Current Strategies in Multiphasic Scaffold Design for Osteochondral Tissue Engineering: A Review

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Abstract

The repair of osteochondral defects requires a tissue engineering approach that aims at mimicking the physiological properties and structure of two different tissues (cartilage and bone) using specifically-designed scaffold-cell constructs. Biphasic and triphasic approaches utilize two or three different architectures, materials, or composites to produce a multilayered construct. This paper gives an overview of some of the current strategies in multiphasic/gradient-based scaffold architectures and compositions for tissue engineering of osteochondral defects. In addition, the application of finite element analysis (FEA) in scaffold design and simulation of in vitro and in vivo cell growth outcomes has been briefly covered. FEA-based approaches can potentially be coupled with computer-assisted fabrication systems for controlled deposition and additive manufacturing (AM) of the simulated patterns. Finally, a summary of the existing challenges associated with the repair of osteochondral defects as well as some recommendations for future directions have been brought up in the concluding section of this paper.

Introduction

Partial- and full-thickness cartilage lesions of the knee are common disorders affecting people of all ages. More than 500,000 procedures related to cartilage injury are performed each year in the US alone.[1] Self-repair of hyaline cartilage is limited and the tissue that forms is usually a combination of hyaline and fibrocartilage, which does not perform as well as hyaline cartilage and can degrade over time.[2–6] Current clinical strategies to repair cartilage include autologous chondrocyte implantation, microfracture and mosaicplasty. However, there is still uncertainty about the quality of the repaired tissue and its ability to restore long-term function.[7]

Osteochondral defects affect articular cartilage as well as the underlying subchondral bone. These defects are often associated with mechanical instability of the joint, and therefore with the risk of inducing osteoarthritic degenerative changes.[8–10] Hence the treatment of chondral and osteochondral lesions is of great interest to orthopedic surgeons. In addition to osteoarthritis (OA), there is an urgent need for more efficient treatment of focal osteochondral injuries arising from sports.[11] A paradigm shift is taking place in orthopedic surgery from using synthetic implants and tissue grafts to a tissue engineering approach, which makes use of biodegradable scaffolds combined with biological molecules or cells to regenerate tissues.[12,13] Tissue-engineering requires scaffolds that balance temporary mechanical function with architectural properties (pore shape, size and interconnectivity) to aid in biological delivery and tissue regeneration.[12,14] In recent years, osteochondral tissue engineering has been the subject of considerable investigation.[15–19] The repair of osteochondral defects requires a tissue engineering approach that aims at mimicking the physiological properties and structure of two different tissues (cartilage and bone) using specifically-designed scaffold-cell constructs. When mono-phasic scaffolds are used, the natural environment is not imitated well for new tissue formation. For such purpose, multiphasic and gradient-based scaffolds have been proposed.[10,11,18,20,21]

In vivo studies have shown that the outcome of repairing articular cartilage defects with tissue-engineered osteochondral composites is better than that of tissue-engineered cartilage.[21,22] For example, an osteochondral composite could be securely implanted by press-fitting into defect without additional fixation.[22] In order to construct tissues with different cell types and/or gradients in mechanical properties, a successful osteochondral scaffold should ideally have two or more regions with different compositions and/or microstructures, including pore size and porosity. An intermediate region between the cartilage- and bone-scaffolds would allow for smooth transition to avoid scaffold delamination while facilitating stress transfer.[23,24]

This paper presents an overview of the most recent multiphasic/gradient-based scaffold architectures and compositions for the repair of osteochondral defects, with a focus on studies published since 2009. First, a brief introductory overview of the current clinical strategies for the treatment of osteochondral defects has been presented. The main body of the paper covers the most commonly used scaffold materials, growth factors and cell types in bone and cartilage tissue engineering, as well as an up-to-date review of the current osteochondral tissue engineering approaches using natural/synthetic gradient-based scaffolds and biological gradients. The application of finite element analysis (FEA) in scaffold design and the simulation of in vitro and in vivo cell growth have been briefly covered. A summary of existing challenges associated with repairing osteochondral defects and some recommendations for future directions have been brought up in the concluding section of this paper.

Clinical strategies for the treatment of osteochondral defects

In articulating joints, the osteochondral interface is the junction between the articular cartilage and the underlying bone. In a review paper, Heinegård and Saxne have discussed the role of the cartilage matrix in osteoarthritis.[25] Figure 1A shows a healthy joint with normal articular cartilage composed of four distinct layers, in which the lower-most layer is in direct contact with the subchondral bone.[25]

The articular cartilage is organized into pericellular, territorial and interterritorial matrices, each located at a specific distance from the chondrocytes (Figure 1A, inset image).[25] Figure 1B depicts an osteoarthritic joint showing cartilage destruction, thicker subchondral bone, and decreased trabecular volume. The cartilage compartments are altered even at early stages of disease and exhibit cloning and multiplication of cells.[25] The change in the cartilage compartments can be verified through immunohistochemistry staining (Figure 1B, inset image, arrow heads).[25] Partial loss of cartilage and alterations in the underlying bone often lead to discomfort, chronic pain, and a reduction of joint movement.[25,26]

Degradation of articular cartilage can also result from traumatic or sport injuries and other inflammatory joint conditions.[27] Surgical treatments have been extensively studied for osteochondral defects.[28–30] The goal of surgical treatments is to prevent further cartilage deterioration and improve joint articulation by restoring the joint surface to as close to its original condition as possible.[29] Some of these treatments are illustrated in Figure 1C-1E.[27] The process of autologous osteochondral transplantation involves the removal and transfer of osteochondral plugs from non–weight-bearing areas to the osteochondral defect (Figure 1C).[27] Besides the limitations caused by donor site availability and morbidity, the space between cylindrical grafts may affect the quality of the repair and lead to poor integration of full thickness gaps.[29–31] Autologous chondrocyte implantation (ACI) has provided a new alternative for the treatment of symptomatic osteochondral defects in young patients.[32] In this method, cartilage is taken from a low-contact area, and then chondrocytes are harvested and cultured in vitro (Figure 1D).[27] Injection of the chondrocytes into the defective area aids cellular adhesion and fills in the defect.[29,33] Theoretically, ACI should produce hyaline-like cartilage rather than fibrocartilage, with subsequent improvement in clinical outcomes.[29]

Marrow-stimulating techniques such as microfracture, drilling, and abrasion arthroplasty (debridement) have also been used for the treatment of osteochondral lesions. These techniques involve penetrating the articular cartilage down to subchondral bone, which allows marrow stromal

cells, platelets and other factors to aid in the repair process (Figure 1E).[27] Bleeding from the subchondral bone promoted by these techniques creates vascular communications to the bone marrow from which pluripotent mesenchymal stem cells (MSCs) are released. The advantages of microfracture over debridement and drilling are the preservation of the subchondral plate and avoiding thermal injury, respectively.[29] In general, cartilage surrounding symptomatic lesions is fibrillated and non-functional. Surgical debridement involves the removal of loose and unstable cartilage to promote the formation of new tissue from the bony base of the debrided lesion.[27,32] In clinical studies, a symptomatic improvement in approximately 50% of the treated patients, with therapeutic effects lasting for about 1 year, has been reported for debridement.[27,34] Therefore, the lack of permanent long-term solution is one of the major limitations of this technique.

A comparison between individuals treated with ACI and microfracture (121 patients at 5 years) has shown that those with onset of symptoms of less than 3 years had better outcomes with chondrocyte implantation than microfracture,[35] although functional outcomes were similar at 12 months and 18 months.[36] A similar process, known as matrix-induced ACI (M-ACI), involves implantation of chondrocytes previously expanded in vitro under special culture conditions into a collagen matrix. Matrix and cells are subsequently fixed in place by fibrin glue and/or sutures.[27] The technique has been reported to be a safe and clinically effective procedure leading to the formation of hyaline cartilage, although it requires a two-stage surgery and is cost-intensive.[37]

Bone and cartilage tissue engineering

Tissue engineering applies the knowledge of biology, cell transplantation, materials science, and bioengineering to construct biological substitutes that can restore and maintain normal function in diseased or injured tissues.[38–40] In this strategy, a biodegradable three-dimensional (3D) porous scaffold is often used as a matrix to support cell adhesion, to guide new tissue formation, and to restore organ function. Tissue engineering is a potential alternative for the treatment of osteochondral

defects, as it can be effectively used to regenerate cartilage, bone and the cartilage-bone interface.[41] Natural and synthetic polymeric biomaterials have been widely used for cartilage tissue engineering. It is well known that cell function on a scaffold is related to the chemical properties of the scaffold material, as the scaffold surface chemistry affects cell adhesion, morphology and activity.[42] Incorporation of calcium phosphate ceramics, e.g., hydroxyapatite (HA), into polymeric biomaterials can result in matrices with improved mechanical strength and better osteoconductivity for bone tissue engineering.[43–46] This section covers the various elements involved in scaffold-based bone and cartilage tissue engineering, including scaffold materials, growth factors and cell types. The following section gives an overview of multiphasic scaffold design and biological gradients considered in recent studies for osteochondral tissue engineering.

Scaffold materials

Biomaterials used in tissue engineering can be categorized into four major groups: natural polymers, synthetic polymers, metallic materials, and inorganic materials such as ceramics and bioactive glasses. Based on the need, multicomponent systems are designed to generate composites of enhanced performance.[47,48] Polymers are indispensable in present tissue engineering concepts. Natural polymers like glycosaminoglycan, collagen, starch, hyaluronic acid, chitosan, alginate, and biodegradable bacterial plastics such as poly(hydroxyalkanoates) (PHA) are excellent biomaterials that support cell adhesion and regeneration while offering biocompatibility. One of the major constraints of natural polymers is that their mechanical properties are weaker when compared to ceramics and metallic materials.[49]

Natural polymers can be easily surface modified with RGD groups containing specific molecular recognition sites in the bulk of the polymer chain, which can support and enhance various cellular activities, including adhesion, cell-cell communication, and proliferation.[50] For example, doping of gelatin in alginate scaffolds has been used in bone and cartilage tissue engineering. Ca-

alginate scaffolds cross-linked with gelatin have been shown to enhance cell adhesion and proliferation of mesenchymal stem cells (MSCs), while promoting the differentiation of MSCs into osteogenic and chondrogenic cell lineages.[51] Chitosan is another widely studied natural biomaterial for cartilage regeneration.[52–54] Its chemical structure is similar to glycosaminoglycans (GAGs) found in the extracellular matrix (ECM) of cartilage. This biomimetic nature has shown an influence on the morphology, differentiation and function of chondrocytes.[55,56]

