Combination of Layer-by-Layer Technique and Nanosphere Lithography to Control Adhesion of Cells*

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Introduction and Objective

The surface properties of implants or tissue engineering scaffolds play an important role to promote adhesion, growth and differentiation of tissue cells. Among many surface properties like wettability, surface charge, etc. also nano-structures have been lately defined as a tool to control the cellular behaviour. Here two methods are presented to generate nano-structures on material surfaces.

- The alternating adsorption of oppositely charged polyelectrolytes (PEL), also called layer-by-layer (LBL) technique, forms self-assembled nanostructures as multilayers. The application of biogenic polyelectrolytes allows mimicking of the extracellular matrix.
- Nanosphere lithography (NSL) generates well-defined nanoparticulate structures of gold depending on the used polystyrene particle size before electron beam physical vapor deposition (EBPVD) of gold. Subsequent modification of gold areas with adhesive cues is possible.

The present work was aimed at the control of cell adhesion by conditions during formation of polyelectrolyte multilayers (PEM). Furthermore, first attempts should be undertaken to obtain well-defined nanoparticulate gold structures. Both techniques shall be combined later to design unique surfaces for the control of cell behavior.

Materials

- Cover slips for microscopy (15x15 mm)
- Gold coated, thiolated sensors for (QCM-D)
- Poly (ethylene imine) (PEI): synthetic polymer, polycation, MW ~750 kDa
- Heparin (HEP): a glycosaminoglycan (GAG), anticoagulant activity, polyanion
- Fibronectin (FN): an adhesive protein with a specific binding domain for heparin
- Human fibroblasts (HF)

Methods

- Layer-by-layer technique (LBL) => control of HEP solution pH value
- Quartz crystal microbalance with dissipation (QCM-D)
- Static water contact angle measurement (WCA)
- Atomic force microscopy (AFM)
- Confocal laser scanning microscopy (CLSM), Immunofluorescence
- Scanning electron microscopy (SEM)

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WCA

- Change in wettability due to the alternating adsorption of PEI and HEP
- HEP layers are more hydrophilic than PEI layers
- Layers from PEM solutions at pH 9.0 are less hydrophilic than those at pH 5.0 or pH 7.0

Discussion

- Surface wettability influenced by dominance of PEI in the outer regions
- High rigidity of HEP layers occurs due to interpenetration and water displacement
- PEI dominates surface structuring due to its mole size
- HEP might penetrate into PEI layers due to lower MW => vermiculate structures
- PEI at pH 5.0 or HEP at pH 9.0 favorable for cell adhesion
- NSL creates highly ordered surfaces for later control of cell adhesion

Conclusion

- PEI and HEP are convenient PELs for multilayer formation
- pH variation leads to different PEM thickness, wettability and adhesion strength of surfaces
- NSL is a simple method to design nano-structured surfaces for control of cell adhesion and growth
- Possible combination of NSL and LBL => more precise control of cellular behavior

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