Mammalian cell adhesion on ordered, chemically modified nanostructures*

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Introduction and Objective

The interplay of topographical, chemical and electrical stimuli maintains regular functions of tissue cells in their natural environment. Mimicking of some, if not all, of these stimuli enables the researcher to control adhesion, growth and functions of cells on materials desired for biomedical application. However, the role of topographical and chemical cues is of utmost importance in most biomaterial applications. We applied a novel 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 (PEM). The application of biogenic PEI and HEP are convenient polyelectrolytes for multilayer formation.
- NSL is a simple method to design nanostructured surfaces for control of cell adhesion and growth.

Materials

- Silicon wafers (15 mm x 15 mm)
- Polystyrene nanoparticles (PS-NP) of different diameter
- Poly (ethylene imine) (P, PEI): 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
- Nanosphere lithography (NSL) => design of distinct nanostructures using various polystyrene nanoparticle (PS-NP) diameters
- Atomic force microscopy (AFM)
- Static water contact angle measurement (WCA)
- Confocal laser scanning microscopy (CLSM), Immunofluorescence studies

Materials

<table>
<thead>
<tr>
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</tr>
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Discussion

- Oscillation in wettability between HEP and PEI
- Outermost HEP layers more hydrophilic than PEI layers
- HEP at pH 5.0 more hydrophilic than at pH 9.0
- Wettability decreased from smallest to largest nanostructures on HEP-terminated PEM

Conclusion

- Always more cells on pH 9.0 than on pH 5.0, especially if no FN was present (not shown)
- Prominent effect of FN on cell count on HEP layers
- Cell count was constant or dropped on PEI layers
- Slight trend of increase in cell number with increase in structure size on PEI layers

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**NSL is a simple method to design nanostructured surfaces for control of cell adhesion and growth.**

**The interplay of topographical, chemical and electrical stimuli maintains regular functions of tissue cells in their natural environment. Mimicking of some, if not all, of these stimuli enables the researcher to control adhesion, growth and functions of cells on materials desired for biomedical application. However, the role of topographical and chemical cues is of utmost importance in most biomaterial applications. We applied a novel combination of nanosphere lithography (NSL) and layer-by-layer (LbL) technique to control cellular behaviour on biomaterial surfaces.**

**Materials**: Silicon wafers (15 mm x 15 mm), Polystyrene nanoparticles (PS-NP) of different diameter, Poly (ethylene imine) (P, PEI), Heparin (H, HEP), Fibronectin (FN), Human fibroblasts (HF).

**Methods**: Layer-by-Layer technique (LbL), Nanosphere lithography (NSL), Atomic force microscopy (AFM), Static water contact angle measurement (WCA), Confocal laser scanning microscopy (CLSM), Immunofluorescence studies.

**Discussion**: Oscillation in wettability between HEP and PEI, Outermost HEP layers more hydrophilic than PEI layers, HEP at pH 5.0 more hydrophilic than at pH 9.0, Wettability decreased from smallest to largest nanostructures on HEP-terminated PEM.

**Conclusion**: Always more cells on pH 9.0 than on pH 5.0, especially if no FN was present (not shown), Prominent effect of FN on cell count on HEP layers, Cell count was constant or dropped on PEI layers, Slight trend of increase in cell number with increase in structure size on PEI layers.