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Bone, Stem Cells and Regeneration: Tissue Engineering Approaches

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Running title: Stem cells and bone regeneration and tissue engineering

Key words: bone tissue engineering, pluripotent stem cells, biomaterials

Key concepts/learning objectives:

Bone/cartilage tissue engineering requires interaction between stem cells and biomaterials.

Choosing the appropriate stem cell populations is extremely important and depends on the biological application studies.

The use of directed differentiation of pluripotent stem cells that mimic developmental processes are an important advance in tissue engineering.

Choosing the appropriate biomaterial with specific tunable properties is essential for supporting stem cell differentiation.

Three-dimensional approaches for tissue formation are important for translational studies.

Defined pre-clinical models are necessary for screening stem cell/biomaterials constructs.

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Abstract

The potential for regenerating bone and cartilage for cell-based therapies has increased markedly in the past decades primarily due to advances in the understanding of molecular controls of embryonic skeletal development, in identification of refined stem cell/progenitor populations that are suitable for tissue regeneration, and in the discoveries of novel biomaterials that can mimic tissue architecture. In particular, modelling how the embryo makes the appropriate molecular decisions to fine tune the development of specific bone and cartilage subpopulations that form the complex skeleton has provided a platform upon which regenerative strategies can be translated to the clinic. It is therefore imperative to understand the basic biology of bone and skeletal tissues and apply lessons from developmental biology to identify the unique and appropriate cell populations that have potent regenerative potential when combined with optimal biomaterials.

1. Bone Biology

1.1 Cells, structure and formation of bone

Bone tissue is comprised organic and inorganic components that consist of bone cells, collagen fibers and crystals of calcium phosphate. Bone tissue can be divided into two structural types: cortical and trabecular bone. Continuous units of osteons form the shell-like cortical bone, while the connecting network of trabeculae constitutes the trabecular spongy bone. The initial formation of bone comes from clusters of highly condensed multipotent mesenchymal and skeletogenic cells derived from embryonic mesodermal precursors, which are called mesenchymal condensations. These well-shaped cell groups containing skeletogenic precursors reside in the future bone area and will be differentiating into either chondrocytes, to form the cartilage anlagen that will remain either as articular cartilage in a joint or undergo endochondral ossification, or directly into bone-forming osteoblasts, to facilitate intramembranous bone formation [1] (Figure 1).

Intramembranous ossification is the major process for the formation of the membranous parts of craniofacial bones, e.g. frontal and parietal bones. Initially, precursors of osteoblasts generated from mesenchymal condensations proliferate and differentiate, then migrate to the outside of the

condensations to form osteogenic fronts. These preosteogenic cells then start to deposit collagenous extracellular matrix and then ossify in a regulated manner to create the final morphological shape [2]. In contrast to intramembranous bone formation, endochondral ossification is a more complex process which requires the formation of an expanding cartilage model that culminates in the differentiation of hypertrophic and calcifying chondrocytes that will be infiltrated by blood vessels, resorbed by osteoclasts and replaced by bone following differentiation of osteoprogenitors and osteoblasts in the primary ossification center, forming the future bone tissue [3].

Many transcription factors and growth factors and signaling pathways are involved in the regulation of bone formation. Members of Transforming Growth Factor beta (TGF-β) and bone morphogenetic protein (BMP) families, guide bone shape, the formation and progression of mesenchymal condensations [4,5], and signaling pathways including Wnt and Notch signaling have additional fundamental roles in cell differentiation and bone development [6,7]. During the development of mesenchymal condensations, the runt domain transcription factor, RUNX2, defines the osteogenic lineage, while the SRY-related high mobility group box gene, SOX9, guides the emergence of the chondrogenic lineage. BMP-mediated continuous expression of SOX9, along with SOX5 and SOX6 will lead the immature cartilage to persistent cartilage, while RUNX2 expressed in late immature cartilage will boost hypertrophy and support the replacement of hypertrophic cartilage, to complete endochondral ossification [8–10].

1.2 Fracture healing of bone

Fracture healing of bone recapitulates many aspects of natural bone formation. It is a process that requires the participation of many types of cells and growth factors that originate from the periosteum, blood vessels, bone marrow and the periphery of the fracture site, including immunoregulatory cells/cytokines, mesenchymal progenitors, proangiogenic factors/angiogenic cells and osteoclasts [11–13]. The way fractures are healed depends on the biophysical surroundings the fracture resides in. For long bones, the process of fracture healing can be histologically divided into four overlapping stages: 1) Inflammation: Shortly after fracture, hemorrhage caused by

breakage of blood vessels at the fracture site forms a hematoma, while inflammatory cells including macrophages, lymphocytes and peripheral stem cells penetrate into the damage site and secrete cytokines and growth factors, all together resulting in an increased tissue volume [14]. 2) Cartilage formation: Mesenchymal progenitor-derived chondrocytes, along with fibroblasts, begin to proliferate and secrete collagen and proteoglycans, to form a soft cartilaginous callus containing proliferating chondrocytes that proceed to hypertrophy and are accompanied by a periosteum response, which initiates early intramembranous ossification. Cells that will form the new blood vessels are also recruited and will differentiate in the peripherals [15]. 3) Primary bone formation: After the apoptosis of chondrocytes, osteoblasts originated from mesenchymal stem cells are recruited from the periosteum and bone marrow, and migrate to the hypertrophic site and engage in the formation of osteoid and subsequent mineralisation. At this stage, ingrowth of blood vessels synchronises with hard tissue deposition which elevates the local oxygen level to stimulate osteogenesis and to form woven bone [16]. 4) Secondary and subsequent bone formation: In this final remodeling stage of fracture healing, osteoblasts continue depositing matrix, while resorptive osteoclasts act in concert with osteoblasts to shape and reconstruct newly formed bone into the final cortical and trabecular bone structures. In addition, vascular remodeling also takes place at this stage to return vessel magnitude and blood flow to a physiological state [14].

As membranous bones, the healing of craniofacial bone fractures is, in principle, through intramembranous ossification. However, early evidence has shown that fracture healing in rabbit/mouse mandibles resembled appendicular bone histologically. Areas of cartilage formation, proliferating osteoblasts, neovascularization and mineralisation were identified sequentially at the fracture site which indicated some similarities to endochondral ossification [17]. In another study, culturing of mesenchymal precursors from human hematoma caused by mandibular fracture showed that these cells lack the ability to differentiate to chondrocytes, which contradicts the endochondral healing process of mandibles [18]. Finally, chondrocytes residing in the mandibular cartilaginous condyle possess the ability to transform into osteoblasts and osteocytes, which account for the formation and repair of the bone tissue of the condylar neck and upper ramus of the mandible [19]. The embryonic development of the mandible through Meckel’s cartilage makes it unique amongst

other craniofacial bones, and its repair is therefore complex [20]. However, these studies highlight the need for understanding the ontogeny of these cell populations in order to inform the best strategies for craniofacial bone fracture healing.

1.3 Bone remodeling

The growth of bone ceases simultaneously when the body stops growing, but static as it seems, bone tissue continuously undergoes the process of rebuilding and restoration, in order to repair damage, sustain the integrity of bone structure and to maintain calcium and phosphate homeostasis. This process is called bone remodeling, which is mainly dependent on the function and interaction of osteoblasts and osteoclasts, the imbalance of which is the cause of many metabolic bone diseases such as osteoporosis and osteopetrosis [21,22].

At the cellular level, bone remodeling takes place in defined structural units referred to as the Basic Multicellular Unit (BMU). The BMU consists of bone-resorbing osteoclasts, bone-forming osteoblasts, osteocytes and bone lining cells. There are three main phases of bone remodeling: 1) Resorption: Recruitment of osteoclast precursors stimulated by, for example, microfractures or alterations in loading sensed by osteocytes, will result in differentiation into multinucleated osteoclasts at the appropriate bone surface to start to dissolve the inorganic and organic components of the bone matrix. 2) Reversal: In this phase, osteoclast activity is terminated, and this region is reported to be populated by a variety of cells that have osteogenic potential (expressing Runx2) as well as having myeloid/macrophage characteristics to scavenge debris and prepare for the next phase [23]. 3) Formation: With the apoptosis of osteoclasts and preparation of the bone surface by resident mononuclear cells, local release of multiple growth factors, including BMPs and members of the TGFβ superfamily will attract mesenchymal progenitors and osteoblasts to the remodeling area, to form new bone matrix and deposit osteoid that will subsequently undergo mineralization. Thus, remodeling will enter a new resting phase and await for the next trigger to initiate remodeling. It is estimated that the rate of bone remodeling in the adult skeleton is approximately 10% per year [24,25].

