

Bone Regeneration and Repair: Current and Future Aspects

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Abstract: *Bone regeneration is a complex, well-orchestrated physiological process of bone formation, which can be seen during normal fracture healing, and is involved in continuous remodelling throughout adult life. However, there are complex clinical conditions in which bone regeneration is required in large quantity, such as for skeletal reconstruction of large bone defects created by trauma, infection, tumour resection and skeletal abnormalities, or cases in which the regenerative process is compromised, including avascular necrosis, atrophic non-unions and osteoporosis. Further understanding in this area could be the key to an improved and integrated strategy for skeletal repair. In the future, control of bone regeneration with strategies that mimic the normal cascade of bone formation will offer successful management of conditions requiring enhancement of bone regeneration, and reduce their morbidity and cost in the long term. Research is ongoing within all relevant fields, and it is hoped that many bone disease processes secondary to trauma, bone resection due to ablative surgery, ageing, and metabolic or genetic skeletal disorders will be successfully treated with novel bone-regeneration protocols that may address both local and systemic enhancement to optimize outcome.*

Keywords: Bone, Regeneration, Repair, Bone Grafts

Myopia

1. Introduction

Bone is a highly dynamic tissue comprising a mineralized extracellular matrix embedded with bone cells, blood vessels, and nerves. Bone contains three main bone-specific cell types: the osteocyte is a mature cell that sits in bone lacunae, communicates with other osteocytes through long cellular processes, senses mechanical stress in bone, and sends signals for bone remodeling as a result of mechanical stress. The responding cells are osteoblasts, cells specialized to secrete the unique collagen-rich extracellular matrix in bone that enables mineralization; and osteoclasts, macrophage-like cells that degrade the bone structure through a combination of localized acidification (removes the minerals) and protease secretion (breaks down matrix). Osteoclasts tunnel through bone and are usually followed close behind by osteoblasts. Bone is in a constant state of remodeling in healthy individuals [1]

Bone possesses the intrinsic capacity for regeneration as part of the repair process in response to injury, as well as during skeletal development or continuous remodelling throughout adult life. Bone regeneration is comprised of a well-orchestrated series of biological events of bone induction and conduction, involving a number of cell types and intracellular and extracellular molecular signalling pathways, with a definable temporal and spatial sequence, in an effort to optimise skeletal repair and restore skeletal function. In the clinical setting, the most common form of bone regeneration is fracture healing, during which the pathway of normal fetal skeletogenesis, including intramembranous and endochondral ossification, is recapitulated [2].

Unlike other tissues, the bone can regenerate and repair itself: in many instances, bone injuries and fractures heal

without scar formation. Nevertheless, in pathological fractures or large and massive bone defects, bone healing and repair fail. Insufficient blood supply, infection of the bone or the surrounding tissues, and systemic diseases can negatively influence bone healing, resulting in delayed unions or non-unions. Bone is the second most commonly transplanted tissue after blood. A bone graft is defined as an implanted material that promotes bone healing alone or in combination with other material(s), through osteogenesis, osteoinduction, and osteoconduction, in combination or alone [3].

Clinical Problems Requiring Replacement of Bone [4]

An important concept to appreciate is that clinical problems are extremely diverse, and that no one approach will likely suit all potential applications. We focus on those applications where the bone will not heal on its own, and some sort of bone graft is typically used. Some of these diverse applications include: 1. Non-union fractures (fractures that fail to heal) 2. Craniofacial reconstruction, 3. Segmental defect due to tumor removal, 4. Augmentation of bone around a hip implant revision (i.e., 25% of hip implants are replacements of an existing implant, as the lifespan of a hip implant is only ~10 years) 5. Reconstruction of bone in the jaw for dental purposes -- loss of teeth can lead to resorption of jaw bone as the bone is not being stressed by chewing.

2. Methods to Augment Deficient Bone

The reconstructive options in the osseous reconstruction of the cranio-maxillofacial skeleton include autogenous bone grafts harvested from local or distant sources [5]. Allogeneic bone from another individual may also be considered, as might xenogeneic bone from another species. Because the possibilities of immunogenic problems exist, such grafts were first treated with a freezing technique. Later other methods to deal with immunogenicity were developed. Alloplasts have

also been developed to replace bone. In addition a number of surgical procedures have been designed to increase the amount of bone available locally without bone grafting [6].