Collagen is one of the most abundant proteins in animal tissues. Its primary function is to provide and maintain structural integrity of the ECM. Being a major component in the ECM of cartilage and bone, collagen is considered as an ideal biomaterial for bone (collagen type I) and cartilage (collagen type II) tissue engineering.[57–59] Experimental studies have demonstrated that chondrocytes maintain their phenotype when cultured in 3D collagen gels.[58] Collagen also plays a vital role in tissue repair and wound healing processes.[58,60] Nevertheless, poor mechanical properties have limited the use of collagen in load-bearing applications.[61] Composites of collagen and bioceramics have been shown to generate scaffolds with improved mechanical properties.[61–63] Immunogenicity, large scale production and purification are major issues that limit the use of collagen in clinical settings.[64]

Synthetic biodegradable polymers used in tissue engineering include polyglycolic acid (PGA), polylactic acid (PLA), polycaprolactone (PCL), poly(L-lactic-co-glycolic acid) (PLGA), polydioxanone (PDO), poly(propylene fumarate) (PPF), polyorthoesters (POE), polyphosphazenes and polyanhydrides.[65–67] The advantages that synthetic biodegradable polymers offer lie in their range of chemistries, ease of processing and controlled molecular weight distribution that can be tailored to the target application.[50] Most synthetic polymers are hydrophobic, and therefore possess lower bioactivity than natural polymers. To overcome this drawback, blends of hydrophobic and hydrophilic polymers can be used to enhance hydrophilicity. Shafiee et al. used nanofibrous scaffolds made of a blend of polyvinyl alcohol (PVA)/PCL for cartilage tissue engineering.[68] PVA was

electrospun with PCL to enhance hydrophilicity and support cell adhesion. Both in vitro and in vivo studies in rabbits suggested that PVA/PCL scaffolds supported the proliferation and chondrogenic differentiation of MSCs and enabled the regeneration of cartilage. Another strategy that can be adopted to enhance physico-chemical properties (e.g., hydrophilicity, bioactivity and elastic modulus) of synthetic polymers is to incorporate bioceramics into these matrices.[69–71]

Bioceramics such as hydroxyapatite (HA) and tricalcium phosphate (TCP) are known to enhance and promote biomineralization, making them suitable for bone tissue engineering.[72,73] When implanted, bioceramics can promote the formation of an apatite layer on their surface (similar to calcium deposition in bone) leading to their integration to the host bone. MSC-seeded porous HA scaffolds have shown good osteoconductive properties after implantation in mice, although various factors such as pore size and porosity of the scaffold may affect bone formation.[74] In addition to being biologically active materials, bioceramic-based scaffolds exhibit suitable stiffness, although they are brittle and cannot resist mechanical stresses. Biodegradability of calcium phosphates can be controlled through Ca/P ratio, although compounds with Ca/P ratio of less than 1 are not suitable for biological implantation. This is due to the higher solubility and speed of hydrolysis with decreasing Ca/P ratio.[75] Controlled degradation profiles can also be obtained by optimizing the porosity of the scaffolds. However, enhanced porosity will result in decreased mechanical properties.[76]

Bioactive glasses constitute another important class of bioceramics for bone regeneration, of which 45S5 Bioglass® is the most representative member.[77] The composition of 45S5 Bioglass® includes 45wt% SiO_2, network modifiers of 24.5 wt% Na_2O and 24.5 wt% CaO, as well as 6 wt% P_2O_5 in order to simulate the Ca/P constituents of hydroxyapatite (HA).[78] Xynos et al. seeded 45S5 Bioglass® substrates with human primary osteoblasts and evaluated them after 2, 6, and 12 days.[79] The results showed the ability of 45S5 Bioglass® to stimulate cell cycling, enhance osteoblast turnover, and produce bone-like tissue in vitro in a relatively short period of time. In vivo studies have demonstrated that bioactive glasses bond with bone more rapidly than other bioceramics.[80]

However, it is difficult to produce porous bioactive glass scaffolds for bone regeneration from 45S5 Bioglass® because it crystallizes during sintering.[80] Some recent reviews have elaborated on the application of bioactive glasses for bone tissue engineering.[80–82]

To improve the mechanical properties of bioceramic scaffolds, biodegradable polymers have been used as coating materials. O'Shea and Miao improved the mechanical properties of porous HA/TCP scaffolds by coating them with PLGA.[64] The coated scaffolds showed about a 10 fold increase in compressive strength when compared with control scaffolds, with a negligible compromise in porosity. In another study, 45S5 Bioglass®-based scaffolds coated with poly(D-L-lactic acid) (PDLLA) improved the compressive strength while retaining the bioactivity of 45S5 Bioglass®-based scaffolds.[83] Some of the recent studies on polymer-coated inorganic scaffolds for bone tissue engineering, including HA, bioactive glass, titanium dioxide (TiO_2), alumina (Al_2O_3), and zirconia (ZrO_2), have been reviewed by Yunos et al.[84]

Growth factors and cell types

A review paper by Martin et al. regrouped osteochondral studies conducted between 1999 and 2006 according to repair strategy (scaffold strategy *vs.* cell strategy).[9] According to the authors, for small and confined osteochondral lesions it might be sufficient to use a cell-free approach with appropriate scaffolds (e.g., adequate biomechanical properties and the capacity to resorb/remodel). Although in the case of more extended injuries, the delivery of growth factors is necessary for local cell recruitment. The use of a cell-based approach becomes mandatory if the wound bed is further compromised.[9] Therefore, in most practical cases the scaffolding material alone cannot initiate biological responses that could support the regeneration process.

For osteochondral tissue engineering, progenitor cells that can differentiate into several different lineages or tissue specific cells, such as chondrocytes and osteoblasts are used.[85–87] In general, chondrocytes are often used for osteochondral constructs implanted in vivo and for further

development in vitro.[88,89] The major drawback when using chondrocytes is their limited number in native tissue and unstable expression of phenotype. Chondrocytes constitute less than 5% of cartilage volume.[90,91] In addition, isolation of chondrocytes is a difficult process as it requires collagenase, which can harm the cells.[92,93] Another source of concern about chondrocytes is that they lose their phenotypic expressions in culture environments.[94–96] This phenomenon was reported by von der Mark et al.[96] Fröhlich et al. studied this phenomenon by quantifying the extent of dedifferentiation using q-PCR on rabbit chondrocytes until passage four.[97] The results indicated that there was a major decrease in aggrecan, collagen type II and type I gene expressions when comparing the freshly isolated chondrocytes to the passage one cells. In addition, the proliferation capacity decreased during cultivation and was accompanied by cell enlargement, which was particularly evident in the third and fourth passages.[97] Yonenaga et al. addressed the difficulties in chondrocyte isolation and seeding through optimizing the collagen concentration.[98] They reported that the cell viability could be increased by up to 10 fold if the tissue was treated with graded doses of collagenase rather than a single concentration (e.g., 1.2 % for 4 h, 0.6% for 6 h, and 0.3% for 24 h).[98]

Sheehy et al. compared the growth of porcine bone marrow mesenchymal stem cells (BM-MSCs) and chondrocytes by seeding the cells onto hydrogel scaffolds and culturing under static and dynamic (rotational) conditions. The scaffolds were analyzed by biochemical analysis, mechanical testing, histology and immunohistochemistry. Chondrocytes appeared to be superior to BM-MSCs in both culture conditions.[99] Moreover, the formation of a more homogeneous tissue in chondrocyte-seeded constructs suggested that dynamic conditions that could be beneficial for chondrocytes might be suboptimal for BM-MSCs.[99] In spite of all these advantages, many researchers prefer to work with MSCs because of their abundance, multipotency and rapid multiplication.[100] Moreover, MSCs can be isolated from various tissues and cultured in chondrogenic, osteogenic or co-culture osteochondral media for clinical applications.[101–105]

In bone and cartilage repair/regeneration, growth factors act as molecular cues that promote cellular maturation and differentiation in a guided manner.[106] The growth factors essential for osteochondral repair are produced intrinsically by the body. However, to reach the goal of tissue regeneration, higher concentrations of these growth factors have to be incorporated locally into/onto the scaffolds. These growth factors are known to promote both in vitro and in vivo tissue regeneration. Major growth factors that contribute to osteochondral tissue engineering include insulin-like growth factor–1 (IGF-1), basic fibroblast growth factor (bFGF), and transforming growth factor-β1 (TGF-β1).[107–110] These growth factors have demonstrated anabolic cellular effects and increased production of matrix molecules. Various growth factors along with their scaffold combination and cell type are listed in Table 1.[111–117]

Cell-free approaches to osteochondral regeneration have also been investigated by a number of research groups. Filová et al. studied the effect of cell-free hyaluronate/collagen type I/fibrin composite scaffolds containing PVA nanofibers enriched with liposomes, bFGF and insulin on the regeneration of osteochondral defects.[118] It was reported that the scaffolds were able to enhance the regeneration of osteochondral defects in minipigs. Cao et al. applied a bilayered construct with or without adipose-derived stem cells (ASCs) to repair full-thickness defects in the patellar groove of rabbits.[119] Utilizing a score ranging between 0 (best) to 20 (worst), they reported that the semi-quantitative score of the cell-based group (4.2 ± 1.2) was significantly better than the cell-free group (13.8 ± 2.5). As mentioned earlier, the success of cell-free approaches depend on the defect size.[9]

Osteochondral tissue engineering

Native tissues are anisotropic and inhomogeneous in nature, composed of different types of cells and extracellular matrices (ECMs) in specific spatial hierarchies.[120] For example, articular cartilage consists of different zones with varying types and orientations of collagen fibers and collagen-binding proteins.[25,121] The molecular organization of articular cartilage is shown in Figure 2A.[25] The

pericellular matrix is the zone where molecules that interact with cell surface receptors are located (e.g., hyaluronan binds the receptor CD44). Next to the pericellular matrix lies the territorial matrix, followed by the interterritorial matrix at the largest distance from the cell.[25] The complex microstructure of cartilage enables proper dissipation of loads throughout the tissue. Similarly, bone has an anisotropic structure due to the spatial differences in the concentration and orientation of its mineral and organic constituents.[121] The hierarchical structure of bone is shown in Figure 2B.[122] During bone formation, collagen molecules assemble into fibrils, which are mineralized via the formation of apatite crystals.[122]

The development of artificial micro- and nanostructures to replicate the complex features of biological tissues can lead to promising biomaterials for tissue engineering. For example, to replicate the extraordinary strength and durability of natural bone, the current trend is to design biomaterials that nearly mimic the structural organization of bone from the nanoscale upwards.[123] Hence, some studies have aimed to produce functionalized scaffolds that can mimic the nanofibrous structure of the natural extracellular matrix (ECM) of biological tissues to enhance cell activation.[124] In recent years, there has been an effort to combine additive manufacturing (AM) and electrospinning (ES) techniques to produce bimodal scaffolds, where micro- and nano-scale features can be combined.[125]

Figure 2C shows the typical process steps for a combined AM-ES technique that makes use of PCL as a scaffold material.[126] An AM system (e.g., melt-dispensing) is used to obtain the micro-sized PCL struts, whereas the ES system is used to generate the interlayered PCL micro/nanofibers. The system can potentially offer additional features, such as dispensing cell-laden hydrogel struts (e.g., alginate). The cross-sectional view of the scaffold in Figure 2C shows the multiple features that can be generated during this process.[126] The bimodal architecture of a 3D scaffold produced by the AM-ES technique is depicted in Figure 2D, showing a PCL microfibrous layer and an electrospun PCL/collagen nanofibrous matrix.[124] These hierarchical constructs contain large pores enabling cell

penetration, while the electrospun fibers effectively increase the surface area available for the adhesion of penetrating cells.[125]

Nanostructured materials with surface properties promoting protein adsorption and favoring cell adhesion have a greater chance of stimulating new bone growth when compared to conventional materials (Figure 2E).[123] This is one of the underlying mechanisms that make nanomaterials superior to conventional materials for tissue engineering applications.[81,123,127] A significant volume of recent publications have been dedicated to understanding and controlling matter on the nanometer scale where unique phenomena enable new functional applications.[128] Elaborating on the hierarchical approaches at macro-, micro-, and nanoscales goes beyond the scope of this review paper. Therefore, the emphasis is placed on macro/microscale features of multiphasic scaffolds, as well as a brief overview of additive manufacturing and computational modeling to optimize these constructs.

