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Platelet lysate coating on scaffolds directly and indirectly enhances cell migration, improving bone and blood vessel formation

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ABSTRACT

Suitable colonization and vascularization of tissue-engineered constructs after transplantation represent critical steps for the success of bone repair. Human platelet lysate (hPL) is composed of numerous growth factors known for their proliferative, differentiative and chemo-attractant effects on various cells involved in wound healing and bone growth. The aim of this study was to determine whether the delivery of human mesenchymal stromal cells (hMSC) seeded on hPL-coated hydroxyapatite/β-tricalcium phosphate (HA/β-TCP) scaffolds could enhance vascularization and bone formation, as well as to investigate the mechanisms by which hMSC participate in tissue regeneration. Our study demonstrates that hPL can be coated on HA/β-TCP scaffolds, which play direct and indirect effects on implanted and/or resident stem cells. Effectively, we show that hPL coating directly increases chemo-attraction to and adhesion of hMSC and endothelial cells on the scaffold. Moreover, we show that hPL coating induces hMSC to produce and secrete pro-angiogenic proteins (placental growth factor and vascular endothelial growth factor) which allow the proliferation and specific chemo-attraction of endothelial cells in vitro, thus improving in vivo neovascularization and new bone formation. This study highlights the potential of functionalizing biomaterials with hPL and shows that this growth factor combination can have synergistic effects leading to enhanced bone and blood vessel formation.

1. Introduction

Large bone defect repair following trauma, pathology or tumor resection presents significant clinical challenges for reconstructive and orthopaedic surgery [1–3]. One common surgical procedure is autologous bone grafting. However, this procedure is associated with potential complications, including chronic pain and risk of infection, as well as being limited by the amount of available bone. This has led to a search for alternative methods using bone substitutes.

Bone tissue engineering using osteoprogenitor cells such as human mesenchymal stromal cells (hMSC) combined with a three-dimensional (3-D) scaffold represents a promising therapeutic approach and provides an alternative to autologous bone grafting for the treatment of bone defects. Several scaffolds are currently available, of either natural or synthetic origin. The combination of these biomaterials with hMSC has been shown to be efficient in the repair of large bone defects in several experimental animal models [4–6].

Nevertheless, bone regeneration is a complex and coordinated physiological process involving a number of molecular, cellular, biochemical and mechanical mechanisms [7–9]. Angiogenesis and osteogenesis are closely related, since the development of a functional microvascular network is an essential prerequisite for successful bone formation [10–13]. A lack of blood vessels in grafted sites is a major issue affecting cell survival and proliferation failure [14]. Indeed, the formation of new vessels is required not only for oxygen, nutrient and soluble factor supply, but also to provide a communicative network between the implant and neighbouring tissues. This vascularization allows scaffold integration with the surrounding host tissues and mobilization of resident cells.

A variety of molecules involved in neo-angiogenesis and osteogenesis have been used to functionalize scaffolds, such as fibroblast growth factor (FGF), insulin-like growth factor (IGF)-1, transforming growth factor (TGF)-β1, vascular endothelial growth factor

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(VEGF) and bone morphogenic proteins (BMPs) [15–19]. These findings demonstrate that a combination of factors further increases bone regeneration compared with single factor stimulation [16,17]. However, a universally applicable combination of growth factors has yet to be established for bone cell therapy.

Platelets contain many growth factors, including platelet-derived growth factor (PDGF), FGF, IGF, platelet-derived angiogenesis factor (PDAF), TGF-β, platelet factor 4 (PF-4), platelet-derived epidermal growth factor (PDEGF) and VEGF [20,21]. These growth factors are known for their growth, differentiative and chemoattractant effects on various cells, like hMSC and endothelial progenitor cells (EPC) [22,23]. Taking into consideration that tissue regeneration involves complex cellular interactions regulated by multiple signals, a combination of a number of these factors could have additive or even synergistic effects leading to enhanced bone and blood vessel formation.

Platelets have been used in therapeutic applications in maxillofacial, oral, plastic and orthopaedic surgery. Their application is in the form of either: (i) platelet-rich plasma (PRP), obtained by concentrating platelets by gradient density centrifugation; (ii) platelet gel (PLG), obtained by treatment of PRP with thrombin, a process known to release bioactive molecules; (iii) releasates prepared from PRP, also called platelet lysate (PL). In combination with autologous bone grafts PRP and PLG have been shown to accelerate bone formation and bone density [24–31], in addition to enhancing revascularization when combined with bone marrow stromal cells [32]. These promising case reports demonstrate the usefulness of platelets for a wide range of clinical applications to improve healing after surgical procedures. In addition, PL obtained by freezing platelets has been demonstrated to deliver more growth factors compared with PRP after aggregation [33]. Recently human PL (hPL) has been suggested as a culture supplement for the expansion and clinical grade production of hMSC [22,33–35] and EPC [36]. Furthermore, we have previously shown that hPL medium can prime hMSC differentiation towards the osteoblastic lineage, which enhances in vivo bone regeneration [22]. A recent study has reported that MSC and PL in a fibrin or collagen scaffold can promote new bone formation around an uncemented hip prothesis [37]. However, these scaffolds are unsuitable for filling large bone defects. To our knowledge no study has investigated the feasibility of coating a bone graft substitute with hPL use in the treatment of large bone defects. Moreover, the mechanisms by which hMSC seeded on hPL-coated hydroxyapatite/β-tricalcium phosphate (HA/β-TCP) scaffolds induce tissue repair have never been addressed.

