Acta Biomaterialia xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Acta Biomaterialia

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

Full length article

Platelet lysate-based pro-angiogenic nanocoatings

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ARTICLE INFO

Article history:
Received 2 September 2015
Received in revised form 10 December 2015
Accepted 15 December 2015
Available online xxxx

Keywords:
Layer-by-layer assembling
Instructive surfaces
Platelet lysate
Growth factors
Angiogenesis
Endothelial cells

ABSTRACT

Human platelet lysate (PL) is a cost-effective and human source of autologous multiple and potent pro-angiogenic factors, such as vascular endothelial growth factor A (VEGF A), fibroblast growth factor b (FGF b) and angiopoietin-1. Nanocoatings previously characterized were prepared by layer-by-layer assembling incorporating PL with marine-origin polysaccharides and were shown to activate human umbilical vein endothelial cells (HUVECs). Within 20 h of incubation, the more sulfated coatings induced the HUVECS to the form tube-like structures accompanied by an increased expression of angiogenic-associated genes, such as angiopoietin-1 and VEGF A. This may be a cost-effective approach to modify 2D/3D constructs to instruct angiogenic cells towards the formation of neo-vascularization, driven by multiple and synergistic stimulations from the PL combined with sulfated polysaccharides.

Statement of Significance

The presence, or fast induction, of a stable and mature vasculature inside 3D constructs is crucial for new tissue formation and its viability. This has been one of the major tissue engineering challenges, limiting the dimensions of efficient tissue constructs. Many approaches based on cells, growth factors, 3D bioprinting and channel incorporation have been proposed. Herein, we explored a versatile technique, layer-by-layer assembling in combination with platelet lysate (PL), that is a cost-effective source of many potent pro-angiogenic proteins and growth factors. Results suggest that the combination of PL with sulfated polyelectrolytes might be used to introduce interfaces onto 2D/3D constructs with potential to induce the formation of cell-based tubular structures.

1. Introduction

The development of tissue engineering constructs containing a functional and mature pre-vasculature is still a major challenge [1–3]. In the absence of such a network, the viability and regeneration potential of thick constructs will be compromised due to the limitation of nutrients and cell debris diffusion. In order to overcome this issue, researchers have been recurring either to material-based and cell-based approaches aiming to create an adequate vasculature inside engineered constructs. Material-based approaches have been focusing on the development of cellular or acellular 3D organized vessel-like structures through microfabrication and customized cell seeding methodologies [4,5]. On the other hand, cell-based approaches have been centered the instruction and activation of the involved angiogenic cells (e.g., endothelial and pericytes) to lead their cellular assembling into stable cellular tubular networks (i.e., tubulogenesis). The specific instruction of endothelial cells (EC) towards the formation of stable tube-like structures (TLS) has been extensively investigated [6,7]. Natural or synthetic extracellular cues such as collagen, fibrin, growth factors (GFs) or similar epitopes, are known to activate specific integrins and tyrosine kinase receptors, efficiently promoting angiogenic cells activation and formation of TLS. However, most of those instructive cues/constructs are frequently obtained from animal-origin and costly sources, or need complicated procedures. The formation of neo-vessels involves a complex crosstalk between several cell types, platelets releasates, extracellular matrix and their secreted pro and anti-angiogenic factors. Vascular endothelial growth factor (VEGF) and fibroblast growth factor b (FGFb) are considered the most potent angiogenic GFs being frequently used to

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http://dx.doi.org/10.1016/j.actbio.2015.12.028
1742-7061/

prepare angiogenic biomaterials. The angiogenesis is initiated and regulated by several cells types, GFs and other bioactive proteins and environmental cues such as hypoxia [8]. The ECs are activated, proliferate, migrate and, in the final stage, their tubular structures are stabilized by pericytes, smooth vascular cells and synthetized ECM [8].

Recent works have been highlighting the importance of the provision of multiple GFs in order to achieve better networks regarding size features and stability [1,9–12]. This has been explored by the combination of multiple recombinant GFs or other cell types able to provide such bioactive moieties to the EC. Platelets, natural players in the healing process, are very attractive sources of multiple GFs, metalloproteinases and other potent regulators of angiogenesis [13].

The ability of platelets derivatives to stimulate ECs proliferation, migration and enhance *in vitro* and *in vivo* angiogenesis has been recently reported [9,14–20]. These features has been mainly attributed to platelet-rich-plasma (PRP). PRP has been mixed with biomaterials [9,18,19], adsorbed onto scaffolds [20], used as PRP-gel [17] or by itself [14–16]. Moreover, reports have shown that PRP, used as extract in GFs-reduced Matrigel, can promote the formation of tube-like structures (TLS) of ECs within less than 24 h, which reinforces its angiogenic potential [14–16]. However, Matrigel or other similar rich basement membranes are from animal sarcoma origin, thus are not considered a suitable option for human application [21].

