Cytokine and Growth Factor Reviews 49 (2019) 59–69

Contents lists available at ScienceDirect

Cytokine and Growth Factor Reviews

journal homepage: www.elsevier.com/locate/cytogfr

Collagen and non-collagenous proteins molecular crosstalk in the pathophysiology of osteoporosis

Caterina Licini[a,b], Chiara Vitale-Brovarone[a,*], Monica Mattioli-Belmonte[b]

[a] Department of Applied Science and Technology, Politecnico di Torino, Corso Duca degli Abruzzi 24, 10129, Torino, Italy
[b] Department of Clinical and Molecular Sciences (DISCLIMO), Università Politecnica delle Marche, Via Tronto 10/a, 60126, Ancona, Italy

ARTICLE INFO

Keywords:
Osteoporosis
Type I collagen
NCPs
Transforming growth factor-β
Insulin-like growth Factor-1
Osteopontin

ABSTRACT

Collagenous and non-collagenous proteins (NCPs) in the extracellular matrix, as well as the coupling mechanisms between osteoclasts and osteoblasts, work together to ensure normal bone metabolism. Each protein plays one or more critical roles in bone metabolism, sometimes even contradictory, thus affecting the final mechanical, physical and chemical properties of bone tissue. Anomalies in the amount and structure of one or more of these proteins can cause abnormalities in bone formation and resorption, which consequently leads to malformations and defects, such as osteoporosis (OP). The connections between key proteins involved in matrix formation and resorption are far from being elucidated. In this review, we resume knowledge on the crosstalk between collagen type I and selected NCPs (Transforming Growth Factor-β, Insulin-like Growth Factor-1, Decorin, Osteonectin, Osteopontin, Bone Sialoprotein and Osteocalcin) of bone matrix, focusing on their possible involvement and role in OP. The different elements of this network can be pharmacologically targeted or used for the design/development of innovative regenerative strategies to modulate a feedback loop in bone remodelling.

1. Introduction

The extracellular matrix (ECM) is an assembly of several components, which work together to provide a wide range of functions. The ECM provides structural support and serves as a repository for growth factors and therefore provides an optimal environment for cells in a context-dependent manner. Of the two main types of proteins that compose bone ECM, type I Collagen is the most abundant, forming about 90% of the matrix. The non-collagenous proteins (NCPs) comprise about 180–200 different molecules, with either structural, signalling or mechanical roles. [1] Hydroxyapatite (HA) constitutes the major inorganic phase in ECM and contains elements such as Ca^{2+} and PO_4^{3-} organised in a crystalline lattice. The mineralization of the bone organic matrix is a multistep process: HA incorporation begins 5–10 days after the ECM deposition during primary mineralization; after few days, this process slows down, and secondary mineralization begins, with an increase in the number, size and guidance of HA crystals [2].

The maintenance of the bone mass depends on the metabolic activity and turnover of the skeleton: bone remodelling involves mainly osteoblasts (OBs) and osteoclasts (OCs) in a series of events at the bone surface. The coupling mechanism between OCs and OBs is the result of paracrine, autocrine and endocrine factors whose effectiveness allows for the correct ECM composition, thus affecting the mechanical, physical and chemical properties of bone tissue (Fig. 1). Bone resorption starts with the recruitment of OC precursors and their differentiation into mature OCs. The next phase is a transition stage, where

Abbreviations: 1,25(OH)$_2$D3, 1,25-dihydroxy vitamin D3; AGEs, advanced glycation end products; ALP, alkaline phosphatase; BGLAP, bone gamma-carboxyglutamic acid-containing protein; BMD, bone mineral density; BMSCs, bone mesenchymal stem cells; CatK, cathepsin K; COL1A1, collagen alpha-1(I) chain; CTx, C-terminal telopeptide; DCN, decorin; DPD, deoxypyridinoline; DPL, deoxypyrrololine; EC, EF-hand Ca^{2+}- binding; ECM, extracellular matrix; FS, follistatin; GAGs, glycosaminoglycans; GLA, γ-carboxyglutamate; HA, hydroxyapatite; HRT, hormone replacement therapy; Hyl, hydroxylysine; IGF-1, insulin-like growth factor-1; IGFBPs, insulin-like growth factor binding proteins; LAP, latency-associated protein; LRRs, leucine-rich repeats; LTBP, latent TGF-β Binding Protein; Lys, lysine; M-CSF, macrophage colony-stimulating factor; MMPs, matrix metalloproteases; NCPs, non-collagenous Proteins; NTx, N-terminal Telopeptide; OBs, osteoblasts; OCN, osteocalcin; OCs, osteoclasts; OCYs, osteocytes; ON, osteonectin; OP, osteoporosis; OPG, osteoprotegerin; OPN, osteopontin; OVX, ovariectomized; PDGF, platelet-derived growth factor; PTH, parathyroid Hormone; PYD, pyridinium; PYL, pyrrole; RAGEs, receptors for advanced glycation end products; RANKL, receptor activator of nuclear factor Kappa-B ligand; RGD, Arg-Gly-Asp sequence; RUNX2, runt-related transcription factor 2; SIBLING, small integrin binding ligand N-glycosylated; SLRP, small leucine-rich proteoglycan; SPARC, secreted protein acidic and rich in cysteine; TβRI, TGF-β receptor type I; TβRII, TGF-β receptor type II; TGF-β, transforming growth factor-β; WT, wild-type

* Corresponding author.
E-mail address: chiara.vitale@polito.it (C. Vitale-Brovarone).

https://doi.org/10.1016/j.cytogfr.2019.09.001
Received 10 June 2019; Received in revised form 12 September 2019; Accepted 12 September 2019
Available online 13 September 2019

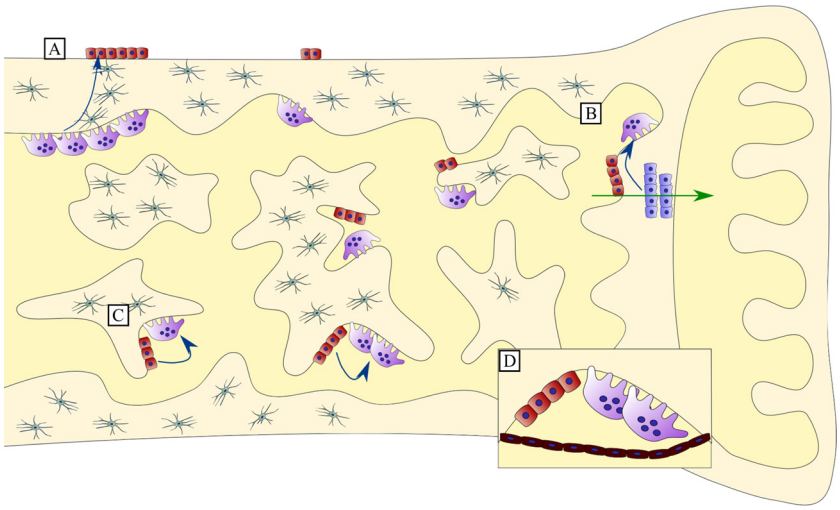

Fig. 1. Intercellular communication between OBs and OCs. A) During the modelling, OCs (in violet) act on different areas of OBs (in red). Osteo-transmitters (blue arrow) communicate through cells, probably *via* the osteocyte net. In this process, the improvement of cortical bone is observed; B) During the growth in length of bone (green arrow), chondrocytes (in blue) of metaphyseal cartilage communicate with OCs present on the trabeculae of newly formed bone; C) During the remodelling process that occurs on the trabecular surface, the communication between OBs and OCs allows the balance between bone formation and bone resorption, through several factors released by OCs and acting on OBs; D) During the remodelling process, a canopy of bone lining cells cover the Bone Remodelling Unit represented by OBs and OCs, forming the Bone Remodelling Compartment (BRC).

osteoprogenitor mesenchymal cells migrate in the resorption site and differentiate into mature OBs. In the final stage of bone resorption, OBs deposit the new bone matrix called osteoid, which will be later mineralized [3].

ECM proteins, which are crucial for bone structure and mineralization, are capable of influencing OB—OC coupling and seem to be implicated in pathogeneses of bone diseases like osteoporosis (OP). OP is characterized by low bone mass and microarchitectural deterioration of the bone tissue which leads to enhanced bone fragility and a consequent increase in fracture risk. The decrease of bone mass is caused by an imbalance between bone resorption and formation, with osteoclastic hyperactivity. [4] Low-density fraction of bone, which corresponds to younger and less mineralized osteons is more frequently observed in fractures of aged patients than in age-matched controls. This is probably related to the higher bone turnover that causes incomplete secondary mineralization [5].

In OP, several morphological changes in bone structure are detectable: a decrease in the cortical and trabecular thickness, an alteration in the transition zones between cancellous and cortical bone, an increase in the diameter of Haversian canals and the number and width of pores of trabecular surfaces are often reported. [6,7] The bone matrix also undergoes important modifications in aging and OP; for instance, the content of HA diminishes, the carbonate/phosphate ratio decreases, and the matrix composition changes both in protein content and chemical structure, leading to an increase in mineral content/matrix ratio [8]. Ovariectomized (OVX) mice showed an increase in the disorder of the bone ECM, with an increased number of random coil structures in proteins [9].

One of the most significant causes of OP is the decrease in oestrogen levels such as in menopause: before its onset, no differences in the bone loss rate between women and men is detectable, but this rate accelerates immediately after menopause. Moreover, women subjected to oophorectomy lose bone mass faster than those undergoing natural menopause. [4] Likewise, long-term glucocorticoid treatment in patients with allergic disorders and chronic diseases (*i.e.* renal, respiratory and neural diseases) could induce OP: dexamethasone has a destructive effect on bone mass [10].

A traditional therapeutic approach in OP is represented using Parathyroid Hormone (PTH): intermittent PTH administration has an anabolic effect, increasing spinal and femoral bone mass in OP and inducing proliferation of OBs and precursor cells. On the contrary, continuous administration of PTH leads to bone catabolism. [11] 1,25-dihydroxy vitamin D3 ($1,25(OH)_2D3$), also named calcitriol, is a physiologic regulator of calcium absorption and its serum levels are diminished in OP. Its long-term administration has been used for treating OP symptomatology, showing an increase in bone mineral density (BMD). However, short-term $1,25(OH)_2D3$ administration induces increased serum levels of bone resorption markers in OP patients as well as in *in vitro* models. [12,13]

The aim of the present review is to resume knowledge on the crosstalk between collagen type I and selected NCPs (Transforming Growth Factor-β, Insulin-like Growth Factor-1, Decorin, Osteonectin, Osteopontin, Bone Sialoprotein and Osteocalcin) of bone ECM, focusing on their possible relationship with OP onset and/or maintenance. These different elements of the ECM network can be pharmacologically targeted or used for the design/development of innovative regenerative strategies to modulate the feedback loop in bone remodelling.

