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Photocrosslinking and 3D Microstructuring of Gelatin for the Generation of Substrates for Artificial Cartilage

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Background and Objectives

The generation of functional tissues on appropriate substrates with well-defined properties has been a major topic in recent scientific research. Three-dimensional (3D) structures have significant effects upon cellular behavior. Therefore, they are essential for the functional cultivation of cells and the optimization of cell substrates.

A promising approach to mimic the behavior of soft tissues in vitro is the use of 3D polymeric hydrogels. Besides synthetic polymers, e.g. PEG, biological materials such as gelatin are at focus for the preparation of hydrogels for tissue engineering approaches due to their ability to promote cell adhesion.

In the present study we describe the development of gelatin-based hydrogels and their characterization with regard to controllable mechanical properties and applicability for microstructuring using multiphoton polymerization (MPP). Both, planar and microstructured gelatin hydrogels were evaluated for their use as cell substrates for porcine chondrocytes with respect to generation of artificial cartilage.

Results

Synthesis of photopolymerizable Gelatin

Methods

→ Preparation of methacrylated gelatin (Gel-MA) by reaction of gelatin with methacrylic anhydride (Fig. 1) [2]
→ Characterization by ^{1}H-NMR- and IR-spectroscopy
→ Degree of substitution: determination of free amino groups with 2,4,6-trinitrobenzenesulfonic acid [3]

Results

✓ Successful methacrylation of gelatin (Fig. 2)
✓ Degree of methacrylation: 95,4% ± 7,8%

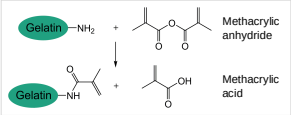

Fig. 1: Methacrylation of Gelatin

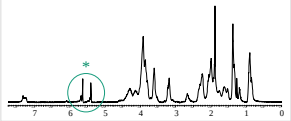

Fig. 2: ^{1}H-NMR spectra of methacrylated Gelatin (Gel-MA) with the two methacrylate signals marked as *.

Preparation and Characterization of Hydrogels

Methods

→ Preparation of Gel-MA hydrogels by photoinduced radical crosslinking
→ Analysis of gel yield and degree of swelling
→ Analysis of viscoelastic properties by oscillatory measurements

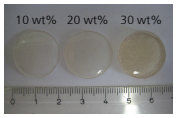

Fig. 3: Gel-MA hydrogels

Results

✓ Successful preparation of planar Gel-MA hydrogels
✓ Increasing gel yield with rising polymer content (Fig. 4A)
✓ Decreasing Degree of swelling with rising polymer content (Fig. 4B)
✓ Decreasing viscoelasticity and increasing stiffness with rising polymer content (Fig. 5A)
✓ Mechanically stable at physiological frequencies (knee) (Fig. 5B) [4]

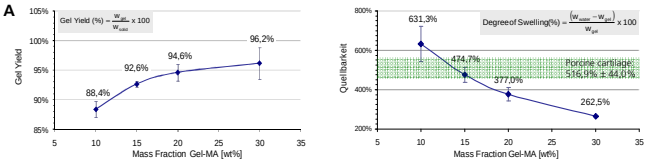

Fig. 4: Gel yield (A) and swelling characteristics (B) of Gel-MA hydrogels of different polymer content (n = 5).

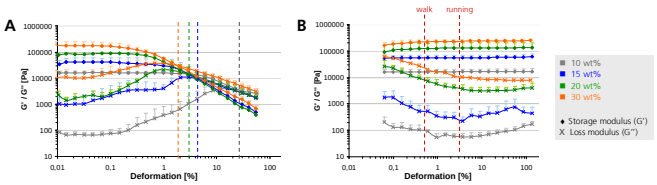

Fig. 5: Amplitude sweep (A) and frequency sweep (B) of Gel-MA hydrogels of different polymer content (F = 2 N, T = 37 ° C, n = 3).

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3D Microstructuring by Multiphoton Polymerization (MPP)

Methods

→ 3D microstructuring of Gel-MA by MPP (Fig. 6)

Results

✓ Successful preparation of micro-structured Gel-MA hydrogels (Fig. 7)

Fig. 7: Microscopic image of 3D micro-structured Gel-MA generated by MPP

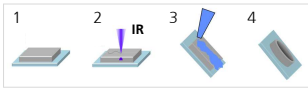


Fig. 6: Schematic depiction of the multi-photon polymerization process:
1. Photo-curable Gel-MA on a glass sub-strate, 2. Photopolymerization induced by tightly focused ultrashort IR laser pulses, 3.+4. Development of the final structure by rinsing with water.

Cell Response to Planar and Microstructured Hydrogels

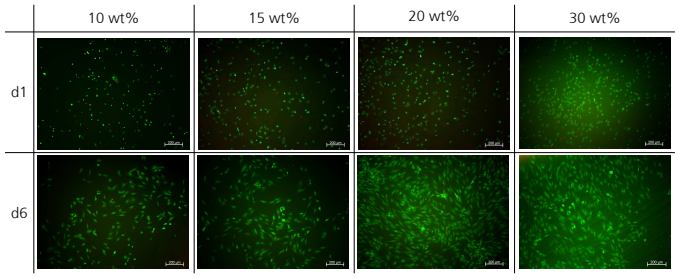
Methods

→ Isolation of primary porcine chondrocytes from porcine knee cartilage
→ Seeding cells on planar and microstructured Gel-MA hydrogels
→ Evaluation of cell adhesion, proliferation and cell substrate interactions

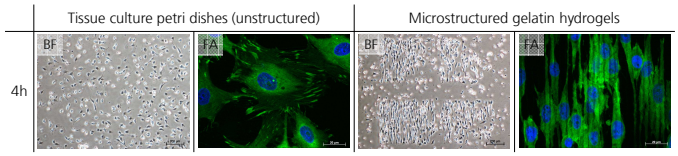
Results

✓ Increased cell adhesion and proliferation on stiffer hydrogels (Tab. 1)
✓ Increased vinculin expression and orientation on Gel-MA lines (Tab. 2)

Tab. 1: Microscopic images of Live/Dead staining (green/red) of porcine chondrocytes on Gel-MA hydrogels of different polymer content after 1d and 6d of cultivation.



Tab. 2: Parallel alignment of chondrocytes (right side) on gelatin lines. BF = bright field, FA = focal adhesions, green: vinculin, blue: nuclei (to be reproduced).



Summary and Perspectives

In this study the fabrication of gelatin hydrogels, their microstructuring by multiphoton polymerization and their usability as cell substrates for primary chondrocytes could be demonstrated. This provides a basis for further approaches generating artificial cartilage based on Gel-MA hydrogels

[1] Flemming RG et al. (1999); Biomaterials **20**:573-585.
[2] Van den Bulcke et al. (2000); Biomacromolecules **1**:31-38.
[3] Habeeb AFSA (1966); Analytical Biochemistry **14**:328-33.
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