3

Signals Between Cells and Matrix Mediate Bone Regeneration

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1. Introduction

Bone regeneration procedures aim at recapitulating optimal wound healing where tissue components are restored to the form and function required for tissue and organ homeostasis (Zohar &Tenenbaum 2005; Bueno &Glowacki 2009; Dimitriou et al. 2011). Examples of ideal bone regeneration include the healing of a healthy tooth extraction socket or a simple bone fracture. This is not the case in non-union fractures, or extensive damage as a result of tumour removal or bone subjected to chemotherapy, where the overall wound healing ability may be compromised (Dimitriou et al. 2011). Bone is a specialized connective tissue consisting of osteoblasts, osteocytes and osteoclasts embedded in a mineralized matrix capable of remodelling, renewing and load bearing. Optimal bone regenerative therapy will enhance mineralized tissue wound healing through enrichment of the wound/bone defect with a matrix scaffold to support the wound, cells that will give rise to osteoprogenitors and inducer molecules, such as growth factors to amplify activity of cells or events responsible for bone formation. New regenerative approaches may include a combination of these factors in part or as a whole. The temporal, spatial activity and maturation of these three components (i.e. cells, matrix and inducer molecules) during bone regeneration has to be a coordinated and integrative process. Delayed, reduced or lack of activity of any of these components may result in repair and not regeneration of a remodelling functional bone. Cell therapy is compared to the gold standard of autogenous bone marrow grafting, which is considered to be enriched with mesenchymal stem cells, osteoprogenitors and inducer molecules; marrow grafting usually offers predicative regenerative approach. Matrix grafting has to offer mechanical support for the regenerative process to interact with the differentiating osteoprogenitor cells and provide the conditions for the cells to deposit host bone matrix. Grafted inducer molecules need to interact with both the developed matrix and differentiating osteoprogenitors to assure bone matrix deposition and mineralization (Figure 1).

Our earlier studies focused on the isolation and differentiation of bone stem cells, osteoinductive cytokines and matrix development and maturation. The spatial and temporal sequence of matrix molecules expression used to sort stem-like cells population, single application of bone morphogenetic protein-7 (BMP7) induced differentiation of these cells to osteoblasts (Zohar et al. 1997a; Zohar et al. 1998; Zohar et al. 1997b). For bone cells to differentiate or for the bone matrix to mature and mineralize, cross talk between matrix and

cells is required to activate bone transcription factors associated with signaling pathways and osteogenic protein expression. Communication between matrix and osteoprogenitor cells is crucial to form a mature, weight-bearing bone. This communication is mediated through secreted growth factors, matrix or matrix associated molecules and activated receptors on the differentiating bone cells.

