Chapter 6
Micro/Nano Scaffolds for Osteochondral Tissue Engineering

Albino Martins, Rui L. Reis, and Nuno M. Neves

Abstract To develop an osteochondral tissue regeneration strategy it is extremely important to take into account the multiscale organization of the natural extracellular matrix. The structure and gradients of organic and inorganic components present in the cartilage and bone tissues must be considered together. Another critical aspect is an efficient interface between both tissues. So far, most of the approaches were focused on the development of multilayer or stratified scaffolds which resemble the structural composition of bone and cartilage, not considering in detail a transitional interface layer. Typically, those scaffolds have been produced by the combined use of two or more processing techniques (microtechnologies and nanotechnologies) and materials (organic and inorganic). A significant number of works was focused on either cartilage or bone, but there is a growing interest in the development of the osteochondral interface and in tissue engineering models of composite constructs that can mimic the cartilage/bone tissues. The few works that give attention to the interface between cartilage and bone, as well as to the biochemical gradients observed at the osteochondral unit, are also herein described.

Keywords Multiscale organization · Multilayer or stratified scaffolds · Biochemical gradients · Osteochondral interface

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6.1 Introduction

The osteochondral (bone to cartilage) interface plays a critical role is the physiology of joints, since it is the anchorage site of hyaline articular cartilage and subchondral bone. In addition, it provides the mechanical structure to support the energy transfer of biomechanical movements from the joint to the skeleton. Unfortunately, damaged osteochondral tissue is difficult to treat due to the poor regenerative capacity of hyaline cartilage. The presence of complex biological and chemical gradients from the cartilage surface to the underlying subchondral bone is also difficult to recover from injury. As a result, interfacial tissue engineering (TE) has focused on overcoming challenges of connecting various dissimilar tissue types in an effort to better match physiological, biomechanical, and biochemical signaling properties [1].

In the development of scaffolds for osteochondral tissue engineering, efforts have been made to develop or improve new or combined processing strategies to obtain repeatable porous constructs with controlled porous morphology, preferably at different scale levels (e.g.. combination of nanoelements and microelements or pores), comprising different materials that are spatially organized and having the capability to deliver relevant molecules such as growth factors in a controlled way [2]. Such scaffolds have been designed to address particular aspects of the osteochondral tissue, namely the vascularization, the deposition of calcium phosphates in predefined regions, the guidance of regeneration certain directions (through gradient delivery of factors or anisotropic porous architecture), the development of different tissues (i.e., osteochondral defects), or the inhibition of calcification and cell adhesion. Recent achievements on the development of osteochondral scaffolds, facing the abovementioned aspects, are described in this book chapter.

6.2 The Multiscale Organization of the Osteochondral Extracellular Matrix

The extracellular matrix (ECM) throughout osteochondral tissue, which is itself secreted and modulated by the encapsulated chondrocytes, presents complex gradients of biochemical cues, such as varying concentrations of glycosaminoglycans and glycoproteins within each region of the tissue, or biophysical (topographical and mechanical) cues, such as nanosized, spatially patterned interactions (with a periodicity of 67 nm) provided by mechanically robust collagen fibers (Fig. 6.1) [3].

Hyaline cartilage is a stratified, multilayered tissue that is anchored to the subchondral bone. Both the phenotype and orientation of the cells (chondrocytes), and the composition and architecture of the extra cellular matrix (ECM) varies substantially along the depth of this complex tissue. The complex architecture throughout the articular cartilage to the subchondral bone interface that constitutes osteochondral tissue spans millimeter (macro) through to nanometer length-scales (Fig. 6.1) [4].

