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REVIEW

The effect of five proteins on stem cells used for osteoblast differentiation and proliferation: a current review of the literature

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Abstract Bone-tissue engineering is a therapeutic target in the field of dental implant and orthopedic surgery. It is therefore essential to find a microenvironment that enhances the growth and differentiation of osteoblasts both from mesenchymal stem cells (MSCs) and those derived from dental pulp. The aim of this review is to determine the relationship among the proteins fibronectin (FN), osteopontin (OPN), tenascin (TN), bone sialoprotein (BSP), and bone morphogenetic protein (BMP2) and their ability to coat different types of biomaterials and surfaces to enhance osteoblast differentiation. Pre-treatment of biomaterials with FN during the initial phase of osteogenic differentiation on all types of surfaces, including slotted titanium and polymers, provides an ideal microenvironment that enhances adhesion, morphology, and proliferation of pluripotent and multipotent cells. Likewise, in the second stage of differentiation, surface coating with BMP2 decreases the diameter and the pore size of the scaffold, causing better adhesion and reduced proliferation of BMP-MSCs. Coating oligomerization surfaces with OPN and BSP promotes cell adhesion, but it is clear that the polymeric coating material BSP alone is insufficient to induce priming of MSCs and functional osteoblastic differentiation in vivo. Finally, TN is involved in mineralization and can accelerate new bone formation in a multicellular environment but has no effect on the initial stage of osteogenesis.

Keywords Dental pulp stem cells · Fibronectin · BMPs · Osteopontin · Tenascin · Bone sialoprotein

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Abbreviations

BMSCs	Bone marrow stromal cells
CaP	Calcium phosphate
ESCs	Embryonic stem cells
ECM	Extracellular matrix
FN	Fibronectin
HA	Hydroxyapatite
MSCs	Mesenchymal stem cells
PEA	Poly(ethyl acrylate)
PLLA	Poly(L-lactic acid)
TG	Tissue transglutaminase
ADSCs	Adipose-derived stromal cells
ASCs	Adult human adipose-derived stem cells
ALP	Alkaline phosphatase
BMPs	Bone morphogenetic proteins
FGF	Fibroblast growth factor
HGF	Hepatocyte growth factor
JNK	c-Jun NH2-terminal kinase
MMP	Metalloproteinase

OA	Osteoactivin
OC	Osteocalcin
PEMF	Pulsed electromagnetic fields
PDGF-AB	Platelet-derived growth factor-AB
PKA	cAMP-dependent protein kinase
PLGA	Poly(lactic-co-glycolic acid)
PTH	Parathyroid hormone
ROK	Rho-associated protein kinase
TGF-beta1	Transforming growth factor-beta1
VEGF	Vascular endothelial growth factor
BSP	Bone sialoprotein
ERRalpha	Estrogen receptor-related receptor alpha
GM-CSF	Granulocyte macrophage colony-stimulating factor
OPN	Osteopontin
BMSC	Bone mesenchymal stem cell
AP1	Activator protein 1
CRE	cAMP response element
FGF2	Fibroblast growth factor 2
FRE	Fibroblast growth factor 2 response element
FSK	Forskolin
HOX	Homeodomain protein-binding site
LPS	Lipopolysaccharide
PDGF	Platelet-derived growth factor
PCL/pHEMA	Polycaprolactone/poly(2-hydroxyethyl methacrylate

Introduction

Bone augmentation is generally carried out using autogenic bone. The ideal bone graft is osteogenic, osteoinductive, and osteoconductive. Osteogenic refers to stem cells or progenitors that are able to form new bone. Osteoinduction is the ability to stimulate new bone formation. Osteoconduction is the ability to support osteogenesis in a vital bone [1]. Autogenous bone is the gold standard. The advantages of autogenous bone graft are the fast angiogenesis from the surrounding original bone [2] and the provision of osteoblasts [3]. However, autogenous bone is not able to fulfill all the requirements for bone regeneration. Discomfort and pain at the donor site of autogenous bone grafting are unavoidable, and donor sites are in limited supply [4]. The large variability in bone defects, the huge biological complexity of bone, the high metabolic activity of bone, and the necessitating vascularization motivate the development of new treatment strategies [5]. Tissue engineering is an alternative to conventional methods for reconstruction. Strategies to engineer bone tissue have been developed using the following three components: scaffold, growth factors, and stem cells. The existence of osteoblasts is important for developing and maintaining the skeleton due to their ability to secret the structural proteins of bone [6].

Stem cells are unspecialized cells. They are characterized by two unique properties, their high self-renewal activity and their multilineage differentiation potential, which make them an ideal source for cellular therapy and regenerative medicine [7]. Mesenchymal stem cells (MSCs) are one type of stem cell and can be isolated from a variety of tissues, such as bone marrow, adipose tissue, dental pulp, etc. MSCs can differentiate into several types of cells, including fibroblasts, adipocytes, osteoblasts, chondrocytes, and skeletal muscle cells [8]. Dental pulp is a highly vascularized tissue and provides another type of MSCs. Dental pulp stem cells (DPSCs) are multipotent stem cells. DPSCs present characteristics of the osteoblastic phenotype, such as osteoblast-like cells, alkaline phosphatase (ALP), collagen I (Col I), osteopontin (OPN), and osteocalcin (OCN). Therefore, DPSCs may be a potential source of osteoblasts that can be used for bone regeneration [9]. DPSCs show good adherence and bone tissue formation on surfaces with micro concavities. SBP-DPSCs are a multipotent stem cell subpopulation of DPSCs. They can differentiate into osteoblasts, synthesize 3D woven bone tissue chips in vitro, and are capable of synergically differentiating into osteoblasts and endotheliocytes [10].

The nature of the surface on which cells are cultured plays an important role in their ability to attach, proliferate, migrate, and function [11]. Components of the extracellular matrix (ECM) are often used to coat glass or plastic surfaces to enhance cell attachment in vitro [11]. Interestingly, which types of proteins are able to coat with scaffolds for enhancing osteoblast attachment. The aim of this review is to determine the relationship between five individual proteins (fibronectin (FN), bone morphogenetic protein (BMP), osteopontin (OPN), tenascin (TN), and bone sialoproteins (BSP)) and osteoblast differentiation in vitro experiments and all in vivo studies were excluded.

Mesenchymal stem cells

Mesenchymal stem cells are multipotent stem cells found in bone marrow stromal cells and other organs. MSCs can differentiate into multiple non-hematopoietic cell lineages, including fibroblasts, adipocytes, osteoblasts, chondrocytes, and skeletal muscle cells [8]. Prospective markers used to distinguish them from other cells and to monitor lineage-specific differentiation are necessary to achieve the full therapeutic potential of hMSCs [12]. The markers used to identify multipotent hMSCs are CD105, CD166, and STRO-1 [13, 14]. Foster et al. [12] studied the dynamic changes undergone by the hMSC membrane proteome before and after short-term osteoblast differentiation and identified 463 proteins. They suggest the increased expression levels of 16 proteins known or implicated in cell adhesion (nine cell matrix adhesion proteins, five hnRNPs, versican, and tenascin) are important for osteoblast adhesion to the underlying matrix in the process of

osteoblast differentiation. Contact with vitronectin and collagen I promotes the osteogenic differentiation of hMSCs, and the extracellular matrix contact alone may be sufficient to induce differentiation in these cells [15]. The Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy proposed three criteria to define a human MSC [16]. First, it must be plastic-adherent when maintained in standard culture conditions. Second, it must be positive for CD105, CD73, and CD90, and negative for CD45, CD34, CD14 or CD11b, CD79alpha, or CD19 and HLA-DR surface molecules. Third, it can differentiate into a plastic-adherent cell when maintained in standard culture conditions [16]. A recent study reported that the hMSC population expressed the human pluripotency surface markers SSEA-3, SSEA-4, and the transcription markers Nanog, Oct3/4, and Sox2 [17].

Dental pulp stem cells (DPSCs)

Gronthos et al. [18] first isolated stem cells from human dental pulp. Laino et al. [19] isolated a selected subpopulation of DPSCs called SBP-DPSCs. In vitro they can form woven bone. The advantage of DPSCs includes their easy collection, which produces very low morbidity and is possible to isolate them, without making a dental extraction just through pulpectomy [10, 219]. The limited ability of dental pulp stromal cells to regenerate hard tissue calls into question their practical application for complete tooth regeneration [20]. Repeated cell passaging may explain the reduction of the osteogenic ability of both bone- and dentinal-derived stem cells [20]. Therefore, it is essential to develop new cell culture methods to harvest the desired cell numbers without obliterating the osteogenic potential [20]. Osteogenesis and angiogenesis mediated by human stromal stem cells from DPSCs may be regulated by distinct mechanisms, leading to the organization of adult bone tissue after stem cell transplantation [21]. CD34(+) cells obtained from dental pulp can be used for engineering bone without the need for prior culture-expanding procedures [22]. Gene expression in osteoblastoids from DPSCs is significantly different from that in osteosarcoma cells, suggesting differences in cell function and activity between these cells [23]. Pluripotent cells isolated from the pulp of human teeth expanded in vitro have been differentiated into osteoblasts, chondrocytes, and adipocytes [24]. Human deciduous dental pulp is an approachable "niche" of stromal stem cells and is an ideal source of osteoblasts as well as mineralized tissue, ready for bone regeneration, transplantation, and tissue-based clinical therapies [25]. DPSCs and stem cells from human exfoliated deciduous teeth (SHED) are not only derived from a very accessible tissue resource but are also capable of providing enough cells for potential clinical applications [24]. SHED show a spindle-shaped morphology, high proliferation rates, and collagen production, resulting in soft tissue formation [26]. In contrast, DPSCs demonstrate reduced proliferation but exhibit an osteoblast-like phenotype, express osteoblast marker genes, and deposit minerals [26].

DPSCs respond to ionizing radiation-induced damage by permanent cell cycle arrest in the G2 phase and stress-induced premature senescence [27]. BMP2-transfected DPSCs show mineralized tissue formation upon ectopic implantation [28]. STRO-1-selected DPSCs show effective hard tissue formation in vivo, and a short in vitro culture period and addition of BMP2 can enhance this effect [29]. STRO-1+ DPSCs consist of several interrelated subpopulations that can spontaneously differentiate into odontoblasts, osteoblasts, and chondrocytes [30]. The differentiation capacity of these DPSCs changes during cell passaging, and DPSCs at the 9th passage restrict their differentiation potential to the osteoblast lineage in vivo [30]. In a pilot study, tooth and bone constructs were prepared from third molar tooth tissue, and iliac-crest bone marrow-derived osteoblasts showed small tooth structures. This tooth structure consisted of organized dentin, enamel, pulp, and periodontal ligament tissues, surrounded by new bone [31].

An in vitro study of MSCs derived from human dental pulp reported that surface microcavities appear to support a more vigorous osteogenic response of stem cells and should be used in the design of therapeutic substrates to improve bone repair and bioengineering applications in the future [32]. Sub-cultured dental pulp cells actively differentiate into odontoblast-like cells and induce calcification in an alginate scaffold [33]. Otaki et al. [34] showed that cultured human adult dental pulp cells produced bone when transplanted into immunocompromised mice. The dental pulp of the erupted molars contains a small population of multipotent cells, whereas the dental pulp of the unerupted molars does not contain multipotent cells but is enriched in osteodentinogenic progenitors engaged in the formation of coronal and radicular odontoblasts [35]. Dental follicle mesenchymal stem cells (DFMSCs) proliferated faster, contained cells that are larger in diameter, and exhibited a higher potential to form adipocytes and a lower potential to form chondrocytes and osteoblasts compared with dental pulp mesenchymal stem cells (DPMSCs) [36]. Stem cells from deciduous teeth, dental pulp, and bone marrow with platelet-rich plasma (PRP), have the ability to form bone, and bone formation with deciduous teeth stem cells (DTSCs) might have the potential to generate a graft between a child and parent [37]. An in vitro study showed that gangliosides play a more important role in regulating osteoblast differentiation of hDPSCs compared to human adipose-derived stem cells (hADSCs) [38].

