Cell Proliferation and Cell Localization Following *In Situ* Immobilization in a 3D Alginate Foam Matrix

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Statement of purpose: The use of 3 dimensional matrixes for cell growth is gaining popularity as a substitute for traditional 2D cell culture methods. Growth in a 3D matrix can, in some instances, approximate cell architecture and cell-cell contact as found in tissues, organs and tumors. We have developed an alginate-based foam matrix for culturing cells in 3D. One significant and very interesting aspect of this matrix is the ability to immobilize cells within the matrix using *in situ* gelation. The ability of alginate to form ionic cross-linked gels is utilized to immobilize cells within the alginate foam matrix. By applying cells suspended in a solution of sodium alginate to a pre-formed alginate foam, calcium ions from the foam will cross-link the added alginate and effectively entrap the cells within the pores of the foam.

Methods: Mouse myoblasts C2C12 (ATCC CRL-1772) were grown as 2D cultures then trypsinized, counted and resuspended in 1.0% sodium alginate in DMEM medium (80,000 and 200,000 cells/mL). 125 µl cell suspension was added to γ-sterilized NovaMatrix 3D foams cut to a size appropriate for use in a 24-well culture plate with resulting cell density of 10,000 or 25,000 cells/ alginate foam. As the cell suspension was absorbed by the foam matrix, the soluble alginate gelled, thereby effectively immobilizing the cells within the foam structure. Cell proliferation was followed by evaluating the total cell count over time. For each time point 3 samples of the alginate foam with *in situ* immobilized cells was de-gelled by incubating in 50 mM sodium citrate solution. Cells were recovered by centrifugation, resuspended and counted using an automatic cell counter (Invitrogen Countess). Cell localization within the alginate foam matrix was visualized by first fluorescently labeling cells using a carboxyfluorescein marker (CellTrace CFSE cell proliferation kit, Invitrogen/Molecular Probes). Cells were identified within the alginate matrix using a Nikon eclipse TE2000-U confocal microscope.

Results: The C2C12 myoblasts proliferated slower when immobilized within the alginate foam matrix as compared to growth in a standard 2D tissue culture plate (Figure 1). Despite the reduced proliferation rate, the cells were viable (as evidenced by trypan blue staining as well as live-dead fluorescence, data not shown).

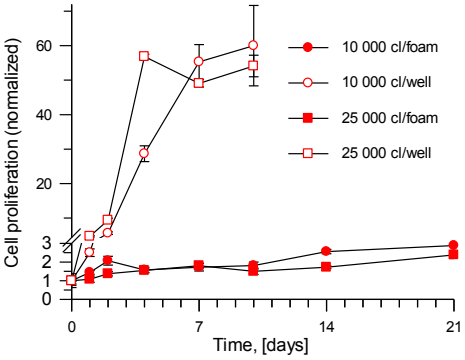

Figure 1: Proliferation of C2C12 myoblasts cultured in 2D (well) or 3D (foam). (n=3± SEM)

For cells immobilized within an alginate gel, the alginate matrix may not provide necessary attachment factors for the cells to retain a high proliferation rate. We have investigated the use of the cell attachment peptide RGD coupled to the immobilizing alginate and its effect on proliferation of cells within the alginate foam matrix. As seen in Figure 2, the proliferation rate for two days in culture of approx. 25,000 immobilized myoblasts was stimulated by the use of RGD-coupled alginate.

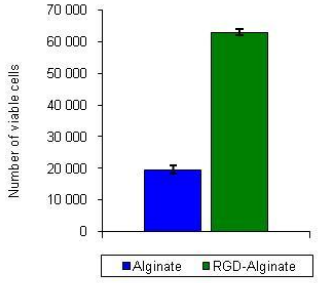

Figure 2: Proliferation of myoblasts in alginate foams for 2 days with regular alginate or RGD coupled alginate as the immobilizing alginate.

Finally, as a demonstration that cells were, immobilized throughout the alginate foam matrix, confocal imaging of fluorescently-labeled cells was used. Figure 3 shows cells immobilized throughout the thickness of the alginate foam.

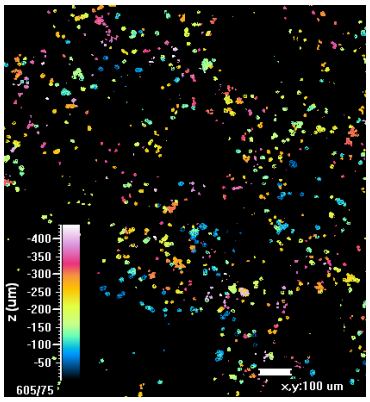

Figure 3: Distribution of immobilized cells throughout the thickness of the foam.

Conclusions: Use of an alginate foam matrix with concomitant *in situ* immobilization of cells results in a 3 dimensional model with the potential to approximate cell growth and architecture within tissues or tumors. The cell proliferation rate can effectively be promoted by addition of cell attachment peptides such as RGD, or limited by subsequent incorporation of stronger gelling ions such as strontium, (cell viability will not be not affected). The elasticity of the NovaMatrix 3D system can be varied by changing the type and concentration of immobilizing alginate as a biomimetic approach for specific cell lines and stem cell differentiation. The immobilized cells can be treated with drugs or other agents in cell survival or cytotoxicity experiments since the alginate matrix is permeable for small and medium sized molecules. Finally, cells can be recovered by de-gelling the alginate matrix with EDTA or citrate, making the NovaMatrix 3D system truly versatile.