CHAPTER

3

Bioinspired materials and tissue engineering approaches applied to the regeneration of musculoskeletal tissues

Márcia T. Rodrigues[a,b,c,*], *Ana I. Gonçalves*[a,b,*], *Pedro S. Babo*[a,b], *Manuel Gomez-Florit*[a,b], *Rui L. Reis*[a,b,c], *Manuela E. Gomes*[a,b,c]

[a]3B's Research Group, I3Bs—Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Parque de Ciência e Tecnologia, Zona Industrial da Gandra, Guimarães, Portugal, [b]ICVS/3B's—PT Government Associate Laboratory, Braga/Guimarães, Portugal, [c]The Discoveries Centre for Regenerative and Precision Medicine, Headquarters at University of Minho, Guimarães, Portugal

1 Musculoskeletal tissues

Musculoskeletal tissues are a complex system which provides form, support, stability, and movement to the body. It is also characterized by natural mechano-responsiveness and an efficient complementarity among muscles, tendons, ligaments, cartilage, and bones necessary for function and coordinated actions both for daily life and the most demanding sports activities. The musculoskeletal system relies on the proper articulation of soft and hard tissues tailored to accomplish specific functions (Fig. 3.1).

*Authors equally contributed to this work.

https://doi.org/10.1016/B978-0-12-816221-7.00003-3

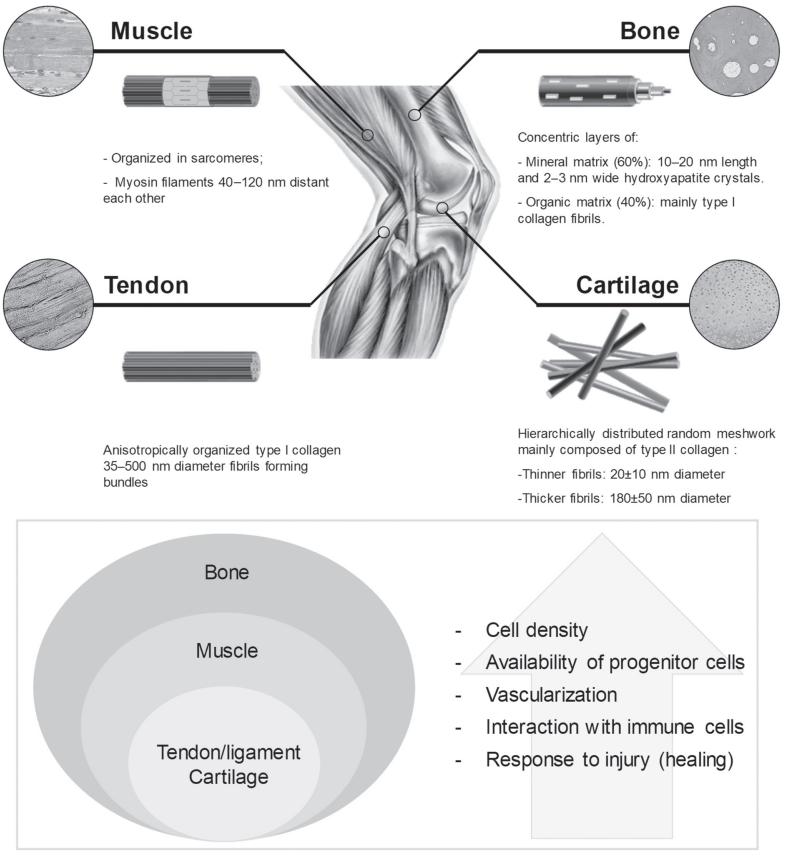

FIG. 3.1 Musculoskeletal system. Representation of the structural organization of the musculoskeletal tissues and summary of their specific features.

Skeletal muscles function to produce force and motion and are responsible not only for the articulated movements that allow the locomotion but also for the movement of internal organs and protection to external shocks. Tendons in the extremities of muscles transmit forces to bones while ligaments keep the stability of the joints and movements between bones. Bones provide structural support, locomotion, and protection of the internal organs as well as in the maintenance of acid-base balance, and calcium, magnesium, and phosphate homeostasis [1]. In joints, the end of the long bones is covered by cartilage, which protects the bones from friction and absorbs the forces over the skeleton. All these tissues work together in continuous adjustment to balance the body during locomotion and movements.

With the increase in life expectancy and the maintenance of an active lifestyle by the aging population, the cumulative musculoskeletal

conditions potentiate disability throughout life. Although the pathological conditions result from a combination of genetic, physiological, environmental, and behavioral factors, aging and the degeneration of tissues and organs have a major impact in increased pain, reduced mobility, and lack of patient's autonomy. Thus, it is imperative to understand the structural characteristics and physiological behavior of the tissues to develop improved tissue engineering and regenerative strategies in order to obtain better therapies.

1.1 Physiology and function

1.1.1 Bone

Bones are mineralized connective tissues that compose the skeleton of vertebrates, harboring the bone marrow which is the primary site of hematopoiesis. The extracellular (ECM) bone matrix is composed of an organic (40%) and an inorganic (60%) phase [1]. The organic matrix is mainly composed of collagenous proteins, predominantly type I collagen, noncollagenous proteins and growth factors [2]. Among the noncollagenous proteins, the osteocalcin, osteonectin, and osteopontin are the most abundant. Osteocalcin is associated with the regulation of the osteoclasts activities and is regarded as a marker of bone formation [3, 4], while osteonectin is related to the osteoblast growth and proliferation, and matrix mineralization [4]. The matrix cohesion and adhesion of bone tissues is maintained by osteopontin [5]. Growth factors and cytokines including insulin-like growth factors (IGFs), transforming growth factor β (TGF-β), bone morphogenetic proteins (BMPs), fibroblast growth factor (FGF), and platelet-derived growth factor (PDGF) are also present in the organic matrix in trace quantities, and modulate cell response to bone remodeling [6]. The organic phase forms a fibrillary matrix over which the mineral phase nucleates. The inorganic material of bone consists predominantly of crystals of a very insoluble calcium phosphate (CaP) salt, the hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] which is responsible for bone stiffness and load-bearing properties. Significant amounts of bicarbonate, sodium, potassium, citrate, magnesium, carbonate, fluorite, zinc, barium, and strontium are also present in bone mineral phase [7].

Morphologically, the bone can be classified into the cortical or trabecular bone. Cortical bone is dense and solid and surrounds the marrow space, whereas trabecular bone is composed of a honeycomb-like network of trabecular plates and rods interspersed in the bone marrow compartment. Regarding the shape and size, bones are classified into four categories: (1) the long bones, including the clavicles, humeri and femurs; (2) the short bones, including the carpal and tarsal bones; (3) the flat bones, including the skull, mandible, and scapulae; and lastly, (4) the irregular bones which include the vertebrae, sacrum, and coccyx [7].

The bone cells are the living elements responsible for bone homeostasis and response to external stimuli and comprise osteoblasts, osteocytes, bone lining cells, and osteoclasts. Osteoblasts, osteocytes, and bone lining cells derive from mesenchymal lineage while osteoclasts are originated from the hematopoietic lineage [1]. Osteoblasts are responsible for the production of the bone matrix constituents [3]. Conversely, the osteoclasts are responsible for bone matrix resorption [4]. Bone health depends on the coordination between the resorption activity promoted by the osteoclasts and the new-bone deposition promoted by the osteoblasts in a process termed *remodeling*. The osteocytes are terminally differentiated osteoblasts that reside within the bone matrix. These are the most abundant bone cells and, among other functions, are responsible for the mechanotransduction events that allow the bone to adapt to mechanical stress [8]. Bone lining cells are mainly metabolically inactive osteoblasts and form the periosteum and endosteum. Besides participating in the osteoclast differentiation [2], the bone lining cells contribute to the exchange of ions between bones and the surrounding tissues [9].

1.1.2 Cartilage

Cartilage is a highly specialized connective tissue composed of a single cell type, the chondrocyte, organized into groups of few cells within an ECM rich in collagen fibrils and proteoglycans. Three types of cartilage have been identified: fibrocartilage, articular, and elastic cartilage, differing in the amounts and organization of collagen and proteoglycan and in function. Fibrocartilage is found for instance at tendon/ligament junction with bone and is designed to stand compressive strength while the principal function of hyaline cartilage, often designed as articular cartilage available at the surface of diarthrodial joints, is to provide a smooth, lubricated surface for articulation and to facilitate the transmission of loads with a low frictional coefficient [10]. The ECM of elastic cartilage found in the trachea and ears is also rich in elastin fibers, which allows tolerating repetitive deformation. Compared to other connective tissues, cartilage has a very slow turnover and a limited capacity to undergo intrinsic healing. Hyaline cartilage is the most prevalent type of cartilage. Since cartilage is avascular and aneural, nutrition to chondrocytes is provided through diffusion, which is assisted by the fluid flow generated in the compression of the articular cartilage or in the flexion of the elastic cartilage.

Articular cartilage comprises four zones. A superficial zone populated with a considerable high number of chondrocytes and where collagen fibers (primarily, type II and IX collagen) are packed tightly and aligned parallel to the articular surface. This zone is in contact with synovial fluid and is responsible for most of the tensile properties of cartilage, which enable it to resist the sheer, tensile, and compressive forces imposed by articulation [10]. Then, a middle zone constituted with collagen organized obliquely into thicker

collagen fibrils with a low density of spherical chondrocytes. Functionally, the middle zone is the first line of resistance to compressive forces [10]. The deep zone is responsible for providing the greatest resistance to compressive forces, given the high proteoglycan content. The chondrocytes are typically arranged in columnar orientation, parallel to the collagen fibers and perpendicular to the joint line [10]. The tide mark distinguishes the deep zone from the calcified cartilage. The calcified layer plays an integral role in securing the cartilage to bone, by anchoring the collagen fibrils of the deep zone to subchondral bone. In this zone, the cell population is scarce and chondrocytes are hypertrophic [10]. The anisotropic nature and unique viscoelastic properties of cartilage in a structure of few millimeters thick highlights the challenges involved in the development of an artificial biomimetic and biofunctional cartilage substitute for regenerative medicine strategies.

1.1.3 Tendon

Tendons and ligaments are similar dense fibrous connective tissues that connect muscle to the skeletal elements (bone) and bone to bone, respectively [11]. These tissues are characterized by the presence of few and dispersed fibroblasts/fibrocytes (ligament) or tenoblasts/tenocytes (tendon) within a collagen-rich ECM, resulting in a dense and hypocellular structure [11]. Tendon ECM is formed from the continual aggregation of the smallest structural unit, collagen, into an increasingly complex architecture [12]. Spontaneous aggregation of multiple collagen molecules results in the formation of collagen fibrils and, in turn, bundles of fibrils form larger primary fiber bundles called fascicles, groups of which associate to form tertiary fiber bundles [12, 13]. These are bound together by a thin layer of connective tissue named *endotenon* that contains blood vessels, lymphatics, and nerves. The multiple fiber bundles and *endotenon* are encompassed by the *epitenon*, which is covered by another layer of connective tissue called *paratenon*.

Tendon tissues are crucial in all joint movements and the limitations of current surgical interventions motivate tissue engineering approaches to build patient-personalized biological substitutes for tendon repair. Despite tendon's hypocellular nature, different cells co-exist constituting a heterogeneous population of tenocytes and tendon stem and progenitor cells [14, 15]. Although the collagen type I is the major component, tendon ECM also includes elastin fibers embedded in a hydrated proteoglycan matrix. The collagen fibers resist to tension forces applied to these tissues, while proteoglycans are responsible for the viscoelastic properties of tendons [16].

