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Biomimetic strategies for fracture repair: Engineering the cell microenvironment for directed tissue formation

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Abstract

Complications resulting from impaired fracture healing have major clinical implications on fracture management strategies. Novel concepts taken from developmental biology have driven research strategies towards the elaboration of regenerative approaches that can truly harness the complex cellular events involved in tissue formation and repair. Advances in polymer technology and a better understanding of naturally derived scaffolds have given rise to novel biomaterials with an increasing ability to recapitulate native tissue environments. This coupled with advances in the understanding of stem cell biology and technology has opened new avenues for regenerative strategies with true clinical translatability. These advances have provided the impetus to develop alternative approaches to enhance the fracture repair process. We provide an update on these advances, with a focus on the development of novel biomimetic approaches for bone regeneration and their translational potential.

Keywords

Fracture repair, biomimetic, endochondral ossification, biomaterials, stem cells

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Introduction

Bone tissue has a remarkable ability to regenerate without forming fibrous scar tissue, due to complex biological processes that recapitulate bone development. However, even with this incredible capacity for regeneration, both external and pathological factors can affect this regenerative pathway, leading to delayed fracture healing and in some cases fracture non-union.[1,2]

A non-union is generally defined by the Food and Drug Administration (FDA) as incomplete healing within 9 months, combined with a lack of radiological characteristics associated with fracture healing observed during the final 3 months.[3,4] Approximately 10% of all fractures in the United Kingdom result in non-union, with the resulting cost to the National Health Service (NHS) ranging from £7000 to £79,000 per patient.[5]

There has been an intense drive towards research focusing on the development of strategies to enhance the fracture-healing process in an attempt to reduce the incidence of failure.[4,6] This review aims to summarise novel developments in the field of skeletal regeneration, with a focus on emerging research mimicking biological processes that underpin bone tissue repair.

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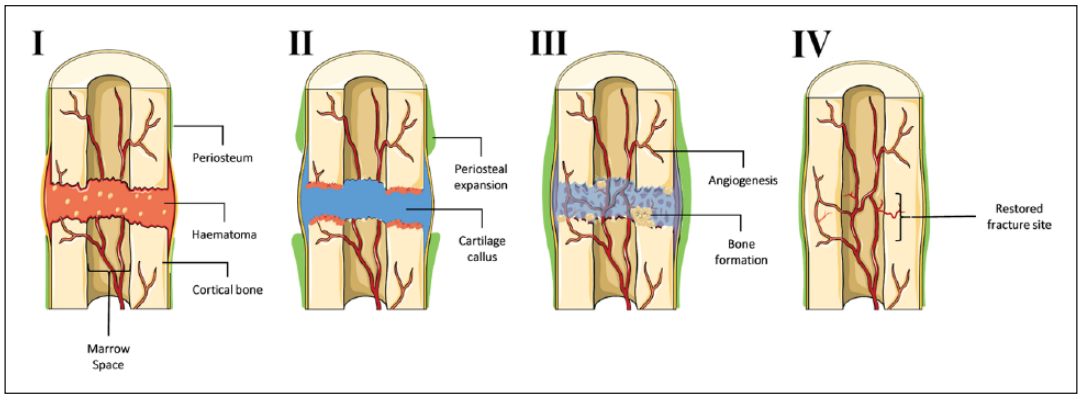

Figure 1. Stages of endochondral ossification during fracture repair. Stage I – haematoma: initial injury leads to the disruption of surrounding blood vessels resulting in the formation of a platelet-rich fibrin clot. Secreted chemokines promote stem cell expansion and localisation to the fracture site. Stage II – soft callus: prolonged hypoxic conditions within the unstable fracture site favour chondrogenic differentiation of stem cells from the periosteum resulting in a cartilage callus. Stage III – hard callus: chondrocytes within the stabilised callus undergo hypertrophy and eventually apoptosis permitting the invasion of blood vessels and woven bone formation. Stage IV – remodelling: woven bone is remodelled into lamellar bone through the synergistic action of osteoblasts and osteoclasts thus re-establishing native bone physiology. Figure generated using the Servier medical art database (http://www.servier.com/Powerpoint-image-bank) and adapted from Roberts et al.[11]

The fracture repair cascade

The biological aspects of skeletal development and healing have been extensively studied. In order to explore advances within the field of skeletal tissue engineering, we first need to understand the complex yet carefully orchestrated process of fracture repair.

