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TISSUE ENGINEERING AND REGENERATIVE MEDICINE

Concise Review: Biomimetic Functionalization of Biomaterials to Stimulate the Endogenous Healing Process of Cartilage and Bone Tissue

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ABSTRACT

Musculoskeletal reconstruction is an ongoing challenge for surgeons as it is required for one out of five patients undergoing surgery. In the past three decades, through the close collaboration between clinicians and basic scientists, several regenerative strategies have been proposed. These have emerged from interdisciplinary approaches that bridge tissue engineering with material science, physiology, and cell biology. The paradigm behind tissue engineering is to achieve regeneration and functional recovery using stem cells, bioactive molecules, or supporting materials. Although plenty of preclinical solutions for bone and cartilage have been presented, only a few platforms have been able to move from the bench to the bedside. In this review, we highlight the limitations of musculoskeletal regeneration and summarize the most relevant acellular tissue engineering approaches. We focus on the strategies that could be most effectively translate in clinical practice and reflect on contemporary and cutting-edge regenerative strategies in surgery. STEM CELLS TRANSLATIONAL MEDICINE *2017;6:2186–2196*

SIGNIFICANCE STATEMENT

Musculoskeletal tissue engineering has been proposed as an innovative therapeutic approach aiming at replacing lost or severely damaged bone, cartilage, ligaments, and tendons. Although 30 years have passed since the field was born, the clinical solutions for musculoskeletal regeneration are still not sufficient to fulfill the demands of the many patients. In fact, only few tissue engineering platforms have been successfully translated to humans. This review gives a brief overview of the current needs of musculoskeletal tissue engineering in order to speed up the transition from "bench to bedside" emphasizing the essential need for a close collaboration between clinicians and tissue engineers.

INTRODUCTION

Amphibians, the most regenerative vertebrates, are capable of limb regeneration after amputation through the formation of a structure called a blastema. The blastema forms through the degradation of the extracellular matrix (ECM) and the subsequent re-organization of the regenerative tissues driven by dedifferentiated cells, along with satellite cells released from skeletal muscle [1]. The blastema grows rapidly by mitosis and its cells re-differentiate into the original tissue pattern [2]. Although mammals do not seem to be able to activate this process in tissue regeneration [3], an endogenous regenerative potential still exists. In all tissues there is a resident population of stem and progenitor cells programmed to respond to certain stimuli to produce replacement cells [4]. These stem cells have been exploited therapeutically with different approaches, especially to boost the repair of bone and cartilage [5].

Stem cells, however, are not the only essential components to restoring mammalian tissues. The ECM is the other integral player that not only offers physical support to the cells, but also serves as a plastic and dynamic structure that controls and directs tissue homeostasis [6]. Furthermore, the ECM is part of a dynamic environment called a *niche* that provides biochemical and biomechanical cues that allow stem cells' survival and define their identity [7]. Depending on external stimuli, the niche finely regulates state of quiescence, self-renewal or active differentiation of stem cells. Moreover, the niche also has an important "shield role" as it protects the residing stem cells from gene mutations, preventing their transformation into cancer cells.

Given these main roles, an ECM that properly responds to the environment could improve tissue restoration. For decades, tissue engineers have tried to develop a stem cell niche replacement by designing and fabricating the "perfect ECM" to improve tissue regeneration.

This review discusses recent developments in biomaterial designs that aim to stimulate the endogenous healing potential of an organism in order to achieve tissue restoration. We will explore the tissue-specific regenerative limitation of musculoskeletal system and highlight different regenerative strategies focusing on cell-free approaches. We will focus our attention particularly on biomimetic tissue engineering strategies aimed toward guiding the endogenous regenerative niche of musculoskeletal tissues [8].

Endogenous Regenerative Potential of Bone

Bone innately regenerates following trauma, typically without scarring. This mainly happens due to a powerful stem cell niche present within the bone [9]. As we have previously anticipated, stem cell niches are not only defined by the cells that inhabit them, but also by the ECM, as the latter plays an important role in the cellular commitment. The protein and proteoglycan composition of a stem cell niche ECM is tissue-specific and differentially modulates the stem cells behavior. It does this through interaction with growth factors (GFs) and bioactive molecules that ultimately determine the fate of the cells [10]. Mesenchymal stem cells (MSCs) are the main players of musculoskeletal tissue building, from bone formation in the embryo to fracture repair and remodeling in an adult [11]. MSCs differentiate toward bone, cartilage, tendon, ligament, and muscle [12, 13]. MSCs can be recruited from multiple tissues surrounding the site of injury including the bone marrow or from other stem cells niches found in compact bone [14, 15], muscle [16] tendons' cuffs [17], and periosteum [18]. However, MSCs alone are not sufficient to repair injured bone, the healing is a regulated process in which resident stromal, progenitor, and inflammatory cells orchestrate a complex signaling cascade that leads to repair and remodeling [19, 20]. If bone can regenerate itself, what are the limitations in bone healing and why do we need tissue engineering strategies to improve bone repair? While we cannot isolate a unique effector for bone healing, we can exploit different strategies that rely either on stem or on inflammatory cells to improve bone regeneration [21].

