Effect of GRGDSP-Alginate in Alginate Foam for 3D Cell Culture Compared to Cells Affinity to FITC-GRGDSP

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Statement of purpose: The use of 3 dimensional 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™. In principle, cells are first suspended in a sodium alginate solution (immobilizing alginate) 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. As alginate is not recognized by cell receptors no interaction would be expected between this synthetic extracellular matrix (ECM) and the entrapped cells. However, some cells require certain attachment factors to be present on the ECM to proliferate. The following experiment follows proliferation of different cell types in alginate foams with and without the adhesion peptide sequence GRGDSP. The results where then compared to cells' ability to bind FITC-labeled GRGDSP to see if the cells need for adhesion peptides and the use of peptide-coupled alginate can be determined before cell culture.

Methods: Various cell lines were first cultured in 2D then prepared as a suspension in 0.5% sodium alginate (immobilizing alginate) in cell culture medium. 125 µl 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. Cell culture media was added after 10 minutes in a CO_2-incubator at 37°C for gelling. The immobilizing alginate used was sodium alginate alone (PRONOVA UP LVG) or a blend of this and GRGDSP-alginate (NOVATACH G RGD, measured ratio peptide:alginate 7.4:1, M_W (alginate): 226 kDa). Cells were isolated from the foam at different time points. Cell harvesting was done by de-gelling the matrix using sodium citrate and cells were counted after trypan blue staining using an automatic cell counter (Countess™, Invitrogen). The cells' ability to bind the peptide sequence GRGDSP was investigated using FITC-LC-GRGDSP (FITC-GRGDSP) from AnaSpec and flow cytometry (Cell Lab Quanta™ SC, Beckman Coulter). A cell pellet of 500 000 trypsinated cells were re-suspended in varying concentrations of 1 ml FITC-GRGDSP and incubated for 30 minutes. The cells were then washed three times with PBS and re-suspended in 1 ml IsoFlow™ sheath fluid (Beckman Coulter) and the intensity of emitted light was measured (Ex/Em=492/516 nm).

Results: Figure 1 shows proliferation curves of cells cultured in NovaMatrix-3D™; C2C12 mouse myoblasts (A), Madin Darby canine kidney (MDCK, B), NIH:3T3 murine fibroblasts (C) and NHIK 3025 human cervical carcinoma (D).

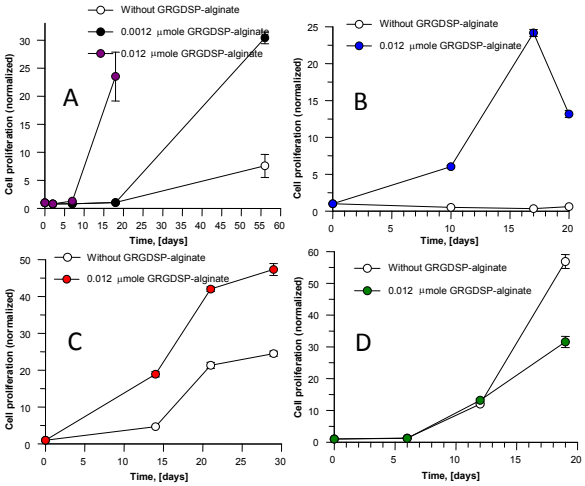

Figure 1. Cell proliferation with and without GRGDSP-alginate. A: C2C12 myoblasts, B: MDCK, C: NIH:3T3 fibroblasts and D: NHIK 3025 cervical carcinoma. (n=1-3 foam discs, ≥ 2counts of each disc ± SEM)

C2C12 and MDCK showed no or little cell proliferation without GRGDSP-alginate and the for the lowest peptide concentration within the first three weeks of culture. GRGDSP-alginate more than doubled the number of NIH:3T3, whereas proliferation of NHIK 3025 did not indicate improved proliferation in presence of peptides. MDCK and NHIK 3025 were incubated in 5 µM FITC-GRGDSP and the results showed 28.5 times increased fluorescence of MDCK compared to non-labeled cells and a 5.6 times increase for NHIK 3025. The intensities of MDCK cells incubated in different concentrations of FITC-GRGDSP are shown in Figure 2.

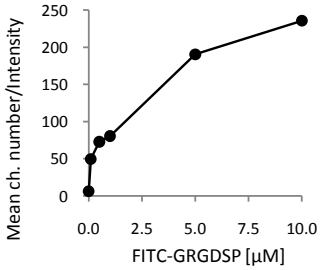

Figure 2. Increased cell fluorescence by treatment with increased peptide concentrations.

Conclusions: For some cell types was the cell proliferation rate effectively promoted by addition of GRGDSP covalently bound to alginate. The differences seen in binding capacity of FITC-GRGDSP may reflect the dependency of attachment peptides for cell proliferation in an inert alginate matrix and the presence of receptors available for binding on the cell surfaces. Evaluating the cells' affinities for different peptide sequences and then covalently couple the relevant sequences onto alginate may be of high value, as this knowledge may be used to optimize the cell culture system and control cell interaction with a synthetic ECM such as the alginate based NovaMatrix-3D™.