Fractures heal through two mechanisms: intramembranous ossification is involved in direct fracture healing and occurs in less than 2% of fractures. It requires rigid fixation with a gap of less than 0.01 mm and begins with the formation of cutting cones near well-defined fracture ends that create longitudinal cavities. Bone is then laid down by osteoblasts bridging the gap and re-establishing the bone's lamellar structure without the formation of a cartilage callus.[1]

Most long-bone fractures, however, heal through the process of indirect fracture healing (Figure 1) driven primarily by endochondral ossification (EO), making it a key area of focus for the development of tissue engineering–based regenerative strategies.[7–10] Unlike direct fracture healing, the process of indirect fracture repair takes place if micro-motion occurs within an unstable fracture site.[1,2]

There are several key steps to the EO process, as illustrated in Figure 1. Many aspects of EO recapitulate skeletogenesis as observed developmentally. It begins with the initial inflammatory response that leads to the formation of a haematoma, thus laying down a template for callus formation. Although it is known that chronic expression of proinflammatory cytokines have a negative effect on bone, the initial secretion of proinflammatory cytokines triggers the repair process. This early inflammatory response is believed to be initiated by the release of platelet-derived interleukin (IL) 1β,[12,13] IL-6,[14,15] tumour necrosis factor-α (TNF-α)[16,17] and IL-17.[13,18,19] These proinflammatory cytokines modulate immune cells and surrounding skeletal stem cell populations.[17,18,20–23] The hypoxic conditions within the haematoma lead to an increase in the expression of pro-angiogenic factors thus promoting vascularisation around the fracture site.[22,24] A plethora of growth factors including transforming growth factor beta-1 (TGFβ1), fibroblastic growth factors (FGFs), bone morphogenic proteins (BMPs), platelet derived growth factor (PDGF) and stromal-derived factor 1 alpha (SDF1α) are involved in the activation and recruitment of skeletal progenitor cells from the periosteum.[23,25–28] It has been suggested that the hypoxic conditions present within the fracture site favour the differentiation of skeletal stem cells towards a chondrogenic phenotype, subsequently producing an avascular cartilage callus.[1,24,29] The fracture callus provides stability while chondrocytes within the fracture callus stop proliferating and become hypertrophic.

This is followed by matrix mineralisation, chondrocyte apoptosis and subsequent degradation/resorption of the cartilage matrix.[1] Through the actions of osteoclasts and osteoblasts, the mineralised callus is replaced by woven bone. The cortical shell provides stability by bridging the bone ends, allowing for limited weight bearing. The final remodelling stage involves the replacement of woven bone with lamellar bone. Although this process is initiated at 3–4 weeks, its completion can take years depending on the age of the patient.[1]

The complex biological processes involved in fracture repair can be affected by a number of factors leading to the disruption of bone healing. Some of these factors include the severity of the fracture that may result in surrounding soft tissue damage and compromised vasculature,

