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Biomimetic materials for controlling bone cell responses

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1. ABSTRACT

Bone defects that cannot “heal spontaneously during life” will become an ever greater health problem as populations age. Harvesting autografts has several drawbacks, such as pain and morbidity at both donor and acceptor sites, the limited quantity of material available, and frequently its inappropriate shape. Researchers have therefore developed alternative strategies that involve biomaterials to fill bone defects. These biomaterials must be biocompatible and interact with the surrounding bone tissue to allow their colonization by bone cells and blood vessels. The latest generation biomaterials are not inert; they control cell responses like adhesion, proliferation and differentiation. These biomaterials are called biomimetic materials. This review focuses on the development of third generation materials. We first briefly describe the bone tissue with its cells and matrix, and then how bone cells interact with the extracellular matrix. The next section covers the materials currently used to repair bone defects. Finally, we describe the strategies employed to modify the surface of materials, such as coating with hydroxyapatite and grafting biomolecules.

2. INTRODUCTION

Bone is a dynamic tissue that plays a crucial role in protecting, shaping the body, ensuring its structural integrity, and enabling it to move. It is also involved in mineral homeostasis and its cavities are filled with bone marrow containing the hematopoietic and mesenchymal stem cell (MSC) niches (1). About 5 to 10% of the 5.6 million fractures that occur each year in the USA result in slow or inefficient bone repair (2). These problems will become more frequent as Western populations become older and more and more people suffer from bone disorders like osteoporosis. Bone defects of a critical size caused by fractures, tumor resection or infection cannot self-repair. They must therefore be filled by grafting or inserting biomaterials during orthopedic surgery.

Biomaterials must have properties similar to those of an autograft, which is the gold standard used by surgeons for bone replacement. They must be readily integrated into the bone, conduct bone signals and induce new bone formation. They also have to be biocompatible and have appropriate mechanical properties. An osseointegrative material must interact strongly with the host bone tissue, while an osteoconductive one must be colonized by host bone cells and blood vessels. Finally, osteoinductive materials must stimulate host MSCs to differentiate into bone-forming cells (3).

Third generation biomaterials of natural or synthetic origin have been developed over the past decade. These can mimic the architecture of bone tissue and its biological properties. They can control the responses of cells that adhere to them through interactions that activate specific intracellular signaling. They may help to replace autografts, which have several drawbacks. Not least is the fact that harvesting autograft requires additional surgery for the patient, placing him/her at increased risk of blood loss, pain and morbidity at both the donor and acceptor sites (3). Added to this, the quantity of bone tissue available is limited, while the shape, structure and resorption rate of the harvested bone may be very different from that of the implant site (4). For example, autografts for nonunions are usually harvested from the iliac crest to limit their structural alteration, but their volume can vary widely depending on the harvest site (anterior or posterior). The volume of anterior iliac crest grafts can range from 5.3 to 72 cm^3 (5,6). The volumes of intramedullary bone grafts harvested from the femur and tibia are larger than those from the iliac crest and they also contained more osteogenic growth factors like the bone morphogenetic proteins (BMPs). Their great disadvantage is that they are made up of nonstructural bone (for review see, 6). Allografts can also be used but they can frequently provoke immune response and be a source of disease, although this last risk is very low (1 in 1.6 million for human immunodeficiency virus) (3,7).

For all the above reasons, this review focuses on the development of biomimetic materials for filling bone defects and promoting bone cell adhesion, survival and differentiation. They may then become the new gold standard for surgeon. We first briefly review bone tissue, with its cells and matrix. We then examine the ways bone cells interact with extracellular matrix (ECM), since most of the new materials will have to mimic these properties. This is followed by a discussion of the biomaterials currently used in bone defect replacement such as bioceramics, polymers and composites. Finally, we examine the strategy used to modify the surface properties of these biomaterials to increase their ability to interact with bone cells and promote new bone formation.

3. BONE TISSUE

Ossification can occur by two processes, endochondral bone formation and intramembranous ossification, that take place in embryonic development. The skeletons of adult humans are made up of compact and trabecular bones, which have different histological organization and porosities (8). Bones are continuously being remodeled by anatomical structures called the basic multicellular units (BMU) that involve two types of specific bone cells, osteoclasts and osteoblasts (9). Osteoclasts are responsible for bone resorption while osteoblasts synthesize the organic bone matrix, the osteoid, and facilitate its mineralization. The osteoblasts become engulfed in mineralized osteoid where they develop into osteocytes (10). These cells are also involved in bone turnover (11).

3.1. Osteoclasts, osteoblasts and osteocytes

3.1.1. Osteoclasts

Mature osteoclasts are multinuclear cells that differentiate from mononuclear myeloid precursors by fusion of cells of the monocyte/macrophage lineage. Osteoclastogenesis depends on two cytokines, macrophage-colony stimulating factor (M-CSF) and receptor activator of nuclear factor kappa beta ligand (RANKL), which is mainly provided by osteoblasts (12). RANKL can also be a soluble protein after its cleavage from the cell surface (13).

RANKL binds to the receptor activator of nuclear factor kappa beta (RANK). This homotrimeric transmembrane protein is a member of the tumor necrosis factor (TNF) receptor superfamily and is located at the membrane of osteoclast precursors. The resulting RANKL-RANK complex promotes the survival and differentiation of the precursor cells (14). RANKL-RANK binding activates a downstream signaling pathway to stimulate several transcription factors such as nuclear factor of activated T cells (NFATc1) that are essential for osteoclast differentiation (15,16). RANKL seems to act in synergy with BMP-2 to increase the differentiation of osteoclasts from bone marrow derived cells in culture (17). However, BMP-2 alone cannot activate the genes encoding osteoclast markers such as NFATc1 (17). RANKL also favors the retention of the osteoclast precursors within the tissue by down-regulating the gene encoding a receptor (S1PR1), so reducing the amount of this protein available to bind the lipid mediator sphingosine-1 phosphate (SP-1) (18). The circulating concentration of SP-1 is high, producing a blood-bone gradient that has a positive chemotaxis effect causing osteoclast precursors to move from the bone to

blood vessels (18). The retention of osteoclast precursors to bone is favored by another SP-1 receptor, S1PR2. S1PR1 activates the small GTPase Rac, while S1PR2 stimulates Rho limiting the positive chemotaxis induced by SP-1 (19).

Several factors, including parathyroid hormone (PTH) and vitamins 1alpha, 25-dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$) increase the expression of the RANKL gene (20). In contrast, osteoprotegerin (OPG), a soluble decoy receptor secreted by osteoblasts, stromal cells and B lymphocytes, inhibits the maturation of osteoclasts by sequestering RANKL. This prevents the subsequent activation of the RANK pathway and bone resorption (21). The RANK/RANKL/OPG system that controls osteoclastogenesis is also regulated by cytokines like interleukins (IL) 1 and 6, and TNFalpha (22).

Mature osteoclasts have a lifespan of about 2 weeks and produce specific proteins such as the calcitonin receptor and H^+-adenosine triphosphatase (22,23). They resorb bone matrix to form Howship lacunae that can be from 40 to 60 microns deep, depending on the age of the individual (24).

3.1.2. Osteoblasts

Osteoblasts develop from MSCs that can also differentiate into adipocytes and chondrocytes in response to specific transcription factors (25). MSCs can be induced to undergo osteogenic differentiation by BMPs, through the runt-related transcript factor 2 (Runx2), while the peroxisome proliferator-activated receptor (PPAR) gamma 2 is involved in their differentiation into adipocyte lineage cells (25,26). Mori *et al.* (2006) found that Runx2 promoted osteoblast-mediated osteoclastogenesis and bone resorption by activating a cyclic AMP protein kinase A pathway that in turn stimulates the RANKL gene (27). However, Kitazawa *et al.* (2008) recently demonstrated that Runx2 has a net negative effect on steady state RANKL gene expression, while it has a positive one in response to 1,25-$(OH)_2D_3$ (28). Other transcription factors such as osterix (Osx) and members of the Dlx homeobox family are also required for MSCs to differentiate into osteoblasts (29). Transfection of Dlx5 into immature chick calvaria cells stimulates their osteogenic differentiation via the synthesis of Runx2 and osteopontin (29).

Differentiation of MSCs to form osteoblasts is controlled by hormones, vitamins and growth factors like fibroblast growth factor (FGF), insulin-like growth factor and BMPs (30). The BMPs, which belongs to the transforming growth factor (TGF) beta family, have been divided into sub-groups based on their amino acid sequences. For example, BMP-2 and BMP-4 form one sub-family. BMPs act on cells as dimers by binding to two specific type I and type II transmembrane Ser/Thr kinase receptors (31). The binding of BMP causes the type II receptors to phosphorylate the type I receptors, which in turn activate the Smad pathway by phosphorylating Smad1, Smad5 and/or Smad8. These phosphorylated proteins, the regulatory Smads, then form a complex with Smad4. This complex can be translocated into the nucleus where it regulates, in association with Runx2, the transcription of target genes like those encoding bone sialoprotein (BSP) and osteocalcin (OC) (32). Cheng *et al.* (2003) used human pluripotent MSCs transfected with a recombinant adenoviral vector encoding BMP (AdBMP) to study the effect of BMP-2 to BMP-15 on osteogenic differentiation (33). They found that AdBMP-2, AdBMP-6 and AdBMP-9 increased the alkaline phosphatase (ALP) activity of both pluripotent C3H10T1/2 mesenchymal progenitors and C2C12 myoblast cells *in vitro* (33). Kang *et al.* (2004) injected transformed C2C12 cells with AdBMPs into mouse quadriceps muscles and showed that AdBMP-6 and AdBMP-9 induced rapid ossification more efficiently than AdBMP-2 or AdBMP-7 (34). Similarly, Wutzl *et al.* (2010) recently showed that a combination of BMP-2, BMP-5 and BMP-6 (100 ng/mL) acted additively on the expression of the gene encoding Osx in mouse bone marrow cells, while they decreased the number of tartrate-resistant acid phosphatase (TRAP)-positive multinucleated cells more effectively than did BMP-2 alone (35). BMPs are therefore potential targets of treatment to enhance the differentiation of MSCs into bone-forming cells. BMP-2 and BMP-7 are currently approved by the USA food and drug administration for certain clinical applications such as spinal fusion and fracture healing (36).