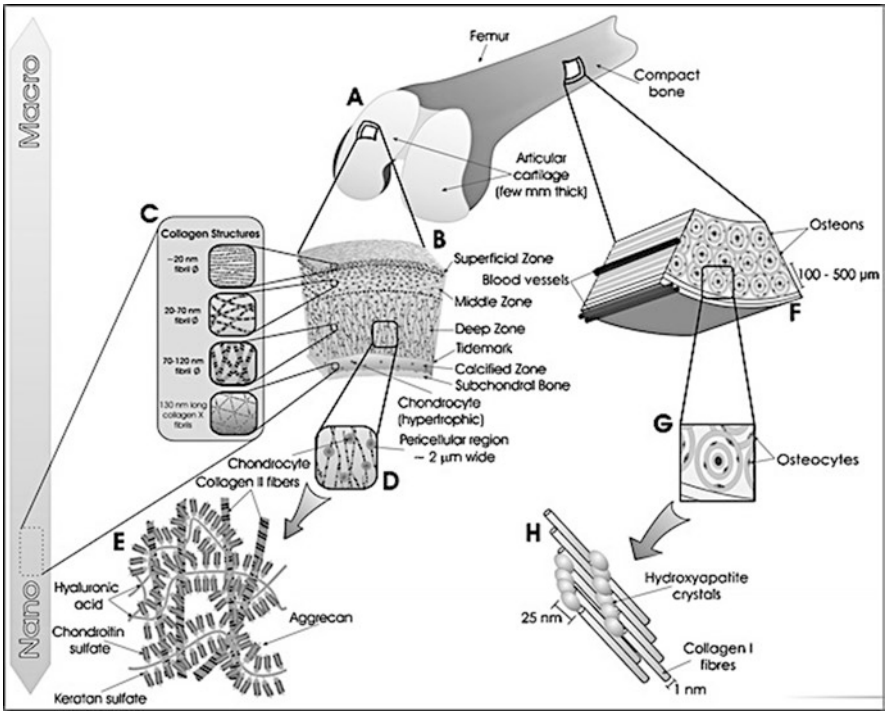

Fig. 6.1 Hierarchical organization of cartilage and bone over different length scales. Articular cartilage forms a wear-resistant, load-bearing surface that covers bone in diarthrodial joints (**a**). It is organized into distinct zones (**b**) where the organization of the collagen structures varies considerably (**c**). Resident chondrocytes (**d**) are surrounded by super-aggregates of aggrecan/hyaluronic acid and macrofibrillar collagen networks (**e**). Bone mineralizes to form a calcified outer compact layer, which comprises many cylindrical Haversian systems or osteons (**f**). The osteocytes within these systems (**g**) are surrounded by the well-defined nanoarchitecture of the ECM—a dense network of aligned collagen I fibers, which provide templates for the self-assembly of hydroxyapatite crystals (**h**) [5]

At the macroscale, adult articular cartilage is a multizonal material in which three layers (i.e., superficial, middle, and deep zones), accounting for different ECM composition, orientation and cell phenotypes, can be distinguished. The uppermost superficial zone of cartilage is characterized by squamous chondrocytes surrounded by collagen fibrils aligning parallel to the articular surface. In the middle/intermediate zone, rounded chondrocytes are embedded in collagen fibrils less organized relative to the surface. In the deep zone, vertical columns of chondrocytes and collagen fibrils are organized perpendicular to the articular surface. The highest concentration of proteoglycans is found in the deep zone [6]. The base of the deep zone displays the *tidemark* that represents the onset of the calcified area, serving as a transitional zone between the soft cartilaginous tissue and underlying hard bone. The calcified area is rich in hydroxyapatite and alkaline phosphatase and poor in chondrocyte number, serves as an interface between the soft cartilage and the subchondral bone and defines a gradient in mechanical properties between these two

tissues [3]. Below the deep zone is the subchondral bone plate. Subchondral bone is a nanocomposite material composed of glycoproteins, such as collagen, laminin, and fibronectin, and hydroxyapatite (HA). Underneath the subchondral bone plate, the subchondral trabecular bone, accounts for a spongy-like structure that is highly vascularized. Trabecular bone is a cellular solid with an interconnected porous structure. The pores have ~1 mm of diameter and the walls (trabeculae) have few micrometres in thickness. The pores appear aligned in the direction of the applied load and the structure is formed by various cell types (i.e., osteocytes, osteoblasts, and osteoclasts), ECM and vasculature (the bone marrow) [3]. Bone is vascularized as well as innervated, and others cells such as neurons and endothelial cells are also present and may play a relevant role in bone biology. In fact, it is generally considered that bone vascularization itself is one of the main reasons for the active self-repair capacity of bone [6].

