

Seeding Strategies for 3D Silk Scaffolds Using Human Mesenchymal Stem Cells

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INTRODUCTION: Initial steps of the cultivation of engineered tissues in bioreactors involve cell seeding of three-dimensional scaffolds. These procedures usually require a high yield, to maximize the utilization of donor cells, a minimal time in suspension for anchorage-dependent and shear-sensitive cells, and spatially uniform distribution of attached cells, for rapid and uniform tissue regeneration. Our hypothesis was that dynamic seeding strategies are superior to static seeding in terms of total cell number, cell viability, attachment to the scaffold, and homogenous distribution of cells as compared to static seeding.

METHODS: Highly porous silk-fibroin scaffolds (8 mm in diameter and 2 mm thick) with pore diameters of 112 μm , 400 μm , and scaffolds with 112 μm at one end and 400 μm at the other, were (i) seeded with human mesenchymal stem cells (MSC) in well-mixed spinner flasks (dynamic seeding) or (ii) MSC were first suspended in a gel –gel is liquid below 20°C - and this cell-liquid suspension was applied to scaffolds and directly transferred to 37°C (static seeding).

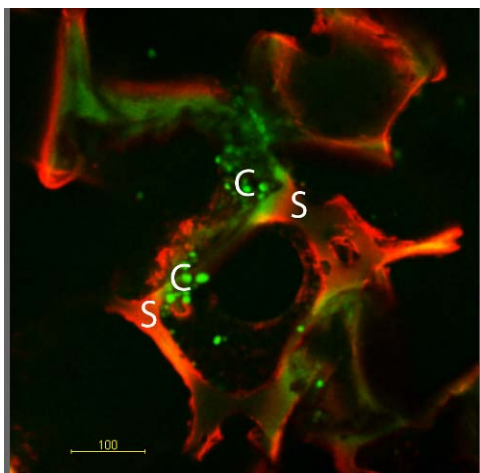


Fig. 1: Dynamic seeding results in direct attachment of cells (C) to the silk lattice (S).

RESULTS: Substantially more cells were deposited on the scaffolds – as determined by total DNA content - using static seeding regimens, and the number of living cells – as determined by MTT transformation – and the ratio of viable to total MSC was the same for both seeding protocols. Pore size did not have significant impact on MSC

number or viability. MSC attached to the scaffold lattice when seeded dynamically (**Figure 1**) whereas statically seeded cells were found in the scaffold's void spaces and entrapped in the gel (**Figure 2**). As determined by live-dead staining and confocal microscopy, all MSCs were alive when seeded dynamically, whereas dead cells – although few in number - were randomly observed and entrapped within the gel matrix between the scaffold rods (**Figure 2**).

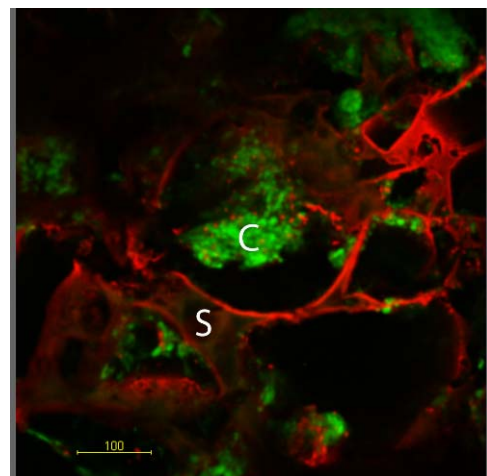


Fig. 2: Statically seeded cells (C) reside in void areas within the silk network (S).

DISCUSSION & CONCLUSIONS: Our hypothesis as mentioned above was not fully confirmed as the same levels of living cells were found using either seeding protocol. However, dynamically seeded cells distributed homogeneously over the scaffold area and cells were attached to the scaffold, whereas static regimens resulted in clusters of cells entrapped in the gel matrix after 2 days.

The proximity of the cells to the scaffold lattice and the proportion of living cells suggests dynamic approaches superior to static seeding for three-dimensional silk scaffolds.