2. Bone repair and tissue engineering

During steady-state physiological skeletal homeostasis, bone remodeling is normally undertaking the role of repairing and renewing bone tissue, however, abnormal physiological or pathological situations like in musculoskeletal genetic disorders, infections, trauma, bone malignancy and other orthopaedic diseases always result in bone defects that exceed the capacity of self-repair. For example, bone loss in the human alveolar ridge caused by periodontitis normally cannot be restored by itself [26], segmental defects in long bones cannot reach full healing even with proper stabilisation [27] and calvarial bone defects considered as critical size defects cannot be cured through endogenous repair mechanisms [28]. To address this clinical dilemma, tissue engineering strategies must be considered, for example, treatments using autograft or allograft cell/tissue transplantation. While autograft approaches are the current “gold standard” for bone repair, autografts are hampered by the limited amount of the desired tissue, extra damage and complications caused by a second surgical procedure, while for allograft procedures, immune rejection continues to be an important concern. Therefore, the concept of “tissue engineering” is extremely important and needs to advance, taking into account the rapid developments in the three main elements that must be considered for cell-based tissue regeneration, namely, choosing the appropriate cell type, identifying relevant growth factor signaling pathways that act on these cell types, and acknowledging the growing importance of bespoke biomaterials and the extracellular environment that can synergise with growth factors and stem cells to enhance tissue growth.

2.1 Growth factors and growth factor-carrying materials

The understanding of bone development and bone remodeling have revealed the vital role of growth factors that regulate specific processes during specific time points (Figure 2). Growth factors including Vascular Endothelial Growth Factor (VEGF), Platelet-Derived Growth Factor (PDGF) and Insulin-like Growth Factor (IGF) have demonstrated their osteoinductive abilities in accelerating the healing process of bone in many critical-sized defect models [29]. However, members of the Bone Morphogenetic Protein (BMP) family remain one of the strongest in dominating the regeneration process, since the early reports that BMPs alone have the ability to induce the formation of ectopic

cartilage and bone in rodents [30]. Indeed, BMP signaling has been reported to affect nearly all processes of bone and cartilage physiology [31]. However, in a tissue engineering setting *in vivo*, the delivery of growth factors has been problematic. Firstly, application and infiltration of growth factors to the specific defect can be obstructed by the anatomical site. Secondly, uncontrolled diffusion, degradation and natural metabolism by the body can make it difficult for the growth factors to reach their effective dose levels to yield a functional and maximal effect. In some instances, it might be necessary to use higher doses in order to maintain a final effective concentration, however, this could result in unexpected physiological or pathological changes. To circumvent this potential problem, carrier materials such as natural polymers have been designed for better controlled delivery and release of growth factors for regeneration of damaged tissue. These biocompatible materials are inspired by the natural cellular extracellular matrix (ECM) and play an important role in the storage and release of growth factors *in vivo*. Many studies have focused on improving growth factor binding to ECM, and the interactions between materials and endogenous matrices at the aimed site to simplify and optimise the delivery of growth factors [32]. However, ECM biomaterials are also heterogeneous in composition and always lead to a mild immune response. Therefore, synthetic polymers with more uniformed components are now routinely used. They can be manufactured into multiple shapes including 3D scaffolds, fibers, sheets and are tunable in microstructures including porosity, orientation, etc. Polylactide, polyglycolide, along with many other polymers have served as carriers of BMPs. However, the disadvantages of using synthetic polymers are their little osteoconductive potential, and can show unmanageable degradation and possible deposition of residues after degradation which can cause inflammation [33].

Calcium phosphates (CaP) such as tricalcium phosphates, hydroxyapatite and biphasic calcium phosphates are another group of materials that have been widely used as bone regeneration materials with and without the addition of growth factors. Sharing the same elements, calcium and phosphorus with natural bone tissue, intramuscularly implanted CaP can induce the formation of ectopic bone in animal models [34]. Indeed, all CaP ceramics are biocompatible. Releasing of calcium and phosphate ions *in vivo* into the microenvironment will enable the precipitation of apatite crystals in the implantation area by elevating the local concentration of ions [35]. Numerous studies

have shown the potential of CaP biomaterials in promoting bone regeneration [36]. The shortcomings of using CaP materials are, first, their osteoconductive properties are highly dependent on various surface parameters including roughness, porosity, pore size, surface charge and energy, which affect the ability of CaP ceramics to absorb adhesive molecules from the surrounding environments [37]. Second, the mechanical properties of CaP materials are relatively high, which are reflected in their high compressive strength, low impact resistance and poor tensile stress. This could obstruct the usage of CaP ceramics in some defect areas that are constantly under physical loading. Finally, another disadvantage is that classical CaP materials are commonly in solid states, some even in solid granules, therefore the application of such materials to small graft areas is difficult, requiring further stabilisation. This is currently being addressed partly by recent progress in new CaP materials, such as low temperature calcium orthophosphate cement, although still not optimal in macroporosity and in liquid/solid phase infusion, they are very flexible in shape that can be applied as injectable materials [38].

2.2 Cell-based bone tissue engineering

Over the past 4-5 decades, scientists and clinicians have used a variety of scaffolds and bone substitutes to facilitate regeneration. Incorporation of osteoinductive growth factors has indeed enhanced local osteogenic activity, but absence of a sufficient endogenous responsive cell population or omitting addition of exogenous cells may result in limited growth and poor cellular circulation inside the scaffold/substitute [39]. Therefore, in cases where the number of cells required are limiting to get the maximum repair potential, and to enhance both osteoconductive and osteoinductive properties of different biomaterials, new approaches for bone tissue engineering using different cell sources are being utilised.

2.2.1 Mature cells

Mature, differentiated cell populations for transplantation into defect sites, has historically been the primary approach of cell therapy used for bone regenerative purposes. This process always requires the harvesting of grafting material, expansion of differentiated cells and the presence of

some scaffold/biomaterials to ensure cell survival and exposure to nutrient diffusion from the surrounding tissues to enhance in tissue growth [40,41]. For example, osteoblasts derived from human mesenchymal stem cells have been supplemented into poly-lactic-co-glycolic acid (PLGA) scaffolds which promoted the healing of rat calvarial defects compared with acellular grafts [42]. Peptide-modified alginate polymers have also been used as a material to show that bone formation was improved by addition of osteoblasts [43]. Studies such as these have demonstrated the value of combining cells with materials, however, generating sufficient numbers of mature cells such as osteoblasts or chondrocytes through harvesting from primary tissue can often be limiting.

2.2.2 Mesenchymal stem cells

With the successful application of transplantation of bone marrow-derived haematopoietic stem cells to treat haematological disorders, other bone marrow-derived cells such as mesenchymal stem cells (MSCs) have similarly been considered a strong candidate for generating specific cell types for tissue regeneration. MSCs are a group of adult multipotent cells derived from various tissues including bone marrow and adipose tissue that possess some self-renewal potential and have the ability to differentiate into bone and cartilage [44–47] (Figure 1). MSCs have previously been loaded onto or mixed with various types of biomaterials, and as regards the composition of the materials, MSC adhesion and differentiation are largely dependent on the type and topography of the material, in terms of porosity, roughness, pattern, etc. [48] (Figure 2). As we gain a greater understanding into the natural properties of ECM produced by specific skeletal tissues, many polymeric materials were designed using techniques such as freeze drying, electrospinning, 3D printing, etc., to mimic the conformation of ECM that will enhance the potential of MSCs to form skeletal lineages [49,50]. Such polymer scaffolds have advantages in that inherently possess porous and fibrous structures, and can be fabricated into different shapes to suit the defects. However, the fabricating process of these polymeric materials typically involves the use of intensive chemicals that can be harmful to cells, therefore encapsulation of stem cells within these materials is difficult to accomplish.

Hydrogels have subsequently emerged as a powerful biomaterial for cell encapsulation. Hydrogels are a high water-content crosslinked hydrophilic polymer network that can be composed

of natural (e.g. collagen, fibronectin, hyaluronic acid/HA) or synthetic (e.g. polyacrylamide/PA, polyethylene glycol/PEG) polymers. The advantages of using hydrogels as stem cell carriers are that they can mimic various ECMs, are biocompatible, and are generally biodegradable with tunable and defined biophysical and biochemical properties [51]. Most hydrogels do not require harsh processes during gelation, which makes them ideal for cell encapsulation. Given these promising properties, hydrogels have recently been used in numerous studies involving MSC-based regeneration.

Because MSCs are naturally residing or circulating within the body or situated locally around a defect area, the simplest strategy for stem-cell based regeneration has aimed to recruit endogenous MSCs to the damaged site. Migration of endogenous MSCs relies on chemokines to ensure efficient homing of stem cells to a specific location or site of injury to divide and respond to local cues to differentiate into the appropriate differentiated cell type. To this end, several recent studies have focused on the chemokine CXCL12/SDF-1 crosslinked within an alginate hydrogel, and implanted into a critical defect in a rat femur, in order to stimulate *in vivo* bone marrow MSCs accumulation and osteogenic differentiation. Although full healing of the segmental bone defect was not achieved, both bone volume and bone mineral density were elevated significantly compared to chemokine-free hydrogels [52]. Additionally, injectable SDF-1-containing chitosan nanoparticles infused into chitosan hydrogels showed that when implanted into a rat critical-sized calvarial defect, the resulting controlled release of SDF-1 showed more regeneration of bone in the defect area [53]. These studies clearly demonstrated the value of site-specific recruitment of endogenous cells using chemokine/hydrogel constructs.

