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Extracellular matrix-mimetic adhesive biomaterials for bone repair

Asha Shekaran[1,2] and **Andrés J. García**[1,3]

[1] Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, GA, USA

[2] Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, GA, USA (2)

[3] Woodruff School of Mechanical Engineering, Georgia Institute of Technology, Atlanta, GA, USA

Abstract

Limited osseointegration of current orthopaedic biomaterials contributes to the failure of implants such as arthroplasties, bone screws and bone grafts, which present a large socioeconomic cost within the United States. These implant failures underscore the need for biomimetic approaches that modulate host cell-implant material responses to enhance implant osseointegration and bone formation. Bioinspired strategies have included functionalizing implants with ECM proteins or ECM-derived peptides or protein fragments which engage integrins and direct osteoblast adhesion and differentiation. This review discusses 1) bone ECM composition and key integrins implicated in osteogenic differentiation, 2) the use of implants functionalized with ECM-mimetic peptides/protein fragments, and 3) growth-factor derived peptides to promote the mechanical fixation of implants to bone and to enhance bone healing within large defects.

1. Introduction

The limited biological performance of current orthopaedic implants, such as joint replacement prostheses, bone screws and bone grafts, presents a large and growing socioeconomic burden in the United States. For example, in 2004, the failure of replacement joints prompted 86,000 revision surgeries for hip and knee arthroplasties at a cost of $3.2 billion, and those surgery numbers are projected to exceed 3.6 million by 2030 [1]. Similarly, the loosening of screws for spinal implants and fracture fixation in osteoporotic patients are major clinical concerns, with high failure rates estimated to be 18–27% [2–4] and 5–23% [5–7] respectively. Furthermore, over 600,000 bone grafting procedures are performed annually in the U.S. to treat non-healing skeletal defects caused by traumatic injury and cancer [8–9]. However, autografts, the gold standard of treatment, are limited by donor site supply and morbidity [10], and allografts are limited by increased resorption, poor mechanical properties and the risk of infection [9–10]. Therefore, there is a significant need for improved orthopaedic materials which promote implant integration into host bone and enhance bone formation.

Bone contains multiple cells types such as osteoblasts, osteoclasts and osteocytes; osteoblasts are the major cell type responsible for bone formation. Osteoblasts differentiate from mesenchymal stem cells and osteoprogenitor cells found primarily in the bone marrow in a multi-step process in which the Cbfa1/Runx-2 transcription factor plays a crucial role [11]. Stem cells differentiate into osteoprogenitors with limited self-renewal capacity, then to pre-osteoblasts with limited proliferation, and finally to mature osteoblasts, which secrete osteoid, the unmineralized organic component of bone matrix. As the deposited osteoid is mineralized, osteoblasts become trapped within lacunae as osteocytes, become bone lining

cells, or die by apoptosis [12]. Biomaterials which can modulate the response of host osteoblast and osteoprogenitor cells to the implant may be crucial to improving the mechanical fixation of implants and osteogenic capacity of bone grafts. For example, implant osseointegration, defined by the enhancement of new bone formation in direct contact with the implant as well as implant fixation within the first 2 years, has been shown to be predictive of the long-term success of implants [13–14]. Therefore, materials that engage osteoblast receptors and induce peri-implant bone formation may effectively address the problems of arthroplasties and screw loosening. Similarly, although bone has an innate capacity to regenerate through intramembranous and endochondral ossification [15], in non- or delayed- unions, biomaterial grafts that augment this healing capacity by upregulating osteoblast-mediated bone formation may present viable alternatives to autografts.

As successful orthopaedic biomaterials must support the adhesion, organization, differentiation and matrix mineralization of osteoblasts and osteoprogenitor cells, many strategies have focused on recapitulating natural biological cues which regulate these processes. Cell fates such as proliferation and differentiation are determined by a complex interplay of signals from the extracellular environment. These signals include (1) insoluble molecules within the extracellular matrix, (2) soluble and/or matrix-associated biochemicals such as systemic hormones or growth factors and cytokines that act locally, and (3) cell-cell receptors (Fig. 1). The ECM itself contains multiple types of insoluble molecules, forming a meshwork of structural proteins to which adhesive proteins, proteoglycans and glycosaminoglycans are associated [16]. This complex biological supramolecular scaffold provides a compelling model for biomimetic strategies which mimic ECM protein, growth factor or hydroxyapatite mineral chemistry or architecture to create a synthetic matrix to control tissue-specific cell responses. Architectural ECM-mimetic approaches include nanofiber scaffolds that recapitulate the structure of proteins within ECM [17], substrates with features which mimic native ECM nanotopography [18], and composites which recreate the mineral content and mechanical properties of bone matrix [19–20]. This review will focus on 1) bone ECM composition and key integrins implicated in osteogenic differentiation, 2) orthopedic biomaterials functionalized with ECM motifs, and 3) growth factor derived peptides.

