Effects of weak static or low frequency magnetic fields on human fibroblasts

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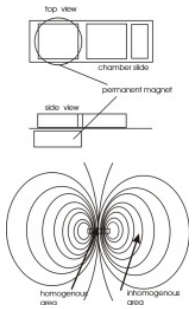
Motivation

- Extern magnetic fields influence the Ca-concentration inside a cell[1]
  - Proteins which are regulating the Ca-ion inside a cell (e.g. troponin) bind to the ions for a certain time inside a cavity which is formed by unfolded proteins.
  - The probability of ion emergence depends on the ion state inside the binding cavity.
  - If an extern magnetic field is applied, the ion state inside the protein changes.
- The dynamic process of building up actin filaments depends on Ca-ion concentration.
- Effects of weak magnetic fields on cell topography were already found[2,3].

Cell culture

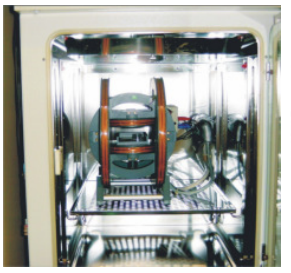
- For all experiments NHDF (normal human dermal fibroblasts) have been used.
- Cultivation from passages 8-20 (with time cells lose their ability for cell division).
- Cells have been cultivated on chamberslides (two separated chambers on one slide) in medium quantum333 (PAA Laboratories).
- 48h under test conditions ⇨ every cell divided once.
- Fixation with methanol (-20°C).

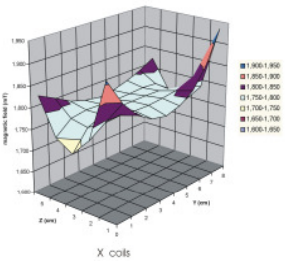
Static Field



A permanent magnet of 0.8 T was situated below the chamberslide so that one part of the slide was in a homogenous area and the other one in a gradient field.

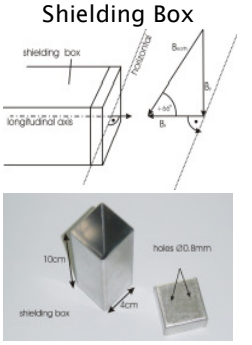
Alternating Field





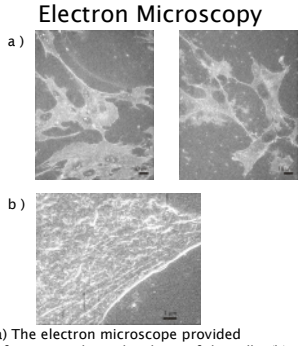
The alternating magnetic field (0.8 mT, 50 Hz) was produced by Helmholtz coils. Experiments were carried out with parallel or perpendicular orientation of the field to the cultivation surface. The homogenous area of the smallest pairs (x-direction) was large enough to position a chamberslide inside[4].

Shielding Box



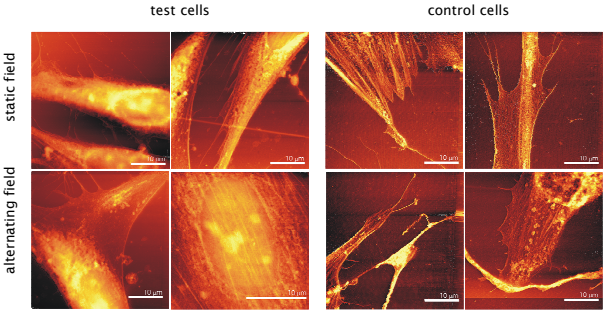
The mumetal box (μ_r= 25000 ⇨ shielding factor: A_{par}=500, A_{ort}=250) decreases the intern field to a maximum value of 180 nT (earth magnetic field 50 µT, angle of hade 66°).