Bone Regeneration Limitations

Although bone remains one of the most-regenerative tissue, there are still many different clinical conditions that require therapeutic support to improve bone repair [9]. The size of the fracture could be so large (i.e., critical size defects) that it results in the physical loss of stem cell niche sites. This type of injury is so severe that even autologous or heterologous grafts would not be sufficient to repair it [22]. The ability of bone to regenerate can be also compromised when the complex repair mechanisms become insufficient either at the fracture site or, more frequently, at the systemic level. An essential aspect to bone regeneration is the inflammatory response and, as a consequence, organisms with compromised immune systems have impaired bone regeneration [21]. Diabetic, immunocompromised, or elderly patients represent a high percentage of the Western countries' populations and are categories that could experience bone regeneration impairment [21].

Biomimetic Approach in Bone Repair

Bone tissue engineering (BTE) platforms have been proposed as a valid alternative to bone grafts. BTE offers several advantages: reduced disease transmission, lower risk of infection or immunogenicity, as well as implant personalization and limitless availability. Although there have been almost three decades of investigations, only few products have been translated to clinical practice [23]. One of the reasons is the complexity of the devices and scaffolds proposed. They serve as a temporary ECM for bone growth and provide specific environment and architectures according to the tissues to be repaired. In addition, they can be combined with drugs such as GFs, bioactive molecules, or antibiotics. The design iterations of a BTE platform have to take into account several variables: (a) the type of materials, (b) the source of chosen materials, (c) the materials' functionalization, and (d) the eventual combination with bioactive molecules/drugs. Moreover, another variable is the possibility to combine the BTE with cells from different sources. All of these iterations are very exciting from an engineering perspective, but they can easily become detrimental when it comes to the regulatory process toward clinical approval.

To speed up the translation process, one strategy could be used to simplify the synthesis and composition of the BTE platform, therefore limiting the associated risks. However, this task is quite difficult to perform. Reducing the variables in the manufacturing and makeup of the device could help, but is it possible to enhance bone regeneration without including cells and osteoinductive factors? In order to achieve efficient bone deposition, the scaffold should be able to support the recruitment, proliferation, and differentiation of infiltrating cells. It should also allow for the formation of new blood vessels throughout the defect, facilitate the deposition on new bone, allow integration with surrounding native tissues, and promote long-term remodeling of the injured site. This list of requirements is extensive and very complex, but an effective solution could be found in nature. Nature often gives the best and simple solutions to complex problems. Biomimicry (the imitation of nature) has significantly contributed to recent advances in biomedical research. The field of tissue engineering has been populated by biomimetic solutions aiming to restore the shape, composition, and finally function of damaged or lost tissue [24].

Biomimetic approaches in BTE have taken cues from the native bone structure to develop bioactive scaffolds [25]. Biomimicry in BTE could be extended to different aspects of bone structure/function including composition, architecture, mechanical properties, and bioactivity.

Mimicking Bone Compositions Through Composite Materials

Human bone is composed of 10%–20% collagen, 60%–70% minerals, 9%–20% water, and small quantities of other proteins, lipids, and polysaccharides [26]. Overall, bone ECM is a complex inorganic–organic nanocomposite material, in which hydroxyapatite [HA, Ca10(PO4)6(OH)2] nanocrystallites and a collagen network are organized in a hierarchical structure [27, 28]. For this reason many BTE biomaterials are mainly fabricated using this structure as template (Fig. 1). However, the most used strategy that became a design principle in biomimetic BTE is the combination between