2. Bone ECM composition and key integrins implicated in osteogenesis

The composition and spatial orientation of ECM varies for each tissue type. These differences in ECM composition/orientation may be useful in tailoring biomaterials to direct tissue-specific cellular responses as each type of ECM molecule may regulate cell differentiation differentially by interacting with specific cell receptors [21]. In bone, the ECM consists of mainly of an organic phase known as osteoid, which constitutes approximately 20% of bone mass, and a mineral phase (Table 1). The organic fraction of bone consists of over 90% type I collagen [22], other minor collagens such as types III and V, and 5% non-collagenous proteins. The non-collagenous proteins in bone include osteocalcin, osteonectin, osteopontin, adhesion proteins such as fibronectin and vitronectin and proteoglycans such as versican, decorin and hyaluronan [23]. The mineral phase of bone is composed of hydroxyapatite, a calcium phosphate compound. The bone matrix also sequesters growth factors, acting as a reservoir for soluble inductive signals such as bone morphogenic protein (BMP).

Bone ECM serves both structural and biological functions, as the mineralized matrix accounts for the tissue's mechanical properties while it also provides chemical cues that regulate bone cells and acts as a reservoir for ions [12]. Collagen fibrils provide tensile strength to bone and are composed of collagen helices that assemble parallel to each other in a regular quarter-staggered pattern, creating 68 nm gaps between adjacent collagen

molecules. Hydroxyapatite crystals, which make up 70% of bone, fill these gaps and are responsible for the compressive strength of bone [12]. Bone ECM also regulates bone cells by providing ECM-integrin bonds that enable the formation of adhesive structures and activate signaling pathways which regulate cell spreading, survival and differentiation. However, as bone biology is not the focus of this article, the reader is referred to the following article [12]. Recreating the biological function of ECM using bone ECM-specific adhesive signals such as collagen I, fibronectin and vitronectin may therefore be a powerful biomaterial strategy to enhance osteogenesis.

Integrins are a family of receptors that primarily mediate adhesion of cells to the extracellular matrix proteins such as collagen and fibronectin [24]. Integrins are heterodimeric transmembrane proteins, each of which consists of α and β subunits. Currently, 8 β and 18 α integrin subunits are known, and these subunits associate to form 24 distinct αβ integrin combinations, each with unique binding characteristics (Fig. 2). X-ray crystallography analysis of integrin structure demonstrates a globular head connected to rod-like tails, and includes a flexible “knee” region that is involved in the activation state of the integrin. Integrins are capable of transducing signals in both directions across the cell membrane. For example, ‘outside-in’ signaling occurs when ECM ligation to integrins trigger intracellular signaling. Conversely, ‘inside-out’ signaling takes place when intracellular signals modulate integrin activation state and thus change its affinity for its extracellular ECM ligand [25]. Upon ECM binding to their extracellular domains, integrins cluster and their cytoplasmic domains associate with both cytoskeletal and intracellular signal transduction molecules. The association of integrins with the cellular signaling network initiates downstream signaling cascades such as the FAK, protein kinase C, Rac, Rho and MAPK pathways. The coordinated clustering of ECM ligands, integrins and cytoskeletal components forms macromolecular aggregates known as focal adhesions on the inside and outside of the cell membrane [26]. Because of the central roles of integrin-mediated adhesion to important cellular responses such as survival, growth, migration and differentiation [25,27–28], materials strategies that harness ECM-integrin interactions may play a key role in eliciting desired cellular responses *in vivo*.

The β1 sub-family integrins are the mostly highly expressed integrins in osteoprogenitors and osteoblasts and the predominant mediators of cell adhesion in these cells [29], although the β3 andβ5 subunits may be expressed as well [30–31]. Alpha subunit expression data has been more inconsistent, with different combinations of α1, α2, α3, α4, α5 and αv subunits having been detected by immunohistochemistry in human and rat bone [30–34]. The expression of the previously mentioned alpha subunits has also variously been determined by flow cytometry, immunoprecipitation, immunocytochemistry and Northern blot analysis on primary bone cultures [29–31,35–39]. Although reports of alpha subunit and integrin heterodimer expression in osteoblasts have sometimes been contradictory, many studies have identified the α1β1, α2β1, α3β1, α5β1,αvβ3 integrins and their subunits in osteoblasts and bone cultures [29,33,37–38]. A few isolated studies have also found osteoblast expression of α6β1 [38,40], α8β1 [41], αvβ1[35] and αvβ5 [29]. Integrin expression studies on osteoprogenitor cells have shown similar profiles as osteoblasts, as Gronthos et al. reported the detection of α1β1, α2β1, α5β1, α6β1, αvβ3 and αvβ5 on STRO-1 expressing human bone marrow stromal cells [42] (Table 2, Fig. 2).

