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SYSTEMATIC REVIEW

A review of biomaterials in bone defect healing, remaining shortcomings and future opportunities for bone tissue engineering

THE UNSOLVED CHALLENGE

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Abstract

Despite its intrinsic ability to regenerate form and function after injury, bone tissue can be challenged by a multitude of pathological conditions. While innovative approaches have helped to unravel the cascades of bone healing, this knowledge has so far not improved the clinical outcomes of bone defect treatment. Recent findings have allowed us to gain in-depth knowledge about the physiological conditions and biological principles of bone regeneration. Now it is time to transfer the lessons learned from bone healing to the challenging scenarios in defects and employ innovative technologies to enable biomaterial-based strategies for bone defect healing. This review aims to provide an overview on endogenous cascades of bone material formation and how these are transferred to new perspectives in biomaterial-driven approaches in bone regeneration.

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Keywords: Biomaterials, Bone, Fracture, Healing phases, Mineralization, Scaffold

Article focus

- Bone healing is still a challenge in today's clinical routine; Biomaterials provide a promising tool to treat bone defects; Understanding the physiology of the bone healing cascade helps in designing new biomaterial approaches

Key messages

- Despite the plethora of available biomaterials to treat bone defects only a small number reached clinical use
- New biomaterial approaches should consider the physiological healing cascade to better harness the endogenous healing potential
- Future biomaterial approaches aim at mimicking biological processes and envision patient specific tailor made productions

Strengths and limitations

- This review addresses a valid clinical problem and summarises the use of biomaterials in the field of bone defect healing. The use of biomaterials is viewed from the current clinical practise, from the research perspective, and concludes with the developments currently arising. Reviewing the current literature and considering the clinic and research approaches to biomaterials for bone regeneration; it becomes apparent that harnessing the endogenous bone healing capacity by mimicking the biology of bone healing could define new approaches for bone defect healing.

Introduction

Modern treatment methods of fracture fixation have reached a high technological standard that ensures methodological soundness of surgery and, globally, a high quality in medical treatment. However, considerable clinical problems are so far unaddressed, with a remaining group of 10% to 20% of delayed or nonunion situations despite all innovative treatment methodologies.[1] Bone loss, defects, lack of vascularization, soft-tissue damage, lack of adequate mechanical stability, infections and tumours remain key challenges for successful bone healing.[2] They hinder or completely prevent clinical recovery, with

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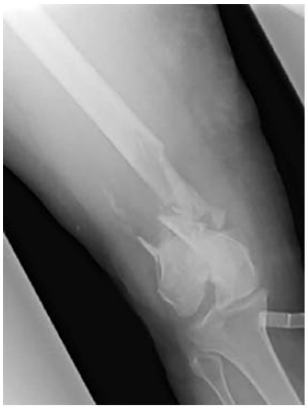
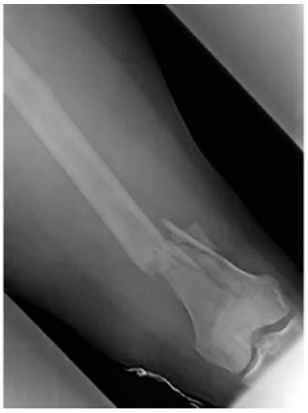


Fig. 1a



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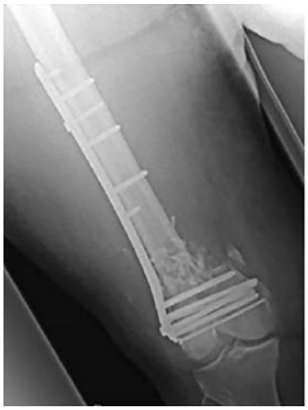


Fig. 1c

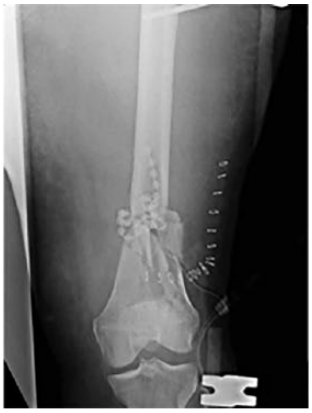
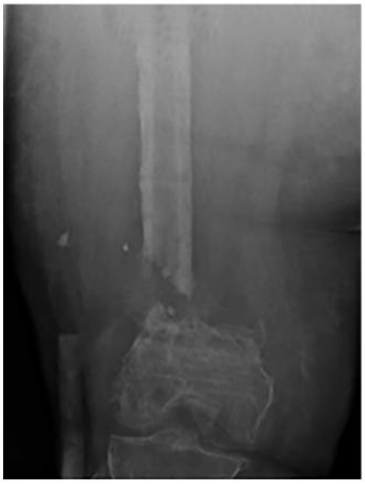


Fig. 1d



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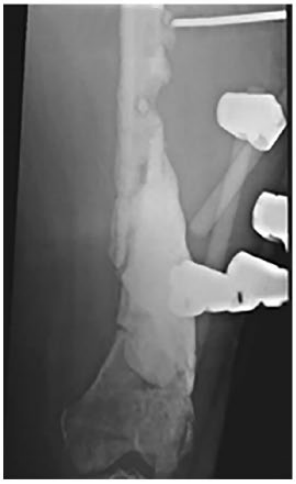


