Acta Biomaterialia 36 (2016) 132–142

Contents lists available at ScienceDirect

Acta Biomaterialia

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

Full length article

RGD and BMP-2 mimetic peptide crosstalk enhances osteogenic commitment of human bone marrow stem cells

I. Bilem [a,b,c], P. Chevallier [a,b], L. Plawinski [c], E.D. Sone [d], M.C. Durrieu [c,*,1], G. Laroche [a,b,*,1]

[a] Laboratoire d'Ingénierie de Surface, Centre de Recherche sur les Matériaux Avancés, Département de Génie des Mines, de la Métallurgie et des Matériaux, Université Laval, 1065 Avenue de la médecine, Québec, G1V 0A6, Canada
[b] Centre de Recherche du Centre Hospitalier Universitaire de Québec, Hôpital St-François d'Assise, 10 rue de l'Espinay, Québec, G1L 3L5, Canada
[c] Institute of Chemistry & Biology of Membranes & Nanoobjects (CNRS, UMR5248 CBMN), Université de Bordeaux, Bordeaux INP, France
[d] Institute of Biomaterials and Biomedical Engineering, Department of Materials Science and Engineering, and Faculty of Dentistry, University of Toronto, Toronto, ON M5S 3G9, Canada

ARTICLE INFO

Article history:
Received 8 December 2015
Received in revised form 19 February 2016
Accepted 17 March 2016
Available online 18 March 2016

Keywords:
Stem cells
Biomimetic materials
Bone tissue engineering
Mimetic peptides
Surface modification

ABSTRACT

Human bone marrow mesenchymal stem cells (hBMSCs) commitment and differentiation are dictated by bioactive molecules sequestered within their Extra Cellular Matrix (ECM). One common approach to mimic the physiological environment is to functionalize biomaterial surfaces with ECM-derived peptides able to recruit stem cells and trigger their linage-specific differentiation. The objective of this work was to investigate the effect of RGD and BMP-2 ligands crosstalk and density on the extent of hBMSCs osteogenic commitment, without recourse to differentiation medium. RGD peptide promotes cell adhesion via cell transmembrane integrin receptors, while BMP-2 peptide, corresponding to residues 73–92 of Bone Morphogenetic Protein-2, was shown to induce hBMSCs osteoblast differentiation. The immobilization of peptides on aminated glass was ascertained by X-ray Photoelectron Spectroscopy (XPS), the density of grafted peptides was quantified by fluorescence microscopy and the surface roughness was evaluated using Atomic Force Microscopy (AFM). The osteogenic commitment of hBMSCs cultured on RGD and/or BMP-2 surfaces was characterized by immunohistochemistry using STRO-1 as specific stem cells marker and Runx-2 as an earlier osteogenic marker. Biological results showed that the osteogenic commitment of hBMSCs was enhanced on bifunctionalized surfaces as compared to surfaces containing BMP-2, while on RGD surfaces cells mainly preserved their stemness character. These results demonstrated that RGD and BMP-2 mimetic peptides act synergistically to enhance hBMSCs osteogenesis without supplementing the media with osteogenic factors. These findings contribute to the development of biomimetic materials, allowing a deeper understanding of signaling pathways that govern the transition of stem cells towards the osteoblastic lineage.

Statement of Significance

For a long time, scientists thought that the differentiation of Mesenchymal Stem Cells (MSCs) into bone cells was dictated by growth factors. This manuscript shed light on other ligands that play a crucial role in regulating MSCs fate. In concrete terms, it was demonstrated that the osteoinductive effect of BMP-2 peptide is 2 folds improved in the presence of adhesive RGD peptide. Compared to previous works highlighting this synergistic cooperation between RGD and BMP-2 peptides, the main strength of this work lies to the use of primitive human cells (hMSCs) and well-defined biomimetic material surfaces (controlled surface roughness and peptide densities). This work provides valuable insights to develop custom-designed *in vitro* cell culture models, capable of targeting the desired cell response.

