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BD Falcon™ Cell Culture Inserts as a Support Substrate for an *In Vitro* Extracellular Matrix System

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Introduction

The purpose of this study was to find a porous membrane substrate which could be used as a support for the production of an extracellular matrix (ECM) by R22 rat aortic smooth muscle cells in culture. To be acceptable for this purpose, a substrate should: (1) provide a surface on which these cells will attach and proliferate to confluence, (2) have sufficient optical clarity to permit phase contrast microscopy of the cultures during growth and subsequent matrix production, (3) retain an attached, uniform and uninterrupted sheet of cell-derived ECM, and (4) be resistant to the reagents used during lysis of the R22 cells and during subsequent microscopic observations.

Materials and Methods

Cell Culture and ECM Production

R22 rat aortic smooth muscle cells were cultured by methods adapted from Jones, et al.[1] Nearly confluent cultures of R22 cells adherent to polystyrene were trypsinized and suspended in MEM supplemented with fetal bovine serum, tryptose phosphate broth, cefotaxime and streptomycin. Cells were plated onto BD Falcon™ Cell Culture Inserts and onto BD Falcon Multiwell tissue culture-treated polystyrene plates at a density of approximately 2.5 x 10^4 cells/cm^2. Inserts were placed in the appropriate wells of BD Falcon Multiwell plates and medium was added to the wells to maintain a level equal to that in the insert. Cultures, incubated at 37°C in 5% CO_2, were examined daily for proper morph-ology and growth pattern. The cells were fed every four days by aspiration of old medium and replacement with fresh medium in both the insert and the surrounding well. When the cells reached confluence and began to synthesize ECM, they were given daily supplements of 50 µg/ml ascorbic acid for eight to ten days until sufficient ECM had been formed for use.

Lysis of R22 Cells and Storage of ECM

To eliminate the R22 cells from the ECM without disrupting the matrix itself, the cells were lysed with 25 mM NH_4OH under microscopic examination. The wells and inserts were then washed three times with sterile water and once with Dulbecco's phosphate-buffered saline (PBS) containing 0.02% NaN_3. The PBS was removed and the wells and inserts were stored at 4°C in sealed bags until ready for use. Immediately before use, the ECM was rehydrated by rinsing three times with sterile PBS.

Microscopy

R22 cells were examined with an inverted phase contrast microscope. ECM from which R22 cells had been eliminated was examined by both phase contrast and by bright field after staining with Trypan blue.

Results and Discussion

Cell Culture

Figure 1 shows R22 cells plated on both (A) BD Falcon Multiwell polystyrene plates and (B) BD Falcon Cell Culture Inserts. There is some loss of clarity on viewing the cells on the inserts, which is more apparent with the 24-well inserts than with the 6-well inserts. This is mostly attributable to distortion from the curved perimeter of the insert membrane and interference from the side walls, both of which were more pronounced in the smaller inserts.

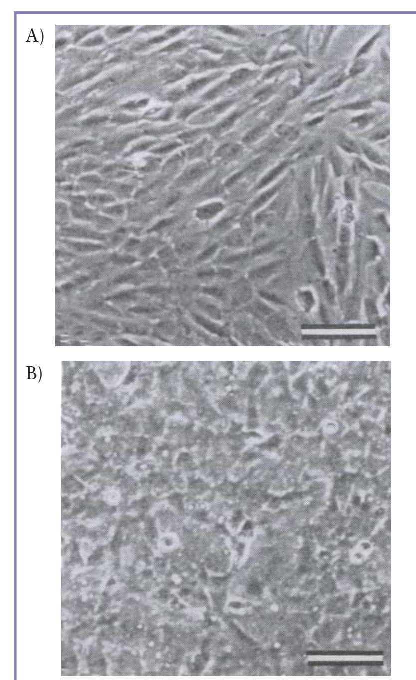

Figure 1*: Phase contrast images of R22 cells growing on (**A**) BD Falcon™ polystyrene Multiwell Cell Culture Plates and (**B**) BD Falcon™ Cell Culture Inserts. Bar = 100 µ.*

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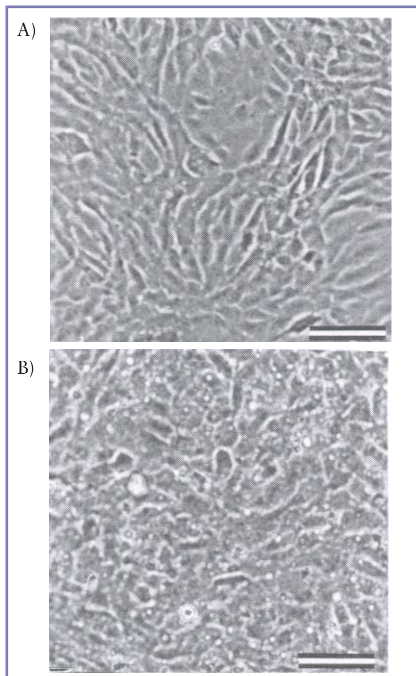