Fig. 1f

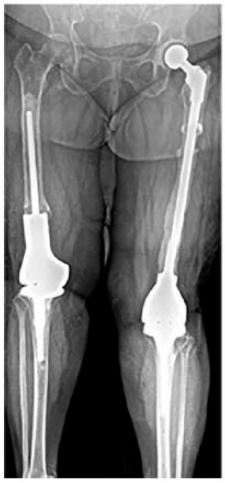


Fig. 1g

Radiological time course of 59-year-old female patient after bilateral open distal femoral fractures (a, c, e: right leg; b, d, f: left leg). a) and b) radiographs at admission with bilateral femoral comminuted fractures. Of note is the bone deficiency on the right femur immediately after injury. c) and d) The status after initial stabilization. Note the shortening and comminution of the right distal femur and the gentamicin beads in the femoral canal of the left femur indicating post-traumatic infection therapy. e) Images after removal of the plate and external stabilization of the right femur. Note the atrophic nonunion, indicating biological inhibition of bone healing. f) Radiographs after removal of avital femoral bone and temporary replacement with antibiotic-loaded bone cement of the left femur. g) Latest radiograph of the follow-up showing both knees replaced with distal femoral replacements; on the left limb, a total femoral replacement was necessary due to the too small residuum of the femur after resecting all infected and dead bone.

few available intervention measures to successfully overcome these challenges.[3] In contrast to pure technological approaches, regenerative medicine may be a game changer and, in combination with technologies in fracture stabilization, may enable innovative solutions for challenging patient settings. Using regenerative medicine approaches, for the first time, we may be able to address effectively the underlying mechanisms of the aforementioned challenges and thereby complement the intrinsic healing capability of the injured bone. In this review we intend to summarize the new and innovative strategies that have emerged from the field of biomaterials in the area of bone regeneration to gain understanding on their potential value and future perspectives.

Remaining clinical challenges

An example of a clinically challenging bone healing situation is given in Figure 1. It shows the time course of treatment in a patient suffering from bilateral open distal femoral fractures after a fall from a height. It demonstrates several possible reasons for non-healing of bone: i) initial loss of bone due to the injury itself; ii) multiple surgical revisions with impairment of vascularization and the local immune competence; and iii) infection with osteitis, osteomyelitis and further bone loss. The patient has been treated with a multitude of different strategies, which represent the current clinical standard, including bone transplantation, local therapy with bone morphogenetic protein (BMP), infection therapy with antibiotic-loaded cement spacers (Fig. 1f), and internal and external fixation procedures. All efforts to fight the infection and regenerate the bone failed. In consequence, bilateral arthroplasty had to be performed after a complete resection of the distal femurs. On the left limb, the entire femur had to be replaced with an implant.

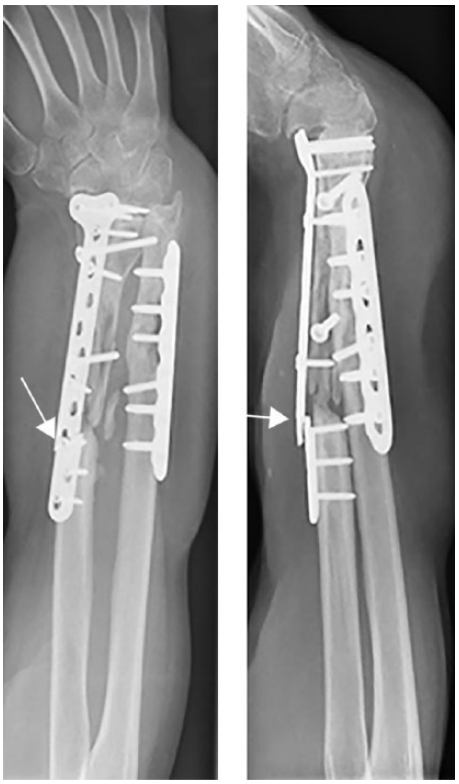


Fig. 2a Fig. 2b

Atrophic pseudarthrosis of the radial diaphysis in a 17-year-old girl. a) Anteroposterior and b) lateral radiograph of the right forearm showing plate breakage at the site of nonunion (arrow and arrowhead). Also seen is an impingement of the carpus due to flexion contracture and a radiocarpal collapse.

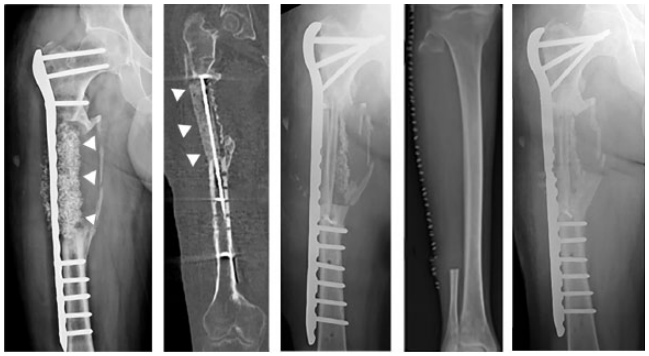


Fig. 3a Fig. 3b Fig. 3c Fig. 3d Fig. 3e

Radiological follow-up of a 44-year-old male patient with infected pseudarthrosis of the right femoral diaphysis and large bone defect after shotgun injury: a) anteroposterior radiograph of the right femur after plate stabilization and implantation of a calcium phosphate bone substitute (indicated by arrowheads) into the defect area without signs of integration or remodelling; b) CT scan after debridement of the defect and removal of most of the calcium phosphate, and implantation of an antibiotic-loaded bone cement spacer (indicated by arrowheads) augmented by a metal nail for local antimicrobial therapy; c) anteroposterior radiograph after removal of the spacer and implantation of a vascularized fibular graft (the fibula was harvested from the ipsilateral leg and fixed with screws into the defect in two parts nurtured by the same artery); d) anteroposterior radiograph from the ipsilateral lower leg demonstrates the donor site morbidity after removal of the fibula; e) anteroposterior radiograph after one-year follow-up. The fibular graft shows good integration and the implant is without loosening or failure.