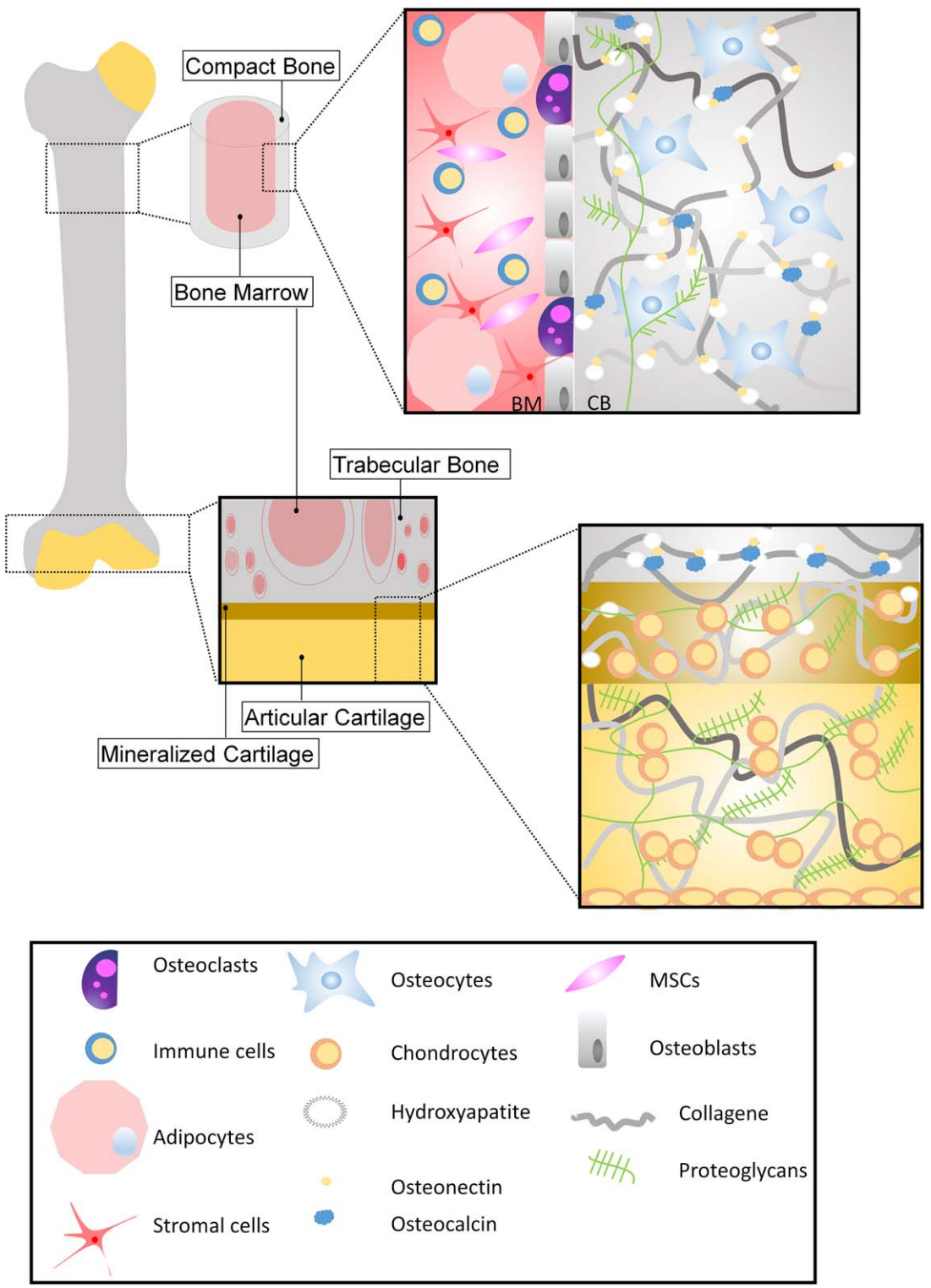

Figure 1. Bone and cartilage structure. The schematic summarizes the architecture of the extracellular matrix (ECM) of bone and cartilage of a long bone. Bone tissue consists mostly of ECM. ECM is composed of osteoid, which represents the organic matrix composed of type I collagen, proteoglycans, and hydroxyapatite, a calcium salt crystal. Cellular components are basically three types of cells: osteoblasts, osteocytes, and osteoclasts. Osteoblasts synthesize matrix and are responsible for its mineralization. They are directly derived from MSCs. Osteocytes are inactive osteoblasts trapped within bone ECM. Osteoclasts are derived from monocytes and they activate bone resorbtion in the continuous remodeling process. Bone marrow is a spongy tissue present in the hollow spaces of bones and consist mainly of hematopoietic, stem, immune, and adipose cells. Articular cartilage is composed of a dense ECM with a bare distribution of chondrocytes. The ECM is principally composed of collagen (type II mostly), and proteoglycans. Articular cartilage is progressively mineralized—like bone matrix—at the junction between cartilage and bone. Abbreviation: MSCs, mesenchymal stem cells.

scaffolds with different GFs [28]. Bone morphogenetic proteins, for example, are the most important GFs in bone restoration and have been clinically used to enhance bone healing for over a decade. Nevertheless, the combination of GFs on the scaffold results in a longer regulatory pathway due to the use of molecules with a biological effect. Moreover, the use of GFs sometimes resulted in some drawbacks due to the uncontrolled release kinetics [29, 30].

As previously anticipated, several mineral phase platforms, with similar compositions to native bone, have been shown to be highly bioactive in vivo without the use of GFs [31–33]. More efficient strategies aim toward reproducing both the organic and mineral components of bone ECM [34]. Both organic and mineral phases have been chosen over a variety of combinations. For instance, the organic phase could be either proteins (collagen/gelatin/chitosan/silk) or small peptides (collagen-mimetic), while other approaches utilize synthetic polymers (polylactide-co-glycolide (PLGA), polycaprolactone (PCL)). At the same time the mineral phase, represented by hydroxyapatite (HA), could vary depending on ion substitutions. Several BTE strategies induced in vivo bone formation with neither GFs nor stem cells in ectopic sites mostly due to the presence of different HA forms. HAs are often, calcium-deficient apatites with many di- and tri-valent ion substitutions (e.g., Zn^{2+}, Sr^{2+}, Mg^{2+}, Mn^{2+}, and CO_3^{2-} ions) [35]. HA—and its functionalization—was shown to be highly osteoinductive in different animal models, even if the exact mechanisms of osteoinduction are still relatively unknown [36–38]. Zinc substituted HA (Zn^{2+}) has been used in combination with collagen scaffolds to induce bone formation both in vitro and in vivo [39], together with its antibacterial properties seems to be a good candidate for BTE. Strontium (Sr^{2+}) is another common HA substituent with osteogenic potential. Similar to Zn^{2+}, showed very promising results when used in BTE [40, 41]. However, magnesium (Mg^{2+}) is one of the most important ion substitutions in an HA lattice, and physiologically present in the early stages of osteogenesis. Several BTE strategies used magnesium substituted HA and showed tremendous osteogenic potential in ectopic site implantations [42, 43] as well as in clinical studies [44].