Multiphasic scaffold architectures

Given the district differences between the hierarchical structures of cartilage and bone, engineering multilayer scaffolds with controlled properties in each layer could allow the replication of the local microenvironment of the osteochondral tissue.[120,129,130] In general, osteochondral tissue engineering strategies can be categorized into monophasic, biphasic, and triphasic depending on the cellular/biological or physical/chemical characteristics of the scaffold (Figure 3).[18] Biphasic and triphasic approaches utilize two or three different architectures, materials, or composites to produce a multilayered construct. A single material can also be used to produce biphasic or triphasic constructs if significant variations in physical properties exist between the different layers.[18] Recently, Martin et al.,[9] Castro et al.,[26] Nukavarapu and Dorcemus,[41] and Keeney and Pandit[131] have discussed the potential of multi-component scaffolds for osteochondral tissue engineering, while emphasizing the challenges in their use for clinical practice. Optimal scaffold design of such constructs is critical for

cell attachment, survival and matrix production. Pore size, pore geometry, overall porosity and material used are all critical factors that influence cell biology.[72]

To satisfy both the mechanical and biological requirements, a wide range of porous scaffold systems with gradient-based porosity and pores size have been developed.[132] In addition, it has been hypothesized that mechanical properties of scaffolds should ideally match or be within the range of actual tissue properties.[38,133] This could enable the scaffold to withstand the physiological loading without failure within the tissue defect,[133] which would mean faster rehabilitation for the patient. Early force transmission through the repair site could stimulate the regenerated tissue with biomechanical properties closely matching those of surrounding native tissue. The rapid restoration of tissue biomechanical function remains an important challenge, emphasizing the need to replicate the structural and mechanical properties of the tissue using novel scaffold designs.[134–137]

In the last decade, the advancement in additive manufacturing (AM) technology has led to the production of free-form porous scaffolds with custom-tailored architecture.[138,139] Therefore, AM technology has become increasingly common in recent years mainly due to its ease of operation as well as its ability to translate a patient's scanned image to a computer-aided design (CAD) model.[24] Some of the enhanced features of current AM technology include the introduction of nano-sized features, as well as the tremendous potential the technology offers for producing functionally graded structures.[24] Effective scaffold design optimization and subsequent fabrication using AM systems would allow meeting the mechanical requirements for faster restoration of tissue function.[24,140,141]

Recent efforts in understanding scaffold architecture-property relationships include a study by Sudarmadji et al. on functionally graded scaffolds (FGS).[142] The team developed a database to correlate the scaffold porosity and the corresponding compressive stiffness. The database included 13 different polyhedral units produced by selective laser-sintering that could be assembled into scaffold structures. The resulting porosity, compressive stiffness and yield strength of the scaffolds varied between 40–84%, 2.74–55.95 MPa and 0.17–5.03 MPa, respectively. This range of stiffness was

reported to closely match the cancellous bone in the maxillofacial region.[142] Nevertheless, in osteochondral tissue engineering the need to properly design the bone/cartilage interface adds to the complexity of the scaffold design strategy. This is mainly because the relationship between scaffold structural parameters and osteochnodral tissue requirements is not well established.[138]

Scaffolds with interconnected unidirectional channels are often used in bone and cartilage tissue engineering, since unidirectional channels may provide a path of least resistance and facilitate in vivo vascularization and the formation of new tissue.[143] Moreover, it has been shown that scaffolds with orthogonal channels can exhibit a larger bone growth area than scaffolds with radially oriented channels.[144] In extrusion-based techniques, a repeating pattern is often used to simplify the deposition process.[145–147] More complex patterns can be obtained by changing the deposition angle between adjacent layers (Figure 4A),[138] also known as honeycomb-like patterns.[148] The use of space-filling curves has also been explored (Figure 4B).[138] Due to the restricting features of extrusion-based techniques, non-intersecting continuous curves are particularly attractive. This includes fractal space-filling curves that can be generated using a simple pattern as a starting point,[138] which then grow through the recursive application of a certain set of mathematical rules.[149]

Multiphasic scaffold compositions

Biomaterial selection is challenging due to the need to satisfy the chemical, morphological, biological and surface requirements for a given application. Many of these properties remain unspecified until the final product is tested in vivo.[150] The scaffold composition plays a vital role in providing a platform for cellular growth. Thus, there is still a constant search for ideal biomaterials. An in-depth understanding of the advantages and drawbacks of potential scaffold materials is required for rational biomaterial selection. A variety of scaffold systems for osteochondral tissue regeneration have been developed to meet the complex functional demands of cartilage and bone tissues, given the distinctive differences in their structural, chemical, and mechanical properties.

Multiphasic/gradient-based strategies that tailor the scaffold composition to the type of regenerated tissues are currently being sought. This involves both natural and synthetic materials, as well as extracellular matrix (ECM)-derived biomaterials.[19,120,151,152] Figure 5 shows some osteochondral scaffold designs featuring cartilage- and bone-specific compartments. One strategy is to engineer two individual cartilage and bone scaffold layers, and then join the two separately fabricated scaffolds by suturing, glue, or simple press fitting. Swieszkowski et al. used biphasic constructs composed of fibrin/PCL or PCL/PCL-TCP phases (Figure 5A).[11] These two phases were fabricated separately and seeded with an appropriate number of cells, and then cultured in chondrogenic and osteogenic media for cartilage and bone regeneration, respectively. Finally, the two phases were integrated into one construct using fibrin glue.[11] However, these methods are limited by the inferior integration between cartilage and bone tissues resulting in the eventual separation of the two tissues.[153]

Interdiffusion of the two layers forming a biphasic osteochondral construct could serve as a means of integrating the chondral and bony phases. Grayson et al. used agarose gel for the cartilage phase of their osteochondral scaffolds, whereas decellularized bone was selected for the bone region (Figure 5B).[154] Agarose was used due to its ability to yield good mechanical properties with immature chondrocytes.[154,155] The rationale for selecting decellularized bone was to provide adequate mechanical properties, osteo-inductive architecture, and biochemical composition.[154] The cell seeded bone scaffolds were overlaid allowing a penetration depth of 500 μm of agarose gel into the bone scaffold, followed by solidification of agarose at room temperature. It was reported that the interface formed in these biphasic constructs upon culturing in an osteochondral bioreactor was different from that of native issue. This study emphasized the need for interface design so as to recapitulate the native interface and investigate the heterogeneous cell-cell communication in this region.[154]

Harley et al. fabricated a series of collagen type I/glycosaminoglycan/calcium phosphate (CGCaP) scaffolds by freeze-drying technique.[63] The composition of the CGCaP suspension, the pore architecture, CaP phase chemistry, as well as the crosslinking density were independently controlled in this study.[63] In addition, the team developed multiphasic osteochondral scaffolds from a mineralized CGCaP suspension and an unmineralized collagen type II/glycosaminoglycan (CG) suspension (Figure 5C).[156] The interdiffusion between the layered suspensions before freeze drying enabled generating an interface zone between the two layers (liquid-liquid-phase cosynthesis). The study did not report cell seeding and growth for these scaffolds. Wang et al. used a similar interdiffusion step to produce biphasic scaffolds with a gradual interface.[157] Articular cartilage extracellular matrix (ACECM) and hydroxyapatite (HA) were used for the two components, leading to a porous, oriented upper layer and a dense, mineralized lower layer (Figure 5D).[157] It was reported that the difference in porosities and pore sizes between the two layers resulted in a low-permeable interface. The scaffolds seeded with rabbit chondrocytes revealed well-distributed cells in the non-mineralized zone, while showing only a few cells adhering to the interfacial zone. No cells entered into the mineralized component, suggesting a cell-barrier layer at the interface.[157] The team proposed further studies to evaluate the potential of these scaffolds for osteochondral tissue engineering.

Miyagi et al. proposed a combination of a β-TCP block with a scaffold-free sheet formed using MSCs for osteochondral regeneration.[104] A similar approach using centrifuged chondrocyte cell sheets had been previously proposed by Niyama et al.[105] It should be noted that the cell-sheet approach has some limitations due to technical challenges in stimulating the differentiation into two respective lineages (osteoblasts and chondrocytes), because only one type of culture medium can be used for a cell sheet.[104]

A combination of additive manufacturing (AM) and electrospinning has been used to produce osteochondral scaffolds with multiscale features and varying compositions.[158] Tuan et al. proposed

biphasic scaffolds comprised of a PCL cartilage phase and a PCL – TCP matrix that served as the bone component.[158] The scaffolds were built using the fused deposition modeling (FDM) process, seeded with MSCs via fibrin encapsulation, and patched with a PCL – collagen 20% electrospun mesh to prevent cell loss and facilitate the diffusion of nutrients from the synovial space. In vivo studies in a pig model indicated favorable outcomes in the cartilage region, with a reduced incidence of fibrocartilage and improved GAG content when compared to cell-free and mesh-free scaffolds. However, besides the implant design, the implantation site appeared to affect the in vivo outcomes (medial condyle vs. patellar groove).[158]

When combined with appropriate growth factors (e.g., TGF-β1), alginate, agarose and chitosan have shown to help with maintaining the spherical morphology of chondrocytes and supporting chondrogenic differentiation and cartilage-specific matrix deposition.[56,159] Jeon et al. developed multiphasic scaffolds comprised of 2% alginate hydrogel and a biphasic PCL scaffold (made by a combined FDM and electrospinning).[159] To integrate the alginate and PCL components, alginate was partially de-cross-linked and press-fitted on top of the biphasic scaffold, which enabled alginate to partially infiltrate the pores of the PCL-FDM scaffolds, and then re-cross-linked. Histological analysis of the constructs implanted subcutaneously in rats showed that some alginate constructs had been separated from the PCL scaffolds possibly due to gradual weakening of the interface region. In another study, a biphasic osteochondral composite was developed by Liu and Jiang, combining a chondral phase composed of chitosan/collagen with a bone-ECM mimicking phase made of β-TCP (Figure 5E).[160] A glue made of cross-linked 1.2 % (w/v) sodium alginate and $CaCl_2$ was used between the chitosan/collagen mixture and the sintered porous β-TCP scaffold, and then the set up was subjected to freeze-drying. The combination of biphasic scaffolds and a double-chamber bioreactor was found to promote cellular proliferation and trigger simultaneous chondrogenic and osteogenic differentiation of MSCs within the porous constructs.