One of the largest unsolved challenges in bone regeneration are defects, as shown in Figures 1a, 1c and 1e. To fill such defects, biomaterials are used to restore both structure and function and, in most cases, provide a replacement for the lost bone. Depending on the location of the bone defect and the type of bone lost (cortical *versus* cancellous), the adequate properties for such biomaterials may vary considerably. The previously mentioned patient lost cortical bone in the right femur and needed a stable replacement of the cortical structure (Fig. 1a). An alternative might have been a soft biomaterial to fill the cortical defect, in which case a stable plate fixation would be required to provide mechanical stability. In such cases, a quick transformation of the softer biomaterial into cortical bone would be needed to enable full mobilization of the patient. In general, bone formation and consolidation needs to precede implant loosening or fatigue failure. If this race is lost, nonunion and failed implants are the consequences of incomplete osteosynthesis, as illustrated in Figure 2.

It is not just trauma but also the loss or resection of bone due to tumour or infection which can cause critical-sized defects. In these cases, bone replacement is one part of the therapy. An ideal biomaterial would serve the purpose of delivering substances, which address the causative disease of the bone loss. This functionalization of biomaterials could be one of the greatest achievements within the scope of modern biomaterial development. In the case of infection, the standard treatment for bone defects is a two-staged or multiple-staged revision surgery usually with the use of antibiotic-loaded bone cement spacers in between stages. Figure 3 shows a patient following treatment for a shotgun fracture of the right proximal femur shaft, who presented with pseudarthrosis with a significant bone defect and infection with a sinus tract. Treatment included an attempt to bridge the defect with a calcium phosphate product, which failed due to the extent of the defect and the accompanying infection. A biomaterial, which would offer both bone regeneration properties for large defects and the elution of antibiotics, would allow a one-stage approach in this case and, furthermore, would help to avoid the donor site morbidity of autologous bone graft (Fig. 3d), as performed here with a vascularized fibular graft. However, autologous bone transplantation is the gold standard for the treatment of bone defects, followed by allogeneic bone, the latter lacking osteogenetic properties but, nevertheless, being almost equally effective in some indications.[4]

However, it is not just severe traumatic events, such as gunshots, which can lead to bone defects. Much more frequent, are bone defects as a consequence of low impact fractures in elderly patients. In such patients, eventually also due to osteopenic weakening of the bone, fractures are frequently combined with a loss in metaphyseal bone substance leading to significant defects. Typical locations of such commuted fractures are the proximal femur, proximal humerus or vertebral body.

Reconstruction of the fractured bones is often challenging due to the limited bone quality in the remaining bone stock, that hinders any rigid fracture fixation by conventional instrumentation. Commonly, such defects are filled with autologous or allogeneic bone and may eventually lead to joint arthroplasty. With an ageing society and an increase in activity even up to older age, methods of improving bone regeneration are urgently needed. Associated with this increase in affected patients are the rising costs of €37 billion for 3.5 million osteoporotic fractures per year in the European Union.[5] With this in mind, one can estimate the immense need for innovative therapies to enable the surgeon to provide fast and efficacious bone regeneration for their patients.

Biomaterial properties

Biomaterials or implant materials are synthetic or non-vital natural materials that are used in medicine for therapeutic purposes and which are placed within the human body or are otherwise in contact with the patient tissue. Within the body, these materials interact in a chemical, physical, or biological way with the recipient's biological system. As these materials often remain within the body, their biocompatibility is a necessary prerequisite. In bone regeneration, biomaterials are used to replace the tissue after disease, injury, or failure due to ageing. As early as the 16th century, Bernardino de Sahagun, then travelling with Hernando Cortez, reported on a treatment for bone nonunion by an Aztec physician using wood that was inserted into the medullary canal to stabilize the limb, wood being a material providing a level of biocompatibility. During the mid 1800s, another material was introduced to stabilize nonunions: ivory.[6] This material offered the desired stability while still having the advantage of slow resorption and degradation. Controlled degradation of a biomaterial is another important property that has to be considered next to its biocompatibility. In any event, the degradation should proceed without the generation of detrimental byproducts that would obstruct the healing process or, at a later stage, initiate complications within the human body. The material best suited in view of degradation within the treatment field of bone is bone itself. Hoglund[7] reported the use of bone transplants to stabilize nonunions in 1917, and today, 100 years later, the reconstruction of large bone defects with either vascularized or non-vascularized autologous fibular grafts is still seen as an effective treatment opportunity.[8] Vascularization is another important aspect to consider in the context of furthering bone formation with biomaterials. As vascularization plays a pivotal role during the healing process,[9,10] it has been proven, that a delayed revascularization is coupled to a delayed bone healing.[11] Therefore, a biomaterial used in the context of bone formation should, if not actively furthering angiogenesis, at least not hinder the naturally occurring revascularization process.

