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Effect of tissue scaffold topography on protein structure monitored by fluorescence spectroscopy

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ABSTRACT

The impact of surface topography on the structure of proteins upon adhesion was assessed through non-invasive fluorescence monitoring. This study aimed at obtaining a better understanding about the role of protein structural status on cell–scaffold interactions. The changes induced upon adsorption of two model proteins with different geometries, trypsin (globular conformation) and fibrinogen (rod-shaped conformation) on poly-L-lactic acid (PLLA) scaffolds with different surface topographies, flat, fibrous and surfaces with aligned nanogrooves, were assessed by fluorescence spectroscopy monitoring, using tryptophan as structural probe. Hence, the maximum emission blue shift and the increase of fluorescence anisotropy observed after adsorption of globular and rod-like shaped proteins on surfaces with parallel nanogrooves were ascribed to more intense protein–surface interactions. Furthermore, the decrease of fluorescence anisotropy observed upon adsorption of proteins to scaffolds with fibrous morphology was more significant for rod-shaped proteins. This effect was associated to the ability of these proteins to adjust to curved surfaces. The additional unfolding of proteins induced upon adsorption on scaffolds with a fibrous morphology may be the reason for better cell attachment there, promoting an easier access of cell receptors to initially hidden protein regions (e.g. RGDS sequence), which are known to have a determinant role in cell attaching processes.

1. Introduction

The physiologic behaviour and morphology of cell tissues depend largely on the degree of interaction between cells and cells with the extracellular media during growth (Ge et al., 2013). In supported cell growth, the interaction of cells with the involving media is mediated by the solid supporting matrices, so called tissue scaffolds, with the interaction being largely influenced by the chemical and topographical characteristics of the scaffold surfaces (Jiang and Papoutsakis, 2013). Optimal tissue scaffolds are those that guarantee a stable cell attachment (Zonca et al., 2013), allowing efficient interactions between cell receptors and specific regions of the adhesion proteins (Imen et al., 2008), which pre-adsorb at the scaffold surface, and simultaneously promote a controlled, non-limited access of the cell to extracellular nutrients and the removal of excreted waste products.

The impact exhibited by the topography of scaffold surfaces on cell growth has been reported by several researchers. It is known that the use of cell supports with regular and aligned channels favours cell orientation, a phenomenon often referred to as contact 'guidance', leading to the formation of well-organized tissues (Morelli et al., 2010; Papenburg et al., 2010a; Truckenmüller et al., 2012). Moreover, several studies have revealed that the use of solid matrices with aligned patterns is also essential to assure adequate cell maturation and the regulation of diverse cell mechanisms (Chew et al., 2008). The presence of topographical induced cell orientation effects has been supported by confocal laser scanning microscopy studies using specifically labelled proteins (Braber et al., 1998; Morelli et al., 2010) and scanning electron microscopy (SEM) analysis (Zhong et al., 2006; Morelli et al., 2010) which revealed that cellular growth and proliferation occur parallel to the long axes of the surface patterns or, in case of scaffold surfaces with fibrous morphology, along the fibres. In contrast, flat or randomly

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patterned surfaces generate non-oriented cell growth leading to less structured cell tissues.

Different hypothesis have been proposed to explain the diverse behaviours observed. Some authors suggested that they may be related to differences in local surface free energy (or surface wettability) that restrains protein and cell attachment to specific surface regions. Others have attributed the preferential cell orientation to the presence of a minimal length for cell attachment of 2 μm that forces cells to attach along the longer axes of surface patterns (Izzard and Lochner, 1976). Alternatively, preferential cell attachment may be correlated with the anisotropic geometry of surfaces (Papenburg et al., 2010b) which forces the orientation of cells due to a required energetic balance between surface stresses and shear free planes (Chen, 2008; Guilak et al., 2009). This effect generates mechanotransduction processes which rule primarily cell shape (Guilak et al., 2009; Horbett, 1994) and then the intrinsic cell signalling mechanisms. These mechanisms are driven by the tensional forces between extracellular matrix (ECM) and cells upon cell attachment to scaffolds, which subsequently regulate cell proliferation and differentiation paths. Also, a recent study from Fujita et al. (2009) revealed that cell protrusions which expand perpendicularly to the nanogroove direction retract more quickly than those that move parallel to it. This effect led these authors to conclude that the cell retraction process may contribute to the cell elongation and alignment in nanogrooved surfaces. The relationship between surface topography and cell growth profile is still unclear; however, it has also been hypothesized that this cell contact guiding effect exerted by specific surface topographies may be directed by the structural status of the different adhesion proteins (e.g. fibrinogen, fibronectin, vibronectin, etc.) that primarily adsorb at the scaffold surface during the growth process.

