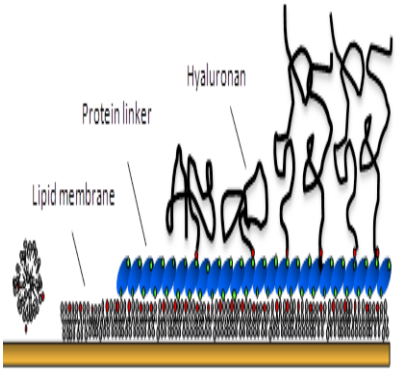

Model System to Reconstruct HA Matrix Assembly

Description

Biological surface science is an interdisplinary field which provides powerful tools to study biological phenomena. Well-defined biomimetic systems can be created thanks to numerous methods of surface fabrication. Information about the structural properties of surface-assembled ultrastructures can be obtained on the micro- and nanoscale by highly sensitive high resolution techniques Kasemo, 2002. A main scientific challenge for surface science is to reproduce the interactions, structures and kinetics of the biological systems in a similar way as it has been done by nature. To reconstitute the biological structure, the molecular building blocks should be brought on the surface in proper order and at the right concentrations. Surface biofunctionalization allows controlling the distribution, surface density and orientation of molecules of interest on the surface of choice.

Definitely, the main advantage of model systems is their simplicity. The reduced complexity compared to the native system allows for highly controlled measurements and to derive quantitative information on specific interactions. On the other hand. the translation of the properties of model systems to the functions of real biological systems remains a bottle neck. Direct comparison of the knowledge gathered in the model systems with the real biological system is essential to fill the gap between the reductionist approach of the models systems and the complexity of the real biological systems. However, starting from a simplified point of view on a specific question, the entire puzzle of the biological assembly can potentially be solved.

Surface-confined model systems are of particular relevance for the investigation of PCM structure. Currently, progress has been made on the understanding of the PCM functions on the cellular and molecular levels, but there is a limited understanding at the intermediate level â?? the supramolecular organization. It remains poorly understood, how different hyaladherins interact simultaneously with HA and how each protein contributes to the organization of cellular coats. A model system displaying surface-bound HA can shed light on these questions and serve as appropriate mimic of PCM, since HA in the PCM also remains attached to the cell surface and is able to self-organize with proteins into different structures.

Figure10 The surface-based model system of HA matrix. The construction of films of end-grafted HA was performed on a solid support (silica) which was first functionalized with a supported lipid bilayer, where some lipids carry biotin groups (red). A monolayer of the linker protein streptavidin (blue) was then immobilized on the surface, in order to attach the next layer â?? end-biotinylated HA chains.

The main strategy for PCM reconstitution is HA immobilization on the surface. Several approaches exist to modify surfaces with HA Morra, 2005. Among them are : passive adsorption on the surface Delpech et al., 1985, formation of polyelectrolyte multilayers, where HA is mixed with chitosan or poly-L-lysine Picart et al., 2002Burke & Barrett, 2003, covalent coupling of HA to a variety of solid supports and functionalized layers, like glass AlbersdÃ¶rfer & Sackmann, 1999, polystyrene Joester et al., 2006 and mixed supported lipid bilayers Benz et al., 2004. Coupling of HA was also performed via the HA binding protein p32 which was grafted to NTA- functionalized supported lipid bilayers (SLB) via a histidine-tag Sengupta et al., 2003. More sophisticated immobilization of HA, with controlled density of anchorage points, was presented by Wolny et al, where HA was immobilized to the SLB via the extracellular part of the cell-surface HA-receptor CD44 Wolny et al., 2010. In all mentioned immobilization strategies, HA was immobilized to the surface via side-grafting, i.e. via numerous grafting points along one HA chain. This type of immobilization results in the formation of thin HA films Sengupta et al., 2003Wolny et al., 2010, in which the exact conformation of the HA chains is not well known because the number and location of binding sites along the HA chains is difficult to control.

Recently, Richter *et al*., Richter et al., 2007presented a model system, where HA chains that were biotinylated at their reducing end were end-grafted to a biotin-functionalized SLB surface via attachment to a linker layer of streptavidin Richter et al., 2007. This approach provides tight control on the attachment of HA to the surface and tunability of the grafting density. In addition, the underlying surface is inert to the nonspecific binding of most proteins, i.e. it provides a passive background. Tunable and well-controlled HA attachment is a high advantage compared to the other immobilization approaches mentioned above. Films of end-grafted HA were intensively used throughout this thesis. Different modifications of the initial model system have been implemented in order to optimize the performance for the studies.

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