Biomimetic surfaces to study carbohydrate-mediated cell trafficking






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The project "GAG2D" exploits nanoscience tools for the development of well-defined biomimetic surfaces for advanced biomolecular and cellular studies. It aims at understanding the role of the extracellular matrix polysaccharides known as glycosaminoglycans (GAGs), in the sequestration and presentation of chemokines, which are important communication molecules between cells.
More precisely, heparan sulfates (HS) are able to bind different chemokines with nanomolar affinity, making it possible, that cells could be guided by gradients of immobilized chemokines at the extracellular matrix surface. The ability to control and characterize the supramolecular presentation of GAGs, *in vivo* or *in vitro*, is to date very limited, and the project aims at alleviating this technological bottleneck.

Interdisciplinary and collaborative, the project is assembling the expertise of four research teams in Grenoble, namely:

- the I2BM team at DCM - which hosts the Chair of Excellence
- the SAGAG team of Hugues LORTAT-JACOB (IBS),
- the IMBM team of Catherine PICART (LMGP) and
- the CREAB team of Roberto CALEMCZUK (INAC/SPrAM).

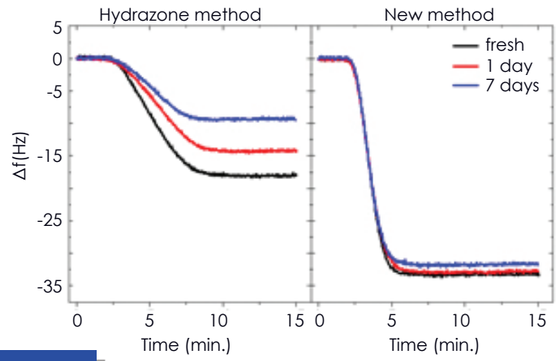

Fig. 7 : Study of stability and reaction yields of biotin-conjugated heparan sulphate (b-HS), prepared either by traditional hydrazone ligation or by the new method. QCM-D frequency shifts (Δf) were measured during exposure of b-HS solutions, previously stored at 4 °C for variable times as indicated, to streptavidin-coated surfaces (0 to 12 min). The frequency shifts relate to the surface density of immobilized b-HS; magnitudes and rates inform about the concentration and degradation of b-HS in the sample solution.

Oxime ligation – an easy applicable method for the terminal functionalization of GAGs

A new one-step ligation method was developed by the DCM and the IBS teams for the terminal functionalization of HS and other GAGs, such as hyaluronan. This method is superior in terms of yield and stability to hydrazone ligation. In particular, stable biotinylation of HS proved critical towards the reliable preparation of glycosaminoglycan-functionalized surfaces. This step represents a milestone in the project "GAG2D", and will find a broad use as a facile method for GAG functionalization.

Quartz crystal microbalance (QCM-D), a surface sensitive analytical technique (available as part of the Nanobio facilities for Characterization of Surfaces and Interactions) was used to monitor the binding of biotinylated HS (b-HS) to streptavidin-coated model surfaces. Fig. 7 shows that both the magnitude of Δf (related to the surface density of b-HS) and the maximal binding rate Δf/Δt (related to the effective concentration of b-HS in the sample solution) decrease appreciably with storage time for hydrazone-ligated b-HS whereas only minor changes were observed for b-HS prepared with the new method. The decreases are caused by the release of biotin upon degradation of b-HS conjugates, demonstrating that conjugates produced via hydrazone ligation degrade substantially within a few hours or days, whereas the conjugates prepared with the new method are virtually stable for at least several weeks. Moreover, comparison of maximal binding rates for freshly prepared samples revealed an approximately 5-time higher effective concentration of b-HS in the sample prepared with the new method, even though the total HS concentration in both samples was the same. This illustrates that the yield of the new ligation method is also considerably improved.

Tailor-made model surfaces that display GAGs, chemokines and integrin ligands

A novel and versatile approach was developed at DCM to create biomimetic surfaces that display HS and other cell membrane or extracellular matrix components (Fig. 8). The surfaces are well defined: HS can be immobilized stably with controlled orientation and lateral mobility, at quantitatively tunable surface densities. The surfaces are multifunctional: they are inert to non-specific binding of most proteins, additional functional groups (e.g. the integrin ligand RGD) can readily be incorporated, and chemokines can be bound and displayed in the native form via the HS chains. The method is versatile: the basic surfaces constitute a molecular printboard to which various molecules with suitable tags can be immobilized. This platform is now available for novel and quantitative biomolecular as well as cellular studies.

