NovaMatrix-3D™ – 3D Cell Culture and Formation of Multi-Cellular Structures in Alginate Foams

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Statement of purpose: The use of 3 dimensional (3D) matrices 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 and an *in situ* gelling cell immobilization technology for culturing cells in 3D, NovaMatrix-3D™. Figure 1 shows the principle of immobilization, where cells are first suspended in a sodium alginate solution then the cell suspension is applied to the alginate foam. *In situ* gelation occurs when calcium ions are donated from the foam cross-linking the added alginate, effectively entrapping the cells within the pores throughout the foam.

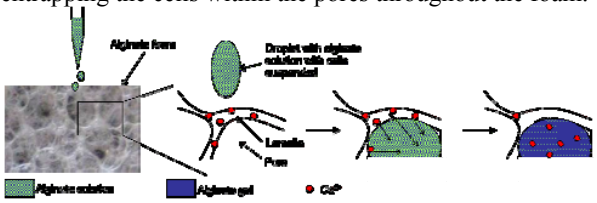

Figure 1. Cell immobilization by *in situ* gelling.

Methods: Various cell lines were first cultured in 2D then prepared as a suspension in 0.5% sodium alginate in cell culture medium. An aliquot of each cell suspension was added to γ-sterilized NovaMatrix-3D™ foams (24-well plate size) at cell densities of 20,000 or 50,000 cells/foam. After 10 minutes gelling in a CO_2-incubator at 37°C cell culture media was added to cover the foams. Cell localization and formation of multi-cellular structures within the foam was visualized using confocal microscopy to identify fluorescently labeled (CellTrace™ CFSE) cells. Cell harvesting was done by de-gelling the matrix using sodium citrate.

Results: Cell distribution 4 days post immobilization throughout the thickness of the foam was demonstrated by confocal imaging of fluorescently labeled NIH:3T3 murine fibroblasts (Figure 2).

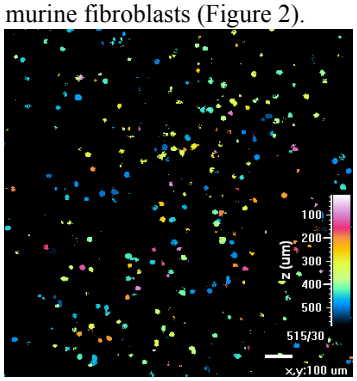

Figure 2. Cell distribution throughout the foam thickness.

By day 21 the NIH:3T3 fibroblasts had formed multi-cellular spheroids (Figure 3 A) and by day 60 C2C12 mouse myoblasts had fused and formed multinucleated myotubes (Figure 3 B). The figures show both the 3D image (left) and the depth localization of the structures within the matrix (right).

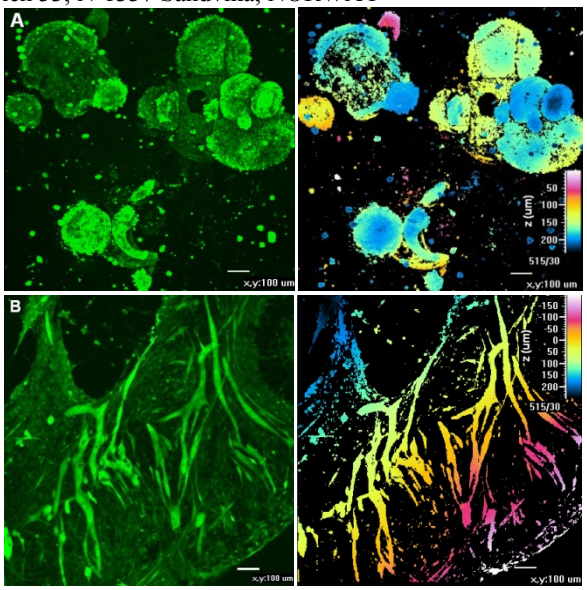

Figure 3. A: Multi-cellular spheroids formed by NIH:3T3 fibroblasts. B: Myotubes formed by C2C12 myoblasts.

Cell structures formed inside the foam matrix may be harvested. Intact multi-cellular spheroids formed by NHIK 3025 (human cervical carcinoma) cells were isolated by de-gelling the surrounding alginate matrix (Figure 4).

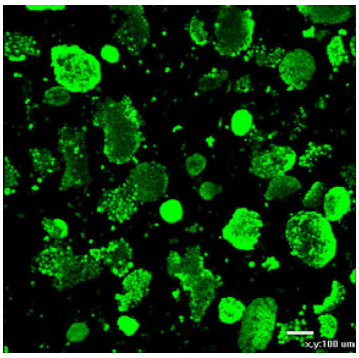

Figure 4. Isolated intact spheroids formed by NHIK 3025 cell from alginate scaffold by de-gelling.

Conclusions: Use of NovaMatrix-3D™ with concomitant *in situ* immobilization of cells results in a 3D model with the potential to approximate cell growth and architecture within tissues or tumors. Cells are uniformly entrapped into the foam matrix by a gentle and fast technique. Cell visualization can easily be performed due to matrix transparency. Cells seem to freely form multi-cellular structures within the matrix and intact structures may easily be isolated under physiologic conditions. 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. The formed structures may also be harvested for further investigation.