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Survey

Tuning cellular responses to BMP-2 with material surfaces

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ABSTRACT

Bone morphogenetic protein 2 (BMP-2) has been known for decades as a strong osteoinductive factor and for clinical applications is combined solely with collagen as carrier material. The growing concerns regarding side effects and the importance of BMP-2 in several developmental and physiological processes have raised the need to improve the design of materials by controlling BMP-2 presentation. Inspired by the natural cell environment, new material surfaces have been engineered and tailored to provide both physical and chemical cues that regulate BMP-2 activity. Here we describe surfaces designed to present BMP-2 to cells in a spatially and temporally controlled manner. This is achieved by trapping BMP-2 using physicochemical interactions, either covalently grafted or combined with other extracellular matrix components. In the near future, we anticipate that material science and biology will integrate and further develop tools for *in vitro* studies and potentially bring some of them toward *in vivo* applications.

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Abbreviations: 2(3)D, two(three) dimensional; ALP, alkaline phosphatase; BMP-2, bone morphogenetic protein; b-BMP2, biotinylated BMP-2; rhBMP-2, recombinant human BMP-2; BMPRI(II), BMP type I (II) receptors; Cdc42, cell division control protein 42 homolog; CS, chondroitin sulfate; C2C12, mouse myoblast cell line; ECM, extracellular matrix; ELISA, enzyme-linked immunosorbent assay; ERK, extracellular signal-regulated kinases; FAK, focal adhesion kinase; FN, fibronectin; GAGs, glycosaminoglycans; GTPase, guanosine triphosphate hydrolase; HA, hyaluronic acid; Hp, heparin; HS, heparan sulfate; Id-1, inhibitor of DNA binding 1; ILK, integrin-linked kinase; LbL, layer-by-layer; LIMK1, LIM domain kinase 1; MAPK, mitogen-activated protein kinase; PI3K, phosphoinositide 3-kinase; PLL, poly-L-lysine; RGD, arginine, glycine, aspartic acid; Rho, ras homolog gene family; ROCK, rho-associated protein kinase; SAM, self assembly monolayer; SAv, streptavidin; SMAD, small mothers against decapentaplegic; TGF-β, transforming growth factor beta; VEGFR, vascular epidermal growth factor.

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1. Introduction

Bone morphogenetic protein 2 (BMP-2) is a multifunctional growth factor belonging to the transforming growth factor β (TGF-β) superfamily. It was identified in the 1970s as an essential molecule for *de novo* bone formation in adult animals [1,2]. Indeed BMP-2 is one of the strongest osteoinductive factors known so far: it initiates the differentiation of mesenchymal stem cells (MSCs) into osteoblasts and chondrocytes *in vivo* and *in vitro* [3], as well as the transdifferentiation of muscle cells into bone cells [4,5].

In view of its osteogenic potential, the clinical use of recombinant human BMP-2 (rhBMP-2), first purified in 1988 by Wang et al. [6], was approved in 2002 by the Food and Drug Administration (FDA) and validated by the European Medicines Agencies. To date, the only FDA-approved material carrier is an absorbable collagen sponge to which a high amount of rhBMP-2 is applied (up to 2.1 mg/level) [7], due to its poor affinity for collagen [8]. In clinical trials, it has been reported that up to 23% of patients suffered complications, such as hematomas and swelling in the neck and throat regions [9], dysphagia and a heightened risk of cancer [10]. In Europe, while the clinical use of rhBMP-2 as an adjunct to standard care has been approved, the increasing number adverse event reports and the growing socio-economic need for bone repair therapies raise the important question of how to develop effective materials which allow the control of the biological responses to BMP-2.

In the last decade, several studies have shown the possibility to deliver BMP-2 from various carrier materials [11–13], especially polymeric materials and ceramics. Since *in vitro* tests were promising and pre-clinical studies are currently being performed, it is likely that future medical devices containing new formulations of BMP-2 will be approved. However, it is still challenging to achieve a controlled presentation of BMP-2, while retaining its activity and minimizing the amount of protein applied locally. Standard biological studies stimulate cells with BMP-2 added to the culture media. In these cases high amounts of the growth factor are needed because of the limited lifetime of BMP-2 in solution. Additionally, this condition does not represent the natural cellular environment, since BMP-2, like other growth factors, is sequestered in the extracellular matrix (ECM) and released upon matrix degradation [14,15]. Thus advanced biomaterials which take into consideration the physical and chemical complexity of the extracellular environmental are being developed. These materials could serve as a tool for biologists to unravel novel biological properties of BMP-2 which could not be explored so far with standard culture methods [4].

A timeline showing a few of the most important findings on BMP-2 in biological and material sciences is shown in Fig. 1. It is noteworthy that approval for the *in vivo* use of BMP-2 (2003) took place before the development of advanced materials able to control and reduce BMP-2 release and before the discovery of new biological functions of BMP-2 such as its influence on the whole human body. Hence, there is now a great need to build an

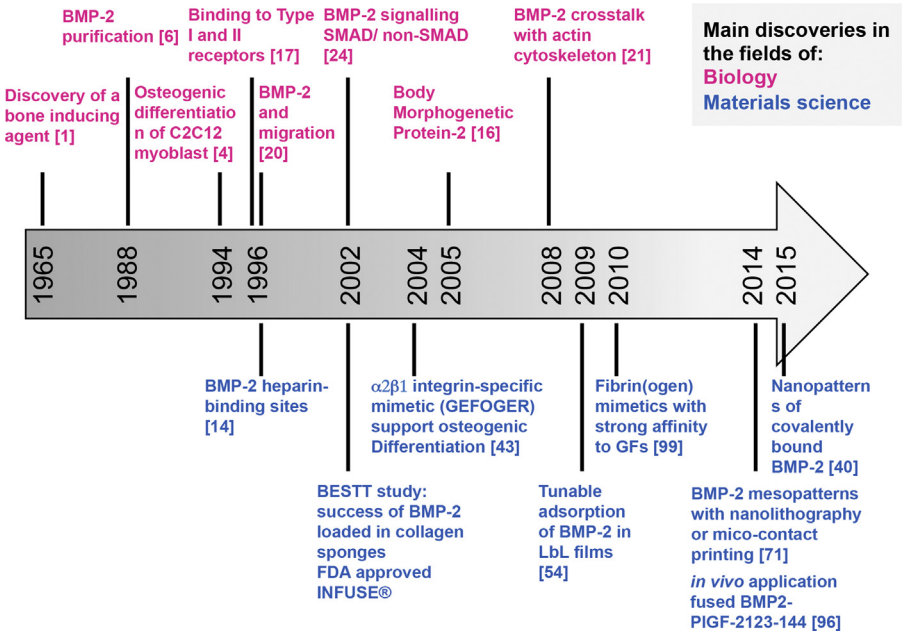

Fig. 1. Time-line showing few of the most important findings on BMP-2 in biology (in red) and in material sciences (in blue). Fundamental biological discoveries such as the influence of BMP-2 in the whole human body and the development of advanced materials able to modulate the physicochemical presentation of BMP-2 followed the approval for the *in vivo* use of BMP-2 in 2002. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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integrative approach including material science, chemistry, engineering, biochemistry and cell biology, and bridge the gap between these different disciplines. From the materials side, researchers could bring innovations in the design of materials for BMP-2 presentation by providing functionalization strategies and characterization methods as well as by developing new tools for the spatial control of BMP-2 delivery using micro- and nanotechnology approaches. From the biochemical and biological standpoint, researcher could provide new tools to produce BMP proteins, engineered mutants or tagged molecules.

In this review, we first summarize the emerging functions of BMP-2 in cell biology and the resulting signaling responses at the interface between cells and their environment. We then present recent developments on engineered surfaces that aim at mimicking the presentation of BMP-2 in its natural environment. Finally, we discuss how specific properties of materials may help in optimizing existing systems or may bring new ideas for the design of innovative delivery systems.

2. Cell responses to soluble BMP-2

Although BMP-2 signaling has historically been linked to bone, the growing number of known BMPs functions in different tissues brought the biology community to coin a new term for all bone morphogenetic proteins: "body morphogenetic proteins" [16]. Fig. 2 schematically illustrates the major steps for the BMP-mediated induction of osteogenic differentiation in bone progenitor cells and myoblasts, which transdifferentiate into osteoblasts upon BMP-2 stimulation [4]. BMP-2, like other members of the TGF-β superfamily, signals upon binding to two types of cell transmembrane serine/threonine kinase receptors, the BMP type I (BMPRI) and type II (BMPRII) receptors. The binding of BMP-2 to BMPRI results in the phosphorylation of SMAD1/5/8, which forms a complex with co-SMAD (SMAD4) and translocates to the nucleus [17]. For transcriptional signaling, this shuttling leads to a subsequent expression of transcription factors such as Id-1 and BMP-2 responsive element, typical markers of osteogenic differentiation [18]. At later time points, alkaline phosphatase (ALP) is expressed after several days, and mineralized matrix deposition is detected after several weeks of culture [3]. Besides the SMAD pathway, gene transcription is induced by BMP-2 *via* non-SMAD signaling as BMP induces the MAPK pathway, which leads to the expression of ALP, osteopontin and collagen I (for details about signaling, see review [19]). Regarding the non-transcriptional signaling mediated by BMP-2, recent studies have shown that BMPRs might control cytoskeletal rearrangements involved in cell migration [20–23]. The regulation of BMP signaling takes place at several levels, from receptor complex formation to crosstalk with other pathways, as will be discussed in the next paragraphs.

2.1. Modulation of BMP-2 signaling at the cell surface

2.1.1. BMP receptor complex formation

Studies from P. Knaus group have indicated that BMP receptors present a distinct mode of oligomerization and activation [24]. For the formation of a functional signaling receptor complex, BMP-2 binds to BMPRI, which is either already organized in a receptor complex with BMPRII, or recruits BMPRII. These modes of oligomerization result in the activation of different signaling pathways: binding of BMP-2 to a pre-formed complex induces the classical SMAD signaling pathway, while ligand-induced oligomerization induces the non-SMAD pathway. So far, these events have been analyzed by applying biochemical separation of detergent-resistant membranes and co-immunoprecipitation methods [25]. There is still little information regarding the spatial arrangement of BMPRs at the nanoscale and the localization of the different complexes in distinct cellular compartments. Only recently was the spatial distribution of BMPRIb and BMPRII visualized using high-resolution imaging techniques. Using two-color Stimulated Depleted Emission (STED) microscopy (Fig. 3A), single BMPRII appear to arrange sparsely, whereas BMPRI assemble in larger clusters comprised of multiple receptors [26]. When BMP-2 was added to the cell culture media, the BMPRII associated with the larger BMPRI assemblies at the cell periphery.