An in vivo study of patients with bilateral bone reabsorption of the alveolar ridge showed that a biocomplex

of DPSCs, progenitor cells, and collagen sponge can completely restore human mandible bone defects and indicated that this cell population could be used for the repair and/or regeneration of tissues and organs [39]. In Matrigel, DPSCs differentiated with osteoblast/osteocyte characteristics and connected by gap junctions, and therefore formed calcified nodules with a 3D intercellular network [40]. Transplantation of human dental pulp cells—expanded ex vivo in the presence of bFGF into subcutaneous immunocompromised mice—revealed the formation of bone, cartilage, and adipose tissue [41]. DPSCs differentiated in a collagen sponge that actively secreted human type I collagen micro-fibrils and formed a calcified matrix containing trabecular-like structures [40]. DPSCs differentiated into osteoblasts, forming a biocomplex made of Biocoral, ECM, and differentiated cells [42]. An in vivo study reported that fibrin allows the growth and differentiation of dental stem cells and can be inserted into small defects and thus appears to be a promising biomaterial for tissue regeneration in the oral cavity [43]. Another study showed that a tissue-engineered bone complex with nano-hydroxyapatite/collagen/poly(L-lactide) (nHAC/PLA), recombinant human bone morphogenetic protein 2 (rhBMP2), and autologous DPSCs might be a better alternative to autologous bone for the clinical reconstruction of periodontal bone defects [44]. Similarly, osteoblast differentiation of DPSCs and BMP procedure was a better and quicker way by challenging stem cells with LST surfaces [45]. Exogenous glucosamine (GlcN) can promote the osteogenic differentiation of human DPSCs, and the underlying mechanism involves a TGF-beta-dependent Smad signal pathway [46]. DPSCs express insulin growth factor-binding protein 5 and can form mineralized matrix nodules that are a feature exclusive to osteoblasts [9]. Exposure of DPSCs cultured in osteogenic medium to vascular endothelial growth factors (VEGF)-A165 for a similar period enhanced cell differentiation toward osteoblasts [47]. The combination of amniotic fluid stem cells (AFSCs) with DPSCs may provide a rich source of soluble proteins that could be useful for bone engineering purposes [48].

Very recently, a new stem population from the human dental pulp of third molars has been isolated with high efficacy of obtaining and characterized [49]. These cells are dental pulp pluripotent stem cells (DPPSCs). These cells express pluripotency markers (due to the culture conditions) such as Oct-4, Lin-28, Sox-2, and Nanog, four factors whose induced expression alone is sufficient to revert human-differentiated cells to a pluripotent phenotype [49]. DPPSCs have been shown to differentiate into cells from the three embryonic layers, endoderm, mesoderm, and ectoderm, thus displaying a potency that was widely thought to be exclusive to embryonic stem (ES) cells and induced pluripotent stem (IPS) cells [50].

Fibronectin (FN)

Fibronectin (FN) promotes cell adhesion [51] and migration [52]. FN enhanced vascular calcification by promoting the osteoblastic differentiation of vascular smooth muscle cells via the ERK signaling pathway [53]. FN-mediated cell spreading and proliferation are dependent on surface energy and establish a new combinatorial approach for screening cellular response to changes in surface energy [54]. FN, an adhesive glycoprotein, and osteonectin, a counter-adhesive protein, are known to be involved in the early stages of osteogenesis [55]. FN adsorption is correlated to human osteoblast adhesion through morphology and actin cytoskeleton formation [56]. The cell morphology is more rounded when the degree of FN-mediated fibrillogenesis of the substrate is lower [56]. The attachment, proliferation, and morphology of pre-osteoblasts were significantly improved on a cyclic potentiodynamic polarization (CPP)-modified surface, which was attributed to the more open conformation of FN on the CPP-modified surface [57]. Type I collagen, FN, and TN-C localized in the dentary of mandibles and tibias during embryonic osteogenesis [58]. bFGF increased Fn expression in rat osteoblasts via the FGFR2/PLCgamma2/PKCalpha/c-Src/NF-kappaB signaling pathway [59]. The mixture of FN and transglutaminase may prove to be a useful treatment for producing increased osteoblast differentiation on scaffolds [60]. Transglutaminase 2-mediated crosslinking enhances the cell-adhesive properties of FN by increasing the molecular rigidity of FN in the extracellular matrix [61]. Evaluation of gene-modified hESCs that were subsequently attached onto FN-coated gold nanoparticles revealed that the un-differentiation marker, Oct-4, was no longer present following electrical stimulation [62]. An in vitro study revealed a novel cell adhesion/survival mechanism in human osteoblasts that requires the association of FN-bound tissue transglutaminase 2 with the cell surface heparin sulphates in a transamidase-independent manner [63]. Osteoblast FN affects osteoblast function. This does not seem to be mediated by the RGD motif of FN. In contrast, liver-derived FN affects bone matrix properties without affecting osteoblast or osteoclast function [64]. The presence of the adsorbed FN layer on calcium phosphate thin films improved MG63 osteoblast cell adhesion and proliferation and promoted early onset differentiation [65]. The study of the influence of a deep pit on the biological activity of FN and FN reorganization was performed on places on 29- and 45-nm deep pit surfaces, and enhanced late matrix production was found [66] (Table 1).

FN monolayer coverage and the root mean square (rms) roughness are similar on –OH and –COOH-terminated self-assembled monolayers with or without calcium phosphate coating. Higher levels of ALP activity, more actin cytoskeleton formation, and more cell growth are obtained

Table 1 Fibronectin (keyword: fibronectin and osteoblast, 2006–2011 and published in English)

Author	Type	Cell	Objective	Result
Hindé et al. [83]	In vitro	MC3T3-E1 osteoblast-like cells	To study MC3T3-E1 osteoblast-like cell behavior on silicon oxide and PLLA substrates with a focus on the influence of the adhesive protein fibronectin and the non-adhesive protein albumin adsorbed on the substrates	PLLA substrates coated with fibronectin and subsequently exposed to albumin exhibited the highest level of cell differentiation, as assayed via alkaline phosphatase activity
Wang et al. [208]	In vitro	Osteoblasts	To investigate the involvement of cell surface receptors and their intracellular signaling molecules to further explore the pathway mediated by this novel TG-FN heterocomplex	A novel cell adhesion/survival mechanism in human osteoblasts requires association of FN bound TG2 with the cell surface heparan sulphates in a transamidase-independent manner
Bentmann et al. [64]	In vivo; transgenic mice		To identify the receptor involved in fibronectin effects on osteoblasts	Osteoblast fibronectin affects osteoblasts function. This does not seem to be mediated by the RGD motif on fibronectin. In contrast, liver-derived fibronectin affects bone matrix properties without affecting osteoblast or osteoclast function
Cairns et al. [65]	In vitro	MG63 osteoblast-like cells	To investigate the role of surface topography in determining the nature of this cell-protein-surface interaction	The presence of the adsorbed FN layer on the CaP thin films improved MG63 cell adhesion, proliferation, and promoted early onset differentiation
Chen et al. [106]	In vitro		To investigate the details of apatite nucleation and growth on the coating layer in Dulbecco's phosphate-buffered saline solutions containing calcium chloride (DPBS) or DPBS with fibronectin (DPBSF)	The presence of fibronectin in the DPBS solutions delayed the formation and affected the morphology of the apatite. Fibronectin incorporated into apatite deposited on the surface of titanium did not affect its biological activity in terms of promoting osteoblast adhesion
Gonzalez-Garcia et al. [66]	In vitro	MC3T3 osteoblast-like cells	To investigate the influence of deep pit on biological activity of FN	FN reorganization only takes places on the 29- and 45-nm-deep pit surfaces, where enhanced late matrix production was found
Pegueroles et al. [70]	In vitro	MG63 osteoblast-like cells	To investigate the early events of bone matrix formation, and specifically the role of fibronectin (FN) in the initial osteoblast interaction and the subsequent organization of a provisional FN matrix on different rough titanium (Ti) surfaces	Osteoblasts deposit FN fibrils in a specific facet-like pattern that is organized within the secreted total matrix overlying the top of the samples. The thickness of this FN layer increased when the roughness of the underlying topography was increased, but not by more than half of the total maximum peak-to-valley distance
Ribeiro et al. [55]	In vitro	MC3T3-E1 osteoblast-like cells	To analyze the influence of two distinct nanophased HA ceramics, (HA725 and HA1000) on FN and osteonectin adsorption	FN as an adhesive glycoprotein and osteonectin as a counter-adhesive protein, are known to be involved in the early stages of osteogenesis. However, the osteoblast adhesion and metabolic activity seemed to be more sensitive to surfaces morphology and roughness than to the type of adsorbed proteins
Zhang et al. [83]	In vitro	hMSC	To investigate the effects of a bio-inspired ceramic surface modified with a novel recombinant protein on surface parameters and cell behavior	The fibronectin module III7-10 and extracellular domains 1 and 2 of cadherin 11 bio-inspired ceramic surface possesses enhanced functionality in adhesion, proliferation and ossification and may be a promising scaffold for tissue engineering
Ball et al. [60]	In vitro		To explore the possibility of controlling cell interaction with biomaterials, tricalcium phosphate scaffolds were modified using the enzyme tissue transglutaminase (tTgase) in conjunction with fibronectin	The mixture of fibronectin and transglutaminase may prove to be a useful treatment for producing increased osteoblast differentiation on scaffolds

Table 1 continued

Author	Type	Cell	Objective	Result
Forsprecher et al. [61]	In vitro	MC3T3-E1 osteoblast	To test the effects of extracellular FN, before and after in vitro crosslinking and polymerization by TG2	TG2-mediated crosslinking enhances the cell-adhesive properties of FN by increasing the molecular rigidity of FN in the extracellular matrix
Muhonen et al. [84]	In vitro	MC3T3-E1 osteoblast	To investigate the effect of two phases of Nitinol with plasma fibronectin and studied if this modifies the proliferation and cell cycle of MC3T3-E1 osteoblasts	Fibronectin improved cell proliferation in both phases, but the effect of fibronectin coating was stronger on the austenite surface. In both Nitinol phases, the proportion of cells in the G(1) phase was observed to grow in the presence of fibronectin. This could indicate cell differentiation on Nitinol
Rico et al. [56]	In vitro		To investigate the influence of surface chemistry and effect on osteoblast adhesion	FN adsorption is correlated with human osteoblast adhesion through morphology and actin cytoskeleton formation. Actin polymerization is in need of the formation of the protein network on the substrate's surface. Cell morphology is more rounded when the degree of FN fibrillogenesis on the substrate is lower
Shahryari et al. [57]	In vitro		To investigate the influence of an electrochemical surface passivation technique (cyclic potentiodynamic polarization, CPP) on the physico-chemical surface properties of SS316LS and its subsequent response to FN and pre-osteoblasts	The attachment, proliferation, and morphology of pre-osteoblasts were significantly improved on the CPP-modified surface, which was attributed to the more open conformation of Fn on the CPP-modified surface. Thus, the CPP surface passivation method was demonstrated to yield a SS316LS surface of enhanced biocompatibility
Toworfe et al. [67]	In vitro	MC3T3-E1	To investigate the effect of silane self-assembled monolayer surfaces on apatite formation, fibronectin adsorption, and osteoblast cell function	FN monolayer coverage and the root mean square (rms) roughness are similar on –OH and –COOH terminated self-assembles monolayers with or without CaP coating, higher levels of ALP activity, more actin cytoskeleton formation, and more cell growth are obtained on –OH– and –COOH–terminated SAMs with CaP coating
Tsai et al. [68]	In vitro	MG-63 osteoblast-like cells	To investigate the morphology of osteoblast on nano-grooved substrates	FN coating initially modulated cellular spreading, length, and orientation on all types of grooved surfaces. After 24 h of culture, the cell morphology was not affected by FN coating on the 250- and 500-nm surfaces, while FN decreased cell alignment on the 90-nm surfaces
Woo et al. [62]	In vitro	hESCs	To investigate the effect of electronical stimulation on the differentiation of hESCs adhered onto fibronectin-coated gold nanoparticles	Evaluation of gene-modified hESCs that were subsequently attached onto fibronectin-coated gold nanoparticles revealed that the un-differentiation marker, Oct-4, was no longer present following electrical stimulation
Schonmeyr et al. [73]	In vitro, in vivo; mice	Bone-firming cells	To study the result of the treatment of HA scaffolds with FN and fetal calf serum	In vitro attachment and proliferation of bone-forming cells on hydroxyapatite is significantly increased by pretreatment with fibronectin/fetal calf serum, but this difference is less profound and not significant in vivo
Sousa et al. [76]	In vitro	MC3T3-E2 osteoblast	To assess the influence of the competitive preadsorption of human serum albumin (HSA) and human plasma FN from binary solutions and 10 % plasma on MC3T3-E1 osteoblast adhesion and morphology on two types of TiO_2 substrates	FN reversibility does not seem to be dependent on the HSA/FN adsorption mass ratio in solution, suggesting that FN competitively adsorbs to TiO_2 in a favorable conformation and does not suffer subsequent conformational changes allowing exchange with other FN molecules in solution