Based on this effect, it is reasonable to speculate that the efficiency of cell anchorage to scaffolds is intimately related to the mode how surface impacts on the structure and molecular flexibility of the adhesion proteins since it may influence the accessibility of cell receptors to protein regions (Imen et al., 2009), such as arginine-glycine-), such as arginine–glycine–aspartic acid (RGD) sequences, which are known to be determinant in cell attaching processes (Horbett, 1994).

The ability and the way proteins adsorb at a given surface depend on their conformation and also the structural status of an adsorbed protein relies on the surface topography. This relationship was clearly demonstrated in the work developed by Roach et al. (2006) which highlights the contrasting effects induced on globular and rod-like proteins upon contacting surfaces with different patterns. These authors have shown that the contact of a rod-like protein, such as fibrinogen, to silica surfaces with accentuated curvature results in a significant change of its secondary structure, characterized by a loss of the α-helix structure in benefit of random coil chain segments, which is related to its capacity to fit the surface. Contrastingly, and due to their inability to adapt to surfaces with high curvatures, globular proteins exhibit an opposite structural behaviour when adsorbed at an identical surface, registering a decrease of the α-helix content with the decrease of surface curvature.

Studies of the impact of surface topography in cell growth have shown that cell spreading, adhesion and differentiation also depend on the dimensions of the surface topographical features (Dalby et al., 2002, 2003), and fibre diameter (Elias et al., 2002) when fibrous scaffolds are used. However, a clear relationship between structural changes induced upon attachment of plasma proteins to the scaffold surface and the degree of cell orientation achieved with different surface topographies has not been established so far.

Fluorescence spectroscopy is a highly sensitive technique capable of providing simultaneously valuable information about protein structure and mobility, which has not been sufficiently explored so far for monitoring of cell growth at tissue scaffolds. Therefore, in this work, a fluorescence monitoring methodology combining data from steady-state fluorescence and fluorescence anisotropy is used to assess the impact of surface topography on the structure and molecular flexibility of proteins. Protein structural changes can be inferred from the fluorescence emission of a protein intrinsic fluorescence probe, tryptophan. The use of tryptophan as a structural reporter excludes the need of protein labelling with an external chromophore. Based on the high sensitivity of the fluorescence emission of tryptophan to changes of the physicochemical properties in its surrounding environment, fluorescence emission allows for in situ monitoring of the changes of protein structure (Portugal et al., 2006, 2007; Guedidi et al., 2012) and the mode that each geometrically different protein interacts to the surface. Fluorescence anisotropy measurements allow for eliciting information about the influence of the different surface topographies on molecular flexibility. The present study was conducted using model proteins with opposite conformational geometries: a globular protein, trypsin (largely used in adsorption studies, presenting a significant fluorescence emission) and a rod-like protein, fibrinogen, which integrates the group of adhesion proteins directly involved in the cell–tissue scaffold interaction process. The two proteins were selected in order to mimic the conformational diversity (globular and rod-like proteins) of the proteins involved in the cell attachment process.

This work aims at showing the potential impact of the structural status of proteins induced by scaffold surface topography on cell attachment using a non-invasive fluorescence monitoring approach. To achieve this goal, a correlation between the topographically induced structural changes of the adhesion proteins and the growth of C2C12 mouse pre-myoblast cells at these scaffolds was established. As described in previous papers from some of the authors of the present work (Haneveld, 2006; Papenburg et al., 2010a; Bettahalli et al., 2012) the use of fibrous scaffolds improves cell–cell interactions while allowing a good spreading of the C2C12 cells.

2. Experimental

2.1. Preparation of protein solutions

Protein solutions of trypsin from bovine pancreas (EC. 3.4.21.4, Ref. T9935 from Sigma–Aldrich), a globular protein with MW of 20.3 kDa (Fig. 1(a)), and fibrinogen from bovine plasma (EC 232-598-6, Ref. F8630, from Sigma–Aldrich), a rod-like protein with MW of 340 kDa (Fig. 1(b)), with respective concentrations of 2 g/L and 0.7 g/L were prepared in 0.1 M Trizma® buffer at pH 8 containing 0.02 M of $CaCl_2$, added to prevent trypsin autolysis. Trizma buffer was prepared using Trizma® hydrochloride (Sigma Aldrich) and Trizma® base (Sigma Aldrich). To avoid protein structural changes due to the physicochemical conditions, both protein solutions were prepared using the solvent media with identical characteristics. Protein concentration was selected in order to be in the range of the typical cell culture media protein content and to assure the detection of fluorescence emission from the protein molecules adsorbed at the scaffold surfaces. Differences in concentrations of both protein solutions were due to the low solubility of fibrinogen in the Trizma buffer used.

2.2. Protein adsorption on PLLA scaffolds with distinct topographies

Trypsin and fibrinogen were separately adsorbed at poly-L-lactic acid (PLLA) scaffolds with different surface patterns: flat, aligned nanogroove pattern and random fibrous surfaces. Surfaces with aligned nano-patterning were obtained by using nano imprint