

## IN VITRO VASCULARISATION OF COLLAGEN-GAG SCAFFOLDS USING MESENCHYMAL STEM CELLS

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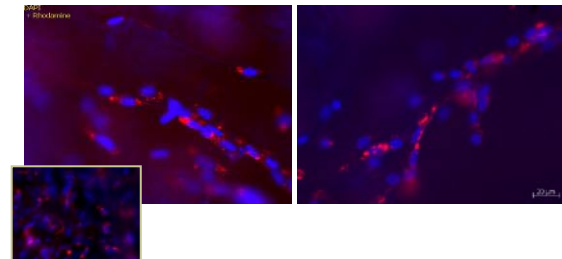
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**INTRODUCTION:** Proximity to a capillary network is essential for survival of most cell types. Consequently, in tissue engineering, implanted cell-seeded scaffold constructs frequently suffer from avascular necrosis before vessel ingrowth from the host can occur. We aim to engineer capillary-like networks in vitro, within a type I collagen-glycosaminoglycan (CG) scaffold optimised for bone tissue engineering, which might then integrate with host vasculature in vivo. Mesenchymal stem cells (MSCs), which have recently shown potential to differentiate towards the endothelial lineage [1-3], were employed as candidate vasculogenic cells, with smooth muscle cells (SMCs) as a potential vessel stabilising element [4].

**METHODS:** Highly porous CG scaffolds (~3 mm thick) were fabricated using a lyophilisation process as previously described [5] and used as 1 cm diameter discs followed by chemically crosslinking (EDAC) [6]. MSCs were extracted from the bone marrow of Wistar rat and expanded in 10% serum-supplemented DMEM according to routine techniques in our lab [7]. SMCs and endothelial cells (ECs) were also employed in some experiments. Four endothelial differentiation media were tested and EC medium from Cell Applications Inc. was optimal for promoting vascular structure formation by MSCs on Matrigel. Red or green fluorescent cell membrane labels (PKH26 and PKH2 respectively, Sigma) were used to facilitate morphological observations. RNA levels were measured by quantitative RT-PCR, normalising target RNA values to rRNA as endogenous reference.

**RESULTS:** Dye-labelled MSCs or ECs were seeded into the CG scaffold and cultured in EC medium (Figure 1). Cord-like structures developed from both cell sources after approx 1-2 weeks and persisted for at least 3-4 weeks. DMEM-cultured MSC constructs did not generate similar structures (Figure 1, inset) and, macroscopically, these constructs underwent

cell-mediated contraction. These observations correlated with a much greater fall in the RNA levels in MSCs for the contractile protein  $\alpha$ -Smooth Muscle Actin ( $\alpha$ SMA) in EC medium than in DMEM, converging towards levels in ECs. A similar trend was seen for Transferrin Receptor (CD73) RNA levels, another marker of undifferentiated MSCs, which fell to a level equal to that in ECs at day 24 of scaffold culture, and Endoglin (CD105) RNA levels also declined in EC medium. The endothelial marker PECAM-1, in contrast, rose in both cell types in EC medium. Thus, the RNA data are consistent with the idea that the MSCs cultured in the scaffold in EC medium are undergoing endothelial differentiation.



*Fig 1: Micrographs from near-surface of CG scaffolds seeded with red-labelled MSCs (left) or ECs (right) in EC medium, day 24.*

**DISCUSSION:** The results presented here show that MSCs can form structures suggestive of early vascular formations in the CG scaffold when cultured in an optimal EC medium. Furthermore, the addition of SMCs as a vessel stabilizing cell source resulted in the formation of larger and more complex vessel structures.

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