Flow Perfusion Bioreactor for Bone and Cartilage Tissue Engineering

Ville V. Meretoja, Rebecca L. Dahlin, Kurtis F. Kasper, Antonios G. Mikos.
Dept. of Bioengineering, Rice University, Houston, Texas.

Introduction: In tissue engineering (TE), scaffolds are necessary for providing a three dimensional architecture on which cells may proliferate and develop into a functioning tissue. Mass transfer limitations in commonly used static cultures, however, usually lead to constructs with viable cells and deposited extracellular matrix on the exterior portion of the scaffold only. Enhanced mass transfer and more uniform cell distribution as well as mechanical stimulation can be provided by flow perfusion bioreactors which pump media through the internal pore network of scaffolds. The aim of this study was to develop a new flow perfusion bioreactor suitable for both cartilage and bone engineering, allowing increased sample through-put and cost efficient use of growth factors within bioreactor cultures.

Methods: Major design requirements for the new bioreactor included reduced medium volume, increased number of TE constructs and decreased contamination risk. To test the new bioreactor, rat and rabbit bone marrow derived mesenchymal stem cells were first expanded for up to 3 passages, seeded at a density of 5-10 x10^6 cells / mL scaffold volume and left to adhere for 24 hours. Scaffolds were then divided into static and flow perfusion groups, and cultures were continued in osteogenic (dexamethasone, ascorbic acid, β-glycerophosphate) or chondrogenic (dexamethasone, ascorbic acid, TGF-β3) conditions for up to 21 days.

Results: The bioreactor consists of a custom made polycarbonate flow chamber and loading cassettes for scaffolds, connected to an external medium reservoir by silicon tubing to form a 20 mL circulation loop per 4 constructs. The scaffolds were press fit into cylindrical loading cassettes and supported by a PEEK mesh to create a straight flow path. All bioreactor parts are autoclavable, and the modular structure reduces the possibility of spreading contamination. A 12-channel peristaltic pump provided identical flow conditions into each perfusion chamber (Figure 1).

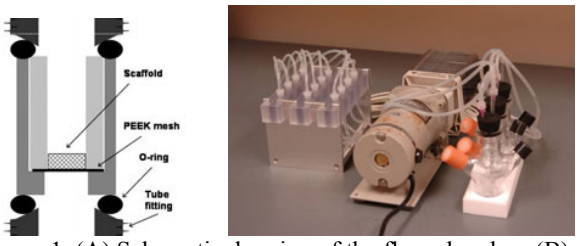

Figure 1. (A) Schematic drawing of the flow chamber. (B) Image of the new flow perfusion bioreactor.

Cultures in the novel bioreactor indicated increased cellularity and differentiation capacity in comparison to static cultures. Histological examination showed highly increased mineralization of osteogenic constructs on electrospun PCL fiber mats (Figure 2A and 2B) and cartilaginous tissue formation throughout 2 mm thick scaffolds with large pores (Figure 2C).

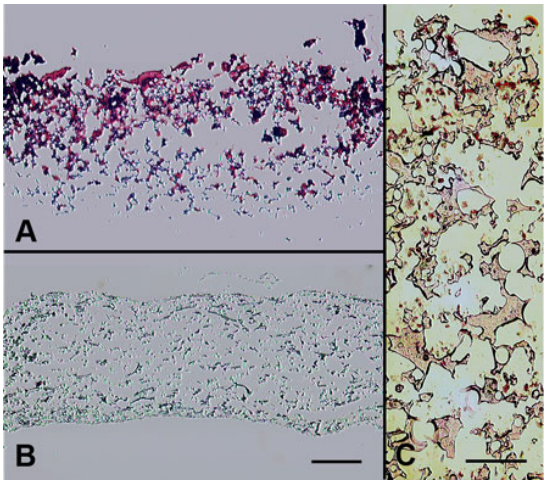

Figure 2. Enhanced mineralization in osteogenic flow perfusion cultures (A) in comparison to static (B) conditions. Alizarin Red staining. (C) Tissue penetration throughout 2 mm thick scaffolds in flow conditions. Safranin O staining. Scale bars = 300 μm.

Flow perfusion also increased the production of glycosaminoglycans (GAG) in chondrogenic cultures (Figure 3).

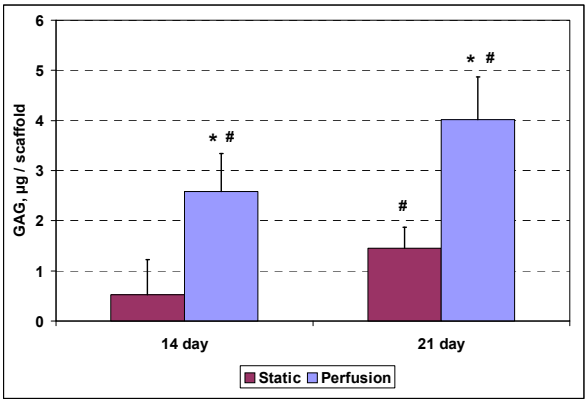

Figure 3. GAG production was increased in perfused chondrogenic cultures. * and # denotes statistically significant difference to the corresponding static culture and to the previous time point, respectively ($p < 0.05$).

Conclusions: The new flow perfusion bioreactor was able to increase both osteogenic and chondrogenic cell responses. Good cell penetration was achieved in flow conditions, and both mineralization and GAG production were increased in comparison to static cultures.