1.2 Response to injury and healing mechanisms

The proportion of the population reporting musculoskeletal conditions increased from 28.0% in 1996–98 to 33.2% in 2009–11. The reality of these figures is only for the US population, suggesting that these figures can be

significantly aggravated [17]. This implies that persons with musculoskeletal conditions accounted for an aggregate economic impact of $367.1 billion in 1996–98 and $796.3 billion in 2009–11, an increase of 117% in real terms [17].

The mechanical forces applied to these tissues can be both protective and detrimental. Although the mechanical stress is critical for the physio-anatomy of healthy tissues, misuse or overuse can inflict pathological alterations in biomolecular and cell-mediated mechanisms, thus contributing to impaired healing.

The healing response of musculoskeletal tissues typically involves an acute inflammatory response with the production and release of several important molecules including cytokines that initiate a healing cascade. In bone, this response is accompanied by the recruitment of mesenchymal stem cells (MSCs) in order to generate a cartilaginous callus that undergoes mineralization and resorption. The callus provides biomechanical stability but requires further remodeling to fully restore the biomechanical properties of normal bone. The healing of bone fractures is a complex and efficient process that occurs without the development of a fibrous scar. Unlike bones, damaged articular cartilage and injured tendon/ligaments have limited intrinsic healing, likely because of the hypocellular nature and the lack of resident vascular and lymphatic vessels that restricts the access to cells and biochemical factors with regenerative action. Moreover, in adult tissues, mature chondrocytes and tenocytes that are responsible for ECM maintenance, have low mitotic and metabolic rates. In general, early-stage lesions in cartilage and tendon are asymptomatic which may relate to the absence of an intrinsic nerve network in these tissues. Moreover, these lesions are still manageable with antiinflammatory and analgesic drugs. When the damage is severe, patients become candidates for surgical interventions, in many cases resourcing to tissue grafts, with variable and often insufficient outcomes in the long term. The dissimilar healing responses of musculoskeletal tissues are thus related to their intrinsic properties and function, influencing tissue adaptation and response to mechanical forces (Fig. 3.2).

Impaired tissue restoration or nonresolved healing favors the progression of lesions into chronic and degenerative conditions, and compromises nearby tissues that can culminate into a total joint destruction. Current strategies for the repair of defects and lesions in musculoskeletal tissues are generally unsatisfactory as the restored tissue does not meet the mechanical biofunctionalities of uninjured tissues.

2 Cartilage regeneration

Cartilage lesions progress from asymptomatic lesions to injuries and diseases such as osteoarthritis (OA) that compromise the entire articular joint. Cartilage injuries are broadly classified as partial thickness or full

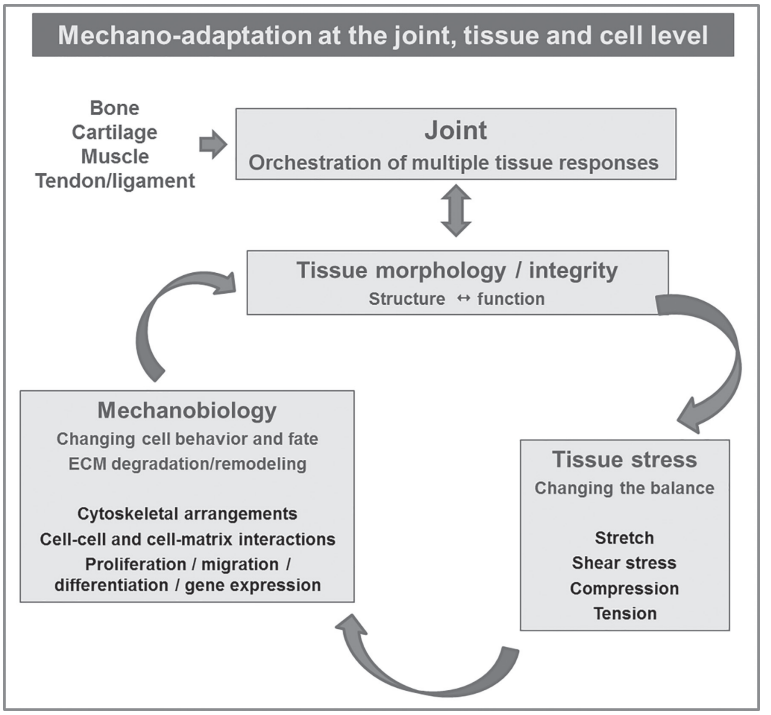

FIG. 3.2 Schematic representation of the articulation of the musculoskeletal tissues from cell to the joint level in the adaptation and response to external mechanical forces.

thickness defects. Partial thickness (chondral) defects as clefts and fissures do not reach subchondral bone and fail to heal spontaneously, while full thickness (osteochondral) defects penetrate to the subchondral bone with a resource to the blood supply and progenitor cells and elicit of an intrinsic repair response. However, the fibrocartilaginous repair tissue formed fails to replace hyaline cartilage in organization and function. Current standard procedures for the treatment of cartilage defects include chondral resurfacing with abrasion, debridement, autologous chondrocyte implantation, and matrix-induced chondrocyte implantation, or osteochondral autologous transplantation. Cartilage surgical procedures can result in short- and mid-term clinical improvement of the joint but do not prevent degeneration of repaired tissue and are a major cause of morbidity of donor tissues, interfering with local biomechanics, and creating the need for replacement of donor cartilage in future years. Thus, the limited success of current treatments and the shortage of cartilage substitutes challenges for innovative approaches to improve cartilage treatments and favor tissue regeneration.

2.1 Scaffold/hydrogel-based approaches

During the last decades, hydrogels have been pursued as preferential candidates for cartilage tissue engineering and regenerative medicine (TERM) approaches. Hydrogels are hydrophilic networks formed by physical and/or chemical crosslinking of polymers providing versatile and highly desirable 3D environments for biological processes (Table 3.1).

Hydrogels proposed for cartilage approaches have been designed with different shapes, complexity (e.g., single and multiple polymers and single and multiple crosslinked networks), and tunable properties (e.g., mechanical properties and responsiveness toward an external stimuli) to support and stimulate cells within a physical matrix [18, 19]. These hydrogels have been developed to act as cell carriers [20] and other therapeutic agents and to study chondrogenesis and associated mechanisms [18, 19]. In large defects, a supportive (hydrogel) matrix may assist the transport of therapeutic cells and enhance tissue regeneration [21] toward a complete integration with surrounding tissues. Furthermore, hydrogels offer the possibility for filling irregular defects using minimally invasive procedures for local and sustained delivery of therapeutics, including cells, drugs, and bioactive molecules such as growth factors and genetic material (revised by Liu et al. [22]) holding the promise for off-the-shelf products to use upon request in patient customized solutions [23].

The advent of personalized medicine brought the attention for bioprinting and plotting technologies to hydrogel fabrication. In the particular case of cartilage, the 3D layered deposition by printing technologies can recreate the tissue-specific anatomic-physiological design in shape and depth-dependent structure with zonal-like hierarchies assisting the fabrication of cartilage constructs with increased biomimicry. Cell-laden printing is thought to significantly enhance the interaction between cells and matrix and improve tissue regeneration. This technology has been explored using different cell types and materials; however, the translation of knowledge from traditional hydrogel fabrication into these inspirational technologies is hampered by the requirement for specific hydrogel properties. Besides a controlled gelation time and swelling or contraction, bioinks require stability and shear-thinning properties to allow their syringe uptake and application as well as self-healing features for a fast reassemble when shear forces are removed. Bioinks are often chosen for their printability but is also important to consider their influence in the biological response. In a study by Daly et al. the phenotype of bone marrow-derived mesenchymal stem cells (BM-MSCs) encapsulated in bioinks produced with different materials [agarose, alginate, gelatin (GelMA), and BioINK] resulted in the development of cartilaginous-like tissues with hyaline-like and fibrocartilage-like structures [24].

TABLE 3.1 Overview on hydrogels for cartilage tissue engineering and regeneration approaches

Material	Manufacturing techniques	Fabrication methods	Advantages	Limitations
Natural derived Chitosan Collagen/gelatin Hyaluronic acid Chondroitin sulphate Alginate Fibrin Keratin	*Fibrous* Electrospinning *Amorphous* Cryogelation Freeze-drying	*Physical methods (responsive to)* Temperature pH Ionic concentration Electric fields Magnetic forces Electrostatic interaction Hydrophobic interactions Hydrogen bonding Stereo-complexation	High water content Similarities to natural ECM Porous framework Hydrogel morphologies Facilitates migration, adhesion, proliferation of cells Inclusion of other materials in the matrix as precursors (hybrid gels), particles (nanogels) Precise deposition of cells (SFF) Control of pore size and geometry (SFF) Minimally invasive procedures in injectable systems Match irregular defects	Unsuitable degradation kinetics Poor control of gelation kinetics Weak mechanical properties and instability (especially physical methods) Toxicity of several cross-linking agents (chemical methods) Requirement for shear-thinning and self-healing properties (bioinks)
Synthetic PEG pNIPAAm OPF	*Organized structure* Solid freeform fabrication (SFF): Bioprinting Plotting	*Chemical methods* Enzymatic crosslinking Schiff base cross-linking Michael additions Click chemistry Photo-crosslinking		

Abbreviations: OPF, oligo(poly(ethylene) glycol) fumarate; PEG, polyethylene glycol; pNIPAAm, poly(*N*-isopropylacrylamide).

Besides the 3D matrix support and stimulation, printing technologies are evolving into more complex and multifactorial systems to meet the natural requirements of tissues and guide the process of regeneration. Zhu et al. investigated the chondrogenic potential of human BM-MSC laden in a stereolithography-based 3D bioprinting matrix fabricated with gelatin and polyethylene glycol diacrylate (PEGDA) [25] incorporating core-shell nanospheres as TGF-β1 carriers. The developed system allowed maintaining cell viability and TGF-β1 bioactivity postprinting. Moreover, TGF-β1 improved the chondrogenic differentiation of MSCs with increased expression levels of Collagen II, SOX-9, and Aggrecan.

In a proof of concept work, distinct cell types associated to cartilage tissue, namely articular cartilage-resident chondroprogenitor cells, BM-MSCs, and chondrocytes, were cultured in gelatin-based hydrogels using multicompartment printed hydrogels to recapitulate the zonal stratification of native cartilage and assess for preferential zonal-like and for cartilage regenerative potential [26]. Despite variations found in the amount and quality of cartilage ECM synthetized by the different bioinks, this study proposes a bioprinted model for the exploitation of cell-cell and cell-matrix interactions within the layered distribution for cartilage regeneration.

2.2 Cell-based approaches using mesenchymal stem cells

MSCs potential for cell-based therapies aiming at cartilage regeneration has been anticipated by numerous studies with different models [18, 21]. Clinical trials aiming at articular cartilage repair, especially focusing on the treatment of OA are still at early stages with preliminary aims to evaluate safety, feasibility, and efficacy (revised by Lee et al. [27]). Overall, these trials indicate pain relief but the renewal or improvement of cartilage tissue shows high degree of variability in human subjects. Recently, intraarticular administration of autologous human BM-MSC entered clinical trials as a safe and feasible procedure with improved outcomes for knee OA [28–30]. Autologous peripheral blood stem cells (AAPBSCs) were also investigated for the treatment of early OA. Three groups of 20 patients were 3 weekly treated with APPBSCs activated with platelet-rich plasma, with or without granulocyte colony-stimulating factor + IA-HA (intraarticular hyaluronic acid carrier) and IA-HA alone (control). Clinical scores showed statistically significant improvements at 6 and 12 months for the AAPBSC groups vs controls. Moreover, the differential effects of stimulated AAPBSCs were noted with an earlier onset of symptom alleviation throughout. At 12 months follow-up, AAPBSC groups also avoided the need for total knee arthroplasty [31].