Electron Microscopy



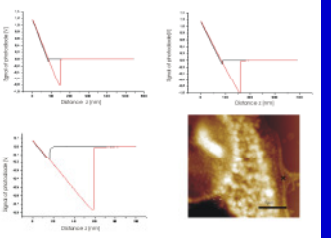
(a) The electron microscope provided information about the shape of the cells, (b) but not further insight into changes of the actin filaments. Probably they have been destroyed during the sputtering process. The shape of the cells does not vary between test and control cells.

Atomic Force Microscopy



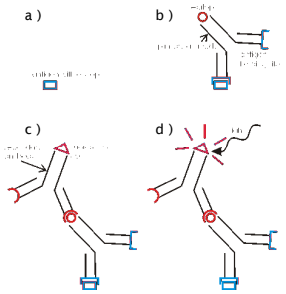
For all AFM images cantilevers with resonant frequency f=17 kHz and spring constant k=0.1N/m have been used in contact mode. Single actin filaments are visible. Comparison of cell height and thickness of the filaments of test and control cells show no significant difference. Signals between two cells are artifacts which occured during the drying process in methanol.

Force-Distance Curves



The force-distance curves have been taken on actin filaments. The black curves represent the cantilever approach to the surface, the red ones the retraction. The linear behavior after jump-to-contact is typical for a hard material, although the filaments have been expected to be smooth. No differences between test and control cells appear. Further investigations will involve liquid environment.

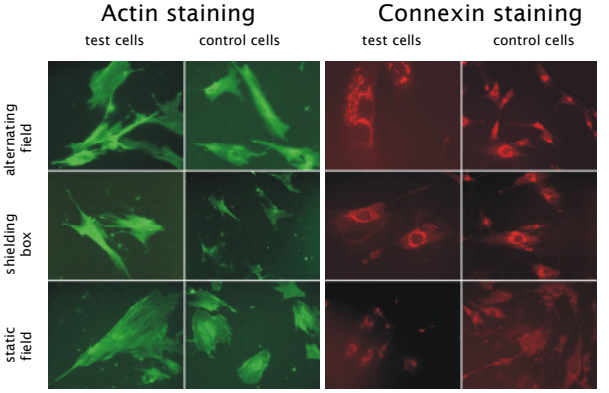
Principle of indirect staining



(a) Actin and connexin proteins are antigens. (b) Washing with primary antibody. (c) Washing with secondary antibody. (d) Proteins are visible in the fluorescence microscope. Indirect staining is more specific, because backround signals are reduced and several secondary antibodies can react with one epitop.

Actin staining

Connexin staining



The actin staining reveals single filaments, but no differences between test and control cells. The changes in Ca-ion concentration were not strong enough. The connexin staining showed proteins around the cell core and not, like expected, at focal contacts. Background staining is not completely avoidable because of van-der-Waals attraction between antibodies.

Conclusions and Outlook

- ◇ No significant differences of cell growth between cultivation under alternating, static and shielded field respectively occur.
- ◇ Possible changes on the molecular level are to small to arouse changes of the cytoskeleton, which can be detected with immunostaining or AFM.
- ◇ Western Blot analyses of connexin and actin expression reveal no quantitative differences between test and control cells.
- → In vivo investigations with fluorescence dyes can provide further insight of the functionality of Ca binding proteins under different magnetic conditions.
- → AFM images in vivo in liquid environment can show the dynamic behavior of cells.
- → Experiments on influences of magnetic particles will be carried out. Magnetic beads, coated with biomolecules (growth factors, proteins etc.), will be used to produce nanostructured surfaces to contact cells in a well-defined manner.

References

[1] V. N. Binhi and A. V. Savin, *Effects of weak magnetic fields on biological systems: physical aspects*, Physics-Uspekhi 46 (2003) 259-291.
[2] S. Pacini, *Effects of 0.2 T static magnetic field on human skin fibroblasts*, Cancer Detection and Prevention 27 (2003) 327-332.
[3] M. Girasole, *Atomic force microscopy study of lymphoblastoid cells under 50-Hz 2-mT magnetic field irradiation*, Appl. Phys. A 67 (1998) 219-223.
[4] ChenSun Gao, SAMS Project 1999-2001, Institut of experimental physics, Saarland University.