Application of functionalized magnetic beads to produce structured surfaces for contacting living cells

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CellPROM Cell Programming by Nanoscaled Devices

CellPROM Project[1]

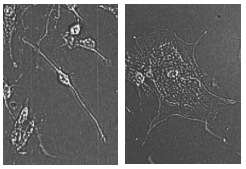
- "reprogramming" individual cells by structured and functionalized surfaces (non-invasive) on an industrial scale.
- first steps in "Tissue Engineering".

Our part:
- developing a variable system for inducing cell differentiation
- avoiding difficult chemical steps for structuring substrates
- delivering a wide range of differnt samples for testing cell behavior as a function of surface topography

Stem cells

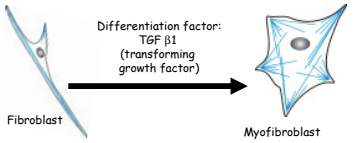
- unspecialized cells or not completely specialized
- no certain function in the living organism
- ability for cell division
- certain physiologic conditions induce to become cells with special functions
  ⇨ differentiation
- adult stem cells: everywhere in the body, mainly in the bone marrow, ethical not critical
- assumtion of repair mechanism: stem cells get signals from damaged tissue and develop into the needed cell type

Cell Model: NHDF



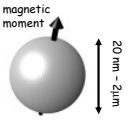
spindle-shaped fibroblast

star-shaped fibroblast (progenitor cell to myofibroblast)

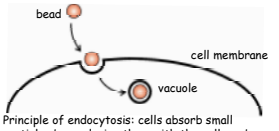


Fibroblasts are part of the connective tissue. Their cytoskeleton is mainly build of actin filaments which give rise to high elasticity and stability. Under influence of TGF β1 diluted in culturing medium fibroblasts undergo a stem-cell-like differentiation to myofibroblasts. These cells express **α** smooth muscle actin, which can be checked by immunostaining. As this differentiation is well characterized and controlable this cell type (NHDF: normal human dermal fibroblast) is a candidate for testing the here introduced method for preparing surfaces.

Beads



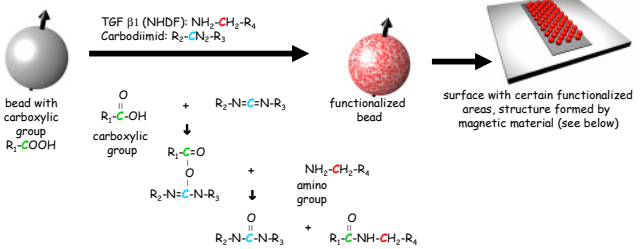
- magnetite
- superparamagnetic or ferromagnetic
- commercial available with different chemical properties (NH_2, $COOH$)



Principle of endocytosis: cells absorb small particles by enclosing them with the cellmembrane

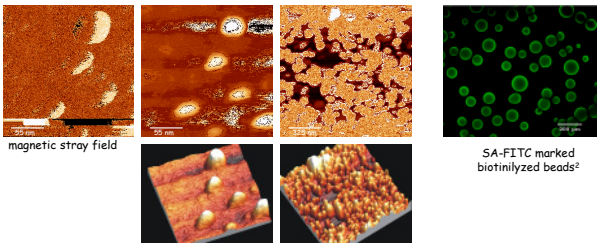
small beads	⇔	large beads
-superparamagnetic -beads itself are a structure		- prevention of endocytosis

