FLUID FLOW PROFILE AFFECTS TISSUE-ENGINEERED CONSTRUCT MORPHOLOGY IN BIOREACTORS

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Introduction

The complex relationship between the hydrodynamic environment in bioreactors and the cultured tissue directly impacts on the design and production of clinically relevant tissue engineered (TE) implants [Hutmacher, 2008]. In this work we employed a cost-efficient computational approach to determine the optimal TE construct location in a perfusion bioreactor by using a volume-averaged computational fluid dynamic simulation (CFD). The location affected the flow profile at the entrance of the TE construct. Subsequently the effect of TE construct positioning during perfusion culture on several important TE construct quality characteristics was experimentally evaluated over time.

Methods

CFD: For the CFD simulation the scaffold was assumed to be a porous medium. The Brinkman equation, a combination between free flow profile (Stokes equations) and porous medium flow (Darcy law) was employed to model flow environment in the bioreactor.

Experimental: Human periosteum derived cells (hPDCS) were culture on titanium alloy (Ti6Al4V) scaffolds in a perfusion bioreactor setup. Cell number was determined via DNA content analysis by using a quantitative DNA assay. For the determination of the extracellular matrix (ECM) quantity and 3D distribution throughout the TE construct contrast enhanced nanoCT (CE-nanoCT) was employed [Kerckhofs, 2013].

Results

From the CFD simulations we determined a critical length (L_{crit}), beyond which a steady state laminar flow profile was reached, for a range of flow rates. Bioreactor cultures were run for TE constructs positioned at the bioreactor chamber entrance (L_o) and beyond the determined L_{crit}. DNA content analysis showed a statistically significant increase for those scaffolds cultured beyond L_{crit} both at culture day 14 as well as day 21. In the case of L_{crit} cultures smaller standard deviations were observed between batches for both time points. Furthermore, CE-nanoCT analysis demonstrated that for TE constructs cultured at L_o a lower quantity of ECM was found and also the ECM distribution was less homogeneous compared to the respective time points of L_{crit} cultures.

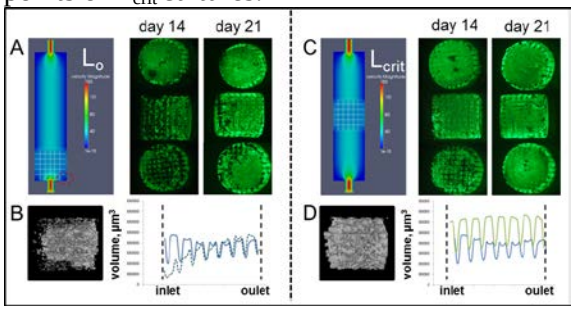

Figure 1:TE construct were positioned at (A) the bioreactor entrance (L_o) and (C) above the CFD determined critical length (L_{crit}). Live/dead visualization of TE constructs for culture day 14 and 21 is shown for both cases for cell distribution evaluation. CE-nanoCT reveals ECM inhomogeneity and lower content for L_o (B) while homogeneity and quantity are increased for L_{crit} (D).

Discussion

The non-uniform flow and shear stress profile developed for TE constructs at L_o did not provide the optimal flow and mass transport environment for the cells, thus resulting in TE constructs of inferior quality. The volume-averaged approach used in this work in combination with the time-dependent TE construct geometric characterization via CE-nanoCT provides crucial input for more accurate computational modeling, that can take into account the gradual change of the internal TE construct properties over time.

References

Hutmacher *et al*, Trends Biotechnol, 26:166-172, 2008.

Kerckhofs *et al*, Eur. Cells Mater, accepted, 2013.