Mature osteoblasts have a lifespan of about 3 months and synthesize the major components of the organic bone matrix, the osteoid, at a rate of 2 to 3 microns per day (22,37).

3.1.3. Osteocytes

There are about 10 times more osteocytes, the terminal differentiation state of osteoblasts embedded within the bone matrix, than there are osteoblasts in adult human bone (11). They are located in lacunae near blood vessels that provide the nutrients and oxygen required for their survival. They communicate with each other and surrounding bone cells by dendritic cell processes that extend from them through the canniculi to form gap junctions with neighboring osteocytes (38). Osteocytes are very long-lived, with a half life of about 25 years (39). However, they can also become senescent and die by necrosis or apoptosis in response to microdamage and bone turnover (40).

Recent studies have highlighted their role in bone remodeling (11,41-43). Heino *et al.* (2009) demonstrated that mechanically damaged MLO-Y4 osteocyte-like cells produce M-CSF and RANKL that promote the differentiation of osteoclast precursors (11). Osteocytes also send signals to osteoblasts. They secrete sclerostin, a soluble glycoprotein belonging to the DAN family that is encoded by the gene SOST. Sclerostin binds to low density lipoprotein receptor-related protein 5/6. This binding inhibits activation of the Wnt pathway and bone formation (16,44). Sclerostin can also inhibit BMP-7 by sequestering it and directing it to breakdown by proteasomes (44).

3.2. Bone Matrix

3.2.1. Osteoid

3.2.1.1. Proteins and proteoglycans

Collagen type I is the most abundant protein of the organic bone matrix. Procollagen type I is a triple helix consisting of two alpha1 chains and one alpha2 chain, each about 300 nm long (about 1000 amino acids) with a

Table 1. Non-collagenous proteins

Protein	Molecular weight (MW) and post-translational modifications	Effects on osteoblasts	Effects on osteoclasts	References
Osteocalcin	- 49 amino acids (5-8 kDa) - 3 vitamin K dependent γ carboxylated glutamic acid (Gla) residues (porcine osteocalcin) - Structure stabilized by Cys_{23}-Cys_{29} disulfide bond - Negatively charged protein surface able to coordinate five calcium ions	↓ Mineralization	↑ Differentiation and maturation of osteoclast precursors by osteocalcin fragment	(52-55)
Osteonectin	- 32-35 kDa - Single chain acidic glycoprotein rich in cysteine - Post-translational glycosylation modifications inducing a MW of about 45 kDa	↑ Mineralization	↑ Cell adhesion	(56,57)
SIBLING proteins				
Osteopontin (BSP-1)	- 301 amino acids (rat) - Post-translational modifications (phosphorylation on serine, O/N-linked glycosylation) inducing a MW of about 60 kDa - RGD cell binding motif	↑ Cell adhesion ↑ Differentiation	↑ Cell adhesion ↑ Bone resorption	(56,58,59)
BSP	- 327 amino acids (33-34 kDa) - Post-translational modifications (serine/threonine phosphorylation, tyrosine sulfation, N/O-linked glycosylation) increasing the MW of BSP up to 75 kDa - RGD cell binding motif	↑ Cell adhesion ↑ Mineralization	↑ Cell adhesion ↑ Differentiation ↑ Bone resorption	(58,60,61)
SLRP				
Type I (biglycan)	- 200-350 kDa - Consist of a leucine rich protein core (45 kDa) with two small chondroitin/dermatan sulfate glycosaminoglycan (GAG) chains	↑ Differentiation ↑ Mineralization	↑ Osteoclastogenesis due to defective osteoblasts	(62)
Type II (decorin)	- 90-140 kDa - Leucine rich protein core (40 kDa) with a chondroitin sulfate/dermatan sulfate GAG chain	↓ Mineralization by modulating collagen assembly		(63)
Adhesive proteins				
Fibronectin	- 2 subunits of about 235-250 kDa linked by 2 disulfide bonds - 3 types of repeating units: 12 type I repeats, 2 type II repeats and 15-17 type III repeats - RGD cell binding motif located in repeat III_{10} and its synergy site PHSRN located in repeat III_9	↑ Cell adhesion ↑ Cell survival ↑ Cell proliferation ↑ Differentiation ↑ Mineralization	↓ Osteoclastogenesis ↑ Bone resorption	(64,65)
Thrombospondin	- 120-150 kDa - RGD cell binding motif	↑ Cell differentiation	↑ Bone resorption	(58)

Adapted from 51

diameter of about 1.5 nm (for review see 45,46). The triple helix can form because the chains contain a $(Gly\text{-}X\text{-}Y)_n$ repeat sequence that places the smallest amino acid Gly at the helix center (45). The C-terminal and N-terminal telopeptides of the procollagen triple helix are cleaved by proteases in the ECM just after its secretion. This cleavage causes the collagen monomer to assemble into fibrils (45,47,48). The collagen monomers in these fibrils are staggered side by side with an axial periodicity (D-band) of about 67 nm between neighbors (48,49). Collagen fibrils are stabilized by intramolecular and intermolecular crosslinks that include covalent and hydrogen bonds between amino acids and pyridoline bridges between lysines and hydroxylysines (45). The deformation state, water content and nanoscale hierarchical level of collagens influence the way they modulate the mechanical properties of bone such as its ductility, flexibility and fracture resistance (50). Gautieri *et al.* (2011) recently found that hydrated collagen microfibrils had a Young's modulus of about 300 MPa when subjected to a small deformation, but a higher tangent stiffness (E=1.2 GPa) when subjected to larger ones (>10%) (50).

Various non-collagenous proteins such as the small integrin-binding ligand N-linked glycoprotein (SIBLING) family of proteins that includes osteopontin (also called BSP-1) and BSP play a crucial role in bone cell functions (Table 1). SIBLING proteins undergo extensive post-translational modification, such as N/O- linked glycosylation, sulfation and/or phosphorylation (59). They are also recognized by specific cell membrane receptors, the integrins, through their RGD tripeptide sequence (Table 2). Huang *et al.* (2008) used sequential extraction to determine the distribution of SIBLING proteins in the inorganic and organic phases of rat bone (66). They found BSP in all bone extracts, suggesting that this protein binds tightly to both type I collagen and hydroxyapatite (HAP) crystals. BSP and osteopontin may be involved in mineralization (61,67,68). Yang *et al.* (2010) recently showed that BSP favors the nucleation of amorphous calcium phosphate (61). In addition, the bones of osteopontin- deficient mice are less resistant to fracture than are those of normal mice because the calcium concentration in their matrixes is more variable (69). Bone osteoid also contains OC, a Gla protein that is synthesized by osteoblasts and regulates mineralization (53,54). The OC fragment corresponding to residues 7-36 of bovine OC (10 ng/mL) can, when combined with M-CSF and RANKL, also increase the differentiation of $Mac\text{-}1^+$ $c\text{-}fms^+$ osteoclast precursors isolated from the bone marrow of femurs of 10

Table 2. Amino acid abbreviations

Code	Amino acid
A	Alanine
C	Cysteine
D	Aspartic acid
E	Glutamic acid
F	Phenylalanine
G	Glycine
H	Histidine
I	Isoleucine
K	Lysine
L	Leucine
N	Asparagine
O	Hydroxyproline
P	Proline
R	Arginine
S	Serine
T	Threonine
V	Valine
Y	Tyrosine

Table 3. Integrin subunits in osteoblasts and osteoclasts

Beta integrin subunits	Alpha integrin subunits	Ligands	Function	References
beta 1	alpha 1	Collagens	↑ Osteoblast differentiation induced by BMP-2 - Involvement in osteoclast adhesion and bone resorption	(74,75)
	alpha 2	Collagens		
	alpha 3	Collagens, fibronectin, thrombospondin		
	alpha 4	Fibronectin		
	alpha 5	Fibronectin		
	alpha 6	Laminins		
	alpha v	Fibronectin		
beta 3	alpha v	BSP, fibronectin, osteopontin, thrombospondin, vitronectin	Osteoblast adhesion and differentiation Blocking antibodies to alphavbeta inhibit osteoblast differentiation induced by BMP-2 Osteoclast adhesion and function Major integrin in mature osteoclasts: - Mice lacking beta3 gene had osteoclast dysfunction (no ruffled border and actin ring formation) leading to osteopetrosis - Overexpression of alphav integrin subunits promotes osteoclast differentiation	(76,77) (78,79)
beta 5	alpha v	Vitronectin	↑ Osteoblast adhesion (*in vitro*) ↓ Osteoclast differentiation - Mice lacking gene encoding beta5 integrin subunit have accelerated osteoclast differentiation	(80)

Adapted from 51

week old male mice (55). Adhesion proteins like fibronectin interact with bone cells via integrins to regulate bone cell activity. They influence the ability of both MSCs and osteoblasts to migrate, proliferate and differentiate (70).

Small leucine-rich proteoglycans (SLRP) like biglycan and decorin modulate HAP crystal growth (71). Decorin, which has a leucine-rich protein core and a chondroitin sulfate/dermatame sulfate glycoaminoglycan chain, is the major proteoglycan in bone. Decorin can link collagen fibrils together at the D-band and thus modulate the assembly of collagen fibrils. This, in turn, regulates matrix mineralization (63).

3.2.1.2. Integrins

The cell alphabeta transmembrane receptors called integrins link the cell cytoskeleton to the ECM proteins (72). Integrins that are bound to ECM proteins initiate intracellular outside-in signaling, but their affinity can also be regulated intracellularly by inside-out signaling. A total of 24 alphabeta integrins have been identified to date, made up of 8 beta subunits and 18 alpha subunits (72). They mainly interact with the RGD cell binding motif of certain ECM proteins (64,73). Some integrins are specific for a given protein. Thus, alpha5beta1 integrins interact with fibronectin, while alphavbeta3 integrins bind fibronectin, vitronectin, BSP and osteopontin. A given protein can also be recognized by several integrins. For example, collagen type I can interact with alpha1beta1, alpha2beta1 and alpha3beta1 integrins (Table 3).