* Corresponding authors at: Institute of Chemistry & Biology of Membranes & Nanoobjects (CNRS, UMR5248 CBMN), Université de Bordeaux, Bordeaux INP, France (M.C. Durrieu) and Laboratoire d'Ingénierie de Surface, Centre de Recherche sur les Matériaux Avancés, Département de Génie des Mines, de la Métallurgie et des Matériaux, Université Laval, 1065 Avenue de la médecine, Québec G1V 0A6, Canada (G. Laroche).

E-mail addresses: Ibrahim.bilem.1@ulaval.ca (I. Bilem), Pascale.Chevallier@crchudequebec.ulaval.ca (P. Chevallier), l.plawinski@cbmn.u-bordeaux.fr (L. Plawinski), eli.sone@utoronto.ca (E.D. Sone), marie-christine.durrieu@inserm.fr (M.C. Durrieu), Gaetan.Laroche@gmn.ulaval.ca (G. Laroche).

[1] Equally contributed.

http://dx.doi.org/10.1016/j.actbio.2016.03.032
1742-7061/

1. Introduction

Mesenchymal Stem Cells (MSCs) are considered as a promising cell source for musculoskeletal regeneration due to their high osteogenic differentiation potential when stimulated with growth factors and specific signaling molecules [1,2]. The first stem cell-based therapies involved the injection of cells directly into bone defect sites. Unfortunately, this approach had limited success due to the high death rate of the cells and their poor engraftment into host tissues [3]. Therefore, much effort has been dedicated to design biomaterials capable of recruiting stem cells, interact with them and drive their fate in a controlled manner towards the osteoblastic lineage.

Up to now, a large panel of natural and synthetic materials has been investigated for bone tissue engineering applications [4–6]. However, no single material fulfills all the criteria of biocompatibility. For example, natural materials have an acceptable level of cytocompatibility but exhibit poor mechanical properties compared to cortical bone [7]. Synthetic materials are more available and their mechanical properties, degradation rate, shape, and composition, etc. can be tailored [8,9]. For example, synthetic hydrogels are usually used to mimic pre-calcified bone tissue of approximately 25–40 kPa of stiffness [10]. Nevertheless, most of synthetic materials are intended to be bioinert and lack regulatory signals required to control cell-biomaterial interactions [7]. To increase materials bioactivity, several recent works have attempted to create a biomimetic microenvironment on conventional synthetic materials by chemically conjugating bioactive ligands or short peptide sequences derived from the Extra Cellular Matrix (ECM) proteins [11].

During the last decades, biomimetic peptides have gained much more notoriety than full-length native matrix proteins due to their straightforward synthesis, high purity, minimal cost and tight control of their conformation and density when grafted onto biomaterials [12]. In fact, several classes of peptides, mimicking properties of the native ECM components, have been designed, synthesized and exploited for their potential to induce desired cell response [13–18]. This has, of course, led to develop strategies for functional peptide sequences conjugation to biomaterials [13,17–19]. In this work, we propose to develop biomaterials functionalized by one or several biomimetic peptides and subsequently to investigate their effect on hBMSCs osteogenic differentiation. One of the most commonly used peptides to functionalize biomaterials are cell adhesion peptides containing the arginine-glycine-aspartic acid (RGD) sequence, which is present in several proteins such as collagen I, fibronectin, bone sialoprotein and osteopontin [20–22]. It was shown in several instances that RGD peptide grafted materials interact with integrin cell surface receptors and enhance adhesion of bone marrow stem cells [21,23]. Moreover, several studies have demonstrated that this peptide is a mild promoter of osteogenic differentiation *in vitro* [24,25] and can stimulate bone formation *in vivo* [26].

