Specific endothelial cell responses to biomimetic surfaces in fibrin gels

Georges Sabra, Patrick Vermette
1. Laboratoire de Bioingénierie et de Biophysique de l'Université de Sherbrooke
Department of Chemical and Biotechnological Engineering, Université de Sherbrooke
2500, blvd de l'Université, Sherbrooke, QC, Canada, J1K 2R1.
2. Research Centre on Aging, Institut universitaire de gériatrie de Sherbrooke,
1036, rue Belvédère Sud, Sherbrooke, QC, Canada, J1H 4C4.

Introduction

In many cases, the survival of tissues produced by tissue engineering methods depends on the vascularization of the scaffold which supplies cells with nutrients and oxygen and allows waste removal. Hence, understanding cell behavior in three-dimensional cultures becomes essential to modulate and to achieve controlled cell responses and consequently better tissue formation and vascularization. Low-fouling surfaces are used to obtain well defined biomimetic surfaces that allow the discrimination between specific and non-specific cell responses[3,4] according to the molecule grafted on the surface. But few studies report the use of such biomimetic surfaces in a 3D environment, where signals also come from the extracellular matrix (ECM) environment.

In this study, we investigate the effects of synthetic GRGDS and CDPGYIGSR synthetic peptides covalently immobilized on low-fouling surfaces on human endothelial cell responses in a fibrin gel.

Methods

1. Surface preparation and peptide immobilization

Borosilicate glass surfaces were modified by cold plasma polymerization of *n*-heptylamine[1]. The amine groups of *n*-heptylamine plasma polymer layer were then used to covalently graft the carboxy-methyl-dextran (CMD) layers by carbodiimide chemistry[2]. Afterwards, synthetic gly-arg-gly-asp-ser (GRGDS) or gly-arg-gly-glu-ser (GRGES) peptides were immobilized on activated CMD surfaces by amide chemistry and CDPGYIGSR by maleimide chemistry. The immobilization of peptides on low-fouling surfaces presents the advantage of preventing non-specific protein adsorption[2] supporting specific cell-bioactive surface interaction[3,4].

2. Cell culture and cell responses

The biomimetic surfaces were embedded vertically in fibrin gels containing HUVEC. This approach was employed to avoid the sedimentation of cells onto the surfaces caused by gravity and to characterize the directional movement of cells toward the surface. Cell responses were analyzed by fluorescence microscopy.

Results

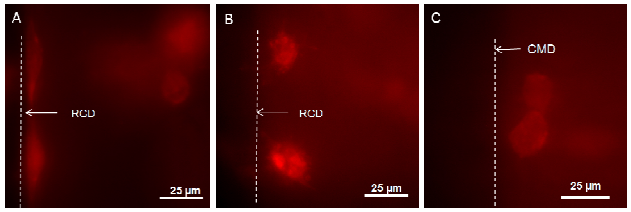

Figure 1. HUVEC responses to CMD and RGD surfaces after 2 hours of incubation (actin stained with red phalloidin).

Figure 1 clearly shows the bioactivity of RGD surfaces compared to the negative CMD control. Note that after 2 hours of incubation, cells in contact with RGD surfaces spread and adhere to them while those in contact with CMD surfaces remain quiescent.

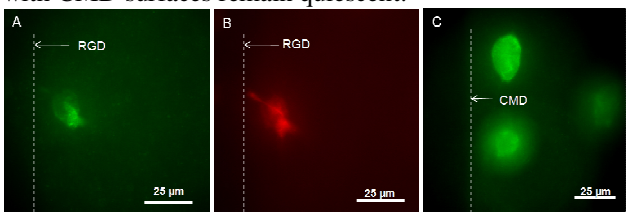

Figure 2. Cell migration toward RGD surfaces after 6 hours of incubation (caveolin-1 immunostained with AF488 and actin with red phalloidin).

Caveolin is polarized in migrating endothelial cells (Fig. 2A). It can be clearly seen that the cytoskeleton makes contact with the surface in Figure 2B, illustrating that cells are attracted by RGD surfaces and migrated toward them. Cells in contact with the CMD surfaces do not exhibit migrating characteristics since caveolin appears well distributed and not polarized (Fig. 2C).

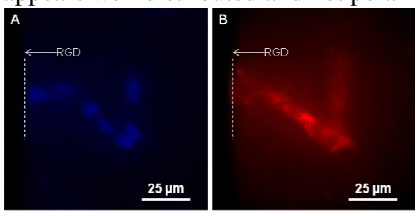

Figure 3. Effect of RGD surfaces on cell recruitment after 24 hours of incubation (Hoescht and red phalloidin staining).

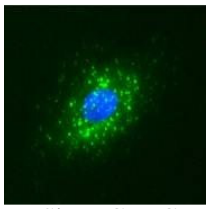

Figure 4. HUVEC adhered to CDPGYIGSR surface. 67-kDa laminin receptor was immunostained with AF488.

Figure 4 shows the specific ligand-receptor interaction.

Since CDPGYIGSR is only present on the surface, but not in fibrin gel, and this peptide specifically bind to the 67-kDa laminin receptor, the biospecificity of the interaction is demonstrated.

Conclusions

The results revealed that cells can respond to a well-defined biomimetic surface in a three-dimensional environment and moreover, biospecific cell responses based on ligand-receptor interaction can be obtained.

References

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