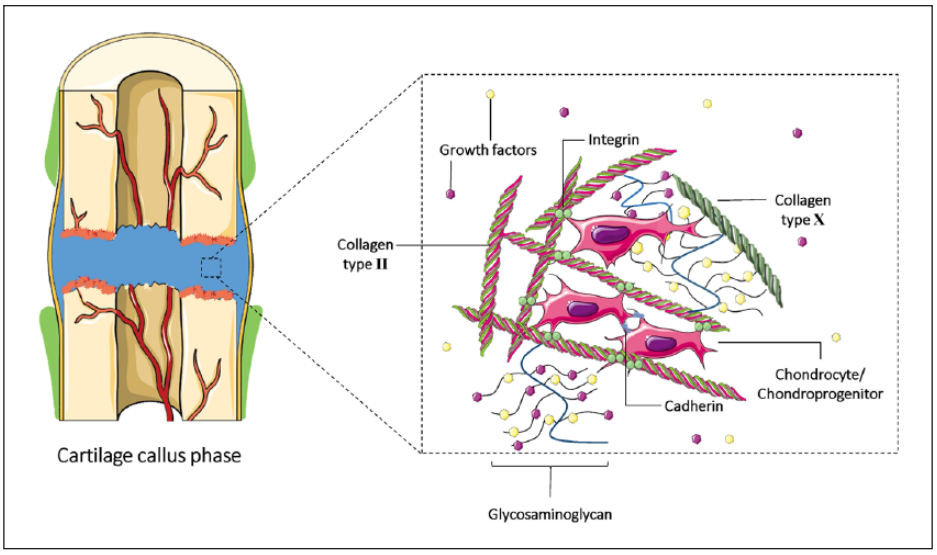

Figure 2. Potential cell–matrix interactions with the soft/hypertrophic callus. Chondrocytes and chondroprogenitor cells within the fracture cartilage callus extracellular matrix (ECM) may be tethered to collagen type II and X through integrins.[40] Cell to cell interactions occur through cadherins. Glycosaminoglycans are bound to the collagen structure and sequester local growth factors within the ECM. Sequestered growth factors interact with chondroprogenitors, activating cell-signalling pathways that promote chondrogenesis, which in turn promotes the expression of matrix remodelling factors.[42] Figure generated using the Servier medical art database (http://www.servier.com/Powerpoint-image-bank).

commonly associated with high impact fractures.[30] However, some of the most common factors are host-associated. These include metabolic diseases such as diabetes,[31] lifestyle choices such as smoking[32] and underlying pathologies such as osteoporosis or the age of the patient which can affect bone quality.[33] Any one of these factors, or a combination of many, can result in the failure of this finely balanced process resulting in a delayed or non-union bone fracture.[34]

Mimicking the fracture repair process as a strategy for non-union repair

As the majority of long-bone fractures heal through the process of EO, which in many respects replicates developmental mechanisms, Lenas and colleagues have defined the concept of 'developmental engineering' as a modality for the creation of reparative implants. This states that tissue development progresses through several intermediates, which can be incorporated into a engineered regenerative strategy.[35,36] As discussed, EO during fracture repair consists of a number of stages (Figure 1), with a key intermediate stage involving the formation of a cartilage callus (soft callus).[37] Understanding the fundamental components that make up the ECM of this tissue is vital in developing substrates capable of partaking in the subsequent stages of EO. Additionally, key regulatory factors that drive cartilage callus formation is vital when considering strategies to mimic its ECM or in priming its progenitor cells.

The ECM is a complex three-dimensional (3D) scaffolding structure particular to each tissue type.[38,39] The dynamic and versatile assembly confers the ability of the ECM to modulate the production, degradation and remodelling of its self-assembled macromolecules, thus supporting the development, function and repair of tissues.[38–40] The cartilage ECM is predominantly composed of collagen, non-collagenous glycoproteins, hyaluronan and proteoglycans[40] (Figure 2). The ECM also maintains a reservoir of growth factors and cytokines that initiate and regulate cell activation and turnover.[40–42] Mimicry of natural processes can assist in improving tissue regeneration strategies and it is likely that using a mimetic ECM will help to achieve this by providing niches for cells to reside and form tissues within.

The concepts relating to the creation of these mimetic niches will be further discussed in the context of progress made in synthetic polymer engineering, natural scaffolds and tissue intermediates composed of stem cells and cell-derived matrix.

Synthetic polymer engineering to mimic cell–matrix interactions

Scaffold design and fabrication have been major areas of research as they are key components within tissue engineering and regenerative medicine. The use of polymers is widespread for the fabrication of tissue engineering biomaterials.[42–45] Scaffold materials can be synthetic or natural, and non-degradable or degradable depending on their

application.[45] Natural polymers were some of the first biodegradable biomaterials to be used clinically[43] due to their bioactivity contributing to the ability to interact readily with cell populations. Natural polymers include proteins such as silk, gelatin, fibrinogen and collagen, and polysaccharides such as amylose, cellulose, chitin, dextran and glycosaminoglycans.[45,46] However, a major limitation of most naturally derived polymers relates to manufacturability and functionalisation. This combined with inconsistent results has led to the investigation of synthetic polymers for tissue engineering purposes. Currently, much research is being focussed on using modified polymers to modulate the interaction of cells with the material in an attempt to replicate cell niches. Major technological advancements in this area include the development of high-throughput screening platforms to define polymer functionalisation and surface topology.