***Figure 2**: Phase contrast images of confluent R22 cells prior to ascorbic acid treatment and matrix production. (**A**) polystyrene and (**B**) BD Falcon Cell Culture Inserts. Bar = 100 µ.*

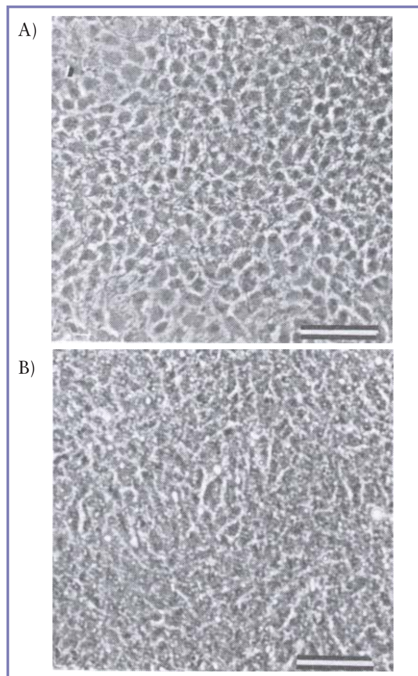

***Figure 3**: Phase contrast images of R22 cells and matrix after eight days of ascorbic acid supplement. (**A**) polystyrene and (**B**) BD Falcon Cell Culture Inserts. Bar = 100 µ.*

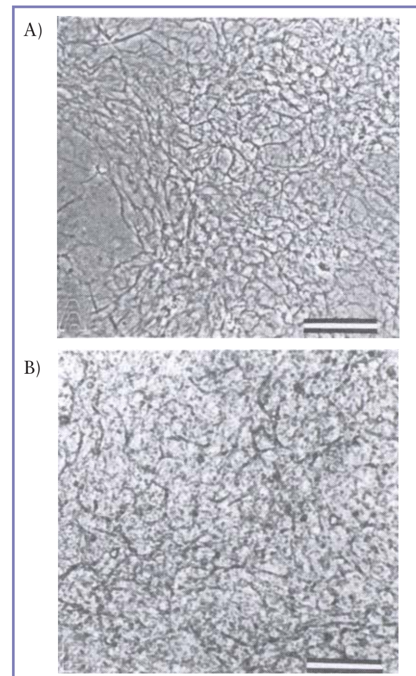

***Figure 4**: R22 ECM after cell lysis, storage and rehydration. (**A**) phase contrast image of matrix on polystyrene and (**B**) bright field image of Trypan blue stained matrix on BD Falcon Cell Culture Inserts. Bar = 100 µ.*

R22 cells attach, spread and proliferate to confluence at the same rate in inserts and in polystyrene wells. When plated at an initial density of 2.5×10^4 cells/cm^2, the cells reach confluence in approximately four days. The appearance of confluent cells, shown in ***Figure 2***, is similar on (A) polystyrene and (B) insert membranes.

Matrix Production

Confluent cells appear to produce ECM of comparable characteristics on polystyrene wells and on insert membranes. The appearance of the ECM produced after eight days of ascorbic acid supplementation is shown in ***Figure 3***; ECM on (A) polystyrene in panel and (B) inserts. Matrix fibers can be observed running throughout the cultures of tightly packed cells. These fibers are somewhat more difficult to discern on the insert membranes and do not photograph as clearly as those on polystyrene plates.

After lysis of the R22 cells with NH_4OH, the remaining ECM is still very similar in appearance on polystyrene wells and on insert membranes, as shown in ***Figure 4***. The insert membranes introduce some interference (B) behind the matrix which is absent from (A) the matrix on polystyrene. ECM on both substrates is not affected by the lysis procedure, multiple rinsing steps, storage at 4°C and rehydration.

We conclude that the BD Falcon™ Cell Culture Inserts are suitable as a porous support substrate for production of ECM from R22 cells. It is noteworthy that this matrix consists of a mixture of components typical of a stromal or interstitial ECM, i.e. types I and III collagen, elastin, proteoglycans and fibronectin. Other protocols for deposition of ECM on multiwell insert membranes have employed solubilized basement membrane matrix, which contains a very different complement of components, including type IV collagen and laminin. We find that the R22 ECM on the BD Falcon inserts provides an ideal model system for study of migration of inflammatory cells through an interstitial matrix. By growing the cells in medium containing appropriate radio-labeled precursors, the components of the ECM can be selectively labeled, and the degradation of ECM can be correlated with the ability of the cells to penetrate the matrix as they migrate to the underlying insert membrane.

Reference

1. Jones, P.A., Scott-Burden, T.S., and Gevers, W. *Glycoprotein, elastin, and collagen secretion by rat smooth muscle cells.* Proc. Natl. Acad. Sci., USA, **76**:353 (1979).

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