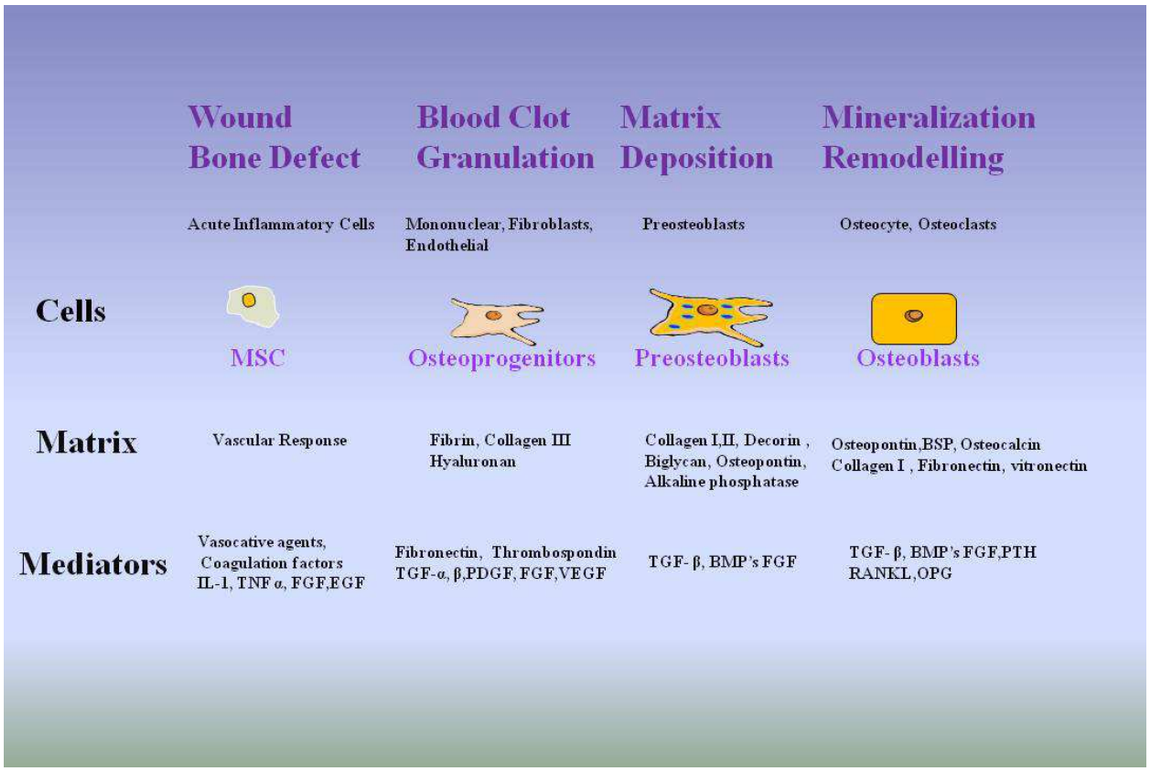

Fig. 1. Wound healing in bone regeneration follows a temporal sequence of ideal healing where a clean wound start healing through bleeding, clot formation and recruitment of mesenchymal stem cells which will differentiated to bone forming cells. The successful differentiation of mesenchymal stem cells to osteoblasts dependent of the temporal and spatial recruitment and expression of cells, matrix and bone related mediators. Matrix would form through adequate blood supply, stable clot formation and deposition of bone matrix that will mineralize. Osteoblasts and osteocytes will differentiate with matrix maturation and will secret mediators and bone specific proteins.

Various animal wound models in number of animal species are used to asses regenerative approaches include rodents, rabbits, sheep, goats, cats, dogs and primates (Gomes &Fernandes 2011; Intini et al. 2007; Kim et al. 2007; Artzi et. al 2003 a,b; Meinig 2002 ; Lemperle et al. 1998). New experimental approaches attempt to regenerate critical-size defects in the affected bone that won't heal without therapeutic intervention. Comparing results between animal models is challenging due to different wound models, different bones used, healing rate, unique animal physiology, whether or not the bone is weight bearing and a variety of protocols. Mice are the animals of choice for transgenic analyses for the significance of the permanent present or absence of one or two molecules (Kim et al.

2007; Masaki &Ide 2007). Large animal models on the other hand are preferred for a slower healing process resembling human physiology; however due to the high cost, control of animals and lower sample number, their use is more limited. The tibia or femur are usually used for the fracture model in a load-bearing area and the calvaria may be used for critical size bony defects in a non-loaded area (Alberius &Gordh 1996; Au et al. 2007; Landry et al. 1996).

Regenerative regimens usually focuses on one of the main components of the missing mineralized tissue: matrix, cells or inducer molecules. While expression patterns were identified for cell differentiation and matrix maturation, ongoing interactions during healing through receptors and signal molecules determines whether the outcome is repair or full regeneration. Thus, evaluating these interactions and the ability of the host wound area to support the process is a major determinant of regeneration. This chapter will focus on the importance of signaling between matrix and bone cells and how growth factors or inducer molecules can mediate this interaction and lead to the regeneration of bone tissue.

2. Bone wound healing

Bone wound healing in primates may involve formation of cartilaginous template, leading to endochondral ossification and/or intramembranous ossification (Dimitriou et al. 2011 ; Javed et al. 2011). Both processes require the commitment of adult stem cells toward bone-forming or osteoprogenitor cells (Figure 1). It is well recognized that adult bone contains a reservoir of mesenchymal stem cells responsible for physiological remodelling of bone and reconstruction during wound healing (Awad et al. 1999; Pittenger et al. 1999). Notably, mesenchymal stem cells are multipotential and capable of differentiation not only to bone forming cells but also to chondrocytes, adipocytes or fibroblasts, as shown in vitro and in vivo studies (Ghilzon et al. 1999; Owen 1988). Commitment of mesenchymal stem cells is thought to be irreversible, and thus signals during the early stages of the wound healing where mesenchymal stem cells differentiation to osteoprogenitors occurs is crucial for bone regenerative process. The ability to induce mesenchymal stem cells to express osteoblastic markers is dependent on transcription of bone-related genes activated by specific signalling, such as wingless-type MMTV integration site (Wnt) family which control osteoblasts differentiation (Hoeppner et al. 2009; Secreto et al. 2009). Important mediators in these pathways activated by Wnt will be the Runx2 (Cbfa1) and Osterix transcription factors. These proteins control expression and repression of genes that will direct the commitment of mesenchymal stem cells toward osteoblasts (Liu, W. et al. 2001). Runx2-deficient mice exhibit neonatal lethality due to absence of bone. In the absence of Runx2 there will be no osteoblast differentiation or ossification. Haploinsufficiency of Runx2 in humans results in cleidocranial dysplasia, a disease characterized by abnormal bone development, formation and decreased bone density (Notoya et al. 2004; Post et al. 2008; Xiao et al. 2004). Cytokines derived from the TGFβ superfamily, such as BMP-4, induce the expression of these transcription factors and thus bone-specific proteins such as alkaline phosphatase (AP), collagen I, bone sialoprotein (BSP), osteocalcin(OCN), osteopontin (OPN), integrin and TGFβ receptors. The expression of these markers serves to ascertain osteoblastic differentiation and evaluate the progression of bone formation. Unfortunately, at present, clear markers to identify and isolate mesenchymal stem cells or osteoprogenitors are not available and the lack of hematopoietic stem cells markers, as well as cellular morphological

characteristics, such as undeveloped cytoplasmic structure, are the only reliable criteria for osteoprogenitors (Belmokhtar et al. 2011; Bernardo et al. 2011; Vater et al. 2011).