2. Type I collagen

Mature type I Collagen molecule is a right-handed triple helix typically composed of two α1 and one α2 chains. (Fig. 2A) Collagen chains are synthetized as procollagen molecules, with N-terminal and C-terminal pro-peptides that are cleaved before the molecule becomes mature. Each mature molecule of 300 nm in length, has two short non-helix regions at both N- (NTx) and C-terminus (CTx), called telopeptides. (Fig. 2A) The molecules of Type I Collagen are parallel to each other in fibrils, overlapping one another by 67 nm distance (D) or multiples, and a gap of 0.6D (*i.e.* 40 nm) is present between the end of a Type I Collagen molecule and the beginning of the next one. [14]

Every chain is constituted by glycine-X-Y repeating sequences, with glycine (Gly) residues in the centre of the triple helix and the X and Y residues on the surface. About 100 proline residues in the Y position, a few of proline (Pro) in X position and about 10 lysine (Lys) residues in the Y position undergo hydroxylation. The hydroxylation of Pro to hydroxyproline (Hyp) is critical to obtain a stable triple helix. The hydroxylation of Lys residues then serves as a substrate for glycosyltransferase and galactosyltransferase that add glucose and galactose molecules to chains. These post-translational modifications occur before the triple helical conformation. Glycosylation decreases the diameter of fibrils, intervening in the packaging of mature molecules into fibrils. [15]

Triple helices are held together by covalent bonds, and collagen cross-links between the different molecules are mainly obtained by enzymatic activity. (Fig. 2B, C) Divalent cross-links between telopeptide and helix are not stable and can be easily reduced. [16] The divalent cross-links between hydroxylysine (Hyl) of a telopeptide and Hyl present in another Type I Collagen helix “maturate” in pyridinium (PYL) and pyrrole (PYD) cross-links, when they bind allysine and hydroxyallysine of a third telopeptide respectively (Fig. 2B, C), which are the predominant cross-links in bone. The interaction of the divalent cross-links between Hyl of a telopeptide and Lys present in another Type I

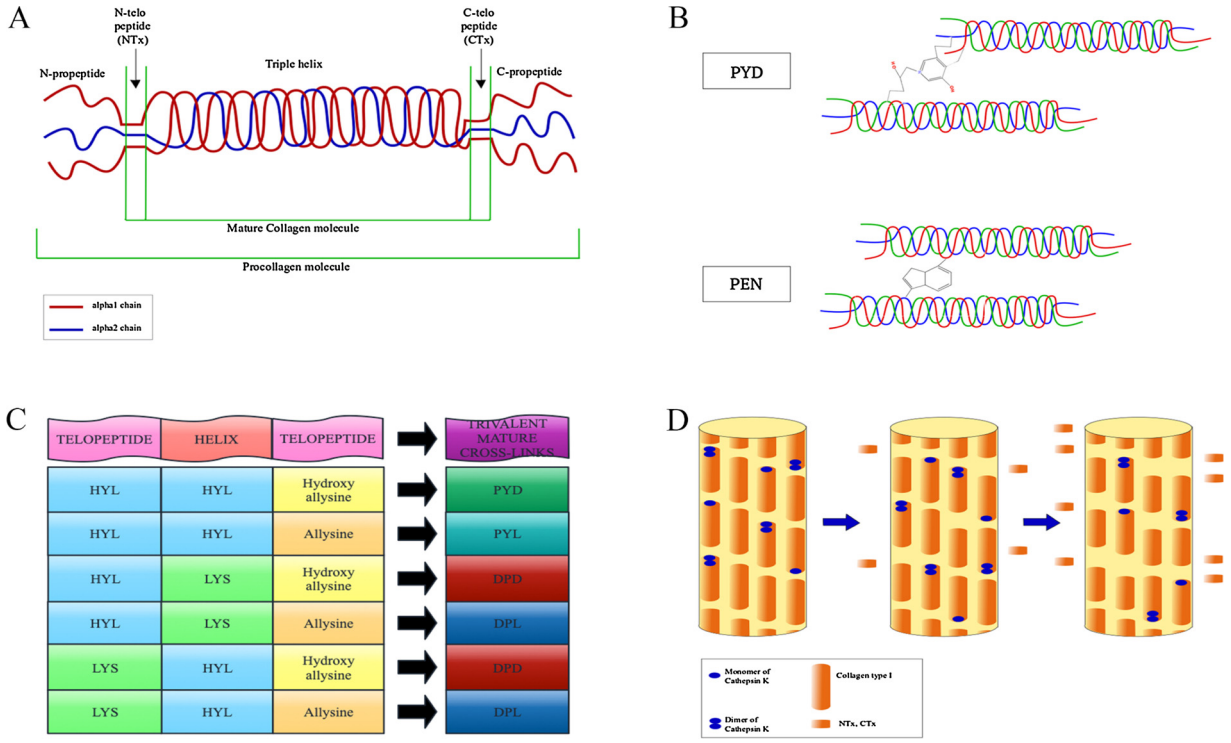

Fig. 2. Type I Collagen structures and digestion by Cathepsin K (CatK).
A) Procollagen and mature Type I Collagen molecules; B) Examples of cross-links between molecules of Type I Collagen: pyridinium (PYD) cross-links between telopeptide- telopeptide- helix are enzymatic mature cross-links, while Pen cross-links belong to Advanced Glycation End Products (AGEs) that are non-enzymatic cross-links caused by senescence and oxidative stress; C) Trivalent mature cross-links flowchart: the different nature of mature cross-links depends on the different chemical groups present in telopeptides and helix. D) Type I Collagen fibrils degraded by CatK: homodimers of CatK bind to the collagen molecules at the gap regions and fragment molecules of type I collagen that release N- and C-telopeptides (NTx and CTx).

Collagen helix, or *vice versa*, with allysine and hydroxyallysine of a third telopeptide lead to deoxypyridinoline (DPL) and deoxypyrrololine (DPD) cross-links respectively. (Fig. 2C) All PYD, PYL, DPD and DPL are mature non-reducible trivalent cross-links between telopeptide, telopeptide and helix and the total quantity of enzymatic cross-link formation is regulated by Lysil- hydroxylase and oxidase enzymatic activity. [16]

The main function of Type I Collagen in bone ECM is structural: its assembly and biochemical features are crucial for bone mechanical properties. Experimental studies on bone showed that the decrease of mature cross-links (*i.e.* PYD, PYL, DPD and DPL) leads to a diminished deflection capacity, bending strength and elastic stiffness. Moreover, bone with denatured Type I Collagen loses toughness and strength compared to that without denaturation. [17] It has been shown that the increase in Lys hydroxylation leads to modifications on cross-links with a reduction in fibril diameters and mineral content [18]. The decrease of enzymatic cross-link content and an increase of hydroxylated Lys residues occurs in OP, suggesting that these biochemical properties could be significant for the onset of this pathology. [5,16,18] Noteworthy, type I Collagen quantity and density decrease in aged bone, but it does not change in OP bone compared to age-matched samples [5,18].

The degradation of Type I Collagen produces molecules, such as NTx and CTx, considered biological markers of high bone resorption and OP (Fig. 2D), since urinary NTx, CTx, DPD, PYD and serum NTx, CTx increase as a consequence of oestrogen deficiency in menopause and OP. [19]

Advanced Glycation End products (AGEs) are the result of spontaneous non-enzymatic glycation - by glucose and reactive products of sugar metabolism - between Type I Collagen molecules that are mainly caused by senescence and oxidative stress. (Fig. 2B) Pen is one of the main intermolecular AGEs present in bone. (Fig. 2B) [18] OP patients showed significant reductions in enzymatic cross-links and a higher amount of AGEs in bone, largely Pen, without alteration of Type I Collagen content compared to age-matched healthy subjects. The ratio of Pen/total enzymatic cross-links is indicated as an index of collagen senescence, and it was shown to be more elevated in bone fractures of OP subjects than in the healthy ones [5].

High bone turnover, oxidative stress and oxidation accelerate AGEs formation and accumulation in OP bone. [5,20] During bone resorption, OCs secrete collagenases like Matrix Metalloproteases (MMPs) and Cathepsin K (CatK), which is the main enzyme that degrades Type I Collagen during bone matrix resorption. CatK binds to the gap regions of collagen molecules at N- or C-terminus, depending on the presence of appropriate glycosaminoglycans (GAGs) and induces the release of NTx and CTx. (Fig. 2D) [21,22] The presence of AGEs modifies the interaction between ECM and bone cells (*i.e.* OBs and OCs): matrix with an increased amount of AGEs shows a high number of resorption pits and pit per area, suggesting their role in increasing bone resorption [20]. AGEs can also alter OBs growth and differentiation, as well as decrease nodule formation during the mineralization phase. *In vitro*, AGEs treatment triggered the AGE-AGE receptor (RAGE) pathway, upregulating the expression of RAGEs mRNA in OBs, and leading to a reduction in the mRNA expression of alkaline phosphatase (ALP) and osteocalcin (OCN). This corroborates the idea that AGEs could contribute to impaired matrix mineralization. [23] Moreover, the observation that RAGEs$^{-/-}$ mice had increased BMD and biomechanical properties in conjunction with a decreased in OCs formation and defects in bone resorption, strengthened the key role of AGEs in bone modelling and remodelling. [24]

Type I Collagen interacts with HA, and mineral deposition occurs on the surface of collagen fibrils, within the collagen holes and the overlap zones. Alteration in Type I Collagen conformation such as AGEs, could hamper HA deposition. Besides, Type I Collagen interacts with specific

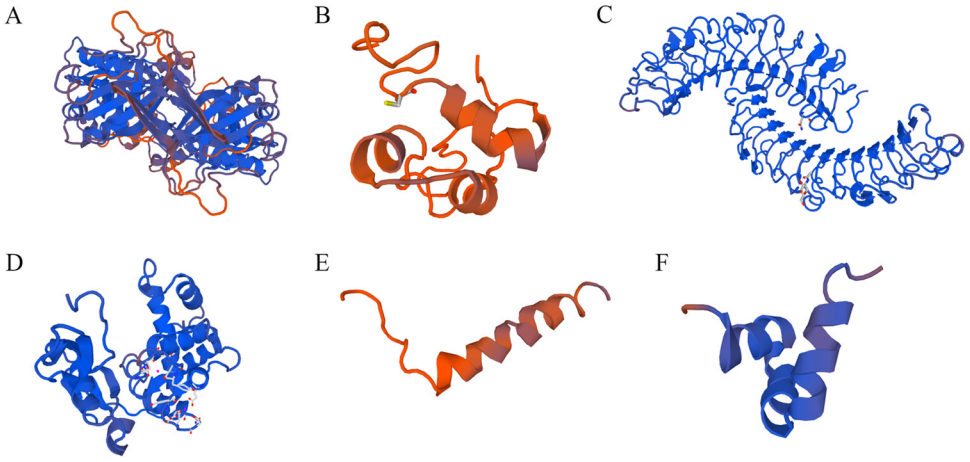

Fig. 3. Predicted secondary structures of the main proteins of the bone ECM derived from SWISS-MODEL database (www.swissmodel.expansy.org).
A) Transforming Growth Factor-beta (TGF-β); B) Insulin-like Growth Factor-1 (IGF-1); C) Decorin (DCN); D) Osteonectin (ON); E) N-terminal domain of Bone Sialoprotein-2 (BSP-2); F) Osteocalcin (OCN).