Other nanostructured biocomposites have been reported to induce bone formation even in the absence of HA. For instance, the group of Gaharwar recently showed that nanostructured hydrogels functionalized with nano-silicate could be osteogenic in vitro. The presence of nano-silicate enhances the mechanical properties of the hydrogel together with bone induction [45]. Nanostructured materials, in general, have proven to be bone biomimetic. The surface roughness of natural bone is around 32 nm, thus, modification of implant topography introducing nano-features has demonstrated enchanced bone formation mimicking the cellular environment compared to micro-features [46]. In addition, the mimicking of the hierarchical structure of bone ECM proved to be a potent osteogenic strategy. The composite HA collagen fibrils show a banding period of 67 nm due to the mineral nanocrystals randomly nucleated on collagen gap zones. Several proposed attempts have been successful, including the pioneering work of Stupp, where in 2001 he produced self-assembling mineralized peptides mimicking bone structure [47]. A more recent and refined study by Wang et al., showed nucleation to be completely directed by the collagen template as it happens in the natural process of biomineralization [48]. The list of nanomaterials in BTE is very long, and includes nanofibers, nanotubes, nanoparticles, or nanostructured hydrogels, all of which have been proposed as a promising candidate to efficiently replace bone defects [49]. However, the nanostructure could also be combined with the macrostructure in order to achieve a better regeneration.

Mimicking Bone Structure

Cell-conductive porosity and pore interconnectivity are both necessary to support early angiogenesis and tissue infiltration within the scaffold. However, a new dogma in the regeneration of critical size bone defects predicts that the close mimicking of the chemical-physical and morphological-structural features of the intact tissue is instrumental in achieving proper bone healing [50]. The complex, hierarchically organized structure of bone from the nano- to the macro-scale accounts for its outstanding mechanical performances (e.g., resistance and flexibility associated with lightness) and is necessary for the transfer of the appropriate mechanical stimuli to the bone cells [51]. Mimicking bone architecture typically results in mimicking the mechanical features of the bone itself, which is a crucial parameter for in vivo osteoinductivity [52]. Furthermore, bone cells and their progenitors are very sensitive to the biomechanical environment, so this aspect is crucial to this type of mimicry [52]. As the complete structure of bone is very complex, there are only few strategies that aim to mimic the overall architecture. Some studies exploit three-dimensional (3D) printing methods to fabricate biomaterials with precisely controlled structures [53]. This technology is relatively new in bone repair and it is still far from having the resolution necessary to mimic bone ultrastructure [54]. So far, the biomimetic architecture achieved by a 3D printer is too basic in comparison with that of natural bone. Moreover, only synthetic polymers [55] have been proved to be reliable with such technology while no natural materials have been successfully used thus far. However, some attempts have been done using 3D printer BTE constructs based on synthetic polymers (hydroxyapatite and either PCL or PLGA), mimicking the structure of the native bone with excellent results [56]. Nonetheless, as there is a huge hope in 3D printing right now, we believe there will be further implementations in the technique that will allow for the use of natural polymers (ECM-based materials) in order to achieve a more osteoinductive BTE biomaterial [57]. To reach this goal, an interesting approach has been developed by Tampieri et al., in which wood was used as a natural template and its chemical composition transformed in mineral phase by a sequence of reactions while maintaining the original micro-structure of wood [58].

Cartilage Endogenous Healing Potential

Unlike bone, articular cartilage (AC) has a very low endogenous healing capacity and is not able to properly regenerate the lesions due to injury or disease [59]. Cartilage tissue, found at the epiphysis of long bones, functions mainly to bear and distribute the loads away from the joints and to reduce the friction during movement [60]. AC unique composition and organization allows for compression and deformation, moreover, it confers the resistance to the loads and shear stresses originated within the movements of the musculoskeletal system in motion [61]. This tissue is avascular, aneural, and alymphatic, it also has a low cell-to-matrix ratio and high water content [62]. The minimally abundant chondrocytes produce a rich an ECM formed mainly by collagen type II fibrils, with lesser quantities of types IX and XI, and proteoglycans, mainly aggrecan, which is bound to hyaluronic acid through linker proteins [63].