A huge step towards implant materials in bone surgeries was triggered by World War I. In the field, gunshot wounds often included shattered bones. A quick and easy method was needed to deal with these injuries. Hey Groves[12] reported on the use of metal rods which were placed in the medullary cavity for stabilization, albeit at the cost of high infection rates. Another disadvantage of metal implants at this time was the body's reaction towards the material which was encapsulated by fibrous tissue. It was not until 1931 when the use of stainless steel nails for fracture fixation was reported that the application of metallic implants started to become universally accepted for the treatment of bone injuries.[13]

Metal delivered one important factor for fracture treatment: the stability needed to ensure that the patient regained the weight-bearing capabilities of the injured limb, a treatment strategy that also today complies with the guidelines of the Arbeitsgemeinschaft für Osteosynthesefragen (AO) for fracture treatment. Initial principles – formulated by the AO to treat bone fractures – were anatomical reduction, preservation of the vitality of bone and soft tissue, proper stability of fracture fragments, and minimization of soft-tissue damage.[14] Where a biomaterial is needed for a bone defect, this material should provide an osteoconductive, osteoinductive or even osteogenic environment.[2]

Osteoconductivity describes the property of a biomaterial structure which provides a framework that favours new bone formation. The biomaterial, therefore, should offer an ideal structural environment for hosting cells relevant in bone healing. As stated above, cadaveric allograft bone, available either with a cancellous or a cortical structure, is considered a biomaterial, offering the ideal osteoconductive structure. Cortical allografts are sometimes used in reconstructing the femur during revision hip arthroplasty.[15] While these allografts offer the structure and stability of bone, this is being offered during the haematoma phase when they are implanted during surgical treatment. During this healing phase the physiologically present structure and stability would therefore differ considerably from that supplied by an allograft material. The matrix formed within the hematoma[2] offers the ideal structure to promote bone healing during the early stages. Consecutively, in some cases an osteoconductive structure should vary from the structure of mature bone to resemble a structure more prone to the bone forming process during the early healing stages. This could for example be a structure that copies the growth plate in a biomaterial targeting the support the process of endochondral bone formation.

Bones major anorganic component is hydroxyapatite (HA) which nucleates from specific zones on the collagen fibres deposited by osteoblasts, the bone forming cells.[16] Subsequently, HA has been widely investigated as a bone substitute and has been proven to have high osteoconductive potential.[17] Studies have shown that HA bone

graft substitutes function as well as bone allografts when considering the long-term clinical outcome, confirming their value as a biomaterial in bone replacement.[18]

Osteoinduction is a stimulating property of a biomaterial that actively induces new bone formation. The first time an osteoinductive factor was reported was probably the injection of 'bone extract' into muscle by Levander in 1934 and the subsequent observation of ectopic bone formation.[19-21] In 1945, such a 'bone extract' was named 'osteogenin' by Lacroix.[22] The bone morphogenetic properties of demineralized, lyophilized bone segments described by Urist led to naming of the bone morphogenetic proteins (BMPs) in 1971.[23,24] Bone morphogenetic protein 2 (BMP2) and BMP7 are the two growth factors which reached clinical approval (in 2000 and 2001, respectively) and have been used as very potent osteoinductive factors to enable bone formation in cases where a nonunion occurred.[25] Neither factor has ever achieved broad and unlimited routine clinical use due to safety concerns that imposed severe restrictions in their clinical application. Recently, BMP7 has been withdrawn from the market.[26] The aforementioned 'bone extract' was probably efficient in regeneration since it also contained and thereby transferred locally BMPs to stimulate bone formation. Nowadays, such approaches would be considered less desirable due to the high risks of pathogen transfection in any clinical setting. The synthetic production of an osteoinductive biomaterial is still considered the preferable approach. Unfortunately, nothing can compare with the BMPs so far. Biomaterials are classified as being osteoinductive when they contain calcium phosphate.[27,28] Also, biomaterials containing alumina ceramic, titanium or glass ceramics were classified as osteoinductive, indicating that the chemical composition of the biomaterial is essential for the osteoinductive properties.[29]

Osteogenesis describes a biomaterial property that actively supports *de novo* bone genesis through the recruitment of osteoblasts. This could be achieved by a biomaterial stiffness that supports osteogenic differentiation,[30] or by providing voids within the biomaterial that are attractive for cell attachment and differentiation,[31] or by providing stress-relaxation patterns that further bone formation,[32,33] or by coupling factors with the biomaterial that attract osteogenic progenitor cells.[34] Other strategies could comprise biomaterials that include cells beneficial for bone formation such as angiogenic progenitor cells[35,36] or drugs to reduce the reactive oxygen species to enhance the survival of mesenchymal stromal cells (MSCs).[37]

In considering biomaterial properties that are beneficial for bone formation, the environment into which the biomaterial is introduced should always be considered as well.

Lessons to learn from endogenous material formation during bone healing

Bone has the unique capability to restore its material basis. Thus, understanding the endogenous strategy of bone formation during successful healing could lead to a blueprint for material scientists in view of the material, cellular and signalling components that are essential to form bone. In the following, we describe the cascades of bone healing as a complex process comprised of several consecutive, partly overlapping phases that lead to a '*restitutio ad integrum*' recovery of form and function. In the majority of cases, bone is able to regenerate. However, even with advanced medical care, too high a percentage of patients with bone injury experience delayed healing or even nonunion. Due to the difficulties in diagnosing delayed bone healing, the numbers are probably even higher. The complexity of the process, and thus the multitude of possible failures to complete the healing successfully, further underscores the problem. Several tissues are involved in the bone healing process: bone; bone marrow; periosteum; endosteum; vasculature; surrounding muscle tissue; and nerve tissue. Many different cells are involved: erythrocytes; granulocytes; macrophages; fibroblasts; fibrocytes; chondroblasts; chondrocytes; osteoblasts; osteocytes; osteoclasts; MSCs; mast cells; megakaryocytes; T cells; B cells; natural killer cells; dendritic cells; haematopoietic stem cells; endothelial cells; and pericytes. This plethora of cells is an indication of the signalling molecules and signalling pathways that govern the healing process. For example, the OPG-RANK-RANK L (osteoprotegerin – receptor activator of nuclear factor kappa B – receptor activator of nuclear factor kappa B ligand) pathway is exploited in bone loss for prevention and treatment.[38] Another pathway that plays a crucial role in bone treatment is the BMP2-transforming growth factor beta (TGF-β)-Smad pathway,[39,40] with BMP2 being one of the growth factors that reached clinical approval for nonunion treatment.[25]