MSCs express alpha5beta1 and alpha2beta1 integrins and smaller amounts of alphavbeta3 integrins (70). Osteoblasts can contain alpha 1-6, alphav and beta1, 3 and 5 integrin subunits depending on their differentiation state (77). Immature osteoclasts do not bear alphavbeta3 but do have alphavbeta5 (81). The alphavbeta3 integrin is the major functional receptor on mature osteoclasts (82).

Osteoblasts in 2D culture, whose integrins bind to ligand, become organized to form several types of adhesions, such as small focal complexes, focal and fibrillar adhesions. Focal adhesions located at the cell periphery anchor actin stress fibers through integrin receptors such as the alphavbeta3 integrins and many

Table 4. Composition of bovine bone matrix

Ion	% wt.
Calcium	34.8
Phosphorus	15.2
Sodium	0.9
Magnesium	0.72
Potassium	0.03
Carbonate	7.4
Fluoride	0.03
Chloride	0.13
Pyrophosphate	0.07
Total inorganic	65
Total organic	25
Adsorbed H_2O	10
Trace element (Sr, Pb, Zn, Cu, Fe…)	

Adapted from 92

intracellular structural proteins like talin and vinculin. Signaling proteins, especially focal adhesion kinase (FAK), are also involved in outside-in signaling (83,84). Osteoblasts can organize fibrillar adhesions made of alpha5beta1 integrins-fibronectin complexes located at the cell centre (85). By contrast, adherent osteoclasts form a unique type of matrix adhesion known as a podosome, which has a dense actin core surrounded by a rosette-like structure containing many proteins including alphavbeta3 integrins, vinculin and actin-associated proteins. Signaling proteins such as tyrosine kinases (c-Src and Pyk2) and RhoGTPases are also involved in podosome formation (86).

The behavior of osteoblasts and osteoclasts depends on these interactions between integrins and their ligands (87). For example, collagen-alpha2beta1 integrin interactions are involved in the differentiation of murine MC3T3-E1 preosteoblasts; they act by activating FAK and extracellular signal-regulated kinase (ERK) (88). Mice lacking the gene encoding the integrin beta3 subunit (beta3$^{-/-}$) have more osteoclasts than normal mice, but they are dysfunctional. They lack a ruffled border and cannot form actin rings (78). Bone marrow macrophages extracted from beta3$^{-/-}$ mice do not differentiate into osteoclasts when cultured under standard osteoclastogenesis conditions (100 ng/mL RANKL and 10 ng/mL M-CSF) (79). But a higher dose of M-CSF (100 ng/mL) restores osteoclastogenesis due to ERK signaling rescue (79). However, the beta 3$^{-/-}$ osteoclasts cannot resorb dentine slices (79). Lane *et al.* (2005) also highlighted the role of the beta5 integrin subunit in osteoclastogenesis *ex vivo* and *in vivo* (80). Bone marrow monocytes/macrophages extracted from ovariectomized three-month old beta5$^{-/-}$ female mice and cultured in medium containing M-CSF and RANKL for 7 days showed more osteoclast differentiation than monocytes/macrophages from wild type mice (80). Deleting the beta5 integrin subunit also increased osteoclastogenesis *in vivo* (80).

Recent studies have also demonstrated that integrins are involved in the activation of growth factors like TGF-beta (for review see 89). The interaction of integrins with specific components of the osteoid strongly influences the efficiency of BMPs. For example, early osteoblast differentiation induced by BMP-2 depends on alpha2beta1 integrins (74). Antibodies that block alphavbeta3 and alphavbeta5 integrins also prevent BMP-2 from stimulating ALP activity in human osteoblasts (77). Su *et al.* (2010) used MC3T3-E1 preosteoblasts to show that the capacity of a recombinant cysteine-rich protein 61 to mediate the synthesis of BMP-2 and thus osteoblastic differentiation depends on alphavbeta3 integrins (90).

Lastly, BMP-2 can influence integrin signaling in human osteoblasts by inducing the phosphorylation of FAK (91). BMP-2 increases also the amounts of several integrins (alphavbeta3, alphavbeta5, alphavbeta6 and alphabeta1) in the membranes of human osteoblasts (77).

3.2.2. Mineral matrix

The mineral components of bone account for 65% of the bone mass (37). It is composed of calcium and phosphate ions combining to form small HAP crystals with some carbonate substitutions. It also contains magnesium, sodium, potassium, fluoride and manganese (Table 4).

3.2.2.1. Matrix vesicles

Calcification begins with the nucleation of HAP crystals within matrix vesicles that bud off from the surface membrane of hypertrophic osteoblasts or chondrocytes (93). These matrix vesicles are spherical bodies of 20-200 nm that are involved in both endochondral and intramembranous ossification (94). Several channels in the matrix vesicle membrane take part in calcification; the annexins allow calcium to enter, while type III Na/Pi cotransporters mediate phosphate entry (95). Calcium binding acidic phospholipids like phosphatidyl serine may also help retain calcium in matrix vesicles (93). $CaPO_4$ minerals precipitate inside the matrix vesicle when the calcium and phosphate ion concentrations exceed their solubility (93). The calcium phosphate crystals growing inside the matrix vesicles eventually perforate their membranes.

The hydrolysis of inorganic pyrophosphate (PPi) to phosphate by tissue non-specific ALP is crucial for the growth of HAP crystals in the matrix vesicles since PPi prevents crystal formation (96). PPi is generated by enzymes like nucleotide pyrophosphatase phosphodiesterase 1 located in the membrane of matrix vesicles (93).

3.2.2.2. Collagen fibril mineralization

Osteoid mineralization begins with nucleation of the HAP crystals and their deposition between the collagen fibrils in the extracellular bone matrix (97). Collagens and certain non-collagenous proteins (eg. BSP) are involved in this process (61).

However, the details of biological mineralization are still unknown (98). Chung *et al.* (2011) recently identified peptides that facilitate the growth of HAP crystals *in vitro* (98). The most effective peptide was composed of 12 amino acids, especially proline and hydroxylated amino acid residues like serine, threonine and tyrosine. These mimic the GPO sequence that is characteristic of the major motif in type I collagen. Nudelman *et al.* (2010) also showed that regions like the

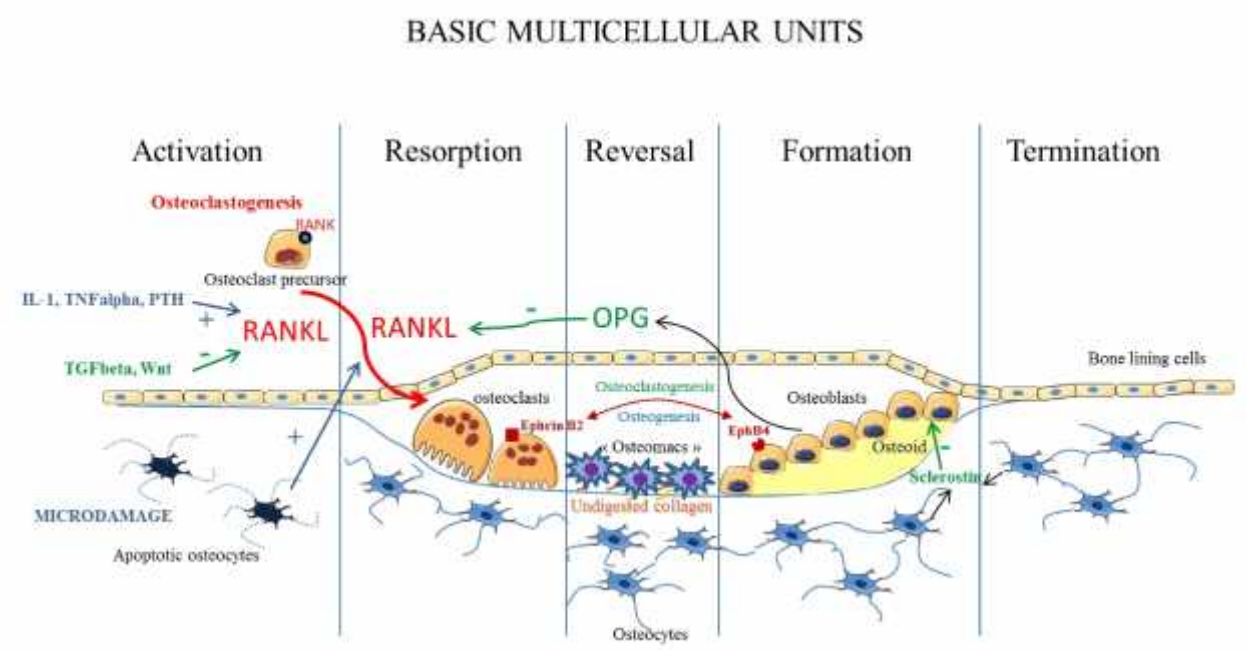

Figure 1. Bone remodeling cycle with active BMU [Illustration adapted with permission from (16) using Servier Medical Art, http://www.servier.fr]. Abbreviations: IL: interleukin, OC: osteocalcin, OPG: osteoprotegerin, PTH: parathyroid hormone, RANK: Receptor Activator of NF-kappaB, RANKL: Receptor Activator of NF-kappaB Ligand, TNF: Tumor Necrosis Factor.

positively charged domain in the C-terminal of collagen molecules favor the infiltration of amorphous calcium phosphate into the fibrils in the presence of inhibitors of HAP nucleation (97). Collagen itself also controls the nucleation of amorphous calcium phosphate to form apatite crystals (97).

3.2.2.3. Effect of ions on bone cell behaviors

Ions like calcium and magnesium that are released by the resorption of bone mineral matrix influence the behavior of bone cells and the growth of HAP crystals (99,100). For example, adding calcium ions to the culture medium decreases osteoclast formation by binding to specific receptors like the calcium sensing receptor (CaSR) (100, 101). However, the role of the CaSRs in bone remodeling is still debated because some osteosarcoma cell lines (MG-63, SaOs-2, U2-Os) and osteoclasts have little or none of this receptor (102). Magnesium-deficient animals also have brittle bones (for review see 99). A recent study showed that magnesium ions may inhibit HAP nucleation and crystal growth by forming magnesium-phosphate ion pairs (103).