The delivery of MSC to the injury site with a carrier system is an important procedure in clinical therapies to avoid cell leakage to other

areas of the joint keeping the therapeutic potential of implanted biological agents where it is needed the most. Recent studies showed the success of magnetic targeting to deliver MSC system for the treatment of focal articular cartilage defects [32, 33], including a preliminary study with human induced pluripotent stem cells magnetically delivered to osteochondral defects in a rat femur model. The latter demonstrated that magnetic forces generated by a neodymium magnet improved the repair of the defects resulting in the formation of hyaline-like cartilage in comparison to non-magnetic actuated models [20]. Interesting though is the fact that only when external magnetic forces were applied, the tumor formation (teratoma) was prevented [20].

In the clinical scenario, a significant improvement was found in the outcome scores of the knee defects in 5 human patients 48 weeks after magnetic delivery of autologous BM-MSC [33], suggesting the safety and efficacy of magnetic targeting as a minimally invasive treatment for cartilage repair.

Magnetic actuation strategies also allow for more sophisticated real-time monitoring of the implanted systems while assisting a remote noninvasive control of therapeutic agents during follow up treatments. The ability to monitoring and control the evolution of tissue regeneration contributes to the possibility for medical intervention at critical stages of the treatment, thus, assisting better outcomes for the patients.

2.2.1 Cell-free therapies

Increasing evidence suggests that the therapeutic efficacy of MSC relies on the paracrine signaling necessary to guarantee proper coordination among cells and to modulate the microenvironment surrounding the cells (revised by [34]). The trophic role of MSCs for cartilage repair was highlighted by Pleumeekers et al. [35] with a coculture of BM-MSC and OA chondrocytes. In this work, the extracellular vesicles (EVs) secreted by MSC showed an antiinflammatory effect on TNF-alpha-stimulated OA chondrocytes, inducing the stimulated OA chondrocytes to produce ECM. EVs and exosomes are part of the cell-to-cell communication and may provide novel opportunities as noninvasive tissue-oriented products of regenerative medicine. Toh et al. also reported that MSC exosome-treated rats displayed accelerated neotissue filling and enhanced matrix synthesis of type II collagen and sulfated glycosaminoglycan (s-GAGs) displaying features that resemble hyaline cartilage [36]. In another study performed in a rodent osteochondral model, human embryonic MSC exosome-treated animals revealed enhanced tissue repair at 2 weeks that persisted and extended to week 12 [37]. At 6 weeks, exosome-treated defects showed improved surface regularity and integration with the host cartilage, hyaline cartilage formation with chondrocytic cells, high expression of s-GAG and type II collagen, and low expression of type I collagen [37].

Vonk et al. also investigated the role of EVs secreted by human BM-MSC in human OA cartilage repair [38]. EVs inhibited TNF-alpha-induced inflammatory effects associated to NF-κB signaling in chondrocytes derived from osteoarthritic patients but favored the production of proteoglycan and collagen II, thus evidencing important regenerative and immunoregulatory properties for the regeneration of OA cartilage [38].

The exploratory findings of these vesicles in cell biology and tissue homeostasis and disease models envision ready-to-use exosomal therapies translation to human patients. EVs and exosomes are acellular products and do not hold the ethical restrictions of a cell-based therapy. Despite the minimal risk of immunogenicity and toxicity as a result from their MSC origin, exosomes and EVs face some concerns on biosafety, efficacy, kinetics, and bio-distribution and clearance that require investigation in future randomized trials.

3 Strategies for bone regeneration

The bone tissue is highly dynamic, responding to mechanical stimuli and displaying a remarkable regenerative potential. However, bone defects beyond a critical size, pathological conditions, such as *osteogenesis imperfecta*, osteoporosis, or autoimmune diseases, and glucocorticoids compromise bone regenerative ability. Moreover, the healing process is not similar for all types of bone, nor follows the same healing fashion. The healing of a long bone defect is generally faster than that of flat bone by approximately twofolds [39]. Unlike the flat bones, the long bone defects undergo endochondral regeneration, recapitulating their embryogenesis [39]. Bone-related diseases or bone loss have usually significant effects on a patient's quality of life. The worldwide incidence of bone disorders and conditions has trended steeply upward and is expected to double by 2020, especially in populations where aging is coupled with increased obesity and poor physical activity.

The ultimate goal of bone regeneration strategies is to provide reliable cost-effective substitutes to autologous bone grafts, the current gold standard treatment of large bone lesions. These substitute biomaterials aim to reproduce the osteogenic properties of bone grafts, while circumventing their associated drawbacks, such as limited availability and requirement for secondary surgery [40].

3.1 Bioinspired materials for bone tissue engineering

In order to mimic bone structure and function, the proper control of micro-architecture, mechanical properties, and degradability of the scaffolds/substrates is of utmost relevance to achieve bone regeneration

(Fig. 3.3A) [48]. For instance, the scaffolds for bone TE should provide an interconnected micro- and macro-porosity (<10 and >100 μm, respectively) for nutrient diffusion and new bone ingrowth [49]. Moreover, the stiffness of the substrates can determine cell fate [50], therefore, the stiffness of the scaffolds should resemble that of natural bone. Finally, biomaterials for bone TE approaches should maintain shape stability during the healing/regeneration process, in order to promote the formation of the anticipated bone volume and not to lose the bridging effect between bone margins. Different biomaterial-based approaches have shown potential to support bone TE; the use of soft (injectable) polymer-based materials that resemble the bone ECM, and the use of stiff scaffolds that mimic the bone mechanical support or a combination of both approaches.

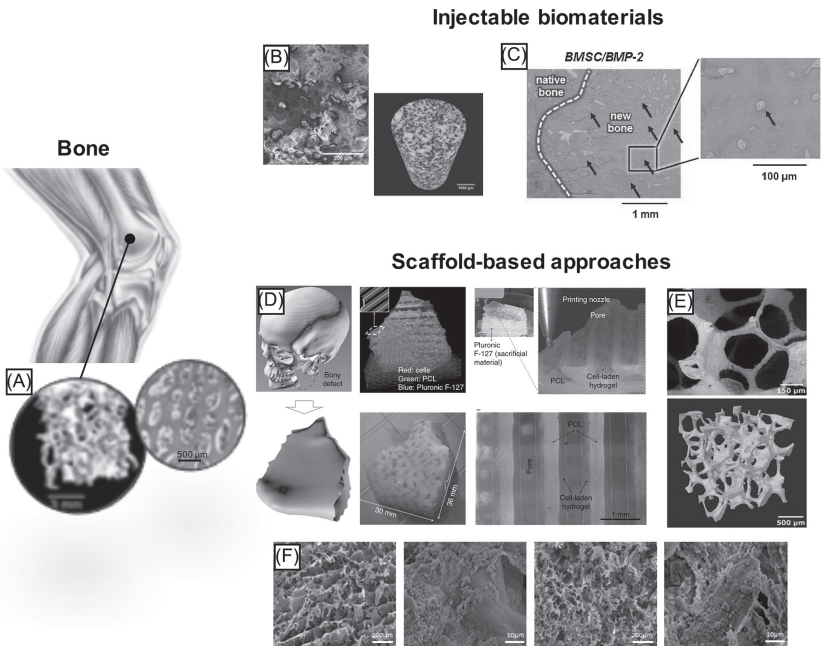

FIG. 3.3 Biomimetic bone tissue engineering strategies: (A) micro-computed tomography (micro-CT) and histological sections images of trabecular bone [41]. Injectable biomaterials: (B) micro-CT images of injectable CaP and hyaluronic acid microparticles loaded with platelet lysate nanocomposite cement [42]; (C) histological sections from the reconstructed bone using an injectable alginate/hyaluronic acid hydrogel loaded with BMP2 [43]. Scaffold-based approaches: (D) biofabrication of mandible bone using PCL and cell-laden hydrogels [44]; (E) micro-CT and SEM images of an ultra-porous TiO_2 scaffold [45]; (F) scanning electron microscope images of collagen/HAp scaffolds derived from marine sources produced using freeze-drying [46]; and (G) digital representation of a human femur and the 3D printed hyperelastic-bone (PCL and HAp) [47]. All figures reproduced with permission from the copyright holders.

3.1.1 Injectable bone substitutes

The injectable biomaterials, capable of setting or crosslinking in situ, have been explored as bone substitutes. Their ability to be injected into complex defects and reshaped to the original bone anatomy before setting is a major advantage over the precasted bone fillers. Therefore, these systems are of particular interest to reduce bone fractures or the filling of bone defects caused by trauma or neoplasia [51]. The injectable calcium phosphate (CaP) cements (CPC) or CaP/polymer composites have found wide applications in bone repair or bone implant fixation [51, 52]. The injectable CPCs have been preferred over the CaP/polymer composites [e.g., polymethyl methacrylate (PMMA)] given their milder curing temperatures, which reduce the local site necrosis [52]. Injectable CPCs are often a two-component system composed by a solid and a liquid phase which, after being mixed, form a self-setting paste. The solid phase usually contains hydroxyapatite and other calcium salts that can originate apatite or brushite cement, depending on the original composition, the first being more soluble than the second (more information in [52]). The similarity with bone mineral matrix composition makes the injectable CPCs highly biocompatible and osteoinductive. Moreover, their compressive strength, ranging from 0.2 to 180 MPa [53], spans that of human trabecular (4–12 MPa) and cortical bone (130–180 MPa) [54].

For filling of bone fractures, or implant fixation [51] the injectable CPCs are good alternatives to bone grafts. Nevertheless, the low bioresorbability and bone ingrowth rates of the injectable CPCs or the polymeric bone cement impair their application for the regeneration of large bone defects. The incorporation of micro- and macro-porosity has been successfully attempted for accelerating the degradation of injectable cement [42] and improving bone tissue ingrowth and osteointegration of the cement. Moreover, the porogenic elements can be used as growth factors' delivery vehicles [42, 55]. In a recent work, Babo and coworkers incorporated platelet lysate (PL) into injectable CPCs, both directly into the cement paste or laden in hyaluronic acid (HA) microparticles [42] (Fig. 3.3B). The incorporation of platelet-rich hemoderivatives, namely PL, into regenerative medicine approaches intends to benefit from their richness in cytokines and growth factors involved in the orchestration of wound healing [56]. The incorporation of PL directly into the cement paste or laden into HA microparticles was shown to modulate the release of specific growth factors and, consequently, the osteogenic potential of the injectable CPCs composites [42]. Conversely, the increase of the porosity makes the cement brittle and decreases the load-bearing capacity [42, 53]. The low stability of these cement composites compromise their therapeutic potential, particularly for flat bone regeneration [57–59].