Beside integrin ligands, growth factors have also been used to improve materials bioactivity due to their ability to stimulate stem cells expansion and differentiation towards a specific lineage [27,28]. For example, Bone Morphogenetic Proteins (BMPs), belonging to the Transforming Growth Factor beta family ((TGF-β), promote the differentiation of mesenchymal stem cells into mineral-depositing osteoblasts [29], via different signaling pathways [30]. Indeed, BMPs interact with their cell surface receptors through non-covalent bonds [31], leading to the phosphorylation of Smad 1, Smad 5, and Smad 8 signaling pathways. Phosphorylated Smads associate with Smad 4, leading to the translocation of this complex from cytoplasm into nucleus [32], which leads to the transcription of genes mediating cell differentiation such as Runx2 and Osterix [33,34]. These growth factors can also trigger the activation of the Mitogen-Activated Protein Kinase (MAPK) pathway which plays a critical role in cell commitment and differentiation into osteoblastic lineage [35,36]. Another pathway that influences osteogenic differentiation mediated by BMPs proteins is the Wnt canonical pathway [37,38]. The activation of this pathway through the binding of Wnt ligands to their Frizzled receptors and LRP5/LRP6 coreceptors stabilizes β-catenin and causes its translocation to the nucleus [39]. This leads to the activation of specific genes like c-Jun that, in turn, influences the early osteoblast differentiation [40].

Among 20 BMPs identified to date, BMP-2 is considered as the most potent one in terms of inducing osteogenesis, hence its widespread use in the clinic for bone therapy [41,42]. For instance, the USA Food and Drug Administration (FDA) approved recombinant human BMP-2 delivered in a collagen scaffold, called Infuse Bone Graft®, as a bone tissue engineering product for spinal fusion surgery [43]. Due to the high cost of BMP-2 and satisfactory clinical outcomes reported using this protein, several peptide sequences derived from the knuckle epitope of BMP-2 protein have been identified, synthesized and used both *in vitro* and *in vivo* [44–46]. It was shown that these BMP-2 peptides also bind to BMP receptors I and II, thus activating specific signaling pathways similarly to the full-length BMP-2 protein [45]. Further studies demonstrated that materials functionalized with BMP-2 peptides induced osteoblastic differentiation of mesenchymal stem cells *in vitro* and bone regeneration *in vivo* [47]. In addition, several literature works have shown that integrins and BMP-2 proteins/peptides cooperate synergistically to up-regulate osteogenic differentiation [44,48–51]. However, most of studies investigated this synergistic effect on committed pre-osteoblasts or in the presence of osteogenic supplements in the medium. For example, we previously determined that the concomitant immobilization of RGD and BMP-2 mimetic peptides on polyethylene terephthalate (PET) surfaces elicit a synergistic effect that positively affects the osteogenic differentiation and mineralization of mouse calvaria-derived pre-osteoblastic cells [44]. Although such animal models are widely used in basic and applied research, cells from mice are likely to behave differently than human cells. Hence the current study objectives to investigate RGD and BMP-2 ligands crosstalk in more physiologically relevant conditions. In fact, we used more primitive cells, hBMSCs, which are likely to differentiate into pre-osteoblastic cells and more mature bone cells. These cells were harvested from patient's iliac crest and cultured in basal medium free of soluble osteogenic factors. Moreover, to facilitate the interactions of grafted ligands and their cell receptors, hBMSCs were plated and maintained on the different materials in serum-free medium for the first 6 h since we can imagine that serum proteins may adsorb on modified surfaces and mask specific cell/material interactions. In this work, three categories of materials chemically modified were synthesized: glass material surfaces grafted with RGD or BMP-2 peptides and bifunctionalized surfaces with both peptides. BMP-2 peptide used in this work was designed by Durrieu's group [44] while fibronectin-derived RGD cell-adhesive peptide was designed by Laroche's group [22]. Both mimetic peptides have been reported as potent effectors of cell differentiation and adhesion, respectively. The surface physicochemical characterization after each step of peptide grafting was achieved by XPS and AFM while the peptide surface density was quantified using fluoro-tagged peptides. The osteogenic differentiation of hBMSCs was then characterized after four weeks of cell culture by fluorescent staining of two specific markers, STRO-1, the best known mesenchymal stem cells marker and Runx-2 which is an earlier osteogenic marker. The expression level of STRO-1 and Runx-2 were evaluated by quantifying the average fluorescence intensity of each marker in hBMSCs

ID	Title	Pages
63	RGD and BMP-2 mimetic peptide crosstalk enhances osteogenic commitment of human bone marrow stem cells	11