2.1 Osteoinduction [7]

Osteoinduction describes a process whereby new bone is produced in an area where there was no bone before, where one tissue or its derivative causes another undifferentiated tissue to differentiate into bone. The phenomenon of osteoinduction was first described in the classic works of Urist. Bone matrix was shown to induce bone formation within muscle pouches of many species of animals. Later a specific extract from bone, a protein now referred to as Bone Morphogenetic Protein (BMP), was identified as that factor which caused the phenomenon.

2.2 Osteoconduction [8]

Osteoconduction describes bone formation by the process of ingrowth of capillaries and osteoprogenitor cells from the recipient bed into, around and through a graft or bioimplant. Therefore the graft or bioimplant acts as a scaffold for new bone formation. Unlike osteoinduction, this process occurs in an already bone containing environment. Osteoconduction describes the facilitation of bone growth along a scaffold of autogenous, allogenic or alloplastic materials.

Local Procedures to Augment Existing Alveolar Bone [5].

Here are a number of techniques, which enable the surgeon to maximize the available bone in the cranio-maxillofacial skeleton without harvesting a bone graft. These techniques serve to minimize reconstructive morbidity, as there is no graft donor site. Osteocondensation is one such technique. It can reshape the morphology of the alveolar bone of the maxilla for example, by compacting it in various directions using the condensing chisels or plungers. The procedure can establish a new contour of the bone being condensed. This allows the clinician who is placing dental implants to more optimally house a dental implant, resulting in better primary stability in areas of poor bone quality. Orthopaedic surgeons have practiced osteocondensation since the early 1960s.

Distraction osteogenesis (DO) of the long bones in growing children has been used for decades to gradually lengthen osteotomized bones without a bone graft. The resulting distraction gap is initially filled with callus, which later matures into bone. DO has also been adapted to the maxillofacial area and special devices and implants are being developed for that purpose [9].

2.3 Autografts [10]

At the present time, autogenous bone grafting is the gold standard by which all techniques of osseous reconstruction of the cranio-maxillofacial skeleton must be judged. Autogenous cancellous bone grafts produce the most successful and predictable results. Free bone grafts act mostly as scaffolds and are thus more osteoconductive than osteoinductive even though osteogenic activity may have remained in the spongy part of the graft. The major disadvantage of autogenous grafts is the need for a second

surgical site and the morbidity resulting from harvesting. The source of autograft, however, is not limitless for the patient.

Potential Autogenous Bone Graft Donor Sites.5, 10

Autogenous bone grafts can be vascularized or non-vascularized. Vascularized bone grafts are much more complex to harvest and have a great deal of donor site morbidity associated with their use. Non-vascularized grafts are considerably simpler to harvest and use if they are placed into a well vascularized recipient bed.

2.4 Allografts.

Allogenic bone is non-vital osseous tissue taken from one individual and transferred to another individual of the same species. There are three forms of allogenic bone: fresh frozen, freeze-dried and demineralized bone matrix (DBM). Fresh frozen bone is rarely used today for the purposes of bony reconstruction in the cranio-maxillofacial skeleton because of concerns related to the transmission of viral diseases [5].

2.5 Xenografts.

Xenogenic bone grafts consist of skeletal tissue that is harvested from one species and transferred to the recipient site of another species. These grafts can be derived from mammalian bones and coral exoskeletons. Bovine derived bone has been commonly used, even though other sources are such as porcine or murine bone are available. Xenogenic bone was popular in the 1960's but fell into disfavor due to reports of patients developing autoimmune diseases following bovine bone transplants. The re-introduction of these products in the 1990's comes after the development of methods to deproteinate bone particles. This processing reduces the antigenicity making implants more tolerable to host tissues [11].

Synthetic Bone Substitutes

Alloplastic bone substitutes are synthetic substances that have been processed for clinical use in osseous regeneration. There are three types of alloplastic substances in clinical use today: hydroxyapatite, other ceramics and polymers. Hydroxyapatite (HA) is a ceramic. HA has several potential clinical applications including the filling of bony defects, the retention of alveolar ridge form following tooth extraction and as a bone expander when combined with autogenous bone during ridge augmentation and sinus grafting procedures. Apart from HA, there are three other types of ceramics: tricalcium phosphate (TCP), bioglasses, and calcium sulphate [12].