Produced by the crosslinking of natural or synthetic polymers, the mesh of the injectable hydrogels closely mimics the ECM of connective tissues. Moreover, the mild conditions used to crosslink the hydrogels and the aqueous environment, allow for the loading and sustained release of biomolecules and cell encapsulation [43, 60, 61]. Jung and coworkers proposed a hybrid hydrogel composed of the natural origin polymers alginate and HA, which gelled in situ with Ca^{2+}, for the delivery of the pro-osteogenic BMP-2 and BM-MSCs [43]. The combined effect of BMP-2 delivery and BM-MSCs promoted the regeneration of a mandibular defect in guinea pigs (Fig. 3.3C) [43]. Other versatile crosslinking chemistries have been proposed for hydrogels production with potential for bone regeneration. In our group, methacrylated glucosaminoglycans have been studied for the production of PL-laden hydrogels [60, 62, 63]. These PL-laden hydrogels were shown to be stable and released growth factors in a sustained manner for long time frames [60, 62, 63]. Moreover, the incorporation of PL into HA hydrogels enhanced the osteogenic differentiation of human dental pulp stem cells [64]. Likewise, synthetic materials have been explored to produce injectable hydrogels for the delivery of BMP-2 [65]. Nanoparticles of the thermosensitive poly(phosphazene) were modified with PEG to enhance the affinity of BMP-2 [65]. The hydrogels produced by thermal actuation over nanoparticles solutions were able to release BMP-2 up to 3 weeks promoting ectopic and orthotopic bone formation in mice [65].

The incorporation of hydroxyapatite nanoparticles into the hydrogel matrices, emulating the bone ECM has also been explored [66]. Hydroxyapatite nanocomposite hydrogels of silk are more osteoinductive and promote larger bone regeneration in preclinical models than the injectable silk hydrogels alone [66].

3.1.2 Scaffold-based approaches

In scaffold-based approaches, the structural characteristics, such as roughness, scaffold porosity, pore structure, and interconnectivity, play a crucial role to provide optimal conditions for bone tissue formation in vitro and in vivo [67]. However, in load-bearing applications, the scaffold is also expected to provide sufficient mechanical support during the bone healing process and substitute the lacking mechanical function of the missing or damaged bone tissue. In order to retain its pore architectural structure under physiological loading and to support and transfer the appropriate mechanical stimulation to the bone forming cells within the scaffold and to the host bone, a porous bone scaffold is required to exhibit initial mechanical strength and stiffness that is comparable to the native bone tissue [68]. Therefore, the ideal scaffold should display good mechanical strength, biocompatibility, osteoconductivity, and optimal size and interconnected porous spaces for the bone cells homing.

Natural and synthetic materials, or their combinations, have been investigated as bone scaffolds (for detailed recent reviews, readers are addressed to Ref. [69, 70]). On one hand, natural polymers, such as collagen, chitosan, silk fibroin, and HA, tend to exhibit excellent biomimicry, biocompatibility, and biodegradability. These materials are particularly suitable to fabricate hydrogels, cryogels, and freeze-dried scaffolds. Nevertheless, the scaffolds created using these methods tend to show limited mechanical properties, which may impair their use in load-bearing bone repair. On the other hand, synthetic polymers, such as polycaprolactone (PCL), poly-lactic acid (PLA), and PEG, offer suitable solutions for mechanical challenges faced by the natural polymers. These have been used to produce bone scaffolds through techniques such as porogen leaching, gas foaming, phase separation, fiber meshing, supercritical fluid processing, microsphere sintering, and 3D printing. Yet, synthetic polymers are characterized by poorer cell attachment properties and slower degradation rates.

Blending of natural and synthetic polymers has offered suitable solutions for the shortcomings of both types of polymers. For example, our group developed blends of starch and PCL or PLA to produce fiber-mesh scaffolds with adequate porosity and mechanical properties to support cell adhesion, proliferation, and differentiation into the osteoblast lineage in vitro and bone formation in vivo [71–74]. In a biofabrication approach, Atala's Group combined the mechanical integrity of PCL with the superior biological performance of cell-laden gelatin/fibrinogen/HA hydrogels to create perfusable constructs using a multihead 3D printer (Fig. 3.3D) [44]. The authors showed the feasibility to produce clinical-relevant size constructs matured into vascularized functional tissues assessed in mandible, calvarial bone, cartilage, and skeletal defects in rodents.

Ceramic scaffolds are typically derived from biocompatible and osteoconductive inorganic materials, such as CaPs, bioglass, and titanium oxide (TiO_2). The chemical composition of CaPs, such as hydroxyapatite (HAp) and tricalcium phosphate (TCP), is close to the inorganic phase of bone, which makes these materials very attractive for bone scaffolds. In this regard, nearly 60% of the commercially available synthetic bone graft substitutes involve ceramic materials [75]. Porous ceramic scaffolds have been classically produced by different methods including bone decellularization, sponge replication, and gas foaming. Furthermore, two recent studies have explored the possibility of 3D print ceramic-based materials (TCP and HAp) [76, 77]. These ceramic inks allowed rapid manufacturing of scaffolds with micro- and macroporosity needed for bone regeneration. Using a biocompatible and bioactive material, Haugen and colleagues developed bone biomimetic ultraporous TiO_2 scaffolds with compressive strength above 2.5 MPa using the foam replication process

(Fig. 3.3E). These scaffolds promoted osteogenic differentiation in vitro and bone formation using different in vivo models [45, 78–80]. Our group has been working with marine species such as coral skeletons, sea urchins, and sponges as biomorphic scaffolds and templates for bone TE, since they represent a promising, inexpensive, and biomimetic alternative to engineered scaffolds [81, 82]. In particular, sponges interconnected porous architecture together with their high content in biosilica have been shown to mimic ideal bone scaffolds and to stimulate bone formation and mineralization [83].

Although ceramic scaffolds might present a biomimetic architecture while exhibiting high stiffness and compressive strength, there is some concern regarding the brittle nature of these materials. Creating composite materials addresses the challenges experienced by single material and has yielded more optimal materials and functionalized scaffolds [69]. Generally, ceramic and bioglass minerals are added to the natural and synthetic polymers to create scaffolds with enhanced mechanical and biological performance. While the presence of CaP-based ceramics in the composites improves compressive strength, degradability rate, and osteogenic capacity of the scaffold, the polymers are credited for maintaining good elastic strength and providing a crosslinking mechanism. Composites such as TCP/polymer, PLA/CaP, HAp/starch, HAp/collagen, and PCL/HAp are frequently reported in the literature [69]. Using novel biomaterials sources, that is, by-products of the fishing industry such as fish skin and bones, collagen/CaP scaffolds produced using freeze-drying supported attachment and proliferation of osteoblast-like cells (Fig. 3.3F) [46]. A very interesting and recent composite biomaterial for bone regeneration is the hyperelastic "bone," developed by Jakus et al. (90 wt% HAp and 10 wt% PCL) [47]. This material was rapidly 3D printed into personalized bone scaffolds with excellent elastic mechanical properties (~32%–67% strain to failure, ~4–11 MPa elastic modulus) (Fig. 3.3G). Furthermore, it induced osteogenic differentiation of human BM-MSCs cultured in vitro without exogenous supplementation of osteo-inducing factors in the medium and supported new bone growth in vivo. More recently, nanocomposites involving biopolymeric matrices and bioactive nanosized fillers have gained a considerable amount of attention due to their capacity to mimic the nano-sized features of the natural bone mineral [84, 85]. Minardi et al. fabricated a biologically inspired nanocrystalline magnesium-doped HAp/collagen type I composite scaffold in order to mimic the composition and structure of the osteogenic niche [86]. These scaffolds increased the expression of osteogenic markers in vitro, when compared with nonmineralized collagen-based scaffolds, and allowed the formation of trabecular and cortical bone in vivo.

4 Tendon regeneration

Tendons and ligaments hold a critical role in the musculoskeletal system, transmitting forces, and stabilizing the joints, being able to withstand the high tensile forces upon which locomotion is entirely dependent [16, 87, 88]. The anatomical location, architecture, and function make these tissues highly prone to injuries with limited endogenous resolution.

The development of engineered functional tendons is greatly dependent on the mimicry of tendon mechanical behavior and structural components in order to recapitulate the tendon matrix toward the development of functional substitutes. Moreover, the replication of native tendon microenvironment, requiring a highly aligned architecture and oriented cell morphology [89] is a key aspect in biomaterials design. Therefore, the challenge to engineer advanced functional biomaterials holding mechanical and structural cues highly depends on the proper combination of material type, processing technique, and structure design. In terms of mechanical properties, tendons exhibit a unique crimp pattern and viscoelastic properties akin to a spring that enables tendon to effectively store and subsequently release mechanical energy [90]. The profile of a typical tendon stress-strain curve is composed of different regions: at the toe region of strain up to 2% the tendon retains a characteristic crimped structure; the linear region in which the strain remains lower than 4% and the tendon behaves in an elastic fashion being able to lengthen its crimped collagen fibers and withstand forces. The linear region is representative of the physiological range of the tendon and the slope of the curve defines the Young's modulus of the tissue. Stretching over 4% results in microscopic tearing and tendinopathy can develop, whereas repeated micro-tears and strain beyond 8%–10% leads to macroscopic failure and tendon rupture [12, 91, 92]. Thus, in order to engineer a tendon mimetic scaffold, the characteristic nonlinear biomechanical behavior of tendons and the characteristic anisotropic hierarchical structure (as described in Section 1.1) must be combined.

4.1 Biomaterial processing technologies to meet tendon function and properties

4.1.1 Fiber-based technologies

The complex fibrous hierarchical structure of tendon instigates engineers in the development of materials that ensure enough strength under uniaxial tension and, at the same time, viscoelastic properties in order to optimize stiffness under different loading environments [93]. A growing number of publications resources to aligned scaffolds that can exert influence on cell morphology and tenogenic differentiation in both in vitro and

in vivo models [94–101]. A recent example of the development of aligned scaffolds for tendon TE was proposed by Zheng and coworkers [102] in which a macroporous 3D aligned collagen/silk scaffold was investigated in a rabbit massive rotator cuff tear model. Aligned collagen/silk scaffolds were fabricated using 12 yarns of silk fibers and also resourcing to a unidirectional freezing technology. These scaffolds presented a profound influence on the cellular morphology and arrangement of rabbit tendon stem/progenitor cells. Moreover, the in vivo performance revealed abundant organized bundles of collagen fibers formed in the outer zone of the macroporous 3D aligned collagen/silk scaffold and evidence of a denser, matured and organized regenerative tissue with cell infiltration [102]. Indeed, aligned fiber-based scaffolds were suggested to guide cell response from repair to healing [95]. Lee et al. also evaluated the effect of fiber diameter of unaligned meshes and fiber alignment on human tendon fibroblast attachment, organization, growth, and phenotype, as models of connective tissue repair and healing. Unaligned fibers with nanometer diameters promoted cell proliferation and matrix deposition as well as the expression and activity of RhoA and Rac1, characteristic of the initial, proliferative phase of wound repair. Moreover, the mature repair model represented by unaligned micron-sized fibers supported cell organization and adhesion, while suppressing cell growth and ECM biosynthesis, indicative of the remodeling phase of tissue repair. The nanofiber model showed matrix alignment as a critical design factor for circumventing scar formation and promoting biological healing of soft tissue injuries [95].

