Application of ferrofluids to develop biocompatible microstructured surfaces and characterization of cell-surface interactions by using SPM methods





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CellPROM Project

- eprogramming" individual stem 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 different samples for testing cell behavior as a function of

Cell Model: NHDF



fibroblast



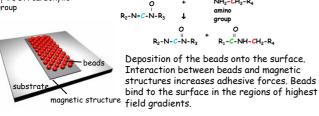
fibroblast (progenitor cell to myofibroblast)

TGF B1 Myofibroblast

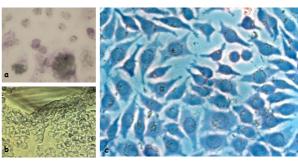
TGF $\beta1$ (transforming growth factor) diluted in culturing medium leads NHDF (normal human dermal fibroblast) to undergo a stem-cell-like differentiation to myofibroblasts. This mechanism is well characterized and therefor a good test candidate for the here introduced surface preparation.

Concept covalent binding lithographic methods and changing of the deposition domain structures protective coating unstructured artificially on the surfaces structured surfaces surfaces induction of differentiation?

Beads magnetic moment Differentiation (growth) factor for example TGF $\beta 1$ for firoblasts - TGF β1 (NHDF): NH₂-CH₂-R₄ - Carbodiimid: R₂-CN₂-R₃ superparamagnetic functionalized bead 0 fluorescent with covalently bound R1-C-OH - magnetite particles in molecules a dextran matrix R,-C=0 - R₁-COOH carboxylic group R2-N=C-N-R2 R1-C-NH-CH2-R4

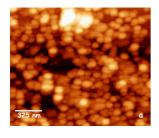


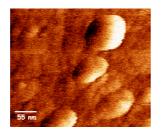
Biocompatibility Tests



- (a) HuTu on Permalloy after three days. The magnetic material peels off under cultivation conditions ⇒ protective coating
- (b) L929 on a garnet film after five days. Cells grow well to confluency. (c) L929 with 100nm beads added to culturing media. Endocytosis: Cells
- collect beads around the core, dependent on size, reactive groups and material of the beads.

Bead Visualization



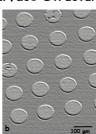


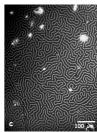
Magnetic force and atomic force microscopy are used to visualize single magnetic beads. The MFM technique gives information about the magnetic properties (stray field) of the beads or magnetic surface structures (not shown).

- a) AFM, Tapping Mode, $f_{resonant} = 70 \text{ kHz}$
- b) MFM, 50 nm mean distance to the surface, same cantilever, coated with 30 nm CoCr

Surface Structuring







- a) Light microscopic images, Beads deposited on glass, structures obtained by using a magnetic stirrer. Turning off the stirrer results in "freezing" of the structure
- b) Magnetic thin films (here Permalloy FeNi 30 nm thick) structured by ebeam-lithography. Circles ϕ 60 $\mu\text{m}.$ c) Garnetfilm : Y Sm Bi Ga Fe
- white/dark contrast ↔ antiparallel magnetization
- beads can be deposited between white and dark areas (field gradient)

Summary

A highly variable set up for a straightforward change of the chemical and topographical properties of a substrate for inducing cell differentiation is presented. The usage of functionalized magnetic beads allows to combine topographical with chemical (biomolecules) conditions.

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