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Tissue engineering approaches for bone repair: Concepts and evidence

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ABSTRACT

Over the last decades, the medical world has advanced dramatically in the understanding of fracture repair. The three components needed for fracture healing are osteoconduction, osteoinduction and osteogenesis. With newly designed scaffolds, *ex vivo* produced growth factors and isolated stem cells, most of the challenges of critical size bone defects have been resolved *in vitro*, and in some cases in animal models as well. However, there are still challenges needed to be overcome before these technologies can be fully converted from the bench to the bedside. These technological and biological advancements need to be converted to mass production of affordable products that can be used in every part of the world. Vascularity, full substation of scaffolds by native bone, and bio-safety are the three most critical steps to be challenged before reaching the clinical setting.

The clinical problem with critical size bone defects

Bone is a very forgiving tissue. It withstands multiple insults and can regenerate itself into a healthy bone. One of the strengths of bone is its ability to build new osteones when the native structure of the bone is injured. The entire process of bone healing is beyond the scope of this paper; however, a basic understanding of the concepts of bone healing is essential for understanding the pathopysiology of critical bone defects. When bone is injured, a gap is created. This gap is filled with necrotic bone debris; blood from broken blood vessels and inflammatory cells, chemo mediated to the damage site by a set of signals, not yet fully understood.[13,20] The healing process is margined by a combination of osteoconduction – a material acting as a scaffold for the new bone to grow into, and osteoprogenitor cells allowing osteoinduction – a combination of signals and cells getting rid of the necrotic bone and putting down the scaffolds for the newly generated bone.[23] However, a gap beyond two and a half times the bone radius (termed as a critical size bone defect) remains a significant clinical problem.[12,36,43] Such defects can be caused by blunt or penetrating trauma,[38] surgical treatment of tumors[12,17] or necrosis caused by radiation,[74] or various chemical substances.[96]

Traditional therapeutic approaches in treating large bone defects include bone grafts[16] and transplants[90] (autologous – from the iliac bone or fibular grafts, allograft – fresh or frozen after cleaning, or xeno-grafts). These grafts are supported by different fixtures, in hope that native bone will bridge the gaps and a boney fusion will occur. Other options include specialised implants that can serve as internal prosthesis (for example, tumour prosthesis after large bone resection, due to bone tumors[91]), shortening of the limb,[44] with or without secondary distraction osteogenesis,[5] bone transport methods (i.e. Ilizarov technique[45]), or in the unfortunate result, an amputation of the involved limb.[42]

The best classic solution for a large bone defect is the use of autologous bone graft.[16] These grafts do not cause immuno-reaction and contain the osteoconductive scaffolds, osteogenic cells and, if preserved, a viable blood supply (via connected arteries[88]). However, the use of bone grafts in clinical practice is limited due to high percentage of donor and recipient site complications.[8] Vascularised grafts need a more extensive surgical team, with an over all, relatively low, artery patency.[81]

The use of allograft or xenografts prevents the problems involved with donor site morbidity, and allows larger substitutes. However, since they undergo sterilisation and purification, allografts and xenografts do not provide osteoinduction signals, and do not have living cells. In addition, they also present the potential risk of viral or bacterial infections and of an immune response of the host tissue after implantation.[59] In addition, full integration of the graft is rare, ending at most cases with only bone substitution at the ends of the grafts, leading to late graft fracture, reported as high as 60% at 10 years.[94]

The use of large prosthesis for bone grafts is a well-known solution. They provide a medial to long term solution, and new coatings provide better osseous coating; however, there is no

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biological interface – once the material gives in (as a result of chronic ware) or loosening occurs, the patient is in need of further surgery. The rate of revision is high, with over 30% of the patients needing revision after 7 years.[63]

Distraction osteogenesis is a well-established technique, by means of which an acute shortening is preformed at the bone defect site, and a slow distraction process is started elsewhere in the bone. This technique takes advantage of the bone's regeneration potential, avoiding the troubles associated with graft's integration.[45] However, it requires extreme patients' compliance, since it takes a long period of time and it is often complicated with infections.[4] In addition, this process calls for a bone that can regenerate; thus if the patient's regeneration capacity is compromised, this technique cannot work. However, in animal models, the chemotherapy, commonly given for cancer, did not effect the cells contraindicating distraction osteogenesis.[87]

In summary, the current situation is suboptimal at best, and a new, better and wider substitution for large bone defects is needed.

As stated above, in order to create new bone (osteogensis) a combination of osteoinduction and osteoconduction is required. One cannot function without the other. In the classic approaches, each part of this equation was separately tackled, however, in the advanced biochemical methods there is a combination of the two, with sophisticated materials integrating osteoconduction and induction allowing a surplus of osteogenesis. In the next sections, we will discuss the concepts and evidence in each of these elements.