Castro et al. have reviewed some other scaffold compositions for the treatment of osteochondral defects.[26] Other recent multiphasic scaffold systems include bilayered chitosan–gelatin scaffolds,[161] trilayered PEG-based hydrogel systems with varying ECM composition,[162] and PCL/alginate scaffold systems.[163] It should be mentioned that material selection for osteochondral regeneration strategies are highly contingent on the specific manufacturing technique employed, and on the way cells are used in each strategy (encapsulated within or seeded on the surface of the scaffold).[26] Another recent review paper gives an overview of in vivo osteochondral repair studies that have been undertaken since 2009.[18]

Bone-cartilage interface design

Natural articular joints are characterized by a strong, stable interface between cartilage and bone. The CaP content gradually decreases from ~75 wt % at the subchondral bone plate to zero in articular cartilage.[156] In addition, collagen type II level decreases gradually from the superficial to the deep calcified zone of articular cartilage, whereas type X collagen and proteoglycan levels increase.[162] These variations lead to an increase in compressive modulus from the superficial to the deep zone. The smooth compositional transition between vascular/mineralized bone and nonvascular/ unmineralized cartilage has a major role in the stability of cartilage-bone interface.[156] Therefore, it is important to reproduce the native architecture and function, as well as the interface zone of the osteochondral tissue. To date, the formation of a stable interface between cartilage and subchondral bone scaffolds remains a significant challenge.[164] Some review papers have briefly covered the recent efforts in interface design for osteochondral scaffolds.[64,129]

Nooeaid et al. developed triphasic scaffolds where the middle layer functioned as an adhesive at the transition zone.[164] The subchondral bone scaffold was composed of porous 45S5 Bioglass® and alginate composites (Alg-c-BG), whereas freeze-dried alginate-based foams (Alg-foam) were used as the cartilage layer scaffolds. The two layers were integrated using an alginate/45S5 Bioglass® hybrid

interface (Figure 5F).[164] To generate the interface layer, 2 w/v% solution of alginate/Bioglass® (1:3 by wt) in DI water was prepared. The solution was brushed on one side of the Alg-c-BG scaffold, and then the Alg-foam was placed on the adhesive coated side of the scaffold and pressed manually. Subsequent immersion in 0.5M CaCl$_2$·2H$_2$O for 24 h enabled crosslinking the Alg-foam and the interface. Delamination did not occur during normal handling for testing or upon immersion in a simulated body fluid (SBF) for 28 days.[164]

Da et al. developed a compact layer made of PLGA/β-TCP as an interface zone between the cartilage and bone phases of their multilayer scaffolds (Figure 5G).[165] The cartilage phase was derived from bovine decellularized articular cartilage ECM. The bone phase was produced by additive manufacturing (AM), and was made of a PLGA/β-TCP skeleton wrapped with collagen Type I. To bond the two phases, the surface of the compact layer was dissolved by application of 1,4-dioxane. Then, the cartilage phase was pressed onto the dissolved compact layer, frozen for 2 h at −80°C, and subsequently lyophilized for 24 h. In vivo results in rabbits revealed superior GAG and collagen content in the compact layer-containing scaffolds compared to the control group.[165] The team suggested that the interface layer could potentially enhance the biomechanical properties of the biphasic scaffolds and the regenerated osteochondral tissue.

Cao et al. proposed layered scaffolds featuring three distinct regions.[119] The upper chondral phase was composed of cross-linked collagen-chitosan, collagen gel and bovine bone morphogenetic proteins (bBMPs). The lower bony phase was composed of collagen-modified bovine cancellous bone, collagen gel and bBMPs. The two phases were separated by an air-dried collagen membrane. The constructs, with or without adipose-derived stem cells (ASCs), were applied to repair full-thickness defects in the patellar groove of rabbits. Implantation of the layered constructs alone did not enhance repair, whereas the contrasts combined with ASCs were found to enhance osteochondral regeneration.[119] Qu et al. developed layered scaffolds composed of polyvinyl alcohol (PVA), gelatin,

nano-hydroxyapatite (nHA), and polyamide6 (PA6).[166] The cartilage layer was made of porous PVA cryogel with pore diameter of 5–40 μm, 70% porosity and 71.6% water content. The bone layer was composed of porous nHA/PA6, with a pore diameter of 100–400 μm and 80% porosity. The interface was made of nonporous PVA. The scaffolds seeded with induced bone mesenchymal stem cells (BMSCs) and implanted at ectopic sites (rabbit muscle pouch) showed a potential to differentially support cartilage and bone tissue generation.[166] The team also reported that the subchondral bone layer was completely integrated with the cartilage layer.

Some studies have developed triphasic scaffolds featuring chemical and morphological gradients by stacking a highly mineralized composite layer made of HA (70%)/collagen (30%), resembling the subchondral bone layer, an intermediate layer with reduced mineralization (as tidemark), and an upper layer made of collagen.[167–172] In a clinical study, twenty seven patients who were affected by osteochondritis dissecans (OCD) of the femoral condyles (average defect size 3.4 ± 2.2 cm^2) were treated with the implantation of triphasic scaffolds (Figure 5H).[168] The treatment results were analyzed using the cartilage standard evaluation form as proposed by the International Cartilage Repair Society (ICRS). A good clinical outcome at 2-year follow-up was reported, despite certain postoperative adverse events such as swelling and stiffness observed in some patients.[167]

Biological gradients

In addition to physical gradients, biological gradients also play a vital role in tissue engineering. Since cartilage and bone have different biochemical, structural, and mechanical microenvironments, osteochondral scaffold designs that do not address such differences suffer from obvious limitations. Multiphasic designs do not necessarily replicate all such parameters; therefore, osteochondral constructs with tissue-specific designs may contribute to the generation of functional osteochondral constructs within a shorter timeframe.[18] The ability to fabricate scaffolds containing systematic gradients in distribution of stimulators can enable simultaneous triggering of osteogenic

and chondrogenic factors and provide additional means for mimicking the important gradients observed in native tissues.[173] However, very few reports are available on gradient-based delivery systems of growth factors for osteochondral tissue engineering.[41,113,116]

Wang et al. used PLGA and silk fibroin microspheres to investigate microsphere-mediated delivery of bone morphogenetic protein-2 (rhBMP-2) and insulin-like growth factor–1 (rhIGF-1) in polymer scaffolds and its impact on osteochondral differentiation of human bone marrow derived MSCs (hMSCs).[117] The growth factors were incorporated in the scaffolds as a reverse gradient combining the two factors, as well as a single concentration gradient. Initially a cylindrical alginate gel was fabricated, and then microspheres were incorporated as gradients. Silk microspheres were found more efficient than PLGA microspheres in delivering rhBMP-2, probably due to sustained release of the growth factor, while less efficient in delivering rhIGF-1, which was attributed to loading efficiency. The shallow growth factor gradients induced non-gradient trends in hMSC osteochondral differentiation. Aqueous-derived silk porous scaffolds were also used by the team to incorporate silk microspheres using the same gradient process. After culturing for 5 weeks in a medium containing osteogenic and chondrogenic components, hMSCs exhibited osteogenic and chondrogenic differentiation along the concentration gradients of rhBMP-2, but not along the rhIGF-1 gradient system. These results suggested that silk microspheres were more efficient in delivering rhBMP-2 than rhIGF-1 for hMSCs osteochondrogenesis.[117]

A hydrogel composite consisting of oligo(poly(ethylene glycol) fumarate) (OPF) and gelatin microparticles (MPs) was used by Guo et al. for osteochondral regeneration.[174] The top layer consisted of rabbit MSCs encapsulated in OPF with either blank MPs or TGF-β3-loaded MPs. In the bottom layer, OPF hydrogel composites with blank MPs were used to encapsulate osteogenically precultured MSCs (0, 3, 6 and 12 days).[174] After cell encapsulation, the bilayered composites were cultured in chondrogenic medium. The results indicated that TGF-β3-loaded MPs could significantly enhance chondrogenic differentiation of MSCs in the chondrogenic layer. Osteogenically precultured

cells maintained their osteoblastic phenotype in the osteogenic layer; however, TGF-β3 showed an inhibitory effect on cell mineralization. In addition, encapsulated cells of different degrees of osteogenic differentiation were found to significantly affect the chondrogenic gene expression of co-cultured MSCs in both the presence and absence of TGF-β3.[174]

Saha et al. used mulberry (Bombyx mori) and non-mulberry (Antheraea mylitta) silk fibroin scaffolds for osteochondral tissue engineering, with and without growth factors.[175] Non-mulberry constructs seeded with hMSCs showed neo tissues containing chondrocyte-like cells after 4 to 8 weeks of in vitro culture, whereas mulberry constructs seeded with hMSCs formed bone-like nodules. The team also conducted cell-free growth-factor guided in vivo studies in order to determine the potential of these scaffolds to attract and differentiate endogenous progenitor cells. The constructs used for in vivo implantation were monophasic in composition, but were coated with TGF-β3 and BMP-2 in their respective cartilage and bone phases, before being assembled using fibrin glue (Figure 5I).[175] The osteochondral defects in the patellar groove of the knee joints of Wistar rats were filled with mulberry or non-mulberry scaffold discs with or without growth factors.[175] Excellent integration of the neo-tissue with the host tissue was reported in all constructs. Therefore, the team proposed the use of multi-layered combination of mulberry and non-mulberry scaffolds, for bone and cartilage respectively, for cell-free osteochondral tissue engineering.[175]