Synthetic polymers such as polyglycolide (PGA), polysulfone (PSU) and polylactic acid (PLA) have been widely investigated in the context of the bone-healing process.[47–50] Furthermore, the composition and applications of these polymers has been extensively reviewed.[51,52] Despite this, limitations remain pertaining to their limited bioactivity, resulting in restricted cell interactions and tissue-forming capacity.[53–55]

Several strategies have attempted to overcome some of these hurdles through surface modifications. Through the use of heparin functional groups, growth factors such as basic fibroblast growth factor (bFGF) can be localised to the material surface, resulting in enhanced cell attachment and proliferation.[56] The modification of surface topography has also shown great promise in enhancing cell attachment and differentiation, with enhanced osteoblastic cell adhesion on specific surfaces observed through the fabrication of nanoscale topography.[57–59] The principle of altering surface topographies has also been applied to the fabrication of PLA/polycaprolactone (PCL) hybrid scaffolds using electrospinning to obtain fibre alignment, demonstrating that this characteristic could enhance chondrogenic differentiation of septum-derived progenitors.[60] Other studies have also reported similar success in directing cells towards the chondrogenic fate through the application of surface modifications.[61–66] Further modulation of methacrylates demonstrated that the use of functional groups such as phosphates and glycosaminoglycans, which are found within native bone and cartilage, induce hMSCs towards an osteogenic or chondrogenic lineage, respectively.[67]

The approach of polymer modification offers great promise towards the development of novel biomaterials that can functionally regulate cellular mechanisms. This increasing need for novel polymers has driven research towards screening methodologies such as polymer arrays. The development of polymer array technologies is aimed at the simultaneous screening of several factors or polymer blends, thus downscaling the resources and time needed for the screening process. One such approach involved the development of materials employing varying physical properties or chemical concentrations[68,69] (the emergence of polymer array technologies has been extensively reviewed elsewhere[70]). Some studies have leveraged recent developments in microfabrication through robotic liquid-dispensing technology to examine various conditions. These polymer arrays have been utilised to investigate the control of cell behaviour, including research into pluripotent stem cells,[71,72] primary articular chondrocytes[73] and for the investigation of pluripotent stem cell interactions when exposed to various glycosaminoglycans and integrins.[74] Polymer array technology has also successfully been used to identify specific materials that can aid in the isolation, expansion and differentiation of human skeletal progenitors.[75,76] It is envisaged that this technology could be used to find polymers that mimic the complex cell–matrix interactions that are observed during the fracture repair process and thus contribute to engineering the endochondral response. Further screening technologies such as the 'TopoChip' utilise unbiased algorithms to fabricate topographies on PLA. This technology successfully allowed for the screening of specific patterns that demonstrate enhanced osteogenic differentiation.[77,78] Further optimisation to the system using a chip carrier has also helped eliminate other variations within the culture system that may influence cell viability and adhesion.[79] Developments of polymer array technologies show great applicability in the field of bone tissue engineering due to their applicability towards materials, biochemical factors and cell populations currently used within the field. This is especially important due to the emergence of novel innovations in additive fabrication methods such as 3D printing, allowing for the rapid and varied fabrication of material structures. It is therefore essential to utilise robust and high-throughput methodologies for material assessment in order to keep pace with ever-increasing knowledge surrounding tissue-formation processes.

3D printing of bone tissue and niches

The field of tissue engineering has strived to mimic the cellular and extracellular bone matrix as a means of restoring bone tissue and improving its functions in vivo. The tissue microenvironment, including bone and cartilage tissue, is a complex 3D structure that provides a template for cell adhesion and initiates bone repair in vivo.[80,81] Microenvironments also permit the regulation of nutrients and molecules and their transport to the innermost regions of the scaffolds to enable cell growth, vascularisation and waste material removal.[81,82] Therefore, the properties of biomaterials including material and cellular composition, pore size, volume and mechanical strength are vital parameters that define their performance. Conventional

fabrication techniques such as chemical/gas foaming,[83] particle/salt leaching[84] and thermally induced phase separation[85] lack the ability to generate the complex 3D structure and material properties needed to replicate biological tissues. In an attempt to achieve better adaptability, the field has utilised additive manufacturing methods such as 3D printing. The feasibility of 3D printing as a means for generating biomimetic scaffolds for fracture repair was demonstrated by Inzana and colleagues who optimised a process for 3D printing of collagen/calcium phosphate.[86] To assess the bone healing performance of the scaffolds, they were implanted into a critically sized murine femoral defect for a duration of 9 weeks. The implants displayed new bone formation, which incorporated the degrading scaffold material.