Following differentiation of osteoprogenitor cells, a stage of the committed cells proliferation and growth cell cycle changes, accompanied by regulation of proliferation-related genes, such as histones, c-myc and c-fos being upregulated; secretion of matrix proteins, such as collagen I, II, III; alkaline phosphatase; fibronectin (Figure 1); as well as cytokines like FGF-2, TGF and BMBs members (Augello &De Bari 2010). Osteoprogenitors mature to secretory osteoblasts with a reduction in mitotic activity and formation of collagenous extracellular matrix (ECM) enriched with bone-specific proteins such as AP, OCN, BSP and OPN. Osteoblasts also secrete osteoprotegerin (OPG) a member of the TNF superfamily to reduce osteoclastic bone resorption by binding with the receptor activator of NF-kappaB ligand (RANKL) (Takahashi et al. 1999). Osteoblasts express receptors to mediate connections between ECM and cells; this connection is primarily mediated through integrins, which will attach to the ECM and intracellular will activate the actin cytoskeleton, initiating cellular signal transduction of proteins such as mitogen-activated protein kinase (MAP kinase) and the SMAD pathway (Blair et al. 2008; Komori 2011). Decrease in matrix formation precedes deposition of hydroxyapatite crystals in the mature collagen organized in a quarter-staggered pattern with 68nm gaps to house hydroxyapatite crystals, which accumulate on the collagen fibers within them and flattening of the active osteoblasts, which may undergo apoptosis or become trapped in the mineralized matrix as osteocytes (Kogianni &Noble 2007).

3. Bone regenerative therapy - Present approaches

There are multiple approaches and various grafting materials available for bone regenerative therapy. The noble regenerative objective is the same for all suggested approaches: living, functional, remodelling bone! Different studies evaluating the success of fracture regeneration or repair estimate the failure rate as 10% or more. Common factors in failure are: lack of vascularity, improper correction, delayed union, non-union and revision surgery (Jones et al. 2000; Lee et al. 2004; Osti et al. ; Parker et al. 2011; Smith, T. O. et al. 2009). The tibia is the most common bone to fracture in children and adults. Corrections that exhibit non-union complications present greater challenge to regenerative therapy (Garrison et al. 2011; Mashru et al. 2005). Other than fixation of fructure, there are also non-invasive approaches used to improve healing, such as electromagnetic field or ultrasound stimulations (Griffin et al. 2011). Distraction osteogenesis is another approach which encourages bone formation through gradual distraction of defect surfaces, requires long treatment, sensitive technique and prolonged healing for the patient; it also serves as a burden to the health system (Heo et al. 2008). Autologous bone marrow grafting is the most predictable approach to achieve regeneration. Bone can be harvested from the iliac crest of the pelvis, or alternatively, reamers can be used to harvest the intramedullary canal of long bones (Hak &Pittman 2010; Valimaki &Aro 2006). If a larger volume of grafts required, allograft or biomaterials are sometimes used in conjunction with autograft.

Present descriptors of grafting materials other than their source (i.e. allograft, autogenous, alloplast, cancellous, cortical), refer to grafts as being capable of osteoconduction, osteoinduction, mechanical support, cell exclusion, cement and filler. Regeneration of bone is a very clear outcome, and unless osteoblast differentiation taking place, new bone matrix

deposition and interaction between the two during de novo bone formation and remodelling, no real regeneration could occur. The assumption that placing an allograft that may contain BMP's, collagen matrix or an even high number of mesenchymal stem cells will result in regeneration in every case cannot be true. Without receptive wound environment where osteoprogenitors have signals for differentiation and deposition of new bone matrix, healing by fibrous or cartilage or adipose tissues may occur. Thus, using terminology like osteoconduction and osteoinduction would only suggest of the potential of regenerative approach or material, but it is not necessarily predictive of the desired outcome in specific host, specific wound, and specific surgical approach. The clinical results suggest variability of wound healing (Garrison et al. 2011).

Since most new bone graft or regenerative product is first tested for its biological activity, rather than focusing on osteoinduction and osteoconduction, this chapter classifies present grafts by their contribution to one of the major missing components of the missing bone: cells, matrix, and mediators (Figure 1). To evaluate the present state of bone regenerative therapy, it is worthwhile to see how each approach can contribute to the restoration of one of these three components.

4. Matrix grafting

Matrix serves as an organized framework for bone as a tissue and organ, offering mechanical support, and facilitate preservation of form and adaptive protection to internal organs through ongoing remodelling (Grabowski 2009; Scott et al. 2008). Osteogenic cells, like most other matrix-associated cells, cannot survive or differentiate without adhesion to their matrix (Popov et al. 2011). Thus, the importance of bone matrix in addition to acting as mechanical scaffold, is to mediate the biological activities of bone cells and signals that maintain homeostasis, remodelling and ability for wound healing. The mature mineralized bone matrix is composed of ~20% organic components, primarily collagens I, III and V and less than 5% noncollagenous proteins. The latter consists of proteoglycans, such as versican, decorin, and hyaluronan, adhesions molecules such as fibronectin and vitronectin, and specialized proteins like OCN, BSP, OPN and cytokines (Nagata et al. 1991). The collagen fibrils structure house the hydroxyapatite crystals which tend to be oriented in the same direction as the collagen fibrils. The collagen network also mediates adhesion to cells primarily through integrin receptors connected to collagen or the associated non-collagenous proteins. A mineralized bone matrix not only increases the mechanical strength of the bone but also act as reservoir for specialized proteins and cytokines, such as BMP's.

