

Cell-Substrata Interactions: Role of Biomaterial Architecture in Regulation of Endothelial Cell Phenotype and Tissue Engineering

Special Interest Group News

Christopher Siedlecki, *Special Interest Group News*

Contributing Editor

Peter Edelman, *Cardiovascular Biomaterials Special*

Interest Group Reporter

Laura Indolfi and Elazer R. Edelman

Harvard-MIT Division of Health Sciences and Technology,
Massachusetts Institute of Technology, Cambridge, Massachusetts,
United States of America

Cell-substrata material interactions

Tissue engineering (TE) is the discipline that supports the controlled *ex vivo* growth of cells and tissues on or within three-dimensional support structures to provide units that replace, repair or regulate biological functions or structures *in vivo*. The use of biomaterials in this field has progressed from simple, passive platforms for cellular support to novel, dynamic substrata capable of influencing cell differentiation and function¹. This article highlights our recent investigation examining how biomaterials substrata affect the physical state and phenotype of three-dimensional vascular endothelial cells (EC).

Three dimensional synthetic constructs mimic natural architectures and cell-substrata relationships, allow cells to grow to precisely controlled density, with precisely regulated secretion and provide a robust system for *in vivo* implantation. Conventional 2D systems frequently distort normal cell behavior as they do not recapitulate faithfully physiologic cell interactions with the surrounding milieu. Interesting, 3D environments influence cells through both the surface material properties and the architectural and topographical cues that are far more complex than those present in 2D systems. Recent studies demonstrated that substratum curvature can direct neurite outgrowth². Similarly, fibroblasts cultured on 3D collagen matrices have distinctly different patterns of morphology and migration compared with 2D counterparts³.

In their native physiologic state *in vivo*, cells may be completely embedded in surrounding matrix, like chondrocytes, or attached to basement membranes with intricate surface properties, like epithelial and endothelial cells. Neither case is a planar, static interaction between cell and underlying surface. The vascular endothelial substratum has a defined contour that is subject to dynamic changes in dimensions and surface properties in response to local hemodynamic forces. Thus, the natural milieu of epithelial cells is not only supportive but unique architecture of the substratum transmits local mechanical cues and more than subtly directs cell biology.

Intriguingly, EC in 3D collagen matrices have a wide spectrum of physiologic activities and attain a phenotype that resembles neither the confluent nor the subconfluent phenotypes classically used to differentiate quiescent and proliferative EC when cultured in 2D collagen-coated systems.

The thesis driving our work is that, when embedded within matrices with specific contours and architecture, EC undergo morphological remodeling, affecting their bioregulatory function. We therefore aim to understand if the natural EC function-matrix structure relationships can be recapitulated in synthetic systems and in return impact their *in vivo* outcome.

While cellular density is the dominant force in 2D cell culture, surface contour is more important in 3D reservoirs. Our investigation of the biology of vascular endothelial cells in tissue engineering scaffolds illustrates this dimension-dependent effect. ECs cultured within 3D porous matrices, whose surface texture, porosity and materials properties mimic natural EC microenvironments, produce a marked difference in cell phenotype, biosecretion and regulatory effect *in vitro* and *in vivo*. Mechanical stimuli are transduced to the cell via receptors, extracellular matrix and the cytoskeleton; when seeded on 2D gelatin-coated tissue culture plates (gTCP) EC are stretched and pulled (Fig. 1a), while within 3D supports they conform to far more energy favorable states (Fig 1b). The nature of the contoured substratum is sensed by the cell; it is

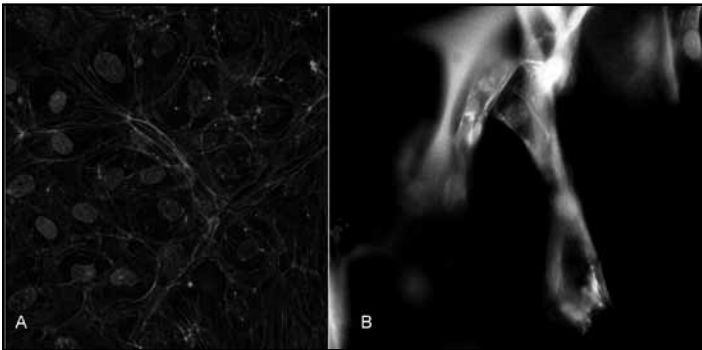


Figure 1: Immunofluorescent images showing cell-substratum interactions. A) EC on 2D collagen-coated plates. B) EC within 3D collagen matrix. Cells are stained for actin (red), and nuclei (blue). Collagen matrix is shown in green.

apparent that cell cytoskeleton bends accordingly with the strut curvatures.

