Effect of Scaffold Architecture and Time on Cell Viability in the Interior of Tissue Engineering Constructs

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Statement of Purpose: Scaffold architecture plays a crucial role in the success of scaffold-based tissue engineering constructs. Important architectural parameters include pore size, porosity and permeability, which affect transport of nutrients, removal of waste as well as cell migration. In this study, cell viability in the scaffold interior was assessed by the ability of cells to take up a viability dye as a function of architecture and time.

Methods: Two different scaffold architectures (types 1 and 2) with the same pore size and porosity but different tortuosities, and thus different permeabilities, were evaluated for cell viability as a function of depth within the construct at day 1. The type 1 architecture was evaluated at day 7 as well. Cylindrical poly(ϵ-caprolactone) (PCL) scaffolds of porosity 48%, channel size 200 microns and height and diameter 1 cm were fabricated using precision extrusion deposition. The bases of the scaffolds were sealed with a layer of PCL and the scaffolds were surrounded with a tubing of low air permeability to allow diffusion of air into the constructs only from the top. Following sterilization and presoaking in fibronectin solution, the scaffolds were statically seeded with 2 million MG63 cells. The constructs were evaluated at days 1 (types 1 and 2) and 7 (type 1) (n = 3) for cell viability as a function of depth. For this purpose each construct was removed from its surrounding ring and soaked in a solution of Calcein AM cell viability dye (Molecular Probes, Eugene, OR) in PBS for the viable cells to take up the dye at the particular time point of interest. The constructs containing the Calcein AM-treated cells were then sectioned vertically using box-cutter blades, into 4 parts. The vertical face of each part was imaged under a fluorescence microscope for identifying cells that had taken up the dye. Regions of the scaffolds were divided into 3 sections: top, middle and bottom, each corresponding to an approximately 3 mm height division of the vertical face. The % area fraction occupied by the live cells per unit area of scaffold in each division was calculated using Vision Assistant 8.0 imaging software (National Instruments, Austin, TX). Statistical significance was determined using one way Anova followed by Student-Newman-Keuls Test.

Results/Discussion:

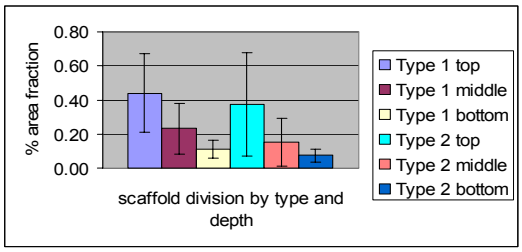

Fig.1. % Area fraction for different regions of scaffolds of type 1 and 2 on day 1. Bar heights indicate mean ± SD.

There were significant differences ($p \leq 0.01$) observed between the top and middle as well as top and bottom sections of both architectures (Fig.1). For both architectures, greatest cell viability, as reflected by the % area fraction occupied by the cells on the scaffolds, was observed at the top, slightly less in the middle and the least at the bottom. This could be attributed to greater availability of oxygen in the top region as the sides and bases of the scaffolds were sealed. However, there were no significant differences observed between the corresponding sections (i.e. type 1 top and type 2 top, etc.) of both architectures, thereby indicating that tortuosity, and thus permeability, does not significantly affect cell viability in the construct interior for the specific architectural parameters selected up to 1 day.

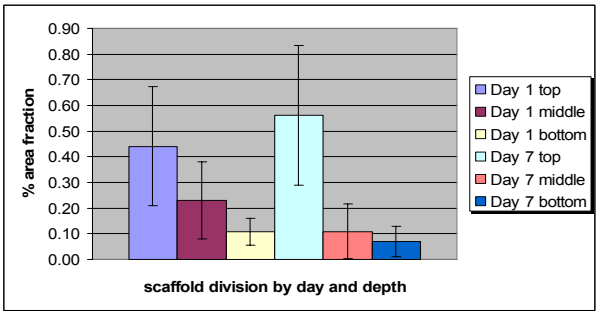

Fig.2. % Area fraction for different regions of scaffolds of type 1 on days 1 and 7. Bar heights indicate mean ± SD.

Significant differences ($p \leq 0.01$) were also observed between the top and middle as well as top and bottom sections on both days for the type 1 architecture (Fig.2). The % area fraction displayed a trend similar to what has been described earlier. However, the values for day 7 top were significantly greater ($p \leq 0.05$) than day 1 top, indicating the possibility of cell proliferation occurring in the region or cells from below migrating to the top. The latter possibility is supported by the fact that the % area fraction for day 7 in the middle and bottom sections was lower than that observed for the corresponding sections on day 1, although not significantly different. Thus, the cells in these regions could have either died probably due to inability of the cells to survive at those depths owing to oxygen diffusion constraints or the cells could have migrated towards greater oxygen supply which is available at the top.

Conclusions: For both architectures and on both days, greatest cell viability was observed at the top, slightly less in the middle and the least at the bottom. Scaffold permeability did not significantly affect cell viability in the construct interior for the specific scaffold architectural parameters considered up to a period of 24 hours. Cell viability increased in the top region but decreased in the middle and bottom regions of the construct over the duration of a week for scaffold architecture of type 1.