Matrix proteins mediate not only maturation and mineralization of bone matrix, but also bone cell differentiation and signalling. Bone cell differentiation is detected through the differential expression of matrix molecules such as collagen OPN, BSP, AP and OCN. Expression of AP, collagen I and OPN are considered an early markers, while BSP, OCN and a second peak of OPN are considered a late mineralization associated marker (Aronow et al. 1990; Binderman et al. 2011; Lynch, M. P. et al. 1995).Our studies of OPN expression, which is not restricted to bone, but can be used as a useful marker for early and late differentiation of osteogenic cells. We isolated a population of small cells that do not express OPN, AP, collagen I and that are enriched with stromal stem cells capable of generating bone, fat and cartilage (Zohar et al. 1998; Zohar et al. 1997b). We have isolated BMP-responsive cells, which will undergo chondrogenic differentiation with continuous

stimulation of BMP-7 or osteogenic differentiation with single dose (Zohar et al. 1998). Thus, evaluating the expression of matrix proteins can help determine the status of mesenchymal stem cells differentiation.

4.1 Matrix-based grafts can be autologous, allogeneic or biomaterials

Autologous bone grafts, such as the marrow graft, marrow aspirates, will contain cancellous and/or cortical or blocks such as vascularised Graft and will carry cells, matrix and potentially inductive molecules (Friedrich et al. 2009; Sotereanos et al. 1997). A vascularised graft will carry blood vessels to enrich the wound with nutrients and soluble mediators, which may support or inhibit bone formation and carry periosteum enriched with osteoprogenitor cells. There is less necrosis of grafted material during healing and vascularised grafts are thought to be a very reliable option for reconstructing non-union or osteonecrosis defects (Friedrich et al. 2009; Gaskill et al. 2009; Sotereanos et al. 1997). The difficulty with all autogenous grafts is the quantity and morbidity, such as non-stress fracture for donor sites (Friedrich et al. 2009). The cancellous or cortical block graft may carry cells and cytokines, and their quantity and effectiveness is related to the age and state of the donor area. Cortical block graft will contain the least amount of cells and mediators and considered to function primarily as scaffolding which is more susceptible to infection and necrosis.

In allogeneic bone matrix grafts, cadaver bone is a common source of allograft. To generate a safe allograft, the bone is subjected to irradiation or freeze-drying and is thus devoid of any cellular components (Nguyen et al. 2007). Allografts are prepared as particulate, morselized or block, with mineralized or demineralized bone particles that are easy to shape and mold. Demineralized bone matrix serves as a natural matrix as well as decellularized matrices that could derive from dura or intestine of various animals (Costain &Crawford 2009; Kligman et al. 2003; Mroz et al. 2006). Allografts have very limited, if any, biological activity and serve primarily as osteoconductive and mechanical support. The main advantage is ample supply (Hamer et al. 1996). Reports of infection transfer, matrix alteration during processing and limited remodelling of the grafted bone reduce the likelihood of full regeneration (Nguyen et al. 2007) unless combined with autologous bone (Matejovsky et al. 2006) to add osteoprogenitors and mediators that can append biological activity to the dead bone particles.

Matrix proteins-based polymers are very popular, as are collagen, fibrin, hyaluronic acid, fibronectin and BSP. These proteins are delivered as membranes, sponges, gels, demineralized bone particles, small intestinal submucosa, dura or even urinary bladder (Chajra et al. 2008; Smith, I. O. et al. 2009; von der Mark et al. 2010). The problem with generating these polymers is fairly low solubility; the organic purified polymers is costly and hard to extract, purify and stabilize; risk of immunogenicity; and variations based on the batch.

Biomaterials and synthetic bone substitutes are currently used as fillers and/or scaffolds for the missing bone structure (Gosain et al. 2009; Healy et al. 1999; Shekaran &Garcia 2010 ; Wojtowicz et al. 2010). The design and fabrication of matrix-based regenerative materials is aimed at restoring the natural bone matrix properties as a whole or in part. Reconstruction of missing bone using matrices involves the planning of macrostructures as well as

microstructure of the engineered matrix (Cholewa-Kowalska et al. 2009; Huang &Miao 2007; Vater et al. 2009). Macrostructures to fill and adapt to the space to assure sufficient quantity and/or provide mechanical support for the surrounding tissue or cells carried. Microstructures of micron or nanotechnology designs of particles or pores are used to encourage cell adhesion, colonization and absorption of proteins or required molecules. An ideal scaffold will have highly interconnected macroporosity to allow host bone tissue and blood vessels to grow into the scaffold (Healy et al. 1999). Popular building blocks are hydroxyapatite (HA), calcium phosphates (CP), tricalcium phosphate (TCP) and bioactive glasses (Behnamghader et al. 2008; Muschler et al. 1996; Valimaki &Aro 2006). They form a carbonated apatite layer when grafted, which is very similar to bone mineral; this will attract attachment of collagen fibres and eventually should be replaced by host tissue, mineralized matrix and cells. Other scaffolds consist of combinations of poly (lactic-co-glycolic acid) (PLGA), alginate and chitosan (Huang &Miao 2007; Jose et al. 2009; Liu, X. et al. 2009; Mishra et al. 2009; Renghini et al. 2009). These polymers can also be used to carry cytokines for controlled release at the wound and/or to carry mesenchymal stem cells. Different studies use different mixtures of these materials or different preparation protocols. The requirement for most preparations is to offer bioactivity and mechanical support. Bioactivity of the scaffold is measured by the number of host cells attached to its surface and interaction with the material to transform them into functional osteoblasts. The mineralized bone matrix will appose directly onto the surface of the material which ideally will have the ability to degrade over time (Holy et al. 2000). It is important that the material will degrade at a rate that allows the newly formed tissue to gradually replace the scaffold, both as a mechanical structure and in terms of space occupied. Finally, and this is where most current materials fail, the material needs mechanical properties that allow the device to be implanted without losing mechanical properties, still allowing sufficient loading of the newly formed tissue (Au et al. 2007; Smit et al. 2010). As of yet, no one has reported a material that fulfills all these requirements. The new scaffolds, usually termed composite scaffolds, maybe coated with proteins to increase cell adhesion, carry cells or cytokines with sustained release (Ameer et al. 2002; Bueno &Glowacki 2009; Gupta et al. 2011 ; Nie et al. 2008).