These tissues, cells, and signalling pathways govern the bone healing process through the haematoma and inflammatory phase that initiates the healing process. This phase ends with a granulation tissue that transforms into a soft callus dominated by cartilage. Chondrocytes become hypertrophic before the callus matures, is mineralized and transformed towards a hard callus. In the hard callus, woven bone develops and the stability of the bone is thus restored. To regain the former mechanical properties, the bone then undergoes remodelling according to the mechanical strains sensed by the tissue upon movement of the limb. Figure 4 shows all decisive phases, their characteristics, and which functions a biomaterial would have to exhibit at the respective timepoints in the healing process.

Inital phase	granulation tissue	Cartilage	Woven bone	Inital phase
Following injury and/or intervention a haematoma forms at the site of injured bone and an inflammatory reaction commences	The haematoma matures towards a granulation tissue - cellular composition changes towards fibroblasts – cellular debris has been removed	Secondary bone healing proceeds through a cartilaginous stage - cartilage provides mechanical stability and precedes bone formation	Woven bone replaces cartilage - mineralization in an unstructured concept not supporting mechanical needs	Bone rearranges according to the mechanical cues
Conditions: • Disrupted blood supply • Hypoxia, low pH,High Na+, High K+ • Inflammatory cytokines	Conditions: • Revascularized area • Initial inflammatory signalling has changed towards M2/Th2	Conditions: • Avascular tissue • Only one cell type - chondroblasts / chonodrocytes	Conditions: • Mixed cell population with osteoblasts, osteoclasts, immune cells, vasculature cells, progenitors replace single cell composition of cartilage.	Conditions: • Mineraliztion takes place in a well vascularized area • Mechanical stability is reacquired.
Processes in normal bone healing: • Void filling - first ECM • Chemotactic signals to draw cells • Cytokine pattern to initiate healing	Processes in normal bone healing: • Mature granulation tisssue represents the stepping stone for the next healing phase • Secured tissue supply • Structured ECM	Processes in normal bone healing: • Chondrocytes expressing Coll II • Become hydrophobic and express Coll X • Transdifferentiate towards osteoblastic cells	Processes in normal bone healing: • Osteoblasts proceed with Coll I apposition • ECM mineralization	Processes in normal bone healing: • Bone rearranges to best adapt to mechanical constraints. • Bone marrow cavity opens • Surplus bone being degraded
Biomaterial support: • Void filling • Providing structure for cells • Containing inflammatory reaction	Biomaterial support: • Newly formed vessels • Cell differentiation towards osteogenic lineage	Biomaterial support: • Revascularization • Steering chondrocytes towards Coll X expression	Biomaterial support: • Provinding space for growing bone tissue • Osteoinductive, osteoconductive, osteogenic	Biomaterial support: • Enables the sensing of mechanical cues • Degrades to allow the bone to take over or fully integrates without stress shielding

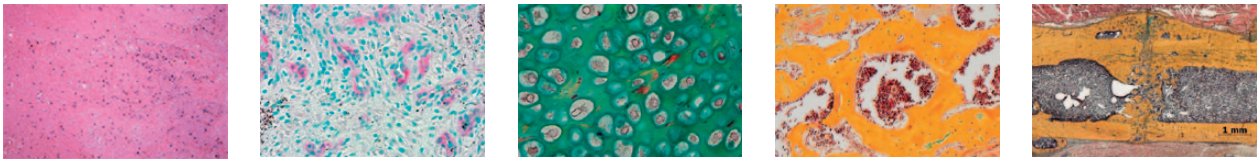


Fig. 4

Consecutive phases of bone healing are described in view of conditions, processes occurring in normal healing and the biomaterial properties which could support these phases. The lower row depicts the healing bone tissue in the different phases. Images from left to right: haematoma, haematoxylin eosin (H&E) staining, one day after injury, sheep; granulation tissue, immune histology for alpha smooth muscle with a methylene green counterstain, seven days after injury, sheep; cartilage, Movat pentachrome staining, 14 days after injury, mouse; woven bone, Movat pentachrome staining, 21 days after injury, mouse; remodelling, Movat pentachrome staining, six weeks after injury, rat.

Initial phase

When designing and applying a scaffold to promote bone healing, one should consider the surrounding in which the scaffold is placed and what purpose the biomaterial inserted into the bone should fulfill. If we think about the early healing phase, a simple, biocompatible and safe scaffold could be a blood clot.[36] Drawing peripheral blood using an anticoagulant, and applying cell or growth factors before adding a coagulant for clot formation describes an easy way to incorporate treatment factors into a scaffold suitable for mimicking the initially formed tissue in a healing bone. Inserting an artificial clot from allogenic peripheral blood into a 5 mm bone defect in a rat femur stabilized with an external fixation system demonstrated that such a biomaterial does not delay the bone healing process (Fig. 5).