The lateral mobility of BMPRI and BMPRII is also very distinct, as shown by single particle tracking experiments: in fact BMPRI is very confined, both in presence or absence of the ligand, whereas the mobility of BMPRII can be either confined or free diffusing [27]. The preformed complex, which triggers the SMAD-dependent pathway, does not require the confined movement of BMPRI, while the non-SMAD seems to be highly dependent on the localization of BMPRI in membrane microdomains. Thus, non-SMAD signaling might require more stable complexes, possibly to allow interaction with other protein complexes, *e.g.* those involved in signaling to the cytoskeleton. To determine BMPR localization, the successful expression of tagged receptors has been possible for over-expression of human influenza hemagglutinin (HA)-tagged BMPRII [28] and it remains very challenging for BMPRI because of its low expression level. Tools are currently lacking in order to combine high-resolution approaches with studies on the dynamics of receptor complex formation and to identify the physical determinants of receptor mobility.

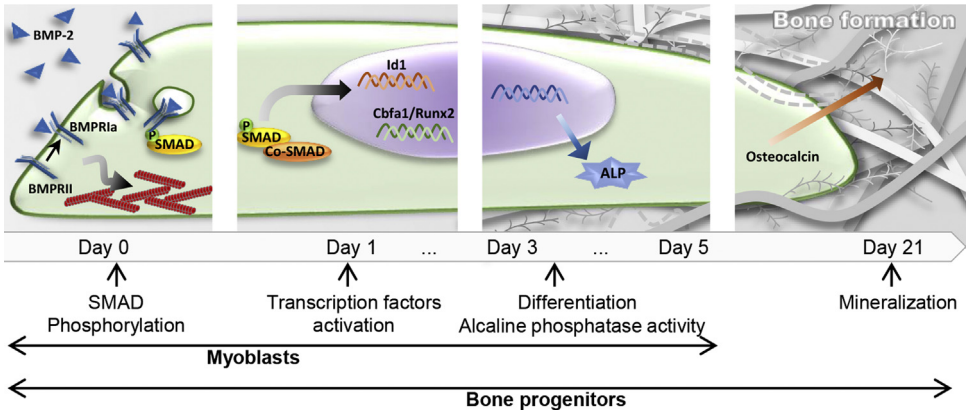

Fig. 2. Schematic representation of the major steps in the differentiation of bone progenitor cells and myoblasts over time. Note that the crosstalk with other signaling pathways and other relevant markers are omitted for simplicity. The relative size of all molecules is not drawn to scale.

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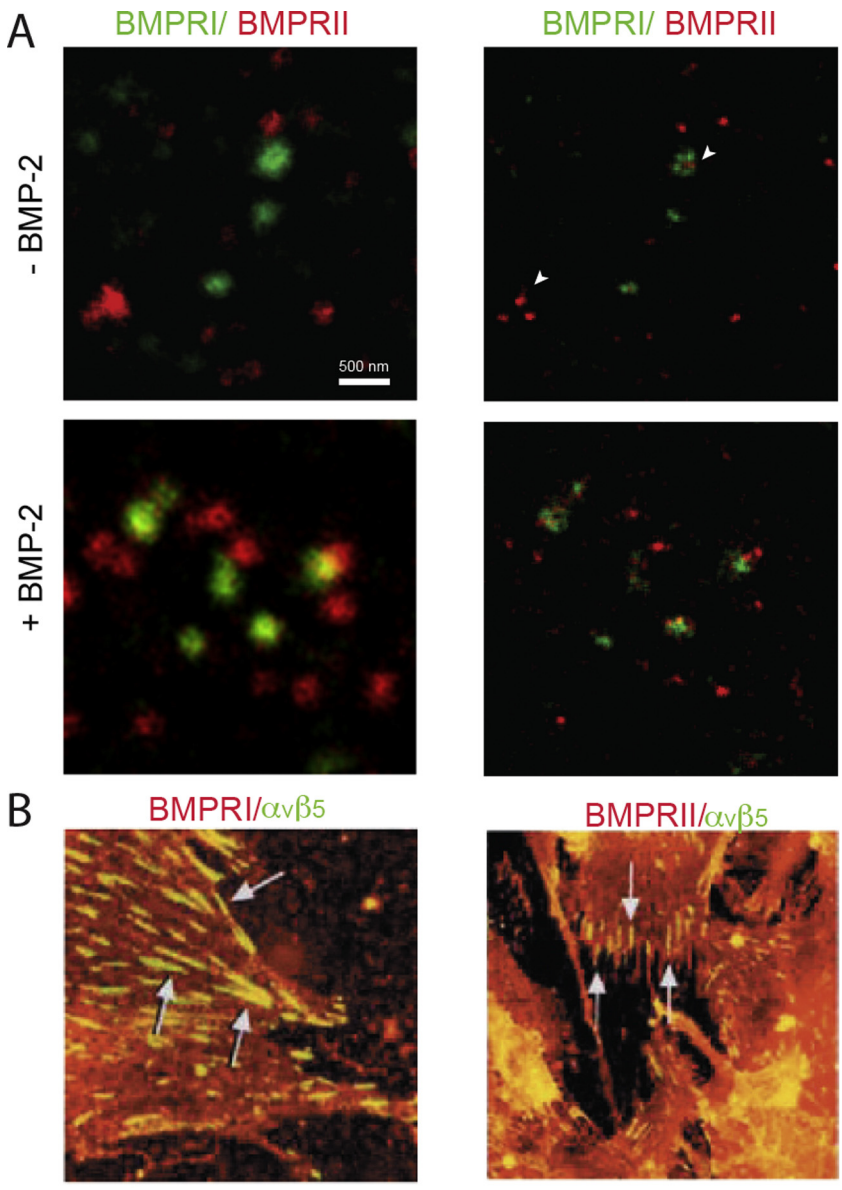


Fig. 3. (A) Confocal microscopy (right) and STED microscopy (left) images of BMPRIb (in green) and BMPRII (in red). In the absence of BMP-2, the two different receptors rarely co-localized (upper white arrowhead) and BMPRII did not cluster (lower arrowhead). When cells were exposed to BMP-2, BMPRII associated with the larger BMPRIb assemblies. This different behavior could not be appreciated with confocal microscopy. Image adapted from [26]. (B) Example of colocalization (indicated by arrows) of BMPRI and BMPRII (red) with $\alpha_v\beta_5$ integrins (green) detected by confocal microscopy. Images adapted from [45]. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2.1.2. Receptor-ligand internalization

For BMP-mediated signaling the receptor complexes are internalized in two possible ways: (i) caveolae pits are formed for BMPRI and recruited BMPRII complex and activate non-SMAD pathways; (ii) clathrin-dependent internalization is required for the preformed receptor complex resulting in the activation of the SMAD pathway [29]. Different points of discussion have been raised regarding clathrin-dependent internalization of the ligand-receptor complex in growth factor signaling. The first point is whether receptor internalization is required for signaling. For tyrosine kinase receptors, such as vascular epidermal growth factor receptor 2 (VEGFR2) and epidermal growth factor receptor, clathrin-mediated endocytosis is important in regulating receptor recycling to modulate the amplitude of biological response [30–32]. VEGFR2 internalization is required for the activation of ERK1/2 signaling but dispensable for other signaling pathways [33]. For serine/threonine kinase receptors, such as BMPRs, recent studies combining confocal and atomic force microscopy (AFM) have indicated that BMP-2 signaling might already start in domains of the plasma membrane outside of clathrin-coated pits, where BMP-2 molecules bind to BMPRIa, which then phosphorylates and triggers SMAD signaling [34]. Regarding downstream signaling events, the treatment of cells with endocytosis inhibitors does not affect SMAD phosphorylation, while the downstream signal propagation is hindered [29,35,36]. Conversely, inhibition of BMP-2 endocytosis by an epigenetic approach actually elevates transcriptional responses [37]. Additionally, dynamin inhibition impairs osteogenic differentiation but does not block completely the transcriptional activation of several other genes, suggesting the presence of alternative SMAD-dependent signaling cascades which are independent of endocytosis [38].

These biochemical approaches to inhibit endocytosis lead to another point of discussion related to growth factor internalization. As of today, it remains elusive whether the ligand has to

remain bound to the receptors and become internalized *via* the clathrin-mediated pathway, or if it would be sufficient to have trafficking of the activated receptors, regardless of ligand internalization. In 1997 Jortikka et al. [36] reported that bonds with carrier materials should not be tight nor in covalent form to allow endocytosis of BMP-2. However, recent studies demonstrated that anchorage of the growth factor to the ECM or to a surface still conveys signaling by prolonged activation of receptors and differential phosphorylation [39,40]. Thus, ligand-receptor interaction at the cell membrane might be sufficient to obtain a sustained signaling response. It remains to be elucidated if a mechanical component causes deformation of the membrane and affects internalization signaling or if co-recruitment of other adhesion receptors such as integrins might occur in these cases where the BMP-2 molecules cannot be internalized.

2.2. BMP-2 signaling in a cell adhesion context

2.2.1. Osteogenic and adhesion signaling crosstalk

Extracellular factors orchestrate the commitment and differentiation of many cell types; in turn, a concerted action of adhesive and growth factor signals regulates adhesion and motility, which are mediated by interactions with the physical and biochemical cues from the environment. The signaling crosstalk between BMP-dependent and integrin-mediated pathways has been explored toward the modulation of both osteogenic differentiation and adhesion to the ECM [41]. Regarding the participation of integrin signaling in the transcription of genes for osteogenic differentiation, the collagen-binding integrins $\alpha_1\beta_1$ and $\alpha_2\beta_1$ regulate BMP-induced differentiation by acting downstream of BMPRI [42,43]. Moreover, following binding to collagen, FAK phosphorylation is necessary for the transcriptional activity of SMAD6 but not for the translocation of SMAD1 [44]. α_v integrin also regulates BMP-dependent osteogenic differentiation [45], and in particular osteoblastic response to CYR61, a bone activator that increases the level of BMP-2 and activities the $\alpha_v\beta_3$ integrin/ILK/ERK signaling pathway [46].

