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Interactions between bone cells and biomaterials: An update

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1. ABSTRACT

As the populations of the Western world become older, they will suffer more and more from bone defects related to osteoporosis (non-union fractures, vertebral damages), cancers (malignant osteolysis) and infections (osteomyelitis). Autografts are usually used to fill these defects, but they have several drawbacks such as morbidity at the donor site and the amount and quality of bone that can be harvested. Recent scientific milestones made in biomaterials development were shown to be promising to overcome these limitations. Cell interactions with biomaterials can be improved by adding at their surface functional groups such as adhesive peptides and/or growth factors. The development of such biomimetic materials able to control bone cell responses can only proceed if it is based on a sound understanding of bone cell behavior and regulation. This review focuses on bone physiology and the regulation of bone cell differentiation and function, and how the latest advances in biomimetic materials can be translated within promising clinical outcomes.

2. INTRODUCTION

People over 65 years old make up the most rapidly growing Canadian population (1). Five million Canadians were over 65 years old in 2011, and this

number is estimated to double by 2036, so that they will account for a quarter of the population (2,3). The World Health Organization (WHO) estimates that the worldwide population aged over 60 will reach 2 billion by 2050 (4). These older people are most at risk of bone disorders like secondary metastasis causing bone cancer or osteoporotic fractures which are leading causes of pain and disability (5). Osteoporosis Canada reports that over 80 percent of the fractures sustained by people over 50 years old are caused by osteoporosis (6). Major bone loss associated with osteoporosis (particularly in vertebral fractures or non-union fractures) can be difficult to repair, so becoming a significant financial burden. The annual cost to Canadians of treating osteoporosis and the resulting fractures could reach 30 billion dollars by 2018 (7).

Bone is the basic support of locomotion system, where muscles, ligaments and tendons are attached to it. It also provides mechanical support and protects vital organs. It contains bone marrow, the main site of hematopoiesis and an important reservoir of minerals (8,9). Osteointegrative, osteoconductive and osteoinductive biocompatible bone substitutes should, ideally, be used to heal fractures and bridge bone losses (8). Osteointegration requires extensive interaction between the bone substitute and the recipient's bone site. Osteoconduction can only occur if the bone substitute is readily colonized by bone cells and blood vessels, while osteoinductive substitute must be able to stimulate host's mesenchymal stem cells (MSCs) from surrounding tissues to differentiate into bone-forming cells (10).

Biological graft such as autografts, the gold standard used by surgeons, and allografts are the most commonly used (10). Autografts use patient's own living bone tissue, while allografts use cadaveric bone. Autografts provide an osteoconductive scaffold, osteogenic cells and osteoinductive growth factors, while allografts have a limited range of these properties (10). However, the size of an autograft is limited by the amount of bone that can be harvested, and 8.5 to 20 percent of cases suffer from postoperative complications like nerve injury, infections, blood loss, morbidity and chronic pain at the donor and/or recipient's sites (10,11). Most grafts are harvested from iliac crests so as to limit structural modification, but their size may vary considerably (5 to 70 cm^2) (12,13). Larger grafts can be taken from the tibia or femur, providing generally greater quantities of osteogenic growth factors such as bone morphogenetic proteins (BMPs) (13). These donor sites do not provide horseshoe shaped grafts consisting of cortical bone (tricortical graft) that give greater structural support and mechanical resistance, unlike the iliac crest. In contrast to autograft bone, allograft is frozen in liquid nitrogen and undergoes a serie of treatments such as purification ($NaHCO_3$, H_2O_2, NaOH), conditioning and sterilization. These treatments have a considerable effect on the biological and mechanical properties of bone tissue (14–16). With modern procurement and sterilization methods for bone tissue, the risk of infection transmission by allografts such as human immunodeficiency virus is estimated to be 1 in 2.8 billion (17,18).

Biomaterials with the same characteristics as bone grafts have to be developed to overcome these problems (limited graft size, morbidity and chronic pain) (19). The biomaterial may be inorganic, like hydroxyapatite (HAP) or titanium (Ti), natural, such as collagen or alginate, or synthetic polymer like polycaprolactone (PCL), polylactate, or even a composite material (8). Inorganic biomaterials like calcium phosphate ceramics have the same physical properties as bone mineral and induce a minimal immune response *in vivo* during implantation. However, the solubility of the calcium phosphate ceramics influences the activity of osteoclasts, the cells responsible for bone resorption (19,20). Other materials, including synthetic polymers, can be broken down over time and replaced by regenerated tissue in the long term (19). However, they interact poorly with bone cells, resulting in the development of third generation biomaterials, such as biomimetic materials (8,21,22).

Several strategies have been used to create biomimetic materials. One of them is to functionalize the biomaterial by adding extracellular matrix (ECM) proteins, recombinant growth factors and/or peptides derived from them in order to mimic bone as close as possible to its physiology (23–28). The therapeutic potential of these biomimetic materials depends on their capacity to control the behavior of MSCs (29). Biomimetic materials must first promote the adhesion of MSCs to their surface and favor the response of these cells to specific growth factors leading to their differentiation into bone-forming cells. The interactions between cell membrane receptor integrins and the proteins/peptides at the surface of the biomimetic material play a crucial role in this phenomenon (30,31).

In the present review, we first describe the principal components of bone and their roles in bone healing and remodeling. We then look at the adhesion of bone cells to ECM and biomaterials and the crucial role of cell-biomaterial interactions in the integration and repair of bone tissue. Finally, the roles of each of these elements will be set in the context of the latest advances in the field of biomimetic materials.

3. BONE CELLS AND THE EXTRACELLULAR MATRIX

3.1. Bone cells

Bone remodeling is a physiological process in which bone resorption is followed by the formation of new bone. The cells responsible for these interrelated processes include the bone-resorbing cells, i.e. osteoclasts, which are derived from hematopoietic

cells of the monocyte-macrophage lineage, and bone-forming cells, i.e. osteoblasts, which differentiate from bone marrow MSCs. Osteoblasts, osteoblast-derived osteocytes and osteoclasts are highly specialized bone cells (32).

Mature osteoblasts have a lifespan of about 3 months, and are protein-secreting cells with a well-developed rough endoplasmic reticulum and a large Golgi apparatus (33). They synthesize the collagen-based matrix, the osteoid, at a rate of 2 to 3 microns^3 per day and mineralize it 10 days after its deposition. As a result, osteoblasts become surrounded by mineralized tissue. Osteoblasts that cover the bone surface become inactive lining cells, or they die by apoptosis. Osteoblasts can control the differentiation of bone resorbing osteoclasts by secreting the receptor activator of NF-kappaB ligand (RANKL) and osteoprotegerin (OPG) (34).

Osteocytes make up 90 to 95 percent of all bone cells and have a half-life of about 25 years (35). They originate from osteoblasts once they have become enclosed within the mineralized tissue. They are interconnected together via adherent and gap junctions. The osteocytes are sensors of bone mechanical stimuli and the microdamage induced by cyclic loading. They may also respond to fluid flow induced by strain in the canaliculi (36). Osteocytes secrete major bone-regulating factors. They are the main source of RANKL in skeletal tissue, and also produce OPG and sclerostin, which influence the activity of other specialized bone cells (37). Bone microdamages may also cause osteocytes to enter apoptosis, which favors the release of chemotactic signals that target osteoclasts (38).

Osteoclasts are large multinucleated cells with diameter about 50 to 100 microns formed by the fusion of monocytes, which are mononuclear cells. Osteoclasts have a lifespan of about 2 weeks. They become activated when they attached to the bone matrix (33,39). Osteoclasts are highly motile and alternate between migratory and bone-resorbing stages, showing remarkable changes in their phenotype during these phases. When adhered to the bone, the osteoclasts become polarized and reorganize their cytoskeleton. A sealing zone is formed by densely packed actin-rich podosomes that delimit the ruffled border, a highly specialized area composed of membrane expansions directed toward the targeted bone surface. The ruffled border is formed by polarized vesicular trafficking and plays a critical role in the degradation of bone matrix through acidification by vacuolar H^+ ATPases and degrading enzymes released by the fusion of secretory lysosomal vesicles such as matrix metalloproteinases (MMP) and cathepsin K, or acid phosphatases such as tartrate resistant acid phosphatase (TRAP). These proteases can degrade the mineralized osteoid to form Howship lacunae 40 to 60 microns deep (40). Osteoclasts also transport vesicles from basal to the apical cell membrane by transcytosis that contain calcium and phosphate ions and hydrolyzed osteoid proteins released during bone resorption (41). In non-resorptive or migrating osteoclasts, the sealing zone switches to a podosome belt, and relaxed osteoclasts are depolarized (42).

3.2. Osteoid and mineral phase

Osteoid accounts for 20 to 25 percent of the bone mass. It is made up of over 90 percent of type I collagen (43,44). Collagen fibrils (15 to 500 nm in diameter) are stabilized by intramolecular and intermolecular crosslinks formed by covalent, electrostatic and hydrogen bonds (45). Collagen fibrils can co-assemble to form collagen fibers of about 10 microns in diameter (45). The structure and the organization of collagens influence the mechanical properties of bone, such as its ductility and fracture resistance (46).

Osteoid matrix also contains around 5 percent of non-collagenous proteins. These are proteins like the small integrin-binding ligand N-linked glycoproteins (SIBLING), such as osteopontin (OPN), matrix extracellular phosphoglycoprotein (MEPE) and bone sialoprotein (BSP) (47). SIBLING proteins undergo extensive post-translational modifications, including N/O- linked glycosylation, sulfation and/or phosphorylation, that influence their function (for review see (48)). They are important in the regulation of bone cell function and matrix mineralization, acting via their RGD sites and their acidic serine-rich and aspartate-rich motif (ASARM) (48–51). Holm *et al.* recently found that the trabecular bones of BSP$^{-/-}$ knockout mice were less well mineralized than those of their wild type controls (52).

Adhesive proteins like fibronectin are also crucial for bone: they interact with cells via integrins to regulate their activity (53,54). Schwab *et al.* recently reported that fibronectin was better than vitronectin for the adherence of human bone marrow MSCs and their differentiation into osteoblasts (53).

Osteoid also contains the small leucine-rich proteoglycans (SLRPs), biglycan and decorin, whose central protein cores are linked by glycosaminoglycans such as chondroitin sulfate (55). Ingram *et al.* showed that biglycan was present in both cortical and trabecular bone, while decorin was mainly located in the canaliculi of osteocytes and in the matrix near the Haversian canals (55). The collagen fibrils are abnormal in decorin$^{-/-}$ and biglycan$^{-/-}$ double-knockout mice (56). SLRPs can regulate the hydrostatic and osmotic pressures as well as the transport of nutrients and growth factors (57). Chen *et al.* used neonatal murine calvarial cells extracted from biglycan$^{-/-}$ knockout mice and adenovirus encoding biglycan to show that this proteoglycan was required for the osteogenic differentiation of calvarial cells induced by BMP-4 (58).