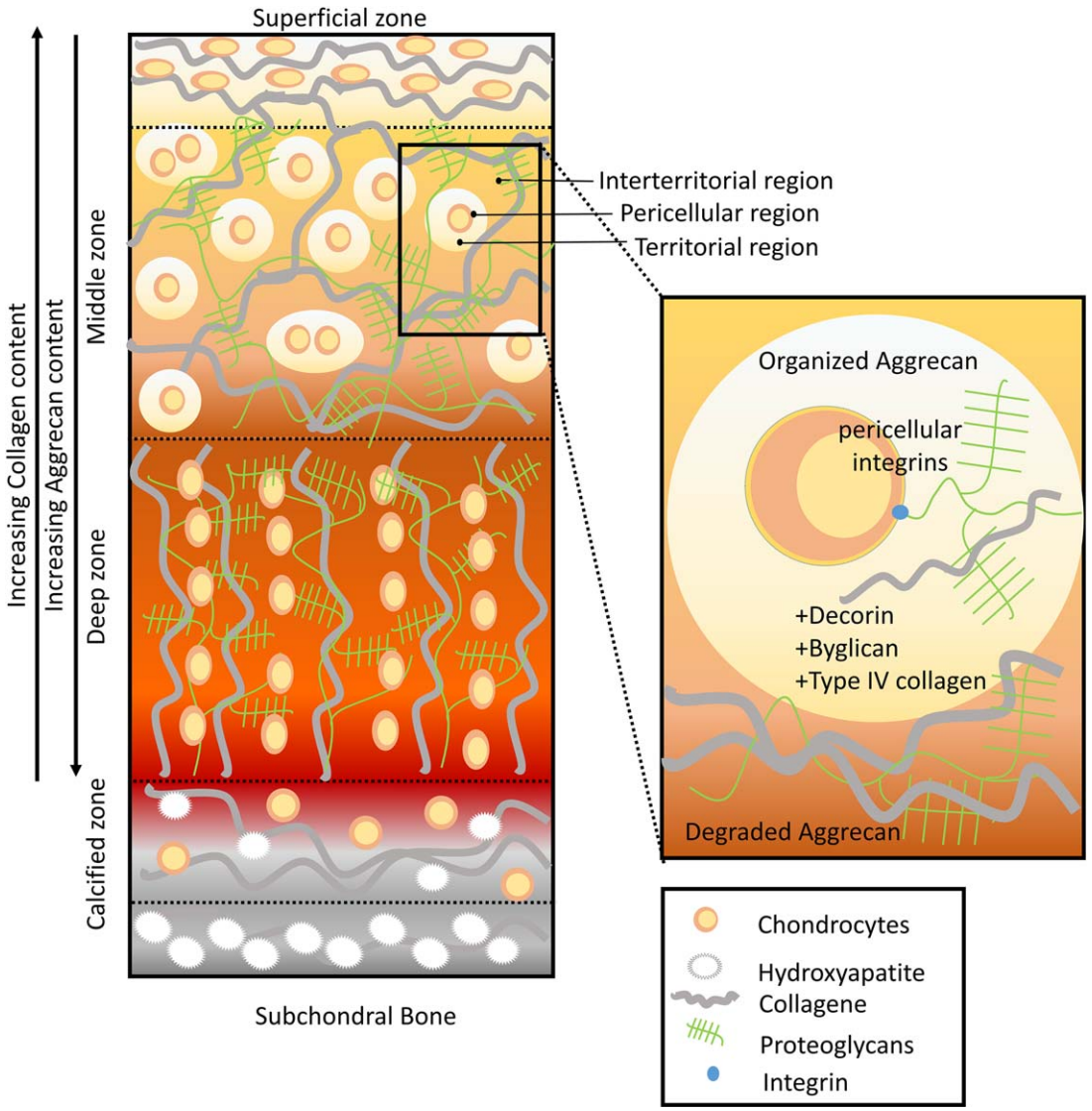

Figure 2. Human articular cartilage. Articular cartilage is divided into four different zones: superficial, middle, deep, and calcified zone. The four zones differ in their collagen, cell orientation, and proteoglycan density. In the superficial zone, collagen fibers are thin and organize themselves parallel to the plane of the articular surface. In the middle zone, the collagen fibers appear more randomly oriented. In the deep zone collagen fibers become thicker and align orthogonally to the superficial zone. At the same time aggrecan density increases from the superficial zone toward the deep zone. Also chondrocyte morphology and density changes depending on the zone. In the superficial zone, chondrocytes are disc-shaped, aligned parallel to the articular surface as well as the collagen fibers. The middle zone is characterized by randomly oriented spherical cells that could be either isolated or in small clusters. In the deep zone chondrocytes are ellipsoid and aligned in columns. Cartilage's extracellular matrix (ECM) is differentially organized around the chondrocytes in pericellular, territorial, and interterritorial region. These different ECM organizations serve to protect the cells and transmit mechanical signals. The pericellular zone is full of proteoglycans (byglicans, aggrecans). The territorial zone also contains thin collagen fibrils. Instead, the interterritorial matrix constitutes 90% of cartilage volume and contains the largest collagen fibrils.

Mature healthy cartilage ECM is very strictly organized and divided into four zones: superficial zone, middle zone, deep zone, and calcified cartilage. These are all clearly differentiated by their cellular organization, ECM components, and orientation (Fig. 2). The ECM composition can also be divided into three categories depending on its proximity to the cellular chondrocyte: the pericellular matrix, the territorial matrix, and the interterritorial matrix. The particular structure and components of these three territories differs again in collagens and proteoglycans content [63]. Softer and proximal to the cells, the pericellular matrix provides cushion to the cells and simultaneously aids their communication with the ECM. Distally to the cells, the matrix composition becomes gradually stiffer with higher collagen content to support the body weight forces [64].

Cartilage's Stem Cell Niche

Cartilage is a tissue of mesenchymal origin and possesses a stem cell niche first described by Dowthwaite et al. [65]. Cartilage chondroprogenitor stem cells (CPSCs) possess trilineage differentiation, self-renewal, colony formation capacity, and express markers common to MSCs [66]. CPSCs have been shown to reside at the

surface of the AC and migrate to the full depth of the tissue during development and to site of injury responding to inflammatory signals in conditions such as osteoarthritis [67]. The appropriate microenvironmental, physical, and biochemical cues are responsible for the differentiation or maintenance of the **stemness phenotype** of CPSCs, features exploited for the novel tissue engineering technologies [68].

In order to tune stem cells, it is important to understand the nature of the cartilage stem cell niche under healthy and diseased conditions. The avascular nature of the cartilage tissue requires that the oxygen, nutrients, and regulatory molecules diffuse primarily from the synovial fluid, aided by the load motion that creates extracellular fluid flow in and out of the tissue [69]. This feature makes the environment become hypoxic and nutrient-deprived. Energy is therefore produced mainly through glycolysis, which acidifies the niche with lactic acid accumulation [67]. This particular niche changes due to age, injury, and early or late stages of osteoarthritis and other inflammatory conditions. It has been shown how CPSCs migrate and differentiate toward these areas of damaged matrix, responding to the inflammatory signals through pGR4 secretion and proliferation [70].