6.3 Scaffold Properties for Osteochondral Tissue Regeneration

In tissue engineering approaches, to restore function or regenerate tissues, one needs a template—a scaffold—that will act as a temporary matrix for cell proliferation and ECM deposition [7]. Moreover, the scaffold also acts as a template for the neo-tissue vascularization and can actively participate in the regenerative process through the release of growth/differentiation factors [8]. In this sense, a 3D scaffold can influence the structure and development of the engineered tissue [9].

The selection of the most appropriate biomaterial to produce a scaffold for bone, cartilage or osteochondral tissue engineering applications is a very important step. The physicochemical properties of the biomaterial will determine, to a great extent, its choice aiming to target a defined tissue composition. After selecting the adequate biodegradable polymer, the next step is to develop or choose an adequate processing method [10]. The selected processing method should not affect the biomaterials properties and characteristics, namely their biocompatibility or chemical properties. The processing method should be accurate and reproducible, regarding pore size, distribution, and interconnectivity. That means that different scaffold batches should exhibit minimal variations in their properties, when processed from the same set of processing parameters and conditions.

Besides the choice of adequate biomaterials and processing method, the architecture of the processed scaffold is an important factor to take into consideration. Indeed, the macrostructural, microstructural, and nanostructural properties of the biomaterials can modulate biological response and the clinical success of the scaffold. Such properties affect cell adhesion, expansion, and also their gene expression and the preservation of their phenotype [7]. In general, all fabrication technologies aim to incorporate a hierarchical element, often in the form of controlled porosity or aligned structures, to imitate the native tissue spatial architecture [11].

Scaffolds should be biocompatible, well integrated in the host's tissue without eliciting an immune response [12]. Scaffolds must possess an open pore, fully interconnected geometry in a highly porous structure with large surface area. This will allow cell in-growth, an accurate cell distribution throughout the porous structure, and will allow the neovascularization of the construct [13]. Porosity and interconnectivity are also important for an accurate diffusion of nutrients, gases and to remove metabolic waste resulting from the cell metabolism. This is of particular importance regarding bone tissue engineering, particularly due to the high rates of mass transfer, even under in vitro culture conditions [14]. The porosity always influences other properties of the scaffolds such as the mechanical stability. This property should always be balanced with the mechanical needs of the particular tissue that is going to be regenerated. Adequate pore size is also important since if the pores employed are too small, pore occlusion by the cells may happen. This will allow cellular penetration, ECM production and neovascularization of the inner areas of the scaffold [15].

The surface properties, both chemical and topographical, can control and affect cellular adhesion and proliferation [16]. Chemical properties are related with the ability of cells to adhere to the biomaterial, as well as with the protein adsorption. Topographical properties are of particular interest when the topic is osteoconduction. The scaffold should also be osteoinductivity that is able to support formation of bone within and/or upon the scaffold [17].

The mechanical properties and biodegradability also have an important role. In vitro, the scaffolds should have adequate mechanical strength to withstand the hydrostatic pressures and to maintain the spaces required for cell in-growth and matrix production. In vivo, and because cartilage and bone tissue are always under continuous stress, the mechanical properties of the implanted construct should ideally be compatible with those of living cartilage and bone, so that an early mobilization of the injured site can be possible. Furthermore, the scaffolds degradation rate must be appropriate to the growth rate of the neotissue, in such a way that by the time the injury site is totally regenerated the scaffold is totally degraded [18].

6.4 Micro/Nano Scaffolds for Bone Tissue, Envisioning Osteochondral Regeneration

Recently, additive manufacturing techniques have been employed to develop hierarchical and functionally graded scaffolds. These technologies are characterized by reproducible and highly organized microarchitecture with patient-specific geometry through precise control over scaffold design and structure (porosity, pore size, and interconnectivity), while allowing for the incorporation of bioactive factors rendering the fabricated scaffolds more biomimetic [19].

