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Investigation of Different Biomaterials for Bone and Cartilage Repair Tissue Engineering

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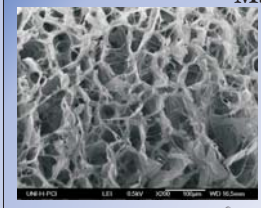
Introduction

One main objective in tissue engineering is the construction of precise scaffolds in order to support and guide cell growth and differentiation. The scaffolds have to be biocompatible, should support cell attachment, growth and differentiation to the desired phenotype.
The aim of the study was the investigation of different biomaterials for bone and cartilage tissue engineering. Cell growth of chondrocytes on different collagen matrices and the cell growth of primary osteoblasts on coated/uncoated Sponceram® was determined. Furthermore, the extracellular matrix synthesis of the chondrocytes was studied, in particular the expression of the typical chondrogenic marker collagen II was analysed.

Material and methods



SEM picture of MATRIDERM® 2 mm .

Collagen scaffold: The materials, developed by Dr. Suwelack Skin & Health Care AG, are currently used for cosmetic and wound healing purposes. The used matrix was MATRIDERM®, which mainly consists of bovine collagen I and small amounts of elastine. For the cell metabolism experiments also MATRIDERM® matrices crosslinked with carbodiimide (EC) or glucose (Glucose I) were used.

Cell culture: Matrices of 2 mm thickness and 6 mm diameter were prepared and desinfected with isopropanol (70 %) for 2-3 hours in a 96-well culture plate. Subsequently, the matrices were incubated over night in DMEM, 10 % FCS and antibiotics (Pen/Strep/Neo) at 37 °C / 5 % CO_2.
Primary chondrocytes were isolated from porcine articular cartilage**.** Cells were cultured with DMEM, 10 % FCS and antibiotics.
For differentiation the chondrocytes were cultivated with DMEM containing 10 % FCS, antibiotics, TGF-β1 (10 ng/ml), dexamethasone (100 nM) and vitamin C (80 µM).

Cell seeding: 5000 cells were seeded onto each matrix in a 96-well plate.

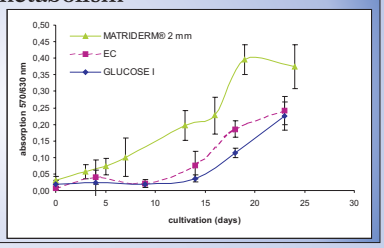
Cell metabolism: Cell viability was assayed using **MTT-test.**

Histological analysis: For histological assessment of the extracellular matrix components (collagen II) the scaffold sections were stained with Haematoxylin & Eosin, Safranin O/light green and Masson Goldner, respectively, using standard protocols (data not shown).
Collagen II was analysed by immunohistological staining with a mouse-anti-collagen II antibody.

Cell metabolism

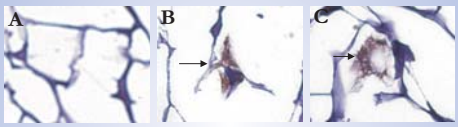
matrix	name
MATRIDERM® + carbodiimide	EC
MATRIDERM® + glucose	GLUCOSE I
MATRIDERM®	MATRIDERM® 2mm

Cell metabolism of chondrocytes on different collagen matrices over a time period of 24 days. Values represents the mean of 4 samples +/-SD.



The cells proliferate on all used matrices. Typical cell growth curves were observed. On MATRIDRM® 2 mm the chondrocytes showed the highest proliferation rate.

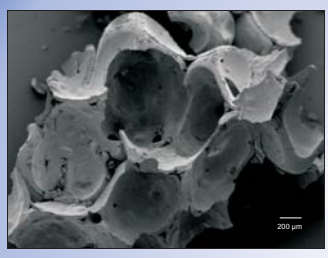
Immunohistological staining



MATRIDERM® 2 mm stained for collagen II visualised by DAB after 19 days (original magnification 400x). A: Matrix without cells. B: Matrix seeded with chondrocytes. C: Matrix seeded with chondrocytes, cultivated 10 days with TGF-β1 (10 ng/ml).

The cells express collagen II, whereas no difference is observed between cells cultivated with or without TGF-β.

Material and methods



Sponceram® (Sp): macroporous ceramic material consists of doped ZrO_2.
Sponceram®(Ha): consists of hydroxyapatite coated Sponceram®

Cell culture: Primary osteoblasts (human) were cultivated with DMEM containing 10 % FCS, antibiotics (Pen/Strep) +/- differentiating conditions (1 µM dexamethasone, 10 mM β-glycerolphosphate, 50 µg/ml ascorbic acid) at 37 °C and 5 % Co_2.

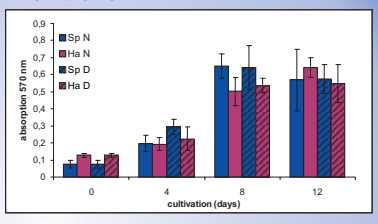
Cell seeding: Scaffolds were incubated for 24 h in DMEM medium (N) or differentiation medium (D), as described above. An excess of osteoblasts was seeded on each matrix in a 96-well plate for 30 min at gentle stirring. Before determination of the **cell metabolism** by **MTT-test** the matrices were placed into a new 96-well-plate.

SEM: Matrices for scanning electron microscopy were fixed in karnovsky buffer at 4 °C over night. Samples were then dehydrated in increasing acetone solutions.

Cell metabolism

SEM picture of primary osteoblasts (human) on Sponceram® 10 days after cultivating under differentiation conditions (1 µM dexamethasone, 10 mM β-glycerolphosphate, 50 µg/ml ascorbic acid).

Osteoblasts grow in a typical flat morphology on Sponceram® with an interconnected network to the adjacent cells.



Cell metabolism of primary osteoblasts on Sponceram® matrices (Sp) and hydroxyapatide coated Sponceram® matrices (Ha) after a time period of 12 days cultivated +/- differentiation conditions (N= DMEM medium, D=differentation medium). Values represents the mean of 5 samples of cultured scaffolds +/-SD.

The cell metabolism assay of the primary osteoblasts of Sponceram® and hydroxyapatite coated Sponceram® shows a fast cell growth on both materials during the first 8 days. After that the viability remains stable.

Conclusion

The results showed that coated and uncoated Sponceram® are both appropriate matrices for the cultivation of primary osteoblats. The applicability of three dimensional collagen matrices for cartilage tissue engineering was proved. The cells grow on all tested materials. For cartilage formation MATRIDERM® 2 mm showed the best results in cell culture. This material has the potential to induce cartilage formation. The immunohistochemistry showed that the chondrocytes differentiate into cartilage on MATRIDERM® 2 mm without the addition of TGF-β1.

Acknowledgement

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