Osteoactive Agents

An osteoactive agent is any material which has the ability to stimulate the deposition of bone. The phenomenon of osteoinduction was first described in the works of Urist and co-workers in. Bone matrix was shown to induce bone formation when implanted within muscle pouches of a number of different species of animals. Urist's group identified a specific extract from bone, a protein now referred to as Bone Morphogenetic Protein (BMP), as that factor which caused the phenomenon. Since then, many other

entities have been found with a variety of effects on bone. These may be classified as osteoinducers, osteopromoters or bioactive peptides [5].

Bone Morphogenetic Protein.

Bone morphogenetic protein (BMP) has been shown to have osteoinductive properties. It is recognized to be part of a larger family of growth factors referred to as the TGF- β superfamily with a 30-40% homology in amino acid sequence with other members in the family. BMP acts as an extracellular molecule that can be classified as a morphogen as its action recapitulates embryonic bone formation. The identifying pattern of the BMP subfamily is their seven conserved cysteine residues in the carboxy-terminal portion of the protein and this is where the unique activity of BMP's is thought to reside [13].

Transforming Growth Factor

The proteins in the family of transforming growth factor β (TGF- β) should be considered as osteopromoters, agents, which enhance bone healing. TGF- β is found in the same supergene family as BMP. TGF- β has been shown to participate in all phases of bone healing. During the initial inflammatory phase TGF- β is released from platelets and stimulates mesenchymal cell proliferation. It is chemotactic for bone forming cells, stimulating angiogenesis and limiting osteoclastic activity at the revascularization phase. Once bone healing enters osteogenesis then TGF- β increases osteoblast mitoses, regulating osteoblast function and increasing bone matrix synthesis, inhibiting type II collagen but promoting type I collagen. Finally, during remodelling it assists in bone cell turn-over [5].

Platelet-Derived Growth Factor

Platelet derived growth factor (PDGF) is angiogenic and is known to stimulate the reproduction and chemotaxis of connective tissue cells, matrix deposition. These properties are all crucial to bone healing. Insulin-like growth factor (IGF) has demonstrated a capacity to increase bone cell mitoses and increase the deposition of matrix. PDGF and IGF have shown an ability to work together during the reparative stages of bone healing. PDGF-IGF impregnated devices have proven to increase bone healing in defects associated with dental implants and teeth [14].

Other Bioactive Molecules

The last category of bioactive molecules is the polypeptide group. They may act as osteoinducers or osteoenhancers. Two short amino acids chain peptides that have demonstrated a bone activity are known as P-15 and OSA-117MV. The P-15 polypeptide was designed to take advantage of a conformational arrangement known as the "beta bend", which was found to have an influence on bone induction and growth when utilized in some in vitro studies. The OSA molecule is even smaller than P-15 and was discovered in relation to the treatment of osteoporosis where OSA's effect is concentrated in areas of high stress [15].

Stem Cells and Hybrid Grafts as Applied Tissue Engineering
The area of tissue engineering has brought to the forefront, the possibilities of hybrids of biomaterials seeded with osteocompetent cells to be used as an implant. The hybrid

could consist of a porous matrix, on which bone marrow cells could grow. The use of bone marrow as the source of cells is logical as bone marrow contains stem cells which have the potential to differentiate along various pathways and lines, including the direction of bone producing osteocompetent cells. Seeding a porous matrix with bone marrow cells could enhance the osteogenic potential of the matrix as a hybrid. Another possibility is the tissue culturing of bone marrow cells to further expand their numbers. Bone marrow derived cells are responsive to the influence of dexamethasone and 1, 25 dihydroxycholecalciferol and can be influenced to differentiate in the direction of bone cells. Human bone marrow cells have been reported to adhere to porous coral matrices and to matrices made of HA and TCP. Osseous cells could be colonized onto or combined with such matrices, producing hybrid grafts [16].

Tissue scaffolds

Scaffolds are the most important issue in tissue engineering and could be divided into two main categories including biological (natural or organic) and synthetic (artificial) materials. The former are natural polymers such as collagen type I or DBM. Porous metals, bioactive glasses, synthetic polymers such as polylactic acid (PLA) and polyglycolic acid (PGA), and calcium phosphate ceramics such as hydroxyapatite (HA) and tricalcium phosphates (TCP) are examples of synthetic materials [16].

Natural-based materials used for tissue scaffolding.