The incorporation of microscale and nanoscale features on a same scaffold can improve both the mechanical properties and tissue regeneration, through toughening mechanics and better cell adhesion, respectively. Particularly, the multiscale network

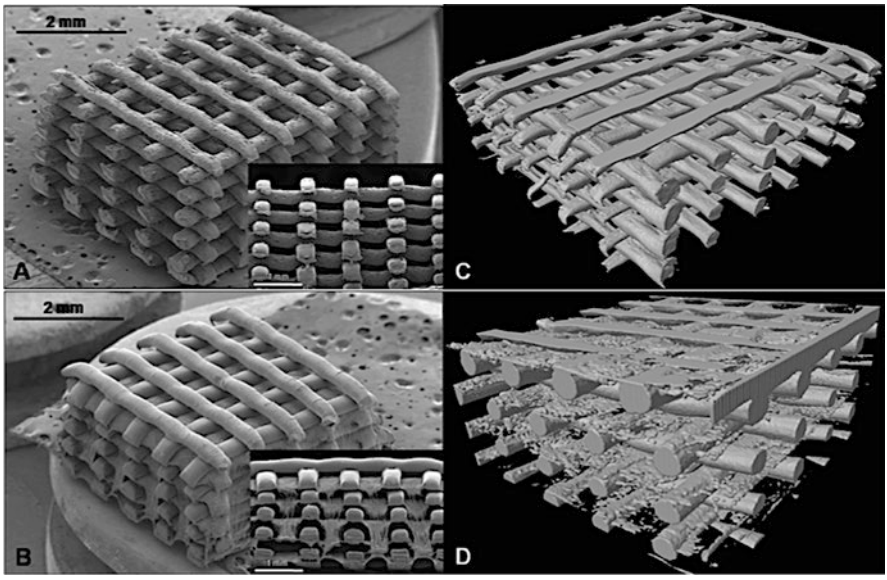

Fig. 6.2 SEM and micron computed tomography analysis of the starch-based rapid prototyped (**a**, **c**) and hierarchical fibrous scaffolds (**b**, **d**). Reproduced with permission from [21]

observed in natural ECMs can be fabricated by the combination of additive manufacturing (AM) and electrospinning (ES) techniques to produce bimodal scaffolds [20]. The resultant multiscale scaffold contained large pore size essential for cell and mass transportation, while the fibrous component provided suitable structures for cell attachment. Moreover, while the 3D rapid prototype scaffold provides structural integrity and mechanical properties, the micro–nano scale of the electrospun fibers mimic the biophysical structure of natural ECM (Fig. 6.2) [21]. Biological results, when human osteobastic cells were dynamically seeded on these hierarchical fibrous scaffolds, showed significantly higher proliferation and maturation. Particularly, scanning electron microscopy (SEM) observation demonstrated that the osteoblastic cells preferentially adhered and spread on the electrospun NFMs, constituting an innovative strategy to enhance cell seeding efficiency/cell adhesion into the microfibrous scaffolds. In a complementary approach, the same hierarchical fibrous scaffolds were able to provide a favorable environment for the proliferation and osteogenic differentiation of human Wharton's jelly derived stem cells [22]. Biochemical data demonstrated that these constructs were in an early mineralization process, because of a significant higher fold change of osteogenic genes typically expressed in the mineralization phase, as well as the identification of calcium and phosphorous elements.

The combination of electrospun nanofibers with microscale to macroscale fibers, processed by other polymer processing techniques (i.e., wet-spinning and fiber extrusion), was been explored by our research group. In a first and simplest approach, electrospun nanofibers were directly deposited over a prefabricated wet-spun microfibrous scaffold [23, 24]. This combined structure was obtained by a two-step methodology

and structurally consist of a nano-network incorporated on a macro-fibrous support. Its biological functionality was demonstrated by the culturing of human osteoblast-like cells, bone marrow stromal cells, and endothelial cells (i.e., human umbilical vein endothelial cells and microvascular endothelial cells) [24–26]. This micro/nano structure was developed to mimic the highly organized fibrous structure of bone tissue, not forgetting the vascular network that is identified as the main pitfall in bone tissue engineering and the major hurdle for the clinical application of engineered constructs.

