BIOENGINEERING FUNCTIONAL ENDOTHELIAL CELL MONOLAYER CULTURED ON DESIGN PEPTIDE HYDROGEL SCAFFOLDS

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INTRODUCTION

The objective of this work is to obtain an *in vitro* model to study endothelial cell function using design biological materials. We have previously described a class of biomaterials that is made from spontaneous assembly of ionic self-complementary peptides [1]. These material scaffolds consist of greater than 99% water content. They form highly hydrated gels when the peptide solution is exposed to physiological media or a saline solution. These materials have been previously used to attach and culture diverse mammalian cells [2]. We hypothesized that these hydrogels mimic the cellular microenvironment and thus enhance cellular function [3]. In this work we describe the use of novel modified self-assembling hydrogels to culture and maintain functional human aortic endothelial cells (HAEC).

MATERIALS AND METHODS

Peptide hydrogel cultures

The cellular scaffolds were prepared by placing 300 µl of 0.5% (5 mg/ml) peptide solution in deionized water into a 0.02 µm transwell insert (Nalge Nunc International, Inaperville, Il). Complete EBM-2 culture media (BioWhittaker, Walkersville, MD) was added to the well bottom (1 ml) to initiate hydrogel formation. HAEC (BioWhittaker, Walkersville, MD) from routine culture conditions were harvested by treatment with trypsin. The released cells were washed with complete culture medium, counted and resuspended in fresh media at a final concentration of $2 \cdot 10^6$ cells/ml. A total of $7 \cdot 10^5$ cells were loaded in each well to study endothelial monolayer formation.

Immunofluorescent analysis

For immunofluorescent analysis HAEC were incubated with a 8 µg/ml solution of DiI-Ac-LDL (Biomedical Technologies Inc., Stoughton, MA) for four hours and detected under a Nikon microscope TE300.

RESULTS AND DISCUSSION

We have observed that HAEC cultured in sub-confluent conditions grow on the surface if the hydrogel forming a stable monolayer (Fig. 1). Since the cell monolayer seals two compartments (hydrogel/culture media) we decided to use this system to study cell-mediated uptake and transport of macromolecules such as LDL through it.

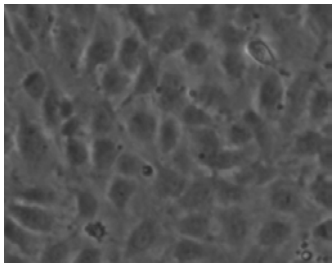

Figure 1. HAEC grown on hydrogel surface

Preliminary results suggest that the cells secrete proteoglycans at the extracellular surface that mediate the internalization of LDL. As a consequence, high levels of cellular vesicle-associated LDL are obtained. We are planing to expand this research by asking the following questions: Can we develop an *in vitro* model to study therapeutic drug transport through the endothelial barrier? Can the model be extended to study transendothelial cell migration such as naïve T cells?

REFERENCES

1. Zhang, S., Holmes, T., Lockshin, C., and Rich, A., 1993, "Spontaneous assembly of a self-complementary oligopeptide to form stable macroscopic membrane", Proc. Natl. Acad. Sci. USA, 90, 3334-3338.
2. Zhang, S., Holmes, T., DiPersio, C., Hynes, R., Su, X., and Rich, A., 1995, "Self-complementary oligopeptide matrices support mammalian cell attachment", Biomaterials, 16,1385-1393.
3. Semino, C.E., 2002, "Can we build artificial stem cell compartments?", *J. Biomed. Biotech.* In press