Conventional fiber fabrication techniques toward the replication of tendon structure relied on spinning-based methods, such as wet- and melt-spinning [103–105]. In recent years, electrospinning systems have been increasingly used for the production of nano- to micro-fibrous anisotropically aligned biomaterials from a wide range of polymer matrices (Table 3.2). Electrospinning technique consists of a capillary system through which the spinning dope solution to be electrospun is forced, a high-voltage source, and a grounded collector [89]. The potential of electrospun nanofiber scaffolds to modulate cells behavior has been extensively reviewed [114–116].

Generally, the produced electrospun fibers are 2D matrices that need to be assembled into hierarchical scaffolds to mimic tendon architecture. One option that has also been considered in tendon TE is the use of textile technologies such as knitting, weaving, or braiding [117, 118] for assembling fibrous structures into 3D tendon mimetic scaffolds. A recent study by Rothrauff et al. provides a representative example of this strategy for tendon and ligament TE [108]. Multilayered scaffolds of aligned electrospun nanofibers were produced using two designs, stacking or braiding. For multilayered scaffold fabrication, the PCL and poly-L-lactic acid (PLLA) nanofibrous sheets were either manually stacked or rolled and braided.

TABLE 3.2 Materials used in electrospinning technique for tendon applications

Material(s)	Study type	Main outcomes	References
Chitosan (CHT) Polycaprolactone (PCL) Cellulose nanocrystals (CNC)	In vitro	The use of CNCs significantly reinforces the mechanical properties of aligned nanofiber scaffolds; The nanotopography and microstructure of hierarchical assemblies of nanofiber threads promoted tenogenic differentiation of hASCs.	[106, 107]
Polycaprolactone (PCL) Poly-L-lactic acid (PLLA)	In vitro	Aligned nanofibrous scaffolds-stacked versus braided: braided constructs upregulated tenogenic differentiation of hMSCs to a greater degree than stacked constructs.	[108]
Polycaprolactone (PCL)	In vitro	Tube-shaped scaffolds with bi-axially aligned fibers based on a "Chinese-fingertrap" design showed nonlinear mechanical response. hMSCs adhered, proliferated, and aligned along fibers. The scaffold geometry encouraged the differentiation of MSCs toward tendon.	[98]
Polycaprolactone (PCL) Poly-lactic acid (PLA)	In vitro	Nanofiber yarns were processed into plain-weaving fabrics, which could guide hASCs and tenocytes alignment and the synthesis of a tendon-like ECM containing oriented fibers of TNMD and COL1 by contact guidance.	[109]
Poly-L-lactic acid (PLLA) Polyethylene oxide (PEO)	In vitro In vivo	Trichostatin A-incorporated aligned fibers modulated HDAC activity, maintaining Scx expression and promoting tenogenesis of TSPCs. Moreover, the in situ implantation study in a rat model further confirmed that the aligned TSA scaffold promoted the structural and mechanical properties of the regenerated Achilles tendon.	[97]
Polycaprolactone (PCL)	In vivo	Fibers were manually twisted into yarns and the effects of gamma and ethanol sterilization were evaluated over a 6-week time period in murine tendon model. Neither techniques had an observable effect on the functionality of the scaffold when compared to the autograft control.	[110]

Polycaprolactone (PCL) Polyamide 6 (PA6) Silica nanoparticles	In vitro	Electrospun bead-on-string fibrous nanocomposite scaffolds (incorporating silica particles) showed greater cell spreading, proliferation, activity, and ECM deposition compared to the pristine polymeric ones.	[111]
Polycaprolactone (PCL) Hydroxyapatite (HA)	In vitro	The hybrid electrospinning and electrospraying process provided an efficient method to achieve a gradient structure with a controllable layer thickness customizable for tendon-bone interface.	[112]
Silk fibroin (SF) Polycaprolactone (PCL)	In vitro In vivo	The combination of biochemical cues from SF and physical cues from fiber alignment had a positive effect on the direction of RDFBs migration, proliferation, and upregulation of gene expression of tendon-specific ECM proteins. Histological and immunohistochemical analysis revealed the production and deposition of collagen and tenascin C in Achilles tendon defect.	[113]

Abbreviations: COL1, collagen type I; ECM, extracellular matrix; hASCs, human adipose stem cells; HDAC, histone deacetylases; hMSCs, human mesenchymal stem cells; RDFBs, rabbit dermal fibroblasts; Scx, scleraxis; TNMD, tenomodulin; TSA, trichostatin A; TSPCs, tendon stem/progenitor cells.

Braiding technique increased suture-retention and tensile strength, but decreased cell infiltration and proliferation compared to stacked constructs. Despite this, both multilayered scaffolds supported tenogenic differentiation of seeded MSCs by expression of tenogenic markers [108].

Recently, our group developed aligned nanofibrous scaffolds [106, 107] aimed at tendon TE using electrospinning technique. Electrospun nanofiber scaffolds combining chitosan (CHT), a natural polymer, and PCL, a synthetic polymer, were reinforced with cellulose nanocrystals (CNCs) [106], and the electrospinning was conducted using a home built disk electrospinning unit. The topography of anisotropically aligned scaffolds, as opposed to randomly oriented scaffolds, promoted a remarkable uniaxial cell orientation and induced elongated tendon cells morphology. Moreover, the incorporation CNCs into electrospun natural/synthetic polymer (PCL/CHT) nanofiber bundles significantly improved mechanical properties in tendon/ligament relevant range ($\sigma = 39.3 \pm 1.9$ MPa and $E = 540.5 \pm 83.7$ MPa, $P < .0001$) [106]. Based on this knowledge, we further developed continuously aligned nanofiber threads (CANT) based on optimized PCL/CHT/CNC formulations and explored their assembly into 3D scaffolds using different textile techniques, including twisting, braiding, and weaving [107]. Briefly, the spinning solution is jetted to the surface of the supporting liquid bath under the high-voltage electric field and the nanofibers formed. CANT, that represent the tendon collagen fibers, are taken up by a roller at a constant speed from the surface of the nanofibers supporting liquid bath, resulting in a continuous thread that is then twisted into yarns, representing tendon fascicles. In the final fabrication step, braiding and weaving textile techniques are used to obtain 3D scaffolds replicating tendon macroscale organization composed of fascicle assemblies. The deposition of aligned tendon-related ECM components by human adipose stem cells (hASCs) suggested that the topography of the woven scaffolds may be inducing a tissue-specific behavior comparable to native tendon cells [107].

4.1.2 3D bioprinting technologies

Bioprinting has been recently defined as the use of computer-aided transfer processes for patterning and assembly of living and nonliving materials with a prescribed 2D or 3D organization to produce bio-engineered structures serving in regenerative medicine, pharmacokinetics, and basic cell biology studies [119], and recently reviewed by others [119–123]. The material must exhibit steady flow until deposition and must rapidly stabilize upon delivery [123]. Finding the materials formulations for the development of bioinks is thus a challenge, even more because it incorporates cells which may impact the biomaterial properties during extrusion.

Bioprinting is a field under development and very few studies have been published envisioning the fabrication of tendon TE scaffolds. However, in

this context, 3D organ printing technology was used by Merceron et al. to fabricate a muscle-tendon unit construct [124]. The combination of polymeric printing patterns (structural component) with cell-laden bio-ink patterns (the cellular component) resulted in customizable hybrid constructs. Thermoplastic polyurethane (PU) and C2C12 myoblasts were used for the muscle side and PCL and NIH/3T3 fibroblasts for the tendon side. These constructs showed over 80% cell viability 1 week after printing and the cells self-organized patterns were consistent with the tissue interface. Anticipating patient-specific therapies, 3D bio-printed scaffold sleeves made of PCL-PLGA-β-TCP were developed as a way of mimicking the actual size and shape of the tendon and bone tunnel. Scaffold sleeves were seeded with MSCs and tested in an anterior cruciate ligament (ACL) reconstruction in a rabbit model for up to 12 weeks [125]. The construct exhibited excellent results in osteointegration enhancement between the tendon and tunnel bone in the ACL reconstruction [125].

The development of a magnetic scaffold by 3D printing was also described by us [96], based on a polymeric blend of starch and PCL (SPCL) and the incorporation of magnetic nanoparticles. The purpose was to combine structural features of the 3D scaffold with mechanomagnetic actuation to improve the differentiation of hASCs to the tenogenic phenotype and assist tendon regeneration. The scaffolds, with aligned fibers, were shown to assist in the tenogenic differentiation of hASCs under magneto-stimulation with evidence of good biocompatibility and integration in an ectopic rat model [96].

4.2 Current applications and clinical potential

Current treatments for tendon injuries, ranging from acute tendon rupture to chronic degenerative tendinopathy, are conservative or via surgical procedures. The first includes the management of pain, using antiinflammatory medication, immobilization, physiotherapy, and commercial treatment modalities, for example, ultrasounds or extracorporeal shock waves [92, 126]. Surgical treatments are mostly considered as last resort when conservative therapy has failed. In these cases, surgeons suture tendon ends, with or without resourcing to autografts or allografts to bridge defects in larger ruptures. Autografts are harvested from a different location in the body leading to donor site morbidity while the use of allografts raises concerns about immune rejection and risks for disease transmission. Moreover, commercial biological or synthetic substitutes have been reported in clinical applications. These scaffolds derived from human acellular tissue GraftJacket [127, 128] or from bovine (TissueMend, OrthoMend, and BioBlanket) and porcine dermis (Restore and Zimmer Patch) have a rich collagenous matrix and have been used for the reinforcement of soft tissues. Examples of synthetic substitutes include Ligament LARS [129],

revised by Batty et al. [130]. On the way to clinical translation, clinical trials are being assigned with alternative substitutes, such as amniotic fluid-derived allograft (NCT03379324), tendon autografts (NCT03073083; NCT03671421), or ArthroFLEX ECM scaffold (NCT03551509). Also, relevant cell-based therapies to address tendon injuries are under evaluation, specifically with MSCs (NCT03449082; NCT03362424).

5 Future perspectives and concluding remarks

This chapter presented an overview of biomimetic strategies for the regeneration of the different musculoskeletal tissues that show unique composition, structure, and function. Nonetheless, these tissues present a continuous structure, rather than being independent tissues, which results in the formation of complex, composite, and graded junctions or interfaces that should also be considered in regenerative approaches. One such example is the tendon-to-bone junction, also called enthesis that involves the transition from an anisotropic fibrous structure with high tensile strength to an isotropic calcified structure optimized for compressive loading. Indeed, this interfacial tissue is essential to ensure smooth mechanical stress transfer between bone and tendon and to assist the integration of potential tissue substitutes. As another example, osteochondral tissues or cartilage-to-bone interfaces contain a fibrous superficial zone that transitions from hyaline cartilage to calcified bone. Given the complex structure and the central role in force transmission, these interfaces are points of high stress concentration and, thus, more prone to fail, which can ultimately result in significant physical and financial burden. Therefore, the engineering of biomaterials that recapitulate the complex tissue interfaces remains among the most unaddressed challenging areas in TERM, demanding for complex integrative multimodal approaches.

Another challenge that needs to be addressed is that the oversimplified ECM produced by the crosslinking of polymers barely emulates the native tissue microstructure. The self-organization of organic polymers into ordered supramolecular assemblies is common to various systems since DNA assembling to collagen fibers elongation. A great interest has been shown in the study of self-assembling low-molecular-weight gelators by noncovalent interactions as molecular scale building blocks to produce biomaterials with defined micro- and nano-architectures and tunable physicochemical properties. This technology entangles the advantages of the injectable therapeutics with the instructive role of the engineered microenvironments. The application of this technology to the regenerative medicine field can produce therapeutics that may well be administrated using minimally invasive procedures, for instance by injection, into a

ruptured tendon, and spontaneously produce aligned fibers bridging the collagen fibers from the defect margins.