For the regulation of adhesion, as of today only few studies have shown the impact of BMP signaling on integrins and integrin-mediated structures. Lai et al. [45] reported that during 4 days stimulation of osteoblasts with BMP-2 in the media, the expression of α_v integrins is increased, BMPRs colocalize with α_v and β_1 integrins in focal adhesions (Fig. 3B) and coprecipitate with these receptors. However, the colocalization pattern with vinculin, a structural protein present in focal adhesions, could not be confirmed by recent studies using high-resolution microscopy [26]. In osteoblasts, BMP-2 enhances the formation of focal adhesions and stress fibers by increasing α_5 and β_1 integrin expression, and triggers migration events by enhancing the incorporation of β_1 integrin into lipid rafts [47,48].

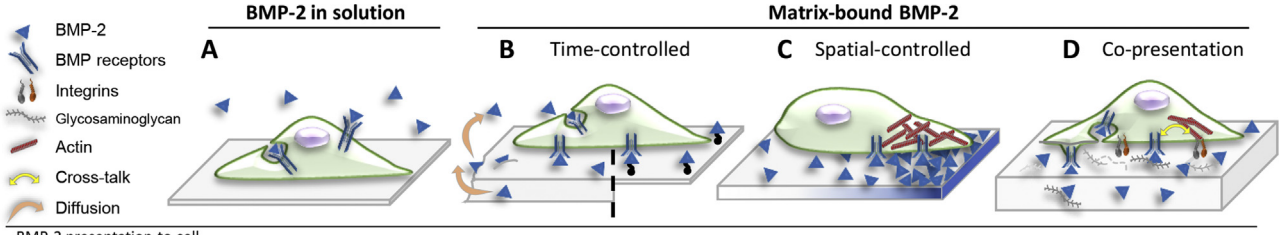
In this context, there are still several key questions that remain unanswered and might add further complexity to the entire picture encompassing BMP and adhesion signaling. First, it should be elucidated where binding sites for integrins and BMPR are located relative to each other within the extracellular matrix. As a consequence, there is the need for a deep understanding of how BMPRs and integrins are spatially organized at the plasma membrane to allow both physical interactions and signaling crosstalk. Finally, it should be determined how multiple pathways modulating adhesion dynamics are regulated spatio-temporally.

2.2.2. Effects of BMP-2 on cytoskeleton assembly and cell migration

The evidence that BMP signaling is involved in the crosstalk with other pathways has brought to attention new functions of BMP-2, which are not necessarily related to its transcriptional signaling pathways. For example, BMP-2 signaling is involved in wound healing and cancer invasiveness by acting on actin cytoskeleton dynamics [49–51]. Upon BMP-binding to the BMPR complex, LIMK1 dissociates from BMPRII and phosphorylates cofilin [52]. The activation of LIMK1 by BMP-2 initiates the signaling to the cytoskeleton in a PI3K-dependent manner; a concomitant activity of Cdc42 is however required [21].

Hiepen et al. [22] have recently shown that a regulatory subunit of PI3K is essential in directed cell migration mediated by BMP-2 at the leading edge of migrating cells. BMP-2 also induces the activation of the p38/MK2/Hsp25 pathway at cortical actin protrusions in migrating cells [23]. To further add complexity, other signaling pathways independent from LIMK1 activation have been identified, where actomyosin assembly is mediated by ROCK1 kinase downstream of Rho GTPases and myosin light chain kinase [53]. Taken together, these studies clearly indicate that BMP-2 participates in the regulation of cell protrusion formation and migration, acting on multiple parallel pathways involved in actin reorganization. However, as for the interaction of the receptors at the plasma membrane, the spatio-temporal aspects of such regulation of signaling to the cytoskeleton still remain unclear.

These new and intriguing functions of BMP-2 are also relevant for the design of biomaterials/implants for the delivery of BMP-2, adhesion being the first step at the interface between cells and artificial materials. In turn, many answers to these open questions might come in the near future with the aid of material science approaches which allow control over the presentation of BMP-2 to cells.



BMP-2 presentation to cell apical (a) - basal (b)	a	a and b	b	b	b
Sustain delivery	-	+	+++	.	++
Internalization of BMP-2	+++	+	-	.	+
Cross-talk possibility	-	+	+	+	+++

Fig. 4. Schematic representation of different material surfaces approaches to control the presentation of BMP-2 at the interface with cells. (A) BMP-2 is added to the cell culture media, which represents the standard stimulation way. (B) BMP-2 either entrapped by electrostatic interactions (left) or chemically bound to the material surface (right). (C) Surface patterning of BMP-2 for the spatial control of BMP-2 presentation. As an example, gradients of matrix-bound BMP-2 are schematically shown. (D) Copresentation of BMP-2 and ECM components.

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3. Mimicking the BMP-2 microenvironment with material surfaces

Several growth factors are present in tissues in a matrix-bound form and released upon matrix degradation [14,15]. The mode of presentation of BMP-2 at the interface with cells might be crucial in modulating its biological activity. For this reason, material surfaces applied to biological studies should mimic the physico-chemical properties of the native ECM, to facilitate and allow predictions of cellular responses. In particular, using materials that enable the control of the amount of BMP-2 on their surface and its local distribution might help in determining the spatio-temporal regulation of BMP-2 signaling pathways.

In comparison with soluble BMP-2 (Fig. 4A), the presentation of the growth factor on material surfaces could be tailored to achieve controlled immobilization and/or release of the protein from the surface (Fig. 4B). This might lead to different signaling kinetics as well as the activation of alternative signaling pathways. Additionally, modifications in surface chemistry which allow the spatial control of BMP-2 (Fig. 4C) could support the quantitative analysis of signaling events. Finally, surfaces where BMP-2 is presented together with ECM components (Fig. 4D) could maintain or even enhance the biological activity of BMP-2 while possessing adhesive properties to allow the growth and colonization of cells.

3.1. Temporal control of BMP-2 activity with material surfaces

In the design of materials aiming at achieving a time-controlled presentation of BMP-2, the growth factor can be immobilized on surfaces either by physical entrapment (*i.e.* electrostatic interaction, hydrophobic effect, hydrogen-bonds) which allows a slow release and internalization of the molecule, or by immobilization through a chemical linker or through biotin-Streptavidin (SAv) binding, which leads to a sustained presentation of BMP-2 (Fig. 4B).

3.1.1. Physical entrapment of BMP-2

The formation of layer-by-layer (LbL) polyelectrolyte multilayer films is a method that allows the entrapment of BMP-2 over a long period of time (Fig. 5A). LbL films are made of poly(L-lysine) (PLL) and hyaluronic acid (HA), which can be stabilized by covalent crosslinking with 1-ethyl-3-(-dimethylaminopropyl)carbodiimide (EDC). The films are post-loaded with BMP-2 by simple diffusion and retain the growth factor for at least 9 days [54]. The amount of retained BMP-2 can be tuned by varying film thickness and the initial concentration of BMP-2 in solution. For instance, a maximal value of $1.42 \pm 0.26\ \mu g/cm^2$ can be trapped in 1.4 μm thick (PLL/HA) films when the initial concentration of BMP-2 in solution is 20 μg/mL. More recently, it was shown that the crosslinking extent of the film allows the control of the amount of BMP-2 remaining in the film after a burst release [55]. This burst release depends on the crosslinking extent (7–11% for the highly cross-linked film in comparison to 62-77% for the low crosslinked films). The final amount of BMP-2 retained in the film varied (between 4 and 14 $\mu g/cm^2$) when the initial concentration was 100 μg/mL. A BMP-2 adsorbed amount of 800 ng/cm^2 was sufficient to trigger SMAD phosphorylation after 4 h and ALP activity at 5 days in C2C12 cells [56]. In addition, BMP-2 loaded on soft films induced adhesion and spreading, in contrast to BMP-2 added in solution. Cells also formed focal adhesions in response to matrix-bound BMP-2, suggesting a possible crosstalk between BMP receptors and adhesion receptors (*e.g.* integrins) [56]. It should be noted that for this type of films a direct comparison of the surface concentration of BMP-2 and soluble concentrations is difficult due to the difference in dimensionality (matrix-bound versus soluble) and molecular diffusion.

The use of temperature-sensitive polymers is another manner to electrostatically entrap BMP-2 which is already applied *in vivo* [57]. The polymers can be formulated in aqueous buffers at a low temperature but become insoluble when delivered to the physiological milieu. A library of temperature-sensitive polymers has been created [58], however only a few of them were able to retain BMP-2 for more than 5 days after the *in vivo* injection.

Entrapment by LbL techniques may be easily adapted for *in vivo* applications and some promising results have already been obtained. Indeed, hydrolytically degradable LbL coating of implants [59] was used to entrap both BMP-2 and VEGF and induced *de novo* bone formation in 4–9 weeks. Interestingly, such surface coatings can be dried and sterilized, all the while preserving BMP-2 bioactivity [55]. Clinical applications of physical entrapment-based materials can be expected in the near future.