Bioactivity of biomaterial can be modified through chemical and physical alterations. Nanotechnology approaches try to mimic cell surface properties through approaches such as controlling space between ligands connected to biomaterials (Smith, I. O. et al. 2009). Using the proper spacing will enable, for example, integrin receptor clustering to enable propagation of signals through ligation. Another line of research focuses on molecules that work in synergy with receptors to promote cell adhesion and differentiation; for example, fibronectin, laminin and BSP contain heparan sulphate binding domains that interact with molecules on the cell surface in conjunction to integrin binding. Thus, using cell membrane molecule, such as syndecan which has three sites of heparan sulphate, would augment ligation of fibronectin or RGD sequence by integrin receptors (Whiteford et al. 2007; Yamada et al. 2010).

5. Cellular grafting

Cellular grafting for bone regeneration is a rapidly developing area. This approach had been used for many years through autologous bone grafting, which contains high numbers of

bone-committed cells in marrow aspirates, or in bone particles or blocks containing cells embedded in their own matrix (Hak &Pittman 2010; Papakostidis et al. 2008; Tiedeman et al. 1995). The objective of new approaches is to obtain an unlimited amount of adult stem cells, comparing new cellular sources to the gold standard of autogenous bone marrow stromal cell, which are considered to be enriched with osteoprogenitors. Notably, the frequency of osteoprogenitors in young rodent marrow is about 0.0005% (Falla et al. 1993) and up to 0.3% in fetal periosteal tissues. Adult marrow shows a reduction of these precursor cells in number and quality (Stolzing et al. 2008). We have used single cell flow cytometric sorting to isolate osteoprogenitors from fetal rodent periosteal tissues. These cells when plated and stimulated exhibit high proliferative capacity and enhanced osteogenic potential. Notably, these cells consisted of only a very small fraction of the fetal bone tissues. Thus, even in young fetal tissues osteoprogenitors consist of only a very small fraction of bone tissue and usually reside in a well-protected niche. Moreover, during seeding, grafting and transfer of cells to the wounded area there is loss of cells through apoptosis or cytotoxic effects of mediators in the wound area (Giannoni et al. 2009). Regeneration efforts focus on the ability to deliver mesenchymal stem cells to the wound, which will differentiate to the osteoblastic lineage. Differentiation requires the commitment of mesenchymal stem cells to osteoblasts, exhibiting bone-specific gene expression. Osteoblast-specific gene expression is a fairly clear analysis of proteins like AP, OPN, BSP, OCN that are selectively expressed in bone. For the mesenchymal stem cells to form new bone and regenerate the wound, cells need to attach, proliferate, differentiate and survive. Mesenchymal stem cells from marrow seem to be the most predictable source for osteoprogenitor cells and a safe autologous grafting. Unfortunately, bone marrow stromal cell consists of heterogenous population that are subject to age changes; not only does their number deplete, but also their quality and ability to generate new bone is reduced (Benayahu 2000; Stolzing et al. 2008; Zhou et al. 2008). Thus, in the aging population where bone wound healing is compromised, harvesting autologous sufficient number of mesenchymal stem cells from marrow may not be that predictable.

Other sources for bone forming cells could be the umbilical cord, peripheral blood, adipose tissue, dental pulp or periodontium (Goodwin et al. 2001; Honda et al. 2011; Rhee et al. 2010; Yamamoto et al. 2007). Human embryonic stem (hES) cells also being considered as an option due to their fast growth and the fact that these cells, if kept as undifferentiated cell lines, are pluripotential and capable of differentiating to many tissue types under the right conditions (Bahadur et al. 2011; Lerou &Daley 2005). The hES has the advantage of unlimited supply, minimal immune response and no need for a second surgical site (Watt &Hogan 2000). Ethical dilemmas, as well as work needed to control their growth in the targeted tissue, seem to be the main concerns limiting their use. Animal experimentations results are inconsistent and complexed by grafted cell death, formation of teratomas and tumours have been observed (Blum &Benvenisty 2008; Brederlau et al. 2006).

Autologous mesenchymal stem cells derived from bone marrow is still the preferred cellular source and iliac crest harvesting is the most common source. The simple approach could be through bone marrow aspirates or the harvest of cancellous bone enriched in osteoprogenitors. These cells can sometimes go through in vitro expansion before being loaded onto a scaffold or other carrier (Bernardo et al. 2011; Caplan &Correa 2011; Kuo et al.