Independently of chemotactic factors, strategies have also focused on the role of metalloproteinases such as MMPs, which are secreted by osteoprogenitors and osteoblasts during bone remodeling and fracture healing, to enable the degradation of matrix, and subsequent invasion and migration of cells (Figure 2). Studies have shown that PEG hydrogels containing MMP-sensitive sequences within the crosslinker and implanting them into a critical-sized rat cranial defect, allowed efficient infiltration of cells that were able to generate bone-like tissue in a manner that correlated with MMP activity [54]. In another study the encapsulation of rat bone marrow MSCs into a poly(lactide-*co*-ethylene oxide-*co*-fumarate) hydrogel, the gelation of which was completed with a

MMP-13 degradable peptide sequence containing crosslinker, showed that MSCs remained viable and motile *in vitro* after 1 week of encapsulation, and 21 days after implantation cells were differentiated and mineralisation was observed at the defect site [55].

Recruitment of endogenous MSCs can be further enhanced within hydrogel materials due to their ability to undergo tunability in their physical and chemical properties, which can provide further stimuli affecting MSC fate decisions. Cell adhesion, proliferation and differentiation requires binding and interaction with ECM, therefore hydrogels have also been adapted to incorporate different types and presentation of binding ligands. For example, human MSCs cultured on vitronectin, collagen I or fibronectin-coated hydrogels, demonstrated that osteogenesis of hMSCs was most prominent on a type-I collagen coated substrate [56]. It has also been demonstrated that ligand spacing is critical and can affect osteogenic differentiation of cells [57]. In another study, a linear and cyclic RGD (Arginine-Glycine-Aspartate), binding peptide at both low and high density were conjugated to substrates and presented to bone marrow MSCs. The results showed that both cyclic and high density of RGD favored osteogenic differentiation of MSCs compared to linear and low density [58]. In addition, integrins and cadherins, which are responsible for cell-ECM and cell-cell interaction, respectively, have also been demonstrated to control MSC fate decision [48,59] (Figure 2). Together, studies such as these highlighted the importance of combining modifiable ECM components within hydrogels to enhance cell differentiation and tissue formation.

In the past decade, significant advances have been made in understanding the role of mechanical properties, especially the stiffness of hydrogels (Figure 2), which has drawn great attention as a major regulator of stem cell fate. Indeed, the elasticity of hydrogels typically ranges from 0.1 kPa to 50 kPa, falls perfectly into the spectrum of natural solid stromal tissues, from adipose to osteoid, and the elasticity of stem cell niches. This expands the usage of hydrogels to recapitulate various physiological conditions during development and repair. The first breakthrough study to show that MSCs can sense and respond to their mechanical surroundings was from Engler et al. [60], in which human MSCs were cultured onto PA gels with varying elasticities to find that MSCs differentiated to either neuronal, myoblastic or osteoblastic cells according to the stiffness of the substrate they were cultured on, with the best conditions for osteogenic differentiation being achieved at high stiffness,

30 kPa [60]. Moreover, that stem cell response to stiffness was represented by the spreading of cells and was mediated by non-muscle myosin II included cytoskeleton [60]. This study not only identified a new perspective of stem cells' ability to act in response to mechanical cues, but also emphasised the critical role of mechanotransduction during stem cell lineage specification. Numerous experiments have since then focused on the relations between elasticity and MSCs' fate [61,62].

In a recent study, by altering the concentration of PEG precursors, a PEG hydrogel with stiffnesses ranging from 6 kPa to 26 kPa was synthesised and plotted using a 2-dimensional microarray approach. Single human MSCs were then seeded onto each PEG hydrogel and confirmed that cell shape and the intensity of osteogenic differentiation was in accordance with the stiffness of gel [63]. In another study, covalently cross-linked collagen-coated polydimethylsiloxane (PDMS) and PA gels at varying stiffnesses from 0.1 kPa to 2300 kPa were seeded with hMSCs but whilst they demonstrated enhanced osteogenic differentiation with increased elastic modulus, this seemed to occur without the dependence on cellular morphology [64]. Modifying these hydrogels further by changing the collagen crosslinking density (protein tethering), showed that longer anchoring distances resulted in upregulated osteogenic differentiation [64] although other evidence suggests that protein tethering did not have a major role in altering MSC differentiation, and substrate stiffness was the only factor that affected MSC fate [65]. Thus, whilst the role of matrix stiffness is certainly established as major regulator of stem cell fate, the exact mechanisms of elasticity-mediated cell differentiation responses remain to be elucidated.

Apart from the studies on 2-dimensional substrates, significant advances have been made recently into 3-dimensional biomaterials, which provide a natural niche for cell growth, and where the many dynamic cellular processes occurring during development can be modelled in a 3D environment. Indeed, hydrogel matrices where cells are encapsulated in 3D have been shown to provide an environment where cells can detect different stiffnesses, adhesive ligands, generate traction forces, and mount an appropriate cellular response to proliferate and differentiate in a 3D environment [66–68]. Attempts at optimising the cellular response in 3D to match more physiological parameters has also taken into account that tissues inside the body are viscoelastic rather than elastic, which means that the resistance generated after the exertion of certain stress is slowly decreased over time (Figure

2). This includes collagen and fibrin fibers, adipose, liver, brain tissue and notably, primary hematoma formed at the fracture site and unmineralised cartilaginous callus during bone regeneration [69]. Indeed, MSCs encapsulated in a viscoelastic alginate hydrogel that undergo stress-relaxation showed that gels with the fastest stress-relaxation time and higher stiffness formed a greater type I collagen-abundant mineralized bone-like matrix, compared to MSCs in a longer stress-relaxing gel or gels with lower stiffness [70]. This was confirmed *in vivo* where MSCs encapsulated in a stress-relaxing hydrogel showed greater bone healing in fast relaxing gels compared to slow relaxing gels indicating a fundamental role of stress-relaxation in the guidance of bone tissue regeneration [71].

Finally, the advantages of further study of the role of ECM degradation has stimulated the usage of bespoke hydrogel constructs under the context of skeletal regeneration. ECM degradation is a key element during physiological and pathological processes, and has become an essential criterion when designing scaffolds for optimized tissue repair [43]. Cell-mediated degradation can in principle affect hydrogel stiffness [72] although the relationship between cross linking density, degradability and stiffness is complex. MSCs-encapsulated in a biodegradable hydrogel not only showed greater ability to retain and maintain stem cells, but the biodegradable properties enabled spatiotemporal control over the slow release of MSCs *in vivo* that other than poor diffusion or death of cells, approximately 10% of the total transplanted MSCs were found in the peripheral of the repair zone in a murine segmental femoral grafting model after 7 weeks [73]. In a 2013 study, ionically crosslinked, cell-degradable and covalently crosslinked non-degradable alginate hydrogels were synthesized and used as carrier materials for human MSCs. MSCs consistently remained rounded in non-degradable gels and underwent adipogenesis, while they spread well and underwent osteogenesis in degradable gels, regardless of stiffness, indicating that degradation enabled cells to pull on the hydrogel and generate traction forces [74]. Recently, an MMP-cleavable methacrylated dextran hydrogel was used to prove that degradation could promote collective cell migration, which is essential for the formation of blood vessels during development and repair [75]. Finally, recent observations using 3D-encapsulated MSCs in HA-based hydrogels demonstrated clearly that cell-mediated ECM synthesis, degradation and remodeling could direct stem cell fate [76]. While there are also studies showing that MSCs can

remodel their surroundings by generating traction forces independently of matrix degradability [77], it is clear that matrix degradation is a parameter that cannot be neglected for optimizing material-based tissue engineering, and the exact mechanisms behind cellular responses to degradability of biomaterials is still being elucidated.

The recent advances in hydrogel and materials technology have opened up new avenues and considerations for tissue engineering, but importantly, have also brought the fields of cell biology and biomaterials together to advance the prospects of cell-based therapy. This has been matched by advances in stem cell technology which have provided novel approaches to identifying optimal cell populations. MSCs have long been the preferred cell type for use in skeletal tissue repair and related studies by virtue of their multipotency and accessibility. However, Pluripotent Stem Cells have recently emerged as attractive cell sources for tissue engineering strategies and for cell-based therapies.