With the intent to reproduce not only the multiscale organization of osteochondral tissue, but also its organic–inorganic composition, it was proposed the development of biphasic scaffolds comprising a polycaprolactone (PCL) cartilage phase and a PCL-tricalcium phosphate (TCP) matrix that served as the bone component. The scaffolds were built using the fused deposition modeling (FDM) process, seeded with mesenchymal stem cells (MSCs) via fibrin encapsulation, and patched with a 20% PCL-collagen electrospun mesh to prevent cell loss and facilitate the diffusion of nutrients from the synovial space [27]. Implantation of such scaffold in a critical size defect, which was created in the medial condyle of the rabbit 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. Furthermore, besides the implant structure and composition, the implantation site appeared to affect the in vivo outcomes (medial condyle vs. patellar groove).

In a similar attempt to create a biphasic scaffold, with a bone and periodontal compartment, FDM was used in addition to an in-house developed melt electrospinning device [28]. Medical grade PCL-TCP membrane scaffolds, acting as the bone compartment, were fabricated using FDM and then coated with calcium phosphate (CaP), while the periodontal compartment was electrospun through a melt electrospinning device. A biphasic scaffold was then assembled by compressing a partially fused CaP-coated bone compartment (FDM scaffold) onto a periodontal compartment (melt electrospun mesh). Subcutaneous implantation of the biphasic scaffold in rats confirmed tissue integration between both compartments, forming a tissue structurally resembling native periodontal tissues, establishing high levels of vascularization and tissue orientation in both bone and periodontal compartments. Despite the dissimilarity between potential tissue engineering applications, this work presents a relevant approach on the development of complex tissues adjacent to bone, such as the osteochondral tissue.

6.5 Micro/Nano Scaffolds for Cartilage Tissue, Envisioning Osteochondral Regeneration

So far, most part of these stratified scaffolds were developed envisioning their application in the regeneration of bone tissue. However, the hierarchical organization of collagen fibers is cartilage has been also addressed by the combination of AM with ES. The first approaches on producing 3D micro–nano fibrous scaffolds involve the

intercalation of electrospun nanofibers, prefabricated or deposited on time, in between rapid prototyped microfibers [29–32]. Briefly, PCL or PCL/collagen nanofiber meshes were directly electrospun over rapid prototyped (i.e., by 3D plotting or by direct polymer melt deposition) microfibers, during shorter deposition periods. Cell culture experiments demonstrated the preferential adhesion of bovine or porcine primary chondrocytes to the electrospun nanofiber matrices, as well as a statistically significant increment of cell proliferation on the micro–nano fibrous scaffolds.

In another attempt, nanostructured porous polycaprolactone (NSP-PCL) scaffold were produced by the combination of rapid prototyping and thermally induced phase separation methods [33]. The NSP-PCL scaffold expresses macro, micro, and nanopores to benefit mechanical strength, chondrocyte adherence, viability, and differentiation. When implanted in an osteochondral rabbit model, the NSP-PCL scaffold design promotes cartilage ingrowth, but not bone ingrowth.

Keeping the combined use of FDM and ES, a multiphasic scaffold was developed comprising a biphasic PCL scaffold which pores were filled with a 2% alginate hydrogel [34]. To integrate the alginate and PCL components, the alginate hydrogel was partially decrosslinked and press-fitted on top of the biphasic scaffold, which enabled alginate to partially infiltrate the pores of the PCL-FDM scaffolds, and then recrosslinked. 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.

Another example is the alternation of electrospun PCL fibers with 3D inkjet printing of rabbit chondrocytes in a fibrin–collagen hydrogel, which resulted in 1 mm thick five-layer tissue constructs [35]. The hybrid scaffold demonstrated enhanced mechanical properties compared to conventional hydrogel constructs generated using inkjet printing alone. Furthermore, these tissue constructs produced cartilage-specific ECM both in vitro and in vivo (subcutaneous implantation in immunodeficient mice), as evidenced by the deposition of type II collagen and glycosaminoglycans. This work demonstrated that the combination of controllable scaffold properties with a cell delivery printing process would enable the production of highly functional tissue constructs.