2.1 β1 integrins

Blocking studies showed that β1-integrin-mediated adhesion contributes significantly to the adhesion strength of human bone marrow cells on fibronectin [43] as well as the adhesion of cells from human bone culture and human bone marrow stromal cells to collagen, laminin and fibronectin [42]. Perturbation of β1-integrin function also inhibits matrix mineralization in human bone marrow cells [42]. In addition, a glucocorticoid-induced reduction of β1

expression is correlated with inhibition of cell adhesion [44]. Transgenic mice expressing a dominant-negative truncated β1 subunit in osteoblasts and osteocytes display reduced bone mass and increased bone porosity [45] as well as an alteration in tibial curvature and femoral torsional strength [46]. The expression of β1 with altered function in the osteoblasts of these transgenic mice also results in impaired adhesion of osteoblasts *in vitro* [45].

2.1.1 α2β1—The α2β1 integrin is implicated in pro-osteogenic pathways as it is highly expressed by osteoblast-like cells and is a primary adhesion receptor used by osteoblast-like cells to adhere to collagen [29], the main organic component of bone. Several studies indicate that the interaction of α2β1 integrin with collagen I is a crucial signal for osteoblastic differentiation and matrix mineralization [47–52]. For example, α2β1-mediated adhesion of mouse MC3T3-E1 pre-osteoblasts to collagen I activates Runx2/Cbfa1, a transcription factor that activates osteoblastic differentiation and matrix mineralization [51–52]. α2β1 ligation to collagen I also induces the phosphorylation of focal adhesion kinase (FAK) and activation of extracellular signal-related kinase (ERK), which has been implicated in the regulation of osteoblast-specific gene expression and matrix mineralization [50–51,53–54]. Furthermore, the collagen – α2β1 integrin interaction promotes an osteoblastic phenotype in rat multipotent bone marrow cells [47,49]. Schneider et al also showed that perturbation of the α2β1 integrin resulted in a 95% reduction mineralization in an osteosarcoma cell line [55].

2.1.2 α5β1—The α5β1 integrin plays an important role in osteogenic differentiation as it is expressed by osteoblasts and osteoprogenitors, and promotes cell survival and matrix mineralization. α5β1 is stably expressed by osteoblasts during varying stages of osteogenesis [41] and is also expressed by bone marrow stromal cells [56]. In addition, α5β1 also mediates cell attachment to fibronectin as well as fibronectin assembly [56]. In mature cells, α5β1 binding is necessary for cell survival and a decrease in α5β1-fibronectin interaction leads to osteoblast apoptosis [57] through a caspase-dependent mechanism [58]. α5β1 may also be involved in mechanical sensing by osteoblasts *in vitro* [59]. Blockade of the α5β1 integrin inhibits bone-specific gene expression and mineralization in rat calvarial cultures [41,60], a rat osteosarcoma cell line [55], human osteoblast-like cells [61] and a mouse immature osteoblast-like cell-line [62]. In human mesenchymal stromal cells (hMSC), priming the α5 subunit with an agonist or overexpression of the α5 subunit increases osteogenic capacity [63], while α5β1 blockade decreases the alkaline phosphatase activity of cells cultured on fibronectin [64].

2.1.3 α1β1 and α3β1—The α1β1 and α3β1 integrins also appear to play important roles in bone healing as α1 integrin knock-out mice display impaired fracture healing [65] and blockade of α3β1 inhibits mineralized nodule formation [41].

2.2 β3 integrins

2.2.1 αvβ3—While engagement of the αvβ3 integrin may support cell adhesion, it has a negative effect on the proliferation and differentiation of osteoprogenitors. Blocking of αvβ3 has been shown to enhance human MSC proliferation on fibronectin and fibronectin fragments [64]. αvβ3 may also inhibit osteoblast differentiation and bone healing *in vivo*. A murine osteoblastic cell line made to overexpress human αvβ3 showed an increase in proliferation rate but a decrease in matrix mineralization [66]. Furthermore, early fracture healing was accelerated in the tibiae of β3-null mice and twenty-three genes related to osteogenesis were upregulated at least two-fold in the β3-null mice [67]. The αvβ3 integrin is also the major integrin receptor expressed by osteoclasts [68] and plays a major role in osteoclast adhesion [69], resorption [70] and sealing zone organization[71].