NCPs playing a primary or secondary role in HA nucleation. [25–28]

3. Transforming Growth Factor-β1

Transforming Growth Factor-β (TGF-β) is a superfamily of proteins, where TGF-β1, TGF-β2 and TGF-β3 are the most similar isoforms, being the former the most abundant in bone ECM. (Fig. 3A) TGF-β precursor is synthesized and then cleaved in a mature TGF-β protein and a latency- associated protein (LAP). A dimer of LAP and a homodimer of TGF-β (25 kDa) remain linked together by disulphide bonds forming a complex of 100 kDa. This complex enhances TGF-β stability and prevents the binding of the protein to its receptors. LAP protein of the complex binds a latent TGF-β binding protein (LTBP) to form the Large Latent Complex (LLC), which interacts with ECM proteins allowing for the storage of TGF-β within the matrix. (Figs. 4 and 5) In the bone matrix, most of the TGF-β is present as a latent form inside the LLC. [29,30]

Molecules, like MMP-2, and MMP-9, could bind LTBPs and activate latent TGF-β by cleavage of the LLC. [31] Furthermore, integrins (mainly $\alpha_v\beta_1$, $\alpha_v\beta_6$) recognise the arginyl-glycyl-aspartic acid (RGD) sequence in LAP and LTBP proteins, enabling the cleavage of LLC and the subsequent release of active TGF-β. [32] This phenomenon can occur when the pH decrease, a condition that occurs under the ruffled border of OCs during bone resorption [30]. (Fig. 4) Active TGF-β binds to a couple of TGF-β receptors type II (TβRII), which are transmembrane serine-threonine kinase proteins. TβRII/TGF-β complex recruits and phosphorylates two TGF-β receptors type I (TβRI), forming a very stable ternary complex [29] The intracellular domain of TβRI transduces the signal, phosphorylating Smad2 and Smad3 proteins that form a complex with Smad4. Smad complex moves into the nucleus, bind to sequence-specific transcription factors and modulate gene expression. [33]

TGF-β plays a central role on OBs and OCs regulation, inducing the migration of bone mesenchymal stem cells (BMSCs) to the bone surface and their differentiation towards OBs by signals at the resorptive site. [34] In cell culture, TGF-β increases OBs proliferation and exerts an anti-apoptotic role: high levels of TGF-β were shown to inhibit cell proliferation and stimulate protein synthesis, mainly Type I Collagen, however, this molecule is not required for the final maturation of OBs [35–37]. TGF-β stimulates OBs to secrete also pro-osteoclastic proteins, like Receptor Activator of Nuclear factor Kappa-B Ligand (RANKL), as well the proliferation and differentiation of OCs, revealing its role in bone resorption [35,38]. TGF-β1 protein influences osteoclastogenesis in a dose-dependent manner: low levels stimulate OCs differentiation with a sufficient macrophage colony-stimulating factor (M-CSF) expression and a high RANKL/Osteoprotegerin (OPG) ratio, while high TGF-β1 levels repress OCs differentiation, repressing both M-CSF and RANKL/OPG ratio [39]. A study in $T\beta RI^{-/-}$ mice confirmed all these data, showing an increased BMD and *Runt-related transcription factor-2* (*Runx2*) expression, with a consequent increase in OBs number and

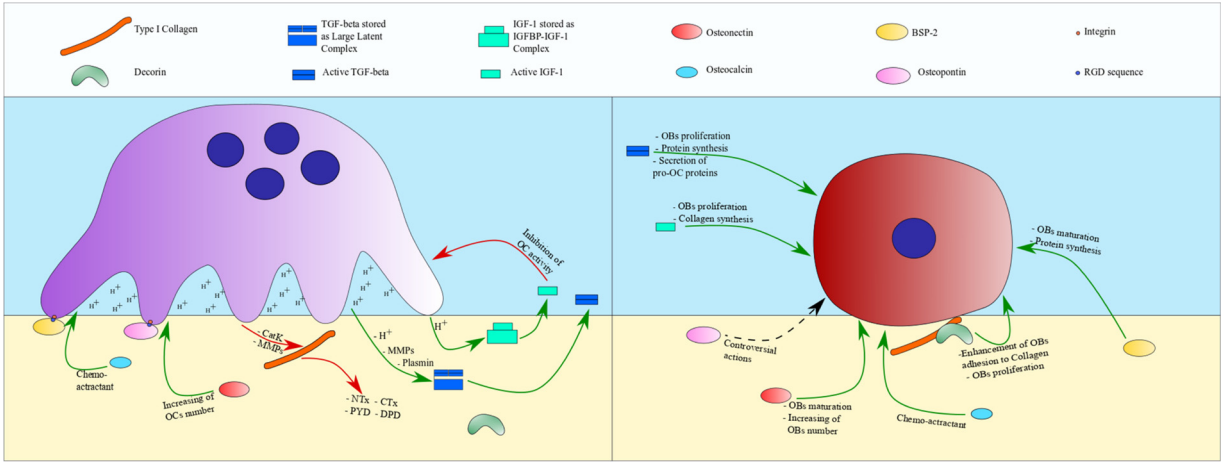

Fig. 4. Representative drawing of the ECM proteins roles on bone resorption and formation.
A) Mutual actions between the ECM proteins and an osteoclast (OC; in violet): OC-matrix interaction, regulation of OC life and activity, protein modifications occurring during bone resorption; B) Functions of ECM proteins on an osteoblast (OB; in red): regulation of OB-matrix adhesion, regulation of OB proliferation and protein synthesis. Green arrows indicate stimulation, red arrows show inhibitory effect and dotted arrow underlines unknown action.

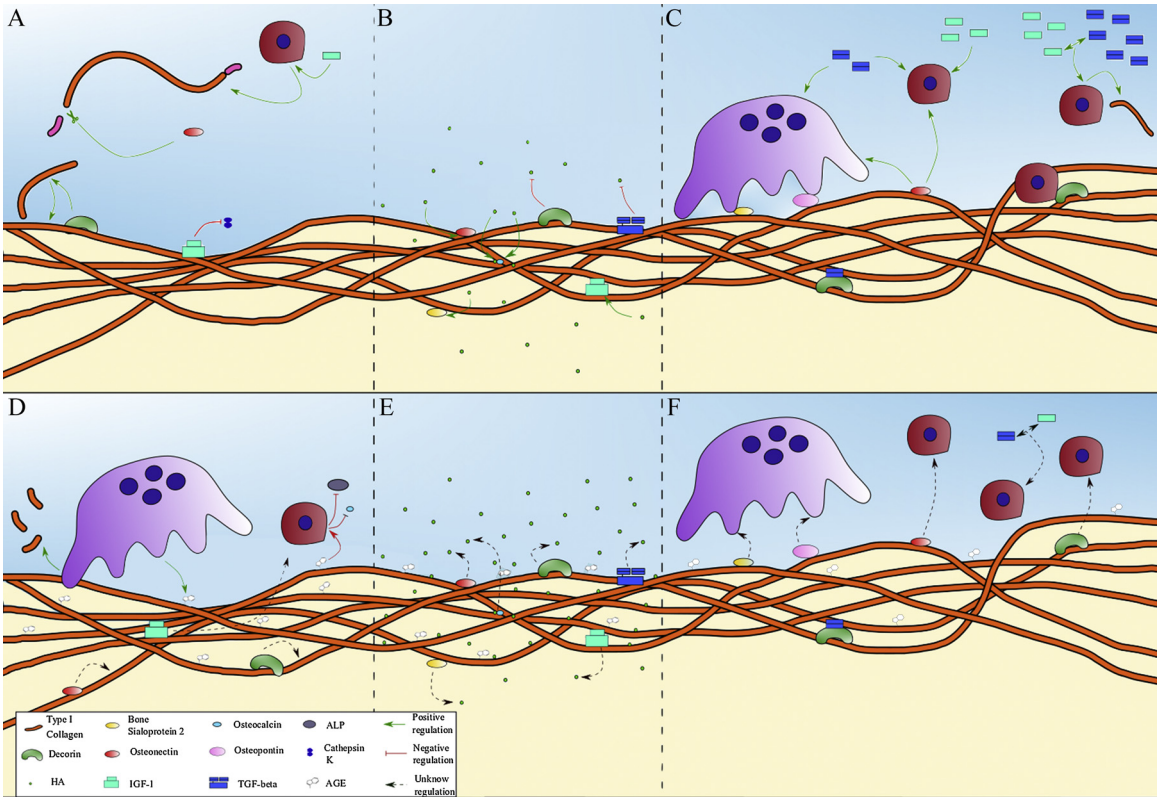

Fig. 5. Protein-protein interactions.
Representative drawing of the role that ECM proteins exert on bone 3D architecture constitution, maintenance, mineralization and on OB—OC coupling in healthy (A, B, C) and osteoporotic (D, E, F) subjects. In healthy bone: A) IGF-1, ON and DCN exert a pivotal role on collagen synthesis, maturation, incorporation in ECM and protection from degradation; B) TGF-β, IGF-1, DCN, ON, BSP-2 and OCN have different functions on mineralization process; C) Different concentrations of TGF-β and IGF-1 and their cooperation act on OB—OC coupling. TGF-β action is influenced also by DCN. ON, OPN and BSP-2 mediate the cell-matrix interaction; In OP onset and/or maintenance: D) The alterations of Collagen structure, like AGE cross-links, stimulate bone resorption by OCs and inhibit ALP and OCN synthesis by OBs. The role of IGF-1, ON and DCN is unknown; E) TGF-β, IGF-1, DCN, ON, BSP-2 and OCN role on mineralization process needs to be defined; F) OPN and BSP-2 mediate OCs adhesion, with contradictory information on bond resorption. The role of DCN, TGF-β and IGF-1 in need to be furtherly investigated.

differentiation as well as in bone formation rate. Reduced expression of *RANKL* mRNA and inhibition of osteoclastogenesis were also detected. [38] The dose-dependent effects suggest that TGF-β availability *in vivo* as active molecule could be tailored based on remodelling needs.

This fundamental role of TGF-β on OBs – OCs coupling suggests that this growth factor could be important in the inception of OP. It was shown that TGF-β reduced the OCs number in OVX rats and that anti-TGF-β1 antibody inhibited OCs apoptosis induced by oestrogen and tamoxifen, suggesting a synergy between TGF-β and oestrogen in inducing OCs death. [40]

In vitro, TGF-β inhibits matrix mineralization and the inhibition of its receptor TβRI increases bone mineralization, proposing an essential role of this factor in mineralization control. [35,36,38]

TGF-β also interacts with other ECM proteins with reciprocal regulation. (Figs. 4 and 5) [30,41–44]

In OP patients no differences were found in serum levels of TGF-β1 compared to controls, whilst TGF-β3 levels increased. [45] Several studies have revealed sequence variations of TGF-β1 gene in OP patients, with polymorphisms associated with low bone mass and higher bone turnover [46]. These initials finding were confirmed by studies on Turkish, Japanese and Italian women. [47–49], whilst no changes in TGF-β1 gene sequence associated with BMD in menopause were observed in Czech Caucasian women [50].

4. Insulin-like Growth Factor-1

Insulin-like Growth Factor 1 (IGF-1) is a small peptide of 7,6kDa, whose three-dimensional structure is represented by three helical segments connected by β-strands. (Fig. 3B) The name is due to its homology with the B chain of insulin and the A chain of proinsulin. [51] Insulin-like Growth Factor Binding Proteins (IGFBPs) bind IGF-1, sequestering the growth factor and preventing its action (Fig. 4); in bone ECM the main members are IGFBP-4 and IGFBP-5. [52]

Not much is known about the distribution of IGF-1 within the bone matrix. In a study on rat tibiae, most of the IGF-1 was detectable on endosteal and endocortical surfaces of trabecular bone, whilst mRNA for IGF-1 was found in areas containing OBs and OCs. [53] IGF-1 is released from ECM during bone resorption, where it promotes the recruitment and the osteoblastic differentiation of BMSCs, and increases the number of functional OBs. (Figs. 4 and 5) IGF-1 can enhance the expression of the early markers of OB differentiation, like RUNX2 and ALP, and inhibit OCN expression, a late marker of OB differentiation. Furthermore, the second wave of IGF-1 expression appeared with the mineralization phase, indicating its role in this process [54]. The lack of IGF-1 causes a decrease in both OB number and activity, with a reduction in bone formation and mineral apposition [55]. In OVX rats treated with intermittent PTH administration, cancellous OBs improved IGF-1 mRNA expression, indicating IGF-1 as a local mediator of PTH-induced bone formation in an OP model [56].