Herein, we propose the reconstruction of angiogenic nanobasement membranes-like constructs by using platelet lysate (PL) – as a source of multiple angiogenic factors-, marine-origin polysaccharides – as stabilizers -, and layer-by-layer assembling (LbL) – for a controlled assembling – Fig. 1a. PL was obtained by lysing human platelet concentrates by freezing-thawing cycles – Fig. 1b.

LbL is a simple and versatile technique comprising the alternated deposition of polyelectrolytes (PEs) interacting by electrostatic, or other types of interactions, and can be performed under mild conditions [22–24]. In order to achieve an efficient EC activation mediated by GFs, their stability, conformation and density presented to the cells must be adequate. Moreover, the type of binding between the GF and their stabilizer will affect the intracellular signal transduction [25] – Fig. 1c. Under this context, several PEs were assembled with PL, and in order to preliminarily assess the pro-angiogenic potential of the nanocoatings, human umbilical vein ECs (HUVECs) adhesion, proliferation, morphology and gene expression were analyzed.

2. Materials and methods

2.1. Materials

Medium molecular weight chitosan (Chi), with a degree of deacetylation of 80% (Sigma Aldrich, MKBB0566), was purified by a re-precipitation method. Briefly, Chi powder was dissolved in 2% (v/v) acetic acid solution with 1% (w/v) concentration. The mixture was stirred overnight at room temperature. The impurities were removed by four filtration cycles. Then, Chi was precipitated by addition of 1 M NaOH while stirring. Final steps consisted on washing Chi with distilled water until reaching a neutral pH and on Chi dehydration rising with ethanol–water mixtures with increasing ethanol content (20–100% v/v). Chi was freeze-dried for 3 days and ground. κ- (Sigma–Aldrich, 22048), ι- (Fluka, 22045), λ-carrageenan (Car; Sigma–Aldrich, 22049), sodium heparin (Hep; Sigma–Aldrich, H3149), sodium alginate (Alg; Sigma Aldrich, 250 cP), and poly(ethyleneimine) solution (PEI; Sigma–Aldrich, P3143) were used as received.

2.2. Materials preparation

2.2.1. Preparation of platelet lysate

Platelet concentrates were obtained from different platelet collections performed at Instituto Português do Sangue (IPS, Porto, Portugal), under a previously established cooperation protocol. The components were obtained using the Trima Accel® Automated Blood Collection System. All the platelet products were biologically qualified according to the Portuguese legislation. The platelet count was performed at the IPS using the COULTER® LH 750 Hematology Analyzer and the sample volume adjusted to 1 million platelet μL^{-1}. The collected samples were subject to three repeated temperature cycles (frozen with liquid nitrogen at −196 °C and heated at 37 °C) and frozen at −20 °C until further use. The remaining platelets were eliminated by centrifugation at

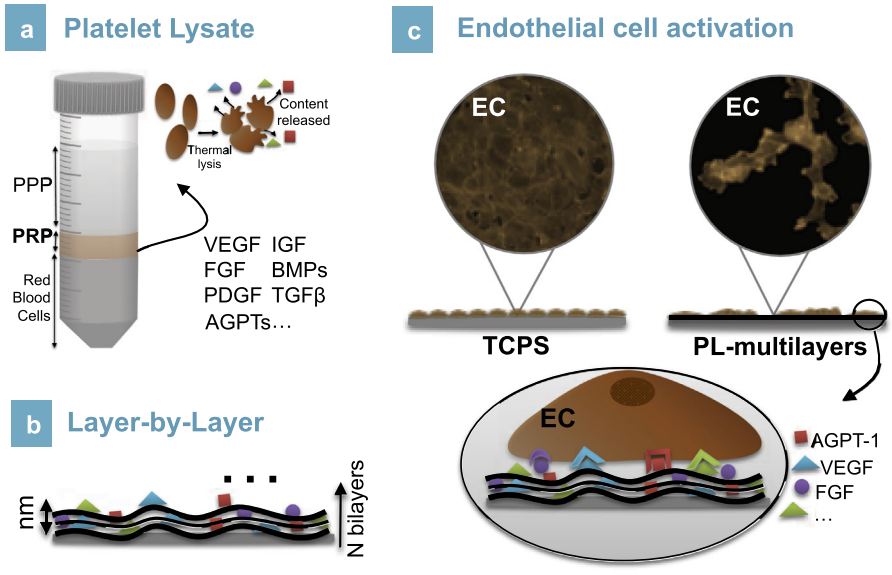

Fig. 1. Schematic representation of the approach followed. (a) PL preparation. (b) Layer-by-Layer assembling onto tissue culture polystyrene (TCPS) surfaces. (c) Culture of endothelial cells (ECs) during 20 h on the nanocoatings and expected interaction through VEGF, FGF, Angiopoietin-1 and other receptors.

Please cite this article in press as: S.M. Oliveira et al., Platelet lysate-based pro-angiogenic nanocoatings, Acta Biomater. (2015), http://dx.doi.org/10.1016/j.actbio.2015.12.028