Implanting a biomaterial is linked to bleeding and subsequent coagulation, a process phylogenetically coupled to an inflammatory reaction.[42] Immune cells are perceived to be the first responders to implanted biomaterials.[43,44] A prerequisite for a successful bone healing is a swift downregulation of the initial pro-inflammatory reaction.[10,45-47] This early immune reaction towards a biomaterial can be detrimental for the healing if it triggers a strong immune response. For example, chitosan, as a biomaterial, induces dendritic cell maturation and activation of adaptive immunity, while this is not the case with alginate or agarose.[48,49] Biomaterials therefore should be tested for their immune response and should create a microenvironment favourable for precursor cells upon implantation. Precursor cells cannot survive within an environment of low pH, and high potassium and sodium concentrations, as seen in pro-inflammatory conditions.[50]

Granulation tissue phase

A highly regulated inflammatory reaction also influences the angiogenic signalling[51] required for the next important step within the regenerative cascade of bone formation: revascularization. Biomaterials can also be specifically

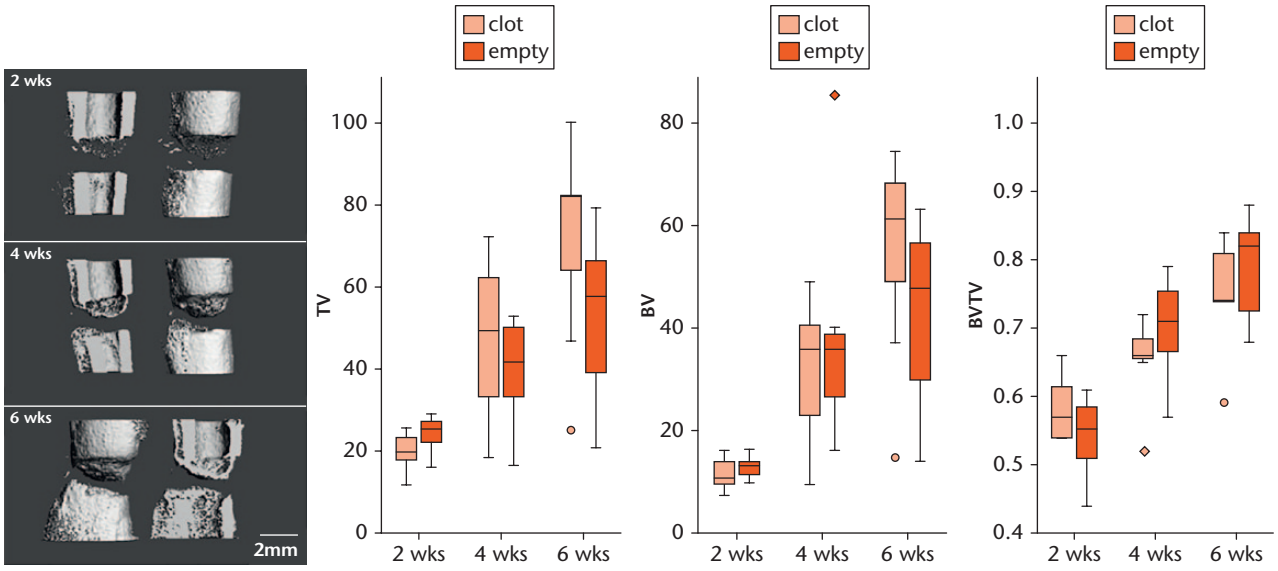


Fig. 5a Fig. 5b Fig. 5c Fig. 5d

In a long bone defect model in a rat femur, gap size 5 mm, stabilized with an external fixator, bone healing was compared between a group with an empty defect and a group receiving an artificial blood clot from allogenic peripheral rat blood (n = 6). Animals were sacrificed after two, four and six weeks, and analyzed with micro-CT to determine the percentage of mineralized tissue. A) 3D images of µCT evaluation, b) tissue volume in mm3 of the volume of interest analyzed by µCT c) bone volume in mm3 of the volume of interest analyzed by µCT d) the quotient of bone and tissue volume of the volume of interest analyzed by µCT. No significant difference was found between the groups, confirming the usability of such a biomaterial to add cells or factors during the early bone healing phase (animal experiments were approved by the local legal representative (G 0428/07) and carried out according to the policies and principles established by the Animal Welfare Act).[41]

designed to support this process. One strategy is to fabricate 3D scaffolds with a functional vasculature to repair large bone defects. Some initial difficulties within these strategies could be overcome with new bioprinting techniques.[52] Another strategy uses the angiogenic potential of specific materials such as silicate ions released from silicon-containing biomaterials[53] or strontium- and cobalt-substituted bioactive glasses.[54] Angiogenesis could also be enhanced by embedding angiogenic factors such as vascular endothelial growth factor (VEGF) within a biomaterial[55] or by encapsulating angiogenic cells.[35,36]

Cartilage phase

Biomaterials supporting the cartilaginous phase in bone healing could be designed by using the growth and differentiation factor 5 (GDF5), which has been identified as being important for this healing phase in bone.[56] Comparing the stiffness in a critical-sized bone defect treated with either BMP2 or GDF5 in a femoral osteotomy model in rats showed that GDF5 did indeed enhance healing compared with an empty control, and that it had not reached the bony consolidation seen in animals treated with BMP2. Histology confirmed that the predominant tissue within the gap consisted of cartilage (Fig. 6).[57]