IGF-1 was shown to exert a pivotal role also in OCs activity. The *in vivo* and *in vitro* treatment with IGF-1 inhibit bone resorption, decreasing OCs number and activity. IGF-1 controls OPG expression in OBs, that works on OCs maturation and function [54,56], and inhibits the resorption induced by continuous administration of PTH and $125(OH)_2D3$ to a culture of foetal mouse long bones, in a dose-dependent manner. [57] Nonetheless, in the culture of unfractionated bone

cells, IGF-1 worked as a supporting factor for OCs recruitment and differentiation, only in the presence of differentiation-inducing factors, like $125(OH)_2D3$. [58]

One of the most significant functions of IGF-1 is the effect on the synthesis of bone ECM proteins: it stimulates type I Collagen synthesis at the transcriptional level and reduces its degradation. Furthermore, the combination of IGF-1, TGF-β and platelet-derived growth factor (PDGF) induced a significant increase in matrix apposition at concentrations which did not work in the case of each factor alone. [30]

A reduction in bone marrow IGF-1 levels was shown in OP individuals, and numerous studies showed that both bone matrix and serum IGF-1 are positively related to bone mass and inversely related to bone loss caused by aging, despite pre- or post-menopausal state and OP. The grade of association was most pronounced before the menopause onset, therefore IGF-1 may be considered as an early marker of bone loss. [59] Studies showed that OP postmenopausal women had also lower serum IGF-1 than those with normal BMD and without fractures. [59] Although the negative relation between IGF-1 and bone loss is unrelated to oestrogen levels, the correlation between serum IGF-1 levels and BMD is gender-specific: men revealed higher IGF-1 concentrations than women with no correlation with BMD, whereas women demonstrated a positive association between the IGF-1 levels and BMD [60].

5. Decorin

Decorin (DCN) is a protein that belongs to small leucine-rich proteoglycan (SLRP) family, which contain a protein core with one or more GAG chains. DCN molecular weight is about 120 kDa with a core (40 kDa) composed of 12 tandem leucine-rich repeat sequences (LRRs) and a single chain of GAG attaches at its N-terminal domain. [61] The three-dimensional structure reveals an arch-shaped molecule, with LRRs organised in parallel β-sheets on the concave surface and short β-strands, α-helices and polyproline II helices on the convex surface. (Fig. 3C) [62]

DCN is highly expressed in the ECM of all Type I Collagen-rich tissues, and it is preferentially found in association with Type I Collagen fibrils. [63] (Figs. 4 and 5) DCN orchestrates the correct collagen fibril assembly, which is important for the collagen cross-linking. Studies showed that the diameter of Collagen fibrils decreased when DCN bound to fibril surface, and its lack causes abnormal Collagen structure without affecting the total protein amount, thus confirming the important role of DCN in Collagen construction [25]. These observations were confirmed in experimental studies on *Dcn*$^{-/-}$ and *byglican*$^{-/-}$ (*bgn*$^{-/-}$) mice that showed a variation on structure and size of collagen fibrils in bone. Double-deficient animals presented a consistent reduction in cortical and trabecular bone mass compared to wild-type (WT) ones. Changes were evident in collagen fibril structure and organization, showing an important synergic effect of both genes that could be crucial in OP disease. [64]

Several *in vitro* and *in vivo* studies investigated the role of DCN in matrix mineralization. Although DCN was detected more in mineralized bone than in osteoid and mainly in calcified cartilage, it was revealed that DCN-rich collagen inhibits calcification and DCN removal promotes calcification. Furthermore, DCN mRNA and protein expression decreased at the onset of matrix mineralization. The mechanism is not well understood, but it was postulated that negative charges of GAG chain could compete with those of HA crystals, inhibiting mineralization. [25,65,66]

Takeuchi et al. demonstrated that DCN was the major binding protein for TGF-β1 among the whole extracted bone matrix proteins. The core of DCN interacts with TGF-β1(Fig. 4), without affecting its activity and therefore the increase in TGF-β1 capability to bind receptors is probably related to its cooperation with DCN. [42]

It was also demonstrated that DCN influences OB behaviour, and *in vitro* studies showed that collagen fibrils containing DCN significantly enhanced focal adhesion and proliferation of OBs, with a critical role exerted by the GAG component of DCN. [67]

6. Osteonectin

Osteonectin (ON) is a 35–45 kDa glycoprotein, also named SPARC (Secreted Protein Acidic and Rich in Cysteine) or BM-40 (Basement-Membrane protein 40). It is composed of three structural domains: the N-terminal domain is a highly acidic domain that binds Ca^{2+} with low affinity; the second one is a follistatin (FS)-like domain; and the third domain at C-terminus is an EF-hand Ca^{2+}- binding (EC) domain that binds Ca^{2+} and various type of collagen, such as Type I Collagen, III, IV and V. (Fig. 3D) [68]

The high expression of ON in bone observed in newly mineralizing bone with no staining in osteoid, suggested that this protein is involved in the initial formation of mineral bone. [26,65] The ON functions are closely related to Type I Collagen. (Figs. 4 and 5) *In vitro* studies suggested that ON is tangled in procollagen processing, regulation of Type I Collagen fibril diameter and their incorporation into ECM. [69] In addition, ON has a higher affinity for calcium than phosphate ions in HA. Type I Collagen is required for ON-mediated mineralization as its presence as well as other ions could induce a change in ON configuration, thus favouring its action as a nucleation site for Ca^{2+} binding. Otherwise, the role of ON in bone matrix mineralization may be related to its high affinity for Ca^{2+}: ON initiates crystallization of HA increasing local Ca^{2+} concentration thus favouring the interaction with Type I Collagen. [26,27] *In vitro,* ON could also stimulate collagenase expression (mainly MMPs), initiating a cascade of events for the ECM remodelling. [70] TGF-β1 regulates ON, increasing its expression in several types of cells, like fibroblasts and osteoblastic rat osteosarcoma cells [43,44].

ON is also important for the formation and maturation of bone cells. It was shown that ON$^{-/-}$ mice revealed a decrease in the number of OB precursors, OBs and OCs. OBs expressed transcripts for bone component, but cells did not reach a fully mature phenotype. The immature phenotype of the cells affected the degradation and replacement of mature collagen, with consequent alterations in bone formation and resorption as well as in degeneration of trabecular bone volume. [71]

The important role in matrix regulation and mineralization makes ON a good candidate for the OP onset. As mentioned above, ON$^{-/-}$ mice manifested fewer OBs and OCs, but the reduction in bone formation exceeded the loss in OCs. The regression in trabecular bone volume and defects in bone architecture imply an important role of ON protein in osteopenia and OP pathologies. [71]

Machado et al. showed that the intermittent treatment with PTH increased the number of OCs and eroded the surface of trabecular bone in ON$^{-/-}$ mice compared to WT. Thus, ON could be involved in modulating the PTH action on bone formation and resorption. [72] Furthermore, ON polymorphisms seem to affect BMD in humans. Delany et al. found that the haplotype commonly associated to a high bone density is mainly expressed in normal subjects than in OP patients, while the expression of ON haplotype associated to low BMD is higher in OP patients than in controls. [73]

7. Osteopontin

Osteopontin (OPN) is a glycoprotein of 44–75 kDa, that belongs to the Small Integrin Binding Ligand N-Glycosylated (SIBLING) protein family. It is expressed by a single-copy gene and then transformed by post-translational modifications. Variations in phosphorylation, glycosylation and sulphation induce conformational changes in OPN determining different functional properties. [74]

Computational prediction of its structure displayed that most of the protein has a highly disordered structure, with short α-helices that represent the only folded organization. [75]

OPN contains a motif constitute by nine consecutive aspartic acid

that binds to HA and an RGD sequence that is central in mediating cell adhesion. [74] It is secreted by OBs at the beginning and the end of their life cycle and TGF-β upregulates OPN mRNA expression [44]. Indeed, its effect on OBs is debated as MC3T3-E1 transfected with OPN cDNA showed a reduction in proliferation and differentiation potential, whilst transfection of OBs harvested from murine bone marrow determine more differentiated and active cells and the formation of mineralization nodules both *in vivo* and *in vitro*. [76,77]

OPN is more prominent in trabecular than in cortical bone and histochemical analyses showed a decreased expression in trabecular bone of subjects affected by OP. It is mainly localized in the mineralized tissue, as cement lines and lamellae, whilst weaker staining is detected in the osteoid portion, suggesting a pivotal role in the mineralization process. [65,79] The role of OPN in mineralization is still unclear. Even though OPN is commonly associated with mineralized matrix, cortical bone of $OPN^{-/-}$ mice showed a higher mineral content and greater crystallinity than the WT ones and, moreover, OBs harvested from $OPN^{-/-}$ mediated more mineral deposition *in vitro*. Additionally, the supplement of OPN peptide to cell culture media reduced the mineralization. [80]

Other studies indicated that OPN post-translational modifications could contribute to mineral modulation. For instance, OPN inhibits the growth of HA crystals, but this effect is reduced by the removing of phosphate and carboxylate groups. Studies indicated that phosphorylated OPN isolated from bone inhibited mineralization *in vitro*, whilst no significant differences in mineralization were evident after OPN dephosphorylation. Surprisingly, the highly phosphorylated form promoted HA formation and growth. [81] Another possible regulatory mechanism of bone ECM mineralization is represented by the interaction between OPN and other proteins such as collagen and osteocalcin (OCN). (Fig. 4) Thrombin digestion of milk OPN generates three OPN fragments, including SKK-fragment. The latter linked to collagen induced HA formation in agarose gel, while it showed an inhibitory effect without collagen. These findings suggested a change of OPN conformation due to Type I Collagen binding that improves HA development. [27] Furthermore, the *in vitro* mineralization process was amplified precoating HA with OCN before adding OPN to culture media, while both OCN and OPN alone inhibited HA formation. [81]

OPN is the target of several enzymes, like MMP-3 and MMP-7, which generate fragments of different molecular weight acting on both unique and overlapping cleavage sites. [78] Circulating OPN acts as cytokine, whilst in bone ECM it mediates cell-matrix interaction influencing bone remodelling in both cases. OCs interact with the RGD-sequence by $\alpha_v\beta_3$ integrins and adhere to ECM by numerous podosomes. OCs express high levels of both OPN mRNA and protein, and the detection of OPN between OCs and mineralized bone matrix suggests that OCs are capable of secreting OPN or, alternatively, may incorporate this protein during bone resorption. [65,82,83]

The role of OPN in bone remodelling is confirmed by studies on OP development. OVX $OPN^{+/+}$ mice lost bone mass, while $OPN^{-/-}$ mice showed higher bone volume than $OPN^{+/+}$ ones. Furthermore, OVX mice had decreased BMD and a reduction in the number and area of resorption pits was observed treating OVX mice with anti-OPN antibody in a dose-dependent manner, a phenomenon probably related to OCs apoptosis. [84] Higher levels of serum OPN were found in post-menopausal women with OP compared to osteopenic and normal ones. The high levels of this cytokine inversely correlated to BMD of the lumbar spine, femoral neck and hip. Plasma OPN decreased in women with post-menopausal OP after the intermittent PTH treatment, with an improvement of BMD. [85]

8. Bone sialoprotein 2

Bone sialoprotein (BSP-2) belongs to the SIBLING family, too. It has an RGD sequence at C-terminus and high glutamic acid content in the N-terminal half of the protein. BSP-2 mediates cell adhesion to ECM by RGD or non-RGD tyrosine-rich sequences. BSP-2 showed numerous post-translational modifications, with the addition of sulphate and phosphate groups as well as carbohydrates, mainly sialic acid, that form a highly dynamic structure without regions of a fixed secondary structure, but with the potential to form α-helixes and β-sheets. (Fig. 3E) [86] The molecular weight of this protein is between 33–80 kDa on SDS-PAGE depending on the carbohydrate content and post-translational modifications [86].