Natural- or biologic-based materials are taken from biologic-based tissues, and xenografts may be the best source for these later products. The advantages of natural-based scaffolds are that they have significantly superior biocompatibility, biodegradability, regenerative characteristics (e.g., osteoinduction, osteoconduction, osteogenesis, and osteointegration) than those of synthetic materials, but their immunological behavior is variable in different species and is also related to the type of application. Collagens are among the most widely present in the human body, providing strength and structural stability to various tissues, from the skin to bone. Collagen (collagen type I), the major organic component of ECM of the bone, is the most popular biologic materials used to produce biologically based tissue-engineered grafts. Chitosan, a linear polysaccharide with many commercial and biomedical uses due to its properties which allow it to rapidly clot blood, has recently gained approval in the USA and Europe for use in bandages and other hemostatic agents, quickly stopping bleeding and reducing blood loss, with a 100% survival of otherwise lethal arterial wounds in swine. Alginate Alginic acid, also called algin or alginate, is an anionic polysaccharide distributed widely in the cell walls of brown algae where, binding with water, it forms a viscous gum. In extracted form, it quickly absorbs water, with a water absorption capacity of 200-300 times its own weight. It is sold in filamentous, granular, or powdered forms. Elastin Similar to collagen, elastin is a key structural protein found in the ECM of most tissues; yet, very little is known about the response of bone cells to elastin or its derivatives. Recently, a novel class of ECM-based composite scaffolds with collagen and a genetically engineered polymer, elastin-like polypeptide (ELP) has been designed and produced [17].

Stem Cells

The combination of stem cells with scaffolds as a polytherapy is a new option. Collagen and demineralized bone powder have been used to produce a novel scaffolds for bone tissue engineering. Human periosteum-derived cells (PD cells) were cultured on this scaffold: the hybrid scaffolds exhibited greater osteoinductive potential than collagen scaffolds. The PD cells with hybrid scaffolds possessed higher ALP activity, calcium deposition, and superior behavior (e.g., attachment, differentiation, and proliferation) than those with collagen scaffolds [18].

3. Discussion

To design and produce an efficient bone graft, the researchers and orthopedic surgeons should have sufficient knowledge of the characteristics of grafts such as osteogenesis, osteoinductivity, and osteoconductivity, and their other advantages and disadvantages. Autografts are the gold standard for bone regeneration. Among the available strategies to improve fracture healing and enhance the outcome of incorporation of the grafts, tissue engineering is a suitable option. An ideal tissue-engineered product should have characteristics similar to those of autografts without their limitations. The bone scaffolds should additionally be highly porous and have pores of suitable sizes at all locations of the scaffold to provide an optimal environment for new bone matrix and bone regeneration [20].

Furthermore, growth factors such as basic fibroblastic growth factor which may affect cell functions, proliferation, or differentiation; healing promotive agents such as hPRP; and also Tarantula cubensis extract can be included in the scaffolds to enhance the healing performance of the injured connective tissues. Agents such as glycosaminoglycans, including hyaluronic acid, chondroitin, and dermatan sulfate have modulatory roles in bone and fracture healing and can improve the quality of the tissue-engineered scaffolds. More recently, zoledronate, simvastatin, and alendronate have been shown to have promising effects on bone healing and regeneration [21].

The vascularity of the scaffold is critical because if not present, the scaffold will undergo ischemia and the cells will die. Therefore, application of growth factors such as VEGF, PDGF, and FGF can be useful to stimulate angiogenesis in the scaffolds and the grafts. A combination of stem cells with scaffolds and healing promotive factors, especially the growth factors, could be one possible strategy providing all the necessary characteristics for bone repair and regeneration [22].

4. Conclusions and Future Aspect

There are several clinical conditions that require enhancement of bone regeneration either locally or systemically, and various methods are currently used to augment or accelerate bone repair, depending on the healing potential and the specific requirements of each case. Knowledge of bone biology has vastly expanded with the increased understanding at the molecular level, resulting in

development of many new treatment methods, with many others (or improvements to current ones) anticipated in the years to come. However, there are still gaps; in particular, there is still surprisingly little information available about the cellular basis for MSC-mediated fracture repair and bone regeneration in vivo in humans. Further understanding in this area could be the key to an improved and integrated strategy for skeletal repair. In the future, control of bone regeneration with strategies that mimic the normal cascade of bone formation will offer successful management of conditions requiring enhancement of bone regeneration, and reduce their morbidity and cost in the long term. Research is ongoing within all relevant fields, and it is hoped that many bone disease processes secondary to trauma, bone resection due to ablative surgery, ageing, and metabolic or genetic skeletal disorders will be successfully treated with novel bone-regeneration protocols that may address both local and systemic enhancement to optimize outcome.

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