The aim of this study was to determine whether the functionnalization of HA/β-TCP scaffolds with hPL, before seeding them with hMSC, could enhance vascularization and bone formation, as well as to investigate the mechanisms by which hMSC participate in tissue regeneration. For this study hMSC were cultured in the absence of osteogenic agents in standard growth medium complemented with foetal bovine serum (FBS) to exclusively evaluate the impact of the hPL coating. The effect of hPL-coated HA/β-TCP scaffolds on chemo-attraction, adhesion, morphology, distribution, gene expression and protein secretion of hMSC was first evaluated in vitro. Then the effects on neovascularization and new bone formation in vivo in a mouse model were studied.

2. Materials and methods

2.1. Mesenchymal stromal cell cultures

hMSC were isolated from bone marrow (3–5 ml) collected from the iliac crest of patients undergoing standard bone marrow transplantation procedures (AP-HP Hôpital Henri Mondor, Créteil, France), after having received their informed consent. Bone marrow aspirates from three healthy donors (15–26 years old) were used. Nucleated cells from fresh bone marrow were seeded at 2×10^5 cells cm^{-2} in 225 cm^2 flasks. hMSC were expanded in α-modified Eagle's medium (αMEM) (PAA, Les Mureaux, France) supplemented with 10% FBS (StemCell Technologies, Grenoble, France) and 0.5% ciprofloxacine (Bayer Pharma, Puteaux, France). The cultures were maintained in a humidified atmosphere with 5% CO_2 at 37 °C and the culture medium was changed twice a week. Upon reaching 80% confluence adherent cells were detached using 1× trypsin/EDTA (PAA, Les Mureaux, France) and replated at a density of 10^3 cells cm^{-2} (passage 1). Cell samples were used to confirm the hMSC characteristics as previously described [22]. Briefly, all the hMSC used in this study were positive for CD90 (clone 5E10), CD105 (clone 266), CD73 (clone AD2) (all from Becton Dickinson and Co., Franklin Lakes, NJ) and negative for CD34 (clone AC136, Miltenyi Biotec, Bergjsch Gladbach, Germany) and CD45 (clone 2D1, Becton Dickinson), and were able to differentiate along the osteogenic, chondrogenic and adipogenic lineages (data not shown).

2.2. Endothelial cell cultures

Human umbilical vein endothelial cells (hUVEC), provided by the team of Dr Llorens-Cortes of the Collège de France (Paris, France), were cultured in Endothelial Cell Growth Medium 2 (PromoCell, Heidelberg, Germany).

Human bone marrow endothelial cells [38], provided by the laboratory of Dr Li of the Université Paris 6 (Paris, France), were cultured in αMEM with 10% FBS.

Endothelial cell cultures were maintained in a humidified atmosphere with 5% CO^2 at 37 °C and the culture medium was changed twice a week.

2.3. Platelet lysate preparation

hPL was obtained from platelet apheresis collections performed at the Etablissement Français du Sang (Rungis, France). All apheresis products were biologically qualified in accordance with French legislation. The platelet count in each product was measured automatically with a ABXpentra $60C^+$ (Horiba ABX, Montpellier, France). A minimum of four different batches were mixed to adjust the concentration to 10^9 platelets ml^{-1}, frozen at −80 °C and subsequently used to obtain hPL containing platelet-released growth factors. Remaining platelet bodies were eliminated by centrifugation at 1400*g*.

2.4. Biomaterial coating and cell seeding

The HA (65%)/β-TCP (35%) scaffolds, provided by Ceraver (Roissy, France), had an average porosity of 65 ± 5% (60 ± 5% macroporosity and 40 ± 5% microporosity) and a specific surface area of 0.8 m^2 g^{-1}. The granules had a diameter of 2–3 mm and weighed 8.0 ± 1 mg). These scaffolds were coated by immersion in hPL (hPL-coated) or left uncoated by immersion in αMEM (control group) in untreated 96-well culture plates at 37 °C overnight. After rinsing in 1× HBSS (PAA) they were loaded with 3×10^5 hMSC or endothelial cells for 3 h. These seeded constructs were then directly placed into untreated 24-well culture plates and cultured in αMEM with 10% FBS at 37 °C for 7 days. Cell-free scaffolds were incubated under similar conditions and served as controls.

2.5. Evaluation of cell number by DNA quantification

Cell seeding efficiency was determined using an indirect method. After 3 h contact with 3×10^5 hMSC or endothelial cells the scaffold was removed and cells that were not adherent on the bone

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