Targeting materials to integrins such as α2β1 and α5β1 which are expressed by osteoblasts-like cells and regulate osteogenesis while preventing interactions with integrins which may

inhibit osteoblastic differentiation such as αvβ3 may be a powerful molecular strategy for developing improved orthopaedic biomaterials.

3. Orthopaedic biomaterials functionalized with ECM motifs (Table 3)

3.1 Full-length natural ECM polymers

Due to the important regulatory role that ECM molecules play on cellular responses *in vivo*, full-length ECM proteins have been studied as potential adhesive scaffolds for bone defect healing and implant integration. These ECM polymers include collagen [72–78], fibrin [79–82], hyaluronic acid [83–86], decellularized matrix [87–88] as well as bone sialoprotein [89] (Table 3). Methods used to functionalize titanium implants with ECM polymers include protein adsorption from solution [75,85], injection of protein solution into a porous implant [74], dip-coating and covalent tethering [73]. For the treatment of bone defects, ECM implants have been used in the form of crosslinked membranes [77], sponges [78], gels [80], demineralized bone particles [87] or cut pieces of small intestinal submucosa [88]. Although naturally derived ECM molecules have demonstrated some degree of success in selected studies [72–73,88], the widespread use of natural ECM macromolecules in orthopaedic applications has been hindered by several factors. First, full-length ECM polymers have low solubility, are costly to extract and purify in large quantities, suffer from batch-to-batch variation and potentially suffer from immunogenicity. Furthermore, it is challenging to modify, characterize and control the presentation of natural ECM biomaterials.

3.2 ECM-derived adhesive peptides/proteins

The above-mentioned limitations of full-length ECM molecules have spurred the use of ECM-derived peptides or recombinant fragments that incorporate the minimal functional sequence of their parent protein [90] in order to convey bioactivity to implant materials. In contrast to ECM polymers, these peptides and protein fragments may be synthesized in larger quantities (and via chemical synthesis or recombinant protein expression), immobilized on non-fouling surfaces at high densities, and may be tailored in composition for specific applications. While natural ECM proteins such as collagens and fibronectin are large macromolecules consisting of thousands of amino acids, only a few short peptide sequences within these polymers serve as integrin recognition and binding sequences that trigger downstream processes such as adhesion, signaling and spreading. For example, in collagens I, II and III, cells bind to the GFOGER [91–92] peptide sequence while in fibronectin, the RGD [93], PHSRN [94], REDV[95], and LDV [96] sequences are responsible for cell binding. As a result, short peptide sequences such as these, as well as ECM-derived protein fragment such as FNIII7-10 are used to biofunctionalize titanium surfaces and bone tissue engineering scaffolds. In addition to the primary sequence of these peptide ligands, the structure or conformation of the ligand is a critical factor in their ability to binding to integrin receptors and trigger signaling pathways. Common peptide/protein fragment functionalization methods for titanium implants include simple adsorption or covalent immobilization onto titanium surfaces. Peptides may be presented on a non-fouling background by covalently tethering them to protein resistant polymer coatings such as poly(ethylene glycol) [97–98]. Peptide modification strategies for bone regeneration within defects include adsorption to polymer scaffolds [99] or bone matrix [100]. Both the tethering density and the stability of these peptide ligands on the implant surfaces are critical considerations in their performance.

3.2.1 RGD—RGD is an adhesive peptide sequence found in many ECM molecules including fibronectin, vitronectin, bone sialoprotein and osteopontin [101]. RGD can bind to multiple integrins suchαvβ3, αvβ1, α8β1, αvβ8, αvβ6, αvβ5 and αIIbβ3. However, for certain integrins, binding to RGD is strongly modulated by another sequence, such as the

PHSRN synergy site for α5β1 [94,102]. Because RGD serves as a potent, promiscuous binding sequence, many biomaterial strategies have incorporated RGD as an adhesive ligand.

The application of linear RGD oligopeptides onto implant surfaces has generally failed to enhance functional osseointegration as determined by bone-implant contact and mechanical fixation in several independent studies [97–98,103–104]. In addition, Bellis and coworkers demonstrated a negative effect for RGD peptides in bone formation and osseointegration responses to hydroxyapatite implants [105]. In contrast to these studies, Soballe and colleagues did report enhancements in osseointegration for implants presenting cyclic RGD peptides [106–107]. However, other studies using cyclic RGD have also failed to show improvements in implant fixation in rat tibiae [75] and canine mandibles [108]. Direct comparison among these contradictory studies is confounded by differences in the presence of a non-fouling polymer coating to prevent non-specific adsorption of plasma proteins, the animal model used, as well as implant surface finish (i.e., roughness). It is worth noting that two studies in which RGD was presented on titanium implants in a controlled fashion from non-fouling background coating demonstrated no improvements in osseointegration [109–110], suggesting that RGD-functionalization is not effective at enhancing implant integration. Fewer RGD modified materials have been tested as bone grafts within defects, but in those studies, RGD does not promote bone formation and repair *in vivo* [111].

