Electrochemical Measurements of Diffusion through Cardiac Muscle Tissue Engineering Scaffolds*

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Cardiomyocytes, the beating cells of the heart, are highly dependent on oxygen for survival and function. Early work in cardiac muscle tissue engineering, in which cells were seeded onto a porous poly(glycolic acid) mesh, revealed that cardiomyocytes only survived within 150 μm of the scaffold's edge due to limitations in oxygen diffusion [1]. To circumvent this problem, perfusion reactors have been used to enhance oxygen transport through scaffolds with increased thickness [2]. However once implanted in the heart, engineered tissues that depend on convective transport will become oxygen limited, jeopardizing cell viability.

Porous poly(ethylene glycol) (PEG) hydrogel scaffolds have been developed using a sphere-templating method [3] to provide paths for the passive diffusion of oxygen and other nutrients, which is expected to enhance survival of the engineered tissue. To create a sphere template, monodisperse uncrosslinked poly(methyl methacrylate) microspheres are packed tightly together and sintered. Sintering fuses the microspheres, which leads to pore interconnectivity. A macromer solution containing PEG dimethacrylate is photopolymerized around the sphere template, followed by dissolution of the template, yielding the porous structure (Figure 1).

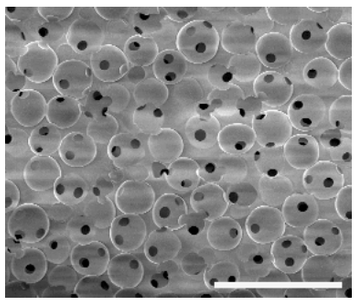

Figure 1. SEM image (bar = 200 μm) shows porous structure of hydrogel (white outlines) and interconnects (black dots).

Diffusion of nutrients occurs through the macroscopic pores, but may also occur through the bulk crosslinked hydrogel. The crosslinking density is readily controlled by the macromer molecular weight and/or the macromer concentration in solution prior to polymerization. This secondary diffusion may be critical once cells and tissue occupy the pore volume.

Scanning electrochemical microscopy (SECM) has been applied to the quantification of diffusion through PEG hydrogel scaffolds using charged and neutral probe molecules. In contrast to previous studies of diffusion in polyacrylate and polyacrylamide gels [4], the crosslinked PEG hydrogels studied here can be significant barriers to the diffusion of small molecules, suggesting smaller connected water domains. This is indicated by a decrease in the current near the hydrogel surface (I) compared to the diffusion-limited current far from the hydrogel surface (I_{lim}). For example, $I/I_{lim} \sim 0.6$ at the surface of a PEG 3000 gel, while $I/I_{lim} < 0.1$ at the surface of a PEG 500 gel (Figure 2). We will discuss results for a series of hydrogels with average macromer molecular weights from 500 g/mol to 3000 g/mol and weight percents from 20 wt% to 65 wt%. Since decreasing the crosslinking density (expected to increase diffusion) results in a decrease in compressive modulus, we seek to tune diffusion characteristics without sacrificing mechanics.

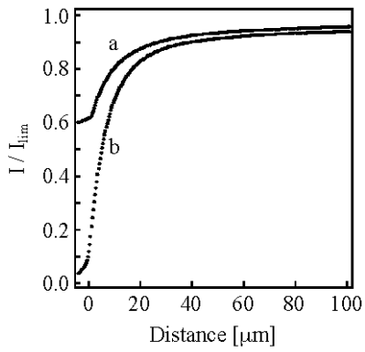

Figure 2. SECM approach curves to (a) PEG 3000 and (b) PEG 500 hydrogel surfaces (65 wt%).

The role of pores, interconnects (tuned by varying the sintering time), as well as the non-porous bulk hydrogel in promoting diffusion to the scaffold interior are examined in a diffusion cell. Preliminary studies indicate that pore topography/size as captured by *in-situ* electrochemical images is consistent with SEM images of dried hydrogels. We expect to isolate the contribution of the macroscopic pores from that of the non-porous regions. These studies will identify the scaffold architectures and compositions most suited to cardiac muscle tissue engineering.

References

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