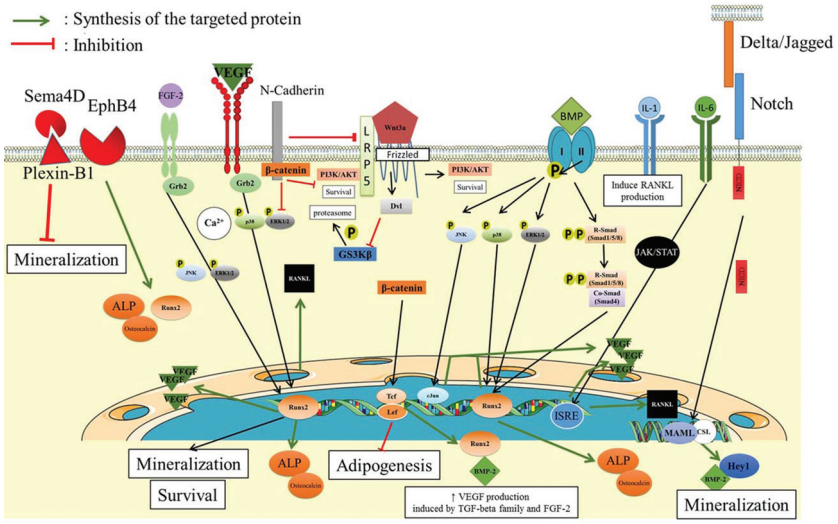

Figure 1. Signaling pathways regulating the differentiation of MSCs into osteoblasts (ALP, alkaline phosphatase; CSL, CBF1 Suppressor of Hairless Lag-1; EphB4, Ephrin type-B receptor 4; Hey, Hes-related with the YRPW motif; MAML, Mastermind-like; NICD, Notch intracellular domain; PI3K, phosphatidyl inositol 3 kinase; Sema4D, semaphorin 4D; VEGF, vascular endothelial growth factor (71–82). [Illustration using Servier Medical Art, http://www.servier.fr].

SLRPs also play a key role in regulating the mechanical properties of bone, especially its poroelasticity (57).

The mineral part of bone is almost entirely made of HAP crystals, constituted of calcium and phosphate ions. It accounts roughly for 65 percent of the bone mass (59). It also contains other ions - fluoride, manganese and magnesium - with some carbonate substitution. Mineralization of the osteoid begins with the nucleation of calcium phosphate followed by crystal growth (60). Nucleation can develop from supersaturated concentrations of calcium and phosphate ions. It occurs in specific vesicles that bud off from the membranes of hypertrophic chondrocytes and osteoblasts and in the interstitial space through the action of specific SIBLING proteins (60). The apatite crystals first form in the gaps between the collagen molecules. Several recent *in vitro* studies have suggested that collagen itself has specific sites rich in charged amino acid that favor crystal nucleation (61–63). Wang *et al.* found that collagen could sequester enough calcium, phosphate and carbonate ions to favor their spontaneous transformation into apatite crystals (64). However, there is still some debate about the results of these studies because most of them used tendon or isolated collagen fibrils as models, which lack the SIBLING proteins.

4. BONE CELL DIFFERENTIATION AND BONE HEALING

4.1. Signaling pathways contributing to osteogenic differentiation of mesenchymal stem cells

The use of MSCs in tissue engineering is a promising strategy to enhance bone healing and regeneration (65). However, fundamental understanding of the osteoblastic commitment capacity of implanted MSCs and its regulation will be critical (65). The differentiation of MSCs into osteoblasts, chondrocytes, and adipocytes is regulated by growth factors, cytokines, hormones and vitamins (Figure 1) (66).

Many growth factors, including fibroblast growth factor (FGF), insulin-like growth factor (IGF), the transforming growth factor-beta family (TGF-beta) and the platelet-derived growth factor (PDGF), are involved in the differentiation of MSCs into osteoblasts (67–70). For example, Baker *et al.* found that bone ossification was abnormally slow in IGF-1$^{-/-}$ mice (68).

FGF and IGF bind to receptors belonging to the tyrosine kinase receptor family, FGF receptor (FGFR) for FGF and IGF type I receptor (IGFIR) for IGF. The receptors bearing their growth factors form dimers that are activated by trans-phosphorylation of their tyrosine residues. These receptors then recruit intracellular adaptor proteins such as growth factor receptor-bound protein 2 (Grb2) and (Src homology 2 domain-containing)-transforming protein (Shc). The son of sevenless (Sos) is then recruited and the extracellular signal regulated kinase 1/2 (ERK1/2) mitogen activated protein kinase (MAPK) cascade is activated. This cascade can trigger the differentiation of MSCs into osteoblasts (83,84).

The BMPs also play a crucial part in bone tissue formation. More than 20 BMPs have been identified to date (23,29). These molecules are synthesized by the MSCs and osteoblasts and are members of the TGF-beta family (85,86). Marshall Urist showed that implanting

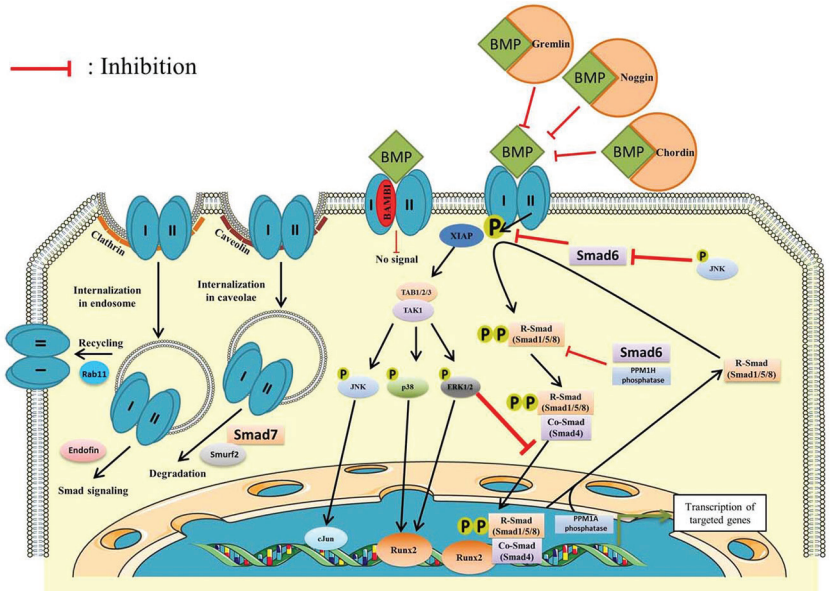

Figure 2. Regulation of BMP-induced signaling (93,94,97,100,101,104,106,109–112). [Illustration using Servier Medical Art, http://www.servier.fr].

demineralized bone in a muscle led to *de novo* bone formation. He also discovered that BMPs gave the organic bone matrix its osteoinductive properties (87). Each kg of demineralized bone matrix contains 1 – 2 microg BMPs (88–90). The osteogenic potential of BMPs has been verified *in vivo* by injecting C2C12 cells transformed with adenovirus encoding BMPs (AdBMP) into mouse quadriceps muscles (91). Kang *et al.* found that AdBMP-6 or AdBMP-9 triggered ossification most rapidly and efficiently, followed by AdBMP-2 and AdBMP-7(91). Like other members of the TGF-beta family, BMPs act on cells by inducing two type I and two type II serine/threonine kinase receptors to form a heterotetrameric complex. A total of 7 type I receptors and 5 type II receptors have been identified to date. They can bind over 30 TGF-beta family ligands and all have similar structures (92). For example, the kinase domains of the BMP type I receptors (BMPR) BMPR-IA and BMPR-IB share 85 percent amino acids homology (92).

There are two pathways involved in BMP signaling, the canonical Smad pathway and a pathway involving TGF-beta activated tyrosine kinase 1 (TAK1) and MAPK (93). In the canonical Smad pathway, after BMPs binding to receptors, the type I receptor is phosphorylated by the type II receptor, which in turn leads to the phosphorylation of Smad1/5/8 (94). The phosphorylated Smad1/5/8 then form a complex with Smad4. This complex is translocated to the nucleus, where it activates the transcription of osteogenic genes like Runx2, osterix (Osx) and osteocalcin (OC) (94–96). Liu *et al.* reported that small interfering RNA (siRNA) against Smad1 reduced the amount of ALP mRNA induced by BMP-2 in MC3T3-E1 preosteoblasts and inhibited matrix mineralization (97).

The canonical Smad pathway is regulated at many levels (Figure 2). The number of available BMP receptors at the cell surface can be modulated by endocytosis. Extracellular regulation occurs when antagonists such as Noggin, Chordin and Gremlin bind to BMPs and inhibit their interaction with their receptors (98–100). The Smad pathway is also regulated by the transmembrane pseudoreceptor BMP and activin membrane-bound inhibitor (BAMBI), which interacts with BMPRI to prevent the transduction of the signal (101). The inhibitory Smads (I-Smad), Smad6 and Smad7, are intracellular regulators of the Smad pathway. They bind to the intracellular domain of type I receptors to form a stable complex that prevents the activation of Smad1/5/8 (102,103). Other intracellular regulators of the Smad pathway are the phosphatases. Protein phosphatase, Mg^{2+}/Mn^{2+} dependent, 1A (PPM1A) dephosphorylates Smad1 and inhibits its BMP-2-induced transcriptional activity (104).

The interaction of a BMP with its receptors can also activate the MAPK signaling pathway (Figure 2). The MAPK pathway is divided into 3 cascades: ERK1/2, p38 and c-jun N-terminal kinase (JNK). The BMPs facilitate the recruitment of a MAPKKK, TAK1, to the type I receptor and then the activation of the 3 MAPK cascades (93). The mechanism by which TAK1 is activated by type I receptors is still unknown. Perhaps the X-linked inhibitor of apoptosis (XIAP) mediates the signal transduction between BMP receptor and TAK1 (105). The phosphorylated ERK1/2, p38 and JNK are then translocated to the nucleus, where they interact with the factors controlling the transcription of specific genes (106). ERK1/2, p38 and JNK can have either

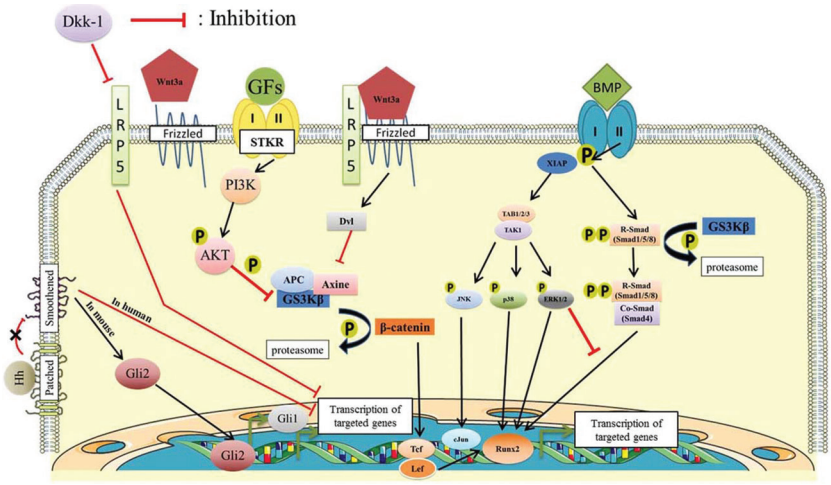

Figure 3. Crosstalk between Wnt and growth factors that regulate osteoblast behavior (APC, adenomatous polyposis coli; Dkk-1, Dickkopf-1; GFs, growth factors; STKR, serine/threonine kinase receptor) (93,94,106,109,119,122–127). [Illustration using Servier Medical Art, http://www.servier.fr].

positive or negative effect on osteoblastic differentiation. Xu *et al.* showed that inhibiting p38 decreased the synthesis of ALP by C3H10T1/2 cells infected with AdBMP-9 (107). Similarly, Lauzon *et al.* found that the inhibition of either JNK or ERK1/2 increased ALP activity in MC3T3-E1 preosteoblasts stimulated with BMP-9 or BMP-2 in the presence of fetal bovine serum (108). Others have found that ERK1/2 regulates the Smad canonical pathway by phosphorylating Smad1 at the linker region, so preventing the complex formed by Smad1 and Smad4 from being translocated to the nucleus (109).