3.2.2 Fibronectin-mimetic protein fragments/peptides—Fibronectin contains both the RGD adhesion site as well as a PHSRN synergy site. α5β1 binds to RGD in the presence of PHSRN in fibronectin with a forty-fold increase in affinity compared to RGD alone [94] (Fig. 3A). Each of these domains independently contributes little to binding, but, in combination, they synergistically bind to α5β1 to provide stable adhesion [102,112]. In contrast, other integrins are unaffected by the synergy site and bind only to the RGD site within fibronectin with a lower affinity than α5β1[113]. Several fibronectin-derived peptides or fragments designed for biomaterial applications therefore recapitulate this interaction between α5β1 and the RGD and PHSRN sites.

3.2.2.1FNIII7-10—Our group has engineered a recombinant fragment of fibronectin, FNIII7-10, which encompasses the 7–10th repeats of native fibronectin and binds specifically to the α5β1 integrin (Fig. 3B). FNIII7-10 enhances both osteoblast adhesion strength and differentiation *in vitro* [110], as well as implant osseointegration in a rat cortical model when compared to titanium implants modified with RGD at an equivalent molar surface density [97]. Furthermore, a simple adsorbed coating this fragment exhibits improved bone apposition and mechanical fixation to bone when compared to full-length fibronectin as fibronectin domains with antagonistic effects are excluded from the fragment [114].

3.2.2.2 FNIII9*-10: Martino et al. investigated the osteogenic potential of human MSCs on surfaces and hydrogels functionalized with full-length fibronectin (FN), fibronectin fragments (FNIII9–10 and FNIII10) and a more α5β1-specific mutated fibronectin fragment (FNIII9*-10) and demonstrated that FNIII9*-10 and FNIII9-10 supported higher MSC differentiation than FN [64]. Interestingly, the level of osteoblastic differentiation for each fragment was correlated with its degree of binding specificity for the α5β1 integrin (FNIII9*-10 > FNIII9-10 > FNIII10), which supports other studies suggesting that α5β1 engagement may enhance osteogenesis[97,110,114].

3.2.2.3 RGD-PHSRN oligopeptides: Synthetic peptides designed to co-present the RGD site and PHSRN synergy sites on the same molecule separated by polyglycine linkers result in increased adhesion and metabolic activity of primary rat calvarial osteoblasts [115] and human osteoblast-like cells [116] *in vitro* when compared to surfaces presenting RGD alone.

However, whether these peptides enhance implant osseointegration and bone formation *in vivo* remains to be established.

3.2.3 Other ECM-derived peptides—Other ECM-derived peptides that have been found to enhance osteoblast adhesion and differentiation *in vitro* include FHRRIKA which is derived from the heparin binding site of bone sialoprotein [117–121], KRSR, which is a heparin binding sequence found on multiple ECM proteins [121–126], the bone sialoprotein derived BSP(278-293) [127], the human vitronectin peptide HVP (351-359) [128–131], an osteopontin derived peptide [132], and a heparin binding peptide, HBP12 [133]. While these ECM derived peptides have shown promise as bone biomaterials *in vitro*, more studies need to be done to demonstrate their osteogenic capacity *in vivo* as well.

3.2.3 Collagen-mimetic peptides

3.2.3.1 GFOGER: The hexapeptide sequence Gly-Phe-Hyp-Gly-Glu-Arg (GFOGER) is found on residues 502 507 of the α1(I) chain of type I collagen and serves as the major recognition site for α2β1 integrin binding [92,134–135]. Our group engineered a Col I-mimetic GFOGER containing peptide, GGYGGGPC$(GPP)_5$GFOGER$(GPP)_5$GPC, which recapitulates the triple helical tertiary structure of native collagen as an adhesive ligand for biomaterials. Surfaces presenting adsorbed or covalently immobilized GFOGER peptide support equivalent levels of α2β1 integrin-mediated adhesion of HT1080 fibrosarcoma and MC3T3-E1 osteoblast-like cells as native collagen I [136] and also promote osteoblastic differentiation of MC3T3-E1 and primary bone marrow stromal cells *in vitro* [137–138]. Furthermore, GFOGER enhances bone repair *in vivo* within rigorous critical-sized rat femur defect models without the delivery of cells or growth factors [139]. GFOGER-functionalized titanium implants also enhance implant integration in a rat cortical model by improving peri-implant bone formation and implant fixation to bone [137–138]. Surprisingly, an *in vitro* study by Hennessy et al. found that adsorption of a different triple-helical GFOGER sequence-containing peptide, GPC$(GPP)_5$GFOGER$(GPP)_5$GPC, did not improve human mesenchymal stem cell adhesion on hydroxyapatite disks [140], although cells cultured on GFOGER-treated tissue culture plastic showed levels of adhesion and spreading equivalent to full-length collagen I. This result contradicts other studies by our group and others which indicate that triple-helical peptides containing the GFOGER sequence support robust cell adhesion [92,135] and differentiation [137] and may possibly be due to low GFOGER adsorption to the hydroxyapatite disks or variations in the primary sequence of the GFOGER peptides used in these studies.