Technological progress in the field is leading to the development of more efficient therapies toward patient-oriented solutions that take into account the individual variability of each patient. Nowadays, precision or personalized medicine concepts are being utilized by TE researchers in the consideration of customized approaches, bearing in mind the healing potential, lifestyle, and outcome expectations. In this sense, stem cells, biomaterial and stimulation technologies, might be tailored in order to meet patient individual requirements. Biofabrication techniques, by which one can control geometry and cellular deposition, together with 3D imaging to screen the anatomical shape, can provide a high level of biomimicry in the construction of complex tissues and organs substitutes. Another aspect is the use of suitable bioactive molecule formulations which can be doped into biomaterials for controlled release in spatiotemporal manner, according to the specific needs of each defect or treatment. The combination of biologically inspired signals with materials science and adequate cellular sources, either autologous strategies or precommitted prone cells, are aspiring approaches that boost precision and personalized concepts in TERM toward the future.

The emergent technologies, including 3D bioprinting, molecular imprinting, and recombinant growth factor delivery have allowed the accumulated knowledge in pathology genesis, healing processes, and the underlying molecular crosstalk. The spatio-temporal control of biochemical and mechanical stimuli delivery has been studied with promising results [62]. Furthermore, the clinical demands for improving biomaterials integration and promote a favorable healing response is a major aim of TERM strategies. At a biomaterial level, it has been suggested the request to identify and modulate biomaterials design characteristics to interact with local cells, while other approaches follow a more biological role with attempts to modulate macrophage polarization due to their pivotal role in healing. It is likely that a successful outcome will be achieved from a combination of both complemented with immune microenvironment engineering, leading to a significant and fast increment in immune-centric approaches in the upcoming years. Insights on immune system mechanisms and the successful interaction and guidance of the physiological cascade toward regeneration will anticipate a revolution of medical procedures and healthcare in general, opening new avenues for cell, gene, and immune-therapies. In the upcoming years, the TERM research lines aiming the regeneration of musculoskeletal tissues are expected to evolve in order to address the specific needs of the patients, having into consideration their healing potential, lifestyle, and outcome expectations, following a precision medicine paradigm.

Thus, despite all the knowledge gathered in the design and development of biomimetic materials to the research on biological processes,

including cellular and tissue responses to the presence of a bioengineered tissue/organ, there are still challenges to be overcome toward complete integration and regeneration. The combinatorial exploitation of materials and architectures to modulate cell responses supported by technological advances and innovative approaches will approximate to the complete regeneration goal meeting architectural, functional, bio-responsive, and personalized therapeutic solutions.

Acknowledgments

Authors acknowledge the FCT Project MagTT PTDC/CTM-CTM/29930/2017 (POCI-01-0145-FEDER-29930) for A.I.G postdoc grant, the FCT Project PTDC/NAN-MAT/30595/2017 (POCI-01-0145-FEDER-30595) for P.S.B. postdoc grant, and for the assistant researcher contract (RL1) of M.T.R from the project "Accelerating tissue engineering and personalized medicine discoveries by the integration of key enabling nanotechnologies, marine-derived biomaterials and stem cells" supported by Norte Portugal Regional Operational Programme (NORTE 2020), under the PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF).

Authors acknowledge the financial support from the European Union Framework Programme for Research and Innovation HORIZON 2020, under the TEAMING Grant agreement No. 739572—The Discoveries CTR and the European Research Council 2017-CoG MagTendon (No. 772817).

References

[1] Morgan EF, Barnes GL, Einhorn TA. The bone organ system: form and function. In: Marcus R, Feldman D, Dempster DW, Luckey M, Cauley JA, editors. Osteoporosis. 4th ed. San Diego: Academic Press; 2013. p. 3–20 [chapter 1].
[2] Florencio-Silva R, Sasso GR, Sasso-Cerri E, Simoes MJ, Cerri PS. Biology of bone tissue: structure, function, and factors that influence bone cells. Biomed Res Int 2015;2015.421746.
[3] Hadjidakis DJ, Androulakis II. Bone remodeling. Ann N Y Acad Sci 2006;1092:385–96.
[4] Clarke B. Normal bone anatomy and physiology. Clin J Am Soc Nephrol 2008;3(Suppl. 3): S131–9.
[5] McKee MD, Nanci A. Osteopontin: an interfacial extracellular matrix protein in mineralized tissues. Connect Tissue Res 1996;35(1–4):197–205.
[6] Linkhart TA, Mohan S, Baylink DJ. Growth factors for bone growth and repair: IGF, TGF beta and BMP. Bone 1996;19(1 Suppl):1S–12S.
[7] Downey PA, Siegel MI. Bone biology and the clinical implications for osteoporosis. Phys Ther 2006;86(1):77–91.
[8] Sims NA, Gooi JH. Bone remodeling: multiple cellular interactions required for coupling of bone formation and resorption. Semin Cell Dev Biol 2008;19(5):444–51.
[9] Currey JD. The structure and mechanics of bone. J Mater Sci 2012;47(1):41–54.
[10] Sophia Fox AJ, Bedi A, Rodeo SA. The basic science of articular cartilage: structure, composition, and function. Sports Health 2009;1(6):461–8.
[11] Rodrigues MT, Reis RL, Gomes ME. Engineering tendon and ligament tissues: present developments towards successful clinical products. J Tissue Eng Regen Med 2013;7(9):673–86.
[12] Walden G, Liao X, Donell S, Raxworthy MJ, Riley GP, Saeed A. A clinical, biological, and biomaterials perspective into tendon injuries and regeneration. Tissue Eng Part B Rev 2017;23(1):44–58.

[13] Liu CF, Aschbacher-Smith L, Barthelery NJ, Dyment N, Butler D, Wylie C. What we should know before using tissue engineering techniques to repair injured tendons: a developmental biology perspective. Tissue Eng Part B Rev 2011;17(3):165–76.
[14] Bi Y, Ehirchiou D, Kilts TM, Inkson CA, Embree MC, Sonoyama W, et al. Identification of tendon stem/progenitor cells and the role of the extracellular matrix in their niche. Nat Med 2007;13(10):1219–27.
[15] Gonçalves AI, Costa-Almeida R, Gershovich P, Rodrigues MT, Reis RL, Gomes ME. Cell-based approaches for tendon regeneration. In: Gomes ME, Reis RL, Rodrigues MT, editors. Tendon regeneration: understanding tissue physiology and development to engineer functional substitutes. Elsevier; 2015. p. 187–203.
[16] Docheva D, Muller SA, Majewski M, Evans CH. Biologics for tendon repair. Adv Drug Deliv Rev 2015;84:222–39.
[17] Yelin E, Weinstein S, King T. The burden of musculoskeletal diseases in the United States. Semin Arthritis Rheum 2016;46(3):259–60.
[18] Amann E, Wolff P, Breel E, van Griensven M, Balmayor ER. Hyaluronic acid facilitates chondrogenesis and matrix deposition of human adipose derived mesenchymal stem cells and human chondrocytes co-cultures. Acta Biomater 2017;52:130–44.
[19] Gentile P, Ghione C, Ferreira AM, Crawford A, Hatton PV. Alginate-based hydrogels functionalised at the nanoscale using layer-by-layer assembly for potential cartilage repair. Biomater Sci 2017;5(9):1922–31.
[20] Kotaka S, Wakitani S, Shimamoto A, Kamei N, Sawa M, Adachi N, et al. Magnetic targeted delivery of induced pluripotent stem cells promotes articular cartilage repair. Stem Cells Int 2017;2017.9514719.
[21] Park YB, Ha CW, Kim JA, Han WJ, Rhim JH, Lee HJ, et al. Single-stage cell-based cartilage repair in a rabbit model: cell tracking and in vivo chondrogenesis of human umbilical cord blood-derived mesenchymal stem cells and hyaluronic acid hydrogel composite. Osteoarthr Cartil 2017;25(4):570–80.
[22] Liu M, Zeng X, Ma C, Yi H, Ali Z, Mou X, et al. Injectable hydrogels for cartilage and bone tissue engineering. Bone Res 2017;5:17014.
[23] Neves LS, Rodrigues MT, Reis RL, Gomes ME. Current approaches and future perspectives on strategies for the development of personalized tissue engineering therapies. Expert Rev Precis Med Drug Dev 2016;1(1):93–108.
[24] Daly AC, Critchley SE, Rencsok EM, Kelly DJ. A comparison of different bioinks for 3D bioprinting of fibrocartilage and hyaline cartilage. Biofabrication 2016;8(4):045002.
[25] Zhu W, Cui H, Boualam B, Masood F, Flynn E, Rao RD, et al. 3D bioprinting mesenchymal stem cell-laden construct with core-shell nanospheres for cartilage tissue engineering. Nanotechnology 2018;29(18).185101.
[26] Levato R, Webb WR, Otto IA, Mensinga A, Zhang Y, van Rijen M, et al. The bio in the ink: cartilage regeneration with bioprintable hydrogels and articular cartilage-derived progenitor cells. Acta Biomater 2017;61:41–53.
[27] Lee WY, Wang B. Cartilage repair by mesenchymal stem cells: clinical trial update and perspectives. J Orthop Transl 2017;9:76–88.
[28] Lamo-Espinosa JM, Mora G, Blanco JF, Granero-Molto F, Nunez-Cordoba JM, Sanchez-Echenique C, et al. Intra-articular injection of two different doses of autologous bone marrow mesenchymal stem cells versus hyaluronic acid in the treatment of knee osteoarthritis: multicenter randomized controlled clinical trial (phase I/II). J Transl Med 2016;14(1):246.
[29] Soler R, Orozco L, Munar A, Huguet M, Lopez R, Vives J, et al. Final results of a phase I-II trial using ex vivo expanded autologous mesenchymal stromal cells for the treatment of osteoarthritis of the knee confirming safety and suggesting cartilage regeneration. Knee 2016;23(4):647–54.
[30] Al-Najar M, Khalil H, Al-Ajlouni J, Al-Antary E, Hamdan M, Rahmeh R, et al. Intra-articular injection of expanded autologous bone marrow mesenchymal cells in