In bone, BSP-2 is localized along cement lines and between collagen fibrils within the mineralized bone and mineralizing osteoid. Staining intensity is greater in the deepest layers of bone ECM than in the superficial ones. [65,86] BSP-2 binds Type I Collagen structure by hydrophobic interactions which are essential to favour HA nucleation [29] and glutamic acid-rich sequences may be of importance in this process [86].

BSP-2 mRNA expression was related to initial formation of mineralized connective tissue during late gestational and early postnatal stages, and bone cells, like the osteocytes (OCYs) recently deposited in osteoid, mature OBs and OCs, are positive for both BSP-2 mRNA and protein. [87]. BSP-2 expression is associated with development and maturation of preOBs to OBs and bone formation, and they are closely related to early-stage OBs markers like *Osterix* (*Osx*) and *Runx2* expression as well as with sites of active bone formation in mature OBs. $Bsp^{-/-}$ mice showed an impaired gene expression for some OB markers, like *Collagen alpha-1(I) chain (Col1a1)*, *Osx*, *Alp1*, *Runx2*, and *bone gamma-carboxyglutamic acid-containing protein (Bglap),* a late-stage marker, and a delay in mineralization of embryonic and neonatal bones. [86–89] (Figs. 4 and 5)

Interactions between matrix BSP-2 and integrins $\alpha_v\beta_3$ are required for OC attachment to ECM (Fig. 3) [83,87], even if OCs from $bsp^{-/-}$ mice did not fully lose the capacity to resorb dentine. The *in vitro* administration of BSP-2 increased resorption area and the number of resorption pits, exerting a significant role in OC differentiation and activity, as shown in $bsp^{-/-}$ mice that exhibited a reduction of the area covered by OCs with a small reduction in cell number. Additionally, bone segments were shorter and hypomineralized, with an improved trabecular bone mass caused by low turnover. These findings propose that BSP-2 is implicated in bone mass regulation and remodelling. [90,91]

Serum BSP-2 level is higher in post-menopausal OP women than in peri-menopausal controls showing a positive correlation with other markers of bone resorption (*i.e.* PYD, DPD, NTx). The treatments with Hormone Replacement Therapy (HRT), Alendronate and combined HRT/Alendronate decrease serum BSP-2 levels. [92] $1,25(OH)_2D3$ suppresses BSP-2 synthesis in rat bone organ cultures, and significantly reduces the mass of mineralized tissues whilst the use of dexamethasone increases BSP-2 mRNA levels. [89]

9. Osteocalcin

Osteocalcin, the most abundant non-collagenous protein in bone ECM, is a 49 amino acid protein of 5–6 kDa characterized by three α-helices and containing three γ-carboxyglutamate (Gla) residues. (Fig. 3F) [93] Gla residues are post-translational modifications occurred after carboxylation reaction by Vitamin K and provide OCN with the ability to bind Ca^{2+} and HA with a high affinity, inducing its conformational changes. All three Gla residues involved in the HA binding are on the surface of α-helix 1. It was speculated that lack of Gla residues on matrix OCN could be involved in OP disease as post-menopausal women with osteoporotic fractures had low levels of circulating Vitamin K, which could influence the γ-carboxylation and bone mineralization consequently. [93]

OCN is produced by OBs during bone formation and is accumulated at the reversal lines in the fully mineralized matrix. Increase in OCN synthesis by OBs was observed during the transition from osteoid to mature mineralized matrix, while low concentrations are shown during

the first mineral formation. MSCs cultured in osteogenic induction showed a peak in *Ocn* mRNA expression after 21 days, and when transfected with siRNA for *Ocn*, these cells showed altered mature mineralization and lower mineral/matrix ratio compared to the control group. Overall, this indicates that OCN is closely associated with progression of the mineralization process. [65,94] Whereas OCN is important for the development of mature mineralized matrix in bone, the lack of OCN leads to an increase in bone formation. Indeed, $Ocn^{-/-}$ mice exhibited more cancellous and cortical bone than the WT ones. [95]

When bound to Ca^{2+}, OCN is unstructured at N-terminus and, from the residue 16, the 3 α-helices are connected by short structures. [93] When OCN binds HA, unbound regions including C-terminus could be oriented and recruits OBs and OCs by receptors for cell adhesion and signalling. The protein acts as chemo-attractant for OC precursors as well as OBs, without mediating the cell bond [94,96]. (Figs. 4 and 5)

In trabecular bone of human femoral heads, a decrease of OCN expression in OCYs/OBs was detected in OP subjects, while an increased OCN expression was observed in samples from patients affected by osteoarthritis compared to healthy controls. [79]

In vitro, $1,25(OH)_2D3$ increases *Ocn* transcription in OBs, acting by different regulation pathway, with a greater stimulatory effect at the first stages of OBs differentiation and a moderate stimulation in cells within mineralized matrix. [97] TGF-β treatment reduced baseline expression of OCN in differentiated OBs [44.] OCN is also capable of interfering with the differentiation of OCs, even if for their final maturation systemic hormones like calcitriol or PTH are required [98].

OCN is considered a marker of OP and several epidemiological studies tried to establish the role of serum and urinary OCN detection as an accurate biomarker of OP with controversial results. Serum OCN levels may not be an accurate indicator of the entity of bone resorption, since OCN is metabolized also by kidneys, liver and lungs. However, studies evidencing the release of OCN by bovine bone slides after OCs culture, the relationship between OCN amount with the presence of PTH in the culture system, and the strong relation between OCN concentration and CTx amount, a common marker of bone remodelling, demonstrates that the main fraction of serum OCN is factually derived from bone resorption. [97–99] Singh et al. demonstrated an inverse relationship between serum OCN level and BMD, with raised OCN levels in post-menopausal women affected by osteopenia and OP compared to women with normal BMD [100]. In this study, the difference between osteopenic and OP women were not statistically significant, suggesting OCN level as a mere marker of low bone mass conditions. On the contrary, Wei et al. indicated that OCN levels were lower in the osteoporotic group than in the osteopenic and normal ones [84], favouring the discussion on the reliability of this detection.

10. Protein-protein interactions: from normal bone biology to osteoporosis development

Bone matrix is the result of a balance between several factors: the proper structure, which confers tensile strength and ductility, the grade of mineralization, which provides stiffness, and the OB—OC coupling, which regulates the relationship between bone formation and bone resorption. ECM proteins and their mutual interactions contribute to the preservation of ECM properties. Type I Collagen constitutes the main component of the structure and some NCPs, like IGF-1, DCN and ON, cooperate to control its synthesis and correct assembly, prevent the degradation, and preserve the three-dimensional structure. NCPs, like TGF-beta, IGF-1, DCN, OPN and OCN, regulate the mineralization process at various stages and in different ways. Also, ON and BSP-2 control mineralization, but they require the interaction with Type I Collagen to bind HA.

ECM proteins affect OBs and OCs life cycle. For instance, OCN is a chemo-attractant for OBs and OCs, while other NCPs directly contribute to cell-matrix bond. In this respect, DCN acts on OB focal adhesion to the matrix, and OPN and BSP-2 contribute to the OC adhesion to ECM through RGD sequences. Other ECM proteins stimulate the proliferation and/or the maturation of OBs and OCs. TGF-beta and IGF-1, released and activated during bone resorption, play a dose-dependent role in OB recruitment, maturation, protein synthesis and matrix formation, and in OC formation and activity. Physiologically, during the different stages of bone remodelling the quantity of active TGF-beta and IGF-1 is adjusted to reciprocal influence the behaviour of each bone cell involved in the process. Interestingly, the collaboration between TGF-beta and IGF-1 enhances matrix apposition, compared to the stimulation operated by each protein alone. TGF-beta action is also improved by DCN-TGF-beta connection that stimulates the capability of TGF-beta to bind its receptors (Fig. 5).

The unbalance of OB—OC coupling and OC hyperactivity, with the consequent loss of bone mass and microarchitectural deterioration, detected in OP me be related to mutual altered signals coming from bone ECM. In OP, biochemical alterations of Type I Collagen such as the decrease of enzymatic cross-links and the increase of AGEs that negatively influence the mechanical properties of bone, the mineralization processes and the cell-matrix interactions are reported. AGEs trigger the AGE-RAGE pathway, inducing the RAGE expression in OBs with the consequent reduction of ALP and OCN production. Additionally, AGEs in matrix favour bone resorption. Although no study associated IGF-1, DCN and ON to Collagen modification observed in OP, their role in the synthesis, assembly and incorporation of Type I Collagen in ECM could be critical in OP onset. Furthermore, ON have a direct role in OB—OC coupling as suggested by the observation in $ON^{-/-}$ mice of a noticeable reduction in bone formation compared to bone resorption (Fig. 5).

A far as the proposed serum markers of low bone mass and/or OP, due to OC hyperactivity, NTx and CTx deriving from Type I Collagen degradation, IGF-1, OPN, BSP-2 and OCN must be considered. Differently, the serum amount of TGF-beta and ON is inefficient to discriminate between the healthy and osteoporotic subjects, even if some polymorphism of both molecules were shown mainly in OP patients' serum.

Therefore, changes in ECM proteins amount influencing the regulatory effect that they exert on OBs and OCs must be considered in OB—OC coupling during OP.

11. Final consideration

Collagenous and non-collagenous proteins in ECM work together to ensure normal bone metabolism. Each protein plays one or more roles in bone metabolism, which is influenced by the stage of the bone remodelling. Anomalies in the amount and structure of one or more of these proteins can cause abnormalities in bone formation and resorption, with consequent defects and malformations, such as OP. To the best of our knowledge, studies investigating bone matrix biology and the role of its proteins, were performed mainly in the past. Current studies are more focused on the identification of markers for the early identification of OP. The connections between key proteins for matrix formation and resorption is far from being elucidated. In this review, we have tried to resume some important networking aspect in bone ECM that could represent starting points for the development of new strategies for the management of OP and other bone diseases.

Transparency document

The Transparency document associated with this article can be found in the online version.

Declaration of Competing Interest

None.

Acknowledgements

The authors are grateful to Dr. Andrell Hosein for her English revision. This project has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement No 681798 - BOOST) (www.ercprojectboost.eu) and by the Italian Ministry of Education, Universities and Research (MIUR), Progetto *FARE Ricerca* in Italia (GRACE). No benefits in any form have been, or will be, received from a commercial party directly, or indirectly, related to the subject of this article.