3.2.3.2 DGEA: The DGEA sequence has been suggested as the α2β1 recognition sequence in type I collagen [141], although a different study failed to demonstrate α2β1 mediated cell responses to DGEA [142]. Soluble DGEA peptide inhibits the osteoblastic phenotype of rat bone marrow stromal cells cultured on type I collagen. DGEA coated hydroxyapatite disks have promoted cell adhesion and upregulated osteoblast marker expression in mesenchymal stem cells *in vitro* [140]. However, surfaces modified with a CCGDGEAG peptide failed to support the adhesion of rat calvarial osteoblasts [143].

3.2.3.3 P15: P15 is a synthetic 15-amino acid peptide derived from the (766)GTPGPQGIAGQRGVV(780) sequence found in the α1(I) chain of type I collagen [144]. Several studies have demonstrated that P15 enhances cell adhesion, osteoblastic gene expression and mineralization on anorganic bone matrix (ABM) *in vitro* [145–146] and accelerates early bone formation in porcine [100] and rat [147] cranial defects. In a head-to-head comparison of DGEA and P15 coated hydroxyapatite disks implanted into rat tibiae, both peptides improved new bone formation, but P15 failed to enhance bone implant contact [140]. P15 peptide-coated ABM has also been used in human periodontal osseous defects [148–149]

resulting in better clinical outcomes than open flap debridement alone, and has also been used in a pilot clinical study for long-bone defects [150]. However, P15-coated ABM has not been compared with ABM alone in these human dental applications to determine the role of P-15 alone on the positive effects observed.

3.3 Growth factor-derived peptides

Many growth factors have a profound influence on bone formation including bone morphogenic proteins (BMPs), fibroblast growth factors (FGFs), insulin-like growth factors (IGFs), platelet-derived growth factors (PDGF), transforming growth factor- β (TFG-β) and epidermal growth factor (EGF) [151]. BMPs in particular have been investigated as bone regenerative therapies as they regulate key steps in the process of bone morphogenesis, such as mitosis, chemotaxis, cartilage induction, osteoblastic differentiation and bone formation [152–154].

3.3.1 BMP (73-92)—P24 is a 24-amino acid peptide, SKIPKASSVPTELSAISTLYLDDD, derived from amino acids 73-92 of BMP-2 which has been shown to enhance ectopic bone formation *in vivo* within poly-lactic-co-glycolic (PLGA) implants [155], hydroxyapatite/ recombinant collagen/poly-lactic acid scaffolds [156] and PLGA/polyethylene oxide-aspartic acid scaffolds [157]. *In vitro* studies with MSCs cultured with osteogenic media containing P24 peptide also showed higher alkaline phosphatase activity than cells in osteogenic media alone [158]. Saito et al. have also shown that the similar 20-amino acid BMP-2 (73-92) peptide, KIPKASSVPTELSAISTLYL, sustains prolonged ectopic bone formation in rat calf muscle [159], and also accelerates bone healing in a rat tibial unicortical defect [160] and a rabbit radial unicortical defect [161].

3.3.2 Osteopromotive Domain (OPD)—Lee et al. identified a peptide sequence derived from BMP-2(30-34), DWIVA, termed the osteopromotive domain, which strongly supports human MSC attachment and enhances the alkaline phosphatase activity of human BMSCs *in vitro* [162].