3.3. Bone remodeling

Healthy human adults renew about 5% of their cortical bone and 20% of their trabecular bone each year (104). This bone remodeling requires the activation of 3 to 4 million BMU per year (22) (Figure 1). Each BMU in trabecular bone is about 50 microns thick, while the BMUs in cortical bone are 80 microns thick and they have a lifespan of 6 to 9 months (22, 24). Drugs like the biphosphonates can reduce the number of BMU and inhibit the activity of osteoclasts (105). There are 5 steps in bone remodeling: the activation, resorption, reversal, formation and terminal phases (16). The bone remodeling cycle takes about 200 days in cancellous bone, but it is less than 120 days in cortical bone (24,106).

During the activation phase, physical and mechanical forces are detected by the osteocytes, which then initiate osteoclast differentiation and maturation. One explanation of osteoclast differentiation states that the apoptotic death of osteocytes reduces the local concentration of TGF beta that inhibits osteoclastogenesis. Another hypothesis is that apoptotic bodies derived from osteocytes directly trigger osteoclastogenesis without any other pro-osteoclastogenic factors (41,42). However, Hedgecock *et al.* (2007) found that there is constant baseline bone remodeling that does not depend on the number of osteocytes becoming apoptotic when there are fewer than 45 apoptotic osteocytes/mm^2 (41). During the activation phase, mature osteoclasts adhere to the bone matrix proteins through their alphavbeta3 integrins to create a sealed zone beneath the cell (104). The resorption phase, which takes about 30 days, is initiated by osteoclasts releasing H^+ generated by carbonic anhydrase II, which dissolves the HAP crystals, together with enzymes like cathepsin K and metalloproteinases (MMP) that degrade osteoid to generate Howship's resorption lacunae (24,107). In the reversal phase, osteoclasts die by apoptosis, while the "osteomacs" remove the indigested bone matrix debris (16). The ensuing formation phase lasts about 150 days, during which preosteoblasts proliferate and differentiate into bone-forming osteoblasts (16,24). These mature osteoblasts synthesize the organic bone matrix and allow its mineralization. The signals that promote the passage

between the resorption and the formation phases are still unknown. Several groups have suggested that osteoclasts and the osteoblasts interact directly through specific membrane receptors (108,109). For example, the binding of the ephrin B2 on osteoclasts to the EphB4 on osteoblasts triggers a two-directional signal that suppresses osteoclastogenesis via the transcription of cFos-NFATc1 and promotes osteogenic differentiation (108). Estrogens are also crucial for bone turnover as they regulate several components of the BMU. They can favor osteoblast commitment and survival (110). Osteoblasts become engulfed within the mineralized matrix as osteocytes during the terminal phase when resorbed bone has been completely replaced by new. They can also die by apoptosis or become bone lining cells. Thus 50-70% of the osteoblasts involved in active BMU in human bone remodeling die during the terminal phase (22,111).

Bone is therefore a mineralized tissue with a complex hierarchical structure whose microscopic and macroscopic organization gives it its specific biological and mechanical properties. Its remodeling requires the cells responsible for bone resorption and formation to be closely regulated, since any imbalance between activities of osteoblasts and osteoclasts can severely impair the quality of the bone. Designers of biomaterials for filling bone defect must therefore try to mimic the hierarchical structure of bone and control the bone cell responses.

4. BONE DEFECT REPLACEMENT

4.1. Calcium phosphate ceramics

The first generation biomaterials with defined physical and mechanical properties were developed to fill the bone defects produced by various disorders. They were designed to supply the mechanical strength needed to compensate for the weakness due to the bone loss or bone fracture and to promote osseointegration. One of the first materials used for this purpose was titanium (Ti), particularly for hip replacements, because of its mechanical properties. But Ti interacts very poorly with the host bone. Therefore, several research teams have also developed calcium phosphate bioceramics that mimic the architectural, mechanical and biological properties of the inorganic bone matrix. These ceramics can be prepared in various shapes like porous scaffold or cement (112). Attention first focused on stoichiometric HAP ($Ca_{10}(PO_4)_6(OH)_2$) since it had a Ca/P ratio of 1.67 that is similar to that of bone calcium phosphate crystals (92). However, the Ca/P ratio in bone can vary from 1.65 to 1.7, depending on its anatomical location and species.

4.1.1. Manufacture process

HAP can be produced by several methods, including hydrothermal reaction, mechanochemical-hydrothermal synthesis, sol-gel synthesis and wet chemical precipitation (113-117). The hydrothermal method is useful since it can produce highly crystalline, homogeneous bioceramics with closely controlled particles size (118). Liu *et al.* (1997) did not observe any decomposition of HAP prepared by this method, although many of the HAP manufacturing processes result in HAP in which local Ca/P ratios may differ from the stoichiometric value (113). For example, CaO and tricalcium phosphate (TCP) phases appear during the sintering step of HAP production due to thermal decomposition. HAP can be decomposed into hydroxyoxyapatite ($Ca_{10}(PO_4)O_x(OH)_{2(1-x)}$) which produces TCP when heated to 1623 K during sintering (119).

4.1.2. Mechanical and biodegradable properties

A major problem with using synthetic apatite ceramics composed of stoichiometric HAP is that its mechanical and biodegradation properties are significantly different from those of biological apatites (92). Indeed, the ratio of Ca/P that directs the synthesis of brushite, TCP, HAP or a mix of them also controls their mechanical properties. For example, Akao *et al.* (1981) have shown that sintered HAP prepared from a ceramic with a Ca/P ratio of 1.69 has better mechanical properties than natural human bone (120). While cortical bone has a compressive strength of 88 to 164 MPa and an elastic modulus of 4 to 12 GPa, sintered HAP at 1200ºC has a compressive strength of about 415 MPa and an elastic modulus of 80 GPa (120).

HAP is also degraded more slowly than the bone matrix. However, increasing the percentage of HAP crystallinity from 79 to 81% using microwave treatment (2.45GHz, 800W for 45min), Farzadi *et al.* (2011) can speed up its resorption rate. CaO, TCP and tetracalcium phosphate can also be used since they are more rapidly dissolved than crystalline HAP (121). Klein *et al.* (1984) showed that keeping beta-TCP with a Ca/P ratio of 1.5 in lactate buffer (0.4M, pH 5.2) for 1 week at 37ºC resulted in it being broken down three times faster than HAP (Ca/P 1.67) (122). Wang *et al.* (2003) therefore incorporated CaO and TCP into well defined HAP microstructures to modulate the degradation rate of this ceramic. Incubating HAP with a Ca/P ratio of 1.62 obtained by adding 36% (wt.) $Ca_3(PO_4)_2$ in PBS (pH 6.8 and 7.0) at 37°C for 15 days caused it to lose weight. By contrast, HAP with a Ca/P ratio of 1.72 obtained by adding 1.79% (wt.) of CaO, surprisingly gained weight (123). Farzadi *et al.* (2011) have also synthesized a biphasic calcium phosphate composite using a compact powder made of HAP and beta-TCP with proportions of HAP from 0 to 100 (wt.%) (121). The degradability of the material when incubated for 14 days in a simulated body fluid at pH 7.25 increased with the proportion of beta-TCP in the HAP (121). *In vivo* experiments on HAP, TCP, and tetracalcium phosphate implants in the femoral condyles of mature Japanese white rabbits revealed that tetracalcium phosphate was better resorbed than TCP and HAP, confirming the *in vitro* results obtained by Ducheyne *et al*. (1993) and De Bruijn *et al.* (1994) (124-126).

The bone mineral matrix also contains several ion substitutions resulting in it containing about 4-8% (wt.) carbonate, depending on the age of the individual (127). The most frequent is the replacement of the phosphate ions in HAP by carbonate; this is called B type carbonation. Replacing the hydroxyl or phosphate ions in HAP with carbonate ($Ca_{9.32}(PO_4)_{4.64}(CO_3)_{1.36}(OH)_2$) produces a scaffold that is more resorbable than HAP after implantation in the femur of a New Zealand White rabbit

for 1 month. In addition, this carbonated HAP also produced more newly formed bone (128).

Therefore, a major challenge in the development of ceramics is to modify the HAP composition with ion substitutions to produce a material that is closer to the composition, degradation rates and mechanical properties of bone HAP. Indeed, the ionic composition of ceramics plays a crucial role, since the release of ions like calcium into the bone ECM modulates bone cell survival, proliferation and differentiation. However, the topography and architecture of the ceramic must also mimic those of bone and favor osteoconduction, and this can influence the mechanical properties of the scaffold.

4.1.3. Cell response to calcium phosphate ceramics

Multiple ionic substitutions (strontium, carbonate, magnesium, potassium, zinc, barium, copper, aluminum, iron, fluorine and chlorine) in HAP have been used to control HAP nucleation rate and its mechanical properties. Replacing some of the calcium ions in HAP with magnesium caused small changes in the Ca/P ratio, which decreased to 1.73. This increased the calcium-containing mineral deposition at 7 days by human preosteoblasts (hFOB 1.19) compared to HAP (Ca/P 1.91) (129). Also introducing magnesium and fluoride into HAP enhanced the hFOB 1.19 cells density after 4h *in vitro*. This effect was only observed in HAP doped with 7.5% mol. Mg/2.5% mol. F or 2.5% mol. Mg/7.5% mol. F (130).

The topography of the artificial material also greatly influences the responses of cells to the ceramic. Rosa *et al.* (2003) showed that HAP samples with different topographies obtained by changing the microporosity (5, 15 and 30%) influenced the proliferation and differentiation of rat bone marrow cells (131). The most irregular HAP surface reduced the proliferation of the rat bone marrow cells and their ALP activity, but did not affect their attachment (131). By contrast, Deligianni *et al.* (2001) observed a significant increase in human MSC attachment to HAP at 18h when its surface roughness was increased from R_a= 0.733 to 4.680 microns (132). They also observed better proliferation at 14 days on a HAP surface roughness of 4.68 microns in comparison to that on a 0.733 microns surface.

The biomaterials used to repair bone loss must favor cell colonization and the transport of nutrients and oxygen within the scaffold. This requires the 3D material structure to have an adequate pore size with interconnections (133). Jones *et al.* (2007) showed that a pore size of 100 microns is required for a cell colonization of HAP scaffolds used to repair a sheep tibial defect (3mm x 3mm) at 4 weeks (133). However, the optimal pore size and interconnections are still being debated. Rose *et al.* (2004) found that the interconnection pore size must be at least 80 microns for colonization of the scaffold by HOS TE85 cells (osteosarcoma) (134). However, Bignon *et al.* (2003) found that the macropore interconnections must be at least 15 microns in order to favor colonization of HAP/beta-TCP by osteoblasts isolated from human cancellous bone at 21 days (135).