moderate and severe knee osteoarthritis is safe: a phase I/II study. J Orthop Surg Res 2017;12(1):190.
[31] Turajane T, Chaveewanakorn U, Fongsarun W, Aojanepong J, Papadopoulos KI. Avoidance of total knee arthroplasty in early osteoarthritis of the knee with intra-articular implantation of autologous activated peripheral blood stem cells versus hyaluronic acid: a randomized controlled trial with differential effects of growth factor addition. Stem Cells International 2017;2017.8925132.
[32] Kamei G, Kobayashi T, Ohkawa S, Kongcharoensombat W, Adachi N, Takazawa K, et al. Articular cartilage repair with magnetic mesenchymal stem cells. Am J Sports Med 2013;41(6):1255–64.
[33] Kamei N, Ochi M, Adachi N, Ishikawa M, Yanada S, Levin LS, et al. The safety and efficacy of magnetic targeting using autologous mesenchymal stem cells for cartilage repair. Knee Surg Sports Traumatol Arthrosc 2018;26(12):3626–35.
[34] Madrigal M, Rao KS, Riordan NH. A review of therapeutic effects of mesenchymal stem cell secretions and induction of secretory modification by different culture methods. J Transl Med 2014;12:260.
[35] Pleumeekers MM, Nimeskern L, Koevoet JLM, Karperien M, Stok KS, van Osch G. Trophic effects of adipose-tissue-derived and bone-marrow-derived mesenchymal stem cells enhance cartilage generation by chondrocytes in co-culture. PLoS One 2018;13(2).e0190744.
[36] Toh WS, Lai RC, Hui JHP, Lim SK. MSC exosome as a cell-free MSC therapy for cartilage regeneration: implications for osteoarthritis treatment. Semin Cell Dev Biol 2017;67:56–64.
[37] Zhang S, Chuah SJ, Lai RC, Hui JHP, Lim SK, Toh WS. MSC exosomes mediate cartilage repair by enhancing proliferation, attenuating apoptosis and modulating immune reactivity. Biomaterials 2018;156:16–27.
[38] Vonk LA, van Dooremalen SFJ, Liv N, Klumperman J, Coffer PJ, Saris DBF, et al. Mesenchymal stromal/stem cell-derived extracellular vesicles promote human cartilage regeneration in vitro. Theranostics 2018;8(4):906–20.
[39] Lim J, Lee J, Yun HS, Shin HI, Park EK. Comparison of bone regeneration rate in flat and long bone defects: calvarial and tibial bone. Tissue Eng Regen Med 2013;10(6):336–40.
[40] Dawson JI, Oreffo RO. Bridging the regeneration gap: stem cells, biomaterials and clinical translation in bone tissue engineering. Arch Biochem Biophys 2008;473(2):124–31.
[41] Vale AC, Pereira MFC, Mauricio A, Amaral P, Rosa LG, Lopes A, et al. Micro-computed tomography and compressive characterization of trabecular bone. Colloid Surf A 2013;438:199–205.
[42] Babo PS, Santo VE, Gomes ME, Reis RL. Development of an injectable calcium phosphate/hyaluronic acid microparticles system for platelet lysate sustained delivery aiming bone regeneration. Macromol Biosci 2016;16(11):1662–77.
[43] Jung SW, Byun JH, Oh SH, Kim TH, Park JS, Rho GJ, et al. Multivalent ion-based in situ gelling polysaccharide hydrogel as an injectable bone graft. Carbohydr Polym 2018;180:216–25.
[44] Kang HW, Lee SJ, Ko IK, Kengla C, Yoo JJ, Atala A. A 3D bioprinting system to produce human-scale tissue constructs with structural integrity. Nat Biotechnol 2016;34(3):312–9.
[45] Tiainen H, Lyngstadaas SP, Ellingsen JE, Haugen HJ. Ultra-porous titanium oxide scaffold with high compressive strength. J Mater Sci Mater Med 2010;21(10):2783–92.
[46] Diogo GS, Senra EL, Pirraco RP, Canadas RF, Fernandes EM, Serra J, et al. Marine collagen/apatite composite scaffolds envisaging hard tissue applications. Mar Drugs 2018;16(8).
[47] Jakus AE, Rutz AL, Jordan SW, Kannan A, Mitchell SM, Yun C, et al. Hyperelastic "bone": a highly versatile, growth factor-free, osteoregenerative, scalable, and surgically friendly biomaterial. Sci Transl Med 2016;8(358):358ra127.
[48] Ma PX. Biomimetic materials for tissue engineering. Adv Drug Deliv Rev 2008;60(2):184–98.

[49] Low KL, Tan SH, Zein SHS, Roether JA, Mourino V, Boccaccini AR. Calcium phosphate-based composites as injectable bone substitute materials. J Biomed Mater Res B 2010;94B(1):273–86.
[50] Frohlich M, Grayson WL, Marolt D, Gimble JM, Kregar-Velikonja N, Vunjak-Novakovic G. Bone grafts engineered from human adipose-derived stem cells in perfusion bioreactor culture. Tissue Eng Part A 2010;16(1):179–89.
[51] Iundusi R, Gasbarra E, D'Arienzo M, Piccioli A, Tarantino U. Augmentation of tibial plateau fractures with an injectable bone substitute: CERAMENT. Three year follow-up from a prospective study. BMC Musculoskelet Disord 2015;16:115.
[52] Lewis G. Injectable bone cements for use in vertebroplasty and kyphoplasty: state-of-the-art review. J Biomed Mater Res B 2006;76B(2):456–68.
[53] Zhang J, Liu W, Schnitzler V, Tancret F, Bouler JM. Calcium phosphate cements for bone substitution: chemistry, handling and mechanical properties. Acta Biomater 2014;10(3):1035–49.
[54] Wagoner Johnson AJ, Herschler BA. A review of the mechanical behavior of CaP and CaP/polymer composites for applications in bone replacement and repair. Acta Biomater 2011;7(1):16–30.
[55] Oortgiesen DA, Walboomers XF, Bronckers AL, Meijer GJ, Jansen JA. Periodontal regeneration using an injectable bone cement combined with BMP-2 or FGF-2. J Tissue Eng Regen Med 2014;8(3):202–9.
[56] Mendes BB, Gomez-Florit M, Babo PS, Domingues RM, Reis RL, Gomes ME. Blood derivatives awaken in regenerative medicine strategies to modulate wound healing. Adv Drug Deliv Rev 2018;129:376–93.
[57] Babo PS, Cai X, Plachokova AS, Reis RL, Jansen J, Gomes ME, et al. Evaluation of a platelet lysate bilayered system for periodontal regeneration in a rat intrabony three-wall periodontal defect. J Tissue Eng Regen Med 2018;12(2):e1277–88.
[58] BP S, Xinjie C, PA S, RR L, JJ A, GM E, et al. The role of a platelet lysate-based compartmentalized system as a carrier of cells and platelet-origin cytokines for periodontal tissue regeneration. Tissue Eng Part A 2016;22(19–20):1164–75.
[59] Babo PS, Carvalho PP, Santo VE, Faria S, Gomes ME, Reis RL. Assessment of bone healing ability of calcium phosphate cements loaded with platelet lysate in rat calvarial defects. J Biomater Appl 2016;31(5):637–49.
[60] Neves LS, Babo PS, Gonçalves AI, Costa-Almeida R, Caridade SG, Mano JF, et al. Injectable hyaluronic acid hydrogels enriched with platelet lysate as a cryostable off-the-shelf system for cell-based therapies. Regen Eng Transl Med 2017;3(2):53–69.
[61] Silva CR, Babo PS, Gulino M, Costa L, Oliveira JM, Silva-Correia J, et al. Injectable and tunable hyaluronic acid hydrogels releasing chemotactic and angiogenic growth factors for endodontic regeneration. Acta Biomater 2018;77:155–71.
[62] Silva ED, Babo PS, Costa-Almeida R, Domingues RMA, Mendes BB, Paz E, et al. Multifunctional magnetic-responsive hydrogels to engineer tendon-to-bone interface. Nanomedicine 2018;14(7):2375–85.
[63] Babo PS, Pires RL, Santos L, Franco A, Rodrigues F, Leonor I, et al. Platelet lysate-loaded photocrosslinkable hyaluronic acid hydrogels for periodontal endogenous regenerative technology. ACS Biomater Sci Eng 2017;3(7):1359–69.
[64] Almeida LDF, Babo PS, Silva CR, Rodrigues MT, Hebling J, Reis RL, et al. Hyaluronic acid hydrogels incorporating platelet lysate enhance human pulp cell proliferation and differentiation. J Mater Sci Mater Med 2018;29(6):88.
[65] Seo BB, Choi H, Koh JT, Song SC. Sustained BMP-2 delivery and injectable bone regeneration using thermosensitive polymeric nanoparticle hydrogel bearing dual interactions with BMP-2. J Control Release 2015;209:67–76.
[66] Ding Z, Han H, Fan Z, Lu H, Sang Y, Yao Y, et al. Nanoscale silk-hydroxyapatite hydrogels for injectable bone biomaterials. ACS Appl Mater Interfaces 2017;9(20): 16913–21.

[67] Karageorgiou V, Kaplan D. Porosity of 3D biomaterial scaffolds and osteogenesis. Biomaterials 2005;26(27):5474–91.
[68] Chan BP, Leong KW. Scaffolding in tissue engineering: general approaches and tissue-specific considerations. Eur Spine J 2008;17(Suppl. 4):467–79.
[69] Fernandez-Yague MA, Abbah SA, McNamara L, Zeugolis DI, Pandit A, Biggs MJ. Biomimetic approaches in bone tissue engineering: integrating biological and physicomechanical strategies. Adv Drug Deliv Rev 2015;84:1–29.
[70] Roseti L, Parisi V, Petretta M, Cavallo C, Desando G, Bartolotti I, et al. Scaffolds for bone tissue engineering: state of the art and new perspectives. Korean J Couns Psychother 2017;78:1246–62.
[71] Gomes ME, Azevedo HS, Moreira AR, Ella V, Kellomaki M, Reis RL. Starch-poly(epsilon-caprolactone) and starch-poly(lactic acid) fibre-mesh scaffolds for bone tissue engineering applications: structure, mechanical properties and degradation behaviour. J Tissue Eng Regen Med 2008;2(5):243–52.
[72] Requicha JF, Moura T, Leonor IB, Martins T, Munoz F, Reis RL, et al. Evaluation of a starch-based double layer scaffold for bone regeneration in a rat model. J Orthop Res 2014;32(7):904–9.
[73] Rodrigues MT, Gomes ME, Viegas CA, Azevedo JT, Dias IR, Guzon FM, et al. Tissue-engineered constructs based on SPCL scaffolds cultured with goat marrow cells: functionality in femoral defects. J Tissue Eng Regen Med 2011;5(1):41–9.
[74] Rodrigues MT, Lee BK, Lee SJ, Gomes ME, Reis RL, Atala A, et al. The effect of differentiation stage of amniotic fluid stem cells on bone regeneration. Biomaterials 2012;33(26):6069–78.
[75] Rezwan K, Chen QZ, Blaker JJ, Boccaccini AR. Biodegradable and bioactive porous polymer/inorganic composite scaffolds for bone tissue engineering. Biomaterials 2006;27(18):3413–31.
[76] Bergmann CJ, Odekerken JC, Welting TJ, Jungwirth F, Devine D, Boure L, et al. Calcium phosphate based three-dimensional cold plotted bone scaffolds for critical size bone defects. Biomed Res Int 2014;2014.852610.
[77] Cox SC, Thornby JA, Gibbons GJ, Williams MA, Mallick KK. 3D printing of porous hydroxyapatite scaffolds intended for use in bone tissue engineering applications. Korean J Couns Psychother 2015;47:237–47.
[78] Gomez-Florit M, Rubert M, Ramis JM, Haugen HJ, Tiainen H, Lyngstadaas SP, et al. TiO_2 scaffolds sustain differentiation of MC3T3-E1 cells. J Biomater Tissue Eng 2012;2(4):336–44.
[79] Tiainen H, Wohlfahrt JC, Verket A, Lyngstadaas SP, Haugen HJ. Bone formation in TiO_2 bone scaffolds in extraction sockets of minipigs. Acta Biomater 2012;8(6): 2384–91.
[80] Haugen HJ, Monjo M, Rubert M, Verket A, Lyngstadaas SP, Ellingsen JE, et al. Porous ceramic titanium dioxide scaffolds promote bone formation in rabbit peri-implant cortical defect model. Acta Biomater 2013;9(2):5390–9.
[81] Granito RN, Custodio MR, Renno ACM. Natural marine sponges for bone tissue engineering: the state of art and future perspectives. J Biomed Mater Res B Appl Biomater 2017;105(6):1717–27.
[82] Dudik O, Leonor I, Xavier JR, Rapp HT, Pires RA, Silva TH, et al. Sponge-derived silica for tissue regeneration bioceramics of deep-sea sponge. Mater Today 2018;21(5): 577–8.
[83] Barros AA, Aroso IM, Silva TH, Mano JF, Duarte AR, Reis RL. In vitro bioactivity studies of ceramic structures isolated from marine sponges. Biomed Mater 2016;11(4).045004.
[84] Pina S, Oliveira JM, Reis RL. Natural-based nanocomposites for bone tissue engineering and regenerative medicine: a review. Adv Mater 2015;27(7):1143–69.
[85] Yi H, Ur Rehman F, Zhao C, Liu B, He N. Recent advances in nano scaffolds for bone repair. Bone Res 2016;4.16050.