Another key step in producing biomimetic grafts is the ability to incorporate a cellular component into fabricated 3D biomaterials. Advances in biopolymer printing technology and materials has allowed for the fabrication of 3D constructs using alginate in combination with chondrocytes or human adipose-derived stem cells aimed towards cartilage tissue regeneration[87,88] and osteochondral tissue fabrication.[89] In particular, cartilage tissue engineering using additive 3D printing has gained momentum due to the limited ability of cells to incorporate into the dense avascular structure of cartilage. This could potentially be overcome through additive manufacturing, and further aided by the incorporation of key chondrogenic factors such as members of the TGF-β superfamily.[90]

Biomimetics through the use of natural target-tissue ECM

The concept of tissue engineering initially focused on developing materials that mimicked mature tissue, with the aim of incorporation into the host and subsequent remodelling, as defined by Langer and Vacanti in 1993.[91] Initial attempts were made through the use of biodegradable scaffolds in combination with adult cells. However, in the context of bone repair, this approach has to date provided no clinically approved therapies. Due to this, current research towards improving the bioactive properties of regenerative implants through the use of naturally occurring target-tissue ECM has emerged. Indeed, the process of xenogeneic tissue decellularisation and its use for tissue engineering strategies within the field of regenerative medicine has been intensively studied.[92–95]

The process of decellularisation aims to remove all immunological components while leaving behind the ECM of the tissue and its associated growth factors, with the intention of maintaining the ECM proteins' complex spatial arrangement.[96] The ECM plays a key role in maintaining cell–matrix interactions that favour native tissue organisation and remodelling.[38] Importantly, the structural and functional proteins in the ECM are well conserved within species. This high level of homology allows these matrices to be implanted in recipients of other species without rejection.[97] The past decade has driven research towards novel biomaterials through the process of organ and tissue decellularisation. Some of the many examples of positive clinical results include the use of FDA-approved decellularised matrices such as porcine heart valves (Synergraft®; Cryolife) and acellular dermis (Alloderm®; LifeCell).[98]

The use of decellularised cartilage has drawn much attention due to its ability to harbour a large quantity of bioactive cues for tissue formation. The interaction of decellularised cartilage with resident cells, several chemotactic stimuli and activation of chondroinductive signalling pathways could result in continuous remodelling of the tissue. Decellularisation of cartilage, however, requires a vigorous protocol due to its dense nature. This is known to reduce the glycosaminoglycan (GAG) content and elasticity of the matrix.[96] Despite this, the use of decellularised and lyophilised cartilage scaffolds has previously demonstrated bone formation in a rabbit model by Gawlitta and colleagues.[99] The study involved coupling pre-primed chondrogenic MSCs with decellularised cartilage scaffolds, and demonstrated effective bone mineralisation when compared to the unseeded decellularised matrix. However, the contributing factors to the endochondral bone formation were unidentified, but could include components within the decellularised cartilaginous matrix, or factors produced by the cells as a result of the cell–matrix interaction.[99] The choice of articular cartilage-derived scaffolds used in the study by Gawlitta et al. do pose limitations due to both tissue physiology and in vivo function. Articular surfaces are formed during pre-natal skeletal development and are highly stable during adult life.[100] Indeed, factors such as chondromodulin 1 (ChM-I) have been implicated in the stability of articular cartilage by inhibiting EO in porcine models,[101] and it has been further suggested that ChM-I functions as an inhibitor of angiogenesis, a process essential to endochondral remodelling.[102,103] Additionally, a plethora of Wnt and BMP signalling modulators have been implicated in articular cartilage stability. Therefore, there is a requirement for a source of chondrogenic ECM-derived scaffolds that is intrinsically primed for endochondral remodelling. Other cartilaginous regions such as costal cartilage provide a promising option, as it gradually undergoes EO well into adult development.[104] Furthermore, a study by Okihana and Shimomura[105] indicated that when devitalised costal cartilage was implanted subcutaneously into rabbit and mouse models it underwent endochondral remodelling.

In summary, innovative decellularisation approaches may allow for the development of methodologies that minimise ECM damage. This, in combination with underexplored and more targeted tissue sources, may be the key to developing viable grafts that are able to mimic the endochondral repair process.