Inserting channels into the scaffold can also improve its osteoconductive properties. A channel with a diameter of 421 microns in the center of a HAP scaffold enhances colonization by HOS TE85 cell at 8 days in DMEM in the presence of 10% fetal calf serum better than does a 170 micron channel (134). In the same way, the incorporation of one-directional channels with a diameter of 500 microns separated from each other by 500 microns in porous HAP with micropores (2-5 microns) and macropores (100-200 microns) increased the colonization of the scaffold by MC3T3-E1 murine preosteoblasts and cell viability at 4h compared to control HAP (136). Lastly, Buckley *et al.* (2010) observed higher oxygen concentrations inside scaffold with channels than in scaffolds without them (136).

The scaffold must be highly porous to support osteoconduction and/or osteoinduction. Okamoto *et al.* (2006) demonstrated that highly porous (70%) HAP implants placed subcutaneously on the back of a male Fisher 344 rat for 1, 2 and 4 weeks had higher ALP activities and OC contents than did similar less porous (50%) HAP implants (137). However, increasing the porosity of ceramics to obtain good osteoconduction and oxygen transport greatly modifies their mechanical properties. Bignon *et al.* (2003) synthesized HAP/beta-TCP (70/30% w/w) scaffolds with different micro- and macroporosities (135). The compressive strength of the HAP/beta-TCP increased from 1.5 to 11GPa when the macropore size decreased from 600-1250 microns to 300-600 microns (135).

Therefore ceramics can interact with the surrounding bone because of their good osseointegrative properties. However, their breakdown rates, osteoconductive and mechanical properties vary depending on their composition, crystal size, shape and structure. Their major drawback is their brittleness, while their resistance to compressive stress is good.

4.2. Polymers

Polymers have been widely used in bone replacement to mimic the osteoid. Both natural derived polymers and synthetic polymers can be used alone or in combination to enhance their osteoconductive, or osteoinductive properties.

4.2.1. Natural polymers

Natural polymers like proteins and GAG have been widely employed as bone substitute because of their osteoconductive and/or osteoinductive properties. They can be prepared as gels, scaffolds or sponges and are easily handled during surgery (138-142).

Collagen is the most abundant protein in bone that can be extracted from other species. This protein is not very antigenic in clinical application because the amino acid sequences of mammalian collagens are very similar (97% homology between the alpha 1 chain of bovine and human type I collagen) (143,144). Another candidate protein is fibrin, which provides the scaffold for blood coagulation and wound healing. Its precursor fibrinogen is

digested by thrombin to give fibrin, which then forms a dense fiber network. Both collagen and fibrin strongly modulate the responses of bone cells. Collagen scaffolds induce the colonization, proliferation and differentiation of bone marrow MSCs (145). Fibrin networks also promote the proliferation of human bone marrow-derived MSC but they do not induce their osteogenic differentiation (146).

GAGs, which are an important component of the ECM, have also been used to promote bone repair. GAGs like hyaluronic acid increase the osteogenic differentiation and migration of rat calvarial-derived MSCs (147). Chitosan, a linear polysaccharide of D-glucosamine and N-acetyl-D-glucosamine obtained by deacetylating chitin, also enhances the proliferation of normal human osteoblast precursors *in vitro* (148).

However, most of these natural polymers are rapidly degraded *in vivo*. Collagen can be broken down by collagenases and MMPs, while fibrin networks are dispersed by plasmin (146). Nevertheless, the collagen or GAG molecules can be crosslinked with chemical agents to partly inhibit their degradation. Jeon *et al.* (2007) showed that reticulation of hyaluronic acid with poly(ethylene glycol) diamine up to a cross linking density of 20% slowed the rate at which the GAG was broken down by hyaluronidase (149).

Natural polymers seem to have some potential for promoting bone regeneration. However, their use has several problems. First, it is difficult to assess the reproducibility of the materials/scaffold; second they may not be pure enough, and still contain cells and debris. But the main problem is that sterilizing natural polymers can modify their biological properties. Noah *et al.* (2002) showed that sterilization with gamma irradiation caused collagen to be broken down more rapidly *in vitro* and inhibited its colonization by human umbilical vein endothelial cells (HUVEC) (140). Endothelial cells are involved in vascularization and play a crucial role in wound healing and bone repair.

Therefore, synthetic polymers have been investigated to obtain a better reproducibility during manufacturing process involving synthesis by condensation or addition.

4.2.2. Synthetic polymers

Polyester polymers like polyglycolides (PGA), polylactides (PLA) and polycaprolactone (PCL) are broken down at different rates, partly because of the hydrolysis of their ester bond. Hurrell *et al.* (2003) observed that PGA incubated for 30 days at 37°C in 0.01M PBS (pH 7.4) lost 50% of its total mass (150). PGA could be a useful drug delivery system because of its degradation properties and its byproducts are glycolic acids, which can be removed from the body by the kidneys. PCL is degraded more slowly than PGA and PLA (2-3 years). Lam *et al.* (2009) observed that a PCL scaffold lost 2% (wt.) after 6 months in PBS at pH 7.4 at 37°C (151).

The crystallinity of polymers can influence their degradation rates. The various types of PLA can be obtained due to the chirality of the monomer. Thus poly(L-lactide) and poly(D-lactide) are semi-crystalline polymers. Proteinase K breaks down poly(L-lactide) more rapidly at 37ºC in Tris-HCl buffer than it does poly(D/L-lactide) (50/50) (152). Mainil-Varlet *et al.* (1997) showed that the crystallinity of PLA increased during breakdown due to reorganization of the macromolecules to form a denser material (153). The crystallinity of poly(D/L-lactide) increased from 30 to 36% after it was incubated for 2 weeks in PBS at 37°C (153).

The synthetic polymers used to repair bone can be processed as films, meshes or 3D scaffolds using techniques like solvent casting, phase separation, electrospinning, freeze drying or gas foaming (139,154). These various polymer architectures have been studied *in vitro* and *in vivo* (155-158). The perforated films of PLA used to treat critical size defects in sheep tibiae induced bone regeneration after 16 weeks (155). A define porous PCL scaffold (pore size 300 microns) was used to treat cranial defects in New Zealand white rabbits. It permitted bone regeneration after 4, 8 and 16 weeks (158).

However, these polymers are mechanically weak. Poly(L-lactide) can be prepared as a cylinder with a bending strength of 141 MPa, which is close to that of bone (100-200 MPa). However, its strength dropped to 87 MPa after it had been implanted in the dorsal soft tissue of Swiss mountain sheep for 6 months and to 52 MPa after 1 year (153).

4.3. Copolymers

Copolymers have been investigated as a way of overcoming the drawbacks of polymer composition, breakdown, and their mechanical properties. The aim is to combine the properties of the different polymers to obtain copolymers with improved ability to repair bone defect. For example, the rapid degradation of PGA or PLA can be reduced by adding PCL to the main chain of the copolymer (Table 5).

5. BIOMIMETIC MATERIALS

Biomaterials can be greatly improved by modifying their surface properties to enable them to interact better with cells. This can be done by depositing a calcium phosphate layer on them or adsorbing/grafting ECM proteins or other specific molecules.

5.1. Ceramics

5.1.1. Coatings

The polymers and metals used as bone substitutes have poor osseointegrative properties. Calcification of the surface of an implanted material can therefore enhance its interactions with the surrounding bone and limit undesired host response (171). Several methods have been used to promote the growth of a calcium layer on materials, including plasma spraying, electrochemical deposition and incubation in simulated body fluid (for review see, 172).

Titanium implants, which possess the appropriate mechanical strength for hip replacement, are usually coated with HAP to improve their osseointegrative properties

Table 5. Copolymers for improving bone cell responses

Copolymers	Structure	Cell Type/Animal Model	Properties	References
MEMBRANE				
Poly(L/DL lactide) (80/20)	Porous membrane of 0.25 mm thickness with a pore size at the surface of 60-70 microns	*In vitro* Rat bone marrow stromal cells	↑ Differentiation (from 5-10 days) ↑ Proliferation (5-20 days)	(159)
Poly(epsilon-caprolactone) doped with Poly(lactide-co-glycolide) (65/35)	Membrane	*In vitro* Human osteoblasts	↑ Proliferation at day 3 and day 7	(160)
Poly(lactide-co-glycolide) grafted hyaluronic acid	Membrane of 33 microns thickness	*In vivo* Sprague Dawley rat	↑ Bone repair in critical bone defect in skull (63% of the defect fill after 12 weeks)	(161)
NANOFIBER				
Poly(epsilon-caprolactone)-chitosan	Nanofibers of 1.06 or 1.29 microns diameter via electrospinning	*In vitro* MSC	↑ Mechanical properties in comparison to PCL ↑ Proliferation at day 3 and day 7 in comparison to PCL	(162)
Poly(epsilon-caprolactone) in combination with poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) (50/50)	Fibers obtained via electrospinning 0.44 to 1.79 microns diameter (depending on polymers concentration from 4 to 14% wt.)	*In vitro* MC3T3-E1	10% wt. surface: - At 6h equivalent numbers of cells compared to tissue culture polystyrene - After 7 days the ALP activity is higher than other formulation between 4 and 14 % wt.	(163)
SCAFFOLD				
Polylactide fiber reinforced poly (epsilon-caprolactone)	Scaffold with pore size varying from 100 to 400 microns	*In vitro* Human marrow stromal cells	↑ ALP released by cells in comparison to tissue culture polystyrene	(164)
Poly(lactide-co-glycolide) (50-50)	Scaffold Fisiograft®	*In vivo* Human jaw	- No inflammatory effect in large defect ↑ Bone growth in the defect within 4 months	(165)
Chitosan/poly(DL lactide-co-glycolide)	Scaffold with a pore size of 249 microns	*In vitro* Without cells	- Bioactivity observed by apatite layer formation with simulated body fluid (pH 7.34) after 28 days	(166)
	Slurry gel	*In vivo* White New Zealand rabbit	↑ Bone filling in femoral condyle critical defects (40% at 30 days and 86% at 90 days)	(167)
Poly(lactide-co-glycolide) collagen (50/50 wt.)	Scaffold with a pore size of 120 microns	*In vitro* Embryonic stem cells	↑ Proliferation after 8 days in comparison to Poly(lactide-co-glycolide) alone	(168)
Poly(L-lactide-co-epsilon-caprolactone) (70/30)	Scaffold with a pore size of 250-300 microns	*In vivo* Dutchmilk goats	- After 1 month bone formation was observed at scaffold/vertebrae interface - After 3 and 6 months fusion rate is 50% (using fusion scoring system)	(169)
Poly(propylene fumarate) (PPF)	PPF rod surrounded by porous PPF with empty PLGA microspheres	*In vivo* Lewis rat	↑ Stabilization of the femoral defect ↓ Bone formation after 12 weeks	(170)

Badr and El Hadary (2007) used a two-step method in which calcium phosphate was first precipitated onto the Ti surface by electroplating (173). The implant was then treated by the hydrothermal method to obtain HAP. This material interacted better with bone than did untreated Ti when implanted in New Zealand rabbit for 8 weeks. The gap was smaller (1.32 microns) for HAP- coated Ti than for Ti alone (2.31 microns) (173).