Table 1 continued

Author	Type	Cell	Objective	Result
Jimbo et al. [79]	In vitro, in vivo; mouse femur	BMSCs	To clarify the role of pFN during osseointegration	The in vivo results showed that faster direct bone formation was seen for the FN-Ti-acryl group compared to the Ti-acryl group. The in vitro results showed that pFN significantly promoted BMSCs chemotaxis, however, had no effect on proliferation or differentiation. The results indicate that pFN regulated chemotaxis of osteogenic cells and coating the implant with pFN enhanced earlier osseointegration
Pugdee et al. [78]	In vitro	MC3T3-E2 osteoblast	To examine the cell attachment and gene expression of MC3T3-E1 cells on FN-immobilized titanium using GeneChip	Cells attached on FN-immobilized titanium at a higher rate than untreated titanium. The immobilization of FN on tresylated titanium promoted early matrix mineralization and bone formation
Sogo et al. [75]	In vitro	hMSCs	To investigate FN-calcium phosphate composite layer on HA	The FN-calcium phosphate composite layer formed on the HA is useful for the enhancement of the spreading and osteogenic differentiation of hMSCs in vitro
Tang et al. [59]	In vitro	Osteoblast	To show basic fibroblast growth factor stimulates fibronectin expression through phospholipase C gamma, protein kinase C alpha, c-Src, NF-kappaB, and p300 pathway in osteoblasts	bFGF increased Fn expression in rat osteoblasts via the FGFR2/PLCgamma2/PKCalpha/c-Src/NF-kappaB signaling pathway
Deligianni et al. [69]	In vitro	Human osteoblasts	To investigate the contribution of fibronectin preadsorption to enhance the osteoblasts' adhesion and strength on hydroxyapatite	Fibronectin preadsorption increased the number of attached osteoblasts on smooth and rough hydroxyapatite substratum at 40 and 62 %, respectively, while it increased osteoblast attachment strength on the smooth and rough substratum at 165 and 73 %, respectively
Ding et al. [53]	In vitro	Vascular smooth muscle cells	To investigated the effect of FN on the calcification of vascular smooth muscle cells	FN enhanced vascular calcification by promoting the osteoblastic differentiation of vascular smooth muscle cells via ERK signal pathway
Kennedy et al. [54]	In vitro	Osteoblast	To study the effect of surface energy on fibronectin-mediated cell response	FN-mediated cell spreading and proliferation are dependent on surface energy and establish a new combinatorial approach for screening cell response to changes in surface energy
Osathanon et al. [80]	In vitro	SaOS-2 human osteoblast-like cells	To compare the early response of human osteoblast-like cells (SaOS-2) on commercially pure titanium (cpTi) and titanium-6-aluminium-7-niobium (Ti-6Al-7Nb)	Ti-6Al-7Nb possess a good potential to support SaOS-2 cells on spreading and fibronectin and osteopontin synthesis, therefore, this material may be one of a candidate material used in implant dentistry
Park et al. [81]	In vivo; rabbit tibiae		To evaluate the bone response around anodized titanium implants treated with FGF-FN fusion protein using the histomorphometric analysis and the removal torque test	FGF-FN fusion protein coating on anodized implants may enhance osseointegration

on –OH- and –COOH-terminated SAMs with calcium phosphate coating [67]. The study of the morphology of osteoblast on nano-grooved substrates showed that FN coating initially modulated cellular spreading, length, and orientation on all types of grooved surfaces [68]. After 24 h of culture, the cell morphology was not affected by FN coating on the 250- and 500-nm surfaces, while FN decreased cell alignment on the 90-nm surfaces [68].

The study of the contribution of FN pre-adsorption on osteoblasts' adhesion and strength on hydroxyapatite found that FN pre-adsorption increased the number of attached osteoblasts on smooth and rough hydroxyapatite substratum by 40 and 62 %, respectively, while it increased osteoblast attachment strength on the smooth and rough substratum at 165 and 73 %, respectively [69]. The investigation of the early events of bone matrix formation, and specifically

the role of FN in the initial osteoblast interaction and the subsequent organization of a provisional FN matrix on different rough titanium surfaces revealed that osteoblasts deposit FN fibrils in a specific facet-like pattern that is organized within the secreted total matrix overlying the top of the samples [70]. An in vitro study proved that a titanium surface treated with fibronectin is biocompatible and accelerates the osseointegration process more than surface treatment with acid and/or immersion in a solution containing fluoride ions [71]. Another recent study also supported that Ti-6Al-4V coated with fibronectin stimulates osteoblast differentiation [72].

The thickness of this FN layer increased when the roughness of the underlying topography was increased, but not by more than half of the total maximum peak-to-valley distance [70]. The in vitro attachment and proliferation of bone-forming cells on hydroxyapatite was significantly increased by pre-treatment with FN/fetal calf serum, but this difference is less profound and not significant in vivo [73]. A study of the influence of two distinct nanophased hydroxyapatite ceramics on FN and osteonectin adsorption also reported that osteoblast adhesion and metabolic activity seemed to be more sensitive to surface morphology and roughness than to the type of adsorbed proteins [55]. The presence of FN in Dulbecco's phosphate-buffered saline solutions containing calcium chloride (DPBS) solutions delayed the formation and affected the morphology of apatite [74].

The FN-calcium phosphate composite layer formed on hydroxyapatite is useful for the enhancement of the spreading and osteogenic differentiation of hMSCs in vitro [75]. FN incorporated into apatite deposited on the surface of titanium did not affect its biological activity in terms of promoting osteoblast adhesion [74]. FN reversibility does not seem to be dependent on the human serum albumin/FN adsorption mass ratio in solution, suggesting that FN competitively adsorbs TiO_2 in a favorable conformation and does not suffer subsequent conformational changes allowing exchange with other FN molecules in solution [76]. The study of the absorption and conformation behavior of biotinylated FN on a TiO surface showed that the conformational change of biotinylated FN on the streptavidin monolayer results in a FN structure similar to the conformation inside the extracellular matrix and therefore explains the higher cell affinity for these surfaces [77]. Cells attached to FN-immobilized titanium at a higher rate than untreated titanium. The immobilization of FN on tresylated titanium promoted early matrix mineralization and bone formation [78]. The in vivo results showed faster direct bone formation for the fibronectin-Ti-acryl group compared to the Ti-acryl group [79]. The in vitro results showed that pFN significantly promoted BMSCs chemotaxis; however, it had no effect on proliferation or differentiation [79]. The results indicate that pFN-regulated chemotaxis of osteogenic cells and coating the implant with pFN enhanced earlier osseointegration [79]. Ti-6Al-7Nb possesses good potential to support SaOS-2 cell spreading and FN and OPN synthesis. Therefore, this material may be a candidate material for implant dentistry [80]. Park et al. [81] evaluated the bone response around anodized titanium implants treated with a FGF-FN fusion protein using histomorphometric analysis and the removal torque test. They showed that FGF-FN fusion protein coating on anodized implants may enhance osseointegration [81].

PLLA substrates coated with FN and subsequently exposed to albumin exhibited the highest level of cell differentiation as assayed by alkaline phosphatase activity [82]. The FN module III7-10 and extracellular domains 1 and 2 of cadherin 11 bio-inspired a ceramic surface with enhanced functionality in adhesion, proliferation, and ossification that may be a promising scaffold for tissue engineering [83]. The investigation of the effect of two phases of Nitinol with plasma FN showed that FN improved cell proliferation in both phases, but the effect of the FN coating was stronger on the austenite surface [84]. In both Nitinol phases, the proportion of cells in the G(1) phase increased in the presence of FN. This could indicate cell differentiation on Nitinol [84].

BMP2

Bone morphogenetic protein-2 (BMP-2) is highly involved in the induction of osteoblast differentiation from mesenchymal cell precursors, as well as in enhancing bone matrix production by osteoblastic cells [85]. The observed functional redundancy of type II BMP receptors in osteoblasts provides novel information about the BMP signaling pathway, which is essential to initiating osteoblast differentiation [86]. The study of three different bone cell samples revealed the possibility that BMP receptor-IB could be a therapeutic target for enhancing bone regeneration in vivo [87]. Non-autologous BMP2 gene-transfected stem cells have potential utility for the enhancement of bone repair and bone regeneration in vivo [88]. The sensitivity of cells to BMP2 correlates with BMP receptor expression [89]. BMP2 stimulated osteoblastic markers faster and to a greater extent than Runx2 [90]. Runx2-engineered cells did not utilize paracrine signaling via secreted osteogenic factors in contrast to cells overexpressing BMP2 [90]. Activation of the PKA pathway may be a key BMP2-activated signaling event that leads to osteogenesis, and the downregulation of PKIgamma may be a prerequisite for PKA activation during the osteoblastic differentiation of precursor cells

[91]. BMP2 induces Runx2-deficient cells to express markers related to osteoblast and chondroblast differentiation using a Runx2-independent pathway, but it failed to induce these cells to differentiate into bone-forming osteoblasts and mature chondrocytes. [92] Runx2 may be important to regulate osterix (Osx) during osteoblast lineage progression. When Runx2 activity is blocked, it inhibits the BMP2-mediated induction of Osx [93]. BMP2-induced Osx expression is mediated by Dlx5 [94, 95] (Table 2).

Different mechanisms for BMP2/4- and BMP6/7-induced osteoblastic differentiation in primary hMSCs exist [96]. The osteogenic sensitivity of muscle progenitors provides mechanistic insight into the variable response of different cell lineages to BMP2 [89]. BMP2 may regulate osteoblast function in part through modulation of beta-catenin signaling [97]. BMP2 enhances dexamethasone/ascorbic acid/glycerolphosphate-induced osteogenic differentiation in mesenchymal bone marrow cells [98]. BMP2 agents interact in various ways and can modify osteoblastic bone formation [98]. Pulsed electromagnetic fields (PEMF) enhance the osteogenic effects of BMP2 on MSCs cultured on calcium phosphate substrates, suggesting that PEMF can improve MSC response to BMP2 in vivo in a bone environment [99]. The down-regulation of microRNA-208 in BMP2-stimulated osteoblast differentiation is an important part of the regulatory machinery involved in early osteogenesis [100]. MC3T3-E1-clone 24 cells can be induced by BMP2 to differentiate into mineralizing osteoblast cultures [101]. BMP2 also stimulates Pi transport activity through a selective increase in the expression of the type III Pi transporter Pit-1. In MC3T3-E1 cells, this effect is mediated by the JNK pathway and plays an essential role in bone matrix calcification induced by BMP2 [102]. Nell-1 and BMP2 synergistically enhanced the osteogenic differentiation of myoblasts and phosphorylation in the JNK MAPK pathway [103]. Glucocorticoids regulate BMP2 via a far-downstream domain, and activation of Smad, not ALP, best predicts the pro-mineralization potential of rhBMPs [104]. BMP2 regulates osteoactivin expression through the Smad1 signaling pathway. The osteoactivin protein acts as a downstream mediator of BMP2 effects on osteoblast differentiation and function. BMP2-induced osteoactivin transcription is differentially regulated by Dlx3, Dlx5, and Msx2 during osteoblast differentiation [105]. Simvastatin can promote osteoblast viability and differentiation via the membrane-bound Ras/Smad/Erk/BMP2 pathway [106]. CYR61 up-regulates BMP2 mRNA and protein expression, resulting in enhanced cell proliferation and osteoblastic differentiation through activation of the α(v)β(3) integrin/integrin-linked kinase/ERK signaling pathway [107]. PDZRN3 plays an important role in negative feedback control of BMP2-induced osteoblast differentiation in C2C12 cells through inhibition of Wnt-β-catenin signaling [108]. In vitro, combined gene therapy with human angiopoietin-1 gene (hAng-1) and hBMP2 using lentivirally co-transfected MSCs is feasible [109]. WISP-1 in pre-osteoblasts has a positive influence on bone cell differentiation and function and may work by enhancing the effects of BMP2 to increase osteogenesis through a mechanism potentially involving binding to integrin α(5)β(1) [110]. An in vitro study using bone marrow-derived MSCs harvested from the iliac crest of three human donors and tuber coxae of three equine donors showed that genetically modified bone marrow-derived MSCs could be used for cell-based delivery of BMPs to a bone formation site [111]. The lipid microtube system is able to provide sustained delivery of biologically active BMP2 and thereby induce osteogenic differentiation [112]. The proteoglycan populations that are induced in C2C12 cells upon osteoblastic differentiation and induced by BMP2 [113]. Specific levels of static stretching force increase cell proliferation and effectively stimulate the osteoblast differentiation of C2C12 cells in conjunction with BMP2 stimulation, thus indicating a synergistic interaction between mechanical strain and cytokine signaling [114].