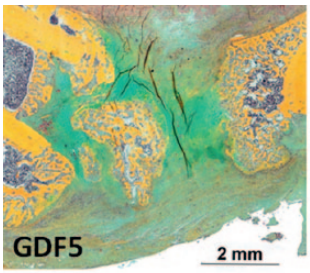
Woven bone phase

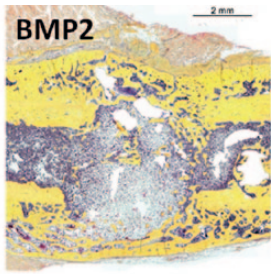
Tissue cells feel and respond to their microenvironment which influences their differentiation and thus tissue development.[58,59] Whether MSCs regenerate bone or instead fail to repair a bone injury by inducing fibrous tissue formation therefore also depends on the tissue stiffness and the oxygen supply (Fig. 7).[60]

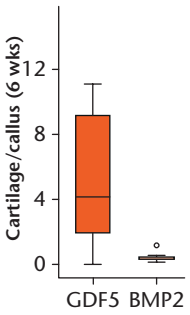
Harnessing extrinsic mechanical signals to influence MSC differentiation towards bone formation has been the goal in several biomaterial designs. Simulating the tissue elasticity of collagenous bone with a relatively stiff matrix induces osteogenic differentiation in naïve MSCs.[61] Using collagen-glycosaminoglycan (CG) scaffolds that mimic the extracellular matrix in structure, composition, and elasticity, a stiffness of 1.5 kPa supported osteogenic differentiation while a less stiff counterpart of 0.5 kPa favoured SOX9 (SRY-type high-mobility group box-9) expression and thus cartilaginous differentiation.[62] Mechanosensing and mechanotransduction are possible due to the cytoskeleton, specifically the interaction between actin, integrins, microtubules, focal adhesion points, and the cytoskeletal pattern.[63-66] All of these involved structures are also essential for cell morphology, which, in turn, is determined by the substrate form. Mesenchymal stromal cells differentiating into osteoblasts depict an elongated and spread form, therefore a surface supporting this cell form should improve osteogenic differentiation of stromal cells.[67] Indeed the lineage differentiation of mesenchymal stromal cells is dependent on the substrate curvature[68] with a convex spherical surface supporting osteogenic differentiation.[69]

Remodelling

Remodelling of bone to adapt optimally to the stresses and strains of load bearing also depends on mechanosensing.







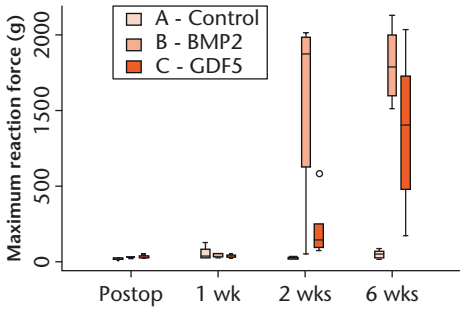


Fig. 6a Fig. 6b Fig. 6c Fig. 6d

a) Growth and differentiation factor 5 (GDF5); b) bone morphogenetic protein (BMP)-2 application. Histological staining was done with Movat pentachrome to depict different tissues: yellow, bone; green, cartilage; orange, muscle; light blue, connective tissue; dark red, bone marrow (adapted from Wulsten et al).[57] c) Graph showing that, when applied within a critical-sized defect in a rat femur osteotomy model, GDF5 with a collagen scaffold favoured cartilage formation within the callus after six weeks, as compared with BMP2. d) Graph showing that tissue stiffness within the defect showed solidification after two weeks with BMP, while GDF5 still had not reached bone stability after six weeks of healing. Control confirms the non-healing of this model without growth factor application.

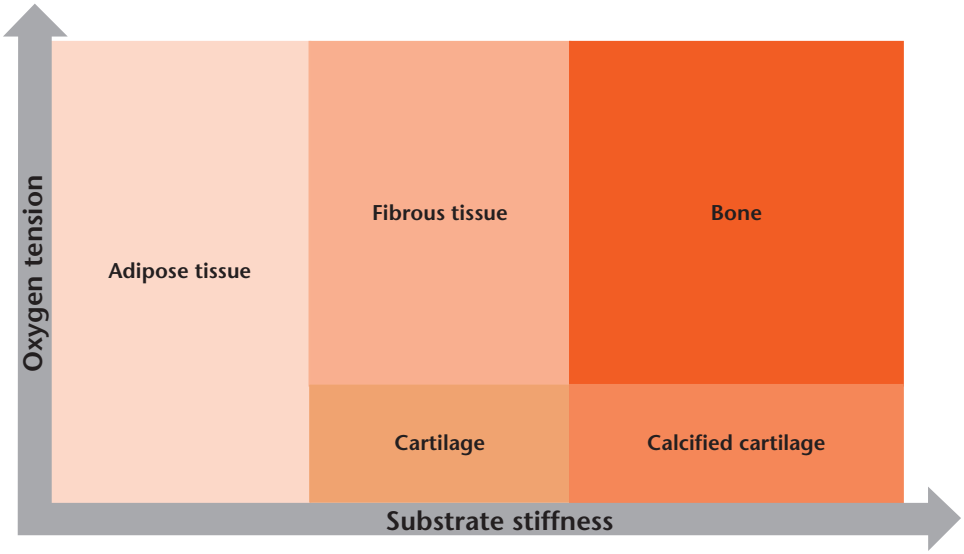


Fig. 7

Mesenchymal stromal cells (MSCs) are able to differentiate into adipose, fibrous or bone tissue depending on the substrate stiffness; a stiffer substrate favours bone formation. Successful bone formation also depends on the oxygen tension. A lack of oxygen leads to cartilage formation. These differentiation properties have prompted numerous research projects in which the substrate qualities were used to steer cell differentiation.