Materials can also be calcified by incubation in simulated body fluid (174-177). Gorna and Gogolewski (2003) observed that polyurethane made of isocyanate with different diol ratios (poly(ethylene oxide) and PCL) can be calcified by incubation for 24 weeks in a simulated body fluid *in vitro* (178). However, the Ca/P ratio of the layer depends on the polyurethane composition.

Mineralization may be challenging because of the surface topography, charges and architecture of the materials. A new method of biphasic calcium phosphate coating has given promising results since the quality of the coated layer seems to be independent of the surface properties of the material (172).

5.1.2 Composite scaffolds

Bioactive glasses or ceramics can also be incorporated into polymers and used to synthesize a composite material that interacts better with bone and promotes bone repair (179-180). Chan *et al.* (2002) studies New Zealand rabbits with a femoral condyle defect implanted for 6 weeks with Bioglass® (179). They found that inserting Bioglass® into a matrix of dextran makes the cohesive properties of the composite material better than those of Bioglass® alone, without altering the ability of bioactive glass to favor bone ingrowth (179). Scotchford *et al.* (2011) showed that implants of PCL/phosphate glass in rat calvaria enhanced bone formation better than did PCL alone (181). Several research groups have also demonstrated that incorporation of an inorganic phase into polymers enhance their cell colonization (182-184). For example, Chen *et al.* (2011) have mixed a solution of PCL (10 wt.%) with different nanoHAP concentrations (0, 25 and 50 wt.%) to prepare nanofibrous membrane by electrospinning. They showed that a combination of nano HAP (50 wt.%) and PCL (10 wt.%) induces the highest proliferation of rabbit MSC after 21 days. They also observed the highest ALP concentration after 21 days compared to PCL alone (183).

Incorporating bioactive glasses like 45S5 can also improve the formation of a HAP layer on the polymer surface (179,185,186). For example, a foam of poly(D/L lactide) containing Bioglass® (45S5) enhances the formation of a HAP layer on poly(D/L lactide) that has been incubated in simulated body fluid for 7 days, which is the first step in obtaining a bioactive compound (185).

However, incorporating ceramics or other materials into polymers can also modify the rate at which the composite is broken down. Implantation for 24 weeks in New Zealand rabbits showed that poly(lactide-*co*-glycolide) PLGA containing HAP (15 wt.%) is broken down more rapidly than PLGA containing carbon fibers (15 wt.%) or pure PLGA (187).

In addition, inserting of HAP into polymers does not provide the expected enhancement in mechanical properties. While some studies have shown that inserting HAP into the polymer matrix increased its compressive strength, others have found that adding HAP to a polymer can decrease its compressive strength (188,189, for review see 190). Moreover, Neuendorf *et al.* (2008) observed a 80% decrease in the strength of the bond between HAP and PCL after a short time in a humid environnement (30h) (191). By contrast, a recent study has shown that the insertion of carbon nanotubes in PCL enhanced the mechanical properties of PCL. Indeed, the elastic modulus increased from 10 to 75 MPa in the presence of 12.5 mg/mL multi-walled carbon nanotubes (192).

5.2. Biomolecules

Materials have been treated with adhesive proteins from the bone ECM or peptides derived from them to obtain a better interaction between the bone and the biomaterial. Peptides derived from growth factors like BMPs have also been used to improve the osteoinductive properties of biomaterials.

5.2.1. Adsorption

5.2.1.1. ECM Proteins and their derived peptides

Specific ECM proteins were first adsorbed onto materials. Osteopontin, fibronectin and vitronectin were used to enhance the cell-material interaction via their integrin receptors, and so obtain better cell survival, proliferation and differentiation (17). MC3T3-E1 preosteoblasts placed on PLA films coated with fibronectin for 3h spread better than the cells on untreated PLA. Cells also grew better on fibronectin-coated PLA (193).

PLA onto which ECM protein has been adsorbed can also prevent cell apoptosis. The activity of caspase 3, an enzyme involved in apoptosis, of cells on composite materials made of PLA and HAP (50/50 wt.%), that adsorbed 2 times more fibronectin and vitronectin than PLA alone, was lower than in cells on PLA (194).

Thus coating biomaterial with proteins can enhance the motility, spreading, survival and differentiation of bone cells. But there are limitations, mainly associated with the purification of the selected proteins, their cost, and their possible immunogenicity.

Adhesive peptides extracted from ECM proteins have been developed to overcome the purification and cost problem. These peptides are selected to have the sequence of the active part of the ECM proteins that bind integrins. The most widely used peptides contain the RGD cell binding motif that is present in several adhesive proteins, including fibronectin, collagen, osteopontin and BSP (73). This tripeptide is recognized by several integrins. Peptides derived from collagen like DGEA, P15 (GTPGPQGIAGQRGVV) and GFOGER that are specifically recognized by the alpha2beta1 integrins have also been widely used for coating both inorganic materials and synthetic polymers (195-197). HAP disks bearing adsorbed DGEA or P15 peptides enhance the adhesion of human bone marrow stromal cells (BMSC), while the long peptide containing the GFOGER sequence ($GGYGGGPC(GPP)_5GFOGER(GPP)_5GPC$) has no effect. DGEA and P15 also promoted the formation of bone in Sprague-Dawley rat tibial bone defects in 5 days. Peptides containing the GFOGER sequence increased bone formation in Lewis rats (197). Wojtowicz *et al.* (2010) have shown that modifying the surface of a material by adsorbing specific peptides onto it is an easy, low-cost way to enhance bone formation without stimulation by other external molecules (197). However, some research groups have also analyzed the influence of BMPs or peptides derived from them adsorbed onto materials on the differentiation of osteoblastic cells and stem cell and bone repair (198,199).

5.2.1.2. BMPs and their derived peptides

BMPs adsorbed onto biomaterials enhance the osteoinductive properties of the materials (198,199). Several teams have adsorbed BMP-2 or BMP-7 onto ceramics of various shapes, from granules to nanoparticles and scaffolds (for review see 172). Adsorption seems to depend on the Ca/P ratio and type of interactions that occur between the ceramics surface and the proteins (200). The carboxylate groups of BMP-7 establish electrostatic attractive forces with the calcium on the HAP surface, while the NH_2/NH_3^+ groups of BMP-7 favor H-bonding with the phosphate on the HAP surface (200).

Xie *et al.* (2010) adsorbed radiolabeled BMP-2 onto nanoparticles of HAP to follow its rate of release. The release reached a plateau after 10 days with about 43% of the initial BMP-2 released (198). Dohnozo *et al.* (2009) also adsorbed various amounts of BMP-2 (0 to 150 micrograms) onto beta-TCP granules (diameter 1 - 3 mm; 75% porosity; pore size: 50 - 350 microns) (201). They found that beta TCP granules bearing BMP-2 induced posterolateral fusion of lumbar vertebrae in New Zealand white rabbits in a time- and dose-dependent manner. The radiological spinal fusion score was significantly higher with beta TCP granules bearing BMP-2 (15, 50 and 150 micrograms) that with an autogenous bone graft (201). Other inorganic materials such as Ti have been also used. Kim *et al.* (2011) modified the surface of Ti with 3-aminopropyltriethoxysilane, which allowed the covalent binding of heparin through carbodiimide chemistry (199). Heparin-grafted Ti was then immersed in a solution of BMP-2 for it to be adsorbed onto the heparin. About 26%

of the BMP-2 (50 ng) was released within 1 day. The presence of BMP-2 on heparin-grafted Ti also favors fibronectin adsorption within 3h and enhances the ALP activity in MG-63 human osteosarcoma cells (199). Significantly more calcium was also deposited in MG-63 attached to heparin-grafted Ti with BMP-2 than in the cells on Ti alone (199). The use of two active molecules (BMP-2 and dexamethasone) adsorbed onto poly(L-lactide-co-caprolactone)/collagen nanofibers also enhanced the osteoblastic differentiation of human MSCs after 14 and 21 days in comparison to the nanofibers alone. The release of the active molecules reached a plateau at 70% within 4 days (202).

Peptides derived from the knuckle epitopes of BMPs that are thought to bind to type II receptors have been also investigated (203,204). A peptide called P4 (KIPKASSVPTELSAISTLYL), corresponding to residues 73-92 of BMP-2, in which the cysteine residues were replaced by serine and the methionine by threonine enhanced the ALP activity of murine C3H10T1/2 cells (205). Peptides derived from BMPs have been adsorbed onto ceramics, natural polymers like alginate, chitosan and collagen, and composite materials (206,207). However, the results obtained with these peptides *in vivo* varied greatly depending on how they were immobilized, their sequence and the biomaterial used (206,208,209). Suzuki *et al.* (2000) found that peptides derived from the knuckle epitope of BMP-2 corresponding to residue 68-87 (NSVNSKIPKACCVPTELSAI) adsorbed onto alginate gels (180 micrograms of peptide per 10 mg alginate) did not induce ectopic bone formation in the calf muscles of Wistar rats (208). However, 5 mg of peptide (73-92) extracted from BMP-2 adsorbed onto porous alpha TCP cylinders (4 mm long and 5 mm in diameter) enhanced the repair of defects in rabbit radial bones within 12 weeks (209).