2011). Gene therapy for insertion or activation of selected genes through transfection or electroporation is often attempted on mesenchymal stem cells (Stender et al. 2007). Due to the morbidity associated with marrow mesenchymal stem cells harvesting, need for a second surgical site, limited amounts of grafting material and lack of mechanical stability in extensive defects composites of mesenchymal stem cells with non-autologous grafting materials are frequently used (Caplan et al. 1997; Dimitriou et al. 2011).

The question is if delivery of bone marrow stromal cell containing stem cells to different wounds will assure a predictable and consistent outcome. Mesenchymal stem cells differentiation, proliferation and survival is dependent on their surrounding matrix, signals to express receptors and secrete signaling molecules. Large size defects with a potentially compromised host may offer a local environment that is not supportive or even inhibitory for bone formation. For example, it has been shown that disruption of integrin activity in mesenchymal stem cells will result in cell death and lack of differentiation (Popov et al. 2011). Various combinations have been prepared in an attempt to find a predictable and consistent graft (Schofer et al. 2011). Notably, at present, even if the number of mesenchymal stem cells is high, without the right matrix and cytokine's support bone differentiation and maturation may not occur.

6. Inducer molecules

The ability of demineralized bone matrix to induce bone formation in the subcutaneous sites of rodents, as reported by Dr. Urist, revolutionized our approach to bone therapy and studies of bone regeneration (Urist 1965; Urist et al. 1967). These studies demonstrated that the non-mineralized fraction of the bone stores molecules that can derive osteogenic differentiation and initiate bone formation in ectopic sites. Factors such as BMP's consist of only a very small fraction of the bone matrix and cannot be purified from bone for scientific or clinical use; however, these factors were cloned and prepared as recombinant molecules or peptides with very potent biological activity (Reddi &Cunningham 1993; Sampath et al. 1992). Inducer molecules can be delivered in a carrier or integrated into expression vehicles through ex vivo transfer to grafted cells, or infected through viruses that will target the tissues; these approaches fall under the category of gene therapy (Table 1)(Franceschi et al. 2000; Mason et al. 1998). Transient transfection and conditional expression approaches achieved in mice and other animals, thorough adeno and lentiviral, as well as non-viral, approaches such as electroporation (Franceschi et al. 2000; Holstein et al. 2009; Kawai et al. 2006). Gene delivery approaches being used in an ex vivo and in vivo gene delivery can also be utilized in humans to deliver genes to marrow stromal cells (Belmokhtar et al. 2011; Chen et al. 2011). Expression control modifications at embryo through transgenic animals or conditional modifications which, dependent on the initiator or temporary gene alteration in adult animals, assist in determining the relative importance of cell, matrix or inducer molecules to mineralized tissue healing. Gene therapy is still not available for regular clinical use, due to inability to assure target of specific cells only and adequate control over the gene transfer transcription, translation and expression in a temporal and spatial manner that will support bone regeneration. Other issues limiting clinical use are concern of viral vectors, control on the expression, immune response and potential for other non-controlled mutations. Moreover, The applications of gene transfer and control in human is not always

as efficient or predictable as shown in rodents or primates in animal experiment models (Gomes &Fernandes 2011; Sharma et al. 2011).

Matrix grafting	Cellular grafting	Inducer molecules	Techniques
Vascularised Graft	mesenchymal stem cells -bone marrow aspirate	(bone morphogenetic proteins (BMPs	Gene therapy-transfection, Transduction
Matrix molecules-Collagen, fibrin, hyaluronic acid, BSP,OPN	Cancellous graft-iliac, distal femur, proximal or distal tibia	platelet-derived growth factor-PDGF, Fibroblast growth factor, Vascular Endothelial growth factor,	Recombinant proteins
Mineral-Hydroxyapatite, β-Tricalcium phosphate(TCP),	Other sources of adult stem cells-peripheral blood adipose	Transforming growth factor's	Peptides
Polymers- poly (lactic-co-glycolic acid) (PLGA), alginate and chitosan	ES-Embryonic stem cells	insulin-like growth factor-I,II	Nanotechnology
Calcium phosphate or sulphate, glass ceramics	Umbilical cord	endothelial growth factor	Cellular in-vitro expansion, differentiation induction,
DBM- Demineralised bone matrix	Dental – follicle, pulp, periodontal	Hormones-parathyroid hormone, Growth Hormone	Scaffolds- Three-dimensional porous scaffolds, coated, biodegradable
cancellous bone allograft		Pepetides- FHRRIKA, FNIII 7-10,P15, DGEA (Asp-Gly-Glu-Ala), RGD, PTH 1-34, and PTH 1-84	Morcellized bone grafting, freeze-drying
Cortical		Denosumab-antibody to RANKL	Purified proteins Membranes, Mesh
Block graft		agonists of the prostaglandin receptors EP2 and EP4	Distraction osteogenesis