2.2.3 Pluripotent Stem Cells

The prospects of using Pluripotent Stem Cells (PSCs) as candidates for creating lineage-specific precursors and differentiated cell types have been greatly enhanced by the ability to direct their differentiation *in vitro* in ways that mimic normal embryonic development [78]. PSCs, including Embryonic Stem Cells (ESCs) and induced Pluripotent Stem Cells (iPSCs), are totipotent cells that can differentiate into all lineages. iPSCs were discovered over a decade ago following the discovery in both mouse and human systems that adult somatic cells could be reprogrammed to a pluripotent state resembling ESCs [79–82]. This offered the potential to generate abundant, defined cell populations from PSCs that are “tissue engineering-ready”, and can be utilised in the repair of a wide range of tissues and organs, including cartilage and bone (Figure 1). Indeed, there have been significant advances in harnessing the potential of ESCs and iPSCs to give rise to translatable and transplantable cells that are defined at the cellular and molecular levels, making them the most promising future candidates for cell-based therapies [83–85].

To recapitulate the various developmental pathways required for induction of specific lineages, many approaches have attempted to harness the complex biochemical and biomechanical cues to

guide and direct PSC differentiation. To facilitate skeletal regeneration, initial efforts were made to generate MSCs from PSCs via the formation of embryoid bodies (EBs) following culture in MSC specific media [86] or culture on feeder cells like murine bone marrow stromals which gave rise to both cell populations with haematopoietic and mesenchymal surface markers [87]. However, though MSCs originated from EBs seeded onto various types of scaffolds including CaP cements led to newly formed bone in different animal bone defect models [88,89], these cell populations are still compromised in purity that have remaining PSCs and the risk of tumor formation [90]. Besides MSCs, studies also tried to generate osteoblast-like cells from PSCs [91], for example by maintaining EBs differentiated from PSCs in osteogenic media [92] or co-culture of PSCs with primary cells isolated from bone tissue [93]. Although upregulation of osteogenic markers and loss of pluripotent markers were observed, these osteoblast-like cell populations still had some potential to cause teratoma formation *in vivo* after long-term culturing *in vitro* [92,94]. To address this, studies have incorporated growth factors or small molecules, for example BMP-2 [95] or adenosine [96], together with biomaterials to more thoroughly direct differentiation of PSCs within biomaterials *in vivo*, and to avoid the formation of teratomas and to achieve better differentiation. Another example is one study where iPSCs-derived osteoblasts and osteoclasts were incorporated together with 3-dmensional synthetic polymeric scaffold for repairing bone defects [97] (Figure 2). However, overall, current MSCs and osteoblast-like cell populations produced from PSCs still need robust methods for purification before they can reliably be put into clinical use.

Before differentiation, PSCs that are maintained in a pluripotent state must be expanded and differentiated efficiently *in vitro*. Traditionally, xenogeneic ingredients as fibroblastic feeder cells or reconstituted substrates like Matrigel are required for the maintenance of PSCs self-renewal [98]. However, these xenogeneic components in culture often lack consistency in composition and can confound the outcome of PSC culturing. To address this, replacing components like recombinant laminin and RGD peptides have emerged to enable xeno-free culture conditions for PSCs. In addition, with the establishment of the niche concept, where stem cells reside naturally *in vivo* (Figure 2), and the elaboration in fabricating biomaterials, novel biophysical properties are being used, with or without a combination of defined chemical factors, to construct an environment suitable for

maintaining PSCs. For example, a polyacrylamide gel at low stiffness was proven to promote homogeneous self-renewal of mESCs [99], and mESCs plated onto PEG hydrogels infused with different growth factors showed that combinations of matrix elasticity and soluble factors can result in altered proliferation and pluripotency [100]. In another study, hiPSCs were seeded onto nano-grooved polyimide to observe structural changes in cell shape and cytoskeleton, which correlated with pluripotency [101]. Such studies clearly demonstrated the sensitivity and flexibility of PSCs to respond to extracellular cues, as well as emphasising the complexity in designing a suitable culturing system for PSCs.

Principles for bone/cartilage regeneration have been guided by understanding the process of bone/cartilage development (Figure 1) and the mechanisms of fracture healing, which have led to various strategies targeting and harnessing different cell phases/populations for tissue restoration. For example, both minimally manipulated iPSCs seeded on a TCP-bonded scaffold [102] and iPSC-derived chondrocytes which possessed an articular cartilage phenotype [103] were showed functionality in regenerating cartilage tissue, confirming the promising prospects of iPSCs in cartilage repair [104]. In addition, lineage tracing of embryonic bone formation through endochondral ossification has enabled the development of protocols to produce transient chondrocytes from PSCs. By treating mESCs with TGFβ in serum-free media, cells underwent chondrogenic differentiation represented by type II collagen expression. Following implantation of Coll 2-expressing mESC aggregates into immunodeficient mice, endochondral ossification and bone formation was observed after three weeks, although there was also some teratoma formation [105]. Although the protocol was proved to be not applicable to human PSCs, this study showed for the first time the possibility of using transient, chondrocytic PSCs to grow bone *in vivo* in a manner mimicking a physiological process, and was thought to be more advanced than traditional approaches to restore bone tissue [106].

During development, not long after formation of the three germ layers (gastrulation), mesoderm will be further divided into different domains depending on their location. Among these regions, paraxial mesoderm will give rise to the formation of muscle, the axial skeleton and axial dermis. Therefore, cell populations that express a distinct paraxial mesodermal profile have been a target

endpoint during the lineage specification of PSCs for skeletal regeneration. Earlier last decade, it was first revealed that the expression of paraxial mesodermal markers in murine and human PSCs was promoted upon treatment of BMP4 and Wnt-agonists, which further expressed upregulated osteogenic markers under osteogenic culture conditions [107]. This population was thus considered to contain possible osteochondroprogenitors (Figure 1). Indeed, PSCs treated with BMP and GSK3β inhibitors led to paraxial mesodermal progenitors that subsequently gave rise to Sox9 expressing chondrocytes after culture in chondrogenic media [108–110]. These populations were proven to be transient chondrocytes where endochondral ossification was confirmed after implantation *in vivo*, although no signs of hypertrophy were observed. Similarly, another study showed successful manipulation of hESCs towards mature chondrocytes by using GDF5 during a stage-specific induction protocol [111]. This protocol also involved combinations of growth factors that mimicked a developmental process resembling primitive streak and mesoderm induction. Further studies presented enhanced chondrogenesis by switching from BMP4 to BMP2 during later stages of induction [112]. Although the exact map of molecular pathways that can master and recapitulate the whole developmental process is still progressing, more recent studies have successfully tuned these chondrocyte-specified cell populations from a transient state to a stable state. Craft et al. showed that by using TGFβ3 to activate the TGFβ signaling pathway in hPSC-derived chondrogenic progenitors, these cell populations were pushed to stable chondrocytes that formed cartilage tissue resembling articular cartilage both *in vitro* and *in vivo* [113]. The latest subsequent experiments confirmed the ability of such stable populations to repair an osteochondral defect in a rat trochlear model [114], with a subset of implantations undergoing endochondral ossification. Taken together, these studies are huge steps forward towards final utilisation of these lineage-committed PSCs in a clinical setting to restore bone/cartilage defects.

Apart from a precisely controlled exposure to biochemical signals, matrix composition and mechanical signals are now widely considered to be strong factors in biasing and directing PSC differentiation and these are important components of the stem cell niche (Figure 2). Learning from vastly explored relationships between biophysical properties of biomaterials and MSCs, bulk stiffness was the first property to be used. For example, PDMS substrates at a stiffness of over 1 MPa favored

the mesodermal and endodermal differentiation of mESCs [115]; hESCs cultured on PA hydrogel preferably underwent mesodermal differentiation at lower stiffness (~0.4 kPa) compare to stiff, through upregulation and localisation of β-catenin at cell-cell contacts [116]. Under a 3D context, it was shown that high stiffness (~1.5 to 6 MPa) promoted mesodermal differentiation of hESCs using a poly(L-lactic acid)/PLGA hydrogel, while intermediate (0.1 to 1 MPa) and low (< 0.1 MPa) stiffnesses led to endodermal and ectodermal differentiation respectively [117]. Besides stiffness, material biochemical composition was also proven to be one of the major factors in directing PSCs differentiation [118]. Interestingly, studies have also shown that under the treatment of TGFβ1, by simply encapsulating EB-derived differentiated mESCs into PEG hydrogels, a 3D environment alone without specified biophysical parameters increased chondrogenesis of cells [119]. In another study, after culture in chondrocyte-conditioned medium, hESC-derived MSCs presented greatly promoted cartilage repair *in vivo* when seeded into a PEGDA hydrogel [120]. Together these studies denoted the importance of three-dimensional structure for stem-cell involved regeneration. These studies clearly demonstrated that biomaterials, rather than simple scaffolds for cells to attach on, harbour properties which should be taken seriously into consideration when designing integrative and reparative regeneration systems containing PSCs.

The current state of art in biochemical and biophysical control over PSCs fate has enabled great progress in the path to generating "tissue-engineering-ready" cell populations, especially in neural [121] and cardiac [122] regeneration. However, in terms of skeletal regeneration, we still need more evidence and experience before we could fully manipulate PSCs for clinical use. To date, the understanding of the molecular mechanisms behind *in vitro* recapitulation of development using PSCs, the hierarchy and relationships between biochemical and biophysical cues controlling PSCs, and efforts and techniques to integrate novel biomechanical properties with biochemical cues into biomaterials that best mimic natural microenvironment, have been a major step forward, however, more studies are urgently needed.

