GUIDED FUNCTIONAL RE-ENGINEERING OF THE MITRAL VALVE LEAFLETS

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Introduction

Mitral valve regurgitation represents the second major valvular disorder in the western world, whereas current strategies for mitral valve reconstruction are imperfect [1]. The aim of this study was to develop a TE substitute for mitral valve leaflet reconstruction using acellular porcine pericardium seeded with porcine mesenchymal stem cells (pMSC).

Methods

Porcine pericardial scaffolds were decellularised as described previously [2]. pMSC were cultured on the mesothelial surface of the scaffolds (3 cm diameter) under static conditions, using 3 different cell densities (2×10^4, 1×10^5 and 2×10^5 cells/cm^2). The seeded scaffolds were analysed by scanning electron microscopy (SEM), H & E staining and live/dead staining at 1, 3 and 7 days (Table 1). Following 3 days of static culture, samples seeded with 1×10^5 cells/cm^2 were cultured dynamically (10% strain) for 1 day in a biaxial strain bioreactor (Fig. 1). Following dynamic conditioning, the samples were assessed for cell viability with live/dead staining and MTT assay, and for extracellular matrix (ECM) integrity with H&E (Fig. 2).

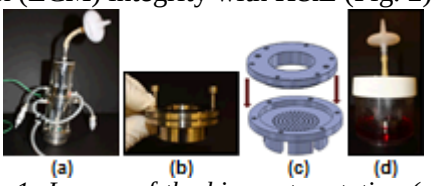

Figure 1: Images of the bioreactor station (a) and tissue loaded on the tissue holders (c, d) and cultured in the culture tubs (d).

Results

The optimum seeding density for acellular pericardial samples was 10^5 cells/cm^2. Samples seeded with this density and maintained statically for 3 days, prior to dynamic conditioning, showed the best cell penetration without a significant disruption in the ECM (Fig. 2). Seeded samples conditioned dynamically for 1 day showed similar levels of viable cells to seeded samples cultured statically for 1 day (Fig. 3 & 4). Cell alignment was also obvious in the dynamically conditioned samples.

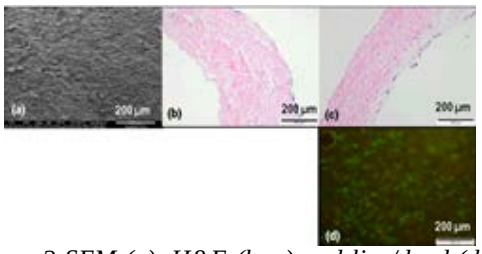

Figure 2:SEM (a), H&E (b, c) and live/dead (d) of scaffolds seeded with pMSCs at 10^5 cells/cm^2 for 24 h (a), 3 (b) and 7 days (c, d).

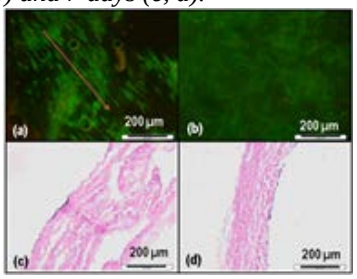

Figure 3:Live/dead (a, b) and H&E (c, d) of seeded samples cultured for 1 day dynamically (a, c) and statically (b,d).Arrow indicates cell directionality.

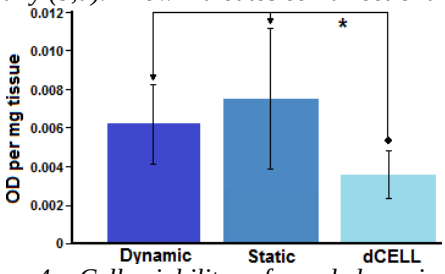

*Figure 4: Cell viability of seeded pericardial scaffolds cultured dynamically and statically after 1day. Decellularised pericardial scaffolds without any cells (dCELL) were used as - control. Means ± 95% C.I., n=4. * indicates significant difference.*

Discussion

Acellular pericardium was shown to be an optimum material for cell repopulation. Reseeded scaffolds were viable after 1 day under 10% dynamic strain. This study provided the basis for optimising the mechano-stimulation of cell-seeded pericardial scaffolds *in vitro* in order to generate heart-valve like tissue.

References

1. Chockalingam *et al*, J Heart Valve Dis, 13(1), 2004.
2. Fisher J *et al*, Matrice decellularisation. Patent PCT/GB02/02341, 2000.