QUANTIFICATION OF THE ADHESION STRENGTH OF ENDOTHELIAL CELLS ATTACHED ON ACELLULAR BOVINE PERICARDIAC TE SCAFFOLDS

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Introduction

Cells within tissues are surrounded by extracellular matrix (ECM) that supports cell adhesion via integrin receptors. In TE scaffolding, the ability of seeded cells to adhere with scaffold material is of paramount importance for potential regenerative cell response to biomechanical stimulation. The strength of seeded cell interactions with extracellular matrix under the application of a shear stress field had been quantified in this work. The two types of decellularized bovine pericardial tissues were compared on the basis of the magnitude of the cell detachment strength.

Methods

By applying a radially increasing shear stress in a spinning disc device, the adhesion strength of aortic bovine endothelial cells cultured for 4 days on bovine pericardial tissues, previously decellularized by detergent (BP1) or enzymatic (BP2) protocols, was determined by measuring the shear stress necessary to detach the cells from their substrate [Pagoulatou, 2012]. A shear stress (τ) field, ranged from zero (center) to a maximum of 119 dyn/cm^2 at edges, was applied to the cells for 10 min. After cells' nuclear staining (DAPI), cell surface density across two rectangular specimen diameters was measured by fluorescence microscopy and averaged. Detachment of the cells was thus determined with respect of τ.

Results

The application of τ resulted in gradually increased cell detachment from the center to the edges, following corresponding increment of shear stress. Supposing no cell detachment at central region (where τ limits to zero) a reduced attached cell surface density, with respect τo 100% at center, was computed. Results showed a gradual, near exponential, decrease (Fig. 1) in cell density from 100% to a minimum 15,53 ± 5,21% for the BP1, lower than that of BP2 substrate where cells were remain attached at edge region with a density of 44,32 ± 7,85% (mean ± SDEV, n=4) at maximum τ (119 dyn/cm^2).

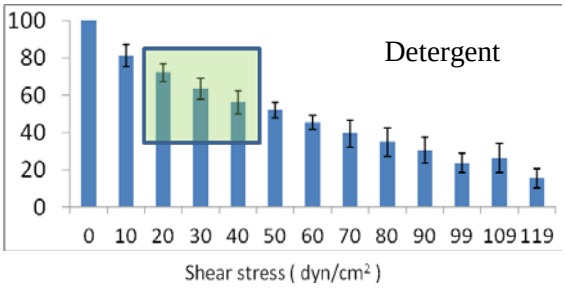

Figure 1. Percentage (%) of the endothelial cells remained attached at the surface of biomaterial after being submitted to a shear stress field.

Discussion

The spinning disc device was adopted to be used in conjunction with a fluoresce microscope, which allows imaging, analyzing and counting of fluorescently stained cells on biomaterials. The proposed method enables an improved quantification of the adhesion strength since the exact number of cells before and after rotation at a given position can be determined. Fine cell spreading and proliferation on biomaterials' surface and good surface cell density (>60%) at physiological shear stress scale (15-40 dyn/cm^2 [Silver, 2006]) were observed and measured. In conclusion, successfully re-endothelialized acellular natural derived biomaterial revealed cell-adhesion properties on biomaterial's surface, which are likely to be favorable to improve neo-tissue regenerative performance of biomaterials.

Reference

Pagoulatou *et al*, J Mater Sci Mater Med, 23:1387-1396, 2012.

Silver *et al*, *Circulation*, 113:2787-2789, 2006.