic waste removal, yet significantly facilitate the cell attachment and intercellular signalling [41-43]. Thus, having a gradient structure allows acquisition of benefits from both small and large pore sizes.

For example, as shown in Figure 4A, the size of the pores on top of the Bone Portion (where it is nearest to the Cartilage Portion) may be small ($\sim 10\text{-}50\text{ }\mu\text{m}$). This not only facilitates cell attachment and intercellular signalling, but also helps preventing the chondrocytes from potential drop into the Bone Portion. The pore size may grow to $500\text{-}900\text{ }\mu\text{m}$ in the bottom of the scaffold in a gradient manner (Figure 4A). Since the bottom of the scaffold is close to the surrounding bone, the cells from the adjacent tissue can have smooth ingrowth into the Bone Portion by taking advantage of successful nutrient delivery and waste removal provided by the large pores. After migration of the cells, they can easily attach to the upper areas of the scaffold with small pore size, which results in effective regeneration of the Bone Portion.

Gradient structure for the Cartilage Portion may also start from large pore size (e.g. $500\text{-}900\text{ }\mu\text{m}$) on top, so the chondrocytes can easily migrate when they are seeded into the Cartilage Portion from the top. As shown in Figure 4A, the gradient configuration continues to the bottom of the Cartilage Portion with the small pore size (e.g. $10\text{-}50\text{ }\mu\text{m}$) so the cells can firmly attach to the scaffold. Having a gradient structure for Bone and Cartilage Portions is not essential in our design, but can be considered as an option to improve the cell seeding efficiency.

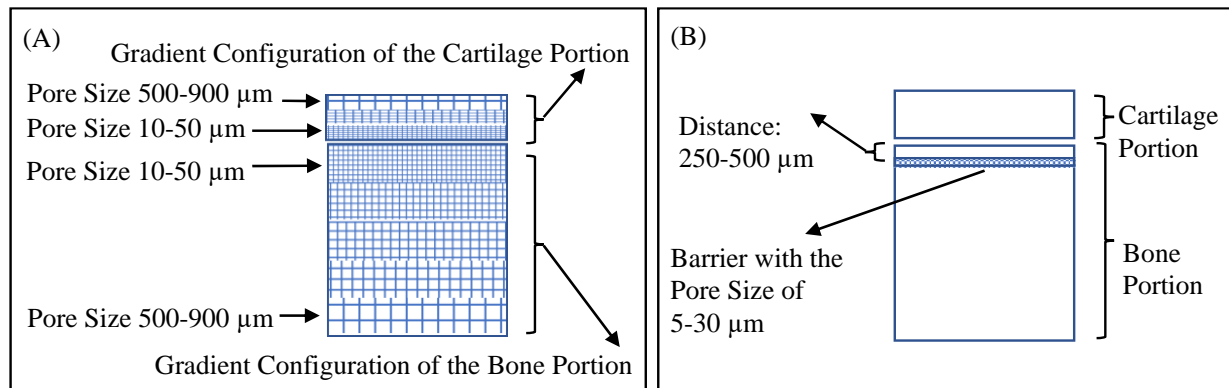


Figure 4: (A) The Cartilage and/or the Bone Portion of the plug may have gradient configuration to enhance the cell seeding efficiency and to prevent the chondrocytes from dropping deep into the Bone

Portion, all while maintaining the contact between chondrocytes and osteoblasts. The suggested pore sizes in different areas of the Bone and Cartilage Portions are indicated in this schematic. (B) The Barrier is an optional part of the plug to substantially avoid fall of chondrocytes to the Bone Portion. A distance (250-500 μm) between the Barrier and the bottom of the Cartilage Portion is necessary to provide a space for communication of osteoblasts and chondrocytes to modulate their phenotypic reactions. Barrier and gradient configuration can be used together in a design, as well.

3.6. Optional Part: Barrier

The kit of the joint plug may further include a Barrier, which is provided in the Bone Portion to substantially prevent drop of chondrocytes from the Cartilage Portion beyond the Barrier (Figure 4B). As described above, the gradient configuration of the Bone/Cartilage Portions can prevent the chondrocytes from a potentially deep fall into the Bone Portion, while maintaining contact between the chondrocytes and osteoblasts. Using a Barrier—a layer with small pore size—in the Bone Portion can provide a similar role but more precisely. It is important to note that communication between osteoblasts and chondrocytes is helpful in regenerating the osteochondral interface on tissue engineered grafts [44]. Basically, investigations show that, under physiological conditions, chondrocytes and osteoblasts may regulate each other's phenotypic reactions and cellular differentiations [44]. Thus, the Barrier must not be designed in a way to block the interaction between the cells in the Bone and Cartilage Portions. More specifically, it should not be placed exactly at the interface of the Bone and Cartilage Portions, because different cell types must be able to still interact with each other. Somewhere inside the Bone Portion and close to the top is a suitable location. As shown in Figure 4B, the suggested site of the Barrier is 250-500 μm below the interface between the Cartilage and Bone Portions. This 250-500 μm distance can provide the space for possible interaction of bone and cartilage cells to modulate their cellular differentiations.

The Barrier can be integrated with the bone scaffold (integral configuration) or can be a separate part (separate configuration). In the integral configuration, it may be a layer of the 3D printed construct of the Bone Portion with the same material composition of the bone scaffold (e.g. TCP/HA). This layer should be 3D printed in a way to have very small pores to avoid easy passage of the chondrocyte beyond the Barrier. In the separate configuration, it can be made of a material different from the material of the Bone Portion (e.g. gelatin/elastin/sodium-hyaluronate or PCL membrane). In this case, the production of the Bone Portion can be divided into 2 steps: 3D printing of the part above the Barrier and 3D printing of the part below the Barrier. After obtaining these three sections (Bone Portion above the Barrier, the Barrier and Bone Portion below the Barrier), they should be engaged by a physical engagement means, such as stitching or a chemical engagement means, depending on their material types. Alternatively, these three sections can be 3D printed together by using a different ink for the Barrier.

If the plug is planning to be used in a way that the autologous cells are seeded to the Bone Portion before the surgery, the pore size of the Barrier can be designed very small (e.g. 5-15 μm), considering that the cells must be seeded to both sides of Bone Portion. However, if seeding the cells to the both sides is not possible, or the plug is going to be inserted into the joint without seeded cells in the Bone Portion, the pore size of the Barrier should be larger (e.g., 15-30), so it does not completely block the migration of osteoblasts

from the bottom of the bone scaffold to the top (does not leave the 250-500 μm with no bone cells). This larger pore size can be considered as a cautionary arrangement for our design, as osteoblasts may always migrate into the Bone Portion from the adjacent bone tissues to the sides of the scaffold.

4. Conclusion

This paper introduces a newly designed joint plug composed of three/four parts: 1) Bone Portion, 2) Cartilage Portion, 3) Film and 4) Barrier (optional). Various aspects of this design were discussed in this manuscript, including the configuration options for surgery and implantation in the body, suggested materials for each part, the cooperative function of the components as a joint plug and options of engagement means between different parts. This paper presents a quick overview of a prototype made as an example of this joint plug.

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Author Contributions statement:

Lobat Tayebi contributes in the design of the work, the acquisition, analysis, interpretation of data and writing of the manuscript.

Zhanfeng Cui contributes in design of the work and interpretation of data

Hua Ye contributes in design of the work, interpretation of data and writing of the manuscript.

All authors have approved the submitted version and have agreed both to be personally accountable for the author's own contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and the resolution documented in the literature.

Competing interests

The authors declare no competing interests

Data availability:

Materials, data and associated protocols are available upon request.

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