4. Conclusions and Outlook

Bone remodeling and host reactions to implants are complex processes in which osteoblasts and osteoprogenitors play important roles. Because host responses to implants are significantly influenced by the protein signals encountered by the osteoprogenitor/osteoblast receptors on the implant surface, biomaterial research efforts have focused on engineering biological recognition into materials using ECM-mimetic peptides and protein fragments. While many of the peptides reviewed here have shown promising results *in vitro*, their efficacy at enhancing bone healing within defects and promoting implant osseointegration must be further demonstrated within clinically relevant animal models (Table 3). Comparing results between studies using different ECM-mimetic peptides is also hindered by variations in peptide deposition method (adsorption/immobilization), the surface on which it is deposited (non-fouling, surface roughness), and the surface or matrix density of the peptide. The most rigorous head-to-head comparison of different peptides in promoting osteoblastic differentiation would study covalently immobilized ECM peptides presented at an equimolar density on a non-fouling background. The improved bone formation and osseointegration outcomes seen with α2β1-specific GFOGER andα5β1-specific FNIII7-10 and FNIII9*-10 suggest that engineering ECM ligands with specificity to integrins or other receptors implicated in promoting osteogenesis may be a valuable orthopaedic biomaterial strategy. We expect that ECM-mimetic bone biomaterial strategies should upregulate osteoblast bone formation *in vivo* by specifically engaging integrins and other receptors that trigger signaling cascades which enhance adhesion, proliferation and differentiation. Therefore, in the case of

ligands which promote bone formation but were not designed with integrin specificity in mind, it may be valuable to charactarize the cell receptors which the ligands engage using antibody blocking studies in order to determine the cellular mechanisms of their effect. Other important future challenges in ECM-mimetic bioadhesion include using synergistic mixed ligand materials to harness integrin cross-talk, combining ECM motifs with surface topography or roughness as well multivalent ligand presentation to promote integrin clustering and signaling. The use of multivalent ECM-derived peptides with nanoscale control of ligand presentation may be a particularly powerful strategy, as we have recently demonstrated that materials functionalized with self-assembled dimeric, trimeric and pentameric constructs of FNIII7-10 on a protein-resistant background enhance *in vitro* cell signaling and differentiation, and improve the mechanical fixation of titanium implants by up to 250% *in vivo* compared to the monomer [163]. Another important ECM-derived peptide strategy includes using matrix metalloproteinase cleavable peptide sequences in combination with ECM peptide motifs to allow cell-mediated degradation and cell invasion into polymer gels with small pore sizes [164–165].

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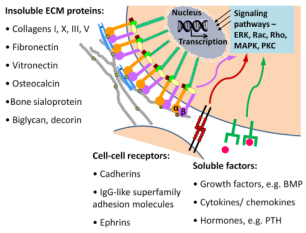

Fig 1.
Bioactive signals found within the extracellular environment in bone.

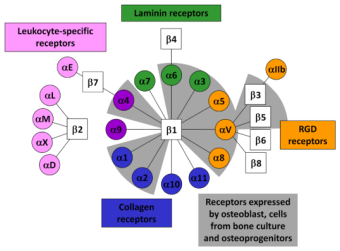

Fig 2.

Integrin alpha and beta subunit combinations, binding specificity and expression in bone cells. Adapted from Hynes[24].

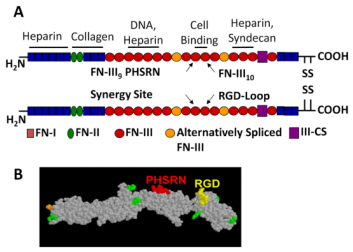

Fig 3.

(A)Structure of plasma fibronectin and location of major binding sites. (B) Space-filling model of $FNIII_{7\text{-}10}$ recombinant fragment of fibronectin.

Table 1

Composition of bone ECM.

		Molecular Weight	Function/regulates	Binds To
Organic (20% of bone mass)	Collagens			
	Type I	Range	Structural protein	Itg, TSP, OSN, OSP, BG, DC, BSP
	Type X	Range	Present in hypertropic cartilage	
	Type III	Range	Col fibril diameter	
	Type V	Range	Col fibril diameter	
	Adhesion proteins			
	Fibronectin	~ 400kD	Adhesion	Itg, Col, heparin,
	Thrombospondin	~ 450 kD	Adhesion, bone formation	Ca, HAP, OSN
	Vitronectin	~ 70kD	Adhesion	Itg, Col, heparin,
	Osteopontin	~44-75kD	Adhesion, proliferation, resorption	Itg
	Osteonectin	~35-45kD	HAP deposition, bone formation	Ca, HAP, Col, TSP
	Osteocalcin	~5kD	Osteoclast activity	Ca
	Bone Sialoprotein	~46-75kD	Adhesion, mineralization	Itg, Col
	Alkaline Phosphatase	~80kD	Mineralization	-
	Proteoglycans			
	Biglycan	~270kD	Col fibril diameter	Col
	Decorin	~150kD	Col fibril diameter	Col, TGF-β
Inorganic (70% of bone mass)	Hydroxyapatite	-	Mechanical strength of bone	-

Itg – Integrins, Col –Collagen, HAP –Hydroxyapatite, Ca –Calcium, TSP –Thrombospondin, OSN – Osteonectin, OSP – Osteopontin, BG – Biglycan, DC–Decorin, BSP – Bone Sialoprotein, TGF-β transforming growth factor- β.