BMPs and other growth factors can act in synergy to trigger the differentiation of MSCs or bone cells. Lauzon *et al.* showed that IGF-2 increased the BMP-9-induced ALP activity in MC3T3-E1 cells (108). FGF2 (or bFGF) is also involved in osteogenic differentiation through its action on the concentration of BMP-2. The concentration of BMP-2 in $FGF2^{-/-}$ mice is drastically decreased leading to reduced bone formation (113). Epidermal growth factor (EGF) also enhances the ALP activity in immortalized mouse embryonic fibroblasts that have been infected with AdBMP-9 (114).

The canonical Wnt/beta-catenin signaling pathways, Notch and Hedgehog (Hh) are also crucial for the differentiation of MSCs into osteoblastic lineage (Figure 3) (115,116). The Wnt/beta-catenin pathway is important for determining the fate of stem cells; it not only favors osteogenic differentiation, it also inhibits adipogenic differentiation (117). Canonical Wnt agonists act on cells by binding to its receptor Frizzled (Fzd) and its co-receptors low density lipoprotein receptor related protein 5 and 6 (LRP5/6). This leads to the recruitment of the Dishevelled (Dvl) protein, which inhibits the phosphorylation of the beta-catenin by glycogen synthase kinase-3 beta (GSK-3 beta) and its subsequent ubiquitination and degradation by proteasomes. The unphosphorylated beta-catenin can then translocate to the nucleus and interact with the transcription factor T-cell factor/lymphoid enhancer factor (TCF/LEF). This complex enables the transcriptional activity of genes encoding proteins like Runx2 and BMP-2 (118–120). Tang *et al.* showed that C3H10T1/2 cells infected with AdWnt-3a increased ALP activity and enhanced the ALP activity induced by AdBMP-9 (121).

Notch is a transmembrane receptor that interacts with the ligands Delta or Jagged present on the surface of neighboring cells. Notch intracellular domain (NICD) is then cleaved by gamma-secretase and moves to the nucleus, where it binds to transcription factors like CBF1, suppressor of hairless (CSL) and the co-activator Mastermind-like (MAML) to stimulate the transcription of genes encoding Hairy enhancer of split (Hes) and Hey (124). Ugarte *et al.* showed that activating the Notch pathway in human MSCs by causing them to overproduce Jagged1 or NICD, induced mineralization and increased their ALP activity and BMP-2 expression, while inhibiting their differentiation into adipocytes (128). However, activating the Notch pathway does not stimulate OC synthesis, which suggests that the Notch pathway induces early osteogenic differentiation but not the formation of mature osteoblasts. The transcription factors like Hes and Hey, which are Notch targeted genes, also influence the responses of cells to BMPs. Sharff *et al.* observed that silencing Hey1 in C3H10T1/2 cells reduced their BMP-9-mediated ALP activity. Infecting the cells with AdRunx2 caused the ALP activity to recover (129). BMP-2 also triggers C2C12 cells to undergo osteogenic differentiation by increasing the expression of genes encoding for ALP and OC, together with increased expression of gene encoding for Hey1 and decreased

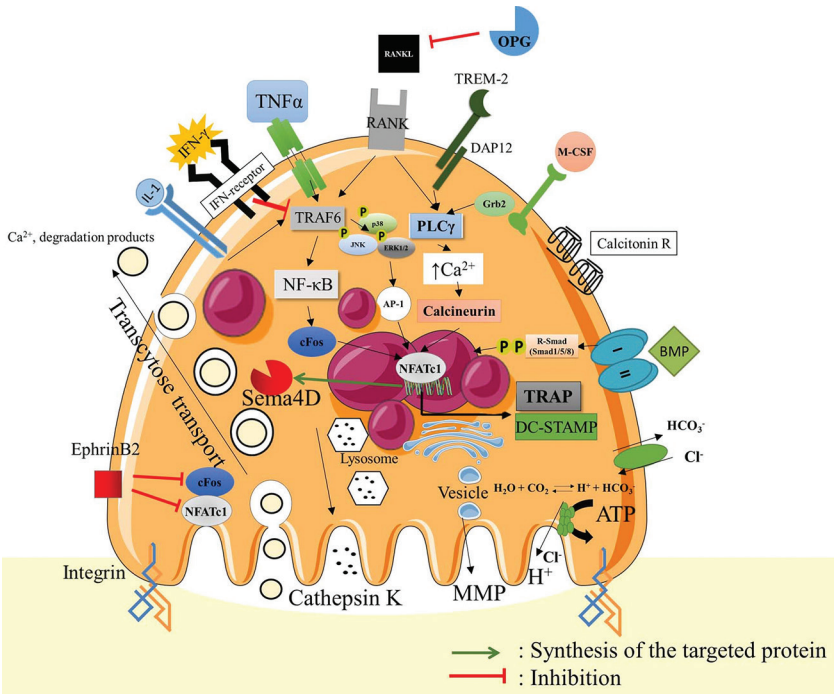

Figure 4. Osteoclast structure and regulation by cytokines and growth factors (AP-1, activating protein-1; PLCgamma, phospholipase C gamma; TRAP, tartrate resistant acid phosphatase) (32,72,140,144,148,149,154–156). [Illustration using Servier Medical Art, http://www.servier.fr].

Hes1 transcripts (130). However, Zamurovic *et al.* found that Hey1 antagonized the transcriptional activity of Runx2 in MC3T3-E1 preosteoblasts stimulated by BMP-2, which led to decreased mineralization (131).

Hedgehog (Hh) is also involved in osteogenic differentiation. It binds to its receptor Patched at the cell surface, which prevents Patched from inhibiting the transmembrane protein Smoothened. This enables Smoothened to activate a signaling cascade leading to stabilization of the transcription factor Gli2. The newly-stabilized Gli2 then activates the transcription of target genes such as that encoding Gli1, which is also a transcription factor that promotes the expression of genes like those encoding ALP and BSP (124,132). The effect of Hh on differentiation depends on the species. Plaisant *et al.* observed that conditioned medium from sonic Hh-secreting cells inhibits the synthesis of ALP, Runx2, osteonectin and OPG by human multipotent stem cells derived from adipose-tissue (122). There is also a crosstalk between Hh and the Wnt pathway. Hu *et al.* showed that inhibiting the Wnt pathway in C3H10T1/2 cells by transfecting the cells with retrovirus encoding Dkk-1 decreased the ALP production induced by a constitutively active Smoothened protein (125). Spinella-Jaegle *et al.* found that stimulating C3H10T1/2 cells with sonic Hh increased the ALP activity induced by BMP-2. However, sonic Hh had no effect on the ALP activity in MC3T3-E1 preosteoblasts induced by BMP-2 (123).

4.2. Osteoclastogenesis

Osteoclastogenesis involves the commitment of hematopoietic precursor cells to the monocyte/macrophage lineage, the fusion of several precursors and their transformation into mature osteoclasts (32). These processes are regulated by two major signaling pathways that are activated by macrophage colony-stimulating factor (M-CSF) and RANKL, a member of the tumor necrosis factor (TNF) ligand superfamily. M-CSF promotes RANK expression and mediates the proliferation of osteoclast precursors and their differentiation and survival. RANKL is crucial for osteoclast differentiation, survival and bone-resorbing activity (Figure 4) (133–135). RANKL also favors the retention of the osteoclast precursors in bone by down-regulating the gene encoding the receptor S1PR1 of the lipid mediator sphingosine-1 phosphate (SP-1), which favors the passage of osteoclast precursors from the bone to blood vessels (136). M-CSF and RANKL are synthesized by osteoblasts, osteocytes, bone marrow stromal cells and lymphocytes in response to stimulation by factors including hormones (parathyroid hormone (PTH), vitamins D), inflammatory cytokines (Interleukin-1 (IL-1), IL-6, TNF alpha, interferon-gamma, IFN gamma) (137–140).

M-CSF binds to the tyrosine kinase receptor c-Fms on osteoclast precursors and causes activated c-Fms to form dimers. These become phosphorylated on their multiple tyrosine residues, enabling them to interact with proteins containing SH2 domains like Grb2 (Y697,

Y974), c-Src (Y559), PI3K (Y721) and to transduce intracellular signaling of M-CSF. PI3K then stimulates Akt, while Grb2 activates the Ras/Raf/MEK/ERK pathways by interacting with Sos (for review see (141,142)). These pathways are mainly involved in the proliferation and survival of osteoclast precursors. However, Amano *et al.* recently demonstrated that the M-CSF-triggered differentiation of murine 4B12 precursor cells (Mac-1(+) c-Fms (+) RANK (+) cells from calvaria of 14-day-old mouse embryos) into osteoclasts also depended on the activation of ERK5 (143).

RANKL acts on osteoclast precursors by binding to its RANK receptor, which, in turn, allows the binding of TNF receptor associated factor-6 (TRAF6) to the intracellular domain of RANK. It induces the activation of several signaling pathways including NF-kappaB, MAP kinases (JNK, ERK1/2, and p38), and also leads to the mobilization of the phosphatidylinositol 3-kinase (PI3K)/Akt pathway (32,144). RANKL may also activate the calcium signals that lead to the activation of another major transcription factor, nuclear factor of activated T cells cytoplasmic 1 (NFATc1), through an immunoreceptor tyrosine-based activation motif-mediated co-stimulatory signaling (145). RANKL-RANK binding induces the hydrolysis of phosphatidylinositol 4,5-bisphosphate by phospholipase C to give inositol-1,4,5-trisphosphate (IP3) and diacylglycerol (DAG) (for review see (146)). The subsequent release of calcium ions from the endoplasmic reticulum induced by IP3 activates calcineurin, which then stimulates NFATc1 by dephosphorylating it (147). NFATc1 activates genes encoding proteins essential for osteoclastogenesis, such as dendritic cell-specific transmembrane protein (DC-STAMP), which is required for cell fusion (148). The activation of NFATc1 by RANKL is amplified by stimulating osteoclast precursors with immunoglobulin-like receptor ligands that bind triggering receptor expressed in myeloid cells 2 (TREM-2) or osteoclast-associated receptors (OSCAR). These receptors become activated by associating with adaptor proteins containing the immunoreceptor tyrosine-based activation motif (ITAM). The ITAM-mediated signal from TREM-2 involves the 12 kDa DNAX-activating protein (DAP12), while OSCAR involves the Fc receptor common gamma (149,150). The adaptors recruit the protein tyrosine kinase Syk, and the resulting signal leads to the release of calcium and activation of transcription factors like NFAT and NF-kappaB. Barrow *et al.* recently reported that OSCAR can bind strongly to type I collagen (151). This interaction promotes osteoclastogenesis (151). The effects of pro-osteoclastogenic factors may also depend on signaling modulators. The recombinant recognition sequence binding protein at the Jkappa site (RBP-J) is a novel transcription factor which inhibits osteoclastogenesis by imposing a requirement of ITAM-mediated co-stimulation for TNF alpha and RANKL signaling (152). Activating RBP-J in osteoclast precursors suppresses the osteoclastogenesis induced by inflammatory stimuli, such as TNF alpha. Conversely, inactivation of RBP-J considerably increases RANKL-independent osteoclastogenesis (153). Finally, the fine-tuning of bone resorption also involves OPG, a secreted decoy RANKL receptor that competes with RANK and inhibits osteoclast differentiation and functions. RANKL signaling can be completely blocked by sequestering RANKL using OPG (32,144). It is also inhibited by cytokines such as IFN gamma (140).