Osteoconduction – mind the gap

The major problem with critical size bone defects is that cells cannot skip from one edge of the bone defect to the other; they need a solid platform on which to build bone and unite the fracture. Fracture ends that are distracted or in motion will not heal, and heterotrophic nonunion will develop.[14] When a critical size bone defect develops, the body cannot heal itself and the gap needs to be surpassed, all the whilst keeping the limb in the proper length and angulations.

Navarro et al.[68] wisely divides materials used in the orthopaedic clinical usage into three generations according to their biofunction: in the first generation, there are bio-inert materials – these materials are used in most cases as fillers for the gap and in the modern scenario they are surrounded by materials that encourage the in-growth of bone. As a result, as in dental implants, a wide variety of surface treatments are applied to most metallic implants before their implantation.[54] The second-generation group of materials include composites that are bioactive and biodegradable. These materials interact with the biological environment around the fracture to enhance the biological response and the tissue/surface bonding. In addition, these materials are bio absorbable and have the ability to undergo a progressive degradation whilst new tissue regenerates and heals. These materials can be metal,[55] ceramics[22] or polymers.[19] The aim is to obtain a material with mechanical properties similar to those of bone, strong enough to allow operating room manipulation that can bond with bone. The material needs a degradation process that matches the healing period of the fracture or lesion. Too fast or too slow degradation pace is not acceptable in the clinical setting.[58]

One of the ongoing challenges is the bonding process,[32] in which the scaffold unites with the adjacent bone. This is achieved by polymers binding at the interface between the organic matrix and the inorganic supplements.[62] This surface modification is achieved by phosphorilation of proteins and peptides on the surface of the implant, as well as by modification of the insert to induce the mineralisation of the endplates and unite with the adjacent bone, via HA layers placed on the outer surface of the implant.[56]

Third-generation materials are designed to stimulate specific cellular responses at the molecular level.[67] The implants are three-dimensional structures that are biodegradable and biocompatible with degraded by-products that are non-cytotoxic. The degradation must happen at the same rate the tissue is repaired. These scaffolds hold a highly interconnected porous network, allowing integration of osteoblasts and osteoclasts. It has been shown that pores sized 100–350 μm are optimal for bone progenitor cells.[79] The mechanical properties of the scaffold must be appropriate to regenerate bone tissue in load-bearing sites.[68]

Many materials have been tried for this group. One of the most commonly used is demineralised bone matrix.[39,86] This provides a solid scaffold and includes bone morphogenetic proteins (BMPs). Other materials are nanocrystalline structures,[84] organic–inorganic composites,[69] nanofibres,[60] biodegradable glass,[26] microspheres,[71] three-dimensional cross-linked hyaluran sponges (ACP) scaffolds,[25] hydroxyapatite,[40] glass micro-beads,[95] hierarchically organised structures and hydrogels containing calcium and phosphate.[92] These materials can be customised to any three-dimensional scaffold needed,[83] or press fitted to the defect to allow maximal surgeon ease.[85]

Despite these advances several main challenges remain. The introduction of blood vessels to the grafts is problematic, thus impairing the integration and migration of the cells to the scaffold site.[33] In addition, the durability of these new composites has yet to be tested and designed in such a way that surgeons will be able to use them freely in a clinical setting.

It is important to remember that most of these materials are still in the *in vitro*/animal model phase, and it will take 10–20 years before they are cleared for everyday use. However, despite these limitations the inclusion of engineers, chemists, physicists and biologists and the constant influence of surgeons can bring great advances, resulting in new and improved materials to our present day operating room.

Osteoinduction – or signals and cells

When a fracture occurs, a set of signals is triggered. These are both local signals and systemic ones; some of these signals are mediated by neuronal impulses,[70] by the haematoma at the site of the fracture[35] and by the trauma caused to the tissues surrounding the fracture.[24] These signals can be divided into two interactive and interchangeable categories: inflammatory signals (i.e. IL-1, IL-6, and TNF-α), and bone building signals (BMPs and WNTs).[24] These factors mitigate the migration of phagocytotic cells to the area of the fracture, removing the necrotic tissue and propagating the in-growth of new blood vessels to the site of the fracture, thus providing nutrients and cells to the fracture site and starting the healing cascade.[20] As stated above, if at the end of the healing process osteo-integration (of the new bone together with the native bone) is not achieved, even with the best type of scaffolds, the chances of long-term success are dismal.[7]

The addition of growth factors such as bone morphogenetic proteins and growth factors to scaffolds or to the area of the bone defects has proven to increase bone formation both *in vitro* and in animal models.[11] However, when converting these studies to humans, the concentrations needed are higher and, at times, supraphysiologic, with possible related side effects and high costs (with a strong industry drive).[3,28] Furthermore, most current clinical techniques for the use of bone growth factors result in fast release of the growth factor shortly after position, with only few clinical studies investigating the long-term release of these factors.[46,48]

An attractive approach for the addition of growth factors to increase bone regeneration is the addition of platelet rich plasma (PRP) to the fracture site.[2] PRP has been shown to enhance osteoid