Controlling Cell Adhesion with Layer-by-Layer Technique and Nanosphere Lithography

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Introduction and Objective

Surface features like chemical composition, wettability, charge, viscoelasticity, and topology are of great importance as any biomaterial interacts with the biological environment through its surface. Different surface modification techniques have been developed to manipulate cell-material-interaction. We applied here a combination of nanosphere lithography (NSL) and layer-by-layer (LbL) technique to control cellular behaviour on biomaterial surfaces.

- 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 alternating adsorption of oppositely charged polyelectrolytes (PEL) forms self-assembled nanostructures as multilayers. The application of biogenic polyelectrolytes allows mimicking properties of the extracellular matrix.

Materials

- Cover slips for microscopy (15x15 mm)
- Gold coated, thiolated sensors; Polystyrene (PS) spheres of different diameter
- Poly (ethylene imine) (PEI): a synthetic polymer, polycation, MW ~750 kDa
- Heparin (H, 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)

Nanostructured surfaces

- Designed nanostructures with NSL and different PS particle sizes
- Particle size of ca. 500 nm favorable for design of regular structured surfaces
- Passivation of glass with PEG and activation of gold with amino groups was achieved

Discussion

- NSL creates highly ordered surfaces for later control of cell adhesion
- 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 molecule size
- HEP might penetrate into PEI layers due to lower MW => vermicate structures
- pH 5.0 => PEI on 1390nm and HEP on 200nm favorable for cell adhesion

Conclusion

- PEI and HEP are convenient polyelectrolytes for multilayer formation
- pH variation leads to different polyelectrolyte multilayer properties
- NSL is a simple method to design nanostructured surfaces for control of cell adhesion and growth
- Combination of NSL and LbL => precise control of cellular behavior
- Freedom of choice for system setup due to variability of design parameters