[86] Minardi S, Corradetti B, Taraballi F, Sandri M, Van Eps J, Cabrera FJ, et al. Evaluation of the osteoinductive potential of a bio-inspired scaffold mimicking the osteogenic niche for bone augmentation. Biomaterials 2015;62:128–37.
[87] Benjamin M, Kaiser E, Milz S. Structure-function relationships in tendons: a review. J Anat 2008;212(3):211–28.
[88] Connizzo BK, Yannascoli SM, Soslowsky LJ. Structure-function relationships of postnatal tendon development: a parallel to healing. Matrix Biol 2013;32(2):106–16.
[89] Domingues RMA, Gonçalves AI, Costa-Almeida R, Rodrigues MT, Reis RL, Gomes ME. Fabrication of hierarchical and biomimetic fibrous structures to support the regeneration of tendon tissues. In: Gomes ME, Reis RL, Rodrigues MT, editors. Tendon regeneration: understanding tissue physiology and development to engineer functional substitutes. Elsevier Publisher; 2015. p. 259–80.
[90] Gonçalves AI, Berdecka D, Rodrigues MT, Reis RL, Gomes ME. Bioreactors for tendon tissue engineering: challenging mechanical demands towards tendon regeneration. In: Cabral JM, da Silva CL, editors. Bioreactors for stem cell expansion and differentiation. CRC Press; 2018.
[91] Wang JH. Mechanobiology of tendon. J Biomech 2006;39(9):1563–82.
[92] Sharma P, Maffulli N. Tendon injury and tendinopathy: healing and repair. J Bone Joint Surg Am 2005;87(1):187–202.
[93] Screen HR. Hierarchical approaches to understanding tendon mechanics. J Biomech Sci Eng 2009;4(4):481–99.
[94] Younesi M, Islam A, Kishore V, Anderson JM, Akkus O. Tenogenic induction of human MSCs by anisotropically aligned collagen biotextiles. Adv Funct Mater 2014;24(36):5762–70.
[95] Lee NM, Erisken C, Iskratsch T, Sheetz M, Levine WN, Lu HH. Polymer fiber-based models of connective tissue repair and healing. Biomaterials 2017;112:303–12.
[96] Goncalves AI, Rodrigues MT, Carvalho PP, Banobre-Lopez M, Paz E, Freitas P, et al. Exploring the potential of starch/polycaprolactone aligned magnetic responsive scaffolds for tendon regeneration. Adv Healthc Mater 2016;5(2):213–22.
[97] Zhang C, Wang X, Zhang E, Yang L, Yuan H, Tu W, et al. An epigenetic bioactive composite scaffold with well-aligned nanofibers for functional tendon tissue engineering. Acta Biomater 2018;66:141–56.
[98] Banik BL, Lewis GS, Brown JL. Multiscale poly-(-caprolactone) scaffold mimicking nonlinearity in tendon tissue mechanics. Regen Eng Transl Med 2016;2(1):1–9.
[99] Wang W, He J, Feng B, Zhang Z, Zhang W, Zhou G, et al. Aligned nanofibers direct human dermal fibroblasts to tenogenic phenotype in vitro and enhance tendon regeneration in vivo. Nanomedicine 2016;11(9):1055–72.
[100] Bosworth LA, Alam N, Wong JK, Downes S. Investigation of 2D and 3D electrospun scaffolds intended for tendon repair. J Mater Sci Mater Med 2013;24(6):1605–14.
[101] Yin Z, Chen X, Chen JL, Shen WL, Nguyen TMH, Gao L, et al. The regulation of tendon stem cell differentiation by the alignment of nanofibers. Biomaterials 2010;31:2163–75.
[102] Zheng Z, Ran J, Chen W, Hu Y, Zhu T, Chen X, et al. Alignment of collagen fiber in knitted silk scaffold for functional massive rotator cuff repair. Acta Biomater 2017;51:317–29.
[103] Enea D, Henson F, Kew S, Wardale J, Getgood A, Brooks R, et al. Extruded collagen fibres for tissue engineering applications: effect of crosslinking method on mechanical and biological properties. J Mater Sci Mater Med 2011;22(6):1569–78.
[104] Kew SJ, Gwyne JH, Enea D, Brookes R, Rushton N, Best SM, et al. Synthetic collagen fascicles for the regeneration of tendon tissue. Acta Biomater 2012;8:3723–31.
[105] Webb WR, Dale TP, Lomas AJ, Zeng G, Wimpenny I, El Haj AJ, et al. The application of poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) scaffolds for tendon repair in the rat model. Biomaterials 2013;34(28):6683–94.

[106] Domingues RM, Chiera S, Gershovich P, Motta A, Reis RL, Gomes ME. Enhancing the biomechanical performance of anisotropic nanofibrous scaffolds in tendon tissue engineering: reinforcement with cellulose nanocrystals. Adv Healthc Mater 2016;5(11):1364–75.
[107] Laranjeira M, Domingues RMA, Costa-Almeida R, Reis RL, Gomes ME. 3D mimicry of native-tissue-fiber architecture guides tendon-derived cells and adipose stem cells into artificial tendon constructs. Small 2017;13(31).
[108] Rothrauff BB, Lauro BB, Yang G, Debski RE, Musahl V, Tuan RS. Braided and stacked electrospun nanofibrous scaffolds for tendon and ligament tissue engineering. Tissue Eng Part A 2017;23(9–10):378–89.
[109] Wu S, Wang Y, Streubel PN, Duan B. Living nanofiber yarn-based woven biotextiles for tendon tissue engineering using cell tri-culture and mechanical stimulation. Acta Biomater 2017;62:102–15.
[110] Bhaskar P, Bosworth LA, Wong R, O'Brien MA, Kriel H, Smit E, et al. Cell response to sterilized electrospun poly(varepsilon-caprolactone) scaffolds to aid tendon regeneration in vivo. J Biomed Mater Res A 2017;105(2):389–97.
[111] Rinoldi C, Kijenska E, Chlanda A, Choinska E, Khenoussi N, Tamayol A, et al. Nanobead-on-string composites for tendon tissue engineering. J Mater Chem B 2018;6(19):3116–27.
[112] Wu Y, Fuh J, Wong YS, Sun J. A hybrid electrospinning and electrospraying 3D printing for tissue engineered scaffolds. Rapid Prototyping J 2017;23(6):1011–9.
[113] Chen CH, Chen SH, Kuo CY, Li ML, Chen JP. Response of dermal fibroblasts to biochemical and physical cues in aligned polycaprolactone/silk fibroin nanofiber scaffolds for application in tendon tissue engineering. Nanomaterials 2017;7(8).
[114] Sankar S, Sharma CS, Rath SN, Ramakrishna S. Electrospun fibers for recruitment and differentiation of stem cells in regenerative medicine. Biotechnol J 2017;12(12).
[115] Brennan DA, Conte AA, Kanski G, Turkula S, Hu X, Kleiner MT, et al. Mechanical considerations for electrospun nanofibers in tendon and ligament repair. Adv Healthc Mater 2018;7(12).e1701277.
[116] Wu Y, Han Y, Wong YS, Fuh JYH. Fibre-based scaffolding techniques for tendon tissue engineering. J Tissue Eng Regen Med 2018;12(7):1798–821.
[117] Barber JG, Handorf AM, Allee TJ, Li WJ. Braided nanofibrous scaffold for tendon and ligament tissue engineering. Tissue Eng Part A 2013;19(11−12):1265–74.
[118] Czaplewski SK, Tsai TL, Duenwald-Kuehl SE, Vanderby Jr. R, Li WJ. Tenogenic differentiation of human induced pluripotent stem cell-derived mesenchymal stem cells dictated by properties of braided submicron fibrous scaffolds. Biomaterials 2014;35(25):6907–17.
[119] Moroni L, Boland T, Burdick JA, De Maria C, Derby B, Forgacs G, et al. Biofabrication: a guide to technology and terminology. Trends Biotechnol 2018;36(4):384–402.
[120] Derakhshanfar S, Mbeleck R, Xu K, Zhang X, Zhong W, Xing M. 3D bioprinting for biomedical devices and tissue engineering: a review of recent trends and advances. Bioact Mater 2018;3(2):144–56.
[121] Bracaglia LG, Smith BT, Watson E, Arumugasaamy N, Mikos AG, Fisher JP. 3D printing for the design and fabrication of polymer-based gradient scaffolds. Acta Biomater 2017;56:3–13.
[122] Lim PN, Wang Z, Wang D, Konishi T, San Thian E. Development in additive printing for tissue-engineered bone and tendon regeneration. Curr Opin Biomed Eng 2017;2:99–104.
[123] Moroni L, Burdick JA, Highley C, Lee SJ, Morimoto Y, Takeuchi S, et al. Biofabrication strategies for 3D in vitro models and regenerative medicine. Nat Rev Mater 2018;3(5).
[124] Merceron TK, Burt M, Seol YJ, Kang HW, Lee SJ, Yoo JJ, et al. A 3D bioprinted complex structure for engineering the muscle-tendon unit. Biofabrication 2015;7(3):035003.

[125] Park SH, Choi YJ, Moon SW, Lee BH, Shim JH, Cho DW, et al. Three-dimensional bio-printed scaffold sleeves with mesenchymal stem cells for enhancement of tendon-to-bone healing in anterior cruciate ligament reconstruction using soft-tissue tendon graft. Arthroscopy 2018;34(1):166–79.
[126] Ho JO, Sawadkar P, Mudera V. A review on the use of cell therapy in the treatment of tendon disease and injuries. J Tissue Eng 2014;5:2041731414549678.
[127] Sharma N, El Refaiy A, Sibly TF. Short-term results of rotator cuff repair using GraftJacket as an interpositional tissue-matched thickness graft. J Orthop 2018;15(2):732–5.
[128] Skedros JG, Henrie TR. Latissimus Dorsi tendon transfer with GraftJacket(R) augmentation to increase tendon length for an irreparable rotator cuff tear. Case Rep Orthop 2017;2017.8086065.
[129] Parchi PD, Ciapini G, Paglialunga C, Giuntoli M, Picece C, Chiellini F, et al. Anterior cruciate ligament reconstruction with LARS artificial ligament-clinical results after a long-term follow-up. Joints 2018;6(2):75–9.
[130] Batty LM, Norsworthy CJ, Lash NJ, Wasiak J, Richmond AK, Feller JA. Synthetic devices for reconstructive surgery of the cruciate ligaments: a systematic review. Arthroscopy 2015;31(5):957–68.