Therefore, biomaterials should be designed to allow a mechanical stimulation and should not shield the bone from mechanical forces completely. Balancing implant stability against stress-shielding is an important factor in the biomaterial design. If a biomaterial is stiffer than bone, the consequent stress-shielding can lead to loosening of the implant[70] due to bone loss at the interface. A possible strategy to counteract this could be a 3D-printed fully porous implant where the material architecture is tuned to reduce bone resorption due to stress-shielding.[71] Mechanical stimulation to increase bone formation has been investigated before and proven to be decisive.[72-75] Due to the positive effect of a mechanical stimulus for bone formation, the concept of early weight bearing after fracture is now already clinical practice, reflecting the need to think beyond the surgical site to achieve good results in fracture and bone healing.[76]

Biomaterials in bone regeneration

There are numerous biomaterials designed for bone regeneration and suitable for cell seeding. While not exhaustive, the following list indicates the multitude of possibilities: polyethersulfone (PES) nanofibres,[77] Gelfoam surgical sponges,[78] polycaprolactone (PCL),[79] hydroxyapatite/tricalcium phosphate (HA/TCP),[80] HA/poly(lactic-co-glycolic acid) (PLGA),[81] HA-coated PLGA/poly(L-lactic acid) (PLLA),[82] PCL functionalized with natural polymer hyaluronan and TCP,[83] decellularized bone scaffolds,[84] calcium phosphate cement (CPC),[85] apatite-coated silk fibroin,[86] fibrin,[87] silk,[88] collagen type I,[89] HA/collagen/

chitosan (CTS),[90] nano HA/methacrylate-co-acryloayl6-aminocaproic acid and gelatin-methacrylate-co-poly-(ethylene glycol)-diacrylate,[91] and calcium sulfate.[92,93] All of these materials have been shown to be beneficial for cell differentiation in terms of bone formation, however, despite this abundance, the biomaterials that are actually used clinically are not diverse at all and rather limited as described above.

Novel strategies in treating bone with biomaterials

New strategies for enhanced biomaterials include bioinspired composite matrices,[94] biogenic hydroxyapatites[95] or biomimetic organoids,[96]. These indicate the development towards biomaterials that copy the naturally occurring tissues/conditions through our advanced understanding of the physiological mechanisms coupled with the highly developed techniques available today. A common problem encountered in using biomaterial is the slow implant integration. Novel enhancing methods of biomaterial integration are therefore a current research goal.[97-100] An emerging strategy is to release drugs loaded on an implanted biomaterial at a defined later timepoint, where they are needed in the treatment regime. This release can be steered through an external signal such as an electrochemically triggered,[101-103] acidity-triggered,[104] pH-triggered[105] or UV-triggered[106] mechanism. Nanotechnology[100,107-109] and the integration of trace elements[110-113] within biomaterials are further strategies to enhance bone formation though biomaterials.

A high number of upcoming strategies will include immune-mediated tissue regeneration driven by biomaterial scaffolds[114] or biomaterials that use the immune reaction to activate a drug release where and when it is needed, circumventing systemic treatment effects.[115] In addition, biomaterials can be harnessed to entrap cells, changing them and subsequently releasing them to fulfil specific functions which they would otherwise not manage.[116] As previously described, the interdependency of the immune and skeletal systems greatly impacts bone regeneration, hence these future biomaterial strategies can be used to support bone formation.

In current therapeutic regimes, we still have to rely on the fixation of bone with non-degradable metal implants. Implant removal is still a problem, in part due to the difficulty of removing the material in cases where the osseointegration was very successful or in the case that the material breaks during removal efforts. Therefore, the development of degradable metals that will provide the initial stability to enable early weight bearing, also providing mechanical stimulation due to the loss of stiffness in consequence of their beginning degradation, and upon bone consolidation a complete degradation to render removal procedures superfluous are underway.[117,118]

A trend that has already reached the clinic is the 3D printing used to produce personalized implants for bone replacement.[119-121] Future efforts will be focused on designing and producing these implants as biomaterials with all of the aforementioned properties, with the final aim being to replace them with vital and fully functional bone.

Combining biomaterials with osteogenic, angiogenic or anti-inflammatory cells to enhance their pro-osteogenic properties provides a powerful therapeutic option. Today, the gold standard to enhance a bone scaffold is still the addition of autologous spongiosa. Harvesting autologous bone graft from the hip is often painful for the patient, and the available bone is also limited. In addition, the autologous material does not always carry a high potential to generate bone, depending on the donor's age and comorbidities. Therefore, further strategies are under investigation. As autologous material cells from peripheral blood,[35,36] platelet-rich plasma (PRP)[122] and RIA aspirate[123,124] are alternatives for autologous bone graft with less patient morbidity, they are, however, less efficient. Consequently, intensive research efforts are underway to harness mesenchymal stromal cells to enhance bone healing. More than 400 clinical trials are currently investigating the potential of these cells for clinical applications.[125]

In conclusion, the data presented in this review consider the current treatment options for problematic bone healing cases. The information presented also provides an overview of the directions in which the field of bone regeneration with respect to biomaterials for enhancing fracture and defect treatment is moving. Considering the endogenous bone healing cascade and possible intervention strategies, embracing regenerative medical approaches could be considered the future in treatment options to enhance bone healing. The reason for this is that knowledge of the biological cues – of what the bone needs in order to heal is rapidly growing. In parallel, the field of biomaterials has evolved and is now able to create solutions that mimic biological processes in an unprecedented way. Furthermore, technical advances in biomaterial science, such as 3D printing, allow the creation of complex biological structures that may drive regeneration and possibly exceed the intrinsic healing capabilities of the body.

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Conflicts of Interest Statement

- None declared