Acta Biomaterialia 42 (2016) 147–156

Contents lists available at ScienceDirect

Acta Biomaterialia

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

Full length article

Improved scaffold biocompatibility through anti-Fibronectin aptamer functionalization

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ARTICLE INFO

Article history:
Received 9 November 2015
Received in revised form 17 June 2016
Accepted 19 July 2016
Available online 20 July 2016

Keywords:
Hydrogels
Biomimetics
Cell culture techniques
Osteoblasts
Cell adhesion

ABSTRACT

Protein adsorption is the first and decisive step to define cell-biomaterial interaction. Guiding the adsorption of desired protein species may represent a viable approach to promote cell activities conducive to tissue regeneration. The aim of the present study was to investigate whether immobilized anti-Fibronectin aptamers could promote the attachment and growth of osteoblastic cells.

Polyethyleneglycole diacrylate/thiolated Hyaluronic Acid hydrogels (PEGDA/tHA) were coated with anti-Fibronectin aptamers. Hydrogel loading and Fibronectin bonding were investigated, through spectrophotometry and Bradford assay. Subsequently, human osteoblasts (hOBs) were cultured on hydrogels for 10 days in 2D and 3D cultures. Cells were monitored through microscopy and stained for focal adhesions, microfilaments and nuclei using fluorescence microscopy. Samples were also included in paraffin and stained with Hematoxylin-Eosin. Cell number on hydrogels was quantitated over time. Cell migration into the hydrogels was also studied through Calcein AM staining. Aptamers increased the number of adherent hOBs and their cytoplasm appeared more spread and richer in adhesion complexes than on control hydrogels. Viability assays confirmed that significantly more cells were present on hydrogels in the presence of aptamers, already after 48 h of culture. When hOBs were encapsulated into hydrogels, cells were more numerous on aptamer-containing PEGDA-tHA. Cells migrated deeper in the gel in the presence of DNA aptamers, appearing on different focus planes. Our data demonstrate that anti-Fibronectin aptamers promote scaffold enrichment for this protein, thus improving cell adhesion and scaffold colonization.

Statement of Significance

We believe aptamer coating of biomaterials is a useful and viable approach to improve the performance of scaffold materials for both research and possibly clinical purposes, because different medical devices could be envisaged able to capture bioactive mediators from the patients' blood and concentrate them where they are needed, on the biomaterial itself. At the same time, this technology could be used to confer 3D cell culture scaffold with the ability to store proteins, such as Fibronectin, taking it from the medium and capture what is produced by cells. This is an improvement of traditional biomaterials that can be enriched with exogenous molecules but are not able to selectively capture a desired molecule.

1. Introduction

Biomaterials, once inserted into the surgical wound, get in contact with blood and spontaneously adsorb plasma proteins [1,2], which are attracted and retained on biomaterial surfaces mostly by weak electrostatic, dipole bonds [3]. The presence of adsorbed proteins triggers subsequent host reactions, such as blood clot for-

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http://dx.doi.org/10.1016/j.actbio.2016.07.035
1742-7061/

mation, inflammation and cell attachment on biomaterials [4]. However, protein adsorption on biomaterials is mostly a haphazard process, which is mainly driven by the chemical and physical characteristics of the material and by protein availability and conformation, and which may result in the adsorption of proteins which do not convey specific or useful stimuli to cells [5,6]. As a consequence the control of protein adsorption through the supply of adequate stimuli can make cell adhesion and colonization of the biomaterial easier, and can also affect subsequent cell behaviour. To this purpose, approaches available in the literature to stimulate cell function and enhance bone formation include the use of chemical and physical treatments, which allow the surface enrichment with functional groups that preferentially bind desired proteins [7–9]. Alternatively, implantable biomaterials can be directly coated with bioactive molecules, which mimic the natural ECM [10–16], or protein fragments, e.g. RGD sequences and other recognition sequences for integrins [17].

A possible alternative approach to enhance the biological activity of a biomaterial is to promote adsorption of bioactive molecules from the host, by means of receptors that can specifically bind to and enrich the biomaterial surface with proteins of interest, to provide an endogenous stimulus to cell colonization. To this purpose, the present study describes the coating of hydrogel scaffolds with ssDNA aptamers (Fig. 1A). Aptamers are small, single stranded biomolecules, typically oligonucleotides, less than 100 residues long [18,19], which specifically bind to a target molecule [20]. Aptamers therefore act as antibodies by binding target molecules, but without some of the drawbacks associated to the use of antibodies, i.e. immunogenicity and low stability.