3. Conclusions and outlook

During the past 20 years, biomaterials-directed tissue regeneration has grown so fast that we have moved from non-oriented simple grafting to the focused and directed biophysical and biochemical manipulation of stem cell behaviour. Hydrogels, along with other biomaterials with unique features, have led to the establishment of various systems that mimic *in vivo* conditions. Indeed, the progress in bioengineering has begun to break the boundaries that separated different types of biomaterials and different approaches have started to merge together, so that single types of materials are able to possess multi-grade biological features [123]. In addition, with advances in the generation and tight control of PSC differentiation, we are becoming more confident in designing and producing cell populations that represent certain timepoints in development or possesses a unique profile that could efficiently facilitate the precise regeneration of tissue within various bioengineering systems. Indeed, many studies are starting to use iPSCs in preclinical reconstructive surgeries, providing strong evidences of their osteogenic potential and supporting for future clinical applications [124]. However, there are still several aspects that remain unsolved. Firstly, for each type of biomaterial, the establishment of gold-standard criteria from their composition and structure to their instructive parameters for stem cells would be extremely beneficial. Secondly, traditional tissue engineering approaches have been trying to decouple soluble cues from insoluble cues for the identification of a single “magic bullet”, but the fact that cells *in vivo* reside in a complex milieu of both soluble/insoluble cues combined with the numerous niche components (Figure 2), and understanding the interplay between these inputs, is being increasingly recognised as a critical barrier that needs to be addressed in a tissue engineering context. Finally, a thorough establishment and understanding of reproducible and tractable protocols for manipulation of specific stem cell populations and differentiation intermediates are of vital need to produce robust “tissue-engineering-ready” populations.

The goal has always been seemingly simple, to create a method that fully repairs and heals to restore tissue function. Clearly this simple goal is rather complex. But by combining novel, smart materials and well-defined cell populations, the field is moving forward rapidly towards achieving cell-based therapies for skeletal and other tissues.

References

1. Berendsen, A. D. & Olsen, B. R. Bone development. *Bone* **80**, 14–18 (2015).
2. Rice, R., Rice, D. P. C., Olsen, B. R. & Thesleff, I. Progression of calvarial bone development requires Foxc1 regulation of Msx2 and Alx4. *Dev. Biol.* **262**, 75–87 (2003).
3. Maes, C. *et al.* Osteoblast Precursors, but Not Mature Osteoblasts, Move into Developing and Fractured Bones along with Invading Blood Vessels. *Dev. Cell* **19**, 329–344 (2010).
4. King, J. A., Marker, P. C., Seung, K. J. & Kingsley, D. M. BMP5 and the Molecular, Skeletal, and Soft-Tissue Alterations in short ear Mice. *Dev. Biol.* **166**, 112–122 (1994).
5. Storm, E. E. *et al.* Limb alterations in brachypodism mice due to mutations in a new member of the TGF beta-superfamily. *Nature* **368**, 639–643 (1994).
6. Zanotti, S. & Canalis, E. Mechanisms in Endocrinology: Notch signaling in skeletal health and disease. *Eur. J. Endocrinol.* **168**, R95–R103 (2013).
7. Wang, Y. Wnt and the Wnt signaling pathway in bone development and disease. *Front. Biosci.* **19**, 379 (2014).
8. Karsenty, G. Minireview: Transcriptional Control of Osteoblast Differentiation. *Endocrinology* **142**, 2731–2733 (2001).
9. Franceschi, R. T., Ge, C., Xiao, G., Roca, H. & Jiang, D. Transcriptional Regulation of Osteoblasts. *Cells Tissues Organs* **189**, 144–152 (2009).
10. Lefebvre, V. & Smits, P. Transcriptional control of chondrocyte fate and differentiation. *Birth Defects Res. Part C Embryo Today Rev.* **75**, 200–212 (2005).
11. Eames, B. F., Fuente, L. D. L. & Helms, J. A. Molecular ontogeny of the skeleton. *Birth Defects Res. Part C Embryo Today Rev.* **69**, 93–101 (2003).
12. Loi, F. *et al.* Inflammation, fracture and bone repair. *Bone* **86**, 119–130 (2016).
13. Einhorn, T. A. & Gerstenfeld, L. C. Fracture healing: mechanisms and interventions. *Nat. Rev. Rheumatol.* **11**, 45–54 (2015).
14. Gerstenfeld, L. C., Cullinane, D. M., Barnes, G. L., Graves, D. T. & Einhorn, T. A. Fracture healing as a post-natal developmental process: Molecular, spatial, and temporal aspects of its regulation. *J. Cell. Biochem.* **88**, 873–884 (2003).
15. Hankenson, K. D., Zimmerman, G. & Marcucio, R. Biological perspectives of delayed fracture healing. *Injury* **45**, S8–S15 (2014).
16. Schindeler, A., McDonald, M. M., Bokko, P. & Little, D. G. Bone remodeling during fracture repair: The cellular picture. *Semin. Cell Dev. Biol.* **19**, 459–466 (2008).
17. Granström, G. & Nilsson, L. P. Experimental Mandibular Fracture: Studies on Bone Repair and Remodellation. *Scand. J. Plast. Reconstr. Surg.* **21**, 159–165 (1987).
18. Hasegawa, T. *et al.* Mandibular Hematoma Cells as a Potential Reservoir for Osteoprogenitor Cells in Fractures. *J. Oral Maxillofac. Surg.* **70**, 599–607 (2012).
19. Hinton, R. J., Jing, Y., Jing, J. & Feng, J. Q. Roles of Chondrocytes in Endochondral Bone Formation and Fracture Repair. *J. Dent. Res.* **96**, 23–30 (2017).
20. Vieira, W. A. & McCusker, C. D. Regenerative Models for the Integration and Regeneration of Head Skeletal Tissues. *Int. J. Mol. Sci.* **19**, 3752 (2018).

21. Owen, R. & Reilly, G. C. In vitro Models of Bone Remodelling and Associated Disorders. *Front. Bioeng. Biotechnol.* **6**, 134 (2018).
22. Nandiraju, D. & Ahmed, I. Human skeletal physiology and factors affecting its modeling and remodeling. *Fertil. Steril.* **112**, 775–781 (2019).
23. Delaisse, J.-M. The reversal phase of the bone-remodeling cycle: cellular prerequisites for coupling resorption and formation. *BoneKEy Rep.* **3**, 561 (2014).
24. Manolagas, S. C. Birth and Death of Bone Cells: Basic Regulatory Mechanisms and Implications for the Pathogenesis and Treatment of Osteoporosis. **21**, 23 (2000).
25. Langdahl, B., Ferrari, S. & Dempster, D. W. Bone modeling and remodeling: potential as therapeutic targets for the treatment of osteoporosis: *Ther. Adv. Musculoskelet. Dis.* (2016) doi:10.1177/1759720X16670154.
26. Kornman, K. S. Mapping the Pathogenesis of Periodontitis: A New Look. *J. Periodontol.* **79**, 1560–1568 (2008).
27. Boer, F. C. den *et al.* New segmental long bone defect model in sheep: Quantitative analysis of healing with dual energy X-ray absorptiometry. *J. Orthop. Res.* **17**, 654–660 (1999).
28. Vajgel, A. *et al.* A systematic review on the critical size defect model. *Clin. Oral Implants Res.* **25**, 879–893 (2014).
29. Majidinia, M., Sadeghpour, A. & Yousefi, B. The roles of signaling pathways in bone repair and regeneration. *J. Cell. Physiol.* **233**, 2937–2948 (2018).
30. Wozney, J. M. The bone morphogenetic protein family and osteogenesis. *Mol. Reprod. Dev.* **32**, 160–167 (1992).
31. Salazar, V. S., Gamer, L. W. & Rosen, V. BMP signalling in skeletal development, disease and repair. *Nat. Rev. Endocrinol.* **12**, 203–221 (2016).
32. Martino, M. M., Briquez, P. S., Maruyama, K. & Hubbell, J. A. Extracellular matrix-inspired growth factor delivery systems for bone regeneration. *Adv. Drug Deliv. Rev.* **94**, 41–52 (2015).
33. Seeherman, H. & Wozney, J. M. Delivery of bone morphogenetic proteins for orthopedic tissue regeneration. *Cytokine Growth Factor Rev.* **16**, 329–345 (2005).
34. Klein, C., de Groot, K., Chen, W., Li, Y. & Zhang, X. Osseous substance formation induced in porous calcium phosphate ceramics in soft tissues. *Biomaterials* **15**, 31–34 (1994).
35. *Advances in Calcium Phosphate Biomaterials*. (Springer-Verlag, 2014).
36. Eliaz, N. & Metoki, N. Calcium Phosphate Bioceramics: A Review of Their History, Structure, Properties, Coating Technologies and Biomedical Applications. *Materials* **10**, 334 (2017).
37. Samavedi, S., Whittington, A. R. & Goldstein, A. S. Calcium phosphate ceramics in bone tissue engineering: A review of properties and their influence on cell behavior. *Acta Biomater.* **9**, 8037–8045 (2013).
38. O'Neill, R. *et al.* Critical review: Injectability of calcium phosphate pastes and cements. *Acta Biomater.* **50**, 1–19 (2017).
39. Agarwal, S., Wendorff, J. H. & Greiner, A. Progress in the field of electrospinning for tissue engineering applications. *Adv. Mater. Deerfield Beach Fla* **21**, 3343–3351 (2009).
40. Zhang, X. *et al.* Periosteal Progenitor Cell Fate in Segmental Cortical Bone Graft Transplantations: Implications for Functional Tissue Engineering. *J. Bone Miner. Res.* **20**, 2124–2137 (2005).