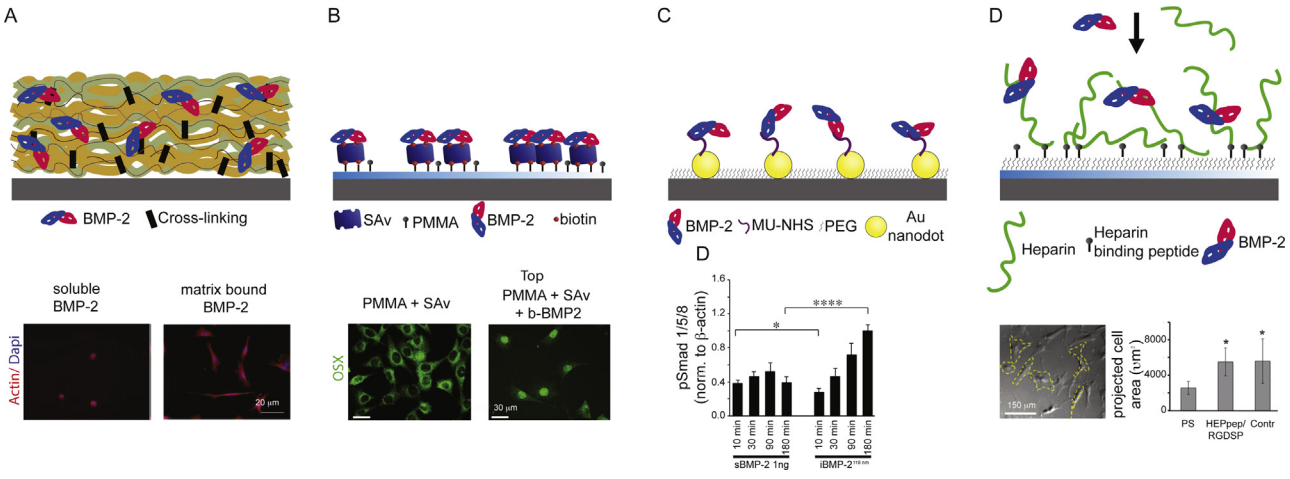

Fig. 5. Examples of material surfaces applied for the control BMP-2 effect on cells. Top: schematic representation of the material design. Down: cellular response to the substrates (A) Electrostatic entrapment on BMP-2 on polyelectrolyte multilayer films. C2C12 cells were plated on LbL soft films containing BMP-2 and stained for actin (red) and nucleus (DAPI, in blue). Fig. adapted from [56]. (B) Immobilization of b-BMP2 on Streptavidin gradient. Immunofluorescence images showing the nuclear translocation of the osteogenic marker Osterix, in cells grown on the BMP-2 modified surfaces. Image adapted from [62]. (C) BMP-2 immobilized to gold nanoparticle arrays produced by block copolymer micellar nanolithography. The histogram shows a comparison of SMAD1/5/8 phosphorylation levels and kinetics in cells stimulated with 1 ng of BMP-2 either added to the culture media or bound to the nanoparticles. Image adapted from [40]. (D) Heparin binding peptides immobilized on a SAM captures endogenous heparin and BMP-2 from serum. The histogram shows that hMSCs area significantly increases in cells adhering to the functionalized surfaces. Image adapted from [85]. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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Since the physical entrapment-based techniques are quite versatile and do not require expensive equipment, they could represent an alternative surface material to study the temporal dependence of BMP-mediated signaling. In addition, the parameters of the microenvironment, such as stiffness or growth factors presentation, can be tuned in order to analyze their effects on the BMP-2 pathway. However the nature of adhesive interactions between cells and LbL films should be clarified in order to be able to distinguish between the mere contribution of BMP-2 to signaling from the possible contribution of adhesive receptors (*e.g.* integrins, HA receptors), which may induce secondary signaling pathways.

3.1.2. Chemical binding of BMP-2

Biotin-Streptavidin (SAv) is the strongest non-covalent bond, which can be used to immobilize a protein on a surface following its biotinylation. This method provides not only a stable binding but also a versatile platform on which it is possible to immobilize different biotinylated compounds [60]. The drawback consists in the need of two grafting steps, *i.e.* fist biotin moieties on the surface and then SAv, before growth factor immobilization. Amino-biotinylated BMP-2 added to culture media exhibits an increase in bioactivity, in contrast to carboxyl-biotinylated BMP-2 [61]. BMP-2 was amino-biotinylated and grafted on a poly(methyl methacrylate) (PMMA) thin film presenting a gradient of SAv in the range of 1.4–2.3 pmol/cm^2 [62] (Fig. 5B). While the SAv concentration was measured by surface plasmon resonance, the binding of a BMP-2 dimer to a single SAv could be only estimated, based on the comparable size of the two proteins. However, this assumption does not consider variations in protein solubility due to aggregate formation, and the presence of non-bound biotin molecules which could change the 1:1 ratio between BMP-2 and SAv. With such an approach, a dose-dependent osteogenic response was measured on the same substrate over a period of 6 days. Neutravidin was used to immobilize biotinylated BMP-2 (b-BMP2) on biotinylated fibronectin (b-FN) [63] for studies on SMAD-dependent signaling and cell migration. By means of Quartz Crystal Microbalance with dissipation monitoring (QCM-D) and ELISA assays, and the amount of immobilized b-BMP-2 was detected on the surface for at least 6 days. While biotinylation is a relatively straightforward method to link proteins, it is however not site-specific and might negatively affect the biological activity of b-BMP2, when biotins are close to the BMPR binding sites. Moreover the immobilization through the small biotin moiety (~1 nm) might constrain BMP-2 spatial conformation, further inhibiting its recognition by cellular receptors.

For decades the use of covalently immobilized growth factors has been a matter of debate because of its negative impact on receptor binding and complex formation, as well as on the internalization of the protein, as discussed in Section 2.1.2. To achieve covalent binding of growth factors to supporting materials, several approaches have been developed and the use of bifunctional linkers, which target either the amino- or the carboxy-groups of the protein, is the most commonly used. Such linkers are either pre-coupled to the growth factor and then immobilized on the surface, or are at first immobilized onto the surface and then the growth factor is immobilized in a second step [64,65]. While the former has the advantage of involving fewer preparation steps, the latter appears to be advantageous to avoid protein denaturation due to unspecific interactions with the material surface [66]. Additionally, the use of molecular linkers, which confer a certain degree of flexibility to the tethered growth factor, may have an impact on the mobility and accessibility of the protein for receptor binding, without loss due to diffusion. BMP-2 has been immobilized covalently to gold surfaces *via* a self-assembled monolayer (SAM) consisting of 11-mercaptoundecanoyl *N*-hydroxysuccinimide ester which binds to the free amine residues of the protein and remains bioactive for a period of 6 days without being internalized [39].

Besides the difficulties in performing and controlling the different steps for the covalent immobilization, as well as in tailoring the immobilization strategies to the specific growth factor, a remaining challenge is to control the exact number of immobilized molecules. Thus, alternative approaches such as protein modification by expression of artificial domains or peptide tags, *e.g.* his-tags, have been also developed [67]. So far, the biological activity of BMP-2 is often affected by such a modification in comparison with the native protein.

3.2. Surface patterning for the spatial control of BMP-2 presentation

To achieve control over the distribution and amount of proteins presented on materials, various strategies for surface patterning at different length scales have been developed over the last two decades. A few examples showing the patterning of BMP-2 from sub-millimeter down to nanoscale are described in the following paragraphs. These approaches may help in the future in improving the design of biomaterials as well as in deciphering BMP-2 signaling pathways (Fig. 4C).

3.2.1. Sub-millimeter patterning of BMP-2

During morphogenesis, an essential long range BMP-2 gradient is formed along the ventral to dorsal axis [68]. *In vitro* mimicry of long-range gradients or spatially organized tissues may help deciphering the pathways of BMP-2 signaling underlying tissue formation and spatial organization. By taking advantage of the natural affinity of FN for BMP-2 (see Section 3.3), Miller et al. created millimeter-sized BMP-2 patterns by printing the growth factor as a "bioink" on fibrin [69]. This technique is versatile as it is possible to form patterns of various sizes and shapes, as well as gradients 1.5 mm long with different amounts of BMP-2 (from ~0.02 to ~2.245 μg/cm^2) that are deposited by overprinting BMP-2 at the same location. These BMP-2 patterns were shown to be bioactive, as assessed by ALP expression in two different cell types, namely C2C12 myoblasts and mesenchymal fibroblasts.

Another strategy consists in using microfluidics in combination with LbL technology to create millimeter-sized gradients of matrix-bound BMP-2 [70]. To this end, a microfluidic chamber was set in contact with a PLL/HA film and a BMP-2 gradient in solution was generated *via* passive flow pumping. As the amount of BMP-2 adsorbed onto the film directly depends on the BMP-2 concentration in solution in the channel [54], a 40 mm-long gradient of matrix-bound BMP-2, ranging from 0.04 μg/cm^2 to 2 μg/cm^2, was thus generated. BMP-2 remained bioactive after 3 days as assessed by ALP activity in C2C12 myoblasts. This matrix-bound BMP-2 enabled the generation of a spatially controlled osteogenic differentiation, confined to the patterned area and dependent on the amount of BMP-2. Such patterns may be further used to create microtissues for studies on the effects of specific gene mutations or drugs on the formation and maintenance of bone tissues.

3.2.2. Micrometer-sized patterns of BMP-2 on surfaces

BMP-2 patterned at the micron scale allows studies on single-cell responses. To this end, by using microcontact printing with a poly(dimethylsiloxane) stamp, Hauff et al. created 25 μm-wide patterned stripes of FN onto which BMP-2 was then immobilized by biotin-neutravidin binding [63]. These patterns are stable for at least one day and b-BMP2 is not released from the stripes. Because of the discrete localization of BMP-2 molecules on the stripes, the amount of the immobilized protein on the surface is relatively high (0.52 μg/cm^2). The grafted BMP-2 triggers SMAD1/5/8 phosphorylation and inhibits myotube formation in C2C12 cells. Interestingly,

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in comparison with samples where BMP-2 was added to the culture medium, SMAD phosphorylation is prolonged over a period of 90 min, leading to a sustained localization of the SMAD complex in the nucleus. In this regard, it remains to be elucidated whether the prolonged SMAD-signaling might impact other BMP-mediated pathways. These patterned stripes served also as platform to study directed cell migration: while the migration velocity seems independent of the immobilization of BMP-2 on the patterned stripes, cells do not show any preference for a direction on the immobilized BMP-2. These results suggest it is not the binding of BMP-2 to the extracellular matrix, but rather the presentation of the proteins in gradients that might be therefore necessary to guide migration. So far, continuous surface chemical gradients of BMP-2 have been applied to study the effects of different amounts of surface-immobilized BMP-2 on cell differentiation [62]. However, such gradients might not be steep enough to induce migratory responses. This still leaves the challenge of creating surfaces that could serve as platforms to decipher the haptotactic function of BMP-2 gradients and to study possible differences with chemotactic gradients in BMP-induced migration signaling.

To uncouple total surface density from localized density of BMP-2, microcontact printing or dip-pen nanolithography were used to produce circular micropatterned islands of BMP-2 having a diameter of 4–5 μm [71]. The latter technique is based on the use of an AFM tip to deposit molecules on the surface as an ink droplet while varying the spacing between the islands. For the chemical binding of BMP-2 to the surface, either a thiolated biotin linker or a thiolated biotin lipid layer was first placed on gold-coated substrates using the micropatterning approaches. Following incubation with SAv, b-BMP2 was immobilized on the patterned regions and remained bioactive. Cell differentiation was comparable to non-patterned BMP-2 on the surface, when taking into account the estimated total surface density of the protein. When considering the impact of the local density of BMP-2 on cell response, these studies suggest that BMPR oligomerization might be favored when the growth factor is presented in discrete regions, thus leading to more efficient signaling, but this remains to be elucidated.