DeltaEF1 acts as a potent inhibitor of BMP2-induced osteogenesis in vitro in part by differentially regulating the AP-1 signaling pathway [115]. Cells contacting 316L steel were exposed to increased concentrations of Ni, which impaired BMP2-induced ALP activity [116]. CCN3 exerts inhibitory effects on BMP2-induced osteoblast differentiation through its involvement in the BMP and Notch signaling pathways [117]. Heparin suppresses BMP2-BMP receptor binding and inhibits BMP2 osteogenic activity in vitro [118]. Gremlin inhibits BMP2 signaling and activity and does not have independent actions on ERK signaling in osteoblasts [119]. The effect of platelet-released supernatant (PRS) on human MSCs could be at least partially mediated by BMP2. Activated autologous PRS could therefore provide an alternative to agents like recombinant bone growth factors by increasing osteoblastic differentiation of bone precursor cells at bone repair sites, although further studies are needed to fully support these observations [120]. Hypoxia enhances BMP2 expression in osteoblasts through an HIF-1alpha-dependent mechanism involving the activation of integrin-linked kinase Akt (1L-6-hydroxymethyl-chiro-inositol-2-[(R)-2-*O*-methyl-3-*O*-octadecylcarbonate]) and mammalian target of rapamycin pathways [121]. Tanshinone IIA enhances the commitment of C2C12 cells to differentiation into osteoblasts and through synergistic cross talk between tanshinone IIA-induced p38 activation and BMP2-induced Smad activation [122]. BMP2 down-regulation of PTHrP could facilitate terminal differentiation of osteoblasts [85]. PTH stimulated BMP2 mRNA expression via the mevalonate pathway and ROK in osteoblastic MC3T3-E1 cells [123]. Adiponectin enhanced BMP2 expression in osteoblastic cells, and the

Table 2 Bone morphogenetic proteins 2 (keyword: BMP2 and osteoblast, 2006–2011 and published in English)

Author	Type	Cell	Objective	Result
Hughes-Fulford and Li [135]	In vitro	MC3T3E1 osteoblast-like cells	To describe the sequential roles of FGF-2 in inducing gene expression, cell growth, and BMP-2 in gene expression and mineralization of bone	The ability of FGF-2 to re-program a mineralizing gene expression profile to one of proliferation suggests that FGF-2 plays a critical role of osteoblast growth in early fracture repair while BMP-2 is instrumental in stimulating mineralization
Kaewrichan et al. [136]	In vivo; nude mice	Marrow stromal cells	To develop culture conditions that permit a rapid increase in the number of marrow stromal cells while retaining or improving their potential for complete differentiation in vivo	FGF2 increased the pool of committed osteoblasts by up-regulating the Cbfa1/Runx2 gene. The later stages of bone formation seemed to be induced by Cbfa1/Runx2-downstream factors such as BMP2, ALP, collagen type I, bone sialoprotein and OC
Keibl et al. [138]	In vivo; femur of male rat	ASCs	To investigate bone healing upon administration of the growth factor BMP-2 embedded with ASCs in a locally applied fibrin matrix	Transplantation of ASC modulated the callus induction by BMP-2 to a normal volume
Lee et al. [139]	In vitro, in vivo	MC3T3-E1 preosteoblasts	To examine a 3D scaffold with embedded growth factor-delivering microspheres	Solid free-form fabrication scaffolds created by microstereolithography were superior to traditional scaffolds produced using a particulate leaching/gas foaming method. The scaffolds that released BMP2 promoted bone formation
Liu et al. [109]	In vitro	Marrow-derived mesenchymal stromal cells	To investigate the osteogenic and angiogenic effects of marrow-derived mesenchymal stromal cells when co-transfected (by means of lentivirus) the human angiopoietin-1 gene (hAng-1) and hBMPs	The combined gene therapy with hAng-1 and hBMP2 using lentivirally co-transfected MSCs is feasible
Liu et al. [109]	In vitro	Osteoblast	To investigate the role of type II BMP receptors in osteoblasts	The observed functional redundancy of type II BMP receptors in osteoblasts is novel information about the BMP signaling pathway essential for initiating osteoblast differentiation
Ono et al. [110]	In vivo; transgenic mice that overexpressed human WISP-1 in preosteoblasts		To determine the function of WISP-1 during osteogenesis, osteogenic bone marrow stromal cells	WISP-1 has a positive influence on bone cell differentiation and function and may work by enhancing the effects of BMP-2 to increase osteogenesis through a mechanism potentially involving binding to integrin $\alpha(5)\beta(1)$
Schofer et al. [146, 147]	In vitro	Mesenchymal stem cell differentiation of osteoblasts	To analyze whether these processes can be remodeled in an artificial PLLA-based nanofiber scaffold	The incorporation of BMP2 into PLLA-collagen type I nanofibers resulted in a decrease in diameter as well as pore sizes of the scaffold. Mesenchymal stem cells showed better adherence and a reduced proliferation on BMP-containing scaffolds
Carpenter et al. [111]	In vitro	Bone marrow-derived mesenchymal stem cells were harvested from the iliac crest of three human donors and tuber coxae of three equine donors	To compare the effect of genetic modification of human and equine bone marrow-derived mesenchymal stem cells with BMP2 or -7 or BMP2 and -7 on their osteoblastogenic differentiation in the presence or absence of dexamethasone	Genetically modified bone marrow-derived mesenchymal stem cells could be useful for cell-based delivery of BMPs to a site of bone formation

Table 2 continued

Author	Type	Cell	Objective	Result
Chen et al. [74, 106]	In vitro	Osteoblasts	To understand the events of osteoblast differentiation induced by statins	Simvastatin can promote osteoblast viability and differentiation via membrane-bound Ras/Smad/Erk/BMP2 pathway. Statins stimulate osteoblast differentiation in vitro and may be a promising drug for the treatment of osteoporosis in the future
Honda et al. [108]		C2C12 mouse mesenchymal progenitor cells	To examine the potential role of PDZRN3 in the differentiation of C2C12 cells into osteoblasts	PDZRN3 plays an important role in negative feedback control of BMP2-induced osteoblast differentiation in C2C12 cells through inhibition of Wnt-β-catenin signaling
Huang et al. [46, 124]	In vitro	Cultured osteoblasts	To investigate the effects of adiponectin on BMPs expression	Adiponectin enhances BMP2 expression in osteoblastic cells, and AdipoR1 receptor, AMPK, p38 and NF-kappaB signaling pathways may be involved in increasing BMP-2 expression by adiponectin
Ishibashi et al. [128]	In vitro	PDL cells, MC3T3-E1 osteoblastic cells	To analyze characteristics unique for PDL at a molecular level	Raise a possibility that PDL cells respond to BMP2 via a unique signaling pathway dependent on endoglin, which is involved in the osteoblastic differentiation and mineralization of the cells
Itoh et al. [100]	In vitro	MC3T3-E1 cells, primary osteoblast cells	To investigate the role of microRNAs to BMP2	The down-regulation of microRNAs-208 in BMP2-stimulated osteoblast differentiation is an important part of the regulatory machinery involved in early osteogenesis
Kawasaki et al. [133]	In vivo; mouse model		To analyze osteogenic properties of HGF, particularly during BMP2-induced bone formation	Treatment with HGF prior to administration of BMP-2 induced cellular proliferation of mouse embryonic fibroblasts and did not influence subsequent osteoblast differentiation induced by BMP-2
Kim et al. [127]	In vitro	C2C12 cells	To demonstrate a stimulatory effect of tanshinone IIA isolated from the root of Salvia miltiorrhiza on the commitment of bi-potential mesenchymal precursor C2C12 cells into osteoblasts in the presence of BMP2	Tanshinone IIA enhances the commitment of C2C12 cells into osteoblasts and their differentiation through synergistic cross talk between tanshinone IIA-induced p38 activation and BMP-2-induced Smad activation
Kim et al. [127]	In vitro	MC3T3-E1 preosteoblasts	To investigate osteoblast responses to high-power laser and combined irradiation with BMP2 treatment	High-power, low-level Nd:YAG laser increased osteoblast activity, very efficiently accelerating mineral deposition. Osteoinductive effect of laser is likely mediated by activation of BMP2-related signaling pathway
Ko et al. [129]	In vitro, in vivo; Sprague–Dawley rats	MG-63 cells	To assess the cytotoxicity of mineral trioxide aggregate (MTA) and BMP2 and the response of rat pulp tissue to MTA and BMP2	The addition of BMP2 had a beneficial effect in vitro, reducing the initial cytotoxicity of freshly mixed MTA. However, the pulp reaction to a combination of MTA and BMP-2 was not significantly better than use of MTA alone
Mandal et al. [125]	In vitro	Mouse 2T3 preosteoblasts	To demonstrate a novel mechanism of BMP-2-induced osteoblast differentiation	A signaling pathway linking BMP-2 stimulated Nox4-derived physiological reactive oxygen species to BMP-2 expression and osteoblast differentiation
Seib et al. [142]	In vitro	C2C12s	To investigate the role of ECM physisorbed BMPs in inducing the differentiation of resident mesenchymal stem cells into osteoblasts	Physisorbed BMP2 is more active than diffusible BMP2. The current clinical practice of immobilizing BMPs on collagen type I scaffolds not only prolongs local delivery of the morphogen but could also enhance biological activity at the cellular level