Stem cells for the creation of bone-forming tissue intermediates

Many of the key developments within the field of tissue engineering centre on the ability of cells to interact favourably with its carrier and mediate tissue formation and integration. It is therefore essential to investigate the interactions of key cell types in any regenerative approach in order to develop effective stem cell–based skeletal regenerative strategies.

Embryonic stem cells

The embryonic stem (ES) cell is derived from an early mammalian embryo and possesses a remarkable potential for differentiation into cell types from all three germ layers as demonstrated by Kaufman and colleagues using mouse embryos, termed ES cells.[106] In vitro culture protocols paved the way for isolation and culture of the first human ES cell in 1998,[107] where it was demonstrated that human ES cells could be kept in culture for up to 5 months followed by subsequent differentiation into all three embryonic germ layers. This facilitated further research into ES cell culture and differentiation programmes. Since then, ES cell research has generated promising results towards the treatment of diabetes,[108] cardiovascular disease[109–111] and musculoskeletal regeneration.[112] In vitro differentiation of murine and human ES cells towards the osteogenic lineage has also been successfully achieved.[113,114] Although multiple studies have shown that ESCs seeded onto scaffolds and primed in osteogenic media do not produce bone in vivo,[115,116] the formation of bone within teratomas aligned with hypertrophic cartilage regions has been observed, indicating the capacity of ES cells to form bone through the developmental process of EO. This potential to form endochondral bone was confirmed with murine ESCs which were seeded onto ceramic scaffolds and differentiated towards a chondrogenic lineage using TGF-β.[115] Furthermore, when implanted in vivo at an ectopic site into nude mice for 21 days, bone formation was observed on every one of the implanted cartilage tissue-engineered constructs (CTECs). Their capacity to form bone was further demonstrated when the CTECs were implanted orthotopically in rats with critical-size cranial defects. Similar results have also been obtained using human ESCs,[117] thus highlighting the ability of ESCs to form bone via the endochondral pathway, hence mimicking both developmental skeletogenesis and fracture repair. Despite this huge potential of ESCs within tissue engineering and especially within the field of bone tissue engineering, their application is restricted by complex culture conditions, ethical constraints related to ESC isolation and their inherent tumour forming capacity.[118,119]

Induced pluripotent stem cells

There was a renewed interest in pluripotent stem cells when Takhashi and Yamanaka broke new ground by re-establishing the principles of developmental biology, which state that somatic cell differentiation is an irreversible process. Transfecting murine and human fibroblasts with the embryonic factors Oct4, c-Myc, Sox2 and Klf4 caused the regression of cell characteristics to a pluripotent, embryonic-like state, leading to them being named induced pluripotent stem cells (iPSCs).[120,121] It was also demonstrated that like ES cells, iPSCs were able to form tissue from all three germ layers; however, iPSCs also had the inherent capacity to form teratomas (tumours) in vivo. Importantly, establishing a route for producing stem cell populations from a patient's own cells overcomes many of the ethical issues faced by ES cell use.

In the context of bone repair, deriving progenitors with bone-forming potential from iPSCs has been an intensively studied area. Recent work has described a xeno-free-defined culture condition for the differentiation of iPSCs into iPSC-derived mesenchymal stem cells (iPS-MSCs), which were able to differentiate into chondrogenic, osteogenic and adipogenic lineages in vitro.[122] Furthermore, when these cells were osteogenically differentiated for a period of 4 days and implanted into calvarial defects in immunocompromised mice, de novo bone formation originating from the implanted iPS-MSCs was observed.[122] A recent study by Shey and colleagues has also demonstrated the efficacy of iPSC-MSCs for the treatment of non-union defects in mice.[123] Furthermore, their chondrogenic differentiation capacity, and therefore their potential application towards endochondral tissue formation, has been demonstrated.[124] The ability to create iPSC-derived cartilage constructs was also demonstrated with optimised culture conditions utilising scaffold-free hyaline cartilage tissue that displayed good integration into surrounding cartilage tissue when implanted, while not forming tumour masses.[125] Although this study was targeted towards the treatment of cartilage defects, it is envisaged that differential stimulation of these cartilage constructs may allow the generation of implants capable of endochondral bone formation. Indeed, iPSCs have been shown to undergo chondrocyte hypertrophy in a similar manner to ESCs.[117] Furthermore, the possibility of direct cellular reprogramming towards chondroprogenitors, a process that takes many of the concepts used to derive iPSCs, has demonstrated the ability of engineered cells to undergo in vitro hypertrophic differentiation. Implants containing these cell populations can drive endochondral bone formation and remodelling post implantation in nude mice.[126]