Additive manufacturing has been explored in recent years to generate biological gradients within tissue engineering scaffolds.[126,163,176,177] In these studies, hydrogel systems have enabled encapsulating cells and growth factors in a multilayer fashion. Fedorovich et al. used a 3D fiber deposition (3DF) technique for the fabrication of cell-laden, heterogeneous hydrogel constructs as potential osteochondral grafts.[177] The team encapsulated and printed fluorescently labeled human chondrocytes and osteogenic progenitors in alginate hydrogel, with different zones for both cell types. Changing the fiber spacing or angle of fiber deposition resulted in scaffolds with different

porosities and elastic moduli. It was reported that distinctive ECM regions were formed in vitro and in vivo according to the anticipated tissue type. Some studies have made use of synthetic biomaterials such as PCL and PLGA to enhance the mechanical stability of biologically-graded constructs.[126,163] In the bioprinting process shown in Figure 5J, the sequential dispensing is repeated to stack synthetic biomaterials and hydrogels, loaded with cells and growth factors, to build multiplayer constructs featuring chemical and biological gradients..[163]

From cartilage/bone interface design perspective, microfluidic systems can offer opportunities for studying cell differentiation and interfacial construction in vitro.[178,179] Figure 5K shows a microfluidic system for generating a gradient-based stem cell-laden hydrogel construct.[178] The system enables different cell culture/differentiation media (OM: osteogenic medium, M: normal medium, CM: chondrogenic medium) to flow into the hydrogel slab, where distinct zones with specialized cell lineages and extracellular matrices can be formed.[178] Shi et al. reported that after 25 days of culture using this microfluidic device, stem cells differentiated into osteoblasts and chondrocytes in their respective zones, while a biological gradient mimicking the bone-cartilage interface was observed in the middle zone of the hydrogel.[178]

Computational scaffold design

Computational methods have been widely used in designing implants for tissue replacement. A recent FEA of implant design has suggested that both the size and material properties of implanted cartilage replacements (ICR) have a major role in the failure of the fibrin glue used to attach the implant to the native tissue.[1] According to this study, increasing the compressive modulus (E) by 25%, with respect to that of native articular cartilage (AC), can reduce the fibrin damage in both the osteochondral and chondral implants, whereas decreasing E by 25% may lead to a higher damage at the interface (Figure 6, A and B).[1] This study also suggested that Poisson's ratio (v) of the ICR might affect the integrity of the fibrin adhesive. While the fibrin surrounding the osteochondral implant

showed less damage at higher value of ν and more damage at a lower values of ν, the simulated trend was the opposite for the chondral implant (Figure 6, C and D). This was attributed to the important role of the collagen network in instantaneous lateral expansion of articular cartilage. Therefore, a less organized network of collagen fibers may result in a lower ν.[1] Similar results could be expected in tissue-engineered constructs, since they lack an organized collagen fiber distribution and have lower collagen content compared to native AC.[1,180]

Finite element modeling tools have been used by many research teams to predict the modulus of 3D scaffolds produced by a variety of fabrication techniques.[181–185] This is particularly important for multi-layer scaffolds used for regeneration of layered tissues consisting of cartilage and subchondral bone.[186] In addition, scaffold design for tissue engineering involves many parameters that directly influence the rate of tissue regeneration throughout the scaffold microstructure. Investigating the effect of each specific scaffold parameter on tissue regeneration using in vitro and in vivo techniques can be costly and time consuming.[187] Therefore, combining finite element modeling tools with mechano-biological models could potentially assist researchers in predicting the outcomes of tissue culture trials. Evaluating the effect of individual factors on cell migration, proliferation and angiogenesis may also help with generating experimentally testable hypotheses regarding optimal scaffold design,[188] while allowing to predict the outcomes of tissue engineering based on in vitro/in vivo conditions.[189] Multiple simulations can be performed in order to identify a topology that would perform well under physiological loading conditions while allowing tissue ingrowth.[138] Then, additive manufacturing (AM) techniques can be used to produce prototypes of the optimized scaffold for experimental testing, enabling researchers to explore more innovative topologies and their resulting effects on mechanical strength and tissue regeneration.[138]

Cahill et al. designed simple CAD models of two scaffold architectures using ABAQUS software.[190] The models were used to estimate the effective and shear moduli of the scaffolds.

Prototypes of the scaffolds were fabricated via selective laser sintering (SLS) and subjected to experimental testing. It was found that the FEA overpredicted the moduli of the scaffolds to different extents in x-y-z directions. For polycaprolactone (PCL) scaffolds, the effective modulus was overestimated by 67%, assuming isotropic properties. For polyamide (PA) scaffolds, the effective modulus under compression was overpredicted by 81% in the x direction, whereas the moduli in the y and z directions were overestimated by 125% and 147%, respectively. The results called for a greater understanding of how the microstructure of the scaffolds, such as surface roughness and microporosity, affected the scaffold properties.[190]

McIntosh et al. performed FEA in order to simulate the properties of three hydroxyapatite (HA) scaffolds of varied properties as they became integrated with surrounding healing bone.[191] The scaffolds were produced using a directed deposition technique followed by sintering. It was found that shear modulus was affected by the geometry of the bone surrounding the scaffold. Whether the bone coated the scaffold or bridged across the pores affected the scaffold's ability to resist shear forces. The instance where bone bridged the pores of the scaffold served to strengthen the system. However, the interaction with the bone geometry did not seem to affect the elastic modulus. Decreasing the elastic modulus of the material used for scaffold fabrication had a greater impact on the overall mechanical properties than did the scaffold porosity.[191]

Melchels et al. generated CAD models of 3D scaffolds with varied architectures (cube, diamond, and gyroid). High-resolution stereolithography was used to fabricate the designed scaffolds made of poly (D,L-lactic acid) (PDLLA) or poly(D,L-lactide-co-ε-caprolactone) P(DLLA-co-CL).[192] The bulk properties of solid materials made by stereolithography were measured and described mathematically using a constitutive model. The model was then implemented into ABAQUS finite element software, which allowed simulating the deformation characteristics of the porous scaffolds.

The simulations suggested that the gyroid structure could provide evenly distributed mechanical stimuli to cells within the scaffold, which would be beneficial for cell growth and differentiation.[192]

Olivares et al. used FEA to optimize the scaffold architecture so as to enhance cell differentiation.[193] To this end, they examined the effect of scaffold microstructure and inlet fluid flow conditions on mechanical stimuli transferred to cells within the scaffold via scaffold deformation. The design variables included varied porosities (55%, 70%) for hexagonal and gyroid architectures, directions of load (longitudinal/transverse for hexagonal) and pore size gradients (radial/longitudinal for gyroid). The simulations suggested that pore size and porosity influenced tissue differentiation, whereas pore shape affected the movement of fluids within the scaffold in addition to the mechanical load distribution. In that respect, the gyroid structure was superior to the hexagonal structure, as fluid flow was more easily distributed through the scaffold. However, the mechanical loading was more homogenous in the hexagonal structure.[193] The simulated results of differentiations for a porosity of 70% under 0.1 mm/s of inlet fluid velocity showed how the tortuosity of the structures influenced the mechanical stimuli (Figure 7). Gyroid structures and the transversal fluid flow on hexagonal structures led to zones with a high percentage of cartilage phenotype differentiation, although they also had some regions with bone phenotype differentiation (shown in a darker color in Figure 7).[193]

Sanz-Herrera et al. combined the macro-scale asymptotic homogenization theory with a micro-scale bone remodeling theory in an FEA performed in ABAQUS.[187] Based on the simulation results, it appeared that a higher modulus for the scaffold led to improved cell differentiation. Higher porosity promoted bone formation due to increased mechanical stimuli, whereas larger pore sizes improved cell migration but also reduced specific surface area for cell adhesion. It was concluded that while the results of the simulations met expectations, multiple factors needed to be improved. For instance, they suggested that random-walk cell crawling be used to model cell migration instead of Fick's Law.[187] Checa and Prendergast used a mechano-biological model to simulate tissue formation and angiogenesis within a porous bone tissue engineering scaffold, while taking into

account the individual cellular processes (e.g., migration and proliferation).[194] The simulation results suggested that the seeding process and mechanical stimulation were key parameters when engineering large bone tissue volumes. Table 2 summarizes some of other recent studies on computational scaffold design.[195–199]

Despite the recent advancements in computational methods applied to tissue engineering, simulation of the in vitro and in vivo outcomes for osteochondral tissue engineering is still premature. The existing theories of cartilage tissue as well as the experimental data available in literature may allow the development of a biphasic model of the tissue behavior.[200] However, one should ideally consider the constitutive modeling of degenerated cartilage, cartilage growth, tissue differentiation models extended based on biphasic mechano-regulation theory, as well as appropriate cell migration/proliferation models and bone remodeling algorithms to properly simulate tissue differentiation during osteochondral defect repair.[200]

Future directions and conclusions

Designing joint-scale osteochondral constructs is driven by consideration of biomedical need, as well as by the customization of size, maturity, and shape. Biological joint replacement can have a huge impact in joints afflicted with osteoarthritis and on the quality of life of patients, while addressing an unmet clinical need.[201] Most current therapeutic tissue engineering treatments are intended primarily for relatively small defects, and are immature compared to native tissue (Figure 8).[201] However, existing treatments using ACI, M-ACI, and small chondral and osteochondral constructs are incrementally shifting towards larger defects and more phenotypically stable and mature tissues.[201] This is because biomechanically mature grafts could contribute to restoring the mechanical environment of the joint from a chronically abnormal state to a healthier state. It remains to be determined how mature joint-scale constructs should be at the time of implantation.[201] Scientific

investigation and engineering design should also look into the creation of complex tissue shapes, multi-tissue units, specialized tissue interfaces, and bioreactor systems for mechanical stimulation.[201]

The local mechanical environment influences many critical steps during bone healing process. Corroborated mechanobiological models have the potential of improving our understanding of basic biology during bone regeneration, and could help to identify areas that need further investigation.[202] In addition to sufficient nutrients and oxygen supply, appropriate biophysical stimuli are needed in bone scaffolds to favor appropriate tissue differentiation.[188] As for cartilage regeneration, it has been hypothesized that the architecture of cell-seeded scaffolds can be manipulated in order to achieve collagen accumulation throughout the scaffold rather than preferentially in the construct periphery. Although the possibility of incorporating sophisticated designs into engineered tissues for clinical application is an open question, such designs may help to better understand basic chondrocyte mechanobiology.[203] In light of this, many recent computational studies have focused on the role of scaffold design on mechanical properties, porosity and cell growth efficiency for tissue engineering of bone and cartilage.[204]