Table 2 continued

Author	Type	Cell	Objective	Result
Su et al. [107]	In vitro	MC3T3-E1 osteoblasts, primary cultured osteoblasts	To investigate the interaction between CYR61 and BMP-2	CYR61 up-regulates BMP-2 mRNA and protein expression, resulting in enhanced cell proliferation and osteoblastic differentiation through activation of the α(v)β(3) integrin/integrin-linked kinase/ERK signaling pathway
Tseng et al. [121]	In vitro	Osteoblastic cells MG-63, hFOB, and bone marrow stromal cells M2-10B4	To investigate the effects of hypoxia exposure on BMP2 expression in cultured osteoblasts	Hypoxia enhances BMP2 expression in osteoblasts by an HIF-1alpha-dependent mechanism involving the activation of integrin-linked kinase/Akt (1L-6-hydroxymethyl-chiro-inositol-2-[(R)-2-*O*-methyl-3-*O*-octadecyl carbonate]) and mammalian target of rapamycin pathways
Verrier et al. [120]	In vitro	hMSCs	To test the effect of platelet-released supernatant (PRS) on human MSCs differentiation towards an osteoblastic phenotype	The effect of PRS on human MSCs could be at least partially mediated by BMP-2. Activated autologous PRS could therefore provide an alternative to agents like recombinant bone growth factors by increasing osteoblastic differentiation of bone precursor cells at bone repair sites, although further studies are needed to fully support our observations
Zhao et al. [154]	In vivo; mandibular defects in rats	bMSCs	To investigate the effects of mandibular defects repaired by a tissue engineered bone complex with beta-TCP and BMP2 gene-modified bone marrow stromal cells (bMSCs)	BMP2 regional gene therapy together with beta-TCP scaffold could be used to promote mandibular repairing and bone regeneration
Zheng et al. [155]	In vitro, in vivo; femoral defects	MC3T3-E1 pre-osteoblasts	To demonstrate that metallic nanosilver particles (with a size of 20–40 nm)-PLGA composite grafts have strong antibacterial properties	Nanosilver of defined particle size is bactericidal without discernable in vitro and in vivo cytotoxicity or negative effects on BMP-2 osteoinductivity, making it an ideal antimicrobial for bone regeneration in infected wounds
Balmayor et al. [156]	In vitro	Osteo/myoblast cell line (C2C12)	To develop and test a novel injectable drug delivery system consisting of starch-poly-epsilon-caprolactone microparticles for inducing osteogenesis and requiring smaller amounts of BMP2	Starch-poly-epsilon-caprolactone microparticles are suitable carriers for the incorporation and controlled release of glucocorticoids and growth factors. Specifically, they reduce the amount of BMP-2 needed and allow more sustained osteogenic effects
Degat et al. [140]	In vitro, in vivo; rats	C2C12 myoblasts	To explore the binding capacity of synthetic heparin-like dextran derivatives to rhBMP2	The formation of the BMP-2/carboxymethylated dextran polymers grafted with high amounts of benzylamide (DMCB) complex may protect the protein from being inactivated. In rats in vivo, DMCB also stimulated ectopic calcification mediated by BMP-2
Hou et al. [126]	In vitro	Osteoblast	To investigate the effect of ultrasound to BMP2 expression	Ultrasound increased BMP2 expression in osteoblasts via the PI3K, Akt, c-Fos/c-Jun, and AP-1 signaling pathway
Johnson et al. [112]	In vitro		To develop sustained release systems	The lipid microtube system is able to provide sustained delivery of biologically active BMP2 and thereby induce osteogenic differentiation

Table 2 continued

Author	Type	Cell	Objective	Result
Kim et al. [114]	In vitro	C2C12 pluripotential myoblasts	To test mechanical stretching enhances osteoblast differentiation in distraction osteogenesis by means of interaction with BMP2 induced cytokine stimulation	Specific levels of static stretching force increase cell proliferation and effectively stimulate the osteoblast differentiation of C2C12 cells in conjunction with BMP2 stimulation, thus indicating a synergistic interaction between mechanical strain and cytokine signaling
Liu et al. [89]	In vitro	Myoblastic murine cell lines, primary cells with osteoprogenitors	To test the sensitivity of cells to BMP2 would correlate with BMP receptor expression	Osteogenic sensitivity of muscle progenitors and provide a mechanistic insight into the variable response of different cell lineages to BMP2
Schofer et al. [145]	In vitro	hMSCs	To analyze the impact of PLLA nanofibers on VEGF and BMP2 gene expression during the time course of hMSC differentiation towards osteoblasts	The PLLA nanofibers have little effect on growth factor production. An enhancement in gene expression of BMP2 and VEGF can be achieved by an incorporation of BMP2 into the PLLA nanofibers
Takase et al. [123]	In vitro	Osteoblastic MC3T3-E1 cells	To examine if PTH affects BMP2 expression and to clarify its involvement of the mevalonate pathway	PTH stimulated BMP-2 mRNA expression via the mevalonate pathway and ROK in osteoblastic MC3T3-E1 cells
Zhang et al. [97]	In vitro	Primary osteoblasts, osteoblast precursor cell lines 2T3, and MC3T3-E1 cells	To investigate the effect of BMP-2 on beta-catenin signaling	BMP2 may regulate osteoblast function in part through modulation of the beta-catenin signaling
Jager et al. [98]	In vitro	Bone marrow-derived human MSCs	To investigate interactions between dexamethasone and BMP2 for an osteoblastic differentiation of MSCs	BMP2 enhances dexamethasone/ascorbic acid/glycerolphosphate-induced osteogenic differentiation in mesenchymal bone marrow cells. Both agents interact in various ways and can modify osteoblastic bone formation
Kanzaki et al. [118]	In vitro	Osteoblast	To investigate the role of heparin in the biological activity of BMP	Heparin suppresses BMP2-BMP receptor binding, and inhibits BMP2 osteogenic activity in vitro
Laflamme and Rouabhia [141]	In vitro	Osteoblasts	To evaluate the effect of BMP2 and BMP7 homodimers and a mixture of BMP2/BMP7 homodimers on osteoblast adhesion and growth following culture on a collagen scaffold	The BMP2, BMP7, and a mixture of BMP2/BMP7 all promoted osteoblast growth on the collagen scaffold, with the mixture of BMP2/BMP7 enhancing the most growth. BMP2, BMP7, and the mixture of BMP2/BMP7 could promote bone regeneration via different mechanisms involving IL-6 and MMP inhibitors
Lavery et al. [96]	In vitro	hMSCs	To evaluate receptor utilization by BMP-2, BMP-4, BMP-6, and BMP-7 in primary hMSCs	Different mechanisms for BMP2/4- and BMP6/7-induced osteoblastic differentiation in primary hMSCs
Luppen et al. [104]	In vitro		To better understand how glucocorticoids regulate BMPs	Glucocorticoids regulate BMP2 via a far-downstream domain, and activation of Smad, not ALP, best predicts the pro-mineralization potential of rhBMPs
Schwartz et al. [99]	In vitro	hMSCs	To test PEMF, enhances osteogenesis of MSCs in the presence of an inductive stimulus like BMP2	PEMF enhances osteogenic effects of BMP2 on MSCs cultured on calcium phosphate substrates, suggesting that PEMF will improve MSC response to BMP2 in vivo in a bone environment
Singhatanadgit et al. [87]	In vitro	Three different bone cell samples	To examine the effects of BMPR-IB knockdown on BMP-induced osteoblast-associated genes	The possibility that BMP receptor-IB could be a therapeutic target for enhancing bone regeneration in vivo

Table 2 continued

Author	Type	Cell	Objective	Result
Samee et al. [132]	In vitro, in vivo	Human periosteum-derived cells were transfected with BMP-2, VEGF, BMP-2 + VEGF	To evaluate the feasibility and efficacy of BMP2 and/or VEGF on periosteal cell differentiation to osteoblasts in vitro and ectopic bone formation in vivo	VEGF might enhance BMP2-induced bone formation through modulation of angiogenesis
Susperregui et al. [85]	In vitro	Pluripotent mesenchymal C2C12 cells	To test BMP2 rapidly down-regulated PTHrP gene expression through a transcriptional mechanism	BMP2 down-regulation of PTHrP could facilitate terminal differentiation of osteoblasts
Van der Zande et al. [130]	In vitro	Osteoblast-like cells from rat bone marrow	To investigate the combined application of TGFbeta-1 and BMP2 to stimulate osteogenic expression in vitro	BMP-2 is the most suitable candidate for osteogenic stimulation of rat bone marrow cells
Zanotti et al. [119]	In vitro	ST-2 murine stromal cell lines, primary cultures of murine calvarial osteoblasts	To determine whether gremlin has direct effects in osteoblasts, independent of its BMP binding activity	Gremlin inhibits BMP-2 signaling and activity, and does not have independent actions on ERK signaling in osteoblasts
Cowan et al. [103, 143]	In vitro	C2C12 myoblasts were transduced with AdLacZ, AdNell-1, AdBMP-2, or AdNell-1 + AdBMP-2 overexpression viruses	To show Nell-1 and BMP-2 synergistically enhanced osteogenic differentiation of myoblasts and phosphorylated the JNK MAPK pathway	Osteochondral specificity of Nell-1 signaling and the potential therapeutic effects of enhanced BMP-2 action with coordinated Nell-1 delivery
Cowan et al. [103, 143]	In vitro	MC3T3-E1 osteoblasts	To examine the utility of microcomputed tomography over conventional techniques in the evaluation of the BMP2 dose response effect in a 3D in vitro culture system and in an established calvarial defect model	The utility of microcomputed tomography analysis as a beneficial addition to existing techniques for objective evaluation of bone tissue engineering and regeneration
Ding et al. [88]	In vivo	MSCs	To investigate the encapsulation of BMP-2 gene-modified MSCs in alginate-poly-L-lysine microcapsules for the persistent delivery of BMP2 to induce bone formation	The nonautologous BMP2 gene-transfected stem cells are of potential utility for enhancement of bone repair and bone regeneration in vivo
Gersbach et al. [90]	In vitro, in vivo	Osteoblast	To investigate the relative efficacy of different strategies for inducing osteoblastic differentiation	BMP2 stimulated osteoblastic markers faster and to a greater extent than Runx2. Runx2-engineered cells did not utilize paracrine signaling via secreted osteogenic factors, in contrast to cells overexpressing BMP-2
Li et al. [144]	In vivo; canine model		To investigate bone regeneration by implantation of ADSCs expressing BMP2	ADSCs modified by the BMP-2 gene can enhance the repair of critical-sized bone defects in large animals
Liu et al. [92, 162]	In vitro	RD-C6 cells	To investigate the molecular mechanism underlying the differentiation of osteoblasts and chondroblasts	BMP-2 induces Runx2-deficient cells to express markers related to osteoblast and chondroblast differentiation using a Runx2-independent pathway, but it failed to induce these cells to differentiate into bone-forming osteoblasts and mature chondrocytes

Table 2 continued

Author	Type	Cell	Objective	Result
Maegawa et al. [134]	In vitro	MSCs from rat bone marrow	To investigate the culture conditions that contributed to extensive osteoblastic differentiation	The number of responding cells or immature osteoblasts was increased by the supplementation of FGF-2 in the early phase of the culture and that these cells can show osteoblastic differentiation, of which capability was augmented by BMP-2 in the late phase
Minamizato et al. [117]	In vitro	MC3T3-E1 osteoblastic cells	To elucidate the role of CCN3/NOV in osteoblast differentiation	CCN3 exerts inhibitory effects on BMP-2-induced osteoblast differentiation by its involvement of the BMP and Notch signaling pathways
Mölders et al. [116]	In vitro	MC3T3-E1 cells	To analyze effects of the Ni-containing steel 316L and major metal constituents thereof on BMP2-induced alkaline phosphatase (ALP)	Cells contacting 316L steel are exposed to increased concentrations of Ni, which suffice to impair BMP2-induced ALP activity. Zn2+, as a competitor of this inhibition, may help to restore normal osteoblastic function and bone development under these conditions
Turhani et al. [137]	In vitro	Osteosarcoma cells (SaOS-2)	To examine whether extracellular matrix compartments and osteoinductive factors could further ameliorate the bioactivity of the scaffold	The combination of collagen type I and exogenous rhBMP-2 did not ameliorate the bioactivity of hydroxyapatite calcified from red algae in the initial period of cultivation
Yang et al. [115]	In vitro	Murine pre-myoblast C2C12 cells	To investigate the role of deltaEF1 to BMP2	DeltaEF1 acts as a potent inhibitor of BMP-2-induced osteogenesis in vitro, in part, by differentially regulating the AP-1 signaling pathway
Gutierrez al. [113]	In vivo	Mouse myoblast cell line C2C12	To analyze and characterize the proteoglycan populations that are induced in C2C12 cells upon osteoblastic differentiation produced by BMP-2	The results are the first biochemical evidence and analysis for the effect of BMP-2 on the synthesis of proteoglycan during osteogenic conversion of myoblasts and suggest a role for decorin in cell response to BMP-2
Seol et al. [148]	In vitro	Osteoblast-like MC3T3-E1 cells	To test BMP2 linked to titanium surfaces	Biochemical modifications of titanium surfaces can enhance the rate of bone healing as compared with untreated Ti surfaces
Singhatanadgit et al. [131]	In vitro	Primary human bone cell	To examined the effects of TGF-beta1, FGF-2, and PDGF-AB on BMP receptor expression and BMP-2-mediated osteoblast functions	Increased BMP receptor-IB by TGF-beta1, FGF-2, and PDGF-AB significantly enhances BMP-2-induced osteogenic functions in vitro, suggesting that they might positively modulate bone formation by up-regulating BMPR-IB in vivo
Suzuki et al. [102]	In vitro	MC3T3-E1 osteoblast-like cells	To study the role of Pi transport in BMP-2-induced matrix calcification	BMP-2 also stimulates Pi transport activity through a selective increase in expression of type III Pi transporters Pit-1. In MC3T3-E1 cells, this effect is mediated by the JNK pathway and plays an essential role in bone matrix calcification induced by BMP-2
Zhao et al. [91]	In vitro	Human MSCs, murine pre-myoblast C2C12 cells	To test that PKA pathway is involved in osteogenesis	Activation of the PKA pathway may be one of key BMP-2-activated signaling events that lead to osteogenesis and that downregulation of PKIgamma may be prerequisite for the PKA activation during the osteoblastic differentiation of precursor cells