References

[1] G.E. Sroga, D. Vashishth, Effects of bone matrix proteins on fracture and fragility in osteoporosis, Curr. Osteoporos. Rep. 10 (2012) 141–150, https://doi.org/10.1007/s11914-012-0103-6.
[2] Y. Bala, D. Farlay, G. Boivin, Bone mineralization: from tissue to crystal in normal and pathological contexts, Osteoporos. Int. 24 (2013) 2153–2166, https://doi.org/10.1007/s00198-012-2228-y.
[3] N.A. Sims, T.J. Martin, Coupling the activities of bone formation and resorption: a multitude of signals within the basic multicellular unit, Bonekey Rep. 3 (2014), https://doi.org/10.1038/bonekey.2013.215.
[4] Assessment of fracture risk and its application to screening for postmenopausal osteoporosis. Report of a WHO Study Group, World Health Organ. Tech. Rep. Ser. 843 (1994) 1–129.
[5] M. Saito, K. Fujii, S. Soshi, T. Tanaka, Reductions in degree of mineralization and enzymatic collagen cross-links and increases in glycation-induced pentosidine in the femoral neck cortex in cases of femoral neck fracture, Osteoporos. Int. 17 (2006) 986–995, https://doi.org/10.1007/s00198-006-0087-0.
[6] C.M. Sprecher, F. Schmidutz, T. Helfen, R.G. Richards, M. Blauth, S. Milz, Histomorphometric assessment of cancellous and cortical bone material distribution in the proximal humerus of normal and osteoporotic individuals: significantly reduced bone stock in the metaphyseal and subcapital regions of osteoporotic individuals, Medicine (Baltimore) 94 (2015) e2043, https://doi.org/10.1097/MD.0000000000002043.
[7] R.M.D. Zebaze, A. Ghasem-Zadeh, A. Bohte, S. Iuliano-Burns, M. Mirams, R.I. Price, E.J. Mackie, E. Seeman, Intracortical remodelling and porosity in the distal radius and post-mortem femurs of women: a cross-sectional study, Lancet 375 (2010) 1729–1736, https://doi.org/10.1016/S0140-6736(10)60320-0.
[8] A.L. Boskey, Bone composition: relationship to bone fragility and antiosteoporotic drug effects, Bonekey Rep. 2 (2013), https://doi.org/10.1038/bonekey.2013.181.
[9] G. Pezzotti, A. Rondinella, E. Marin, W. Zhu, N.N. Aldini, G. Ulian, G. Valdrè, Raman spectroscopic investigation on the molecular structure of apatite and collagen in osteoporotic cortical bone, J. Mech. Behav. Biomed. Mater. 65 (2017) 264–273, https://doi.org/10.1016/j.jmbbm.2016.08.030.
[10] H. Ren, D. Liang, X. Jiang, J. Tang, J. Cui, Q. Wei, S. Zhang, Z. Yao, G. Shen, S. Lin, Variance of spinal osteoporosis induced by dexamethasone and methylprednisolone and its associated mechanism, Steroids 102 (2015) 65–75, https://doi.org/10.1016/j.steroids.2015.07.006.
[11] S. Kousteni, J.P. Bilezikian, The cell biology of parathyroid hormone in osteoblasts, Curr. Osteoporos. Rep. 6 (2008) 72–76.
[12] J.E. Zerwekh, K. Sakhaee, C.Y.C. Pak, Short-term 1,25-dihydroxyvitamn D3 administration raises serum osteocalcin in patients with postmenopausal osteoporosis, J. Clin. Endocrinol. Metab. 60 (1985) 615–617, https://doi.org/10.1210/jcem-60-3-615.
[13] I. Zofkova, M. Hill, Long-term 1,25(OH)2 vitamin D therapy increases bone mineral density in osteopenic women. Comparison with the effect of plain vitamin D, Aging Clin. Exp. Res. 19 (2007) 472–477.
[14] D.J. Prockop, K.I. Kivirikko, L. Tuderman, N.A. Guzman, The biosynthesis of collagen and its disorders (first of two parts), N. Engl. J. Med. 301 (1979) 13–23, https://doi.org/10.1056/NEJM197907053010104.
[15] P.C. Trackman, Enzymatic and non-enzymatic functions of the lysyl oxidase family in bone, Matrix Biol. 52–54 (2016) 7–18, https://doi.org/10.1016/j.matbio.2016.01.001.
[16] M. Saito, K. Marumo, Effects of collagen crosslinking on bone material properties in health and disease, Calcif. Tissue Int. 97 (2015) 242–261, https://doi.org/10.1007/s00223-015-9985-5.
[17] X. Wang, R.A. Bank, J.M. TeKoppele, C.M. Agrawal, The role of collagen in determining bone mechanical properties, J. Orthop. Res. 19 (2001) 1021–1026, https://doi.org/10.1016/S0736-0266(01)00047-X.
[18] H. Oxlund, L. Mosekilde, G. Ortoft, Reduced concentration of collagen reducible cross links in human trabecular bone with respect to age and osteoporosis, Bone 19 (1996) 479–484.
[19] S. Vasikaran, R. Eastell, O. Bruyère, A.J. Foldes, P. Garnero, A. Griesmacher, M. McClung, H.A. Morris, S. Silverman, T. Trenti, D.A. Wahl, C. Cooper, J.A. Kanis, IOF-IFCC Bone Marker Standards Working Group, Markers of bone turnover for the prediction of fracture risk and monitoring of osteoporosis treatment: a need for international reference standards, Osteoporos. Int. 22 (2011) 391–420, https://doi.org/10.1007/s00198-010-1501-1.
[20] X.N. Dong, A. Qin, J. Xu, X. Wang, In situ accumulation of advanced glycation endproducts (AGEs) in bone matrix and its correlation with osteoclastic bone resorption, Bone 49 (2011) 174–183, https://doi.org/10.1016/j.bone.2011.04.009.
[21] A.H. Aguda, P. Panwar, X. Du, N.T. Nguyen, G.D. Brayer, D. Brömme, Structural basis of collagen fiber degradation by cathepsin K, Proc. Natl. Acad. Sci. U. S. A. 111 (2014) 17474–17479, https://doi.org/10.1073/pnas.1414126111.
[22] H.P.H. Liang, J. Xu, M. Xue, C.J. Jackson, Matrix metalloproteinases in bone development and pathology: current knowledge and potential clinical utility, Metalloproteinases Med. (2016), https://doi.org/10.2147/MNM.S92187.
[23] S. Franke, H. Siggelkow, G. Wolf, G. Hein, Advanced glycation endproducts influence the mRNA expression of RAGE, RANKL and various osteoblastic genes in human osteoblasts, Arch. Physiol. Biochem. 113 (2007) 154–161, https://doi.org/10.1080/13813450701602523.
[24] K.-H. Ding, Z.-Z. Wang, M.W. Hamrick, Z.-B. Deng, L. Zhou, B. Kang, S.-L. Yan, J.-X. She, D.M. Stern, C.M. Isales, Q.-S. Mi, Disordered osteoclast formation in RAGE-deficient mouse establishes an essential role for RAGE in diabetes related bone loss, Biochem. Biophys. Res. Commun. 340 (2006) 1091–1097, https://doi.org/10.1016/j.bbrc.2005.12.107.
[25] Y. Mochida, D. Parisuthiman, S. Pornprasertsuk-Damrongsri, P. Atsawasuwan, M. Sricholpech, A.L. Boskey, M. Yamauchi, Decorin modulates collagen matrix assembly and mineralization, Matrix Biol. 28 (2009) 44–52, https://doi.org/10.1016/j.matbio.2008.11.003.
[26] R.W. Romberg, P.G. Werness, P. Lollar, B.L. Riggs, K.G. Mann, Isolation and characterization of native adult osteonectin, J. Biol. Chem. 260 (1985) 2728–2736.
[27] A.L. Boskey, B. Christensen, H. Taleb, E.S. Sørensen, Post-translational modification of osteopontin: effects on in vitro hydroxyapatite formation and growth, Biochem. Biophys. Res. Commun. 419 (2012) 333–338, https://doi.org/10.1016/j.bbrc.2012.02.024.
[28] G.S. Baht, G.K. Hunter, H.A. Goldberg, Bone sialoprotein-collagen interaction promotes hydroxyapatite nucleation, Matrix Biol. 27 (2008) 600–608, https://doi.org/10.1016/j.matbio.2008.06.004.
[29] E. Piek, C.H. Heldin, P. Ten Dijke, Specificity, diversity, and regulation in TGF-beta superfamily signaling, FASEB J. 13 (1999) 2105–2124.
[30] J. Pfeilschifter, L. Bonewald, G.R. Mundy, Characterization of the latent transforming growth factor beta complex in bone, J. Bone Miner. Res. 5 (1990) 49–58, https://doi.org/10.1002/jbmr.5650050109.
[31] S.L. Dallas, J.L. Rosser, G.R. Mundy, L.F. Bonewald, Proteolysis of latent transforming growth factor-beta (TGF-beta)-binding protein-1 by osteoclasts. A cellular mechanism for release of TGF-beta from bone matrix, J. Biol. Chem. 277 (2002) 21352–21360, https://doi.org/10.1074/jbc.M111663200.
[32] M. Shi, J. Zhu, R. Wang, X. Chen, L. Mi, T. Walz, T.A. Springer, Latent TGF-β structure and activation, Nature 474 (2011) 343–349, https://doi.org/10.1038/nature10152.
[33] A. Nakao, T. Imamura, S. Souchelnytskyi, M. Kawabata, A. Ishisaki, E. Oeda, K. Tamaki, J. Hanai, C.H. Heldin, K. Miyazono, P. ten Dijke, TGF-beta receptor-mediated signalling through Smad2, Smad3 and Smad4, EMBO J. 16 (1997) 5353–5362, https://doi.org/10.1093/emboj/16.17.5353.
[34] Y. Tang, X. Wu, W. Lei, L. Pang, C. Wan, Z. Shi, L. Zhao, T.R. Nagy, X. Peng, J. Hu, X. Feng, W. Van Hul, M. Wan, X. Cao, TGF-beta1-induced migration of bone mesenchymal stem cells couples bone resorption with formation, Nat. Med. 15 (2009) 757–765, https://doi.org/10.1038/nm.1979.
[35] S. Ehnert, J. Baur, A. Schmitt, M. Neumaier, M. Lucke, S. Dooley, H. Vester, B. Wildemann, U. Stöckle, A.K. Nussler, TGF-β1 As possible link between loss of bone mineral density and chronic inflammation, PLoS One 5 (2010) e14073, , https://doi.org/10.1371/journal.pone.0014073.
[36] M. Kassem, M. Kveiborg, E.F. Eriksen, Production and action of transforming growth factor-beta in human osteoblast cultures: dependence on cell differentiation and modulation by calcitriol, Eur. J. Clin. Invest. 30 (2000) 429–437.
[37] R.L. Jilka, R.S. Weinstein, T. Bellido, A.M. Parfitt, S.C. Manolagas, Osteoblast programmed cell death (apoptosis): modulation by growth factors and cytokines, J. Bone Miner. Res. 13 (1998) 793–802, https://doi.org/10.1359/jbmr.1998.13.5.793.
[38] K.S. Mohammad, C.G. Chen, G. Balooch, E. Stebbins, C.R. McKenna, H. Davis, M. Niewolna, X.H. Peng, D.H.N. Nguyen, S.S. Ionova-Martin, J.W. Bracey, W.R. Hogue, D.H. Wong, R.O. Ritchie, L.J. Suva, R. Derynck, T.A. Guise, T. Alliston, Pharmacologic inhibition of the TGF-β type I receptor kinase has anabolic and anti-catabolic effects on bone, PLoS One 4 (2009) e5275, https://doi.org/10.1371/journal.pone.0005275.
[39] M. Karst, G. Gorny, R.J.S. Galvin, M.J. Oursler, Roles of stromal cell RANKL, OPG, and M-CSF expression in biphasic TGF-beta regulation of osteoclast differentiation, J. Cell. Physiol. 200 (2004) 99–106, https://doi.org/10.1002/jcp.20036.
[40] D.E. Hughes, A. Dai, J.C. Tiffee, H.H. Li, G.R. Mundy, B.F. Boyce, Estrogen promotes apoptosis of murine osteoclasts mediated by TGF-beta, Nat. Med. 2 (1996) 1132–1136.
[41] P.J. Fagenholz, S.M. Warren, J.A. Greenwald, P.J. Bouletreau, J.A. Spector, F.E. Crisera, M.T. Longaker, Osteoblast gene expression is differentially regulated by TGF-beta isoforms, J. Craniofac. Surg. 12 (2001) 183–190.
[42] Y. Takeuchi, Y. Kodama, T. Matsumoto, Bone matrix decorin binds transforming growth factor-beta and enhances its bioactivity, J. Biol. Chem. 269 (1994) 32634–32638.
[43] T. Fujita, H. Shiba, M. Sakata, Y. Uchida, T. Ogawa, H. Kurihara, Effects of transforming growth factor-beta 1 and fibronectin on SPARC expression in cultures of human periodontal ligament cells, Cell Biol. Int. 26 (2002) 1065–1072.
[44] M. Noda, G.A. Rodan, Type beta transforming growth factor (TGF beta) regulation of alkaline phosphatase expression and other phenotype-related mRNAs in osteoblastic rat osteosarcoma cells, J. Cell. Physiol. 133 (1987) 426–437, https://doi.org/10.1002/jcp.1041330303.