The bone–cartilage interface in the osteochondral region resists remarkably high shear stresses under in vivo loading conditions and rarely fails.[205] In particular, a stress concentration exists at the tidemark interface between the mineralized articular calcified cartilage (ACC) and the unmineralized hyaline articular cartilage (HAC). A better understanding of load transmission and mechanical properties across the osteochondral region would enable a more efficient engineering of replacement materials.[205] Additive manufacturing (AM) technologies have shown promise in developing scaffolds with optimal architectures for regeneration of multiple tissues within a single construct.[153] Further advancements in fabrication methods would pave the way to creating biomimetic constructs satisfying the load-bearing requirements for osteochondral constructs and successful growth of various tissue types for the treatment of osteochondral defects. In addition, a better understanding of the osteochnodral tissue requirements as well as scaffold architecture-

property relationships could contribute to optimal design of the bone/cartilage interface zone by AM technologies. Despite the advancements in cartilage and bone tissue engineering, the true challenge in osteochondral repair lies in the comprehension of the bone-cartilage interface and its combined yet separate mechanical properties, structure, and biology.[41]

Finally, in designing osteochondral grafts animal studies are considered to be an important validation step. The implantation site has shown to affect the in vivo outcomes of engineered osteochondral constructs.[158] For example, subcutaneous environment differs considerably from the orthotopic environment.[206] This includes the absence of mechanical cues, such as hydrostatic pressure and dynamic compression, which have been shown to influence the endochondral phenotype of MSCs and matrix production.[206–208] Moreover, efficacy-driven guidelines could only be established from prospective, randomized clinical trials.[9] This is primarily due to the highly different biochemical and biomechanical milieu in animal and human joints. It should be noted that young individuals affected by traumatic injuries or by osteochondritis dissecans are the main patient population targeted for the treatment with engineered osteochondral grafts. Therefore, future studies should look into the possibility of extending the same paradigm to the treatment of joint pathologies in the aging population.[9]

Abbreviations

3D: Three-dimensional

3DF: 3D Fiber Deposition

AC: Articular Cartilage

ACC: Articular Calcified Cartilage

ACECM: Articular Cartilage Extracellular Matrix

ACI: Autologous Chondrocyte Implantation

Alg: Alginate

ASC: Adipose-derived Stem Cells

AM: Additive Manufacturing

bBMP: Bovine Bone Morphogenetic Protein

bFGF: Basic Fibroblast Growth Factor

BG: Bioglass$^{®}$

BMDC: Bone-marrow-derived cell

BMP: Bone Morphogenetic Protein

rhBMP-2: Bone Morphogenetic Protein-2

BM-MSC: Bone Marrow Mesenchymal Stem Cell

BMSC: Bone Mesenchymal Stem Cell

CAD: Computer-Aided Design

CG: Collagen type II/Glycosaminoglycan

CGCaP: Collagen type I/Glycosaminoglycan/Calcium Phosphate

CM: Chondrogentic Medium

ECM: Extracellular Matrix

ES: Electrospinning

FEA: Finite Element Analysis

FDM: Fused Deposition Modeling

FGS: Functionally-Graded Scaffolds

GAG: Glycosaminoglycan

rhIGF-1: Insulin-like Growth Factor−1

hMSC: Human Mesenchymal Stem Cell

HA: Hydroxyapatite

HAC: Hyaline Articular Cartilage

ICR: Implanted Cartilage Replacement

ICRS: International Cartilage Repair Society

IGF-1: Insulin-like Growth Factor−1

μ-CT: Microcomputed Tomography

M: Normal Medium

Matrix-induced Autologous Chondrocyte Implantation (M-ACI)

MP: Microparticle

MSC: Mesenchymal Stem Cell

nHA: Nano-hydroxyapatite

OA: Osteoarthritis

OCD: Osteochondritis Dissecans

OM: Osteogenic Medium

OPF: Oligo(poly(ethylene glycol) fumarate)

PA: Polyamide

PA6: Polyamide 6

PCL: Polycaprolactone

PDO: Polydioxanone

PEG: Polyethylene glycol

PLA: Poly(lactic acid)

PLLA: Poly(L-lactic acid)

PLGA: Poly(lactic-co-glycolic acid)

PDLLA: Poly(D,L-lactic acid)

P(DLLA-co-CL): poly(D,L-lactide-co-ε-caprolactone)

PHA: Poly(hydroxyalkanoates)

PLCL: Poly(lactide-co-caprolactone)

POE: Polyorthoesters

PPF: Poly(propylene fumarate)

PVA: Polyvinyl alcohol

SBF: Simulated Body Fluid

SFF: Solid Free-form Fabrication

SLS: Selective Laser Sintering

TCP: Tricalcium Phosphate

TGF-β1: Transforming Growth Factor−β1

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TABLE I. Summary of growth factors used in osteochondral tissue engineering.

Growth factor	Scaffolds	Experimental design	Outcomes
TGF-β1	Hyaluronan–gelatin composite sponge	In vitro using bone marrow mesenchymal progenitor cells	Enhanced type II collagen-rich extracellular matrix production by cells (Angele et al., 1999)[111]
TGF-β3	Injectable oligo (poly(ethylene glycol) fumarate) and gelatin microparticles	In vitro using rabbit marrow mesenchymal stem cells	TGF-β3 significantly stimulated chondrogenic differentiation of MSCs (Guo et al., 2010)[112]
TGF-β1 + IGF-1	Gelatin – PEG scaffolds	In vivo using rabbit osteochondral defect model	Neo-surface repair, surface morphology, cartilage thickness, chondrocyte clustering, and the chondrocyte/glycosaminoglycan production were increased (Holland et al., 2007)[113]
TGF-β2 + BMP-7	Polycaprolactone	In vitro using adipose stem cells	Improved differentiation of adipose stem cells to chondrogenic lineage (Im & Lee, 2010)[114]
BMP-2	Polycaprolactone	In vitro using primary chondrocytes	Promoted cartilage matrix production (Jeong et al., 2012)[115]
TGF-β1 + BMP-2	Poly(D,L-lactic-co-glycolic acid) microspheres	In vivo using rabbit knee defect model	Enhanced production of cartilage layer with high content of glycosaminoglycan content and integration with the surrounding cartilage and underlying bone (Mohan et al., 2011)[116]
rhBMP-2 + rhIGF-1	Polylactic-co-glycolic acid and silk fibroin microspheres in alginate gels	In vitro using human mesenchymal stem cells (hMSCs)	Osteogenic and chondrogenic differentiation were clearly observed (Wang et al., 2009)[117]

TABLE II. Summary of some other recent studies on computational scaffold design.

Investigator	Methods	Results of study
Byrne et al. (2007)[195]	The study used a 3D FEA model of a poroelastic scaffold infiltrated by tissue, and simulated a vertical pressure to test the effects of porosity and dissolution rate of the scaffold on bone formation.	At low loading sites, high porosities and medium dissolution rate resulted in the greatest amount of bone. Lower porosities and dissolution rates were recommended under high loading.
Kelly & Prendergast (2006)[196]	A mechanoregulation model was used to simulate stem cell differentiation and growth within scaffolds exposed to strain and fluid flow. A homogenous linear poroelastic model and an inhomogenous model (chondral and bone phases) were used.	The simulations suggested that optimal stiffness and permeability could be estimated for a scaffold, which could contribute to promoting desired stem cell differentiation.
Khayyeri et al. (2010)[197]	A mechanoregulation model was used to determine stem cell differentiation over time when the scaffold stiffness was varied. Angiogenesis, cell differentiation, and cell migration were taken into account, although scaffold degradation was not.	Larger pore size was beneficial to bone growth and vascularization. Material stiffness from 1 to 1000 kPa did not contribute to tissue differentiation, whereas a stiffness greater than 10 MPa increased bone and cartilage formation.
Milan et al. (2009)[198]	The scaffold architecture was converted into a 3D FEA model via micro-computed tomography (μCT). The model was used to simulate stress response to 5% compression at a 1 s^{-1} strain rate.	The results suggested that applying 5%-compressive loading on the scaffolds generated a shear strain that stimulated osteogenesis (51% of the surface).
Sandino et al. (2010)[199]	FEA model of scaffolds was used to simulate 0.5% and 1% compressive strain with varied states of stem cell preseeding. The study used previously validated mechanoregulation model to account for cell migration, differentiation, and angiogenesis.	Vascularization was predominant in external pores. Compressive strain of 0.5% produced favorable mechanical stimuli within 70% of the pore volume. Increasing the strain to 1% reduced osteogenesis.

FIGURE 1. (A) A healthy joint with normal articular cartilage. The inset image shows four distinct layers of articular cartilage as well as its pericellular, territorial and interterritorial matrices.[25] (B) An osteoarthritic joint that shows partial loss of cartilage, subchondral bone thickening, as well as the alterations in cartilage matrices (inset image).[25] Some of the surgical procedures for the treatment of osteochondral defects include: (C) autologous osteochondral transplantation,[27] (D) autologous chondrocyte implantation,[27] and (E) microfracture.[27] Reproduced with permissions from Nature Publishing Group and Wiley Periodicals.

FIGURE 2. (A) The extracelular matrix surrounding chondrocytes in a healthy articular cartilage, which consists of pericellular, territorial, and interterritorial matrices.[25] (B) The hierarchical structure of bone ranging from the macroscale skeleton to nanoscale collagen (green) and hydroxyapatite (red).[122] (C) Schematic of the fabrication steps for cell-laden biomodal scaffolds produced by additive manufacturing and electrospinning.[126] (D) Top view of a bimodal scaffold composed of microfibers and electrospun nanofibers.[124] (E) Schematic of the mechanism by which nanomaterials may be superior to conventional materials for bone regeneration, through promoting protein adsorption and favoring cell adhesion.[123] Reproduced with permissions from Nature Publishing Group, Royal Society of Chemistry and Elsevier Ltd.

FIGURE 3. Various osteochondral scaffold design approaches.[18] Reproduced with permission from Wiley Periodicals.

FIGURE 4. Lay-down patterns with (A) honeycomb pores and (B) Hilbert recursive curve.[138] Reproduced with permission from Elsevier Ltd.

FIGURE 5. Some multiphasic and gradient-based scaffolds for osteochondral tissue engineering; (A) A fibrin/PCL and a PCL/PCL-TCP scaffold;[11] (B) an agarose/decellularized bone scaffold;[154*] (C) a biphasic scaffold composed of collagen type II -glycosaminoglycan (CG) and mineralized CG (CGCaP);[156] (D) a scaffold made of articular cartilage ECM/hydroxyapatite (HA);[157*] (E) a chitosan-

collagen/β-TCP scaffold;[160] (F) A trilayered scaffold made of 45S5 Bioglass® and alginate;[164] (G) A trilayered scaffold made of bovine decellularized articular cartilage ECM, PLGA/β-TCP wrapped with collagen type I, and a compact PLGA/β-TCP layer as an interface;[165*] (H) A trilayered scaffold made of HA and collagen with different compositions in each layer;[168] (I) a trilayered silk fibroin scaffold loaded with different growth factors in each layer;[175*] (J) schematic of a bioprinting process that makes use of synthetic polymers and hydrogels encapsulating cells and growth factors;[163] (K) schematic of a microfluidic device for generating a gradient-based stem cell-laden hydrogel slab (OM: osteogenic medium, M: normal medium, CM: chondrogenic medium).[178] Reproduced with permissions from Elsevier Ltd., Wiley Periodicals, and IOP Publishing (* denotes Open Access).