AdipoR1 receptor, AMPK, p38, and NF-kappaB signaling pathways may be involved in increasing BMP2 expression through adiponectin [124]. A signaling pathway linking BMP2-stimulated Nox4-derived physiological reactive oxygen species to BMP2 expression and osteoblast differentiation [125]. Ultrasound increased BMP2 expression in

osteoblasts via the PI3K, Akt, c-Fos/c-Jun, and AP-1 signaling pathways [126]. A high-power, low-level Nd:YAG laser increased osteoblast activity, very efficiently accelerating mineral deposition. The osteoinductive effect of the laser is likely mediated by activation of a BMP2-related signaling pathway [127]. This raises the possibility that PDL cells respond to BMP2 via a unique signaling pathway that is dependent on endoglin, which is involved in the osteoblastic differentiation and mineralization of the cells [128]. The addition of BMP2 had a beneficial effect in vitro, reducing the initial cytotoxicity of freshly mixed mineral trioxide aggregate (MTA). However, the pulp reaction to a combination of MTA and BMP2 was not significantly better than use of MTA alone [129]. BMP2 is the most suitable candidate for osteogenic stimulation of rat bone marrow cells when compared with transforming growth factor beta-1 or COLLOSS E (a bone-derived collagen product containing a variety of naturally occurring growth factors) [130].

Increased BMP receptor-IB by TGF-beta1, FGF-2, and PDGF-AB significantly enhances BMP2-induced osteogenic functions in vitro, suggesting that these proteins might positively modulate bone formation by up-regulating BMPR-IB in vivo [131]. Vascular endothelial growth factor (VEGF) might enhance BMP2-induced bone formation through modulation of angiogenesis [132]. A study in a mouse model reported that treatment with HGF prior to administration of BMP2 induced cellular proliferation of mouse embryonic fibroblasts and did not influence subsequent osteoblast differentiation induced by BMP2 [133]. The number of responding cells or immature osteoblasts was increased by the supplementation of FGF2 in the early phase of the culture and these cells showed osteoblastic differentiation, which was augmented by BMP2 in the late phase [134]. In an in vitro study of MC3T3E1 osteoblast-like cells, FGF2 played a critical role in osteoblast growth in early fracture repair, while BMP2 was instrumental in stimulating mineralization [135]. In the same way, an in vivo study showed that FGF2 increased the pool of committed osteoblasts by up-regulating the Cbfa1/Runx2 gene. The later stages of bone formation seemed to be induced by Cbfa1/Runx2-downstream factors such as BMP2, ALP, collagen type I, bone sialoprotein, and osteocalcin [136]. The combination of collagen type I and exogenous rhBMP2 did not ameliorate the bioactivity of hydroxyapatite calcified from red algae in the initial period of cultivation [137]. The investigation of bone healing upon administration of the growth factor BMP2 embedded with adult human adipose-derived stem cells (ASCs) in a locally applied fibrin matrix indicated that transplantation of ASC modulated the callus induction by BMP2 to a normal volume [138]. In vivo, MC3T3-E1 pre-osteoblasts formed a 3D scaffold with embedded growth factor-delivering microspheres exhibited that released BMP2 and promoted bone formation [139]. The formation of BMP2/carboxymethylated dextran polymers grafted with high amounts of benzylamide (DMCB) complex may protect the proteins from being inactivated. In rats in vivo, DMCB also stimulated ectopic calcification mediated by BMP2 [140]. BMP2, BMP7, and a mixture of BMP2/BMP7 all promoted osteoblast growth on a collagen scaffold, though the mixture of BMP2/BMP7 enhanced growth the most. BMP2, BMP7, and the BMP2/BMP7 mixture could promote bone regeneration via different mechanisms involving IL-6 and MMP inhibitors [141]. Physisorbed BMP2 is more active than diffusible BMP2. The current clinical practice of immobilizing BMPs on collagen type I scaffolds not only prolongs local delivery of the morphogen but could also enhance biological activity at the cellular level [142]. Cowan et al. used BMP2 (doses of 30–240 ng/mm) grafted into 5-mm critical-sized rat calvarial defects, and increased bone regeneration was observed in a dose-dependent manner, with higher doses of BMP2 inducing greater bone area, volume, and density [143]. Adipose-derived stromal cells (ADSCs) modified by the BMP2 gene can enhance the repair of critical-sized bone defects in large animals [144]. An enhancement in the expression of BMP2 and VEGF can be achieved by incorporation of BMP2 into PLLA nanofibers [145]. The incorporation of BMP2 into PLLA-collagen type I nanofibers resulted in a decrease in the diameter and pore size of the scaffold. MSCs showed better adherence and reduced proliferation on BMP-containing scaffolds [146]. The combination of PLLA scaffolds and BMP2 increased bone regeneration in vivo better than PLLA alone [147]. BMP2 linked to titanium surfaces can enhance the rate of bone healing compared with untreated Ti surfaces [148]. One study showed a BMP2 chondroitin sulphate nanocomplex effect in holding BMP2 on an apatite-coated Ti surface, and osteoblast proliferation was faster in the Ti(C)-HA-BMP2 [149]. Heparin enhanced BMP2-induced osteogenesis on apatite-coated titanium without the loss of BMP2 activity [150]. Grafting heparin and immobilizing BMP2 on Ti surfaces inhibited inflammation and promoted osteoblast function [151]. Likewise, gentamicin and a bone morphogenetic protein 2 (BMP2)-delivering heparinized-titanium implant enhanced osseointegration [152]. Another study demonstrated that co-delivery of PDGF-BB and BMP2 using heparinized-titanium enhanced osteoblast function and osteointegration [153]. A tissue-engineered bone complex with a beta-TCP scaffold and BMP2 gene-modified bone marrow stromal cells was used to promote mandibular repair and bone regeneration [154]. Nanosilver particles of defined size (20-40 nm) attached to PLGA composite grafts have strong antibacterial properties, in vitro and in vivo cytotoxicity and no effects on BMP2 osteoinductivity, making it an ideal antimicrobial for bone regeneration in infected wounds [155]. A novel injectable drug delivery system consisting

of starch-poly-epsilon-caprolactone microparticles induced osteogenesis and reduced the amount of BMP2 needed, allowing more sustained osteogenic effects [156].

Osteopontin

Osteopontin (OPN) was initially isolated from the bovine bone cortex. It was cloned for the first time in 1986. It is expressed in bone and tooth [157]. OPN has an important role in the effects of unloading-induced alterations on the differentiation of bone marrow into osteoblasts and osteoclasts [158]. Similarly, Li et al. [159] demonstrated that OPN, a matrix protein found in mineralized tissues and one that is pivotal in modulating osteoclast functions, was present in increased concentrations in Nf1± osteoblasts. The addition of an OPN-neutralizing antibody to Nf1± osteoblast-conditioned media diminished the gain in bioactivity of osteoclast functions, including osteoclast migration and bone resorption [159]. Local feedback regulation by the bone matrix protein OPN also plays a significant role in the regulation of parathyroid hormone (PTH) actions [160]. OPN is an estrogen receptor-related receptor alpha (ERRalpha) target gene whose promoter is regulated by ERRalpha in a cell context-dependent manner. A predicted silencing mutation in AF2 or a more flexible helix 12 increases ERRalpha transcriptional activity, effects that have implications for ERRalpha as a therapeutic target in bone [161]. The specific binding of OPN to collagen I may naturally orient OPN, thus influencing osteoblast adhesion [162] (Table 3).

OPN deficiency enhanced the direct anabolic action of a prostaglandin E receptor agonist locally injected onto the parietal bone in inducing new bone formation [163]. OPN inhibits mineralization in bone and urine [157]. Another study similarly reported that the acidic OPN serine- and aspartate-rich motif inhibits mineralization by binding to hydroxyapatite in a phosphorylation-dependent manner [164]. A study in human fetal osteoblasts (hFOB 1.19) to explore the osteoblastic cellular response to the physicochemical characteristics of fluoridated hydroxyapatite reported that sintered fluoridated and calcined hydroxyapatite composites could enhance OPN and COL I gene expression after a 6-day culture ($p \leq 0.05$) [165]. Otherwise, sintered hydroxyfluorapatite composites inhibited the expression of cells [165]. Sintered fluoridated and calcined hydroxyapatite composites with both OH and OH···F bands were bioactive bone graft materials [165]. The early human response to systemic endotoxemia boosts OPN levels and modifies bone biomarkers, indicating a decrease in the lytic activity of osteoclasts accompanied by an increase in the activity of immature osteoblasts [166].

An in vivo study reported that the secretion of granulocyte macrophage colony-stimulating factor (GM-CSF) and OPN by immunocompetent cells such as macrophages and dendritic cells plays a role in the maturation of dendritic cells and the differentiation of odontoblasts, respectively, in regenerated pulp tissue following tooth transplantation [167]. OPN promotes osteoblast and osteoclast adhesion, differentiation, and function [168, 169]. A study comparing the cell binding ability of adsorbed BSP and OPN specifically bound to hydroxyapatite concluded that there is a preference for cellular binding to HA with adsorbed BSP compared to OPN, but this preference is not statistically significant [170]. Another study reported that OPN is more important than BSP for osteoblast adhesion to the collagen matrix [171]. In MC3T3-E1/C4 osteoblastic cells, surfaces coated with oligomerized OPN and BSP promoted cell adhesion better than surfaces coated with the monomeric form of the proteins [172]. An investigation of the effect of inorganic pyrophosphate on osteoblast function and matrix mineralization found that inorganic pyrophosphate prevents mineralization in MC3T3-E1 osteoblast cultures by at least three different mechanisms that include direct binding to growing crystals, induction of OPN expression, and inhibition of tissue-nonspecific alkaline phosphatase activity [173]. Ti-6Al-7Nb possesses good potential to support SaOS-2 cell spreading and fibronectin and OPN synthesis; therefore, this material may be a candidate material used in implant dentistry [80]. Pre-adsorption of osteopontin on the HA particles of a degradable PDLLA/HA composite enhances the composite's osteoconductive properties when used as a coating on a commercial titanium implant but there were no differences in the gap [174].