Limitation in Regeneration

The presence of CPSCs at different repair sites account for tissue regenerative capabilities. Unfortunately, the regeneration is normally inadequate as it lacks the fundamental requirements of successful cartilage healing: ECM composition and organization and bioactivity [71]. The anatomist William Hunter first said in 1743: "... ulcerated cartilage... when destroyed, it is never recovered" [72]. This dogma has remained true until now because nothing has achieved perfect regeneration, which has rendered prosthetics as the gold standard for treatment of critical size defects, with all their associated complications. The interesting attempts of autograft or allograft have the limitations of creating new defects and producing immunogenicity, increasing the need for research strategies ready to be translated into medical clinical trials [73].

There are three main limitations of cartilage regeneration in comparison to other tissues with much better natural regeneration capacity. First, the cartilage tissue has a low cellular number together with a characteristic faint metabolic activity, exemplified with a low ECM turnover [67]. Second, the access to nutrients is limited and molecules only diffuse through synovial fluid. Third, cartilage exists in a biomechanically harsh environment with simultaneous high compression, tensile forces, and friction [74].

Many efforts are needed to overcome these obstacles since we are far from a real solution to ameliorate cartilage repair and its post-traumatic degenerations [75]. Regeneration strategies are currently focused on simultaneously optimizing scaffolds, cells, bioactive molecules, and environmental forces [61, 69, 76, 77]. Hence, tissue-engineering strategies are aimed to develop biomimetic tissues that recapitulate the biological, structural, and functional features of native cartilage, increasing its ability to withstand and adapt to the highly loaded environment of the joint.

Cell Free Biomaterials for Endogenous Potential

Despite the many attempts to solve the challenge of cartilage regeneration, only a scarce amount of studies have advanced to the clinical stage [73]. Here, we highlight only the relevant acellular strategies, provided that they offer a greater advantage in comparison to the cellular approaches in terms of handling, time, cost, and regulation [77].

Within this field, aside from overcoming the traditional hurdles of mechanics, integration, and biodegradation, new research studies are trying, with some success, to recruit progenitor cells to repair, direct differentiation, reorganize the ECM, and modulate inflammation [78, 79]. To do so, biomimicry of the native cartilage tissue seems to be the appropriate approach [80].

There is a wide variety of synthetic polymeric, polyesters, polyurethanes, and natural biological materials (e.g., collagen, chitosan, fibroins, and hyaluronan) [81] that provide different advantages and disadvantages in terms of use, structure, and functionalization [61]. These materials are currently being investigated in the form of hydrogels, meshes, or foam-like structures (9), alone or in combination [64]. They can be physically modified to change their properties with compression, UV treatments, or fiber orientation, and chemically modified with the addition of molecules like proteins, GFs, peptides, or glycosaminoglycans [80, 82–86]. 3D printing is also showing some progress toward aiding the fabrication of scaffolds [87].

Differently, part of the scientific community has been focused on research toward bioinspired and biomimetic cell free nanotherapeutics, used as nanotechnology for drug delivery. This includes technologies such as virosomes, liposomes, and exosomes among others [88]. Exosomes, first discovered in 1981 [89], are extracellular membrane-bound vesicles secreted by most cell types. They originate from the formation of multivesicular bodies and are released upon exocytosis [90]. Their expression of surface ligands and receptors confers their biological activity [91]. Exosomes derived from MSCs have been shown to possess cardioprotective, neuroprotective, and osteoregenerative effects [91–93]. In a rat osteochondral defect model, MSC exosomes promote osteochondral regeneration [94]. Moreover, Liu et al. showed promising cartilage regeneration in vivo by fabricating a gelatin hyaluronic acid hydrogel combined with encapsulated stem cell exosome vesicles [95]. Despite of these significant results, there still lacks standardized methods to produce, isolate, and purify exosomes in sufficient amounts [90].

Currently, the trend is to use combinatorial technologies to improve the characteristics of a single material. Kim et al. combined hyaluronic acid fibers and poly (ε-caprolactone) fibers with transforming growth factor-β3 for an increased histological score with higher collagen type II production in a porcine model [96]. Immunomodulation has also been achieved using a collagen scaffold combined with resveratrol, an anti-inflammatory and immunomodulatory drug. Authors demonstrated how this anti-inflammatory scaffold, once implanted in a rabbit osteochondral region, revealed remarkable anti-inflammatory and regenerative properties in comparison to an untreated control [97]. The group of Elisseeff showed successful prospective in cartilage repair with the use of scaffolds integrated with anti-inflammatory drugs [98]. They implanted 3,4,6-*O*-Bu_3GlcNAc-loaded poly(lactic-co-glycolic acid) microfiber scaffolds into rats in order to assess its ability to modulate the inflammatory reaction through b cells. Although the work has not been applied to an osteoarthritic model, there are high hopes that this device could be useful to decrease the inflammatory environment of osteoarthritis and ameliorate cartilage regeneration.

If we widen our scope to find more relevant clinical studies, biphasic osteochondral scaffolds allow for the fabrication of bigger scaffolds, facilitating the fixation of the material, thus having success at the implantation in vivo [99]. However, the ultimate goal is to achieve early repair of the cartilage tissue to stop the development of osteoarthritis and any damage that also occurs to the subchondral bone [71].