3.2.3. Nanoscale surface patterning of BMP-2

Materials which allow the control of cell responses at the nanoscale are of special interest, being at the length scale of BMP-2 and BMPRI and II interactions. Nanoscale modifications of surfaces carrying BMP-2 have been applied to study the influence of substrate modifications on osteogenic differentiation by changing their physicochemical properties [72], or for determining the effect of the surface density of BMP-2 on cell signaling [40]. In the first case, nanogrooves and nanodots ranging between 150 and 300 nm and 460 nm in size, respectively, consisting of polyurethane acrylate and coated with poly(glycidyl methacrylate), were functionalized with BMP-2 peptides. The presence of nanoscale features on the surface improves calcium deposition and the expression of osteogenic markers, which are even enhanced in presence of BMP-2 peptides. Better tuning of the nanostructure size to allow the formation of focal adhesions [73] and quantifying the amount of BMP-2 peptides immobilized on the surface may help to further use these new nanotopography tools to study BMP-mediated signaling

To achieve control over BMP-2 surface density, gold nanostructured substrates produced by block copolymer micellar nanolithography were recently applied as a substrate for the immobilization of BMP-2 using a bifunctional linker, as described in Section 3.1.2, now coupled to gold nanoparticles (Fig. 5C) [40]. The coupling of BMP-2 heterodimers to every single nanoparticle on the surface was detected and quantified at the single molecule level by AFM, thus making it possible to experimentally determine the amount of immobilized growth factor on the surface. Additionally, with this nanopatterning technique it is possible to vary the amount of immobilized BMP-2 by varying the interparticle spacing and to achieve the controlled immobilization of amounts which are below the lowest value reported previously (31 ng/cm^2) [74]. Interestingly, the bioactivity of the immobilized protein shows a characteristic regulation of SMAD phosphorylation levels and kinetics, which differs from those triggered by BMP-2 added to the cell culture medium. In fact, when BMP-2 is immobilized on the surface, regardless of the amount used (ranging from 0.2 to 3.3 ng/cm^2), SMAD phosphorylation onset is delayed but then is still maintained over a long period of time (180 min). Additionally, while the lowest amount of BMP-2 added to the culture media is not sufficient to activate the SMAD complex, the corresponding concentration immobilized on the surface leads to a remarkable SMAD phosphorylation. This study indicates that the sustained presentation rather than the amount of BMP-2 regulates SMAD-signaling, suggesting a different temporal regulation of BMP-mediated signaling pathways when the growth factor cannot be internalized. One hypothesis is that the immobilization might affect lateral receptor mobility and oligomerization on the one hand. On the other hand, when the receptors cannot be internalized in a complex with the ligands, the number activated receptors and their internalization rates might be different than those in presence of BMP-2 in the media.

3.3. Materials inspired by the interaction of BMP-2 with ECM components

One of the ECM functions is to serve as a reservoir of growth factors *via* a large variety of interactions (for example electrostastic, hydrogen-bonds, hydrophobic, Van der Waals). This type of interaction is important for growth factor release in soluble phase, orientation and therefore signaling. ECM presents epitopes which bind growth factors to limit their diffusion and maintain their activity locally. Therefore, the incorporation of BMP-2 binding sites of the ECM on materials would permit BMP-2 sequestration in a non-covalent manner [75] (Fig. 4D).

3.3.1. Modulation of the activity of BMP-2 bound to glycosaminoglycans

Glycosaminogycans (GAG) are major polysaccharide components of the ECM. These biopolymers can be divided into four groups: HA, the only not sulfated, heparan sulfate (HS), chondroitin sulfate (CS) and dermatan sulfate (DS). GAGs bind growth factors with a low binding constant [76,77], mainly due to electrostatic interactions. It has been shown that BMP-2-GAG binding could either up- or downregulate BMP-2 cellular activity [78–80]. Ruppert et al. [14] demonstrated that the BMP-2 homodimer has an heparin-binding site at its N-terminus. The binding seems to be due to the interactions between the basic residues of the Hp-binding site and the sulfate groups presented on GAGs. In particular, it has been demonstrated by surface plasmon resonance that GAGs alter the binding between BMP-2 and its receptor IA in a sulfation-dependent manner [81].

Hp can be used as a material coating to present BMP-2 to cells. For example titanium substrates modified with Hp to present BMP-2 promote osteoblast function, osteointegration, and bone regeneration *in vitro* and *in vivo* [82,83]. Resorbable polymer poly(L-lactic acid) and poly(ε-caprolactone) films, covalently functionalized with oriented Hp, linked *via* reductive amination, immobilize BMP-2 and improve cell attachment and proliferation [84]. However, the surface functionalization with Hp is only qualitative: with these materials it is not possible to achieve a precise quantification of both GAG and growth factor and to characterize the BMP-2 release during cell culture. Moreover,

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changes in mechanical properties after Hp coating might also influence cell behavior by changing cell-substrate forces and activating cytoskeleton rearrangements.

A different way to exploit the use of surfaces functionalized with Hp to bind growth factors has been proposed by Hudalla et al. [85] (Fig. 5D). Here a SAM presenting Hp-binding peptides and RGD peptides was used to specifically bind the endogenous Hp, which is complexed with the growth factors present in the cell medium. Thanks to the inert SAM background, these surfaces avoid the non-specific binding of other components of the serum and reduce the need of high non-physiological concentrations of growth factors. Human MSCs plated on SAM substrates show an enhancement of the BMP signaling pathway, and therefore an enhanced cellular proliferation and osteogenic differentiation. Hp and HS have been extensively used in 3D scaffolds due to their synergistic effect on BMP-2 activity. Other reviews extensively report the use of Hp for drug delivery and for *in vivo* applications [75,86]. Even though promising for clinical applications, these approaches do not provide structural and stoichiometric information of the GAG/BMP-2 binding. The combined effects of GAGs on BMP-2 cellular responses could lead to the hypothesis that HS proteoglycans function as co-receptors for BMP-2. Thus, it is crucial to get clear information on the GAGs structural and conformational modifications after BMP-2 binding. Functionalized surfaces together with surface-sensitive techniques could provide useful tools for answering this question.

A detailed surface-based study was proposed by the Svedhem group [87] using CS, an important structural ECM component. CS, covalently attached to supported lipid bilayers, binds BMP-2 and cells spread in response to BMP-2. Although the bioactivity of BMP-2 in these conditions was not verified, this type of model assembly opens new possibilities for the study of BMP-2 interactions with biopolymers in controlled environments.

HA is a GAG which possesses highly interesting physical and mechanical properties. By interacting with water molecules, HA provides the tissue with the ability to resist compression stresses [88]. HA alone has a positive effect on cell proliferation and upregulates osteogenic markers [89]. Several studies have described that BMP-2 can be trapped in HA crosslinked gels [8,90–92], and its retention can be improved using different strategies. For instance, Kisiel et al. [93] pre-complexed BMP-2 with DS or with Hp to increase its affinity for HA. Thus it is possible to load three times more pre-complexed BMP-2 in HA hydrogels than free BMP-2. The retention of the pre-complexed BMP-2 is significantly higher than free-BMP-2 on HA gels after 30 days. Alternatively, HA can be chemically modified to better retain BMP-2. For instance, bisphosphonates can be grafted onto HA, which leads to a 8-fold increase in the retention capacity for BMP-2 in comparison with pure HA gels [94]. HA can also be associated in LbL with positively-charged polypeptides to create thin self-assembled films that can be deposited on material surfaces, as described in Section 3.1.1 [54]. This presentation mode maintains the biological activity of BMP-2, as confirmed by SMAD phosphorylation. Interestingly, the phosphorylation signal is increased in cells cultured on the matrix-bound BMP-2 soft films in comparison with the stiff ones [56]. Indeed these biomimetic substrates combine both physical and chemical cues, thereby opening new possibilities to investigate the importance of BMP-2 in mechanotransduction. In fact, BMP signaling appears to be closely connected to mechanotransduction pathways at several levels. During embryonic development, for example, both BMP-2 gradients and mechanical signals such as tissue stiffness and compressive forces contribute to tissue polarity and patterning [95], although a deep understanding of the exact mechanisms is still missing.

3.3.2. Co-presentation of BMP-2 and cell binding motifs

Many efforts have been taken to engineer the environment so that it is supportive of both adhesion and differentiation in a controlled manner. However, the presentation of multiple and defined cues at the cell-material interface is still a challenge and so far the main focus has been on the effects on long-term responses and *in vivo* applications, whereas information on the signaling pathways and crosstalk is still missing. An emerging approach is the co-presentation of integrin-binding motifs and BMP-2 (Fig. 4D). Here, adhesion peptides such as RGD or collagen peptides are immobilized on the material surface to induce integrin-mediated adhesion [96–99].

Several studies from the Hubbell group have demonstrated that BMP-2 binds to ECM proteins like FN [100], tenascin C [101], fibrinogen, but not to collagen I [97,102]. In particular, $FNIII_{12-14}$ binds BMP-2 and other growth factors in a promiscuous manner, with a K_D in the nanomolar range and without affecting the biological activity of the growth factors [100]. Engineered substrates made with fibrin molecule carrying a peptide containing $FNIII_{12-14}$ permit a greater retention of the factors with respect to normal fibrin matrices. Fibrin matrix itself and its heparin-binding domain could promiscuously bind several growth factors, including BMP-2 [102]. Fibrin-synthetic matrices presenting both the fibrin heparin-binding domain inside a polymeric scaffold and growth factors, like fibroblast growth factor-2 (FGF-2) and Platelet-derived growth factor-2 (PDGF-2), have been successfully tested *in vivo*. The proximity between the RGD motif present in $FNIII_{9-10}$ and the growth factor binding site on $FNIII_{12-14}$ serves as rationale for the use of such peptides to allow synergy with BMP-2 and potentiate bone formation [100]. The synergistic interaction between immobilized collagen I and BMP-2 in osteogenic differentiation of MSCs has recently been investigated using a microcontact printing platform [103].

To achieve more defined responses, the immobilization of adhesive motifs and BMP-derived peptides on material surfaces have been also performed. The immobilization of BMP-peptides has been applied to various materials, including polymers and hydrogels, but here we will focus on two examples where the molecules have been grafted onto 2D surfaces. Zouani et al., [104] grafted RGD and BMP-2 mimetic peptides on polyethylene terephthalate to enhance osteogenic differentiation. The impact on osteogenic differentiation of the co-presentation of RGD and BMP-bioactive peptides carrying an azide group has been also investigated at concentration gradients on self-assembled monolayers generated by UVO treatment [105]. Osteopontin and BMP-2-derived motifs have been also immobilized by engineering a cysteine residue and 12-aminoacid stretch switch tag to address the C-terminus of the peptides [106]. These strategies rely on the use of BMP-derived peptides based on the sequence of the *knuckle* epitope of a BMP-2 monomer comprising the low affinity site for binding to BMPRII [107]. However, this is in contrast with biochemical studies showing that two *knuckle* epitopes should be present on one BMP-2 molecule in order to achieve receptor activation, since depletion of a single epitope results in complete loss of ALP activation [108]. This leaves the question whether the surface immobilization strategies might unveil otherwise masked activities of the BMP-2 molecule which are not possible to investigate with BMP-2 in solution.