Tenascin

Tenascin (TN) is an extracellular matrix protein that is secreted from different connective tissue cells. TN-C expression is regulated by mechanical stress. It shows highest expression in connective tissue surrounding tumors, in wounds and in inflamed tissues [175]. TN contributes to connective tissue function in many different ways [175]. TN-C seems to influence the function of other adhesion proteins such as adhesin and syndecans. In addition, it also has repulsive (anti-adhesive) interactions [176–178]. TN-C expression exhibits an interesting mechanism of activation by certain growth factors and through the application of mechanical stress to a tissue or to cells in culture [179]. TN distribution changes with development and growth [180]. TN is important for osteoblast adhesion to the underlying matrix in the process of osteoblast differentiation [12]. A mouse study showed that TN-W is expressed in osteoblasts at the edge of the developing bone domain prior to mineralization in mouse fetuses. TN-W is involved in osteoblast maturation (i.e., mineralization) [181]. TN-W can accelerate the formation of

Table 3 Osteopontin (keyword: osteopontin and osteoblast, 2006–2011 and published in English)

Author	Type	Cell	Objective	Result
Saito et al. [167]	In vivo	Cells extracted-mouse molar	To clarify the expression of GM-CSF and OPN in the process of reparative dentin formation by allogenic tooth transplantation using in situ hybridization for OPN and immunohistochemistry for GM-CSF and OPN	The secretion of GM-CSF and OPN by immunocompetent cells such as macrophages and dendritic cells plays a role in the maturation of dendritic cells and the differentiation of odontoblasts, respectively, in the regenerated pulp tissue following tooth transplantation
Addison et al. [164]	In vitro	MC3T3-E1 osteoblast	To examine the role of OPN acidic serine- and aspartate-rich motif and its interaction with PHEX enzyme	OPN acidic serine- and aspartate-rich motif inhibits mineralization by binding to hydroxyapatite in a phosphorylation-dependent manner
Grimm et al. [166]	In vivo; healthy men		To investigate the changes in biochemical parameters of bone turnover following human endotoxemia, an experimental model of self-limiting systemic infection and inflammation	The early human response to systemic endotoxemia boosts OPN levels and modifies bone biomarkers, indicating a decrease in the lytic activity of osteoclasts, accompanied by an increase in the activity of immature osteoblasts
Wu et al. [165]	In vitro	Human fetal-osteoblast (hFOB 1.19)	To explore the osteoblastic cellular response to physicochemical characteristics of fluoridated hydroxyapatite	Sintered fluoridated- calcined hydroxyapatite composites could enhance OPN and COL I gene expression after 6-day culture ($Pp < 0.05$). Otherwise, sintered hydroxy fluorapatites composites inhibited the expression. Sintered fluoridated-calcined hydroxyapatite composites with both OH and OH···F bands were bioactive bone graft materials
Li et al. [159]	In vitro	Osteoblast	To identify whether haploinsufficiency of Nf1 (Nf1±) osteoblasts and their precursors secrete cytokines that have a central role	OPN, a matrix protein found in mineralized tissues and pivotal in modulating osteoclast functions, was present in increased concentrations in Nf1± osteoblasts. Addition of OPN neutralizing antibody to Nf1± osteoblast conditioned media diminished the gain in bioactivity on osteoclast functions, including osteoclast migration and bone resorption
Bernards et al. [170, 171]	In vitro	MC3T3-E1 cell	To compare the cell binding ability of adsorbed BSP and OPN specifically bound to hydroxyapatite	There is a preference for cell binding to HA with adsorbed BSP as compared to OPN, but not to a statistically significant level
Bernards et al. [170, 171]	In vitro	MC3T3-E1 cell	To examine and compare the orientation of BSP under similar circumstances with OPN	OPN is more important than BSP for osteoblast adhesion to the collagen matrix
Ono et al. [160]	In vivo; parathyroid hormone receptor (PPR) transgenic mice		To examine the effects of deficiency of the bone matrix protein osteopontin (OPN) on the systemic effects of PTH specifically within osteoblastic cell lineages	Local feedback regulation by the bone matrix protein OPN also plays a significant role in the regulation of PTH actions
Zirngibl et al. [161]	In vitro	Rat osteosarcoma ROS17/2.8 cells, non-osteoblastic (HeLa) cell lines	To investigate whether the transcriptional regulation by ERRalpha of the gene for OPN	OPN is an ERRalpha target gene whose promoter is regulated by ERRalpha in a cell context-dependent manner and that a predicted silencing mutation in AF2 or a more flexible helix 12 increases ERRalpha transcriptional activity, effects with implications for ERRalpha as a therapeutic target in bone

Table 3 continued

Author	Type	Cell	Objective	Result
Addison et al. [173]	In vitro	MC3T3-E1 osteoblast	To investigate the effect of inorganic pyrophosphate on osteoblast function and matrix mineralization	Inorganic pyrophosphate prevents mineralization in MC3T3-E1 osteoblast cultures by at least three different mechanisms that include direct binding to growing crystals, induction of OPN expression, and inhibition of tissue-nonspecific alkaline phosphatase activity
Ishijima et al. [158]	In vitro	Bone marrow cells obtained from hind limb bones of OPN−/− mice	To obtain further insight into the role of OPN in mediating mechanical stress effect on bone	OPN has an important role in the effects of unloading-induced alterations of differentiation of bone marrow into osteoblasts and osteoclasts
Kato et al. [163]	In vivo; wild-type mice		To test whether deficiency of OPN, a secreted phosphorylated protein, could modulate the effects of prostaglandin E receptor agonist	OPN deficiency enhanced the direct anabolic action of prostaglandin E receptor agonist locally injected onto the parietal bone in inducing new bone formation
Liu et al. [92, 162]	In vitro	Osteoblast MC3T3-E1	To attempt to control the orientation/conformation of bone OPN via its specific interactions with type I collagen	The specific binding of OPN to collagen I may naturally orient OPN, thus influencing osteoblast adhesion
Osathanon et al. [80]	In vitro	Human osteoblast-like cells (SaOS-2)	To compare the early response of human osteoblast-like cells (SaOS-2) on commercially pure titanium (cpTi) and titanium-6-aluminium-7-niobium (Ti-6Al-7Nb)	Ti-6Al-7Nb possess a good potential to support SaOS-2 cells on spreading and fibronectin and OPN synthesis, therefore, this material may be one of a candidate material used in implant dentistry

new bone in a complex multicellular environment [182]. It has no effect on the initial stage of osteogenesis in bone marrow cells [183]. TN-W is a novel marker of pre-osteoblasts in the early stage of osteogenesis, and TN-W inhibits the cell proliferation and differentiation of pre-osteoblasts mediated by canonical Wnt signaling [183]. TN promotes osteoblast differentiation [6]. It is also associated with the process of cartilage development. During development, TN is strongly expressed in the interface zone between musculoskeletal elements, such as myotendinous junctions and insertions of ligaments and tendons of bone [184]. In mature bone, TN-C expression is seen at the pericellular space surrounding some osteocytes and at articular cartilage [185]. It is absent from the matrix surrounding proliferating and hypertrophic chondrocytes, but remains in a restricted distribution in peripheral epiphyseal cartilage [185]. TN-C is known to be induced in inflammation, though the increase in peri-implantitis was less than expected. In the context of peri-implantitis, TN-C might be a marker of bone remodeling rather than inflammation and infection [186] (Table 4).

Juhasz and colleague showed that increased levels of TN, matrix metalloproteinase 9-positive cells (MMP-9), and proliferative activity of lesions and decreased levels of apoptosis. They concluded that TN and MMP-9 may be key molecules of bone destruction during the progression of cholesteatoma [187]. A study of rat tibias by Sasano et al.[58] indicated that type I collagen, fibronectin and TN-C localized to the dentary of mandibles and tibias during embryonic osteogenesis. TN-C localized to the perichondral mesenchymal tissue.

Bone sialoprotein

Bone sialoprotein (BSP) is one of the major non-collagenous glycosylated phosphoproteins of the extracellular matrix in bone [188]. It is a mineralized tissue-specific protein that is expressed in differentiated osteoblasts and appears to function in the initial mineralization of bone [189]. The BSP nucleating motif may help nucleate an amorphous calcium phosphate cluster, which ultimately converts to hydroxyapatite crystal formation [190]. As one of the non-collagenous proteins in the extracellular bone matrix, BSP promotes osteoclast adhesion, differentiation, and function [168, 191]. BSP can inhibit human bone marrow stem cell proliferation and enhance their osteogenic differentiation and mineralization [192]. BSP can stimulate osteoblast differentiation through RGD-mediated cell interactions to promote mineralization [193]. BSP may serve as a matrix-associated signal that directly promotes osteoblast differentiation, resulting in the increased production of a mineralized matrix [193]. In MC3T3-E1/C4

Table 4 Tenascin (keyword: tenascin and osteoblast, 2006–2011 and published in English)

Author	Type	Sample	Objective	Result
Juhasz et al. [187]	In vitro	Cholesteatoma tissue samples	To identify factors that could play important role during the invasion of the disease	TN and MMP-9 can be key molecules of bone destruction during cholesteatoma progression
Mikura et al. [181]	In vivo	Mouse	To investigate the roles of tenascin-W in osteogenesis	Tenascin-W is expressed in osteoblasts at the edge of the developing bone domain prior to mineralization in mouse fetuses. Tenascin-W is involved in osteoblast maturation (i.e., mineralization)
Meloty-Kapella et al. [182]	In vitro	Avian osteoblasts	To examine possible roles for tenascin-W in osteogenesis	Tenascin-W can accelerate the formation of new bone in a complex multicellular environment
Zhang et al. [20]	In vitro	Rat BMSCs	To investigate the effect of uniaxial stretching on the orientation and biological functions of BMSC	Cyclic stretching promotes the synthesis of collagen types I and III and tenascin-C by the rat BMSC
Kimura et al. [183]	In vivo	ATDC5 osteo-chondroprogenitors	To identify a cDNA encoding mouse tenascin-W (TN-W) upregulated by bone morphogenetic protein (Bmp)2	TN-W is a novel marker of preosteoblasts in early stage of osteogenesis, and that TN-W inhibits cell proliferation and differentiation of preosteoblasts mediated by canonical Wnt signaling

osteoblastic cells, surfaces coated with oligomerized OPN and BSP promote cell adhesion better than surfaces coated with the monomeric form of the proteins [172]. However, a previous in vitro study suggested that OPN is more important than BSP for osteoblast adhesion to the collagen matrix [171]. There is a preference for cell binding to hydroxyapatite with adsorbed BSP compared to OPN, but this is not statistically significant [170]. In an in vivo study, BSP, but not OPN, played a role in primary bone formation and mineralization of newly formed bone during the process of cortical bone healing [194]. Increased expression of BSP in osteoblast cells can increase expression of the osteoblast-related genes Runx2 and Osx as well as alkaline phosphatase and osteocalcin and increase matrix mineralization [195]. Nevertheless, BSP overexpression decreased the osteoblast population, increased osteoclastic activity, and led to an uncoupling of bone formation and resorption, which in turn resulted in osteopenia and mild dwarfism in mice [196]. It is likely that BSP deficiency impairs bone growth and mineralization concomitant with dramatically reduced bone formation [197]. The absence of BSP delays bone repair at least in part by impairing both new bone formation and osteoclast activity [198]. In a mouse study, the lack of BSP affected both osteoclast formation and activity [199]. PDGF-BB stimulates human BSP transcription by targeting the CRE1, CRE2, AP1(3), and SSRE1 elements in the human BSP gene promoter [200]. IGF-I stimulates BSP transcription by targeting FRE and HOX elements [201]. FGF2 stimulates BSP gene transcription by targeting the FRE and AP1/GRE elements in the rat BSP gene promoter [202]. Sodium phosphate glass type 25 stimulates BSP transcription by targeting FRE and HOX elements in the proximal promoter of the rat BSP gene [203]. Butyric acid also increases the transcription of the BSP gene mediated by FRE in the rat BSP gene promoter and induces osteoblast activity in the early stages of bone formation [204]. Kaempferol increased BSP gene transcription mediated through inverted CCAAT, CRE, and FRE elements in the rat BSP gene promoter, and could induce osteoblast activities in the early stage of bone formation [205]. *P. gingivalis* lipopolysaccharides increased BSP gene transcription mediated through CRE and FRE elements in the rat BSP gene promoter [206]. IL-11 stimulated BSP transcription by targeting CRE, FRE, and HOX sites in the proximal promoter of the rat BSP gene [189]. Another study found that FSK and FGF2 stimulated BSP transcription in DU145 human prostate cancer cells by targeting the CRE1 and CRE2 elements in the human BSP gene promoter [207]. $Ca(OH)^2$ stimulated BSP transcription by targeting the CRE1, CRE2, and FRE elements in the human BSP gene promoter [208]. In rat osteoblast-like ROS17/2.8 cells, CO_2 laser irradiation increased BSP transcription via FRE in the BSP gene promoter [209] (Table 5).