Table 2

Role of key integrins on function of osteoblasts, osteoblast-like cells or osteoprogenitors.

Beta integrin subunits	Alpha integrin subunits	ECM ligand	Expression references	Functions in osteoblasts (OB), OB-like cells, or osteoprogenitors	Function references
Beta 1	Alpha 1	Col I, LN	[29–30], [32], [34–35], [42]	Differentiation	[48], [65]
	Alpha 2	Col I, LN	[29–30], [32], [34], [39], [42]	Adhesion, differentiation	[47–52], [54–55]
	Alpha 3	FN, TN, Col	[29–30], [32], [34–35], [37], [39], [41]	Differentiation	[41]
	Alpha 4	FN	[30–31], [33]	-	-
	Alpha 5	FN	[29–30], [33–35], [37], [41– 42]	Adhesion, survival, differentiation, mechanical sensing,	[41], [57], [59–64]
	Alpha 6	LN	[37], [40], [42]	-	-
	Alpha 8		[41]	-	-
	Alpha v	FN, VN	[33], [35], [39]	-	-
Beta 3	Alpha v	FN, VN, BSP,	[29–32], [35], [37], [41], [42]	Adhesion, inhibits differentiation, inhibits proliferation	[64], [66–67]
Beta 5	Alpha v	OPN, TN VN	[29], [35], [42]	-	-

Table 3

ECM polymers, ECM-derived peptides and growth factor-derived peptides used in bone biomaterials.

Types	Source	Itg. Spec.	MW (kD)	Sequence	References				
					In vitro	In vivo			
					Adhesion/ diff.	Ectopic bone	Cranio-facial defect	Long bone defect	Implant integration
Full-length natural polymers									
Collagen	Animal		~300				[78]	[76–77]	[72–75]
Fibrin	Animal		~340					[79–82]	
DCM	Animal						[87]	[88]	
BSP	Animal		~30–80		[89]				
ECM-derived peptides									
RGD (linear)	Many	αvβ3 , others	~1						[104–105], [109]
RGD (cyclic)	Many	αvβ3, others	~1						[75], [103], [106–108]
GFOGER	Col I	α2β1	~13.5	GGYGGGPC(GPP)$_5$GFOGER(GPP)$_5$GPC/GPC(GPP)$_5$GFOGER(GPP)$_5$GPC	[136–138], [140]			[99]	[138]
P15	Col I	α2β1	~1.5	GTPGPQGIAGQRGVV	[140], [144], [146]		[100], [147–149]	[150]	
DGEA	Col I		~0.5	DGEA	[47], [142]				
FNIII7-10	FN	α5β1	~55		[97], [110], [114]				[110], [114]
FNIII9-10	FN	α2β1			[64]				
RGD-PHSRN	FN		~1.5	RGDG$_{13}$PHSRN/ G$_3$PHSRNG$_6$RGDG	[115–116]				
FHRRIKA	BSP		~1	CGGFHRRIKA	[117–121]				
KRSR	FN, VN, BSP,TN, OPN		~0.5–0.9	KRSR/ KRSRGYC	[122–126]				
BSP (278– 293)	BSP		~2	YESENGEPRGDNYRAYC	[127]				
(351–359) HVP	VN		~1	FRHRNRKGY	[128–129], [131]				[128], [130]
ODP	OPN		~1.5	DVDVPDGRGDSLAYG	[132]				
HBP12	Heparin		~1.5	VRRSKHGARKDR	[133]				
Growth-factor derived peptides									

Types	Source	Itg. Spec.	MW (kD)	Sequence	References				
					In vitro	In vivo			
					Adhesion/ diff.	Ectopic bone	Cranio-facial defect	Long bone defect	Implant integration
p24	BMP-2 (73–92)		~2.5	SKIPKASSVPTELSAISTLYLDDD/KIPKASSVPTELSAISTLYL	[157–158]	[155–157], [159]		[155,] [160–161]	
OPD	BMP-2 (30–34)		~0.5	DWIVA	[162]				

Itg. Spec.– Integrin Specificity, MW (kD) –Molecular Weight (kiloDaltons), Adhesion/Diff. –Adhesion/ Differentiation, HA –Hyaluronic Acid, DCM –Decellularized Matrix, BSP –Bone Sialoprotein, Col I –Collagen I, FN –Fibronectin, VN –Vitronectin, TN –Thrombospondin, OPN – Osteopontin, BMP –Bone Morphogenic Protein.