THE EFFECT OF PORE SIZE ON PERMEABILITY AND CELL ATTACHMENT IN COLLAGEN-GAG SCAFFOLDS

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Introduction

The ligand density of scaffolds for use in tissue engineering applications determines the biological activity of the scaffold. This density is determined by the composition of the scaffold and by the specific surface area of the scaffold which is a function of the mean pore size of the scaffold. It has been hypothesized that a critical range of scaffold pore sizes exists for tissue engineering applications. If the pore size lies outside of this critical range, the number of cells bound to the scaffold may be insufficient to lead to the desired cellular attachment for tissue formation. Scaffold permeability or hydraulic conductivity is also important because it controls the diffusion of nutrients and waste inside the scaffold, and the biophysical stimuli within the scaffold during mechanical loading. Such properties influence cellular biosynthesis and, inevitably, the overall success of the tissue-engineered construct. The objectives of this study were to produce collagen-glycosaminoglycan (CG) scaffolds with different mean pore sizes and to investigate the influence of pore size on permeability and on cell adhesion and viability.

Material and methods

CG scaffolds were created using an established freeze-drying technique [1] with the final temperature of freezing varied (T_f = -10, -20, -30, -40ºC) to produce a homologous series of scaffolds with a constant composition and solid volume fraction (0.005) but with four distinct pore sizes (150, 121, 110, 96 microns) [1,2]. The scaffolds were then dehydrothermally crosslinked for 24 hours in a vacuum oven.

A device was constructed to measure Darcy's permeability ($k = Ql/\Delta PA$) in the scaffolds at different levels of compression. 13mm^2 samples (3.5 mm wet thickness) of each pore size were submerged in saline solution for 24 hours prior to testing. The scaffold was clamped down with a silicone spacer (10mm diameter) onto a stainless steel mesh. The mesh supported the scaffold over an 8mm diameter brass tube, through which saline solution flowed. Permeability was measured at compressions of 0, 14, 29, and 40% for each of the four pore sizes.

MC3T3-E1 mouse clonal osteogenic cells were cultured and viable cell number was determined prior to seeding. 6 x 10^6 cells were seeded onto the four scaffold types and maintained in culture for 24 and 48 hours; at the end-point, the remaining viable cells were counted to determine the percent cell attachment.

Results

The permeability value of the CG scaffold was found to be on the order of 10^{-10} m^4/Ns. Permeability was found to increase with increasing pore size, and decrease with increasing compression ($p<0.05$). The permeability at zero compression of the smallest scaffold pore size, 96 μm, was 0.6 ± 0.12 m^4/Ns while the largest pore size, 150 μm, was 1.39 ± 0.51 m^4/Ns. Compression caused the permeability of the 150 μm scaffold to decrease to 0.46 ± 0.23 m^4/Ns at 40% compression.

Mean pore size in the four scaffolds resulted in a significant difference in cell attachment at both 24 and 48 hours. In the scaffolds with the smallest mean pore size (96 μm) over 40% of cells seeded attached to the scaffolds in both the 24 hour and 48 hours groups compared to approximately 20% of cells that remained attached to the scaffolds with the largest mean pore size (150 μm). However, there was no significant change in cell attachment between 24 and 48 hours for any group.

Discussion

The results reveal that permeability is directly related to pore size and indirectly related to compression. The scaffold's high permeability is beneficial for cell seeding, allowing the cells to diffuse into the centre of the scaffold which gives this scaffold a distinct advantage over other scaffolds used in tissue engineering applications. Varying the final temperature of freezing during scaffold manufacture produces a homologous series of scaffolds with a statistically significant difference in pore size between scaffolds. The cell seeding experiments showed that mean pore size had a significant difference in cell attachment in the different scaffolds ($P<0.05$). The fraction of cells attached to the CG scaffold decreases with increasing mean pore diameter. These results are consistent with

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scaffold ligand density being determined by pore size with an increase in density causing increased cell attachment.

References

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