6.6 Micro/Nano Scaffolds for Osteochondral Regeneration

Despite the large amount of works reporting the development of micro–nano scaffolds for bone or cartilage tissue engineering approaches, few works addressed the repair of osteochondral defects. Scaffolds targeting the repair of full-thickness osteochondral tissue have combined diverse types of materials such as hydrogels or porous sponges, mimicking the “articular cartilage region,” with porous or fibrous rigid scaffolds (made from polymeric or inorganic ceramic-type materials (or combinations of both)) to mimic the “bone region” [36]. As an example, porous bilayered scaffolds produced by freeze-drying and salt leaching techniques, and built up by fully integrating a silk fibroin (SF) layer and a silk-nanoCaP layer, were

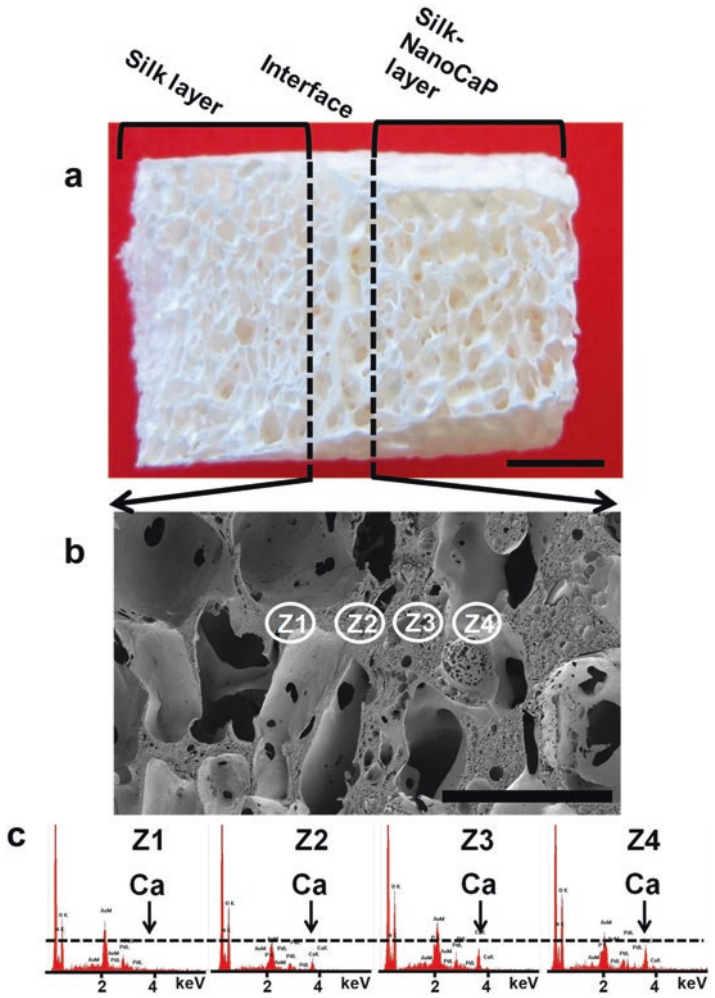

Fig. 6.3 The interface of the bilayered scaffolds. (**a**) Macroscopic image of the bilayered scaffolds (scale bar: 3 mm). (**b**) SEM image of the interface region in the bilayered scaffold (scale bar: 500 μm). Z1, Z2, Z3, and Z4 indicate different regions from the silk layer to the silk-nanoCaP layer, around the interface area. (**c**) EDX elemental analysis of calcium ions in Z1, Z2, Z3, and Z4 regions [37]