Osteoclastogenesis is also regulated by BMPs like BMP-2 (157–159). Thus, the differentiation of bone marrow cells into osteoclasts requires less RANKL (half optimal dose) when BMP-2 is present in the culture medium (159). The extracellular inhibitor of BMP-2, Noggin, severely impairs the osteoclastogenesis induced by RANKL and the BMP type II receptors BMPRII is required for osteoclastogenesis *in vitro* (157,159). Using human mononuclear leukocyte suspensions isolated from umbilical cord blood, Fong *et al.* showed that BMP-9 could protect osteoclasts against apoptosis via a decrease in caspase-9 activation (160). BMP-9 also enhanced the bone resorption by mature osteoclasts (160).

4.3. Bone healing

Bone healing can be divided into two processes, one not excluding the other: intramembranous and endochondral healing. Intramembranous process occurs in fractures that heal by first intention. Fracture sites should have no defect and should be mechanically stable (described in more details in the next section). The healing of larger bone fractures involves both endochondral bone formation and intramembranous healing. Endochondral healing (second intention) occurs in four phases: inflammation and hematoma formation, fibrocartilagenous callus formation, bony callus formation, and finally bone remodeling (for review see (161)). The periosteum is important for bone healing especially during endochondral healing since it provides chondrogenic lineage precursor cells (162). Each phase requires different cytokines, growth factors and vitamins. Growth factors like BMP, TGF-beta, IGF, FGF, PDGF and VEGF all are involved in bone repair (Figure 5) (163). The PTH is also important in bone healing process since it can enhance bone formation by increasing cartilage volume as well as bone mineral density (for review see (161)).

Several types of cells are also involved in the endochondral bone healing. Macrophages are recruited during hematoma formation/inflammation to remove damaged cells and tissue. They secrete cytokines that foster the recruitment (infiltration) and stimulation of leukocytes to the injury site (168,169). Prostaglandin E2, which is present at the fracture site, can also recruit T lymphocytes during this step (170). The second phase of bone healing is the formation of the fibrocartilagenous callus. The chondrogenic lineage precursor cells in the periosteum move to the injury site,

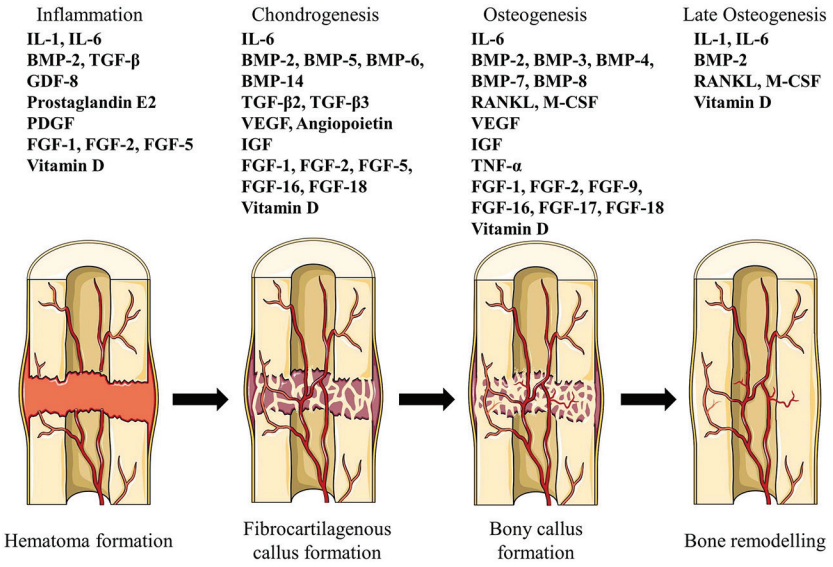

Figure 5. Endochondral healing process following bone fracture (161,163–167). [Illustration using Servier Medical Art, http://www.servier.fr].

where they differentiate into mature chondrocytes under the influence of BMP-2, which is present throughout bone healing (167). Other cytokines and growth factors also promote the differentiation of the precursor cells into mature chondrocytes. TGF-beta3 also facilitates the differentiation of stem cells into chondrogenic lineage (TGF-beta3-BMP2 synergy) (171). FGF-2 can enhance and control the proliferation of the chondrocytes and osteoblast progenitors during each of the bone healing step (165). The transition between the fibrocartilagenous and bony callus phases is promoted by angiogenesis and the differentiation of precursor cells into mature osteoblasts. VEGF and angiopoietin are involved in the vascularization of the callus and the final bone formation/mineralization (161).

Finally, the last stage of bone healing is bone remodeling, which can be subdivided in five distinct phases: quiescence, preosteoclast recruitment and osteoclast differentiation, bone resorption, preosteoblast recruitment and osteoblast differentiation which ultimately lead to the formation of structured bone. The first step is osteoclastogenesis and two hypotheses have been advanced to explain osteoclast activation. The first is that osteoclastogenesis is activated by the apoptotic bodies of the osteocytes while the second is that the death of osteocytes leads to a reduction in the amount of TGF-beta (172–174). The activated osteoclasts then start to resorb bone (155,156). Resorption takes up to 30 days, while bone formation and the recruitment of osteoblasts and osteoblast precursors can take 150 days. The cell signaling that triggers the passage from bone resorption to formation is not yet fully understood. Mature osteoblasts then synthesize the bone matrix and allow its mineralization.

The various cells involved in bone remodeling communicate with each other in several ways throughout the process. Osteoclasts synthesize Sema4D that inhibits the formation of the bone matrix by osteoblasts (72). Sema4D binds to the Plexin-B1 receptors on the osteoblasts and these then activate RhoA to prevent the matrix mineralization induced by IGF-1 (72).

5. CELL ADHESIONS

5.1. Types of cell adhesions

Interactions between cells and ECM or biomaterials are essential for tissue integrity and repair. They influence cell survival, proliferation, differentiation and migration. The integrins are the main heterodimeric alpha beta transmembrane glycoproteins cell receptors involved in the adhesion of cells to ECM proteins and biomaterials (175). There are presently 18 alpha and 8 beta subunits that assemble to form 24 distinct integrins. They interact with adhesive proteins from the ECM via specific amino acid sequences. The most common site on ECM proteins to which integrins bind is the tripeptide sequence Arg-Gly-Asp (RGD). It is found on type I collagen, OPN, BSP, thrombospondin and fibronectin (176). Alphav beta3 integrins bind to several proteins, including fibronectin, vitronectin and BSP, while alpha5 beta1 integrins interact specifically with fibronectin. Collagen type I also interacts with alpha1 beta1 and alpha2 beta1 integrins (175,177).

5.1.1. Osteoblasts

Osteoblasts can bear alpha1, alpha2, alpha3, alpha4, alpha5, alpha6, alphav, beta1, beta3 and beta5 integrin subunits depending on their stage of differentiation. Alpha2, alpha3, alpha5, alphav, beta1 and beta3 subunits are present on osteoblasts, alpha2

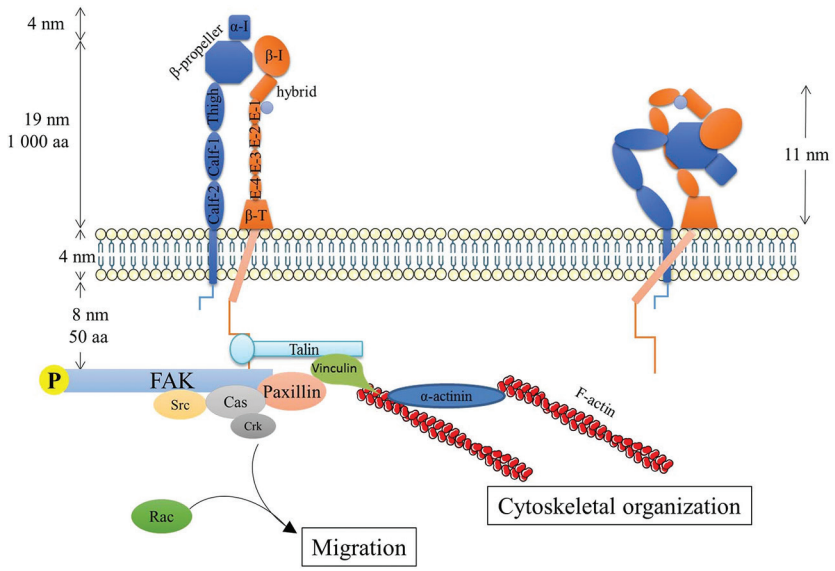

Figure 6. Activation of integrin and focal adhesion organization (E1, Epidermal Growth Factor domain 1; Beta T, beta tail) (199,200). [Illustration using Servier Medical Art, http://www.servier.fr].

integrin subunit is found only on cells close to the bone surface while alphav beta3 integrins are found mainly on osteocytes (54).

Osteoblasts can adhere to 2D systems *in vitro* via integrin adhesion architectures that are organized as focal complexes, focal adhesions and fibrillar adhesions, but the morphology, size and subcellular distributions of these cell adhesion sites differ (178). Focal complexes that are 0.1 to 2 microns in diameter are the main precursors of focal adhesions. They recruit vinculinand phosphoproteins and exert stress between 1 and 3 nN/micron^2. Focal adhesions are elongated streak-like structures (3 to 10 microns) that are often located at the cell periphery (179). They anchor bundles of actin stress fibers (F-actin) through a plaque made up of several proteins including integrins and structural proteins. Integrin-mediated adhesions called adhesomes are composed of more than 180 components (180). Adhesomes contain actin-associated proteins (tensin, vinculin, alpha actinin), adaptor proteins (vimentin, Shc-transforming protein 1) and signaling proteins (tyrosine or serine/threonine kinases and members of the Rho GTPases) (181). Three proteomic studies on the composition of adhesomes in cells attached to fibronectin and control cells on matrices like poly-L-lysine have identified more than 700 components (182–184). These studies also found new focal adhesive proteins such as actin linkers (adducing) and ubiquitin ligase proteins. However, only 63 proteins associated with integrin-mediated adhesion are common to all three studies, perhaps because of the cell types and substrata used (182–185). Robertson *et al.* recently used proteomic and phosphoproteomic methods to analyze the components of integrin-mediated adhesions that attached A375-SM human melanoma cells to polystyrene (PS) surfaces coated with fibronectin or control PS surfaces coated with transferrin (186). They found more than 1170 proteins in adhesomes, including 499 phosphoproteins. Hoffman *et al.* also recently used fluorescence cross-correlation spectroscopy and fluorescence recovery after photobleaching and found that some components of the adhesome existed as cytoplasmic pre-assembled complexes that were available for rapid assembly and adhesion site formation (187). Clearly, further investigations are required to give a better picture of the composition and how the adhesome is assembled.