Biomaterial coating with aptamers is a less explored field in the literature, but with a promising potential. Hoffmann et al. pioneered the field in 2007 by grafting aptamers against endothelial precursor cells on vascular prosthesis grafts to sort them from the bloodstream [21]. Similarly, Chen et al. in 2012 designed an artificial ECM by grafting aptamers against surface cell receptors on PEG-based hydrogels and thus dramatically improved cell adhesion to the substrate [22]. For the present report, hyaluronic acid-based hydrogels, commonly used for stem cells culture, were functionalized with aptamers against Fibronectin. Fibronectin was chosen as a target for the present report because it is a widely available protein in plasma, so it would readily be available after implant insertion, in a hypothetical surgical scenario. Moreover Fibronectin plays a pivotal role in wound healing by providing a substrate for cell attachment during the formation of granulation tissue [23], and because of that it has been proposed as a coating for implantable biomaterials [24–26]. Although the use of anti-Fibronectin aptamers has been previously shown to serve as a tool to inhibit cell adhesion by impeding the interaction of integrins with cell binding domains [27], this is the first report of aptamers directed against Fibronectin to improve cell adhesion on a scaffold.

Our work shows that aptamers against proteins with adhesive properties such as Fibronectin can increase scaffold colonization by cells, enhancing their adhesion and growth both in 2D and in 3D cultures *in vitro*.

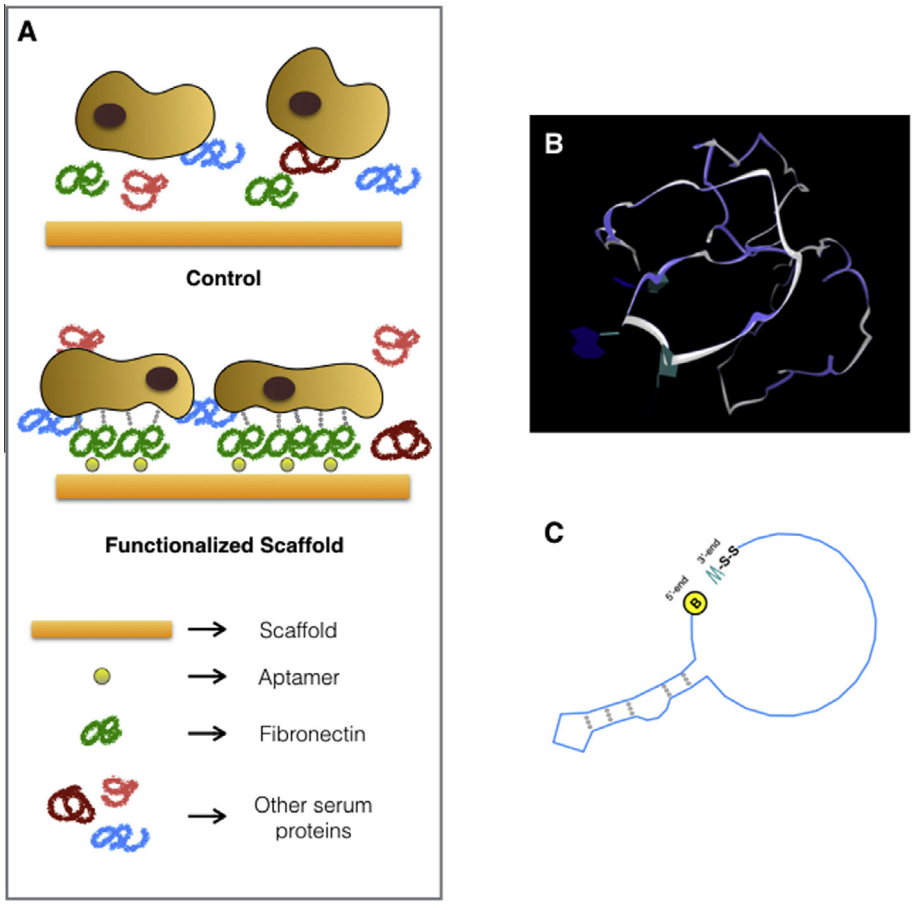

Fig. 1. (A) Diagram representing the rationale for aptamer-coated scaffold to retain specific target proteins. Un-coated scaffolds adsorb proteins from the extracellular environment based on their availability and their chemistry, mostly due to aspecific weak bonds (Control). Aptamers specifically retain target proteins and enrich scaffolds for the desired biological stimulus (Functionalized Scaffold). (B) 3D rendering and (C) 2D reconstruction of anti-Fibronectin aptamer (Courtesy of Dr. Rafal Drabek).