A major problem with adsorption is that the surface properties of the material modulate the conformation of adsorbed proteins and peptides. These conformational changes can prevent the peptides interacting with their receptors. Shen *et al.* (2008) used molecular dynamics and steered molecular dynamic simulations to analyze the adsorption of the 10^{th} type III module of fibronectin (FN-III10) onto HAP (001) (210). The adsorption occurred mainly by electrostatic interaction between the COO^- and NH_3^+ groups of specific amino acids and the charged HAP surface. The RGD loop at the C-terminus of the module could undergo major changes of conformation that depended on the starting orientation of the FN-III10 and the conformation of arginine during adsorption. Some of these changes could prevent it interacting with integrins (210).

Klee *et al.* (2003) showed that cell attachment or proliferation can be modulated by the method used to immobilize protein onto the material surface (211). While both physical adsorption and covalent binding of fibronectin to a poly(vinylidenefluoride) surface promoted the attachment of human osteoblasts extracted from the iliac crest, osteoblasts proliferated better on covalently bound fibronectin. Lastly, the density of the protein or peptide adsorbed on materials also depends on the surface topography, charge and wettability of the material, and there is therefore little control on this process.

To complicate matters even further specific adsorbed proteins can be replaced by other molecules in the medium (for review see 212). Thus there is considerable scope for further studies on the covalent binding of proteins and peptides to the surfaces of biomaterials.

5.2.2. Covalent binding

The density of biomolecules on materials can be controlled by grafting. Several techniques use specific chemical groups on the surface of the polymers. The four chemical groups are: -OH, $-NH_2$, -SH and -COOH. These groups can be reacted with the side chains of some amino acids or the C- or N-terminals of a peptide. For example, an amide bond can be formed between the $-NH_2$ group at the end of a biomolecule and a -COOH on the surface of some biomaterials. Similarly, a thiol group on a biomaterial can interact with the SH group of cysteine to form disulfide bridge.

Chemical agents like 1 ethyl-3-[3dimethylaminopropyl] carbodiimide hydrochloride can be used to improve reactions between these groups and enhance the specificity and efficiency of the reaction (213). Heterobifunctional crosslinkers like sulfosuccinimidyl 4-(*N*-maleimidomethyl)-1-cyclohexane carboxylate can be used to graft peptides via the -SH group of cysteine to an $-NH_2$ on the surface of biomaterials (214). The design of these crosslinker, including such features as their length, is still difficult because the linked biomolecule must remain active (215).

However, peptides and proteins are complex molecules with many side chain groups that can interfere with their attachment, or the functionalization of a support material. The side chains of amino acids like arginine and aspartic can be protected and then deprotected to ensure that they do not compete with the grafting procedure done (216,217). Rezania and Healy (1999) developed another strategy (218). They added a CGG sequence to the N-terminal of their selected peptides containing RGD and used the thiol group of cysteine for functionalization (218).

5.2.2.1. ECM proteins and their derived peptides

Proteins like collagen and fibronectin have been covalently bound to support materials to promote a specific type of functionalization. The oxidation of PLA by ozone can be used to covalently bind type I atelocollagen (a soluble form of collagen) to a support surface. Suh *et al.* (2001) found that osteoblasts isolated from rat calvaria attached to collagen-PLA produced more collagen and had greater ALP activity than did osteoblasts on untreated PLA (219). However, it is difficult to graft entire proteins because functionalization can alter their orientation, conformation and activity (220,221). Because of this, short peptides derived from ECM proteins with specific sequences have been grafted onto biomaterials (218,222).

Nevertheless, since proteins often have several active sites, a combination of peptides could be used to mimic this specificity. For example, fibronectin has two active sequences that are required for its binding to alpha5beta1, the RGD and PHRSN motifs. It has been suggested that grafting peptides having both the RGD and PHRSN motifs onto a biomaterial will enhance their biological activity. Benoit and Anseth (2005) have grafted peptides containing the RGD and PHRSN motifs separated by 13 glycine residues ($RGDG_{13}PHRSN$), to mimic the 40 Å between these motifs in native fibronectin, onto a poly(ethylene)glycol (PEG) hydrogel (223). The grafted peptides enhanced the cytoskeletal organization in osteoblasts from neonatal rat calvaria *in vitro,* while osteoblasts on control ungrafted PEG remained unorganized (223). The osteoblasts on the peptide-grafted PEG also synthesized more ALP than did osteoblasts on PEG alone (223). The response of endothelial cells to such grafted peptides is also important because vascularization has a great influence on bone healing. HUVEC cells attached to a Langmuir Blodgett film, a PEG chain bearing GRGDSP and PHSRN peptides separated by a distance that mimicked that of native fibronectin, spread significantly better than cells on PEG bearing GRGDSP peptides (224).

Two other peptides derived from the laminin, YIGSR and IKVAV can also be grafted onto polymers to favor cell attachment. For example, adipose-derived stem cells became attached to PCL to which were grafted YIGSR and IKVAV. However, significantly more adipose-derived stem cells became attached to PCL bearing IKVAV than to PCL (225). Adipose-derived stem cells are of great interest because they can be easily harvested by liposuction. Cowan *et al.* (2004) found that these cells in a HAP-coated PLGA scaffold induced bone formation in critical size mouse calvarial defects *in vivo* (226).

Cell adhesion can also be regulated using peptides derived from the heparin-binding sites on ECM proteins, such as KRSR and FHRRIKA that bind to cell transmembrane heparan sulfate proteoglycans. Kim *et al.* (2010) showed that human osteoblasts proliferated better on silk fibroin nanofibers bearing KRSR and RGDS immobilized via amide bonds than did cells onto silk fibroin alone (227). Rat calvarial osteoblasts also proliferated more readily on Ac-CGGFHRRIKA-NH_2 covalently linked to an *N*-isopropylacrylamide hydrogel than did cells on untreated gel (228). However, the degree of ECM mineralization produced by rat calvarial osteoblasts cultured for 12 days on poly(acrylamide-*co*-ethylene glycol/acrylic acid) interpenetrating polymer network bearing both Ac-CGGFHRRIKA-NH_2 and Ac-CGGNGEPRGDTYRAY-NH_2 peptides was similar to that produced by osteoblasts cultured on Ac-CGGNGEPRGDTYRAY-NH_2 alone (229).

5.2.2.2. BMPs and their derived peptides

BMPs have been covalently bound to various materials, including Ti, GAG, and synthetic polymers, and successfully used both *in vitro* and *in vivo* (208,230-232). BMPs or peptides derived from them that are covalently bound to materials may have improved osteoinductive properties than adsorbed BMPs or peptides (208,232). Zhang *et al.* (2010) compared BMP-2 adsorbed and covalently bound to a PCL scaffold. They measured the amount of BMP-2 at the surface, the release profiles and amounts of mRNAs encoding osteogenic markers in rat BMSCs cultured on them (232). There was significantly more (38% of the initial dose) immobilized BMP-2 after covalent binding than after adsorption (9% of the initial dose). Adsorbed BMP-2 was released faster than covalently bound BMP-2. The genes encoding ALP and OC in BMSCs adhering to PCL bearing covalently-bound BMP-2 were more active than these genes in BMSCs growing on native PCL (232).

Peptides derived from the knuckle epitope of BMP-2 and BMP-7 can also be grafted onto the surface of biomaterials like alginate to promote the osteogenic differentiation of adhering cells (230,233). Suzuki *et al.* (2000) found that human BMP-2-derived peptides (residues 68-87) covalently linked to alginate hydrogel (20 micromol/L of peptide per 1g gel) induced the ectopic calcification of Wistar rat muscle *in* vivo within 8 weeks, while peptides simply adsorbed onto the hydrogel (180 micrograms per 10 mg alginate) did not (208). P4 peptide (KIPKASSVPTELSAISTLYL) covalently bond to alginate gel (25 mg P4/g gel) also promoted ectopic calcification in the calf muscles of male Wistar rats (205). Similarly, peptides containing part of the sequence corresponding to the knuckle epitope of BMP-7 (0.1 mmol/L) that were covalently bound to self-assembled monolayers on glass coverslips enhanced the transcription of the BSP gene and favored Ca^{2+} deposition in calvaria cells from fetal Sprague-Dawley rats (234).

5.2.2.3. Adhesive peptides and BMPs

The impact of biomaterials bearing adhesive peptides on the behavior of bone cells, particularly osteoclasts, in response to growth factors such as BMPs is not completely understood (235,236). However, Lai and Cheng (2005) demonstrated crosstalk between integrins and BMP signaling in osteoblastic cells (77). Tamura *et al.* (2001) studied MC3T3-E1 preosteoblasts expressing antisense FAK mRNA and found that FAK must be activated for BMP-2 to induce osteoblastic differentiation through Smad1-dependent transcription (237). Combining adhesive peptides and entire BMPs can improve the osteogenic differentiation of stem cells *in vitro* and bone repair *in vivo* (236). Park *et al.* (2010) showed that human bone marrow MSCs embedded in PLGA microspheres functionalized with RGD peptides and BMP-2 underwent osteogenic differentiation; the genes encoding Cbfa1, type I collagen and BSP were activated and the proteins they encoded were more abundant (236).