The formation of focal adhesion sites depends on the activation state of the integrins, and this implies a change in their conformation (Figure 6). The binding of structural proteins such as talin to the cytoplasmic tail of the beta integrin subunits allows the integrin head region to be repositioned to point away from the cell surface. The cytoplasmic domain of each beta integrin subunit is therefore a major factor in establishing the connections between the cell cytoskeleton and the ECM. Integrin-linked kinases (ILK) that interact with beta1 integrin subunits are also central to integrin signaling (188). The interaction of talin with vinculin then promotes the clustering of activated integrins (189). The cytoplasmic domains of beta integrin subunits also contain one or two conserved Asn-Pro-X-Tyr, or Asn-Pro-X-Phe motifs that interact with phosphotyrosine proteins such as focal adhesion kinase (FAK) (190). FAK is a non-receptor protein tyrosine kinase that possesses three domains, an N-terminal ezrin radixin moesin (FERM) domain, a central kinase catalytic domain and a C-terminal

FAT domain. FERM is involved in protein-protein interactions (191–193) (for review see (194)). It interacts especially with the phenylalanine 596 in the central catalytic domain of FAK, thus inhibiting the kinase (195). The FAK kinase contains three tyrosines (Y397, Y576 and Y577); their phosphorylation is essential for the activation of FAK (196). Integrin clustering first causes the rapid autophosphorylation of FAK at Y397. FAK phosphorylated on Y397 then interacts with proteins like the Src-family kinases that phosphorylate other tyrosine residues in FAK such as the two tyrosine residues in the catalytic loop of the kinase domain (Y576 and Y577) so increasing the FAK kinase activity. These kinases also phosphorylate two proteins that interact with FAK, Crk-associated substrate (CAS) and paxillin (197). Paxillin contains many tyrosine and serine/threonine phosphorylation sites and is believed to modulate both cell adhesion and growth factor signaling pathways (198). Paxillin activates some members of the RhoGTPases family such as Ras-related C3 botulinum toxin substrate 1 (Rac1) and cell division cycle 42 (Cdc42) by recruiting Pak-interacting exchange factor and guanine exchange factor (GEF). These members of the RhoGTPases family are involved in cell adhesion and organization of the cytoskeleton (181). Their activation depends on GEF catalyzing the exchange of a GDP for a GTP on the RhoGTPases proteins. Inactivation of the RhoGTPases is mediated by a GTPase activating protein (GAP) that favors the hydrolysis of GTP to GDP. FAK-Src complex acts via p190RhoGAP to keep a RhoGTPase called RhoA inactive during the early steps of cell adhesion and spreading. In contrast, Cdc42 and Rac1 are active: Cdc42 regulates the formation of filopodia while Rac1 regulates membrane ruffling lamellipodia. RhoA becomes activated by GEFs like p115RhoGEF and p190RhoGEF at a later step in cell spreading, which favors the formation of actin stress fibers and the maturation of focal adhesions. Stimulation of RhoA suppresses the activity of Rac1 (181).

Fibrillar adhesions, consisting of tensin-alpha5 beta1 integrin complexes that bind to fibronectin, are more centrally located in the cell than are focal adhesions. The formation of fibrillar adhesions is closely linked to the capacity of cells to polymerize fibronectin to form ECM fibrils (201,202). The translocation of fibrillar adhesions is highly directional, proceeding centripetally from the cell periphery towards the center and is always aligned along the long axis of the focal adhesion. Multi-ligand alphav beta3 integrins remain within focal adhesions, while alpha5 beta1 integrins are translocated at 6.5 microns/h parallel to the actin microfilaments in fibrillar adhesions (201,202). Lin *et al.* recently reported that osteoblasts at 12 hours organize endogenous fibronectin into fibrils on self-assembled monolayers bearing amine groups, while those bearing hydroxyl and methyl groups limit such fibronectin fibril formation (203).

However, it is known that the components of 2D and 3D adhesions differ considerably: 3D adhesions used mainly alpha5 beta1 integrins instead of alphav beta3 and had a low FAK phosphorylation (204).

5.1.2. Osteoclasts

Osteoclasts contain alpha2, alpha5, alphav, beta1 and beta3 integrin subunits. However, the adhesion receptor alphav beta3 is the major integrin expressed by osteoclasts and a marker of the osteoclast phenotype. The engagement of alphav beta3 integrin leads to the formation of a multi-molecular complex that includes c-Src and PI3K (205). In addition to its role in cell-matrix attachment, alphav beta3 is also involved in other aspects of osteoclast biology, and osteoclasts that lack alphav beta3 are dysfunctional (206,207). The bone matrix mediates anti-apoptotic signals via integrins, and alphav beta3 occupancy promotes osteoclast survival (208). In contrast, unoccupied alphav beta3 sites may induce osteoclast apoptosis via caspase-8 activation, and in the absence of alphav beta3 (beta3$^{-/-}$ knockout mice), delayed cell death occurs as a result of caspase-9 activation (209,210).

Upon adhesion, osteoclasts reorganize their cytoskeleton and form podosomes which are small adhesion structures (1 micron diameter) with a dense actin core surrounded by a rosette-like structure containing proteins such as alphav beta3 integrins, structural focal adhesive proteins (talin and vinculin), actin-associated proteins (gelsolin and alpha-actinin), tyrosine kinases (c-Src and Pyk2) and small GTPases. RhoGTPases have been involved in the organization of F-actin cytoskeleton in different cell types, and in mature osteoclasts Rac, Cdc42 and Rho regulate the podosome formation into the sealing zone (211–213). The podosome core also contains large amounts of proteins involved in actin polymerization. Thus, the Arp2/3 complex that is activated by Wiscott-Aldrich syndrome protein (WASP) takes part in the formation of the actin podosome core, while the formin protein initiates the unbranched F-actin strands and the formation of stress fibers (214). Cortactin, which interacts with the Arp2/3 complex, is also involved in the formation and stabilization of the branched actin network (215). By contrast, formation of the ruffled membrane involves vesicular trafficking regulated by the Rab family of GTPases, particularly lysosomal Rab7 (216).

Osteoclasts are capable of adhesion on different substrates by distinct F-actin structures. When osteoclasts adhere on plastic or glass, podosomes form clusters organized in a podosome belt around the cell, in which podosomes can be individualized (Figure 7). Upon adhesion to bone or mineralized ECM, podosomes undergo major reorganization as part of the osteoclastic terminal differentiation into bone resorbing cells. In non-active (non-resorbing) unpolarized osteoclasts,

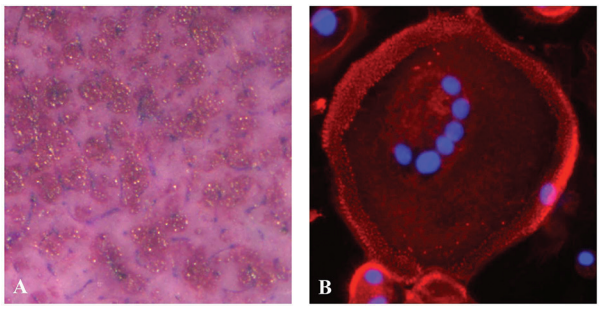

Figure 7. Bone resorption and actin ring formation. Mature osteoclasts were obtained from cord blood monocytes in long-term cultures in presence of MCSF and RANKL. The cells were allowed to settle either on devitalized bovine bone slices or plastic. (A) Bone resorption appears as dark areas after toluidine blue staining, with a bright aspect under epi-illumination. (B) Cell cultured on plastic and F-actin, found in actin ring, was stained with fluorescent-labeled phalloidin (red) and nuclei with DAPI (blue).

podosomes are dispersed on the adhesion surface or organized in a loose belt of podosomes (216). When osteoclasts are activated to resorb bone, they strongly attach to the bone matrix by densely packed F-actin rich podosomes to form the sealing zone that delineates the cellular area in contact with the bone surface where the ruffled membrane enlarges and beneath which the resorption lacuna occurs (42,217). Some studies suggest that a podosome belt prefigures the sealing zone but this is rather controversial (217–220). Microtubules, made up of alpha- and beta-tubulin heterodimers in filamentous networks, also regulate the podosome organization at the end of osteoclast maturation (217). Podosomes are also involved in the migration of osteoclasts, perhaps through a saltatory mode (221). Touaitahuata *et al.* suggested recently that adhesion is essential for osteoclast differentiation while podosome formation is not (219).

5.2. Influence of ECM-integrin interactions on cell behavior

Integrins that are involved in the interactions between cells and the ECM may also influence the responses of cells to growth factors such as BMPs, VEGFs, PDGFs and IGFs (222–225). Integrins and growth factors influence each other in many ways. Integrins can be co-localized with growth factor receptors. Lai and Chen demonstrated that specific integrin subunits (alphav, beta1, beta3, alphav beta3, alphav beta5, alphav beta8) were located with type I and type II (BMPRI and BMPRII) BMP receptors (222). They also showed that blocking the alphav integrin subunits with antibodies decreased the ALP activity induced by BMP-2. Goel *et al.* found that IGFIR was located together with beta1A integrins at focal adhesion points in GD25 cells (224). Interactions that involve the insulin receptor substrate-1 (IRS-1) maintain IGFIR in an activated state that favors cell proliferation and tumor growth. In contrast, beta1C integrin subunits that form a complex with Gab1/Shp2 promote the recruitment of Shp2 to IGFIR, which then leads to dephosphorylation of the receptor. This inactivation of IGFIR slows down cell proliferation and thus inhibits tumor growth (224).

Integrin also act on growth factor signal transduction by recruiting adaptors to the plasma membrane or specific signaling proteins involved in the adhesome. Integrins can induce the phosphorylation of growth factor receptors in the absence of their ligand (226). Veevers-Lowe *et al.* found that the PDGF receptor beta in human bone marrow MSCs attached to fibronectin via their alpha5 beta1 integrins was already phosphorylated, without any growth factor stimulation (226). This activation is essential for MSC migration (226). The N-terminal FERM domain of FAK also interacts with growth factor receptors such as those of PDGF (226–229). In addition, Tamura *et al.* demonstrated that FAK was essential for the differentiation of MC3T3-E1 preosteoblasts induced by recombinant human BMP-2 (230). No ALP activity was detected in FAK-deficient cells treated with BMP-2.

These versatile integrins also modulate the internalization and degradation rate of growth factor receptors. Reynolds *et al.* showed that the alphav beta3 inhibitor stimulated tumor growth and tumor angiogenesis by recycling the VEGFR2 internalized in endosomes back to the plasma membrane and by decreasing VEGFR2 degradation (223). Finally, integrins stimulate the synthesis of growth factors. Mai *et al.* reported recently that silencing the gene encoding for the beta1 integrin subunit inhibited the synthesis of BMP-2 and the differentiation of MC3T3-E1 preosteoblasts into osteoblasts by preventing activation of the gene encoding ALP (231).