Table 1. Classification of Grafting

At present, about 20 BMPs have been identified, with about eight having osteogenic effects: BMP-2, 3, 4, 6, 7, 8, 12, 14. BMP-7 or OP-1 is the subject of many studies approved for clinical use and exhibits a very potent osteoinductive effect in vivo and in vitro. BMP-7 effects on mesenchymal stem cells include increased migration, differentiation and induction of bone formation through endochondral as well as intramembranous ossification (Giannoudis et al. 2009). Many other cytokines are the subject of ongoing investigations and use such as platelet-derived growth factor (PDGF), transforming growth factor-β (TGF β), insulin-like growth factor-I and II (IGF), vascular endothelial growth factor (VEGF), endothelial growth factor (EGF), parathyroid hormone (PTH), growth hormone (GH) and fibroblast growth factor (FGF). Some are prepared as synthetic peptides where only the active sequence is synthesized; often the peptide will be more potent that the whole molecule. Examples of these peptides include PTH [PTH(1-34); Forteo (or teriparitide) and PTH 1-84, P24 is a 24-amino acid peptide derived from BMP2 capable of induction of ectopic bone (Lin et al. 2010; Wu et al. 2008). The growth factors that are approved for clinical use in human and received the Food and Drug Administration (FDA) approval for bone regeneration are BMP-2, 7 and PDGF-BB (Caplan &Correa 2011; Kanakaris et al. 2008; Lynch, S. E. et al. 2006; Mulconrey et al. 2008). These growth factors will predictably stimulate bone formation, and when compared to the gold standard of autologous bone grafting, these growth factors meet the expectations of inducing bone regeneration in a high percentage of the clinical cases (Garrison et al. 2011). Advantages include ample supply, convenient grafting carriers, osteoinduction, no need for a second surgical site and no significant immune responses. The reported concerns are no cellular component, no osteoconduction support, lower mechanical strength of the newly formed bone, expensive and variability in induction. These growth factors are carried or released by various materials that may alter their effects and potency (Nauth et al. 2011). It is beyond the scope of this chapter to describe the molecular mechanism known for each of these growth factors or the expression of their receptors and associated signaling pathways. Each of these growth factors is a subject of numerous clinical trials and reports and suggestions on its most potent use for bone regeneration. Their effects are dependent on the availability of cells, the expression of the appropriate receptors and biological half-life at the bone defect.

Bone formation can also be induced by non-growth factor molecules, such as matrix components or proteins that will encourage mesenchymal stem cells cell adhesion, migration, proliferation, differentiation and survival (Popov et al. 2011). Matrix components like collagen will not only induce bone cells directly but also their ability to bind other potent molecules, such as growth factors, thrombospondin, decorin, biglycan, OPN, OCN, BSP, fibronectin, vitronectin and hydroxyapatite (Bentley &Tralka 1983; Ber et al. 1991; Bergmann et al. 1990). Control of expression of receptors to mediate bone matrix adhesion would be another approach, through antibodies or fragments that will induce their expression; for example, the Denosumab human monoclonal antibody that inhibits osteoclastic activity through binding to RANKL and safe even for systemic use (Miller 2009).

Matrix proteins can be used as purified proteins or synthetic peptides. Purified collagen is one source of a primary matrix molecule derived from human, bovine or porcine sources as purified fibrillar collagen or composite with other minerals that can be use to fill

defects or mixed with other grafts (Gleeson et al. 2011; Muschler et al. 1996; Thula et al. 2011). Proteoglycans, such as hyaluronan, can be purified from the human umbilical cord, cultures of cells or bacteria. The non-collagenous proteins of the bone, such as OPN, BSP and OCN, can also be purified and used to coat biomaterials, or mixed with grafting materials. The use of synthetic peptides as a whole molecule or just active sequence is a more accurate approach, as it may may be missing post-translational modifications found on the native purified protein. It would be a cleaner and safer product as far as immune reactions or carrying impurities for clinical use. Recombinant molecules and synthetic peptide technologies are becoming more popular as well as more accurate, pure and have reduced variability in mediating osteogenic cell adhesion and bone formation. RGD (arginine - glycine - aspartate) is a well-characterized sequence in number of matrix proteins including fibronectin, OPN, BSP and vitronectin that mediate attachment of osteogenic cells to integrin receptors (Hsiong et al. 2009; Pallu et al. 2009). RGD will usually ligate αVβ3-integrin, but also αvβ1, α8β1, αvβ8, αvβ6, αvβ5, and αIIbβ3. RGD being synthesised as linear as well as cyclic peptide as some studies also suggest that the cyclic form may offer better presentation that is more potent in inducing osteoblastic differentiation (Hsiong et al. 2009). Collagen I adheres to bone cells via α2β1 integrin receptor (Mizuno et al. 2000) through DGEA (Asp-Gly-Glu-Ala) motif. Its recognition sequences and competition for this association with DGEA peptide could inhibit osteoblastic differentiation (Takeuchi et al. 1996). Fibronectin fragments FNIII 7-10, α5β1 integrin specific enhanced osteoblastic differentiation in bone marrow stromal cells and can upregulate adherence to titanium implants (Petrie et al. 2008). P15 is a 15-amino acid sequence derived from Collagen I, α1 chain and in clinical use (Gomar et al. 2007; Pettinicchio et al.). P15 enhances osteoblastic cell adhesion and differentiation to osteoblasts. Other peptides will be FHRRIKA, derived from the heparin binding site of BSP, human vitronectin peptide HVP (351-359) and osteopontin-derived peptides (Healy et al. 1999; von der Mark et al. 2010).