4. Concluding remarks and perspectives

Recently new aspects in BMP-mediated signaling have been unraveled, pointing out the need to design and develop new approaches for BMP-2 delivery. In view of future clinical applications, some critical questions regarding BMP-2 presentation and functions remain to be solved in order to provide innovative

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solutions for bone tissue engineering. It is therefore important to engineer materials that can present BMP-2 in a spatially and temporally controlled manner.

In this review, we have shown that several technical solutions have now been developed to present BMP-2 in a controlled manner to cells, using either covalent grafting, physical entrapment or interactions with ECM components, which precisely tune the activity of BMP-2 and control its orientation. The use of two-dimensional surfaces offers the advantage of being controllable with surface-sensitive techniques and compatible with high resolution microscopy. Some of the technical approaches here described, such as physical entrapment of BMP-2 and GAG-based materials, might be soon applied to scaffolds for tissue engineering applications. *In vitro* studies with BMP-2-presenting surfaces could allow the deciphering of hidden biological functions of BMP-2. For instance, materials on which BMP-2 and ECM ligands (adhesion ligand and/or GAGs) are co-presented in a spatially controlled manner could provide important information on the crosstalk between adhesion (*e.g.* integrins) and BMP-2 signaling pathways. Super-resolution microscopy techniques could be helpful to clarify the interactions at the cell membrane between BMP-2 and its receptors, explaining the dynamics of receptor recruitment and mobility, as well as the architecture of receptor complexes. By combining the spatio-temporal control over BMP-2 presentation on surfaces and high-resolution imaging techniques it should also be possible to elucidate the regulation of BMP-2 receptor endocytosis and its impact on signaling pathways. Certainly there is a need to develop labeling strategies to track BMP-2, as well as BMPRs, without affecting their biological activity and signaling kinetics. Recent attempts have shown that BMP-2 activity is significantly slower when fluorophores are coupled to the growth factors [109]. Thus, the development of new biochemical tools becomes essential: for example, the conjugation of BMP-2 to various types of linkers should be in a site-specific manner, to permit the control of its orientation, once grafted on surfaces, and to improve the bioactivity of covalently-grafted BMP-2. Moreover, biochemical and structural studies at the molecular level could also help in improving our knowledge of the mechanisms of BMP-2 binding to GAGs and to ECM proteins, which is largely incomplete at present.

In conclusion, innovative solutions in bone regenerative medicine are needed to repair critical bone defects. Surface materials with controlled delivery and presentation of BMP-2 can be used to direct cell signaling for bone repair. In the future, through a joint effort from material and biological sciences, it should be possible to further improve the presentation of BMP-2 at the cell surface. The knowledge gained from *in vitro* studies, using well-defined materials platforms, may open new ways for regenerative therapies.

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References

[1] M.R. Urist, Bone: formation by autoinduction, Science 150 (1965) 893–899.
[2] M.R. Urist, H. Iwata, P.L. Ceccotti, R.L. Dorfman, S.D. Boyd, R.M. McDowell, et al., Bone morphogenesis in implants of insoluble bone gelatin, Proc. Nat. Acad. Sci. U. S. A. 70 (1973) 3511–3515.
[3] H.M. Ryoo, M.H. Lee, Y.J. Kim, Critical molecular switches involved in BMP-2-induced osteogenic differentiation of mesenchymal cells, Gene 366 (2006) 51–57.
[4] T. Katagiri, A. Yamaguchi, M. Komaki, E. Abe, N. Takahashi, T. Ikeda, et al., Bone morphogenetic protein-2 converts the differentiation pathway of C2C12 myoblasts into the osteoblast lineage, J. Cell Biol. 127 (1994) 1755–1766.
[5] A. Asakura, M. Komaki, M. Rudnicki, Muscle satellite cells are multipotential stem cells that exhibit myogenic, osteogenic, and adipogenic differentiation, Differ. Res. Biol. Divers. 68 (2001) 245–253.
[6] E.A. Wang, V. Rosen, P. Cordes, R.M. Hewick, M.J. Kriz, D.P. Luxenberg, et al., Purification and characterization of other distinct bone-inducing factors, Proc. Nat. Acad. Sci. U. S. A. 85 (1988) 9484–9488.
[7] M. Geiger, R.H. Li, W. Friess, Collagen sponges for bone regeneration with rhBMP-2, Adv. Drug Deliv. Rev. 55 (2003) 1613–1629.
[8] H.D. Kim, R.F. Valentini, Retention and activity of BMP-2 in hyaluronic acid-based scaffolds *in vitro*, J. Biomed. Mater. Res.. 59 (2002) 573–584.
[9] E.J. Carragee, E.L. Hurwitz, B.K. Weiner, A critical review of recombinant human bone morphogenetic protein-2 trials in spinal surgery: emerging safety concerns and lessons learned, Spine J. 11 (2011) 471–491.
[10] R. Fu, S. Selph, M. McDonagh, K. Peterson, A. Tiwari, R. Chou, et al., Effectiveness and harms of recombinant human bone morphogenetic protein-2 in spine fusion: a systematic review and meta-analysis, Ann. Intern. Med. 158 (2013) 890–902.
[11] G. Schmidmaier, P. Schwabe, C. Strobel, B. Wildemann, Carrier systems and application of growth factors in orthopaedics, Injury 39 (Suppl. 2) (2008) S37–S43.
[12] W.J. King, P.H. Krebsbach, Growth factor delivery: how surface interactions modulate release *in vitro* and *in vivo*, Adv. Drug Deliv. Rev. 64 (2012) 1239–1256.
[13] K.W. Lo, B.D. Ulery, K.M. Ashe, C.T. Laurencin, Studies of bone morphogenetic protein-based surgical repair, Adv. Drug Deliv. Rev. 64 (2012) 1277–1291.
[14] R. Ruppert, E. Hoffmann, W. Sebald, Human bone morphogenetic protein 2 contains a heparin-binding site which modifies its biological activity, Eur. J. Biochem./FEBS 237 (1996) 295–302.
[15] S.R. Frenkel, P.B. Saadeh, B.J. Mehrara, G.S. Chin, D.S. Steinbrech, B. Brent, et al., Transforming growth factor beta superfamily members: role in cartilage modeling, Plast. Reconstructive Surg. 105 (2000) 980–990.
[16] A.H. Reddi, BMPs: from bone morphogenetic proteins to body morphogenetic proteins, Cytokine Growth Factor Rev. 16 (2005) 249–250.
[17] F. Liu, A. Hata, J.C. Baker, J. Doody, J. Carcamo, R.M. Harland, et al., A human Mad protein acting as a BMP-regulated transcriptional activator, Nature 381 (1996) 620–623.
[18] T. Katagiri, M. Imada, T. Yanai, T. Suda, N. Takahashi, R. Kamijo, Identification of a BMP-responsive element in Id1, the gene for inhibition of myogenesis, Genes Cells 7 (2002) 949–960.
[19] C. Sieber, J. Kopf, C. Hiepen, P. Knaus, Recent advances in BMP receptor signaling, Cytokine Growth Factor Rev. 20 (2009) 343–355.
[20] M. Lind, E.F. Eriksen, C. Bunger, Bone morphogenetic protein-2 but not bone morphogenetic protein-4 and -6 stimulates chemotactic migration of human osteoblasts, human marrow osteoblasts, and U2-OS cells, Bone. 18 (1996) 53–57.
[21] C. Gamell, N. Osses, R. Bartrons, T. Ruckle, M. Camps, J.L. Rosa, et al., BMP2 induction of actin cytoskeleton reorganization and cell migration requires PI3-kinase and Cdc42 activity, J. Cell Sci. 121 (2008) 3960–3970.
[22] C. Hiepen, A. Benn, A. Denkis, I. Lukonin, C. Weise, J.H. Boergermann, et al., BMP2-induced chemotaxis requires PI3K p55gamma/p110alpha-dependent phosphatidylinositol (3,4,5)-triphosphate production and LL5beta recruitment at the cytocortex, BMC Biol. 12 (2014) 43.
[23] C. Gamell, A.G. Susperregui, O. Bernard, J.L. Rosa, F. Ventura, The p38/MK2/Hsp25 pathway is required for BMP-2-induced cell migration, PLoS One 6 (2011) e16477.
[24] A. Nohe, S. Hassel, M. Ehrlich, F. Neubauer, W. Sebald, Y.I. Henis, et al., The mode of bone morphogenetic protein (BMP) receptor oligomerization determines different BMP-2 signaling pathways, J. Biol. Chem. 277 (2002) 5330–5338.
[25] L. Gilboa, A. Nohe, T. Geissendorfer, W. Sebald, Y.I. Henis, P. Knaus, Bone morphogenetic protein receptor complexes on the surface of live cells: a new oligomerization mode for serine/threonine kinase receptors, Mol. Biol. Cell 11 (2000) 1023–1035.
[26] R.G.A. Medda, E.A. Cavalcanti-Adam, Challenges in imaging cell surface receptor clusters, Opt. Laser. Eng. 76 (2016) 3–8.
[27] A. Guzman, M. Zelman-Femiak, J.H. Boergermann, S. Paschkowsky, P.A. Kreuzaler, P. Fratzl, et al., SMAD versus non-SMAD signaling is determined by lateral mobility of bone morphogenetic protein (BMP) receptors, J.Biol. Chem. 287 (2012) 39492–39504.
[28] B. Marom, E. Heining, P. Knaus, Y.I. Henis, Formation of stable homomeric and transient heteromeric bone morphogenetic protein (BMP) receptor