41. Xie, C. *et al.* Structural Bone Allograft Combined with Genetically Engineered Mesenchymal Stem Cells as a Novel Platform for Bone Tissue Engineering. *Tissue Eng.* **13**, 435–445 (2007).
42. Laboratory of Stem Cells, Institute of Cell Biology, College of Life Sciences, Zhejiang University, 388 Yuhangtang Road, Hangzhou, Zhejiang 310058, PR China *et al.* Reconstruction of rat calvarial defects with human mesenchymal stem cells and osteoblast-like cells in poly-lactic-co-glycolic acid scaffolds. *Eur. Cell. Mater.* **20**, 109–120 (2010).
43. Alsberg, E. *et al.* Regulating Bone Formation *via* Controlled Scaffold Degradation. *J. Dent. Res.* **82**, 903–908 (2003).
44. Oryan, A., Kamali, A., Moshiri, A. & Eslaminejad, M. B. Role of Mesenchymal Stem Cells in Bone Regenerative Medicine: What Is the Evidence? *Cells Tissues Organs* **204**, 59–83 (2017).
45. Garg, P. *et al.* Prospective Review of Mesenchymal Stem Cells Differentiation into Osteoblasts. *Orthop. Surg.* **9**, 13–19 (2017).
46. Cui, D. *et al.* Mesenchymal Stem Cells for Cartilage Regeneration of TMJ Osteoarthritis. *Stem Cells Int.* **2017**, 5979741 (2017).
47. Richardson, S. M. *et al.* Mesenchymal stem cells in regenerative medicine: Focus on articular cartilage and intervertebral disc regeneration. *Methods* **99**, 69–80 (2016).
48. Dalby, M. J., Gadegaard, N. & Oreffo, R. O. C. Harnessing nanotopography and integrin–matrix interactions to influence stem cell fate. *Nat. Mater.* **13**, 558–569 (2014).
49. Szpalski, C., Wetterau, M., Barr, J. & Warren, S. M. Bone Tissue Engineering: Current Strategies and Techniques—Part I: Scaffolds. *Tissue Eng. Part B Rev.* **18**, 246–257 (2012).
50. Preethi Soundarya, S., Haritha Menon, A., Viji Chandran, S. & Selvamurugan, N. Bone tissue engineering: Scaffold preparation using chitosan and other biomaterials with different design and fabrication techniques. *Int. J. Biol. Macromol.* **119**, 1228–1239 (2018).
51. Naahidi, S. *et al.* Biocompatibility of hydrogel-based scaffolds for tissue engineering applications. *Biotechnol. Adv.* **35**, 530–544 (2017).
52. Cipitria, A. *et al.* In-situ tissue regeneration through SDF-1α driven cell recruitment and stiffness-mediated bone regeneration in a critical-sized segmental femoral defect. *Acta Biomater.* **60**, 50–63 (2017).
53. Mi, L., Liu, H., Gao, Y., Miao, H. & Ruan, J. Injectable nanoparticles/hydrogels composite as sustained release system with stromal cell-derived factor-1α for calvarial bone regeneration. *Int. J. Biol. Macromol.* **101**, 341–347 (2017).
54. Lutolf, M. P. *et al.* Synthetic matrix metalloproteinase-sensitive hydrogels for the conduction of tissue regeneration: Engineering cell-invasion characteristics. *Proc. Natl. Acad. Sci.* **100**, 5413–5418 (2003).
55. He, X. & Jabbari, E. Material Properties and Cytocompatibility of Injectable MMP Degradable Poly(lactide ethylene oxide fumarate) Hydrogel as a Carrier for Marrow Stromal Cells. *Biomacromolecules* **8**, 780–792 (2007).
56. Chastain, S. R., Kundu, A. K., Dhar, S., Calvert, J. W. & Putnam, A. J. Adhesion of mesenchymal stem cells to polymer scaffolds occurs via distinct ECM ligands and controls their osteogenic differentiation. *J. Biomed. Mater. Res. A* **78A**, 73–85 (2006).
57. Lee, K. Y. *et al.* Nanoscale Adhesion Ligand Organization Regulates Osteoblast Proliferation and Differentiation. *Nano Lett.* **4**, 1501–1506 (2004).

58. Kilian, K. A. & Mrksich, M. Directing Stem Cell Fate by Controlling the Affinity and Density of Ligand-Receptor Interactions at the Biomaterials Interface. *Angew. Chem. Int. Ed.* **51**, 4891–4895 (2012).
59. Cosgrove, B. D. *et al.* N-cadherin adhesive interactions modulate matrix mechanosensing and fate commitment of mesenchymal stem cells. *Nat. Mater.* **15**, 1297–1306 (2016).
60. Engler, A. J., Sen, S., Sweeney, H. L. & Discher, D. E. Matrix Elasticity Directs Stem Cell Lineage Specification. *Cell* **126**, 677–689 (2006).
61. Li, L., Eyckmans, J. & Chen, C. S. Designer biomaterials for mechanobiology. *Nat. Mater.* **16**, 1164–1168 (2017).
62. Shin, J.-W. & Mooney, D. J. Improving Stem Cell Therapeutics with Mechanobiology. *Cell Stem Cell* **18**, 16–19 (2016).
63. Gobaa, S. *et al.* Artificial niche microarrays for probing single stem cell fate in high throughput. *Nat. Methods* **8**, 949–955 (2011).
64. Trappmann, B. *et al.* Extracellular-matrix tethering regulates stem-cell fate. *Nat. Mater.* **11**, 642–649 (2012).
65. Wen, J. H. *et al.* Interplay of matrix stiffness and protein tethering in stem cell differentiation. *Nat. Mater.* **13**, 979–987 (2014).
66. Huebsch, N. *et al.* Harnessing traction-mediated manipulation of the cell/matrix interface to control stem-cell fate. *Nat. Mater.* **9**, 518–526 (2010).
67. Benoit, D. S. W., Schwartz, M. P., Durney, A. R. & Anseth, K. S. Small functional groups for controlled differentiation of hydrogel-encapsulated human mesenchymal stem cells. *Nat. Mater.* **7**, 816–823 (2008).
68. Huebsch, N. *et al.* Matrix elasticity of void-forming hydrogels controls transplanted-stem-cell-mediated bone formation. *Nat. Mater.* **14**, 1269–1277 (2015).
69. McDonald, S. J. *et al.* Early fracture callus displays smooth muscle-like viscoelastic properties ex vivo: Implications for fracture healing. *Journal of Orthopaedic Research* https://onlinelibrary.wiley.com/doi/abs/10.1002/jor.20923 (2009) doi:10.1002/jor.20923.
70. Chaudhuri, O. *et al.* Hydrogels with tunable stress relaxation regulate stem cell fate and activity. *Nat. Mater.* **15**, 326–334 (2016).
71. Darnell, M. *et al.* Substrate Stress-Relaxation Regulates Scaffold Remodeling and Bone Formation In Vivo. *Adv. Healthc. Mater.* **6**, 1601185 (2017).
72. Sarem, M. *et al.* Interplay between stiffness and degradation of architectured gelatin hydrogels leads to differential modulation of chondrogenesis in vitro and in vivo. *Acta Biomater.* **69**, 83–94 (2018).
73. Hoffman, M. D., Van Hove, A. H. & Benoit, D. S. W. Degradable hydrogels for spatiotemporal control of mesenchymal stem cells localized at decellularized bone allografts. *Acta Biomater.* **10**, 3431–3441 (2014).
74. Khetan, S. *et al.* Degradation-mediated cellular traction directs stem cell fate in covalently crosslinked three-dimensional hydrogels. *Nat. Mater.* **12**, 458–465 (2013).
75. Trappmann, B. *et al.* Matrix degradability controls multicellularity of 3D cell migration. *Nat. Commun.* **8**, 371 (2017).

