495f Seeding of Scaffolds for Tissue Engineering in a Flow Perfusion Bioreactor Improves Efficiency and Cell Distribution

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Bone tissue engineering seeks the creation of ex vivo generated grafts to replace damaged or lost bone. Bone grafts have been generated under a variety of culturing conditions, including static and dynamic systems by seeding and culturing osteoblastic cells on porous scaffolds. Scaffold seeding plays a major role in the development of bone grafts, determining initial cellularities and cell spatial distribution throughout the scaffold, as well as affecting cell-cell and cell-matrix interactions. Drop-wise addition of thick cell suspensions is an example of a static technique. Spinner flask and rotating wall vessel bioreactors have been used as dynamic seeding systems. However, improvement of seeding efficiency and spatial homogeneity is desirable. Flow perfusion bioreactors have been demonstrated to suppress most of these limitations. In this system, the scaffold is confined in a flow chamber so as to force the flow through the porous network and not around it. In this study, we evaluate the effect of flow perfusion on seeding efficiency and spatial distribution of osteoblastic cells in fibrous polymeric matrices (20, 35 and 50 um) and foams prepared by salt leaching. Flow perfusion bioreactors are also used for long term culture of osteoblastic cells; thus, performing seeding in this system has the advantage of diminishing risks of contamination. All scaffolds were seeded statically or in a flow perfusion bioreactor. Perfusion yielded the highest efficiency, and the initial cellularity was preserved after applying flow, while a significant number of statically seeded cells was lost after shearing. Thus, flow perfusion resulted in the most efficient seeding method. Cell surface density increased with inoculation cell number and then plateau, demonstrating a saturation of the surface. Oxygen plasma treatment of the fibers greatly improved seeding efficiency, but patterns on cell attachment were the same. The number of cells attached to the scaffold after perfusion seeding decreased at greater flow rates, but continued to yield higher seeding efficiencies than the static technique. Having similar porosity and dimensions, fibrous matrices yielded higher cell surface densities than porous foams. Histological analysis demonstrated that flow perfusion seeding produced a more homogeneous cell spatial distribution throughout the foams. In conclusion, we have demonstrated that flow perfusion improves scaffold seeding for tissue engineering not only in terms of seeding efficiency and spatial distribution, but also it enhances the attachment strength of the cells.