FIGURE 6. (A) Simulation of damage distribution at the end of loading for osteochondral and chondral implants for different values of compressive modulus; (B) time-history of damage dissipation energy normalized by surface area of adhesive; (C,D) corresponding results for different values of Poisson's ratio.[1] Reproduced with permission from Elsevier Ltd.

FIGURE 7. Color map of the perfusion stimuli (0.1 mm/s) on surface areas for different scaffold architectures. (a,d,e) gyroid structures; (b,c) hexagonal structures.[193] Reproduced with permission from Elsevier Ltd.

FIGURE 8. Developmental progression of biomimetic tissue engineering therapies for articular cartilage repair. Chondro-Gide®, ChondroCelect®, DeNovo®ET, and NeoCart® are products of Geistlich Pharma AG (Wolhusen, Switzerland), TiGenix (Leuven, Belgium), ISTO Technologies, Inc. (St. Louis, Missouri), and Histogenics Corporation (Waltham, Massachusetts), respectively.[201] The image was reproduced with permission from Elsevier Ltd.
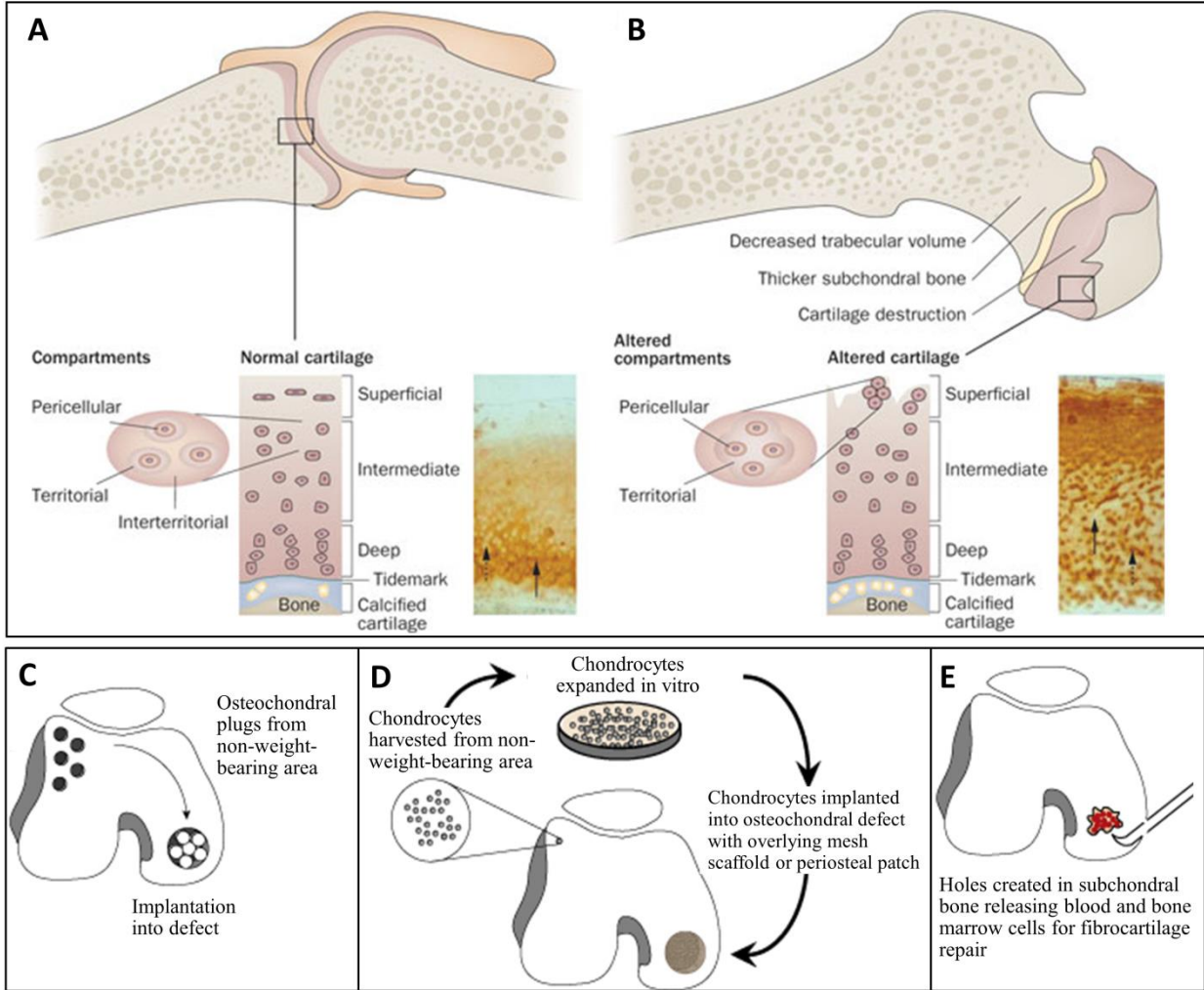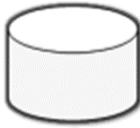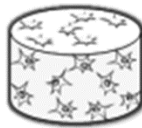
FIGURE 1
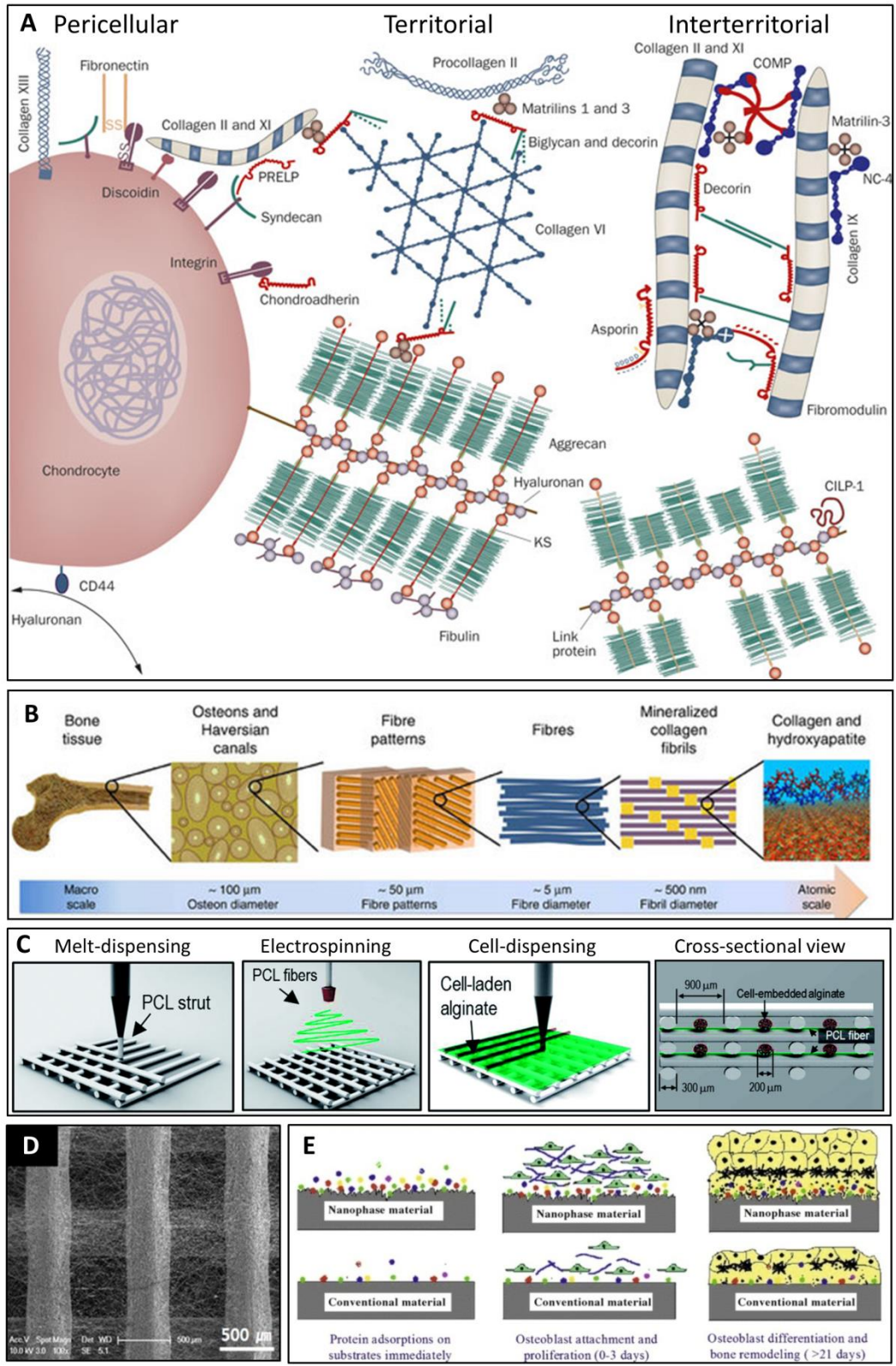
FIGURE 2
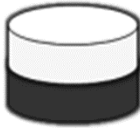
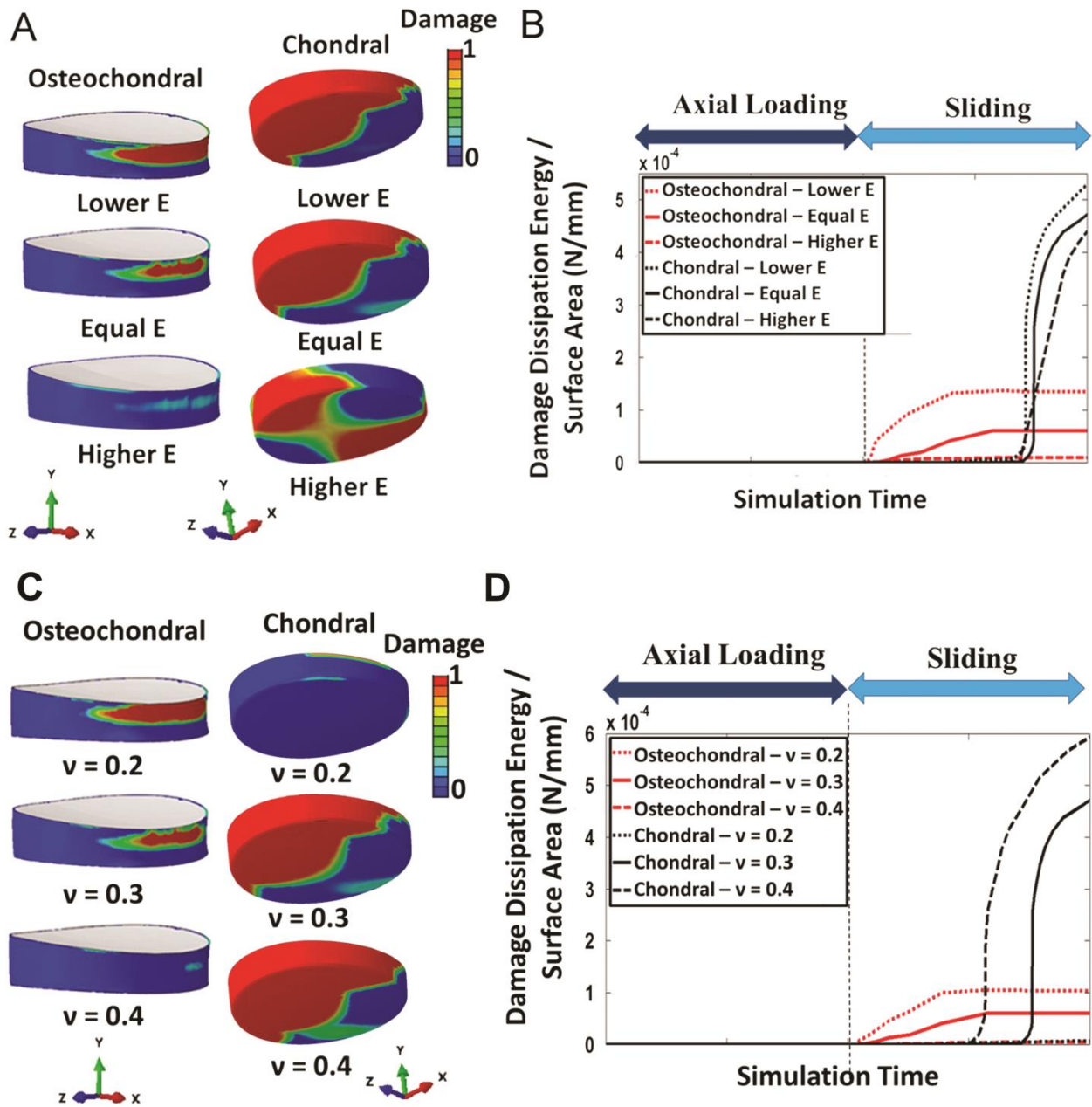|	Physical / Chemical		Cellular / Biological	
Monophasic	One material with one porosity and overall architecture		One cell type with no variation in overall biological environment	
Biphasic	Two different materials or a material with two layers of significantly different porosity, interconnectivity, micro- or macro-architecture		Two different cell types (or one cell type with two different pre-differentiation) or two different biological environment created by the addition of growth factors or bioactive peptides	
Triphasic	Three different materials or a material with three layers of significantly different porosity, interconnectivity, micro- or macro-architecture		Three different cell types (or one cell type with three different pre-differentiation) or three different biological environment created by the addition of growth factors or bioactive peptides	