Clinical Applications of Emerging Cell-Free Technologies

In this section, we will discuss the present state of clinical utilization of cell-free biomaterials to address bone and cartilage defects in symptomatic patients. There is a clinical need to regenerate bone to improve and accelerate fracture healing. This technology has implications for routine fractures, non-unions, and critical-sized bone defects, any of which may occur following trauma or infection. It is also the foundational component for osteochondral defects where there is loss of both subchondral bone and the overlying AC (i.e., degenerative lesions, such as in osteoarthritis). Defects on the AC are common clinical observation. They have been shown to be present in 63% of symptomatic patients undergoing knee arthroscopy [100]. As AC lesions have limited spontaneous healing and have a propensity for progressing to osteoarthritis, addressing lesions of the AC can be particularly challenging.

Clinical Impact of Cell-Free Approach for Treatment of Bone Defects

The treatment strategies for osseous defects include osseous autograft transplantation, allograft transplantation or augmentation, and cell-free scaffold implantation. Limited by donor-site morbidity, autograft transplantation has significant clinical utility for small defects secondary to trauma, degenerative disease (e.g., osteoarthritis), iatrogenic (e.g., total joint arthroplasty revision), or infection. The iliac crest is the most common source for osseous cortical, corticocancellous, and cancellous grafts with high MSC concentration. Local autografts may also be obtained for a variety of fractures (e.g., distal radius for scaphoid non-union, calcaneus for fifth metatarsal non-union), arthroplasty (e.g., proximal femoral or acetabular/pelvic bone loss in total hip arthroplasty revision, distal femoral and/or proximal tibial bone loss in total knee arthroplasty revision), degenerative joint disease (e.g., full thickness AC defect with subchondral bone loss requiring restoration of articular surface contour), or infectious problems (e.g., osteomyelitis, septic arthritis) in the extremities. In the U.S., widespread availability of allograft allows osseous transplant or augment for joint-, whole bone-, and whole limb-specific purposes. Disease transmission, cost, immune response, and compromised healing are factors associated with limitations in allograft bone transplant.

There are clear disadvantages to both autograft and allograft cell-based osseous transplantation for bone defects. This creates a significant clinical need for cell-free scaffold implantation areas of bone loss. The "holy grail" of bone regeneration and remodeling for any size, location, or chronicity of defects has many general requirements. The product should be an off-the-shelf tool, possible inexpensive and moldable. Moreover, the bone scaffold should (a) support early cells' infiltration, (b) avoid the host reaction, and (c) have ability to be completely regenerated by normal host bone (with subsequent remodeling to the correct shape and corticocancellous architecture without over- or under-growth). Unfortunately, no publications exists that have successfully utilized a cell-free osseous scaffold in human trials with at least a short-term follow-up.

Table 1. Available scaffolds for articular cartilage repair.

Biologic scaffolds
Protein-based matrices
1. Collagen
2. Fibrin
3. Gelatin
Carbohydrate-based matrices
1. Hyaluronic Acid
2. Chitosan
3. Agarose and alginate
Combinations: Synthetic scaffolds
1. Polylactic Acid
2. Polyglycolic Acid
3. Polylactide-co-glycolide
4. Polycaprolactone

Clinical Impact of Cell-Free Approach for Treatment of Cartilage Defects

The treatment strategies for chondral and osteochondral defects include prosthetics, allograft reconstructions, biodegradable scaffolds, and tissue-forming cell therapies, or some combination of these. Each has its own advantages and limitations. Despite advances in the field of cartilage repair, orthopedic surgeons report significant challenges to overcome in order to consistently achieve good, long-term clinical outcomes in patients [101]. Prosthetics, such as total knee arthroplasty, can provide significant symptomatic relief, but they also wear over time and are not appropriate in early stages or in younger patients. In countries with advanced tissue banking, such as the U.S., fresh allografts can be used to reconstruct damaged articular surfaces [102]. However, they are expensive and have limited global availability, in additions to concerns regarding the potential for immunologic or infectious sequelae [103]. Attempts to use allograft tissues in a decellularized manner have resulted in unacceptable failure rates [104].

The "perfect" chondrogenic material should be implantable in a single-stage procedure that can lead to regeneration of the complex multi-layered architecture of the osteochondral unit. Moreover, this material should allow for a good incorporation with the native bone and surrounding cartilage, be readily available ("off-the-shelf"), and be sufficiently strong to allow for normal mechanical function within the joint (allows for early weight-bearing) during the process of regeneration. Biomimetic scaffolds can potentially provide a cost-effective, off-the-shelf treatment option that can be manufactured to exact specifications. 3D printed scaffolds may be useful to match the size, depth, and location in a joint in which they are to be used [105]. Controversy still exists as to whether cartilage treatments with or without cells is likely to yield better clinical results and there is no study that directly compares the same scaffold with and without the addition of cells

Table 2. Summary of acellular scaffolds listed in ClinicalTrials.gov for treatment of chondral or osteochondral defects in the knee.

