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Photopatterned chitosan surfaces for spatially controlled immobilization of different cell types

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ABSTRACT

One of the major goals in biomaterials science is to accurately control cellular interactions with implantable surfaces. The control of interfacial phenomena by adequate surface modification approaches has become a challenge and the goal of many studies. The decoration of the surface with signaling molecules, proteins or small peptides can be used to control and direct cell responses to biomaterials surfaces. Our objective is to create a phototriggerable system that allows spatiotemporal control of the attachment of selected cell types using a combination of co-immobilized antibodies that address individual phenotypes.

INTRODUCTION

To control temporally and spatially cell adhesion to the surfaces different strategies have been reported. Most of works report thermally (Okano, Yamada et al. 1995), photochemically (Mrksich, Dillmore et al. 2004) (del Campo, Petersen et al. 2008) and electrochemically (Yeo, Hodneland et al. 2001) responsive surfaces and materials to manipulate cell adhesion. Light controlled cellular adhesion requires the use of a photosensitive molecule that will mediate cell attachment. In this work was used photosensitive biotin. This molecule is protected in the active site preventing binding to streptavidin (SAv). After irradiation protecting groups are released and the biotin is able to couple with SAv. The immobilized SAv was herein used to couple biotinylated antibodies. The main objective is to control cells that attach on a surface, using antigen recognition.
For this study were investigated the use of CD31 and CD105 to control the attachment of adipose stem cells (ACSs) and human umbilical vein endothelial cells (HUVECs) onto a chitosan substrate. This is a preliminary stage toward the development of a technology allowing temporally and spatial control of seeding of different cell types in the same surface.

MATERIALS AND METHODS

Surface modification

Chitosan films were modified with caged biotin using EDC/NHS coupling. 1mg of caged biotin was dissolved in a mixture of DMF, EtOH EDC and NHS to activate the carboxylic groups in the caged biotin. This mixture was incubated with the chitosan membranes for 3h. After immobilization membranes were washed in DMF and dried.

Production of SAv micropatterns
Chitosan films coupled with caged biotin were irradiated at λ = 365nm through a mask. To access the bioactivity of the biotin after irradiation, samples were incubated with fluorescently labeled SAv (10µg/ml).
Membranes were washed with PBS and imaged using fluorescence microscopy.

In vivo experiments- cell micropatterns

For the cell experiments, irradiated samples were incubated with purified SAv.
Biotin-conjugated mouse anti-human antibodies were then added and incubated with the surfaces.
To generate patterned cultures of endothelial and stem cells were used CD105 and CD31 antibodies.

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DISCUSSION

Substrates modified with the photosensitive biotin were irradiated at a wavelength of 365nm.

Photosensitive biotin contains two photoremovable groups covalently bound to its active site. (Figure 1)

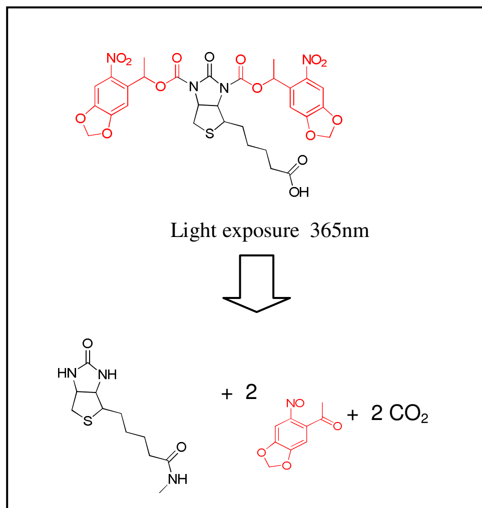

Figure 1: Light irradiation releases the cages from the biotin structure and restores its ability to bind to SAv

Irradiation cleaves the Nvoc group and liberates the reactive group from the biotin. Selective activation of the biotin on surface was accessed by incubation of these surfaces with fluorescently labeled SAv.

Results from fluorescence microscopy reveal that SAv bound selectively to the exposed regions, demonstrating the possibility of regioselective uncaging and immobilization. (Figure2)

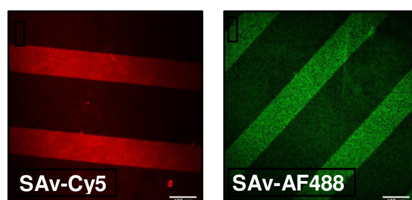

Figure 2: Fluorescence image of patterned substrates after coupling of fluorescently labeled SAv

For the cell experiments purified Sav was coupled to the active biotin on the surface. Biotin-conjugated mouse antihuman antibodies were used for testing surface modification. The ability of the patternd antibodies to attach cells selectively on the surface was tested in a cell adhesion assay with ASCs and HUVECs. Stem cells isolated from adipose tissue from present a CD45-CD31-, CD34+, CD105+ surface phenotype. HUVECs display a endothelial phenotype; CD146+, CD31+, CD105+, CD141+. To access the influence of the antibody on surface in cell attachment were used biotin conjugated CD31 and CD105 antibodies.

Figures 3 presents the attachment of the ACSs and HUVECs on the chitosan surfaces patterned with antibodies after 24h in culture.

It can be seen from Fig. 3 that, after 24 h culture, compared with the CD31 surface (Fig. 3A) the attachment of ACSs on the CD105 antibody coated surface (Fig. 3B) is significantly increased. However it was not possible to distinguish the pattern of antibodies on the surface.

In the case of HUVECs that express both antigens, cells attach in both surfaces. It seems that CD31 is highly expressed and cells are almost confluent in the surface after 24h (Fig. 3C). From the surfaces modified with CD105 (Fig. 3D) it was the only one where was possible to clearly see the patterned antibodies. We are currently optimizing antibody coupling conditions in order to obtain defined cells patterns.

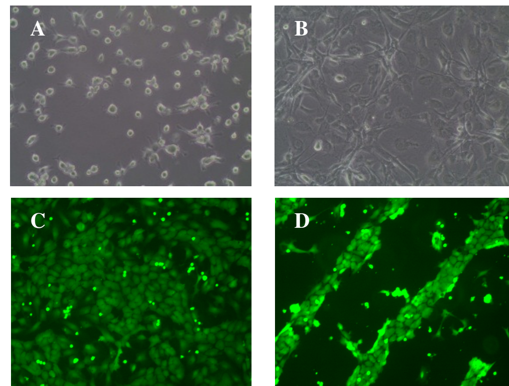

Figure 3: Cell attachment on substrates with patternd antibodies. A) ASCs on CD31, B)ASCs on CD105, C)HUVECs on CD31, D) HUVECs on CD105 Image show cells after incubation 24h.

CONCLUSION

In the presented work were developed chitosan membranes conjugated with caged biotin that allowed light-triggered coupling of SAv and biotinylated antibodies that recognize specific cell phenotypes and allow formation of cell patterns. There is still on going the optimization of antibody coupling, cell recruitment and pattern formation. These patterns will be used to

study co-cultures with predefined positional control of the different cell types.

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