FIGURE 3
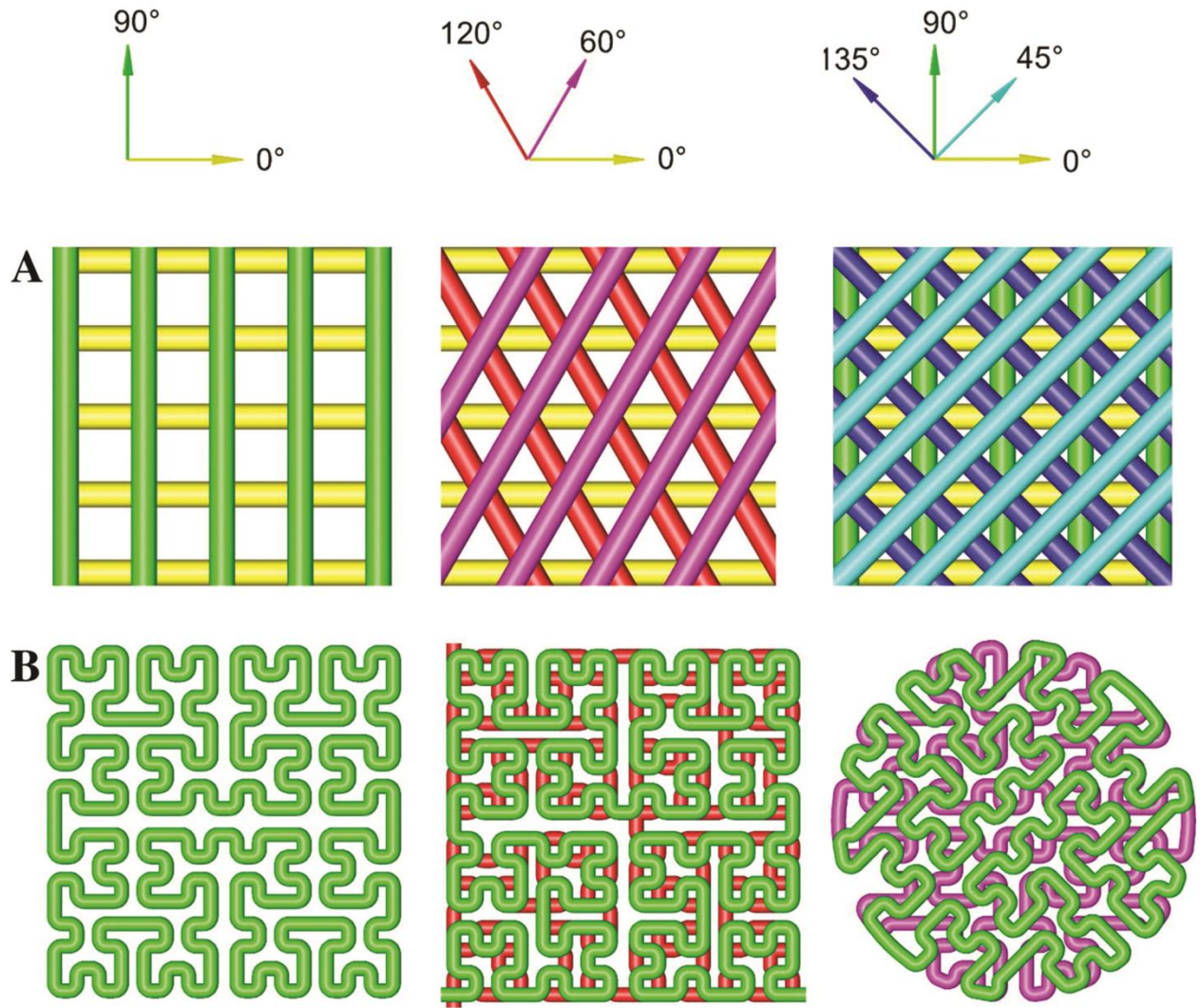
FIGURE 4
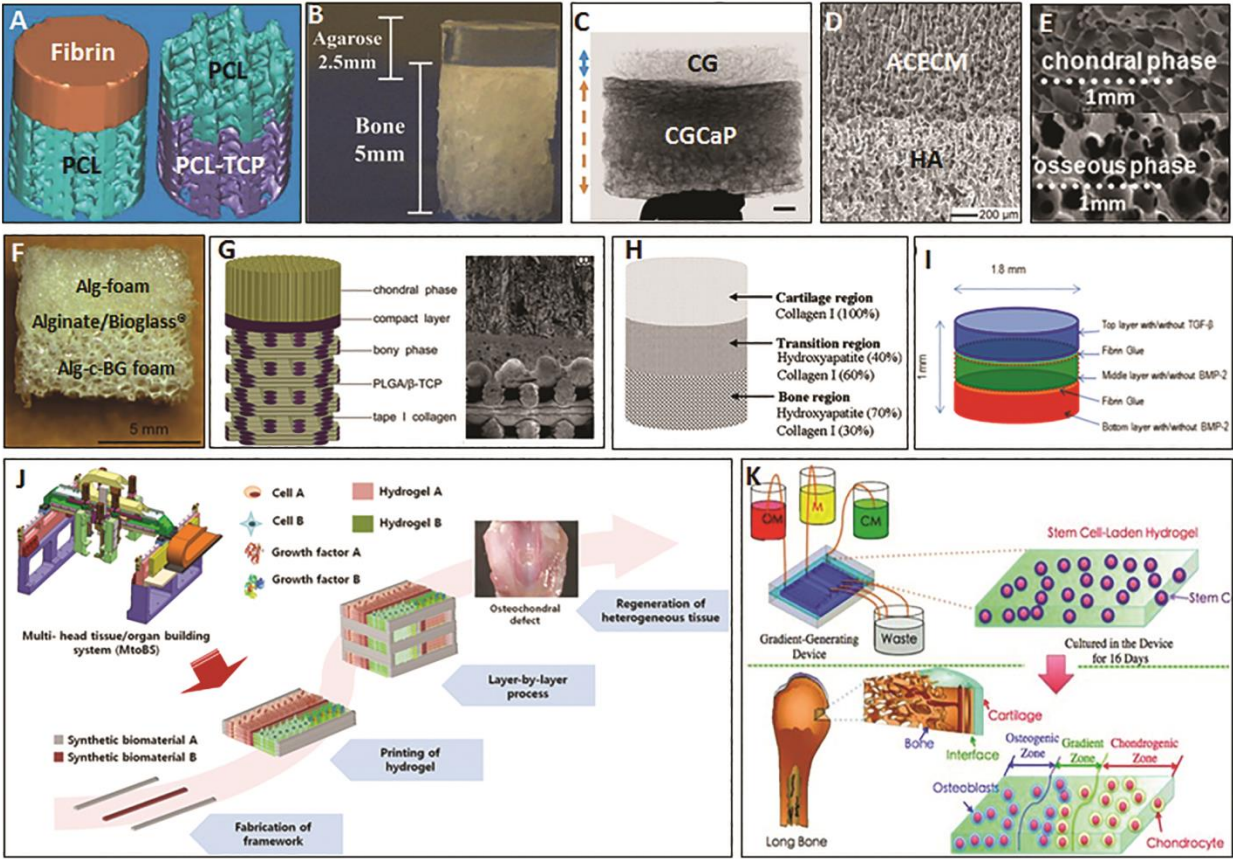
FIGURE 5

FIGURE 6

FIGURE 7

FIGURE 8