However, growth factors also regulate the synthesis of specific integrins. Mena *et al.* observed that stimulating human umbilical vein endothelial cells (HUVECs) with VEGF-A increased the production of alpha5 and beta1 integrin subunits (232). Similarly, our research group has shown that a peptide derived from the knuckle epitope of BMP-9 increased the amount of alphav integrin subunits in the plasma membrane of MC3T3-E1 preosteoblasts attached to PS coated with peptides derived from BSP (233). Lai and Chen also showed that stimulating human osteoblasts (HOB19) with BMP-2 led to the synthesis of alphav, beta1 and beta3 integrin subunits in human osteoblasts (222). As BMP-2 increased the amount of alphav beta3 integrins in human osteoblasts, it favored their adhesion to OPN and vitronectin (222).

The crosstalk between integrin and growth factor signaling pathways coordinates MSCs and bone cells behaviors. Researchers that develop biomimetic materials must therefore be aware of such complex interactions. They have to get a better understanding of the influence of biomimetic materials on the ability of cells to respond to growth factors, cytokines and hormones.

6. BIOMIMETIC MATERIALS

6.1. Materials functionalized with proteins

The biomaterials used in bone repair can be inorganic materials, natural polymers, synthetic polymers, or even composites. But not all bone biomaterials currently in use are osteoconductive and osteoinductive (for review see (8,234)). Biomaterials can be functionalized with ECM proteins such as fibronectin, collagen, OPN and BSP to increase their interactions with cells (53,235). The proteins can be immobilized on the surfaces by non-specific adsorption (physisorption) or by covalent binding (for review see (8)). Schwab *et al.* tested fibronectin, vitronectin and OPN adsorbed onto PS. Fibronectin and OPN promoted greater hMSC proliferation in comparison with the control after 5 and 10 days in standard growth medium (53). In addition, MSCs attached to fibronectin in osteogenic medium contained more BSP and Runx2 after 5 days, while MSCs attached to OPN contained these markers only after 10 days in culture. Cells on vitronectin contained slightly more osteogenic markers, Runx2 and BSP, after 10 days in osteogenic medium than did cells in standard growth medium. Fibronectin and OPN induced MSCs in osteogenic medium to deposit more calcium than did cells on vitronectin (53).

Several studies have shown that the density and conformation of the proteins adsorbed or grafted onto the surface can strongly affect cell behavior (236,237). Faia-Torres *et al.* used a gradient of fibronectin (48-213 ng/cm²) on PCL surfaces and showed that human bone marrow MSCs incubated with intermediate densities of fibronectin for 7 days proliferated most rapidly. On the other hand, cells incubated for 7 and 14 days in lower and higher densities of fibronectin had more ALP activity and collagen type I synthesis than did hMSCs on intermediate fibronectin densities (237). Lin *et al.* also used self-assembled monolayers with terminal −OH, $-CH_3$, and $-NH_2$ groups to evaluate the conformation of adsorbed fibronectin (203). They showed that fibronectin adsorbed onto self-assembled monolayers with terminal -OH groups had more accessible cell-binding domains than did the fibronectin on surfaces with terminal $-CH_3$ and $-NH_2$ groups. The more accessible cell binding domains of fibronectin adsorbed onto surfaces with terminal -OH groups led to greater amount of vinculin and tensin and favored initial cell adhesion at 2h as shown by an increased area of focal and fibrillar adhesions in primary osteoblasts from rat calvaria (203).

The use of proteins extracted from the ECM presents other challenges. There can be batch-to-batch variations (238). ECM proteins which are extracted and purified from non-human species can increase the risk of undesirable immune responses and infections (238). Peptides derived from proteins that contain the sequence recognized by specific cell membrane receptors have therefore been developed (Table 1). A major advantage of these peptides is that they are readily synthesized and purified, which reduces their production costs (239).

6.2. Homogeneous peptide-modified surfaces

One of the most frequent sequences in many proteins is the RGD peptide. A RGD sequence derived from the BSP (CGGNGEPRGDTYRAY, pRGD) grafted to PCL films induced the formation of focal adhesions and organization of the actin cytoskeleton in MC3T3-E1 preosteoblasts placed in serum-free medium for 1h. The cells also had a greater cell surface area than those on the negative peptide CGGNGEPRGETYRAY (240). More importantly, only the MC3T3-E1 preosteoblasts attached to PCL functionalized by pRGD responded to BMP-2 (100 ng/mL). Shin *et al.* also used a composite scaffold of poly (L-lactide) (PLLA) and biphasic calcium phosphate (BCP) grafted with an RGD peptide to study the impact of adhesive peptides on the behavior of hMSCs (241). The cells on the composite scaffold modified with the RGD peptide for 24h contained actin stress fibers and paxillin at the focal adhesions while those on the unmodified scaffold had none. There were also more spread cells on the RGD-modified scaffold. The hMSCs on the RGD-modified scaffold for 7 days had more ALP activity and, after 14 days, greater mineralization than did cells on the unmodified scaffold or PLLA grafted with RGD (241). Since type I collagen is the major component of bone osteoid, several peptides derived from this protein such as DGEA and GFOGER have been developed (242–244). The research group of Garcia has shown that GFOGER-coated PCL scaffolds significantly improved the repair of femoral bone defects in rats, 12 weeks after implantation (244). Furthermore, Mehta *et al.* showed that the ALP activity of rat MSCs trapped in an alginate hydrogel containing a DGEA peptide (H_2N-GGGGDGEASP-OH) for 7 days was greater in comparison with the controls (alginate alone or in combination with RGD) (242). Their production of OC and their mineralization were also increased (242).

Other peptides that could improve the adhesion, proliferation and differentiation of bone cells are those containing a heparin-binding site that interacts with heparan sulphate proteoglycans at the cell surface (245,246). One peptide sequence that binds to heparan sulphate proteoglycans is Lys–Arg–Ser–Arg (KRSR), which is found in proteins such as fibronectin, vitronectin and BSP (247). Sun *et al.* have shown that MC3T3-E1 preosteoblasts on titanium dioxide (TiO_2) anodized nanotubes functionalized with KRSR had more vinculin at focal points and a better organized actin cytoskeleton than did those on nanotubes functionalized with the negative peptide KSRR or the control nanotubes (246). The osteogenic differentiation of MC3T3-E1 preosteoblasts attached to TiO_2 anodized nanotubes functionalized with KRSR was also better, as shown by the increased concentrations of mRNA

Table 1. Effect of ECM-derived peptides on the behavior of bone cells

Protein	Peptide sequence	Negative control	Culture system	Effect	References
Laminin	CRARKQAASIKVAVSADR (IKVAV)		*In vitro* (Adwipose derived stem cells (AdSC) from human tissue)	-↑ Number of AdSC onto PCL-IKVAV after 24 h and 48 h compared with PCL alone	(256)
	DLTIDDSYWYRI (alpha2 chain)	Scramble peptide	*In vitro* (Human osteosarcoma cells)	-↑ Number of cells on Ti-coated with the peptide after 1 day compared with Ti alone -↑ ALP activity of cells onto Ti-coated with the peptide after 1 day compared with Ti alone. No difference after 3 days (Ti, Ti-coated with positive or negative peptide)	(257)
	DLTIDDSYWYRI (alpha2 chain)	Scramble peptide	*In vitro* (MG63 osteoblast like cells)	-↑ number and area of cells on PS coated plates with laminin/PS coated with the peptide compared with PS coated with BSA/PS coated with a scramble peptide after 1h in serum-free medium -↑ osteogenic markers for differentiation (BSP, OC) of cells on Ti disk coated with the peptide after 7 days compared with Ti alone -↑ ALP activity of cells on Ti disks coated with the peptide after 3 days compared with Ti alone -↑ number of cells on Ti disks coated with the peptide after 1h compared with Ti alone	(258)
		Scramble peptide	*In vivo* (New Zealand white rabbits)	-↑ bone area for Ti implant coated with the peptide after 1 week compared with Ti alone. No difference after 2 and 4 weeks (Ti, Ti coated with positive or negative peptide) -↑ collagen deposition for Ti implant coated with the peptide after 1 week compared with Ti alone. No difference after 4 weeks (Ti, Ti coated with positive or negative peptide) -↑ ALP activity for Ti implant coated with the peptide after 4 weeks compared with Ti alone or Ti with negative peptide	
BSP	CGGNGEPRGDTYRAY	CGGNGEPRGETYRAY	*In vitro* (MC3T3-E1)	-Cytoskeletal organization of cells on PCL films functionalized with the peptide after 1h compared with PCL alone or with negative peptide in serum-free medium -↑ area of cells on PCL films functionalized with the peptide after 1h compared with PCL alone or with negative peptide in serum-free medium	(240)
	NGVFKYRPRYYLYKHAYFYPHLKRFPVQ		*In vitro* (Human muscle-derived stem cells)	-↑ ALP activity and calcium deposits in a dose-dependent manner of peptide derived from BSP after 14 days in osteogenic medium -↑ mRNAs of osteogenic genes (ALP, type I collagen, OC, Run×2) was a dose-dependent manner of peptide derived from BSP after 14 days in osteogenic medium	(259)

(Contd...)

Table 1. *(Continued)*

Protein	Peptide sequence	Negative control	Culture system	Effect	References
Fibronectin	RGD-PHSRN K3G4RGDS and K3G4PHSRN		*In vitro* (MC3T3-E1)	-↑ number of cells on PHSRN-RGD Ti surface after 4h compared with RGD or PHSRN Ti surfaces alone -No difference in term of cellular differentiation (ALP activity) after 14 days	(260)
Collagen type I	GGGGDGEASP		*In vitro* (Primary rat MSCs)	-↑ ALP activity of cells trapped in hydrogel modified with DGEA peptide after 7 days compared with unmodified hydrogel -↑ number of cells in hydrogel modified with DGEA after 30 days compared with hydrogel modified with RGD or a mixture of RGD and DGEA -↑ collagen type I in cells trapped in hydrogel modified with DGEA after 30 days compared with hydrogel modified with a mixture of RGD and DGEA -↑ OC production and mineralization in cells trapped in hydrogel modified with DGEA after 30 days compared with unmodified hydrogel or hydrogel modified with RGD or a mixture of RGD and DGEA	(242)
	GGYGGGPC (GPP) 5GFOGER (GPP) 5GPC		*In vitro* (hMSCs)	-↑ migration of cells on GFOGER-coated random PCL nanofiber after 48h compared with uncoated or collagen-coated meshes. No difference between GFOGER- and collagen-coated aligned meshes -↑ ALP activity of cells on GFOGER-coated random meshes after 21 days compared with uncoated or collagen-coated meshes. ↑ ALP activity of cells on GFOGER-coated aligned meshes compared with collagen-coated meshes -↑ calcium w by cells on GFOGER-coated random meshes after 21 days compared with uncoated or collagen-coated meshes. No difference between GFOGER- and collagen-coated aligned meshes	(243)
	RGD		*In vitro* (MC3T3-E1)	-↑ ALP activity and calcification on Ti-RGD after 28 days compared with Ti alone in differentiation-inducing medium	(261)
			In vitro (hMSCs)	-↑ actin cytoskeletal organization and paxillin at focal adhesions in cells onto PLLA/BCP scaffold modified with RGD after 24h compared with unmodified scaffold -↑ number of cells and their spreading on PLLA/BCP scaffold modified with RGD after 24h compared with unmodified scaffold -↑ ALP activity after 7 days and mineralization after 14 days in cells onto PLLA/BCP scaffold modified with RGD compared with unmodified scaffold	(241)
	KRSRGYC	KSRRGYC	*In vitro* (MC3T3-E1)	-↑ focal adhesions, actin fibers and number of cells on KRSR-TiO2 anodized nanotubes compared with KSRR-TiO2 anodized nanotubes or nanotubes alone after 4 h -↑ ALP activity and OC, BSP, OPN, Run×2, Osx and ALP mRNAs for cells on KRSR-TiO2 after 7 and 14 days compared with KSRR-TiO2 anodized nanotubes or nanotubes alone	(246)

(Contd...)