Functionalization



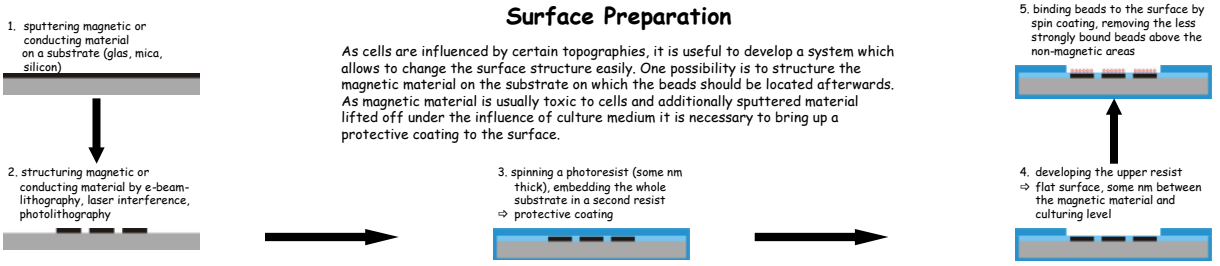
Covalent binding of biomolecules gives the guarantee that not many of the molecules get in solution with the culturing medium. For example the TGF β1 reacts with the carboxylic group of the beads through an intermediate step with a carbodiimid.

Bead Visualization



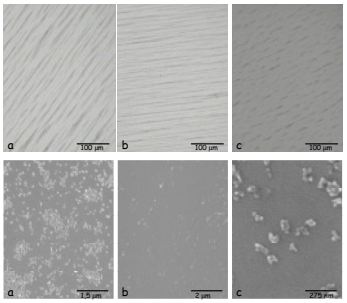
It´s not possible to visualize single small beads in a light microscope. Therefor magnetic force and atomic force microscopy are used. While AFM is sensitive to short range forces (mainly van-der-Waals, -> topographical information), the MFM technique gives information about the magnetic properties of single beads or whole structures.
Beside this, SEM allows a good overview over a large area of the substrate (see below). Fluorescence microscopy is suitable for larger beads about several microns in diameter.

Surface Preparation



As cells are influenced by certain topographies, it is useful to develop a system which allows to change the surface structure easily. One possibility is to structure the magnetic material on the substrate on which the beads should be located afterwards. As magnetic material is usually toxic to cells and additionally sputtered material lifted off under the influence of culture medium it is necessary to bring up a protective coating to the surface.

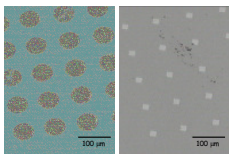
Surface Structures



Beads (100nm) were solved in H_2O dest. and brought to the surface in a droplet. A magnetic stirrer under the substrate delivered a rotating field. Depending on rotation velocity (increasing from a to c) different structures formed (space between two lines, thickness and lenght of lines). Turning off the stirrer causes "freezing" of the structure.
(light microscopic images)

In correlation to different concentrations of beads in the droplet for spin-coating, structures from monolayers (rather high concentration) up to very low concentration of biomolecules can be obtained (a,b). Additionally the SEM allows for further insight into the bead structure (c). This confirms that the "diameter" is just an avaeraged value.
(SEM images)

It´s evident that not only chemical porperties of surfaces have influence on cells but also the topography.
⇨ "contact guidance"[3,4]
To produce structures, there are several possibilitis:
1. Structuring the magnetic material during the production of the surface. The functionalized beads are brought to the surface and bind by magnetic interaction to the areas formed by magnetic material (see right side)
2. Not structuring the magnetic material, just use as amplification of interaction forces between beads and surface. Structures are obtained by handling the beads in different concentrations and various external fields.



Structures of magnetic material obtained by e- beam lithography: circles, ϕ 60 µm and squares, 20x20 µm, both 30 nm high. The beads are supposed to bind to the magnetic areas.

Summary

A proposal how to produce a system, which allows easy changing of the chemical and topographical properties of a substrate for inducing cell differentiation is delivered. The idea, which is mainly based on the usage of functionalized magnetic beads will be tested on the differentiation step of fibroblasts to myofibroblasts, using TGF β1 as differentiation factor. To combine chemical signals (biomolecules) with topographical information some ways for structuring the substrates have been established. On the one hand influencing the beads with external magnetic fields allows the production of large structured areas with simple geometry. On the other hand methods like e-beam-lithography deliver more complex structures and smaller areas.

References

1 : www.cellprom.net
2: in collaboration with H. Bohn: *Rastersondenmikroskopische Charakterisierung biologischer Partnersysteme,* Diplomarbeit H. Bohn (2003), Uni Saarland, AG Prof. Dr. Hartmann
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