76. Ferreira, S. A. *et al.* Bi-directional cell-pericellular matrix interactions direct stem cell fate. *Nat. Commun.* **9**, 4049 (2018).
77. Ho, F. C., Zhang, W., Li, Y. Y. & Chan, B. P. Mechanoresponsive, omni-directional and local matrix-degrading actin protrusions in human mesenchymal stem cells microencapsulated in a 3D collagen matrix. *Biomaterials* **53**, 392–405 (2015).
78. Murry, C. E. & Keller, G. Differentiation of Embryonic Stem Cells to Clinically Relevant Populations: Lessons from Embryonic Development. *Cell* **132**, 661–680 (2008).
79. Takahashi, K. & Yamanaka, S. Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors. *Cell* **126**, 663–676 (2006).
80. Takahashi, K. *et al.* Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors. *Cell* **131**, 861–872 (2007).
81. Yu, J. *et al.* Induced Pluripotent Stem Cell Lines Derived from Human Somatic Cells. *Science* **318**, 1917–1920 (2007).
82. Park, I.-H. *et al.* Reprogramming of human somatic cells to pluripotency with defined factors. *Nature* **451**, 141–146 (2008).
83. Martello, G. & Smith, A. The Nature of Embryonic Stem Cells. *Annu. Rev. Cell Dev. Biol.* **30**, 647–675 (2014).
84. Rowe, R. G. & Daley, G. Q. Induced pluripotent stem cells in disease modelling and drug discovery. *Nat. Rev. Genet.* **20**, 377–388 (2019).
85. Shi, Y., Inoue, H., Wu, J. C. & Yamanaka, S. Induced pluripotent stem cell technology: a decade of progress. *Nat. Rev. Drug Discov.* **16**, 115–130 (2017).
86. Lee, E. J. *et al.* Novel Embryoid Body–Based Method to Derive Mesenchymal Stem Cells from Human Embryonic Stem Cells. *Tissue Eng. Part A* **16**, 705–715 (2009).
87. Trivedi, P. & Hematti, P. Simultaneous generation of CD34+ primitive hematopoietic cells and CD73+ mesenchymal stem cells from human embryonic stem cells cocultured with murine OP9 stromal cells. *Exp. Hematol.* **35**, 146–154 (2007).
88. Villa-Diaz, L. G. *et al.* Derivation of Mesenchymal Stem Cells from Human Induced Pluripotent Stem Cells Cultured on Synthetic Substrates. *STEM CELLS* **30**, 1174–1181 (2012).
89. Rana, D., Kumar, S., Webster, T. J. & Ramalingam, M. Impact of Induced Pluripotent Stem Cells in Bone Repair and Regeneration. *Curr. Osteoporos. Rep.* **17**, 226–234 (2019).
90. Zou, Q. *et al.* Development of a Xeno-Free Feeder-Layer System from Human Umbilical Cord Mesenchymal Stem Cells for Prolonged Expansion of Human Induced Pluripotent Stem Cells in Culture. *PLOS ONE* **11**, e0149023 (2016).
91. Zhu, H., Kimura, T., Swami, S. & Wu, J. Y. Pluripotent stem cells as a source of osteoblasts for bone tissue regeneration. *Biomaterials* **196**, 31–45 (2019).
92. Sottile, V., Thomson, A. & McWhir, J. In Vitro Osteogenic Differentiation of Human ES Cells. *Cloning Stem Cells* **5**, 149–155 (2003).
93. Ahn, S. E. *et al.* Primary bone-derived cells induce osteogenic differentiation without exogenous factors in human embryonic stem cells. *Biochem. Biophys. Res. Commun.* **340**, 403–408 (2006).
94. Kuznetsov, S. A., Cherman, N. & Robey, P. G. In Vivo Bone Formation by Progeny of Human Embryonic Stem Cells. *Stem Cells Dev.* **20**, 269–287 (2010).

95. Levi, B. *et al.* In vivo directed differentiation of pluripotent stem cells for skeletal regeneration. *Proc. Natl. Acad. Sci.* **109**, 20379–20384 (2012).
96. Kang, H., Shih, Y.-R. V., Nakasaki, M., Kabra, H. & Varghese, S. Small molecule–driven direct conversion of human pluripotent stem cells into functional osteoblasts. *Sci. Adv.* **2**, e1600691 (2016).
97. Jeon, O. H. *et al.* Human iPSC-derived osteoblasts and osteoclasts together promote bone regeneration in 3D biomaterials. *Sci. Rep.* **6**, 1–11 (2016).
98. Azarin, S. M. & Palecek, S. P. Matrix Revolutions: A Trinity of Defined Substrates for Long-Term Expansion of Human ESCs. *Cell Stem Cell* **7**, 7–8 (2010).
99. Chowdhury, F. *et al.* Soft Substrates Promote Homogeneous Self-Renewal of Embryonic Stem Cells via Downregulating Cell-Matrix Tractions. *PLOS ONE* **5**, e15655 (2010).
100. Ranga, A. *et al.* 3D niche microarrays for systems-level analyses of cell fate. *Nat. Commun.* **5**, 4324 (2014).
101. Abagnale, G. *et al.* Surface Topography Guides Morphology and Spatial Patterning of Induced Pluripotent Stem Cell Colonies. *Stem Cell Rep.* **9**, 654–666 (2017).
102. Uto, S., Nishizawa, S., Hikita, A., Takato, T. & Hoshi, K. Application of induced pluripotent stem cells for cartilage regeneration in CLAWN miniature pig osteochondral replacement model. *Regen. Ther.* **9**, 58–70 (2018).
103. Diederichs, S., Klampfleuthner, F. A. M., Moradi, B. & Richter, W. Chondral Differentiation of Induced Pluripotent Stem Cells Without Progression Into the Endochondral Pathway. *Front. Cell Dev. Biol.* **7**, 270 (2019).
104. Yamashita, A. *et al.* Considerations in hiPSC-derived cartilage for articular cartilage repair. *Inflamm. Regen.* **38**, 17 (2018).
105. Jukes, J. M. *et al.* Endochondral bone tissue engineering using embryonic stem cells. *Proc. Natl. Acad. Sci. U. S. A.* **105**, 6840–6845 (2008).
106. Tam, W. L., Luyten, F. P. & Roberts, S. J. From skeletal development to the creation of pluripotent stem cell-derived bone-forming progenitors. *Philos. Trans. R. Soc. B Biol. Sci.* **373**, 20170218 (2018).
107. Sakurai, H. *et al.* In vitro modeling of paraxial mesodermal progenitors derived from induced pluripotent stem cells. *PloS One* **7**, e47078 (2012).
108. Umeda, K. *et al.* Human chondrogenic paraxial mesoderm, directed specification and prospective isolation from pluripotent stem cells. *Sci. Rep.* **2**, 455 (2012).
109. Zhao, J. *et al.* Small molecule-directed specification of sclerotome-like chondroprogenitors and induction of a somitic chondrogenesis program from embryonic stem cells. *Development* **141**, 3848–3858 (2014).
110. Craft, A. M. *et al.* Specification of chondrocytes and cartilage tissues from embryonic stem cells. *Development* **140**, 2597–2610 (2013).
111. Oldershaw, R. A. *et al.* Directed differentiation of human embryonic stem cells toward chondrocytes. *Nat. Biotechnol.* **28**, 1187–1194 (2010).
112. Wang, T. *et al.* Enhanced chondrogenesis from human embryonic stem cells. *Stem Cell Res.* **39**, 101497 (2019).