Mesenchymal stem cells

One of the most desirable properties when choosing a cell type for bone tissue engineering is the ability to isolate tissue-specific regenerative cell populations. As previously discussed, ESCs provide a highly malleable cell source, and the development of iPSCs has further advanced the field of personalised medicine through the generation of pluripotent populations from somatic cell sources. However, despite

these developments, the most popular cell type still employed for the development of skeletal regeneration strategies is the mesenchymal stem cell (MSC). MSCs are widely recognised for their ability to differentiate towards osteochondral lineages[127,128] and can be derived from several tissue sources. The most commonly used source of MSCs is the bone marrow, from which cells are isolated through the extraction of a bone marrow aspirate.[129] Another common source of MSCs is adipose tissue from which MSCs (perycites) are isolated from digested fat tissue.[130]

Caplan first coined the term MSC in 1991.[131] It was, however, in the 1960s and 1970s that seminal studies by Friedenstein isolated mesenchymal stromal cells and revealed their osteogenic potential by heterotopic transplantation.[132,133] Since then, MSCs have been defined by the minimal criteria of positive expression of CD105, CD73 and CD90 and negativity for CD45, CD34, CD14, CD79α and HLA-DR.[134] MSCs have been extensively used and reviewed for their clinical applicability within the field of bone tissue engineering.[135,136] In relation to the treatment of bone fracture repair, it has been shown that the delivery of allogenic BM-MSCs in combination with demineralised bone matrix enhances fracture healing in clinical models of diabetes mellitus in rats.[137] Similar results are also demonstrated by several other studies incorporating MSCs with scaffolding material for the treatment of fractures, particularly in their proposed use in non-union fractures.[138–140]

Until now, the treatment of large bone defects has largely relied on approaches that aim to harness the intramembranous pathway of bone regeneration. However, as discussed previously, recapitulation of EO may be more efficacious. Martin and colleagues applied this approach by creating cartilage constructs in vitro using human BM-MSC pellet cultures. Hypertrophy within these engineered constructs was induced through the withdrawal of TFG-β and the introduction of β-glycerophosphate with thyroxin. The resulting constructs displayed increased collagen type X deposition, typical of hypertrophic cartilage. Upon implantation in immunocompromised mice, these engineered hypertrophic cartilage constructs formed bone around the periphery at 4 weeks, with extensive endochondral bone formation after 8 weeks.[7] More recent work has also illustrated that the addition of anti-inflammatory/tissue repair macrophages may further enhance the cartilage-forming capacity of BM-MSCs.[141] This highlights the potential role of inflammatory cells during the fracture repair process; however, further orthotropic investigations are required to establish their clinical significance.

The periosteum – mimicking the master regulator of fracture repair

Despite the immense progress directed towards the design of fracture repair implants, the integration of all vital tissue properties and functions into a single system remains a major research challenge. With this in mind, the ability to mimic cell–matrix interactions with novel biomaterials, developed using natural matrices or engineered through systematic screening of polymers and surface topography of key relevance. However, if these systems are to be combined with cells, they should also be carefully selected to represent those modulating the fracture repair process.

The periosteum is a highly vascularised connective tissue that envelops the bone surface of long bones.[142] It serves as a biophysical barrier to modulate the environmental conditions on the bone surface. The periosteum is composed of two distinct layers: an outer fibrous layer composed of fibroblastic cells in a collagen and elastin matrix, and an inner cambium layer, which provides a niche for a range of cell types, including fibroblasts and stem/progenitor cells.[142,143] Recent prominent evidence has documented the regenerative potential of periosteal tissues and the functional capacity of periosteum-derived cells (PDCs) in the bone-healing process.[144,145] The periosteum is predominantly responsible for 90% of cartilage and woven bone formation in the early fracture callus, with its removal significantly attenuating bone repair.[143,146] In this respect, the periosteum has drawn great attention in pre-clinical bone tissue engineering approaches.[11] The regenerative potential of the cells contained within the periosteum has been further demonstrated in vivo where they play a role in direct bone formation, as well as in chondrogenesis and EO.[147]

Consequently, great efforts have been made to target and isolate PDCs as a cell source for bone tissue engineering purposes. Previous work has shown that once these cells are inoculated into nude mice in scaffold[144] and scaffold-free systems,[147] they give rise to bone and cartilage tissue. Indeed, murine PDCs expanded in the presence of FGF demonstrated an enhanced capacity for in vivo bone production mediated by BMP-2 via the endochondral pathway, a characteristic unique to PDCs. Although culture-expanded PDCs have increased our knowledge and understanding of the periosteum, in vivo targeting of these cells within their niche, or replication of this tissue and therefore mimicking the role of the periosteum are both attractive avenues for further investigation.

