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New Biomaterials for cartilage Tissue Engineering

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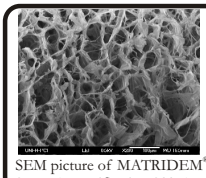
1 Introduction



SEM picture of MATRIDEM® 2 mm (magnification 200 x).

The aim of the study was to compare the proliferation and differentation of chondrocytes on different collagen scaffolds. Furthermore, extracellular matrix synthesis was studied, inparticular the expression of the typical chondrogenic marker collagen II was analysed.

2 Material and methods

Collagen scaffold: The materials, developed by Dr. Suwelack Skin & Health Care AG, are currently used for cosmetic and wound healing purposes. The used matrix for the scaffolds was MATRIDEM®, which mainly consistis of bovine collagen I and small amounts of elastine. For the experiments some of the MATRIDEM® matrices are crosslinkend with carbidiimide (EC) or glucose (Glucose I).

Cell culture: Scaffolds of 2 mm thickness and 6 mm diameter were prepared and sterilized with isopropanol (70 %) for 2-3 hours in a 96-well culture plate. Subsequently, the scaffolds 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**.** 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).

3 Material and methods

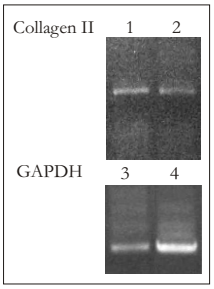
Cell seeding: 5000 cells were seeded onto each scaffold in a 96-well plate.

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

Hisological 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, respectivly, using standard protocols.
Collagen II was analysed by immunohistological staining, with a mouse-anti-collagen II antibody.

RT-PCR: Specific primers for collagen II were used. Sense primer : 5'-TTC AGC TAT GGA GAT GAC AAT C-3', antisense primer: 5'-AGA GTC CTA GAG TGA CTG AG-3' .
35 PCR cycles were used with 45 s at 94 °C and 45 s annealing/extension at 56 °C.
GAPDH: sense primer: 5'-ACC ACA GTC CAT GCC ATC AC-3' , antisense primer: 5'- TCC ACC ACC CTG TTG CTG TA-3'. 33 PCR cycles were used with 45 s at 94 °C and 45 s annealing/extension at 63 °C.

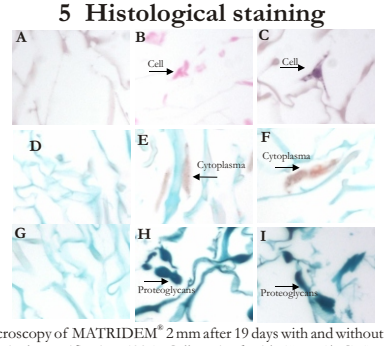
4 RT-PCR



Expression of the extracellular matrix protein collagen II in comparison to GAPDH of chondrocytes on MATRIDERM® 2 mm. Lane 1 and 2 collagen type II. Lane 3 and 4 GAPDH expression. Lane 2 and 4 represent the expression of the chondrocytes without differentation supplements. Lane 2 and 3 represents the gene expression of the cells cultivated two days with TGF- (10 ng/ml).

The results of the RT-PCR showed that the addition of TGF- does not increase the expression of collagen II as the chondrogenic marker. Thus the structure of MATRIDEM® 2 mm induces chondrogenic differentation.

5 Histological staining

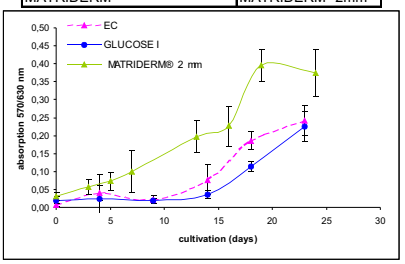


Microscopy of MATRIDEM® 2 mm after 19 days with and without cells (original magnification 400x). Cells stained with H&E (A-C), Masson Goldner (D-F) and Safranin O/light green (G-I).
A, D, G: Matrix without cells. B, E, H: Matrix seeded with cells. C, F, I: Matrix seeded with cells cultured 9 days with differentiation medium (10 ng/ml TGF-beta).

The H&E staining showed that the cells grow on the matrix fibres (B and C). The cytoplasma is stained red with Masson and Goldner (E and F). The green color of the Safranin O staining represents the expression of the proteoglycans (H and I). There is no difference observed between cells cultivated with and without TGF

8 MTT

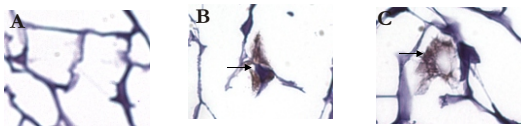
matrix	name
MATRIDERM® + carbodiimid	EC
MATRIDERM® + glucose	GLUCOSE I
MATRIDERM®	MATRIDERM® 2mm



Viability of chondrocytes on different collagen scaffolds over a time period of 24 days. Values represent the mean of 4 samples +/- SD.

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

9 Immunohistological staining



MATRIDERM® 2 mm stained for collagen II visualized 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 (100 ng/ml).

The cells express collagen II, wich is expressed in the cytoplasma in the extra cellular matrix. There is no difference observed between cells cultivated with or without TGF-

10 Conclusion

The in vitro cell culture tested collagen scaffolds for chondrocytes are three-dimensional structures, which can be used for cartilage tissue engineering. The cells grow on the matrix fibres and the materials have the potential to induce cartilage formation. The MTT test showed that the chondrocytes are viable and proliferate on the scaffolds. MATRIDEM® 2 mm was the best of the in cell culture tested matrices. The RT-PCR and the immunohistochemistry showed that the chondrocytes differentiate into cartilage on MATRIDEM® 2 mm without the addition of TGF-β1 .

Acknowledgement

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