GRGD peptides (1.62 pmol/cm^2) and P4 (KIPKASSVPTELSAISTLYL) (5.2 pmol/cm^2) immobilized on poly(lactide-co-ethylene oxide fumarate) hydrogel acted synergistically to enhance the ALP activity and calcium content of Wistar rat BMSCs (235). Moore *et al.* (2011) recently immobilized azide GRGD and P4 peptides (N_3-KIPKASSVPTELSAISTLYL) on self-assembled monolayers using click chemistry (238). They

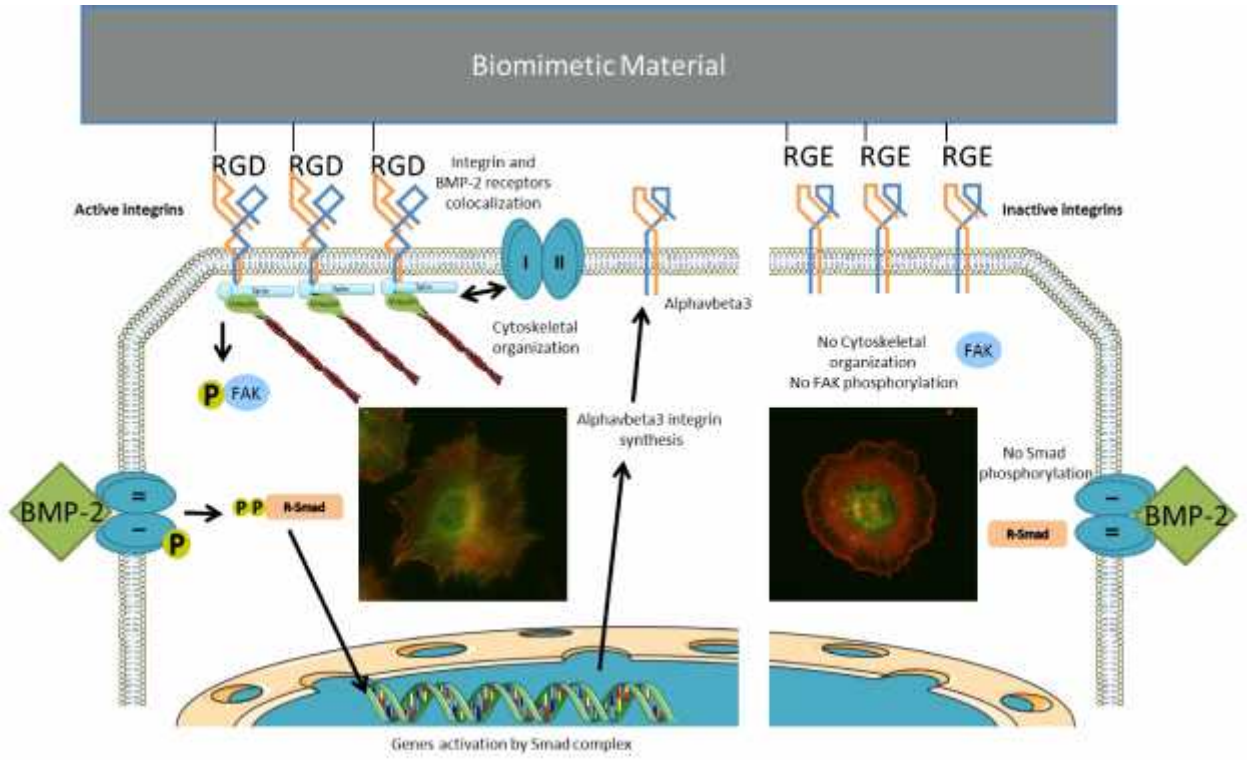

Figure 2. Influence of biomimetic materials functionalized by RGD peptides on BMP-2 signaling [Illustration using Servier Medical Art, http://www.servier.fr].

found that combining RGD and P4 (65 pmol/cm^2 of each peptide) did not increase the transcription of Runx-2, but significantly enhanced the expression of the gene encoding BSP. It also favored the formation of small mineralization at 21 days.

However, the adhesive peptides that direct interactions with specific integrins play an important role in bone cell responses. We have shown that peptides derived from BSP or collagen strongly influence the ability of preosteoblasts to respond to BMP-2 and a peptide derived from BMP-9 (239,240) (Figure 2).

Several obstacles must be overcome before of such materials functionalized by biomolecules can be used in clinical applications. Hennessy *et al.* (2008, 2009) studied the efficiency of peptides like RGD, DGEA and P15 and showed that cell adhesion is drastically decreased by the presence of serum (196,241). Other molecules can also mask the specific biomolecules on the support surface. Non-fouling molecules like PEG must be used to control the non-specific adsorption of proteins (242). The density of the grafted molecules on the support surface is also important for the cells/biomaterial interaction and subsequent cell behavior (243,244). Brinkerhoff and Linderman (2005) have developed a model to show that the spaces between each RGD grafted onto the material surface must be similar to the distance between the RGD binding sites of neighboring integrins to enable adjacent integrins and the RGD ligands to interact (243). Comisar *et al.* (2011) recently used nanopatterns of RGD peptides to develop a correlative model for testing their influence on the organization of bound integrins, FAK phosphorylation, the spreading of MC3T3 cells, and their osteoblastic differentiation (244). They observed that modifying the RGD nanopatterns affected the number of integrins bound as well as their cluster formation. The concentration of OC, a marker of osteoblastic differentiation, was higher when the clusters of integrins were large and heterogeneously distributed (244). However, Hsiong *et al.* (2008) demonstrated that the influence of the RGD density on cells depended on their differentiation state (245). Nanopatterning RGD affected the proliferation of only MC3T3-E1 preosteoblasts; it had no effect on less well differentiated cells like those of a mouse bone marrow stromal line (245).

Rezania and Healy (2000) demonstrated that grafted peptides must be present at a minimum density before it can have a significant impact on cell differentiation (246). When the density of Ac-CGGNGEPRGDTYRAY-NH_2 peptides was 0.62 pmol/cm^2 mineralization was better than when its density was lower (246). Ochsenhirt *et al.* (2006) also observed that increasing the GRGDSP density on a PEG surface that was covered by RGD from 10 to 100% enhanced the adhesion of HUVEC (224).

The selection of the peptides used to functionalize biomaterials must also overcome another problem, the degradation of the grafted molecule. One way of limiting the breakdown of selected peptides and

increasing their stability could be to make them cyclic. Bogdanowich-Knipp *et al.* (1999) showed that cyclic RGD (CRGDFPenicillamine) is 30-times more stable than the linear RGDF at pH 7 and 50°C (247). However, the spatial organization of the peptides also modified their specificity for integrin. The linear peptide ($(C_{18})_2$GRGDSP) interacted with the beta1 integrin subunits while the peptide with a loop ($(C_{18})_2$GRGDSP$(C_{18})_2$) was recognized by the alphavbeta3 integrins (224).

Finally, all the biological benefits of bound proteins and peptides can be seriously impaired by the process of sterilization. Huebsch *et al.* (2005) studied the efficiency of sterilization by UVC and ethanol to remove *E. coli* on *N*-isopropylacrylamide hydrogels grafted with RGD peptides (Ac-CGGNGEPRGDTYRAY-NH_2) (248). Exposure to UVC, even for 15h, did not stop the bacteria proliferating, whereas the hydrogel by ethanol did. The ethanol could also be completely removed after treatment and did not affect the peptide, while exposure to UVC for 1 to 12h broke it down (248). However, ethanol can be used only for *in vitro* studies and is not considered to be a sterilization tool because some viruses and bacterium spores are resistant. High energy radiation also seems to alter the stability of the peptides and proteins used in biomimetic materials (140,249).

6. CONCLUSION

Biomaterials have evolved from "inert supports" to third generation biomaterials over the past thirty years. This latest generation of biomaterials, which includes biomimetic materials developed to replace autografts, the current gold standard, are designed to favor the interaction of bone cells and biomaterials, osteoinduction and have appropriate mechanical properties.

While the results obtained with composite materials produced by coating HAP or immobilizing biomolecules are promising, there are still several drawbacks. The specificity, density, stability, and bioactivity of the immobilized biomolecules remain challenging. Modifying the structures of peptides to increase their stability can have a great impact on their specificity and activity. Further studies are also required to better understand the crosstalk between integrin and growth factor cascades if we are to enhance the biological properties of biomimetic materials. One potentially fruitful way to enhance cell adhesion, differentiation and function within biomaterial scaffolds may be the activation of multi-signaling acting in synergy during the cell-biomaterial interaction.

Finally, most published studies have focused on the interactions between materials and bone-forming cells, but bone remodeling depends on a balance between the activities of osteoblasts and osteoclasts. We need a clearer picture of how all bone cell types are formed and function when they are in contact to biomimetic materials.

7. PERSPECTIVES

Most studies on biomimetic materials have used 2D systems. We now need to use more complex shapes (3D) of composite biomimetic materials to mimic the structural architecture of bone with appropriate biological and mechanical properties. The vascularization that influences the availability of oxygen and nutrients as well as the mechanical stress within the scaffold may also strongly affect the responses of bone cells to biomimetic materials. Bioreactors for culturing cells within 3D scaffolds will help to develop the biomimetic materials that will be the future gold standard.

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Abbreviations: $1,25\text{-}(OH)_2D_3$: 1alpha, 25-dihydroxyvitamin D_3, AdBMP: recombinant adenoviral vector encoding BMP, ALP: alkaline phosphatase, BMP: bone morphogenetic protein, BMSC: bone marrow stromal cell, BMU: basic multicellular unit, BSP: bone sialoprotein, CaSR: calcium sensing receptor, ECM: extracellular

matrix, ERK: extracellular signal regulated kinase, FAK: focal adhesion kinase, FGF: fibroblast growth factor, FN-III10: 10^{th} type III module of fibronectin, GAG: glycoaminoglycan, HAP: hydroxyapatite, HUVEC: human umbilical vein endothelial cells, IL: interleukin, M-CSF: macrophage-colony stimulating factor, MMP: matrix metalloproteinase, MSC: mesenchymal stem cell, NFATc1: nuclear factor of activated T cells, OC: osteocalcin, OPG: osteoprotegerin, Osx: osterix, PCL: poly(caprolactone), PEG: poly(ethylene glycol), PGA: poly(glycolide), PLA: poly(lactide), PLGA: poly(DL-lactide-*co*-glycolide), PPAR: peroxisome proliferator-activated receptor, PPF: Poly(propylene fumarate), PTH: parathyroid hormone, RANK: receptor activator of NF-kappaB, RANKL: receptor activator of NF-kappaB ligand, Runx2: runt-related transcript factor 2, SIBLING: small integrin-binding ligand N-linked glycoprotein, SLRP: small leucine-rich proteoglycans, SP-1: sphingosine-1 phosphate, S1PR1: sphingosine-1 phosphate receptor 1, TCP: tricalcium phosphate, TGF: transforming growth factor, Ti: titanium, TNF: tumor necrosis factor, TRAP: tartrate-resistant acid phosphatase

Key Words: BMP, Bone cells, Bone substitute, Ceramics, Extracellular matrix, Integrin, Osteoinduction, Osteoconduction, Peptide, Polymer, Review

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