Runx2 and HDAC3 repress BSP gene expression so that this repression is suspended upon osteoblastic cell differentiation [210]. Chlorpromazine suppresses BSP gene transcription through tyrosine and MAP kinase-dependent pathways, and the effects are mediated by CRE and FRE elements in the proximal promoter of the BSP gene [211]. The same group of researchers also reported that LPS suppresses BSP gene transcription

Table 5 Bone sialoprotein (keyword: bone sialoprotein and osteoblast, 2006–2011 and published in English)

Author	Type	Cell	Objective	Result
Forsprecher et al. [172]	In vitro	MC3T3-E1/C4 osteoblastic cell	To examine effects of tissue transglutaminase-mediated crosslinking and oligomerization of OPN and BSP on osteoblast cell adhesion	Surfaces coated with oligomerized OPN and BSP promote MC3T3-E1/C4 osteoblastic cell adhesion significantly better than surfaces coated with the monomeric form of the proteins
Li et al. [207]	In vitro	Human prostate cancer DU145 cells	To investigate the effects of cAMP and FGF2 on BSP	FSK and FGF2 stimulate BSP transcription in DU145 human prostate cancer cells by targeting the CRE1 and CRE2 elements in the human BSP gene promoter
Sasaki et al. [209]	In vitro	Rat osteoblast-like ROS17/2.8 cells	To investigate the effects of CO_2 laser irradiation on BSP gene transcription	CO_2 laser irradiation increases BSP transcription via FRE in the rat BSP gene promoter
Wang et al. [63, 189, 208]	In vitro	Rat BSP gene promoter	To analyze the effects of IL-11 on the expression of the BSP gene in osteoblast-like cells	IL-11 stimulates BSP transcription by targeting CRE, FRE, and HOX sites in the proximal promoter of the rat BSP gene. Moreover, phospho-CREB1, c-Fos, c-Jun, JunD, Fra2, Dlx5, Msx2, Runx2, and Smadl transcription factors appear to be key regulators of IL-11 effects on BSP transcription
Wang et al. [63, 189, 208]	In vitro	Human osteoblast-like SaOS-2 cells	To detail the mechanism involved in the mineralization induced by Ca(OH)(2)	Ca(OH)(2) stimulates BSP transcription by targeting the CRE1, CRE2, and FRE elements in the human BSP gene promoter
Xia et al. [192]	In vitro	Human BMSCs	To investigate the effects of recombinant human BSP on the proliferation and osteodifferentiation of human BMSCs	BSP is capable of inhibiting hBMSCs proliferation and enhancing their osteogenic differentiation and mineralization in the presence of osteogenic medium
Boudiffa et al. [199]	In vivo	Mice	To investigate the effect of BSP deficiency to osteoclastogenesis and mineral resorption	Lack of BSP affects both osteoclast formation and activity
Chan et al. [214]	In vitro	Murine preosteoblastic cell line (MC3T3-E1)	To evaluate modification of PCL/pHEMA surfaces with BSP	Modification of surfaces with BSP significantly enhanced osteoblastic cell attachment and spreading, without compromising proliferation
Li et al. [206]	In vitro	Rat osteoblast-like ROS17/2.8 cells	To investigate the effects of *P. gingivalis* lipopolysaccharide on BSP transcription	0.1 microg/ml suppressed, and 0.01 microg/ml *P. gingivalis* lipopolysaccharide increased BSP gene transcription mediated through CRE and FRE elements in the rat BSP gene promoter
Monfoulet et al. [194]	In vivo	Mouse femur	To compare the roles of BSP and OPN in the repair process	BSP, but not OPN, plays a role in primary bone formation and mineralization of newly formed bone during the process of cortical bone healing
Schaeren et al. [215]	In vivo, in vitro	BMSC and nude mice	To test whether synthetic polymer-based porous scaffolds could support ectopic bone formation by human BMSC if coated with BSP	BSP coating of a variety of substrates is not directly associated with an enhancement of osteoprogenitor cell differentiation in vitro or in vivo, and that presentation of BSP on polymeric materials is not sufficient to prime BMSC functional osteoblastic differentiation in vivo
Wang et al. [203]	In vitro	Osteoblast-like ROS 17/2.8 cells	To investigate the effects of inorganic polyphosphate on BSP	Sodium phosphate glass type 25 stimulates BSP transcription by targeting FRE and HOX elements in the proximal promoter of the rat BSP gene
Yang et al. [190, 204, 205]	In vitro	Rat osteoblast-like ROS17/2.8 cells	To investigate the regulation of BSP transcription by butyric acid	Butyric acid increases the transcription of the BSP gene mediated through FRE in the rat BSP gene promoter, and induces osteoblast activity in the early stage of bone formation

Table 5 continued

Author	Type	Cell	Objective	Result
Yang et al. [190, 204, 205]	In vitro	Rat osteoblast-like UMR106 cells	To investigate the regulation of BSP transcription by kaempferol	Kaempferol increased BSP gene transcription mediated through inverted CCAAT, CRE, and FRE elements in the rat BSP gene promoter, and could induce osteoblast activities in the early stage of bone formation
Gordon et al. [195]	In vitro	Primary rat bone osteoblastic cells	To determine the molecular mechanisms responsible for the BSP-mediated increase in osteoblastic differentiation	Increased expression of BSP in osteoblast cells can increase expression of the osteoblast-related genes Runx2 and Osx as well as alkaline phosphatase and osteocalcin and increase matrix mineralization
Malaval et al. [198]	In vivo	Mice femur	To report that absence BSP impair cortical defect repair	The absence of BSP delays bone repair at least in part by impairing both new bone formation and osteoclast activity
Mezawa et al. [200]	In vitro	Osteoblast-like SaOS-2 and ROS17/2.8 cells	To determine the molecular mechanisms PDGF regulation of human BSP gene transcription	PDGF-BB stimulates human BSP transcription by targeting the CRE1, CRE2, AP1(3), and SSRE1 elements in the human BSP gene promoter
Bernards et al. [170, 171]	In vitro	MC3T3-E1 cell	To compare the cell binding ability of adsorbed BSP and OPN specifically bound to hydroxyapatite	There is a preference for cell binding to HAP with adsorbed BSP as compared to OPN, but not to a statistically significant level
Bernards et al. [170, 171]	In vitro	MC3T3-E1 cell	To examine and compare the orientation of BSP under similar circumstances with OPN	OPN is more important than BSP for osteoblast adhesion to the collagen matrix
Graf et al. [216]	In vitro	Osteoblast	To compare BSP as a surface-coating material against the major organic and inorganic components of bone, collagen type I, and hydroxyapatite (TICER)	BSP precoating of the rough TICER implant surface enhanced the osteoinductive effect much more than did collagen precoating
Malaval et al. [197]	In vivo	BSP(-/-) mice	To investigate the role of BSP in bone formation and osteoclastogenesis	BSP deficiency impairs bone growth and mineralization, concomitant with dramatically reduced bone formation
Takai et al. [202]	In vitro	Osteoblastic cell line ROS17/2.8	To report that AP1 binding site overlapping with glucocorticoid response element (GRE) AP1/GRE in the rat BSP gene promoter is another target of FGF2	FGF2 stimulates BSP gene transcription by targeting the FRE and AP1/GRE elements in the rat BSP gene promoter
Valverde et al. [196]	In vivo	Homozygous transgenic mouse	To determine the effects of BSP overexpression in bone metabolism	Overexpression of BSP decreased osteoblast population and increased osteoclastic activity and leads to an uncoupling of bone formation and resorption, which in turn results in osteopenia and mild dwarfism in mice
Gordon et al. [193]	In vitro	MC3T3E1 cell	To demonstrate that BSP can stimulate osteoblast differentiation through RGD-mediated cell interactions to promote mineralization	BSP may serve as a matrix-associated signal directly promoting osteoblast differentiation resulting in the increased production of a mineralized matrix
Lamour et al. [210]	In vitro	Osteoblast-like SaOS-2 cells	To investigate the role of Runx2 in the regulation of BSP expression	Runx2 and HDAC3 repress BSP gene expression and that this repression is suspended upon osteoblastic cell differentiation

Table 5 continued

Author	Type	Cell	Objective	Result
Karadag and Fisher [213]	In vitro	BMSCs and pre-osteoblasts	To investigate the effect of BSP to osteogenic cell migration through basement membrane and collagen matrices	Pre-osteoblasts and their BMSC precursors may use MMP-2/BSP/integrin complexes to disrupt matrix barriers during migration to their final destinations in vivo
Kato et al. [212]	In vitro	Osteoblast-like ROS 17/2.8 cells	To determine the molecular mechanisms involved in the suppression of bone formation	LPS suppresses BSP gene transcription through protein kinase A and tyrosine kinase-dependent pathways and that the LPS effects are mediated through CRE and FRE elements in the proximal BSP gene promoter
Nakajima et al. [211]	In vitro	Osteoblast-like ROS17/2.8 cells and rat stromal bone marrow cells (SBMC-D8)	To investigate the effect of chlorpromazine on BSP gene transcription	Chlorpromazine suppresses BSP gene transcription through tyrosine and MAP kinases-dependent pathways and that the chlorpromazine effects are mediated by CRE and FRE elements in the proximal promoter of the BSP gene
Nakayama et al. [201]	In vitro	Osteoblast-like SaOS-2 and rat stromal bone marrow (RBMC-D8) cells	To determine the molecular mechanism of IGF-I regulation of osteogenesis	IGF-I stimulates BSP transcription by targeting the FRE and HOX elements in the proximal promoter of BSP gene
Wang et al. [188]	In vivo	Rats	To investigate the potential role of BSP in more complex in vivo environments	BSP stimulates calcification and osteogenesis in a site-specific manner, and that local environment and the specificities of responding cells may play critical roles in the function of BSP in vivo

through protein kinase A and tyrosine kinase-dependent pathways, and the LPS effects are mediated through CRE and FRE elements in the proximal BSP gene promoter [212]. A study of the effect of BSP on osteogenic cell migration through basement membrane and collagen matrices showed that pre-osteoblasts and their BMSC precursors may use MMP-2/BSP/integrin complexes to disrupt matrix barriers during migration to their final destinations in vivo [213].

Modification of PCL/pHEMA surfaces with BSP significantly enhanced osteoblastic cell attachment and spreading without compromising proliferation [214]. In contrast, BSP coating of a variety of substrates is not directly associated with an enhancement of osteoprogenitor cell differentiation in vitro or in vivo, and presentation of BSP on polymeric materials is not sufficient to prime BMSC functional osteoblastic differentiation in vivo [215]. An investigation comparing BSP as a surface-coating material against the major organic and inorganic components of bone, collagen type I and hydroxyapatite (TICER) showed that BSP pre-coating of the rough TICER implant surface enhanced the osteoinductive effect much more than collagen pre-coating [216]. BSP is osteoinductive when coated onto femoral implants [217]. BSP enhanced the osteoinductive effect of both smooth and rough surface implants [218].

Conclusions and future directions

In conclusion, the data in this review suggest that pretreatment of biomaterials with FN in initial phase of osteogenic differentiation, improved results for all types of surfaces including titanium and polymers, providing an ideal microenvironment that enhances the adhesion, morphology, and proliferation of pluripotent cells and multipotent. Likewise, in the second stage of differentiation, surface coating with BMP2 decreases the diameter and pore size of the scaffold, inducing better adhesion and reduced proliferation of BMP-MSCs. Surfaces coated with oligomerized OPN and BSP promote cell adhesion, but it is clear that the polymeric coating of BSP alone is insufficient to induce priming MSC and functional osteoblastic differentiation in vivo. Finally, TN is involved in mineralization and can accelerate new bone formation in a multicellular environment but has no effect in the initial stage of osteogenesis.

Further studies are necessary to assess the outcome of surface modification of biomaterials and titanium with these proteins, which could lead to selective therapeutic strategies in the field of dental implant and orthopedic surgery and more comparative studies on osteogenic capacity of PDLSC with DPSC also necessary due to the expression levels of many bone markers in the PDLSC in non-differentiated

state and logically it will be easier to differentiate it to a tissue which is close and of the same embryonic layer, such as bone tissue.

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