[45] D.J. Grainger, J. Percival, M. Chiano, T.D. Spector, The role of serum TGF-beta isoforms as potential markers of osteoporosis, Osteoporos. Int. 9 (1999) 398–404.
[46] B.L. Langdahl, M. Carstens, L. Stenkjaer, E.F. Eriksen, Polymorphisms in the transforming growth factor beta 1 gene and osteoporosis, Bone 32 (2003) 297–310.
[47] S. Tural, G. Alayli, N. Kara, B. Tander, A. Bilgici, O. Kuru, Association between osteoporosis and polymorphisms of the IL-10 and TGF-beta genes in Turkish postmenopausal women, Hum. Immunol. 74 (2013) 1179–1183, https://doi.org/10.1016/j.humimm.2013.03.005.
[48] Y. Yamada, A. Miyauchi, J. Goto, Y. Takagi, H. Okuizumi, M. Kanematsu, M. Hase, H. Takai, A. Harada, K. Ikeda, Association of a polymorphism of the transforming growth factor-beta1 gene with genetic susceptibility to osteoporosis in postmenopausal Japanese women, J. Bone Miner. Res. 13 (1998) 1569–1576, https://doi.org/10.1359/jbmr.1998.13.10.1569.
[49] F. Bertoldo, L. D'Agruma, F. Furlan, F. Colapietro, M.T. Lorenzi, N. Maiorano, A. Iolascon, L. Zelante, V. Locascio, P. Gasparini, Transforming growth factor-beta1 gene polymorphism, bone turnover, and bone mass in Italian postmenopausal women, J. Bone Miner. Res. 15 (2000) 634–639, https://doi.org/10.1359/jbmr.2000.15.4.634.
[50] J.A. Hubacek, M. Weichetova, R. Bohuslavova, Z. Skodova, V. Adámkova, J.J. Stepan, Genetic polymorphisms of TGF-beta, PAI-1, and COL1A-1, and determination of bone mineral density in Caucasian females, Endocr. Regul. 40 (2006) 77–81.
[51] A.M. Brzozowski, E.J. Dodson, G.G. Dodson, G.N. Murshudov, C. Verma, J.P. Turkenburg, F.M. de Bree, Z. Dauter, Structural origins of the functional divergence of human insulin-like growth factor-I and insulin, Biochemistry 41 (2002) 9389–9397.
[52] S. Mohan, Y. Nakao, Y. Honda, E. Landale, U. Leser, C. Dony, K. Lang, D.J. Baylink, Studies on the mechanisms by which insulin-like growth factor (IGF) binding protein-4 (IGFBP-4) and IGFBP-5 modulate IGF actions in bone cells, J. Biol. Chem. 270 (1995) 20424–20431.
[53] D.A. Lazowski, L.J. Fraher, A. Hodsman, B. Steer, D. Modrowski, V.K. Han, Regional variation of insulin-like growth factor-I gene expression in mature rat bone and cartilage, Bone 15 (1994) 563–576.
[54] L. Guerra-Menéndez, M.C. Sádaba, J.E. Puche, J.L. Lavandera, L.F. de Castro, A.R. de Gortázar, I. Castilla-Cortázar, IGF-I increases markers of osteoblastic activity and reduces bone resorption via osteoprotegerin and RANK-ligand, J. Transl. Med. 11 (2013) 271, https://doi.org/10.1186/1479-5876-11-271.
[55] K.E. Govoni, J.E. Wergedal, L. Florin, P. Angel, D.J. Baylink, S. Mohan, Conditional deletion of insulin-like growth factor-I in collagen type 1alpha2-expressing cells results in postnatal lethality and a dramatic reduction in bone accretion, Endocrinology 148 (2007) 5706–5715, https://doi.org/10.1210/en.2007-0608.
[56] P. Watson, D. Lazowski, V. Han, L. Fraher, B. Steer, A. Hodsman, Parathyroid hormone restores bone mass and enhances osteoblast insulin-like growth factor I gene expression in ovariectomized rats, Bone 16 (1995) 357–365.
[57] A. Staal, W.M. Geertsma-Kleinekoort, G.J. Van Den Bemd, C.J. Buurman, J.C. Birkenhäger, H.A. Pols, J.P. Van Leeuwen, Regulation of osteocalcin production and bone resorption by 1,25-dihydroxyvitamin D3 in mouse long bones: interaction with the bone-derived growth factors TGF-beta and IGF-I, J. Bone Miner. Res. 13 (1998) 36–43, https://doi.org/10.1359/jbmr.1998.13.1.36.
[58] H. Mochizuki, Y. Hakeda, N. Wakatsuki, N. Usui, S. Akashi, T. Sato, K. Tanaka, M. Kumegawa, Insulin-like growth factor-I supports formation and activation of osteoclasts, Endocrinology 131 (1992) 1075–1080, https://doi.org/10.1210/endo.131.3.1505451.
[59] J. Liu, H. Zhao, G. Ning, Y. Chen, L. Zhang, L. Sun, Y. Zhao, M. Xu, J. Chen, IGF-1 as an early marker for low bone mass or osteoporosis in premenopausal and postmenopausal women, J. Bone Miner. Metab. 26 (2008) 159–164, https://doi.org/10.1007/s00774-007-0799-z.
[60] E. Barrett-Connor, D. Goodman-Gruen, Gender differences in insulin-like growth factor and bone mineral density association in old age: the Rancho Bernardo Study, J. Bone Miner. Res. 13 (1998) 1343–1349, https://doi.org/10.1359/jbmr.1998.13.8.1343.
[61] A. Oldberg, P. Antonsson, J. Moses, L.A. Fransson, Amino-terminal deletions in the decorin core protein leads to the biosynthesis of proteoglycans with shorter glycosaminoglycan chains, FEBS Lett. 386 (1996) 29–32.
[62] R.V. Iozzo, L. Schaefer, Proteoglycan form and function: a comprehensive nomenclature of proteoglycans, Matrix Biol. 42 (2015) 11–55, https://doi.org/10.1016/j.matbio.2015.02.003.
[63] P. Bianco, L.W. Fisher, M.F. Young, J.D. Termine, P.G. Robey, Expression and localization of the two small proteoglycans biglycan and decorin in developing human skeletal and non-skeletal tissues, J. Histochem. Cytochem. 38 (1990) 1549–1563, https://doi.org/10.1177/38.11.2212616.
[64] A. Corsi, T. Xu, X.D. Chen, A. Boyde, J. Liang, M. Mankani, B. Sommer, R.V. Iozzo, I. Eichstetter, P.G. Robey, P. Bianco, M.F. Young, Phenotypic effects of biglycan deficiency are linked to collagen fibril abnormalities, are synergized by decorin deficiency, and mimic Ehlers-Danlos-like changes in bone and other connective tissues, J. Bone Miner. Res. 17 (2002) 1180–1189, https://doi.org/10.1359/jbmr.2002.17.7.1180.
[65] P. Derkx, A.L. Nigg, F.T. Bosman, D.H. Birkenhäger-Frenkel, A.B. Houtsmuller, Ha.P. Pols, J.P.T.M. van Leeuwen, Immunolocalization and Quantification of Noncollagenous Bone Matrix Proteins In Methylmethacrylate-Embedded Adult Human Bone In Combination With Histomorphometry, Bone. 22 (1998) 367–373, https://doi.org/10.1016/S8756-3282(97)00299-8.
[66] Y. Mochida, W.R. Duarte, H. Tanzawa, E.P. Paschalis, M. Yamauchi, Decorin modulates matrix mineralization in vitro, Biochem. Biophys. Res. Commun. 305 (2003) 6–9.
[67] T. Douglas, U. Hempel, C. Mietrach, M. Viola, D. Vigetti, S. Heinemann, S. Bierbaum, D. Scharnweber, H. Worch, Influence of collagen-fibril-based coatings containing decorin and biglycan on osteoblast behavior, J. Biomed. Mater. Res. A. 84 (2008) 805–816, https://doi.org/10.1002/jbm.a.31501.
[68] E. Hohenester, P. Maurer, R. Timpl, Crystal structure of a pair of follistatin-like and EF-hand calcium-binding domains in BM-40, EMBO J. 16 (1997) 3778–3786, https://doi.org/10.1093/emboj/16.13.3778.
[69] B.S. Harris, Y. Zhang, L. Card, L.B. Rivera, R.A. Brekken, A.D. Bradshaw, SPARC regulates collagen interaction with cardiac fibroblast cell surfaces, Am. J. Physiol. Heart Circ. Physiol. 301 (2011) H841–H847, https://doi.org/10.1152/ajpheart.01247.2010.
[70] K. Jacob, M. Webber, D. Benayahu, H.K. Kleinman, Osteonectin promotes prostate cancer cell migration and invasion: a possible mechanism for metastasis to bone, Cancer Res. 59 (1999) 4453–4457.
[71] A.M. Delany, I. Kalajzic, A.D. Bradshaw, E.H. Sage, E. Canalis, Osteonectin-null mutation compromises osteoblast formation, maturation, and survival, Endocrinology. 144 (2003) 2588–2596, https://doi.org/10.1210/en.2002-221044.
[72] L. Machado do Reis, C.B. Kessler, D.J. Adams, J. Lorenzo, V. Jorgetti, A.M. Delany, Accentuated osteoclastic response to parathyroid hormone undermines bone mass acquisition in osteonectin-null mice, Bone. 43 (2008) 264–273, https://doi.org/10.1016/j.bone.2008.03.024.
[73] A.M. Delany, D.J. McMahon, J.S. Powell, D.A. Greenberg, E.S. Kurland, Osteonectin/SPARC polymorphisms in Caucasian men with idiopathic osteoporosis, Osteoporos. Int. 19 (2008) 969–978, https://doi.org/10.1007/s00198-007-0523-9.
[74] M. Keykhosravani, A. Doherty-Kirby, C. Zhang, D. Brewer, H.A. Goldberg, G.K. Hunter, G. Lajoie, Comprehensive identification of post-translational modifications of rat bone osteopontin by mass spectrometry, Biochemistry. 44 (2005) 6990–7003, https://doi.org/10.1021/bi050109p.
[75] C. Dianzani, E. Bellavista, J. Liepe, C. Verderio, M. Martucci, A. Santoro, A. Chiocchetti, C. Luca Gigliotti, E. Boggio, B. Ferrara, L. Riganti, C. Keller, K. Janek, A. Niewienda, C. Fenoglio, M. Sorosina, R. Cantello, P.M. Kloetzel, M.P.H. Stumpf, F. Paul, K. Ruprecht, D. Galimberti, F. Martinelli Boneschi, C. Comi, U. Dianzani, M. Mishto, Extracellular proteasome-osteopontin circuit regulates cell migration with implications in multiple sclerosis, Sci. Rep. 7 (2017), https://doi.org/10.1038/srep43718.
[76] W. Huang, B. Carlsen, G. Rudkin, M. Berry, K. Ishida, D.T. Yamaguchi, T.A. Miller, Osteopontin is a negative regulator of proliferation and differentiation in MC3T3-E1 pre-osteoblastic cells, Bone. 34 (2004) 799–808, https://doi.org/10.1016/j.bone.2003.11.027.
[77] H. Kojima, T. Uede, T. Uemura, In vitro and in vivo effects of the overexpression of osteopontin on osteoblast differentiation using a recombinant adenoviral vector, J. Biochem. 136 (2004) 377–386, https://doi.org/10.1093/jb/mvh136.
[78] R. Agnihotri, H.C. Crawford, H. Haro, L.M. Matrisian, M.C. Havrda, L. Liaw, Osteopontin, a Novel Substrate for Matrix Metalloproteinase-3 (Stromelysin-1) and Matrix Metalloproteinase-7 (Matrilysin), J. Biol. Chem. 276 (2001) 28261–28267, https://doi.org/10.1074/jbc.M103608200.
[79] C. Tarquini, R. Mattera, F. Mastrangeli, S. Agostinelli, A. Ferlosio, R. Bei, A. Orlandi, U. Tarantino, Comparison of tissue transglutaminase 2 and bone biological markers osteocalcin, osteopontin and sclerostin expression in human osteoporosis and osteoarthritis, Amino Acids 49 (2017) 683–693, https://doi.org/10.1007/s00726-016-2290-4.
[80] E. Holm, J.S. Gleberzon, Y. Liao, E.S. Sørensen, F. Beier, G.K. Hunter, H.A. Goldberg, Osteopontin mediates mineralization and not osteogenic cell development in vitro, Biochem. J. 464 (2014) 355–364, https://doi.org/10.1042/BJ20140702.
[81] W.N. Addison, F. Azari, E.S. Sørensen, M.T. Kaartinen, M.D. McKee, Pyrophosphate inhibits mineralization of osteoblast cultures by binding to mineral, up-regulating osteopontin, and inhibiting alkaline phosphatase activity, J. Biol. Chem. 282 (2007) 15872–15883, https://doi.org/10.1074/jbc.M701116200.
[82] M.A. Horton, M.A. Nesbit, M.H. Helfrich, Interaction of osteopontin with osteoclast integrins, Ann. N. Y. Acad. Sci. 760 (1995) 190–200.
[83] M. Nagao, T.N. Feinstein, Y. Ezura, T. Hayata, T. Notomi, Y. Saita, R. Hanyu, H. Hemmi, Y. Izu, S. Takeda, K. Wang, S. Rittling, T. Nakamoto, K. Kaneko, H. Kurosawa, G. Karsenty, D.T. Denhardt, J.-P. Vilardaga, M. Noda, Sympathetic control of bone mass regulated by osteopontin, Proc Natl Acad Sci U S A. 108 (2011) 17767–17772, https://doi.org/10.1073/pnas.1109402108.
[84] B. Zhang, J. Dai, H. Wang, H. Wei, J. Zhao, Y. Guo, K. Fan, Anti-osteopontin monoclonal antibody prevents ovariectomy-induced osteoporosis in mice by promotion of osteoclast apoptosis, Biochem. Biophys. Res. Commun. 452 (2014) 795–800, https://doi.org/10.1016/j.bbrc.2014.08.149.
[85] Q. Wei, L. Huang, X. Tan, Z. Chen, S. Chen, W. Deng, Serum osteopontin levels in relation to bone mineral density and bone turnover markers in postmenopausal women, Scand. J. Clin. Lab. Invest. 76 (2016) 33–39, https://doi.org/10.3109/00365513.2015.1087045.
[86] K. Vincent, M.C. Durrant, A structural and functional model for human bone sialoprotein, J. Mol. Graph. Model. 39 (2013) 108–117, https://doi.org/10.1016/j.jmgm.2012.10.007.
[87] E. Holm, J.E. Aubin, G.K. Hunter, F. Beier, H.A. Goldberg, Loss of bone sialoprotein leads to impaired endochondral bone development and mineralization, Bone. 71 (2015) 145–154, https://doi.org/10.1016/j.bone.2014.10.007.
[88] J.A.R. Gordon, C.E. Tye, A.V. Sampaio, T.M. Underhill, G.K. Hunter, H.A. Goldberg, Bone sialoprotein expression enhances osteoblast differentiation and matrix mineralization in vitro, Bone. 41 (2007) 462–473, https://doi.org/10.1016/j.bone.2007.04.191.
[89] J. Chen, H.F. Thomas, J. Sodek, Regulation of bone sialoprotein and osteopontin mRNA expression by dexamethasone and 1,25-dihydroxyvitamin D3 in rat bone organ cultures, Connect. Tissue Res. 34 (1996) 41–51, https://doi.org/10.3109/