Table 1. *(Continued)*

Protein	Peptide sequence	Negative control	Culture system	Effect	References
	KRSRC	RKSRC	*In vitro* (Primary human osteoblast, NHOsts)	-↑ number of cells onto calcium aluminate functionalized with KRSRC compared with calcium aluminate alone or with the negative peptide, from 1 day to 7 days -Spread and elongated cells on calcium aluminate functionalized with KRSRC after 4 days compared with calcium aluminate alone where cells were rounded	(262)
	GGGGGGGKRSR		*In vitro* (hMSCs)	-↑ number of cells on HAP coated with KRSR after 1h compared to HAP alone -Combination with RGD peptide had no effect on the number of cells -Cells slightly spread on HAP coated with KRSR or in combination with RGD after 1h compared with HAP alone where cells were rounded	(263)
	GGGGGGGFHRRIKA		*In vitro* (hMSCs)	-↑ number of cells on HAP coated with FHRRIKA after 1h compared with HAP alone -Combination with RGD peptide had no effect on the number of cells -Cells slightly spread on HAP coated with FHRRIKA or combined with RGD after 1h compared with HAP alone where cells were rounded	(263)

AdSC: Adipose derived stem cells, BSA: Bovine serum albumin

encoding osteogenic markers (ALP, BSP, Runx2, OPN, Osx and OC) and ALP activity after 7 and 14 days.

There are many other challenges in the use of peptides-functionalized surfaces, such as the way they are immobilized on the surface to retain their bioactivity, their density and conformation (248). Rezania and Healy compared the effect of peptides derived from BSP (CGGNGEPRGDTYRAY) immobilized on a quartz surface at densities from 0.01 to 3.8 pmol/cm² on the osteoblastic differentiation of rat calvaria osteoblast-like cells (249). A density of at least 0.62 pmol/cm² of BSP peptides was necessary to increase the mineralization in rat calvaria osteoblast-like cells.

The conformation of the grafted peptide also influences cell behavior. The RGD peptide can be used in a linear or cyclic conformation. Cyclic RGD peptide is more stable than the linear peptide at physiological pH (250). Kilian *et al.* also showed that the cyclic RGD (RGDfC where f is a phenylalanine residue with a D configuration) enhanced focal adhesion and increased MSC spreading more than did the linear RGD (GRGDSC) (251). The cyclic RGD peptide could also enhance the affinity of cells for the alphav beta3 integrin better than did the linear one (252,253).

Another important challenge is the sterilization of biomimetic materials that use bioactive molecules. The main sterilization methods used are moist heat, dry heat, gamma radiation, ultraviolet radiation, hydrogen peroxide, and ethylene oxide (254,255). RGD peptide dissolved in ultrapure water is completely broken down after exposure to UVC for 12h, while ethylene oxide sterilization alters proteins by forming adducts between the ethylene oxide and the protein methionine and cysteine residues (254,255).

6.3. Mixed peptide surfaces

6.3.1. Combinations of adhesive peptides

It is possible to immobilize a combination of peptides derived from different ECM proteins on the biomaterial surface so as to mimic more precisely bone physiology and so improve the interaction between the material and the cells. The frequently used RGDS peptides have been immobilized together with KRSR peptides on silk fibroid nanofibers (264). This combination increases the proliferation of human osteoblasts over that of cells on nanofibers of silk fibroid alone. However, the potential of RGD and KRSR peptides combinations for bone application may be controversial. A combination of GCRGYGRGDSPG and GCRGYGKRSRG peptides on a titanium implant has no greater synergistic effect on bone formation in maxillae of miniature pigs than does KRSR alone (265).

The two immobilized peptides can also be derived from the same protein. Fibronectin possesses

a synergistic site PHSRN in its $FNIII_9$ domain that enhances the interaction between the RGD sequence in its $FNIII_{10}$ domain and alpha5 beta1 integrin (266). Chen *et al.* developed a simple technique for immobilizing the bioactive fibronectin motifs, RGD (KKKGGGGRGDS) and PHSRN (KKKGGGGPHSRN) on Ti surfaces modified with (3-chloropropyl) triethoxysilane (CPTES) (260). This combination of RGD and PHSRN peptides enhanced the adhesion of MC3T3-E1 preosteoblasts more than did either of the peptides alone, but there were no difference in the osteoblastic differentiation after 14 days (260). The best proportion between the two peptides that favors the desired cell response and tissue repair must be determined. Nakaoka *et al.* used alginate gels functionalized with different ratios of RGD (GGGGRGDSP) and PHSRN (GGGGPHSRN) peptides to show that cells seeded on alginate containing 67 percent RGD and 33 percent PHSRN peptides had more OC than did cells on gels containing 11 percent RGD and 89 percent PHSRN (267). The two bioactive RGD and PHSRN motifs can be combined to create a longer peptide and so overcome the peptide ratio problem. Benoit and Anseth developed a peptide containing the RGD and PHSRN motifs separated by 13 glycine residues ($RGDG_{13}$PHSRN) (268). This peptide grafted on a poly (ethylene) glycol (PEG) hydrogel favored the organization of the actin cytoskeleton in osteoblasts from neonatal rat calvaria more than did ungrafted PEG.

Peptides derived from the adhesive protein fibronectin can be immobilized with peptides extracted from type I collagen so as to better mimic the properties of the bone matrix. Reyes *et al.* immobilized the peptide GFOGER ($GGYGGGPC(GPP)_5GFOGER(GPP)_5GPC$) that targets the integrin alpha2 beta1 with a fragment of fibronectin containing the sequences RGD and PHSRN recognized by alpha5 beta1 integrins (269,270). This strategy activates specific integrins and improves both the adhesion of human fibrosarcoma cells and the activation of their FAK more than any of the peptides alone. Visser *et al.* designed a peptide containing the decapeptide collagen-binding (CBD) motif derived from the von Willebrand factor with an RGD motif at the C-terminus (WREPSFMALSGRGDS) (271). Absorbable collagen type I sponges (ACSs) functionalized with CBD-RGD increased the ALP activity of rat spinal bone marrow MSCs by day 10 and enhanced mineralization of the matrix on day 21. Moreover, *in vivo* experiments in rats showed that with the injection of ACS functionalized by CBD-RGD in combination with BMP-2 formed ectopic bone after 21 days compared with ACSs containing BMP-2 alone (271).

These strategies can also be used to improve growth factor efficiency. The CBD motif has been used to develop chimeric recombinant proteins such as CBD-TGF-beta fusion proteins that enhance the delivery of the growth factor to the cells (272).

6.3.2. Mixed adhesive peptides with BMPs or their derived peptides

Adhesive peptides can be used in combination with growth factors such as the BMPs to develop synthetic osteoinductive materials (Table 2). Shekaran *et al.* designed a PEG gel functionalized with the GFOGER peptides ($GGYGGGP(GPP)_5GFOGER(GPP)_5GPC$) using maleimide chemistry in combination with embeded BMP-2 (0.03, 0.06 and 0.3 microg per 1.5 microL of hydrogel) (273). Implanting these hydrogels in defects in the radius bone of B6129SF2/J wild type male mice resulted in better bone reconstruction in the presence of BMP-2 (mineral density and bone volume) than did implants of hydrogel without BMP-2.

Both adhesive peptides and BMPs can be covalently bound to surfaces or scaffolds. He *et al.* functionalized a poly(lactide-ethylene oxide fumarate) (PLEOF) hydrogel with GRGD peptides, one peptide derived from residues 162−168 of OPN and another derived from residues 73−92 of BMP-2 (24). Each peptide was covalently bound to the PLEOF polymer using three types of chemistry. The GRGD peptide contained an acrylamide function which bound to the crosslinker N,N-methylenebis(acrylamide) during the formation of the covalent gel, the OPN used an oxime reaction involving the $–O-NH_2$ function on the OPN peptide with the aldehyde group from the hydrogel, while the BMP-2-azide derived peptide was attached via a click chemistry involving the propargyl function of the hydrogel. He *et al.* used bone marrow MSCs isolated from young adult Wistar rats to show a greater increase in the ALP activity (14 days) and calcium content (28 days) in MSCs on this hydrogel than in cells seeded on hydrogels containing RGD alone or RGD plus BMP-2 (24). The combination of the 3 peptides (RGD, BMP-2 and OPN) also improved the production of vasculogenic markers like PECAM-1 and VE-cadherin *in vitro* (24). Osteogenic and vasculogenic differentiation could greatly enhance the process of bone repair, as good vascularization of the biomaterial delivers more oxygen and the nutrients necessary for the proper functioning of bone cells. However, the size of the protein (for example BMP) used in combination with short adhesive peptides can block the interaction between the cells and the short peptides.

BMPs can be replaced by short derived peptides to overcome this problem. These peptides can mimic the knuckle epitope of the BMPs and interact with type II receptors such as BMPRII (274). Our research group has developed a peptide derived from BMP-9 (pBMP-9) based on the studies of Suzuki *et al.* and Saito *et al.* on peptides derived from the knuckle epitope of BMP-2. We have shown that pBMP-9 (Ac-CGGKVGKACCVPTKLSPISVLYK-NH2) promoted the differentiation of murine MC3T3-E1 preosteoblasts in the same way as the entire protein BMP-9 (275–277). It also induced woven bone formation when injected,