developed for osteochondral tissue regeneration (Fig. 6.3) [37]. The silk-nanoCaP layer of the bilayered scaffolds promoted better osteogenesis differentiation of rabbit bone marrow mesenchymal stromal cells under osteogenic conditions as compared with the SF layer. Furthermore, these scaffolds allowed tissue ingrowth and induced only a very weak foreign body reaction when subcutaneously implanted in rabbit. When implanted in a rabbit knee critical defect, the bilayered scaffolds supported cartilage regeneration in the top silk layer, and encouraged large amounts of

subchondral bone ingrowth and angiogenesis in the bottom silk-nanoCaP layer. Using a different processing approach, taking advantage of the sol-derived 70S bioactive glass and of silk fibroin (Indian non-mulberry *Antheraea assama*), a bilayer electrospun mats were proposed to the repair of osteochondral defects [38]. In vitro biological studies revealed that the biphasic mats presented spatial confinement for the growth and maturation of both osteoblasts (MG63 cell line) and chondrocytes (primary porcine ear-derived chondrocytes).

Despite these particular studies based on the use of silk fibroin as biomaterial scaffold, most of the reports relies on the use of additive manufacturing techniques for the production of 3D osteochondral scaffolds [39]. The addition of multiple printing techniques and novel scaffold designs may give rise to advanced 3D printing technologies capable of fabricating higher quality scaffolds for cartilage tissue engineering. Specifically, a multi-head tissue/organ building system (MtoBS) that enabled dispensing of biologically relevant biomaterials, such as PCL and alginate hydrogel, was developed to manufacture 3D tissue and organs [40]. Envisioning the building of an osteochondral tissue, PCL and two alginate solutions with osteoblastic and chondrocytic cell lines were sequentially dispensed, keeping their viability up to 7 days. Considering these promising results, the MtoBS, which overcomes the drawbacks of current cell printing technology, constitutes an interesting method for dispensing multiple cells and biomaterials for heterogeneous tissue regeneration.

Another approach to generate osteochondral scaffolds also relies on the combination of novel nano-inks, composed of organic (i.e., chondrogenic transforming growth-factor beta 1) and inorganic (i.e., nanocrystalline hydroxyapatite) bioactive factors, with advanced tabletop stereolithography 3D printing technology [41]. A series of hierarchical constructs were successfully fabricated which closely mimic the native 3D extracellular environment, with nanocomponents, microarchitecture, and spatiotemporal controlled release of bioactive cues [1]. Experimental data demonstrated that these osteochondral scaffolds promote human bone marrow-derived MSCs adhesion, proliferation, and osteo and chondral differentiation. Also with the attempt to control the release kinetics of transforming growth factor-β3 (TGF-β3) and/or insulin-like growth factor-1 (IGF-1), biodegradable bilayered oligo (poly(ethylene glycol) fumarate) composite hydrogels were developed aiming to mimics the distinctive hierarchical structure of native osteochondral tissue [42]. It was achieved higher amounts of active TGF-β3 released when it was incorporated with gelatin microparticles, as compared to gel phase loading. Single delivery of IGF-1 showed higher scores in subchondral bone morphology, as well as chondrocyte and glycosaminoglycan amount in adjacent cartilage tissue of a rabbit full-thickness osteochondral defect model after 12 weeks, when compared to a dual delivery of IGF-1 and TGF-β3. The lack of synergy between IGF-1 and TGF-β3, regardless of TGF-β3 release kinetics, demonstrates that the dual delivery of GFs does not necessarily confer an improved healing response over the single delivery of GFs in vivo.

In another work that combines 3D bioprinting with multi-nozzle electrospinning, osteochondral scaffolds with multiscale structures are capable of controlling release of multiple biomolecules, namely gentamycin sulfate (GS) and desferoxamine (DFO) [43]. Blend electrospun GS/polyvinyl alcohol (PVA) and coaxial electros-

pun core PVA-DFO/shell PCL fibers were deposited in between gelatin/sodium alginate struts. The composite scaffold showed its potential to delivery multiple biomolecules with various release profiles over space and time, achieving functional gradient osteochondral scaffolds. In another dual-release approach, a hybrid twin-screw extrusion and electrospinning process was developed for generating osteochondral tissue engineering scaffolds with controlled gradations of concentrations of insulin and β-GP) [44]. In this demonstrative study, the concentration of insulin increased from one side of scaffold to the other, whereas β-GP phosphate concentration decreased. The use of both insulin and β-GP at graded concentrations led to the differentiation of human adipose-derived stromal cells (ADSCs) in a location-dependent manner: higher chondrocytic cell counts and increasing total collagen deposition with increasing concentration of insulin, and different extents of mineralization generated by the β-GP concentration distribution.

