Stable Feeder- and Xeno-free Surfaces for Long-term Growth of Undifferentiated Human Embryonic Stem Cells

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Statement of Purpose: Stem cells hold enormous potential for application in regenerative medicine and tissue engineering[1]. Both human embryonic stem cells (hESC) and human induced pluripotent stem cells are difficult to culture and grow while maintaining an undifferentiated state. Currently used techniques of culture on mouse embryonic fibroblast feeder layers or on a layer of Matrigel, a gelatinous protein mixture secreted by mouse tumor cells, introduce xenogeneic proteins of unknown composition, and Matrigel may vary from one lot to the other. Approaches for producing feeder-free culture surfaces composed of well defined human components are in their infancy. Most rely on physical adsorption of proteins, which provides limited long-term stability.

Here we describe a compositionally defined matrix with long-term stability that supports hESC expansion. It consists of a cross-linked hydrogel with carboxylic functional groups, fabricated by initiated Chemical Vapor Deposition (iCVD) situated on silicon rubber membrane substrates. This is the first report of iCVD coatings used for hESC growth. The advantages of this approach are that (i) it is possible to coat silicon rubber without modifying its oxygen permeability. However, hESC do not attach and grow on uncoated silicon rubber surfaces. Silicon rubber membranes were selected for this study because recent reports show that control of oxygen level at reduced levels can aid directed differentiation. [2] (ii) iCVD allows easy copolymerization with monomers bearing useful functional groups like carboxylic groups that can be easily functionalized with peptides and proteins (iii) iCVD-prepared hydrogels can have a tunable cross-linking density that makes them stable under sterilization conditions and repeated use (iv) iCVD allows easy tuning of the functional group density on the surface which directly relates to the density of peptides or proteins covalently bonded on the surface.

Methods: The bottom of the wells of 24-well tissue culture treated plates was removed and replaced with silicon rubber sheets and sterilized as described by Powers et al. [2]. The interior silicon rubber surfaces were coated with five different polymer coatings that varied in the density of carboxylic groups, using iCVD. Their ability to promote attachment and proliferation of undifferentiated hESC was tested. After depositing the coating, the plates were sterilized, in 70%ethanol for 1h and dried overnight under a germicidal UV lamp. Human fibronectin was then covalently bonded to the polymer coating. Finally cells were seeded and enumerated as described in [2].

Results: By changing the vapor feed ratio of the monomers used in the iCVD process, we were able to modify the density of -COOH groups on the surface. The -COOH groups were functionalized by reaction with $-NH_2$ groups of proteins in order to covalently bond proteins to the surface. The formation of amidic (-CONH) groups detected by FT-IR analysis demonstrated that the surface containing the lowest percentage of COOH groups was the surface that bound the least amount of proteins, while the surface that had the highest surface density of COOH groups was the one that bound the most protein. The density of protein on the surface influenced the cell attachment and proliferation. Cell attachment was monitored with DAPI, which stains the nuclei, and pluripotency was monitored with the transcription factor OCT4, which is a marker for the undifferentiated stem cells (Figure 1).

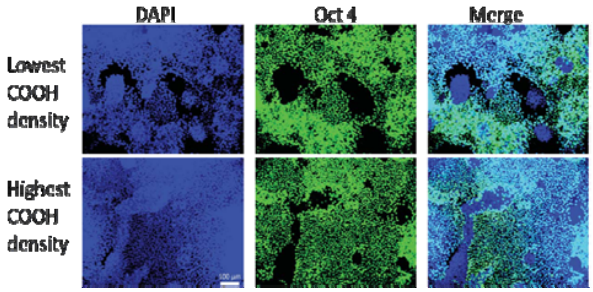

Figure 1. Micrographs of H7 cells cultured on two iCVD coated silicon rubber surfaces showing expression of hES cell markers: DAPI and OCT4 and a merged image.

When the density of COOH groups (and consequently of protein) on the surface was the highest, the cells formed agglomerates but did not form a uniform monolayer. When the density of the COOH groups on the surface was the lowest, the cells formed a uniform monolayer but did not form a confluent surface. The loss of confluency and monolayer formation resulted in spontaneous differentiation. For the intermediate densities of COOH groups, the cells formed a uniform confluent monolayer on the surface. OCT4 expression was seen in the majority of the seeded cells, which means that only few cells were differentiating. The differentiated cells are observed in the DAPI and OCT4 merged images. Through more than 5 passages, hES cells seeded on the coated surfaces displayed a normal karyotype and retained pluripotency.

Conclusions: The use of iCVD to coat silicon rubber surfaces and promote embryonic stem cell adhesion is a novel approach to the goal of providing stable, xeno-free environments. iCVD coatings give stable surfaces that survived sterilization and allowed cell proliferation for more than 5 passages. The stem cells expressed pluripotency markers and a normal karyotype after propagation on this synthetic surface. The intermediate densities of protein covalently bonded to the surface gave the best results in terms of cell attachment. The definition of this synthetic approach for stem cell culture is important for future scale-up and use of stem cell culture in actual biomedical applications.

References: [1] Daley GQ. New England Journal of Medicine 2003;349(3):211-212
[2] Powers DE. et al., Biotechnol. Prog. 2010; 26:805-818