Multipotent adult progenitor cells (rMAPCs):
The imaging of cell differentiation and the influence of nanostructured and functionalized surfaces

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CellPROM Cell Programming by Nanoscaled Devices

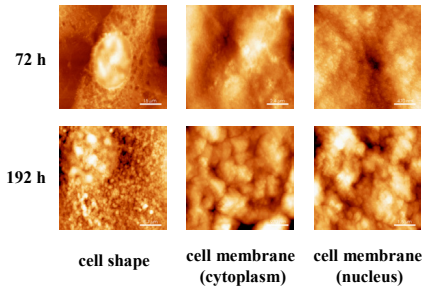
Introduction

Multipotent adult progenitor cells (MAPCs), characterized by Verfailles *et al.* in 2002, are a subpopulation of mesenchymal stem cells. MAPCs can be expanded for > 120 population doublings. They proliferate without obvious signs of senescence. These cells, isolated from human or rodent bone marrow, have the capability to differentiate into most of the mesodermal cell types, neuroectoderm-like and hepatocyte-like cells. Mouse (m)MAPCs can also be injected in blastocysts and contribute to most somatic cell lineages, even to cell types of the brain.

Motivation

A main ambition of the study is to figure out if the differentiation process of rMAPC and other cell lines can be influenced by the use of nanostructured and functionalized surfaces of different materials. The changes of the cell membrane and cell morphology during differentiation are characterized by microscopic methods. First steps of this investigation are culturing and imaging rMAPCs on different materials as glass or metals and the monitoring of the differentiation process. The second step involves the use of artificially modified surfaces, for example structured by electron beam lithography or magnetic beads.

rMAPC before and after reaching confluence



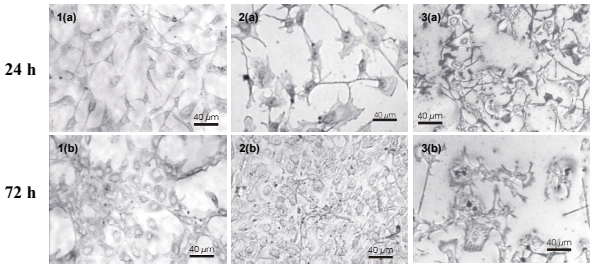
Sample preparation

rMAPCs were grown for 8 days on fibronectin (5ng/ml)-coated glass coverslips under standard culturing conditions (37°C, 5% CO_2, MAPC-medium). Cells were fixed with 70 %, 80 % and 99 % ethanol for 10 minutes each. Images were taken by atomic force microscopy (NSC18/AIBS cantilever, tapping mode). Confluence was reached after 72-96 h.

Experimental Results

The obtained images show no significant difference in all scanned cell-membrane areas before and after confluence. The confluent cells could be seeded again and they proliferate even several days after start of cultivation.

rMAPC growth on different unstructured and unfunctionalized materials



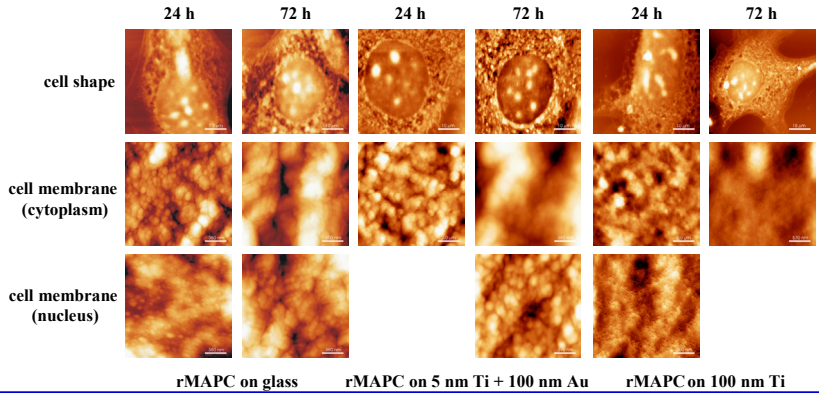
rMAPCs were grown for one or three days respectively on different materials. Cells were fixed as mentioned above.

According to recent investigations cell morphology depends on the chosen substrate material. In the present experiment the cells were cultivated on glass, gold and titanium. The latter two materials were deposited by dc-magnetron sputtering on a glass substrate with film thicknesses of approx. 100 nm for both metals.

Material	Cell shape	Confluence
glass	fibroblast-like	after 3 days
gold	more broadened	after 2-3 days
titanium	more broadened compared to gold	no confluence apoptosis

AFM investigations of rMAPC growth on different substrates

The sequence on the right side represents the growth process of rMAPC on different materials 24 h and 72 h after seeding. By comparing the obtained AFM images it becomes obvious that there is no significant difference in the scanned membrane areas of the fixed cells. Only a change in cell shape could be detected. The rMAPCs grown on gold surfaces show a more broadened shape than the cells cultivated on glass coverslips. The cells cultivated on titanium show an even more broadened appearance. Furthermore the rMAPCs grown on gold for 24 h show more distinct filopodium-like structures compared to the cells grown on the other substrates.



Further Reading

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2. Y. Jiang *et al.* Neuroectodermal differentiation from mouse multipotent adult progenitor cells. Proc Natl Acad Sci U S A. 2003 Sep 30;100 Suppl 1:11854-60. Epub 2003 Aug 18.
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4. M.J. Dalby *et al.* Fibroblast reaction to island topography: changes in cytoskeleton and morphology with time. Biomaterials 24 (2003) 927-935

Acknowledgements

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