113. Craft, A. M. *et al.* Generation of articular chondrocytes from human pluripotent stem cells. *Nat. Biotechnol.* **33**, 638–645 (2015).
114. Department of Orthopedic Surgery, Boston Children's Hospital, Enders 260, Mailstop 3096, 300 Longwood Avenue, Boston, MA 02115, USA. *et al.* Effective repair of articular cartilage using human pluripotent stem cell-derived tissue. *Eur. Cell. Mater.* **38**, 215–227 (2019).
115. Dept Materials, Royal School of Mines, Imperial College, Prince Consort Road, London SW7 2BP, U.K. *et al.* Substrate stiffness affects early differentiation events in embryonic stem cells. *Eur. Cell. Mater.* **18**, 1–14 (2009).
116. Przybyla, L., Lakins, J. N. & Weaver, V. M. Tissue Mechanics Orchestrate Wnt-Dependent Human Embryonic Stem Cell Differentiation. *Cell Stem Cell* **19**, 462–475 (2016).
117. Zoldan, J. *et al.* The influence of scaffold elasticity on germ layer specification of human embryonic stem cells. *Biomaterials* **32**, 9612–9621 (2011).
118. Dickinson, L. E., Kusuma, S. & Gerecht, S. Reconstructing the Differentiation Niche of Embryonic Stem Cells Using Biomaterials. *Macromol. Biosci.* **11**, 36–49 (2011).
119. Hwang, N. S. *et al.* Effects of Three-Dimensional Culture and Growth Factors on the Chondrogenic Differentiation of Murine Embryonic Stem Cells. *STEM CELLS* **24**, 284–291 (2006).
120. Hwang, N. S. *et al.* In vivo commitment and functional tissue regeneration using human embryonic stem cell-derived mesenchymal cells. *Proc. Natl. Acad. Sci.* **105**, 20641–20646 (2008).
121. Maclean, F. L., Rodriguez, A. L., Parish, C. L., Williams, R. J. & Nisbet, D. R. Integrating Biomaterials and Stem Cells for Neural Regeneration. *Stem Cells Dev.* **25**, 214–226 (2015).
122. Okano, S. & Shiba, Y. Therapeutic Potential of Pluripotent Stem Cells for Cardiac Repair after Myocardial Infarction. *Biol. Pharm. Bull.* **42**, 524–530 (2019).
123. Madl, C. M., Heilshorn, S. C. & Blau, H. M. Bioengineering strategies to accelerate stem cell therapeutics. *Nature* **557**, 335–342 (2018).
124. Fliefel, R., Ehrenfeld, M. & Otto, S. Induced pluripotent stem cells (iPSCs) as a new source of bone in reconstructive surgery: A systematic review and meta-analysis of preclinical studies. *J. Tissue Eng. Regen. Med.* **12**, 1780–1797 (2018).
125. Marie, P. J., Haÿ, E. & Saidak, Z. Integrin and cadherin signaling in bone: role and potential therapeutic targets. *Trends Endocrinol. Metab.* **25**, 567–575 (2014).
126. Kołodziejska, B., Kaflak, A. & Kolmas, J. Biologically Inspired Collagen/Apatite Composite Biomaterials for Potential Use in Bone Tissue Regeneration—A Review. *Materials* **13**, 1748 (2020).
127. Peng, Y. *et al.* Degradation rate affords a dynamic cue to regulate stem cells beyond varied matrix stiffness. *Biomaterials* **178**, 467–480 (2018).
128. Paiva, K. B. S. & Granjeiro, J. M. Chapter Six - Matrix Metalloproteinases in Bone Resorption, Remodeling, and Repair. in *Progress in Molecular Biology and Translational Science* (ed. Khalil, R. A.) vol. 148 203–303 (Academic Press, 2017).
129. Madl, C. M. & Heilshorn, S. C. Engineering Hydrogel Microenvironments to Recapitulate the Stem Cell Niche. *Annu. Rev. Biomed. Eng.* **20**, 21–47 (2018).

Figure legends:

Figure 1: Basic ontogeny and differentiation of chondrogenic and osteogenic lineages from mesodermal precursors cells towards tissue formation. Specification of mesoderm in the embryo during gastrulation yields a population of many lineages, including those harbouring potential for osteochondral cell differentiation. This early population is typically represented by expression of the essential master chondrogenic (SOX9) and osteogenic (RUNX2) transcription factors which are thought to be co-expressed at this stage. Commitment to specific chondrogenic and osteogenic lineages and maturation to tissue formation is generally driven by growth factors of the TGFβ, BMP and Wnt families in species- and tissue-specific combination as indicated. The goal of cell-based therapies is to direct differentiation of appropriate stem cells and precursors in a defined manner to expand populations that are stable and suitable for transplantation. While adult stem cells such as MSCs can provide populations of progenitors with limited osteogenic and chondrogenic potentials, pluripotent stem cells (PSCs) are emerging rapidly as an important source of readily expandable populations of early precursors that can be directed efficiently to any lineage, including bone and cartilage. All stem cells will require specific biochemical signals as well as biomechanical and chemistry considerations. See also text and glossary for specific details.

Figure 2: Niche interactions essential in bone physiology and regenerative strategies. The interactions of stem cells with their niche are essential for driving differentiation and ultimately, maturation and regeneration. There are a multitude of niche interactions shown, all of which have the potential to modulate stem cell behaviour. One goal of stem cell-based tissue engineering applications is to recapitulate some of these niche interactions through engineered biomaterials and hydrogel constructs. Specific cell-niche interactions vary in a tissue-specific manner, therefore a thorough knowledge of the development and molecular interactions of the tissue of choice (eg. bone, cartilage) is essential. Red boxes show niche components that are important in normal bone physiology as well as being essential parameters/factors for stem cell fate decision during design of biomaterials for bone regeneration: eg. Cadherins/integrins/notch signaling [6,48,59,125], Collagen and topography of biomaterials [48,126], Growth factors [13], bone matrix/materials degradation [127,128], osteoblast-osteoclast coupling and remodeling [22], and mechanobiology parameters, i.e. matrix stiffness, stress relaxation [60,70]. Modified from Madl and Heilshorn. 2018. Annu. Rev. Biomed. Eng. 20:21–47 (see ref. 129). See also text and glossary for specific details.

Glossary

Articular cartilage: Thin layer of hyaline cartilage that lines the ends of bones allowing articulation of joints.

Biomaterials: A group of engineered materials designed to interact with biological conditions for therapeutic or diagnostic use.

Bone/cartilage cells: Osteoblasts – cells that make bone; Osteoclasts – cells that eat (resorb) bone; Osteocytes – cells embedded inside bone that detect mechanical loads; chondrocytes – cells that make cartilage.

Bone/cartilage growth factors: Examples of growth factors and signaling pathways affecting bone and cartilage cell differentiation and function, include BMP, TGFβ, Wnt, Notch, cadherins, IGF, and PDGF. See text for details.

Calvaria: The top of the skull, skull cap.

Cartilaginous condyle: The secondary cartilage tissue residing in mandible, which is unique in its appearance and function compared to primary cartilaginous tissue.

Chemokines: A family of small cytokines or proteins secreted by cells that have the ability to induce chemotaxis.

Chitosan: A linear polysaccharide produced by deacetylation of chitin, a derivative of glucose. Chitosan is water-soluble and bio-adhesive and can be fabricated into multiple types of bio-scaffolds.

Cortical bone *(also called compact bone):* The dense outer layer of bone eg. long bones, that protects the internal bone marrow space. Makes up ~80% bone mass.

Critical (sized) defect: A loss of eg. bone tissue of certain size that cannot heal spontaneously without intervention. Critical sizes vary between species and location of defect.

Crosslink: Bonds linking one polymer to another, consisting of covalent and ionic bonds. A crosslinker is the chemical or biological agent that can link one polymer to another.

Embryoid bodies (EBs): 3-dimensional cell aggregates from pluripotent stem cells.

Embryonic stem cells (ESCs): Pluripotent stem cells derived from the inner cell mass of a pre-implantation embryo.

Endochondral ossification: One relatively complex process of bone formation during development where bone tissue is formed via a cartilage template. Long bones and vertebrae typically form via this process.

Extracellular Matrix (ECM): Non-cellular fibrous network composed of macromolecules like collagens and glycoproteins that provides structural support and is essential for tissue/cell morphogenesis, differentiation and homeostasis.

Gastrulation: Stage during early embryonic development that specifies the three embryonic germ layers, endoderm, ectoderm, mesoderm, creating a gastrula.

Induced pluripotent stem cells (iPSCs): Pluripotent stem cells created by nuclear reprogramming of somatic cells.

Intramembranous ossification: One relatively simple process of bone formation during development where bone tissue is formed directly from mesenchyme. Parts of the skull typically form via this process.

Mechanotransduction: A mechanism by which cells convert mechanical stimuli into electrochemical and biological activities.

Mesenchymal stem cells: Multipotent 'adult' stem cells found in tissues like bone marrow and adipose tissue that can differentiate into skeletal cell types, eg. bone, cartilage.

Natural polymers: Polymeric materials composed of organic macromolecular matter of plant, animal and microbes.

Osteoid: The matrix material produced by osteoblasts during bone formation which is mainly composed of collagen and is unmineralised.

Osteon *(also called Haversian System):* The basic cylindrical structural unit of cortical (compact) bone, consisting of a central canal containing blood vessels and nerves and surrounded by concentric rings of bone.

Paraxial mesoderm: Together with intermediate and lateral plate mesoderm, subtypes of mesoderm that contributes to all connective tissues.

RGD (motif): The amino acid sequence Arginine (R)-glycine (G)-aspartate (D), originally identified in the ECM protein fibronectin that functions in mediating cell attachment. RGD is used as one of the key sequences in designing adhesive ligands for cell attachment in biomaterials.

Stiffness: The extent to which an object resists deformation in response to a specified stress. Commonly defined by the elastic modulus.

Stress relaxation: The resistance generated within an object/substance after the exertion of certain stress is slowly dissipated over time. It is one characteristic of viscoelasticity.

Synthetic polymers: Polymeric materials composed of man-made macromolecular matter.

Teratoma: Tumour consisting of several cell types that represent the three embryonic germ layers, endoderm, ectoderm, mesoderm.

Trabecular bone *(also called spongy/cancellous bone):* The structural network of bone found typically inside long bones that provides important structural and metabolic function. Makes up ~20% bone mass.

Transcription factors - Sox9, Runx2: Master genes that control commitment to the chondrogenic (Sox9) and osteogenic (Runx2) lineages.