Very recently, a biomimetic osteochondral scaffold with continuous multilayer architecture and gradient composition, made of PCL and hydroxyapatite (HA)/PCL microspheres, was also produced via selective laser sintering technique [45]. In vitro and a rabbit osteochondral defect model demonstrated that the multilayer scaffold could successfully induce the formation of multiple tissue types, including articular cartilage and subchondral bone. Due to its controllable forming process, flexible structural design, tailored composition and tunable biomechanical properties, this multilayer scaffold provides a successful platform for the enhanced repair of osteochondral defects.

6.7 Concluding Remarks and Future Trends

The repair of osteochondral defects requires a tissue engineering approach that aims at mimicking the physiological properties and structure of two different tissues (i.e., cartilage and bone) using specifically designed scaffold–cell constructs [39]. Furthermore, the transitional zone between these two tissues, i.e., the tidemark, should also be considered in this approach. While polymeric (or even composite) materials offer many possibilities to the field of tissue engineering, they inherently lack the plethora of biological cues provided by the native tissue microenvironment, through cell–ECM and cell–cell communication that facilitate tissue remodeling and repair.

Engineering interactions between culturing cells and biomaterial scaffolds (the "interactome") through mimicry of the hierarchical nature of the native ECM is potentially of great relevance to eliciting control over the molecular and structural cues capable of determining cell fate decisions (e.g., cell migration, proliferation, differentiation, and apoptosis) and neo-tissue formation to achieve functional osteochondral tissue repair [3]. Therefore, in the recent past, multilayered or stratified scaffolds targeting osteochondral repair consisted of only two distinct zones resembling the bone–cartilage interface either chemically, mechanically, or structurally [36]. There are multiple ways to achieve stratification and gradient-based composition. One sim-

ple approach is to build composite scaffolds through multilayered scaffold design, to generate structural templates for the cartilaginous layer, the tidemark and calcified cartilage, and the subchondral bone [46]. Such complex but necessary structure is usually accomplished by using two or more different materials. However, these approaches lacked the ability to mimic the architecture of articular cartilage, leading to isotropic cartilaginous tissues that fail to resemble the structure and depth-dependent characteristic of native tissue, and consequently, its mechanical properties.

The scientific literature has shown that the structural stratification alone is not sufficient for establishing effective transition between two tissues as different as cartilage and bone, prompting the need to also establish biochemical gradients, particularly in the interface region [47]. Such biochemical gradients can be achieved by embedding the growth factors, non-growth factor inductive agents (e.g., hydroxyapatite) and other signaling molecules (therapeutic drugs, genes) into the scaffold. Indeed, osteochondral scaffolds are being designed to facilitate tissue-specific growth-factor delivery, mimic connective tissue ECM, be chondroinductive or osteoinductive, and recapitulate the stratified nature of the osteochondral tissue through multiphasic designs [36].

Despite the success of numerous proof-of-concept studies, it is not clear from an accumulation of successes and failure paradigms that the herein described approaches recapitulate the intricate hierarchical organizations of physical structures found in native ECM environments [11]. From our perspective, the successful development of an osteochondral tissue engineering strategy is dependent on the specific biological mechanisms under investigation, which determine the level of complexity. This may vary from the simplest coculture systems to complex bioreactors to generate close-to-native osteochondral constructs, which may have the capability of incorporating other joint tissues, such as the vasculature. Therefore, future attempts to replicate the biological organization of cartilage interfaced with bone may be achieved by recapitulating in vitro key aspects of the in vivo developmental biology.

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