Indeed, the fracture-healing process can be enhanced by mimicking periosteum–bone interactions. We have previously shown that human PDCs (hPDCs) that are seeded onto natural decellularised/devitalised bone matrix have the ability to undergo endochondral bone formation resulting in the formation of a bone organ containing a haematopoietic compartment.[144] This process is driven by early PKC, BMP and Wnt signalling[148] and can be further enhanced through the expansion of periosteal stem cells in humanised conditions.[149] This inherently shows that the closer that the biological systems are mimicked, the more

efficacious the system. Further work on this cell population has displayed the importance of mitogenic growth factors such as BMPs[150] and cell–cell interactions[151] as key regulators of the in vivo response to periosteal cell implants. Interestingly, combinatorial screening of growth factors involved in skeletogenesis reveals specific conditions that can direct PDCs towards stable cartilage[152] and biphasic tissues with the potential capacity for osteochondral repair.

The fracture-healing site is known to be intrinsically responsive to key growth factors such as BMP, β-catenin/wingless-related factors (Wnt), TGFβ1 and FGFs which are released by cells to recruit and trigger hPDC in the periosteum. Further to this, we have reported the importance of BMP, β-catenin/Wnt, cAMP Response Element-Binding Protein (CREB), TGFβ, Endothelial Growth Factor (EGF) and Extracellular Signal-Regulated Kinase (Erk) signalling to bone formation from periosteal cells.[153] Integrating the bioactive cues found within the niche of periosteal progenitor cells has been proposed as a vital step in developing a successful transplantable scaffold that promotes bone regeneration. Indeed, this may be achieved through the use of native decellularised matrix, for which proof of principle confirming the biocompatibility of decellularised periosteum has been delivered.[154]

Outlook and perspectives

As a means of repairing critical-sized bone defects, tissue engineering aims to recapitulate the biological processes involved with the formation of the various tissues that modulate the fracture-healing process. The development of scaffolds that possess the necessary cell-interaction properties and biological cues to ensure cellular survival, proliferation and differentiation of either native or infiltrating cells is progressing. Multiple efforts have succeeded with a plethora of studies yet to follow; however, a number of limitations have hindered the field of fracture repair tissue engineering. These include ensuring the engineered scaffolds are able to maintain their functional characteristics post implantation for the duration of the remodelling process,[155] and lack of suitable biomaterials and characterised cells. The mechanical properties of the scaffold are critical to the regulation of mechano-transduction and cellular behaviour, affecting the cells' potential to differentiate, develop and regenerate components that are key to the bone remodelling process.[156] Many tissue-engineered constructs have demonstrated potential regenerative capabilities in vitro; however, upon implantation into in vivo models, this regenerative capacity can be lost, predominantly as a result of insufficient vascularisation and integration/remodelling of the scaffold with recipient tissue. Previous studies have therefore suggested allowing cells to form a microvasculature in the scaffold prior to implantation to improve vascularisation.[157] Furthermore, the production of a biomimetic scaffold or niche may be subjective due to the age-related bone repair processes that occur in vivo. Implanting a model within a young individual may result in a rapid healing, yet the same scaffold may demonstrate the contrary in elderly patients. An aspect quite often neglected is the maintenance of the cell characteristics to provide a pool of progenitors capable of directing the full cascade of tissue repair. The use of stem cells in bone implants is a promising approach; however, knowledge gaps related to isolation, expansion, differentiation and tissue integration are likely to limit short- to mid-term clinical translation. It is proposed herein that the creation of a tissue module that can stimulate the environment prior to implantation, the formation of a material that can attract endogenous cells through specific cell-interaction motifs, or the in vivo targeting of stem cell niches are promising solutions for cases of delayed or non-union bone fractures.

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