Product name	Company	Composition	Identifier
TruFit	Smith & Nephew, (Andover, MA)	Biphasic poly[D,L-lactide]/glycolide and calcium sulfate polymer	NCT01246635
Chondromimetic	Orthomimetics (Cambridge, U.K.)	Biphasic scaffold composed of collagen, calcium phosphate, and glycosaminoglycans	NCT01209390
MaioRegen	FinCeramica Faenza S.p.A., (Faenza, Italy)	Tri-layered scaffold. Type I collagen in the chondral layer, and differing concentrations of collagen and HA in the middle and deep layers	NCT01282034
"BiCRI" BiPhasic Cartilage Repair Implant	Exactech Taiwan Ltd. (Gainsville, FL)	Bi-phasic scaffold. Unknown composition	NCT01477008
HYTOP	TRB Chemedica AG (Germany)	Bi-layer bioresorbable matrix. Upper layer purified porcine splint-skin, and lower layer of collagen fleece containing hyaluronan (HA)	NCT01791062
BioMatrix CRD	Arthrex, (Naples, FL)	Bi-layer scaffold with a top layer of type I collagen and a subchondral layer composed of β-Tricalciumphosphate with PLA at the ratio of 80%–20%	NCT02309957
BST-CarGel	BioSyntech, (Quebec, Canada)	Chitosan-glycerol phosphate-based hydrogel scaffold whose active component is a polyglucosamine thrombogenic polysaccharide	NCT02981355
GelrinC	Regentis, (Haifa, Israel)	Hydrogel composed of polyethelyne glycolated fibrinogen which polymerizes upon contact with ultraviolet light	
Chondro-Gide	Geistlich Pharma AG (Wolhusen, Switzerland)	Bi-layer type I/III collagen membrane	NCT02993510
Agili-C	CartiHeal Ltd. (Kfar Saba, Israel)	Bi-phasic implant. The bone phase is composed of calcium carbonate in an aragonite crystalline form, and the cartilage phase is composed of modified aragonite and HA	NCT01471236
CartiFill	Sewon Cellontech, (Seoul, Korea)	Atelocollagen, highly purified porcine derived type I collagen modified by removal of telopeptide	NCT02685917

Abbreviations: CRD, Cartilage Repair Device; HyA, hyaluronic acid; PLA, polylactic acid.

[106]. Cell-free treatments have the significant advantage of avoiding cell manipulation and the regulatory hurdles that come with it and rely on the presence of endogenous cells of the native tissues. Some scaffolds are designed to use microfracture as a source for influx of cells. The clinical challenge is how to attract, activate, and direct their differentiation into the desired tissue. The scaffold needs to support or direct their production of matrix and lead to mature tissue that integrates with both the subchondral bone and the surrounding cartilage [107]. A single-stage scaffold implantation that does not require cell-expansion is ideal from both a regulatory approval process and from a patient acceptance standpoint [108].

There are four primary materials currently used in cartilage scaffolds: protein polymers, carbohydrate polymers, synthetic or artificial polymers, and composites [109, 110]. The composites are often constructed in bi- or tri-layered constructs that are tuned to direct cells to form AC on the surface and bone on the deep surface [111] (Table 1). In addition, hydrogels have emerged as a promising scaffold due to their highly tunable mechanical properties and ability to entrap cells or other materials. They also have the potential to be injected into sites of injury [101, 112, 113]. The ability to recruit cells and direct their differentiation can be modulated using molecules, such as GFs, either bound to the polymer or released in a controlled fashion [114].

While there are several studies showing clinical success with expanded cells cocultured in a scaffold matrix (e.g., "MACI" Vericel, Cambridge, MA) [109, 115], there are only a few cell-free scaffolds presently available for clinical or investigational use in humans (Table 2). While initial reports with scaffolds such as the "TrueFit" (Smith & Nephew, Andover, MA) plug showed questionable integration and clinical results [116], others such as "MaioRegen" (FinCeramica, Faenza, Italy) have advanced to a multi-center clinical study after initial published success [117]. Many barriers including cost, regulatory, insurance, and logistical issues still exist in attempting to bring such treatments to clinical practice [118]. Table 2 is a summary of cell-free scaffolds involved in human trials that can be accessed via ClinicalTrials.gov.

Conclusion

Physicians have an immediate clinical need for biomaterials that will help treat patients with AC defects in their weight bearing joints as well as for critical size defects in bone. It is unclear whether acellular or cell-seeded scaffolds are most likely to be successful. In the authors' opinion, the key in either scenario is to activate those pathways that lead to regeneration, and to do so while blocking the inflammatory and reparative pathways that presently lead to scar formation, failure of integration, and failure to generate native tissue. To that end, it may not be necessary to populate biomaterials with a finite number of specific cells, but rather to provide the surrounding tissues with the appropriate signals to recruit those cells. Either way, it is not just the presence of cells but the ability to direct them into a specific pathway that is going to be required for true regeneration. This makes understanding of these activation pathways one of the critical directions for research. The mechanical properties of the scaffold alone can induce some of these responses. Moreover, multi-layered composite scaffolds with varied materials, pore sizes, and inductive GFs seem to hold significant promise. Making these scaffolds with sufficient initial stability, strength, and integrity to function under high loads in a weight bearing joint while retaining the ability to bioabsorb over an appropriate time course remains a challenge.

The use of 3D printing allows for more complex designs to be developed reproducibly, which will be critical for clinical applicability. There are still many open challenges in musculoskeletal repair; however, tissue engineering aligns well with conventional orthopedic practice in order to improve the final healing. Although the transition of tissue engineering platforms from bench to bedside could be very draining, the multidisciplinary approach involving material scientists, biologists, engineers, and clinicians seems to be a winning strategy in order to speed up the translational process.

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Author Contributions

F.T., G.B., P.M., J.H., and E.T.: manuscript writing, final approval of the manuscript.

Disclosure of Potential Conflicts of Interest

F.T. has compensated employment. P.M. has stock options in Orthobullets.com. J.H. is a consultant Smith and Nephew, Ossur, NIA Magellan, Arthroscopy Journal and has research funding from Smith and Nephew. The other authors indicated no potential conflicts of interest.

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