The Effects of Plasma-Polymerized Surface Interactions on R1 Mouse Embryonic Stem Cell Differentiation

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Statement of Purpose: Plasma polymerized tetraglyme coatings have previously been studied for their non-fouling and biocompatibility properties due to their inhibition of non-specific protein binding[1]. If the ultimate goal is the development of an artificial blood vessel surface, then this coating could be used to seed stem cells that will differentiate into a lumenal layer of endothelial cells (EC). In this study, we explore how embryonic stem (ES) cell differentiation patterns are affected by surface interactions with various plasma-processed materials.

Methods: In order to identify a surface as a potential scaffolding material for ES cells in the development of an artificial blood vessel, at least two design criteria must be met. First, the ES cells must adhere to the surface. Second, the surface must facilitate, rather than inhibit, the process of vasculogenesis. ES cells were plated on the following four surfaces to compare their effectiveness: 1.) glass cover slips onto which a coating of tetraglyme was deposited by plasma polymerization[2], 2.) glass cover slips onto which a coating of tetraglyme was deposited by plasma polymerization and then exposed to a UV light treatment that has been known to increase polymer cross-linking, 3.) vacuum gas plasma treated polystyrene (Falcon), and 4.) unprocessed control glass coverslips (Corning).

Two different cell lines were plated onto each of the four surfaces: R1 mouse ES cells, and control rat aorta endothelial cells. An equal number of R1 mouse ES cells were plated on the four surfaces and allowed to differentiate. All cells were grown in DMEM + 15% FBS, to which no exogenous growth factors were added.

In order to characterize the progression of differentiation of the ES cells, each sample was fixed (with 4% paraformaldehyde) three and seven days after cells had been plated on each surface, and then stained for immunofluorescence analysis. Two genetic markers were used for the antibody staining procedure. PECAM (platelet endothelial cell adhesion molecule: CD31) is an early marker for endothelial cell differentiation. vWF (von Willebrand Factor) is a cytoplasmic protein only expressed in mature endothelial cells.

Results/Discussion: It has been previously reported[3] by using an RT-PCR/southern hybridization blot analysis that ES cells in embryoid bodies first express PECAM near day 5 of differentiation and will first express vWF near day 11 of differentiation. ES cells that were plated on tetraglyme and tetraglyme+UV surfaces showed expression of PECAM after 3 days and vWF after 7 days of being plated on the surface as shown in Figure 1 above.

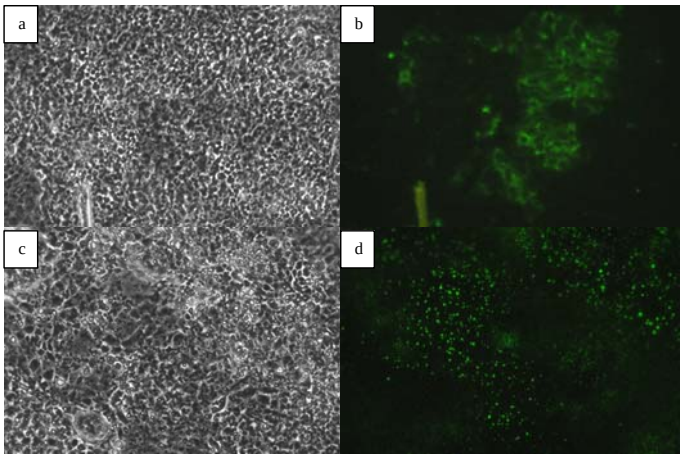

Figure 1*: a.) phase image of R1 ES cells plated on tetraglyme surface for 3 days, b.) antibody staining of (image a) shows expression of PECAM, c.) phase image of R1 ES cells plated on tetraglyme surface for 7 days, d.) antibody staining of (image c) shows expression of vWF.*

Surface	Antibody	Days Post-plating	positive wells / total wells
polystyrene	PECAM	3	0 / 3
polystyrene	vWF	7	0 / 2
glass coverslips	PECAM	3	0 / 3
glass coverslips	vWF	7	0 / 3
tetraglyme	PECAM	3	3 / 5
tetraglyme	vWF	7	2 / 2
tetraglyme+UV	PECAM	3	3 / 5
tetraglyme+UV	vWF	7	2 / 3

Table I: *ES cells plated on tetraglyme and tetraglyme+UV surfaces express PECAM and vWF earlier than on the polystyrene and glass control surface.*

Conclusions: All cell lines that were plated adhered to each of the four surfaces. Due to the differences seen between the antibody staining results for each of the four surfaces, it was concluded that the nature of the surface does influence R1 stem cell differentiation. As shown in Table I, the positive results seen by ES cell derivatives precociously expressing the vWF and PECAM genetic markers on the tetraglyme and tetraglyme+UV surfaces *suggest* a directed differentiation of ES cells into endothelial cells.

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References:

[1] Shen M. J. Biomat. Sci., 2002, 13:367-390
[2] Pan V. Plasmas & Polymers, 2004, **7**,:171-183
[3] Vitett D. Blood, 1996, 88:3424-3431