ARTIFICIAL EXTRACELLULAR MATRICES CAN BE USED FOR IN VITRO CONTROL OF STEM CELL DIFFERENTIATION

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INTRODUCTION

A major goal in tissue engineering is the molecular engineering of synthetic biological materials capable of supporting growth and functional differentiation of cells and tissues in a controlled manner. We have previously described a class of biomaterials that is made from spontaneous assembly of ionic self-complementary peptides that form highly hydrated gels (>99% water) and have been previously used to attach and culture diverse mammalian cells [1, 2]. We hypothesize that 3-dimensional scaffold would mimic the stem cell compartment and thus enhancing functional stem cell differentiation [3]. In this work we investigated the capacity of a three-dimensional peptide hydrogel culture system to promote functional differentiation into pancreatic β-cell type of a clonal epithelial stem cell line (Lig-8), derived from adult rat liver.

MATERIALS AND METHODS

Peptide scaffold cultures and differentiation protocol

Trypsinized Lig-8 cells were washed with complete culture medium were resuspended in 10% sucrose at final concentration of ~2,000,000 cells/ml, and loaded (50 µl) into a transwell insert (clear polyester membrane, 6.5 mm diameter, 0.4 µm pore size, Costar). When required, type I laminin (Lam I) was added to the cell suspension (50 µg/ml final concentration). At this point, an equal volume of liquid RAD16-I peptide solution in deionized water (1% w/v, sequence: Ac-RADARADARADARADA-$CONH_2$, SynPep Corporation, Dublin, California) was added to the cell suspension by pipetting up and down several times to mix thoroughly. Regular culture media (DMEM high-glucose, 25 mM, without sodium pyruvate/10% dialyzed fetal bovine serum/penicillin-streptomycin) with or without nicotinamide (10 mM), containing EGF (0.8 ng/ml), FGFb (0.5 ng/ml), or a mix of both (0.8 ng/ml EGF and 0.5 ng/ml FGFb), was added to the well bottom (0.5 ml) to initiate immediate scaffold formation. Then, the scaffolds cultured for 8 days in standard cell culture conditions.

Induction of insulin release by glucose and other agonist

Cells were incubated in Krebs-Ringer buffer in the presence of agonsts of insulin release such as glucose (0-25 mM), carbachol (100 µM; Sigma), tolbutamide (10 µM; Sigma) and 3-isobutil-1-methylxanthine (IBMX) (100 µM; Sigma), in conjunction with low glucose (5 mM). After incubation, the supernatant was removed and stored at -80°C for later analysis. An ELISA kit specific for rat insulin detection (Alpco, Windham, NH) was used to analyze the supernatant for insulin release. The assay was performed according to the directions of the manufacturer. Total protein levels were determined using a protein detection kit (Bio Rad) to normalize insulin amounts according to total protein levels in each well. Assays were performed in triplicate and standard deviation was calculated.

RESULTS AND DISCUSSION

In regular dish cultures, Lig-8 cells express the primitive hepatocyte marker α-fetoprotein and the definitive endoderm marker HFN3β. However, after culturing Lig-8 cells in peptide hydrogels, they assemble into spheroid-clusters (150-250 µm size) that up-regulate expression of the transcription factor C/EBPα and insulin**.** Moreover, after growing Lig-8 cells in hydrogels containing type I laminin in the presence of EGF, FGFb, and nicotinamide, we obtained cellular clusters three to four times larger (500-700 µm) similar to pancreatic islets. These clusters secrete insulin in response to physiological changes of external glucose and other agonists, such as tolbutamide, 3-isobutyl-1-methylxanthine (IBMX), and carbachol. We propose that the peptide hydrogel system can be rationally "decorated" to provide a unique microenvironment conducive to enhanced transit adult stem cell differentiation into pancreatic β-cell type.

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