03008209609028892.
[90] S. Razzouk, J.C. Brunn, C. Qin, C.E. Tye, H.A. Goldberg, W.T. Butler, Osteopontin posttranslational modifications, possibly phosphorylation, are required for in vitro bone resorption but not osteoclast adhesion, Bone 30 (2002) 40–47.
[91] L. Malaval, N.M. Wade-Guéye, M. Boudiffa, J. Fei, R. Zirngibl, F. Chen, N. Laroche, J.-P. Roux, B. Burt-Pichat, F. Duboeuf, G. Boivin, P. Jurdic, M.-H. Lafage-Proust, J. Amédée, L. Vico, J. Rossant, J.E. Aubin, Bone sialoprotein plays a functional role in bone formation and osteoclastogenesis, J. Exp. Med. 205 (2008) 1145–1153, https://doi.org/10.1084/jem.20071294.
[92] M. Shaarawy, M. Hasan, Serum bone sialoprotein: a marker of bone resorption in postmenopausal osteoporosis, Scand. J. Clin. Lab. Invest. 61 (2001) 513–521.
[93] J.R. Cairns, P.A. Price, Direct demonstration that the vitamin K-dependent bone Gla protein is incompletely gamma-carboxylated in humans, J. Bone Miner. Res. 9 (1994) 1989–1997, https://doi.org/10.1002/jbmr.5650091220.
[94] Y.-T. Tsao, Y.-J. Huang, H.-H. Wu, Y.-A. Liu, Y.-S. Liu, O.K. Lee, Osteocalcin mediates biomineralization during osteogenic maturation in human mesenchymal stromal cells, Int. J. Mol. Sci. 18 (2017), https://doi.org/10.3390/ijms18010159.
[95] P. Ducy, C. Desbois, B. Boyce, G. Pinero, B. Story, C. Dunstan, E. Smith, J. Bonadio, S. Goldstein, C. Gundberg, A. Bradley, G. Karsenty, Increased bone formation in osteocalcin-deficient mice, Nature 382 (1996) 448–452, https://doi.org/10.1038/382448a0.
[96] J.D. Malone, S.L. Teitelbaum, G.L. Griffin, R.M. Senior, A.J. Kahn, Recruitment of osteoclast precursors by purified bone matrix constituents, J. Cell Biol. 92 (1982) 227–230.
[97] L. Carvallo, B. Henríquez, R. Paredes, J. Olate, S. Onate, A.J. van Wijnen, J.B. Lian, G.S. Stein, J.L. Stein, M. Montecino, 1alpha,25-dihydroxy vitamin D3-enhanced expression of the osteocalcin gene involves increased promoter occupancy of basal transcription regulators and gradual recruitment of the 1alpha,25-dihydroxy vitamin D3 receptor-SRC-1 coactivator complex, J. Cell. Physiol. 214 (2008) 740–749, https://doi.org/10.1002/jcp.21267.
[98] M. Ishida, S. Amano, Osteocalcin fragment in bone matrix enhances osteoclast maturation at a late stage of osteoclast differentiation, J. Bone Miner. Metab. 22 (2004) 415–429, https://doi.org/10.1007/s00774-004-0503-5.
[99] K.K. Ivaska, T.A. Hentunen, J. Vääräniemi, H. Ylipahkala, K. Pettersson, H.K. Väänänen, Release of intact and fragmented osteocalcin molecules from bone matrix during bone resorption in vitro, J. Biol. Chem. 279 (2004) 18361–18369, https://doi.org/10.1074/jbc.M314324200.
[100] S. Singh, D. Kumar, A.K. Lal, Serum osteocalcin as a diagnostic biomarker for primary osteoporosis in women, J. Clin. Diagn. Res. 9 (2015) RC04–RC07, https://doi.org/10.7860/JCDR/2015/14857.6318.

Dr. Caterina Licini is a Research Assistant at Politecnico di Torino, Department of Applied Science and Technologies (DISAT), working in collaboration with Department of Clinical and Molecular Sciences (DISCLIMO), Università Politecnica delle Marche, Ancona. She obtained her PhD in Human Health at Università Politecnica delle Marche, in 2018. The research group's current interest is focused on ERC BOOST project, funded by European Union's Horizon 2020. The aim of the project is to develop a 3D-printed scaffold targeted to osteoporosis, mimicking chemical, structural and topographical features of healthy bone. Co-author of 7 scientific publications in International Journals

Prof. Chiara Vitale-Brovarone is Full Professor in Materials Science and Technology at Politecnico di Torino where she leads the IRIS group (Improving Regeneration by Intelligent Scaffolds). Her research interests are mainly related to the development of innovative biomaterials ranging from the macro to the nanoscale (3D-scaffolds, functionalised micro and nanoparticles, resorbable fibers, injectable cements, resorbable polymeric microcapsules…) and to 3D scaffold biofabrication (3D printing and electrospinning) for bone, nerve and skin regeneration. Master Degree in Materials Engineering in 1997, Ph.D in Materials Engineering at Politecnico di Torino in 2001, she has won fellowships for research stays at the Ecole de Chimie de Montpellier in France and at the Lawrence Berkeley National Laboratory, California, USA. She has authored 155 papers on international journals, h-index 35 receiving about 3500 citations. She has coordinated the EU-funded projects (MATCH, BIORESS) and she has been Team leader for the EU project RESTORATION. At present, she is coordinating the H2020 projects MOZART, funded in the NMP-6-2015 call and GIOTTO funded in the call NMBP-22-2018. She is the Principal Investigator of the ERC-CoG-2015 grant BOOST and of the GRACE project funded by the Italian Minister for Research (FARE Ricerca in Italia).

Prof. Monica Mattioli-Belmonte holds a PhD degree in Cytomorphology (1995). From 1996–1998, she worked, as a post-doc at the Institute of Normal Human Morphology, Ancona University, Italy. From 2000–2004, research fellow at the same Institute. From 2005–2016, Assistant Professor of Histology (05/H2) at the Department of Clinical and Molecular Sciences (DISCLIMO), Università Politecnica delle Marche, Italy. Qualification for the roles of Associate Professor and Full Professor of Anatomy (05/H1) within the area of Anatomy and Histology (05/H MIUR Legislative Decree 855 - 30/10/15) in 2015. From 2016, Associate Professor of Histology (05/H2) at DISCLIMO, Università Politecnica delle Marche. In 2017, qualification for the roles of Full Professor of Histology (05/H2). From 2018, Vice-Director of DISCLIMO. Research work focus on morpho-functional evaluation of toxicity and/or biocompatibility of materials and 3D structures mainly in orthopedics and dentistry. Member of the Editorial Board of JBCP, IJTE and WJSC. Member of the Italian Society of Anatomy and Histology, Italian Society of Histochemistry, Italian Society of Biomaterials (Vice-president) and Italian Orthopedic Research Society. Co-author of more than 150 scientific publications (orcid.org/0000-0002-2087-2776; Scopus h-index 32; h-index 10 75; 3498 citations)