Most of these peptides and growth factors show great promise in in vitro studies and great potential in human trials and therapy (Bosetti et al. 2007; Nauth et al. 2011; Rose et al. 2004). Unfortunately, the animal and human analyses seem to exhibit wide variability (Faour et al. 2011; Giannoudis &Dinopoulos 2010; Papakostidis et al. 2008; Shekaran &Garcia 2011). An important factor in the application of these peptides and growth factors is the delivery system, as are the biochemical properties of the surrounding matrix and accessibility of the cells and the relevant receptors for their signaling.

The nature of the biomaterial, the surface to be coated or the carrying polymer, scaffold or gel will have an impact on the availability of the inducer or the ligand used to attach the differentiating bone cells. A common problem will be the hydrophobic surfaces of biomaterials, which will be covered by plasma and absorb abundant proteins such as albumin. This will make any ligand attached to the biomaterial less accessible, while more hydrophilic surfaces, such as culture dishes coated with ECM proteins, will encourage cell adhesions. Nanotechnology used to space ligands, such as RGD, affects cells adhesion, clustering and increases affinity between ligand and the receptors through both chemical and physical modifications. These approaches will enable osteoprogenitors to differentiate and migrate in the desired direction (Hirschfeld-Warneken et al. 2008). Designs aimed at

creating the right topography of the biomaterial, as well as chemical alteration of serine residues or energy molecules such as purines that will change the availability of the inducer, will have impact on the ability of osteoprogenitors to differentiate (Costa et al. 2011; Mager et al. 2011; Vater et al. 2009).

7. Concluding remarks

The number of bone regeneration tools is growing every day, some but not all of which are listed above (Table 1). Unfortunately, there is no single tool available that can predictably match the gold standard of autologous marrow bone grafting. To restore the missing bone matrix, cells and inducer molecules need to act in a synergistic manner. Indeed, the new regenerative approaches are based on composite grafting, including matrix replacement, mesenchymal stem cells and inducer molecules. Most composites grafts focus on merging osteoconductive scaffolds with osteoinductive agents, such as BMP, or with cells (Bueno &Glowacki 2009; Lin et al.2010). Nanotechnology improves matrix characteristics for cell adherence, survival and differentiation, delivery vehicle for cell, proteins or gene carriers also improve macro mechanical properties (Shekaran &Garcia 2010; Smith, I. O. et al. 2009; Zhang et al. 2007). The research of forming a scaffold with organic and non-organic parts, which is mechanically strong, bioresorbable, carries inducer molecules and cells, and will adhere to the newly forming bone and still be affordable, is challenging. These are hard objectives to achieve. At present, a composite graft that can match the success of autologous marrow bone grafting does not exist.

The question is whether our quest for an ideal composite graft that will fit and regenerate most, if not all, bone wounds in every host is a realistic one. This chapter classified the three main components needed to restore missing bone tissue and outlined some of the tools and techniques(Figure 1). It is unlikely that composite grafts will be successful as autogenous grafting without having individual “custom made composite graft”. We can mix autogenous marrow aspirates with the scaffold, but still most of the grafted components will not derive from the host. Host factor variables should dictate our regenerative approach for supplementing either matrix, cellular and inductive molecules at the right composition to increase our success. Bony defects are rarely uniform and healing patterns may vary, especially in human subjects. Other than local factors, host factors such as age, medications and chronic conditions may impact wound healing in general. Our future ability to design and adapt our regenerative tools may aid in boosting critical wound healing factors required in a compromised site or individual.

A different approach is suggested, in which the clinical team will be able to identify the difficulties associated with particular wounds, such as size, mechanics, blood supply and whether or not the bone is load bearing. Host factors to be considered include age, medications and other systemic conditions that may compromise wound healing. Based on these analyses of the available tools (Table 1), a list will be presented to the lab with physical, chemical and inductive requirements. An individual composite graft will be constructed for the wound that will meet and boost the particular requirements of the specific wound. With advancement of clinical diagnosis and scientific and biotechnological tools, this approach may be more predictable in achieving bone regeneration.

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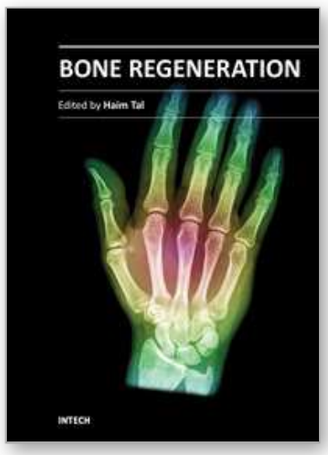

Bone Regeneration
Edited by Prof. Haim Tal

ISBN 978-953-51-0487-2
Hard cover, 340 pages
Publisher InTech
Published online 04, April, 2012
Published in print edition April, 2012

Bone is a specialized connective tissue, most prominently characterized by its mineralized organic matrix that imparts the physical properties that allow bone tissue to resist load, to support functional organs, and to protect highly sensitive body parts. Bone loss and bone damage may occur as a result of genetic conditions, infectious diseases, tumours, and trauma. Bone healing and repair, involves integrative activity of native tissues and living cells, and lends itself to the incorporation of naturally derived or biocompatible synthetic scaffolds, aimed at replacing missing or damaged osseous tissues. There are several modalities of bone regeneration including tissue engineering, guided bone regeneration, distraction ontogenesis, and bone grafting. This book concentrates on such procedures that may well be counted among the recent outstanding breakthroughs in bone regenerative therapy.

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