Please cite this article in press as: E. Migliorini, et al., Tuning cellular responses to BMP-2 with material surfaces, Cytokine Growth Factor Rev (2015), http://dx.doi.org/10.1016/j.cytogfr.2015.11.008

complexes regulates Smad protein signaling, J. Biol. Chem. 286 (2011) 19287–19296.
[29] A. Hartung, K. Bitton-Worms, M.M. Rechtman, V. Wenzel, J.H. Boergermann, S. Hassel, et al., Different routes of bone morphogenic protein (BMP) receptor endocytosis influence BMP signaling, Mol. Cell. Biol. 26 (2006) 7791–7805.
[30] A.V. Vieira, C. Lamaze, S.L. Schmid, Control of EGF receptor signaling by clathrin-mediated endocytosis, Science (New York, NY) 274 (1996) 2086–2089.
[31] A. Sorkin, Z. von, M. astrow, Endocytosis and signalling: intertwining molecular networks, Nat. Rev. Mol. Cell Biol. 10 (2009) 609–622.
[32] A. Eichmann, M. Simons, VEGF signaling inside vascular endothelial cells and beyond, Curr. Opin. Cell Biol. 24 (2012) 188–193.
[33] M. Gourlaouen, J.C. Welti, N.S. Vasudev, A.R. Reynolds, Essential role for endocytosis in the growth factor-stimulated activation of ERK1/2 in endothelial cells, J. Biol. Chem. 288 (2013) 7467–7480.
[34] J. Bonor, E.L. Adams, B. Bragdon, O. Moseychuk, K.J. Czymmek, A. Nohe, Initiation of BMP2 signaling in domains on the plasma membrane, J. Cell. Physiol. 227 (2012) 2880–2888.
[35] Y.G. Chen, Endocytic regulation of TGF-beta signaling, Cell Res. 19 (2009) 58–70.
[36] L. Jortikka, M. Laitinen, T.S. Lindholm, A. Marttinen, Internalization and intracellular processing of bone morphogenetic protein (BMP) in rat skeletal muscle myoblasts (L6), Cell. Signalling 9 (1997) 47–51.
[37] C. Rauch, A.C. Brunet, J. Deleule, E. Farge, C2C12 myoblast/osteoblast transdifferentiation steps enhanced by epigenetic inhibition of BMP2 endocytosis, Am. J. Physiol. Cell Physiol. 283 (2002) C235–C243.
[38] E. Heining, R. Bhushan, P. Paarmann, Y.I. Henis, P. Knaus, Spatial segregation of BMP/Smad signaling affects osteoblast differentiation in C2C12 cells, PLoS One 6 (2011) e25163.
[39] T.L. Pohl, J.H. Boergermann, G.K. Schwaerzer, P. Knaus, E.A. Cavalcanti-Adam, Surface immobilization of bone morphogenetic protein 2 *via* a self-assembled monolayer formation induces cell differentiation, Acta Biomater. 8 (2012) 772–780.
[40] E.H. Schwab, T.L. Pohl, T. Haraszti, G.K. Schwaerzer, C. Hiepen, J.P. Spatz, et al., Nanoscale control of surface immobilized BMP-2: toward a quantitative assessment of BMP-mediated signaling events, Nano Lett. 15 (2015) 1526–1534.
[41] S.H. Kwon, T.J. Lee, J. Park, J.E. Hwang, M. Jin, H.K. Jang, et al., Modulation of BMP-2-induced chondrogenic versus osteogenic differentiation of human mesenchymal stem cells by cell-specific extracellular matrices, Tissue Eng. Part A 19 (2013) 49–58.
[42] A. Jikko, S.E. Harris, D. Chen, D.L. Mendrick, C.H. Damsky, Collagen integrin receptors regulate early osteoblast differentiation induced by BMP-2, J. Bone Miner. Res. 14 (1999) 1075–1083.
[43] C.D. Reyes, A.J. Garcia, Alpha2beta1 integrin-specific collagen-mimetic surfaces supporting osteoblastic differentiation, J. Biomed. Mater. Res. Part A 69 (2004) 591–600.
[44] Y. Tamura, Y. Takeuchi, M. Suzawa, S. Fukumoto, M. Kato, K. Miyazono, et al., Focal adhesion kinase activity is required for bone morphogenetic protein-Smad1 signaling and osteoblastic differentiation in murine MC3T3-E1 cells, J. Bone Miner. Res. 16 (2001) 1772–1779.
[45] C.F. Lai, S.L. Cheng, Alphavbeta integrins play an essential role in BMP-2 induction of osteoblast differentiation, J. Bone Miner. Res. 20 (2005) 330–340.
[46] J.L. Su, J. Chiou, C.H. Tang, M. Zhao, C.H. Tsai, P.S. Chen, et al., CYR61 regulates BMP-2-dependent osteoblast differentiation through the {alpha}v{beta} 3 integrin/integrin-linked kinase/ERK pathway, J. Biol. Chem. 285 (2010) 31325–31336.
[47] T. Sotobori, T. Ueda, A. Myoui, K. Yoshioka, M. Nakasaki, H. Yoshikawa, et al., Bone morphogenetic protein-2 promotes the haptotactic migration of murine osteoblastic and osteosarcoma cells by enhancing incorporation of integrin beta1 into lipid rafts, Exp. Cell Res. 312 (2006) 3927–3938.
[48] A.K. Shah, J. Lazatin, R.K. Sinha, T. Lennox, N.J. Hickok, R.S. Tuan, Mechanism of BMP-2 stimulated adhesion of osteoblastic cells to titanium alloy, Biol. Cell/under Auspices Eur. Cell Biol. Organiz. 91 (1999) 131–142.
[49] A. Moustakas, C.H. Heldin, Dynamic control of TGF-beta signaling and its links to the cytoskeleton, FEBS Lett. 582 (2008) 2051–2065.
[50] D. Padua, J. Massague, Roles of TGFbeta in metastasis, Cell Res. 19 (2009) 89–102.
[51] S. Ehata, Y. Yokoyama, K. Takahashi, K. Miyazono, Bi-directional roles of bone morphogenetic proteins in cancer: another molecular Jekyll and Hyde? Pathol. Int. 63 (2013) 287–296.
[52] V.C. Foletta, M.A. Lim, J. Soosairajah, A.P. Kelly, E.G. Stanley, M. Shannon, et al., Direct signaling by the BMP type II receptor *via* the cytoskeletal regulator LIMK1, J. Cell Biol. 162 (2003) 1089–1098.
[53] G. Konstantinidis, A. Moustakas, C. Stournaras, Regulation of myosin light chain function by BMP signaling controls actin cytoskeleton remodeling, Cell. Physiol. Biochem. 28 (2011) 1031–1044.
[54] T. Crouzier, K. Ren, C. Nicolas, C. Roy, C. Picart, Layer-by-layer films as a biomimetic reservoir for rhBMP-2 delivery: controlled differentiation of myoblasts to osteoblasts, Small 5 (2009) 598–608.
[55] R. Guillot, F. Gilde, P. Becquart, F. Sailhan, A. Lapeyrere, D. Logeart-Avramoglou, et al., The stability of BMP loaded polyelectrolyte multilayer coatings on titanium, Biomaterials 34 (2013) 5737–5746.
[56] T. Crouzier, L. Fourel, T. Boudou, C. Albiges-Rizo, C. Picart, Presentation of BMP-2 from a soft biopolymeric film unveils its activity on cell adhesion and migration, Adv. Mater. 23 (2011) H111–H118.
[57] N. Saito, T. Okada, H. Horiuchi, N. Murakami, J. Takahashi, M. Nawata, et al., Biodegradable poly-D,L-lactic acid-polyethylene glycol block copolymers as a BMP delivery system for inducing bone, J. Bone Joint Surg. Am. 83 (Suppl. 1) (2001) S92–S98.
[58] H. Uludag, T. Gao, T.J. Porter, W. Friess, J.M. Wozney, Delivery systems for BMPs: factors contributing to protein retention at an application site, J. Bone Joint Surg. Am. 83 (Suppl. 1) (2001) S128–S135.
[59] N.J. Shah, M.L. Macdonald, Y.M. Beben, R.F. Padera, R.E. Samuel, P.T. Hammond, Tunable dual growth factor delivery from polyelectrolyte multilayer films, Biomaterials 32 (2011) 6183–6193.
[60] E. Migliorini, D. Thakar, R. Sadir, T. Pleiner, F. Baleux, H. Lortat-Jacob, et al., Well-defined biomimetic surfaces to characterize glycosaminoglycan-mediated interactions on the molecular, supramolecular and cellular levels, Biomaterials 35 (2014) 8903–8915.
[61] H. Uludag, J. Golden, R. Palmer, J.M. Wozney, Biotinated bone morphogenetic protein-2: *In vivo* and *in vitro* activity, Biotechnol. Bioeng. 65 (1999) 668–672.
[62] A. Lagunas, J. Comelles, S. Oberhansl, V. Hortiguela, E. Martinez, J. Samitier, Continuous bone morphogenetic protein-2 gradients for concentration effect studies on C2C12 osteogenic fate, Nanomed. Nanotechnol. Biol. Med. 9 (2013) 694–701.
[63] K. Hauff, C. Zambarda, M. Dietrich, M. Halbig, A.L. Grab, R. Medda, et al., Matrix-immobilized BMP-2 on microcontact printed fibronectin as an *in vitro* tool to study BMP-mediated signaling and cell migration, Front. Bioeng. Biotechnol. 3 (2015) 62.
[64] V. Luginbuehl, L. Meinel, H.P. Merkle, B. Gander, Localized delivery of growth factors for bone repair, Eur. J. Pharm. Biopharm. 58 (2004) 197–208.
[65] K.S. Masters, Covalent growth factor immobilization strategies for tissue repair and regeneration, Macromol. Biosci. 11 (2011) 1149–1163.
[66] R. Goncalves, M.C. Martins, M.J. Oliveira, G. Almeida-Porada, M.A. Barbosa, Bioactivity of immobilized EGF on self-assembled monolayers: optimization of the immobilization process, J. Biomed. Mater. Res. Part A 94 (2010) 576–585.
[67] K. Kashiwagi, T. Tsuji, K. Shiba, Directional BMP-2 for functionalization of titanium surfaces, Biomaterials 30 (2009) 1166–1175.
[68] M.C. Ramel, C.S. Hill, The ventral to dorsal BMP activity gradient in the early zebrafish embryo is determined by graded expression of BMP ligands, Dev. Biol. 378 (2013) 170–182.
[69] E.D. Miller, J.A. Phillippi, G.W. Fisher, P.G. Campbell, L.M. Walker, L.E. Weiss, Inkjet printing of growth factor concentration gradients and combinatorial arrays immobilized on biologically-relevant substrates, Comb. Chem. High Throughput Screening 12 (2009) 604–618.
[70] J. Almodovar, R. Guillot, C. Monge, J. Vollaire, S. Selimovic, J.L. Coll, et al., Spatial patterning of BMP-2 and BMP-7 on biopolymeric films and the guidance of muscle cell fate, Biomaterials 35 (2014) 3975–3985.
[71] S. Oberhansl, A.G. Castano, A. Lagunas, E. Prats-Alfonso, M. Hirtz, F. Albericio, et al., Mesopattern of immobilised bone morphogenetic protein-2 created by microcontact printing and dip-pen nanolithography influence C2C12 cell fate, RSC Adv. 4 (2014) 56809–56815.
[72] M.J. Kim, B. Lee, K. Yang, J. Park, S. Jeon, S.H. Um, et al., BMP-2 peptide-functionalized nanopatterned substrates for enhanced osteogenic differentiation of human mesenchymal stem cells, Biomaterials 34 (2013) 7236–7246.
[73] E.A. Cavalcanti-Adam, A. Micoulet, J. Blummel, J. Auernheimer, H. Kessler, J.P. Spatz, Lateral spacing of integrin ligands influences cell spreading and focal adhesion assembly, Eur. J. Cell Biol. 85 (2006) 219–224.
[74] V. Karageorgiou, L. Meinel, S. Hofmann, A. Malhotra, V. Volloch, D. Kaplan, Bone morphogenetic protein-2 decorated silk fibroin films induce osteogenic differentiation of human bone marrow stromal cells, J. Biomed. Mater. Res. Part A 71 (2004) 528–537.
[75] G.A. Hudalla, W.L. Murphy, Biomaterials that regulate growth factor activity *via* bioinspired interactions, Adv. Funct. Mater. 21 (2011) 1754–1768.
[76] T. Maciag, T. Mehlman, R. Friesel, A.B. Schreiber, Heparin binds endothelial cell growth factor, the principal endothelial cell mitogen in bovine brain, Science (New York, NY) 225 (1984) 932–935.
[77] M.A. Nugent, E.R. Edelman, Kinetics of basic fibroblast growth factor binding to its receptor and heparan sulfate proteoglycan: a mechanism for cooperactivity, Biochemistry 31 (1992) 8876–8883.
[78] X. Jiao, P.C. Billings, M.P. O'Connell, F.S. Kaplan, E.M. Shore, D.L. Glaser, Heparan sulfate proteoglycans (HSPGs) modulate BMP2 osteogenic bioactivity in C2C12 cells, J. Biol. Chem. 282 (2007) 1080–1086.
[79] W.J. Kuo, M.A. Digman, A.D. Lander, Heparan sulfate acts as a bone morphogenetic protein coreceptor by facilitating ligand-induced receptor hetero-oligomerization, Mol. Biol. Cell 21 (2010) 4028–4041.
[80] D.S. Bramono, S. Murali, B. Rai, L. Ling, W.T. Poh, Z.X. Lim, et al., Bone marrow-derived heparan sulfate potentiates the osteogenic activity of bone morphogenetic protein-2 (BMP-2), Bone 50 (2012) 954–964.
[81] V. Hintze, S.A. Samsonov, M. Anselmi, S. Moeller, J. Becher, M. Schnabelrauch, et al., Sulfated glycosaminoglycans exploit the conformational plasticity of bone morphogenetic protein-2 (BMP-2) and alter the interaction profile with its receptor, Biomacromolecules 15 (2014) 3083–3092.
[82] S.E. Kim, C.S. Kim, Y.P. Yun, D.H. Yang, K. Park, S.E. Kim, et al., Improving osteoblast functions and bone formation upon BMP-2 immobilization on titanium modified with heparin, Carbohydr. Polym. 114 (2014) 123–132.