Intriguingly, EC in 3D collagen matrices have a wide spectrum of physiologic activities and attain a phenotype that resembles neither the confluent nor the subconfluent phenotypes classically used to differentiate quiescent and proliferative EC when cultured in 2D collagen-coated systems.

It is important to notice that cells in 2 and 3D are sensing the same surface chemistry, collagen substrata, therefore divergence in cell biology can be attributed to differences in topographical properties. ECs within collagen-based matrices secrete higher level of antiproliferative, antithrombotic, and anti-inflammatory factors with significantly reduced expression of adhesion (CD58, ICAM-1, VCAM-1, P- and E-selectin) and costimulatory (CD80, CD86, CD40) molecules (Fig. 2)⁴. Matrix-embedded ECs (MEEC) are characterized by a highly immunoregulatory phenotype that cannot be attained by free floating cells or cells on flat planar surfaces.

The regulatory, anti-proliferative and anti-inflammatory effects of cell embedded within 3D matrices persist when implanted *in vivo* – positively affecting the healing process of damaged tissues. Every aspect of vascular repair is modulated by MEEC implanted externally (perivascular) adjacent to an area of vascular injury, imposed for example by superficial injury of balloon denudation or complex insult of flow and compliance mismatch seen with arteriovenous (a-v) anastomoses. Two months after a-v anastomosis, perivascular EC implants virtually eliminated the classic and significant intimal hyperplasia seen in control group (Fig. 3)⁵. Moreover, evidence of inflammation was not detected in any of the venous sections of MEEC-treated group (Fig. 3, small boxes). The effect persisted long after the matrices had been cleared; these matrix formulations degrade over 4-6 weeks and are not detectable thereafter, yet they induce long-term healing – allowing for true repair rather than temporary pharmacologic poisoning of cell responses.

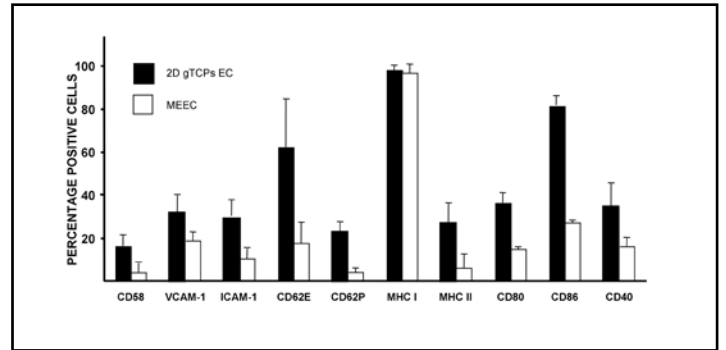


Figure 2: EC embedded within 3D porous collagen matrices exhibit reduced expression levels of adhesion, MHCII and costimulatory molecules when compared to EC on tissue culture plates coated with the same collagen material.⁴

Taken together, these functional, immune and morphological related findings suggest that EC phenotype can be modulated in response to mechanical stimuli induced by the surrounding physical environment, eliciting attenuation of the immune response and inducing vascular healing through cell-substratum interaction. The confluence of cell and molecular biology with materials science has spawned the field of tissue engineering and in doing so has increased our understanding of how the biochemical, biomechanical and biological aspects can control cells physiology. Our work and that of others suggest that the physical properties of the substratum are as important as the biochemical microenvironment. A greater understanding of these processes will add to fundamental understanding of cell and immune biology and may be useful in creating powerful tools for regenerative medicine.

Acknowledgement:

This work was supported in part by grants from the NIH (GM 49039).

References

- Orsi S et al. *Biomater*;31(3):570-576.
- Smeal RM, Tresco PA. *Exp Neurol* 2008;213(2):281-292.
- Zaman MH et al. *Proc Natl Acad Sci U S A* 2006;103(29):10889-10894.
- Methe H, Edelman ER. *Transplant Proc* 2006;38(10):3293-3299.
- Nugent HM et al. *J Vasc Res* 2002;39(6):524-533.

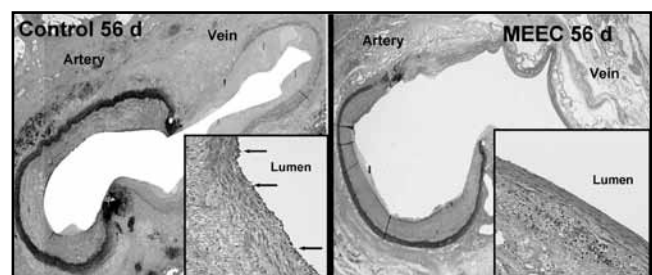


Figure 3: 3 Cross-sections of the anastomotic sites, 2 months after surgery. When compared to control anastomoses (A) perivascular endothelial cell implants (B) reduced intimal hyperplasia. Reproduced with permission of S. Karger AG, Basel from Ref. 5