Table 2. Targeting specific integrins to improve cell response to growth factors

Integrins	Growth factor	Culture system	Behavior	References
Beta1	BMP-2	*In vitro* (MC3T3-E1)	-Blocking the beta1 integrin subunit↓the BMP-2 transcription and secretion induced by mechanical stimulation -BMP-2 induces MC3T3-E1 differentiation (↑ in Run×2, Osx and ALP mRNA at 12h)	(231)
	BMP-2	*In vitro* (MSCs)	-↑ beta1 synthesis on alumina surface functionalized with BMP-2 after 2 days compared with alumina alone	(284)
Alpha2 Beta1	BMP-2	In vivo (B6129SF2/J wild-type mice)	-Scaffold targeting alpha2 beta1 integrin loaded with BMP-2 permitted better bone healing than scaffold targeting the integrin alone	(273)
	BMP-2, BMP-7	*In vitro* (hMSCs from healthy and osteoporotic patient)	↓ in alpha2 integrin subunit was concomitant with a ↓ in cell migration induced by the BMPs compared with control	(285)
Alpha5 Beta1	BMP-2	*In vitro* and In vivo (MSCs, Sprague-Dawley rats)	Fibronectin derived fragments: -↑ cell adhesion over hyaluronic acid hydrogel alone -↑ bone formation *in vivo* than hyaluronic acid hydrogel alone	(286)
	VEGF-A (20 ng/mL)	*In vitro* (HUVEC)	-↑ in amounts of alpha5 and beta1 integrin subunits (2-4 h) -↑ in COX-2 mRNA induced by VEGF-A	(232)
	IGF-2	*In vitro* (hMSCs)	-Silencing alpha5 and beta1 integrin subunits ↓ IGF-2 synthesis -Alpha5 beta1↑IGF-2 signaling via FAK and PI3K pathways	(287)
Alphav Beta3	VEGF	*In vitro* (HUVEC)	-↑ beta3 integrin phosphorylation in the presence of VEGF -Used antibodies blocking alphav and beta3 integrin subunits to inhibit VEGFR-2 phosphorylation	(288)
	FGF-1	*In vitro* (NIH3T3)	-FGF-1 bound directly to alphav beta3	(289)
	FGF-1	*In vitro* (NIH3T3, HUVEC)	-FGF-1 formed ternary complex with the integrin alphav beta3 and the FGFR needed to maintain Erk1/2 phosphorylation	(290)
	IGF-1 (200 ng/mL)	*In vitro* (OSE-3T3)	-IGF-1 promoted Cbfa1 activity using the PI3K pathway -Used an inhibitor of alphav beta3 occupancy to inhibit Cbfa1 activity induced by IGF-1	(291)
	BMP-2	*In vitro* (MC3T3-E1)	-rCYR61 influenced the differentiation of MC3T3-E1 preosteoblasts by BMP-2 via the alphav beta3 integrin -↑ in rCYR61 induced an↑in BMP-2 synthesis	(292)
	pBMP-9 (400 ng/mL)	*In vitro* (MC3T3-E1)	-pBMP-9 induced↑in alphav integrin subunit in cell membrane -pBMP-9 induced more↑in ALP activity after 24 h than in unstimulated cells	(233)
	RGD+pBMP-2	*In vitro* (hMSCs)	-↑ mineralization of hBMSCs after 14 days more than pBMP-2 or RGD alone	(279)
pBMP-2: peptide-derived from BMP-2				

together with chitosan, into the quadriceps of mice (278). However, the early differentiation of murine MC3T3-E1 preosteoblasts seems to depend on the type of integrins involved in cell adhesion. Marquis *et al.* found that murine MC3T3-E1 preosteoblasts in the presence of pBMP-9 had enhanced ALP activity when they were adhered to polystyrene coated with BSP-derived peptides, while cells on peptides DGEA-coated polystyrene targeting alpha2 beta1 integrins showed no such increase (233).

Selection of the adhesive peptides to combine with peptides derived from BMPs is therefore critical. He *et al.* immobilized the adhesive peptides GRGD (1.62 pmol/cm^2) and peptide P4 derived from BMP-2 (KIPKASSVPTELSAISTLYL) (5.2 pmol/cm^2) on PLEOF hydrogels (27). This combination acted synergistically on the commitment of rat MSCs to osteoblast lineage by increasing the ALP activity and stimulating better mineralization of the matrix

than did peptides grafted alone (27). These same two peptides have been used to functionalize self-assembled monolayers using click chemistry, to create a concentration gradient (279). The combination of equal amount of GRGDS and P4 (65 pmol/cm^2 of each peptide) synergistically up-regulated the number of BSP transcripts in human bone marrow stromal cells and mineralization of their matrix without any osteogenic supplements (279). Peptides derived from the knuckle epitope of BMP-2 (KIPKACCVPTELSAISMLYL), BMP-7 (TVPKPSSAPTQLNAISTLYF) and BMP-9 (KVGKASSVPTKLSPISILYK) and adhesive peptide (GRGDSPC) have been grafted onto the surface of polyethylene terephthalate (PET) to investigate the differentiation of mouse MC3T3-E1 preosteoblasts (280). These modified PET increased the cell contents of Runx2 transcripts and the production of ECM. A recent study that evaluated the synergistic effect of a peptide derived from BMP-7 (GQGFSYPYKAVFSTQ) and a cyclic RGD peptide grafted to a quartz substrate on the behavior of MSCs found that these two peptides increased the ALP activity and the matrix mineralization (281).

Another recently developed strategy uses self-assembled peptides that mimic the matrix (for review see (282)). These peptides can have a beta sheet motif and even a coiled-coil motif to assemble alpha helices into ordered structures. The osteoblastic differentiation of C2C12 cells can be increased by peptides amphiphiles TSPHVPYGGGS that bind with high affinity to BMP-2 (283).

We therefore should investigate combinations of several BMPs and growth factors with adhesive peptides so as to develop osteoinductive biomimetic materials that act in synergy to favor bone healing. This will be challenging because growth factors can trigger antagonistic intracellular signaling (8). BMP-2, BMP-7 and BMP-9 are not members of the same BMP subfamily and they interact with different type I receptors. They therefore activate different non-canonical pathways. Our research group has recently demonstrated that BMP-2 and BMP-9 did not act synergistically when used to treat MC3T3-E1 preosteoblasts seeded on PCL films functionalized with a peptide derived from BSP due to different level of activation of the canonical Wnt pathway (21).

7. CONCLUSION

Bone healing process involves complex interactions between several cell types and signaling molecules. Autografts, the current gold standard for repairing bone defects, have several limitations. Therefore, biomaterials functionalized with adhesive peptides that favor bone cell attachment have been combined with growth factors, especially BMPs and their derived peptides, in order to optimize bone healing. However, it is essential to understand the crosstalk between the integrins, which interact with these adhesive peptides, and growth factors so as to understand the signaling that will direct cell behavior such as the ability of stem cells to differentiate into mature osteoblasts. While it is true that using combinations of adhesive peptides and BMPs or their derived peptides to create biomimetic materials has given promising results, challenges remain, especially questions of peptide density, conformation, graft stability and sterilization methods.

8. PERSPECTIVES

We still face numerous challenges as we strive to create osteoinductive biomimetic materials in 3D using bioactive molecules since most current studies are performed in 2D cultures. Moreover, most studies have focused on the impact of BMPs on bone cell responses despite the fact that many other growth factors, such as FGF and VEGF, are involved in bone healing. We need a combination of several growth factors that together optimize the action of bone substitutes. Finding such a combination will not be easy, but will promise great benefits for the patients.

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Abbreviations: ACS: Absorbable collagen type I sponge, AdBMP: adenovirus encoding BMP, AdSC: adipose derived stem cell, ALP: alkaline phosphatase, AP-1: activating protein-1, APC: adenomatous polyposis coli, ASARM: acidic serine-rich and aspartate-rich motif, BAMBI: BMP and activin membrane-bound inhibitor, BCP: biphasic calcium phosphate, beta T: beta tail, BMP: bone morphogenetic protein, BMPR: BMP receptor, BSA: bovine serum albumin, BSP: bone sialoprotein, CAS: Crk-associated substrate, CBD: decapeptide collagen-binding, Cdc42: cell division cycle 42, CPTES: (3-chloropropyl) triethoxysilane, CSL: CBF1, Suppressor of Hairless, Lag-1, DAG: diacylglycerol, DAP12: 12 kDa DNAX-activating protein, DC-STAMP: dendritic cell-specific transmembrane protein, Dkk-1: Dickkopf-1, Dvl: Dishevelled, E1: epidermal Growth Factor domain 1, ECM: extracellular matrix, EGF: epidermal growth factor, EphB4: Ephrin type-B receptor 4, ERK1/2: extracellular signal regulated kinase 1/2, F-actin: filamentous actin (stress fiber), FAK: focal adhesion kinase, FERM: N-terminal ezrin radixin moesin, FGF: fibroblast growth factor, FGFR: FGF receptor, Fzd: Frizzled, GAP: GTPase activating protein, GEF: guanine exchange factor, GFs: growth factors, Grb2: growth factor receptor-bound protein 2, GSK-3 beta: glycogen synthase kinase-3 beta, HAP: hydroxyapatite, Hes: Hairy enhancer of split, Hey: Hes-related with the YRPW motif, Hh: Hedgehog, hMSC: human MSC, IFN gamma: interferon-gamma, IGF: insulin-like growth factor, IGFIR: IGF type I receptor, IL: interleukin, ILK: Integrin-linked kinase, IP3: inositol-1,4,5-trisphosphate, IRS-1: insulin receptor substrate-1, I-Smad: inhibitory Smad, JNK: c-jun N-terminal kinase, KRSR: Lys–Arg–Ser–Arg, LRP5/6: low density lipoprotein receptor related protein 5 and 6, MAML: Mastermind-like, MAPK: mitogen activated protein kinase, M-CSF: macrophage-colony stimulating factor, MEPE: matrix extracellular phosphoglycoprotein, MMP: matrix metalloproteinases, MSC: mesenchymal stem cell, NFATc1: nuclear factor of activated T cells cytoplasmic 1, NICD: Notch intracellular domain, OC: osteocalcin, OPG: osteoprotegerin, OPN: osteopontin, OSCAR: osteoclast-associated receptors, Osx: osterix, pBMP-2: peptide derived from BMP-2, pBMP-9: peptide derived from BMP-9, PCL: polycaprolactone, PDGF: platelet-derived growth factor, PEG: poly (ethylene) glycol, PET: polyethylene terephthalate, PI3K: phosphatidyl inositol 3 kinase, PLCgamma: phospholipase C gamma, PLEOF: poly(lactide-ethylene oxide fumarate), PLLA: poly (L-lactide), PPM1A: protein phosphatase, Mg^{2+}/Mn^{2+} dependant, 1A, PS: polystyrene, PTH: parathyroid hormone, Rac1: Ras-related C3 botulinum toxin substrate 1, RANKL: receptor activator of NF-kappaB ligand, RBP-J: recombinant recognition sequence binding protein at the Jkappa site, RGD: Arg-Gly-Asp, Sema4D: semaphorin 4D, Shc: Src homology 2 domain-containing, SIBLING: small integrin-binding ligand N-linked glycoproteins, siRNA: small interfering RNA, SLRP: small leucine-rich proteoglycans, Sos: son of sevenless, SP-1: sphingosine-1 phosphate, STKR: Ser/Thr kinase receptor, TAK1: TGF-beta activated tyrosine kinase 1, TCF/LEF: T-cell factor/ lymphoid enhancer factor, TGF-beta: transforming growth factor-beta, Ti: titanium, TiO_2: titanium dioxide, TNF: tumor necrosis factor, TRAF6: TNF receptor associated factor-6, TRAP: tartrate resistant acid phosphatase, TREM-2: triggering receptor expressed in myeloid cells 2, VEGF: vascular endothelial growth factor, WASP: Wiscott-Aldrich syndrome protein, WHO: World Health Organization, XIAP: X-linked inhibitor of apoptosis

Key Words: Alpha Integrin Subunit, Adhesion, Angiogenesis, Apoptosis, Beta Integrin Subunit, Biomaterial, Biomimetic, BMP, Bone, Bone Remodeling, Bone Substitute, Collagen, Differentiation, ECM, Extracellular Matrix, Fibrillar Adhesion, Fibronectin, Focal Adhesion, Focal Complex, Growth Factor, GTPase, Healing, Integrin, Interaction, Kinase, MAPK, Osteoblast, Osteoclast, Osteoinduction, Osteoconduction, Osteocyte, Peptide, Polymer, Proliferation, Receptor, RGD, Scaffold, Signal Transduction, Smad, TGF, Vascularization, VEGF, Wnt, Review

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