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[83] S.E. Kim, S.H. Song, Y.P. Yun, B.J. Choi, I.K. Kwon, M.S. Bae, et al., The effect of immobilization of heparin and bone morphogenic protein-2 (BMP-2) to titanium surfaces on inflammation and osteoblast function, Biomaterials 32 (2011) 366–373.
[84] U. Edlund, S. Danmark, A.C. Albertsson, A strategy for the covalent functionalization of resorbable polymers with heparin and osteoinductive growth factor, Biomacromolecules 9 (2008) 901–905.
[85] G.A. Hudalla, N.A. Kouris, J.T. Koepsel, B.M. Ogle, W.L. Murphy, Harnessing endogenous growth factor activity modulates stem cell behavior, Integr. Biol. 3 (2011) 832–842.
[86] S.E. Sakiyama-Elbert, Incorporation of heparin into biomaterials, Acta Biomater. 10 (2014) 1581–1587.
[87] N. Altgarde, J. Becher, S. Moller, F.E. Weber, M. Schnabelrauch, S. Svedhem, Immobilization of chondroitin sulfate to lipid membranes and its interactions with ECM proteins, J. Colloid Interface Sci. 390 (2013) 258–266.
[88] K. Haxaire, Y. Marechal, M. Milas, M. Rinaudo, Hydration of hyaluronan polysaccharide observed by IR spectrometry. II. Definition and quantitative analysis of elementary hydration spectra and water uptake, Biopolymers 72 (2003) 149–161.
[89] X. Zou, H. Li, L. Chen, A. Baatrup, C. Bunger, M. Lind, Stimulation of porcine bone marrow stromal cells by hyaluronan, dexamethasone and rhBMP-2, Biomaterials 25 (2004) 5375–5385.
[90] E. Martinez-Sanz, D.A. Ossipov, J. Hilborn, S. Larsson, K.B. Jonsson, O.P. Varghese, Bone reservoir: injectable hyaluronic acid hydrogel for minimal invasive bone augmentation, J. Control. Release 152 (2011) 232–240.
[91] M. Kisiel, M. Ventura, O.P. Oommen, A. George, X.F. Walboomers, J. Hilborn, et al., Critical assessment of rhBMP-2 mediated bone induction: an *in vitro* and *in vivo* evaluation, J. Control. Release 162 (2012) 646–653.
[92] G. Bhakta, B. Rai, Z.X. Lim, J.H. Hui, G.S. Stein, W. van, A.J. ijnen, et al., Hyaluronic acid-based hydrogels functionalized with heparin that support controlled release of bioactive BMP-2, Biomaterials 33 (2012) 6113–6122.
[93] M. Kisiel, A.S. Klar, M. Ventura, J. Buijs, M.K. Mafina, S.M. Cool, et al., Complexation and sequestration of BMP-2 from an ECM mimetic hyaluronan gel for improved bone formation, PLoS One 8 (2013) e78551.
[94] G. Hulsart-Billstrom, P.K. Yuen, R. Marsell, J. Hilborn, S. Larsson, D. Ossipov, Bisphosphonate-linked hyaluronic acid hydrogel sequesters and enzymatically releases active bone morphogenetic protein-2 for induction of osteogenic differentiation, Biomacromolecules 14 (2013) 3055–3063.
[95] J. Kopf, P. Paarmann, C. Hiepen, D. Horbelt, P. Knaus, BMP growth factor signaling in a biomechanical context, Biofactors 40 (2014) 171–187.
[96] A.J. Garcia, C.D. Reyes, Bio-adhesive surfaces to promote osteoblast differentiation and bone formation, J. Dent. Res. 84 (2005) 407–413.
[97] M.M. Martino, P.S. Briquez, E. Guc, F. Tortelli, W.W. Kilarski, S. Metzger, et al., Growth factors engineered for super-affinity to the extracellular matrix enhance tissue healing, Science 343 (2014) 885–888.
[98] A. Shekaran, J.R. Garcia, A.Y. Clark, T.E. Kavanaugh, A.S. Lin, R.E. Guldberg, et al., Bone regeneration using an alpha 2 beta 1 integrin-specific hydrogel as a BMP-2 delivery vehicle, Biomaterials 35 (2014) 5453–5461.
[99] K. Lee, E.A. Silva, D.J. Mooney, Growth factor delivery-based tissue engineering: general approaches and a review of recent developments, J. R. Soc. 8 (2011) 153–170.
[100] M.M. Martino, J.A. Hubbell, The 12th–14th type III repeats of fibronectin function as a highly promiscuous growth factor-binding domain, FASEB J. 24 (2010) 4711–4721.
[101] L. De Laporte, J.J. Rice, F. Tortelli, J.A. Hubbell, Tenascin C promiscuously binds growth factors *via* its fifth fibronectin type III-like domain, PLoS One 8 (2013) e62076.
[102] M.M. Martino, P.S. Briquez, A. Ranga, M.P. Lutolf, J.A. Hubbell, Heparin-binding domain of fibrin(ogen) binds growth factors and promotes tissue repair when incorporated within a synthetic matrix, Proc. Nat. Acad. Sci. U. S. A. 110 (2013) 4563–4568.
[103] G. Rasi, S. haemi, B. Delalat, X. Ceto, F.J. Harding, J. Tuke, N.H. Voelcker, Synergistic influence of collagen I and BMP 2 drives osteogenic differentiation of mesenchymal stem cells: a cell microarray analysis, Acta Biomater. (2015) .
[104] O.F. Zouani, C. Chollet, B. Guillotin, M.C. Durrieu, Differentiation of pre-osteoblast cells on poly(ethylene terephthalate) grafted with RGD and/or BMPs mimetic peptides, Biomaterials 31 (2010) 8245–8253.
[105] N.M. Moore, N.J. Lin, N.D. Gallant, M.L. Becker, Synergistic enhancement of human bone marrow stromal cell proliferation and osteogenic differentiation on BMP-2-derived and RGD peptide concentration gradients, Acta Biomater. 7 (2011) 2091–2100.
[106] E.A. Mitchell, B.T. Chaffey, A.W. McCaskie, J.H. Lakey, M.A. Birch, Controlled spatial and conformational display of immobilised bone morphogenetic protein-2 and osteopontin signalling motifs regulates osteoblast adhesion and differentiation *in vitro*, BMC Biol. 8 (2010) 57.
[107] J. Nickel, M.K. Dreyer, T. Kirsch, W. Sebald, The crystal structure of the BMP-2: BMPR-IA complex and the generation of BMP-2 antagonists, J. Bone Joint Surg. Am. 83 (Suppl. 1) (2001) S7–S14.
[108] P. Knaus, W. Sebald, Cooperativity of binding epitopes and receptor chains in the BMP/TGFbeta superfamily, Biol. Chem. 382 (2001) 1189–1195.
[109] H. Alborzinia, H. Schmidt-Glenewinkel, I. Ilkavets, K. Breitkopf-Heinlein, X. Cheng, P. Hortschansky, et al., Quantitative kinetics analysis of BMP2 uptake into cells and its modulation by BMP antagonists, J. Cell Sci. 126 (2013) 117–127.

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