FURTHER READING

N. S. Baranova, E. Nilebäck, F. M. Haller, D. C. Briggs, S. Svedhem, A. J. Day and R. P. Richter
The Inflammation-Associated Protein TSG-6 Cross-Links Hyaluronan via Hyaluronan-Induced TSG-6 Oligomers
J. Biol. Chem. 286(29)25675-86 (2011)

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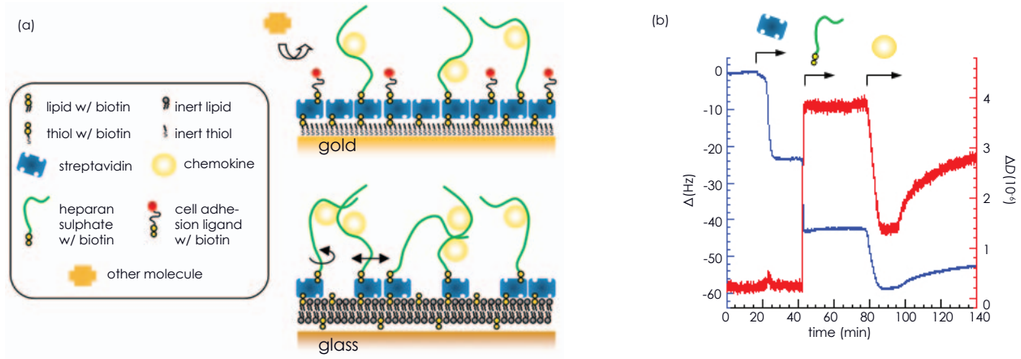

Fig. 8 : (a) Sketch of developed surface functionalization approaches, based on streptavidin-coated self-assembled OEG monolayers (top) and supported lipid bilayers (bottom).
(b) Assembly of a functional surface on a self-assembled OEG monolayer, followed by QCM-D. Start and duration of each sample incubation step is indicated by arrows. The binding kinetics and stability of the biomolecular layers are assessed in real time. A negative frequency shift (Δf) indicates binding of molecules; the dissipation shift (ΔD) is insightful for the viscoelastic properties of the surface-confined film.

Cells recognize and respond to the presentation of chemokines through GAGs

The well-defined biomimetic surfaces displaying the chemokine SDF-1a through its native ligand HS are now being used as substrates for cellular studies with two distinct cell lines known to be sensitive to SDF-1a: T-lymphocytes (of interest in the context of the immune response), and myoblasts (for muscle development and regeneration).

T-lymphocytes adhere to surfaces where SDF-1a is specifically bound to HS, to an extent comparable to the positive control, i.e. fibronectin (Fn) coated surfaces. In contrast, cell adhesion to HS-coated surfaces lacking SDF-1a or to HS-free surfaces in the presence of SDF-1a in solution was comparable to the negative control, i.e. bovine serum albumin (BSA) coated surfaces, indicating that the non-specific adhesion of T-lymphocytes to the model surfaces is not significant. The specific recognition of HS-bound chemokines demonstrates that the project's surface-functionalization strategy is a useful platform to create well-defined models of the endothelial cell surface for future cellular studies on the role of GAGs in chemokine-mediated cell migration.

Myoblast adhesion to this model surfaces was assessed by quantifying cell spreading in terms of the average surface area occupied per cell (Fig. 9). The cell area was significantly increased on surfaces displaying HS-bound SDF-1a as compared to HS-coated surfaces lacking SDF-1a or surfaces exposing bare streptavidin (SAv) without HS or SDF-1a.
Moreover, the myoblasts were found to adhere strongly and selectively to surfaces displaying the tripeptide sequence RGD, a known ligand for integrin cell adhesion receptors. Interestingly, the morphology of the adhered cells was distinct with HS-bound SDF-1a as compare to RGD, revealing that distinct mechanisms are involved in cellular recognition and internal signalling.

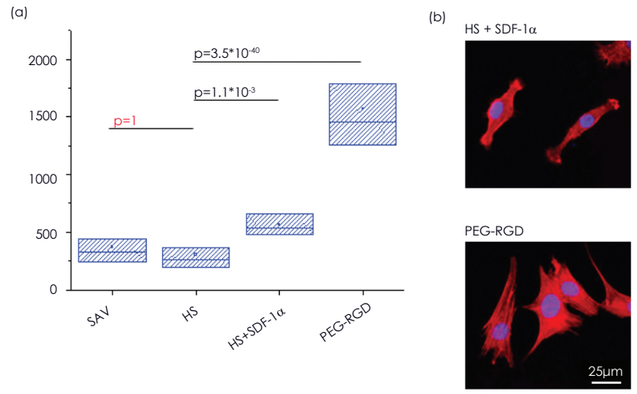

Fig. 9 : Adhesion and spreading of C2C12 myoblasts. Cell adhesion and spreading were observed 4 h after plating on surfaces with different functionalization. (a) Box plot of the cell area with results of an ANOVA test.
(b) Representative micrographs of fixed cells with the cell nuclei labeled in blue and actin in red reveal distinct cell morphologies on surfaces presenting HS-bound SDF-1a or RGD, respectively.

Significant progress has been made in the preparation of well-defined surfaces that reconstitute GAGs and other cell membrane and extracellular matrix components (lipids, proteins) in vitro into multifunctional model surfaces that recapitulate selected aspects of the in vivo situation. Thanks to state-of-the-art surface biofunctionalization strategies, and a toolbox of surface-sensitive in situ analysis techniques, the model surfaces can be tailored with tight control on the orientation, surface density and lateral mobility of the exposed biomolecules. Such model surfaces are now being used for novel and quantitative biomolecular and cell biological studies, to understand the role of GAGs in cell adhesion and trafficking, and in cell-cell and cell-ECM communication in general. They also open novel avenues for the development of biosensor applications, and for the control of cellular fate.

FURTHER READING

S. Attili and R. P. Richter.
Self-assembly and elasticity of hierarchical proteoglycan-hyaluronan brushes
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