UV-polymerisierbare Biopolymere zur Herstellung von Gewebemimetika mittels 3D-Inkjet-Druckverfahren am Beispiel von Knorpel

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Abstract

Die chemische Funktionalisierung von Gelatine ermöglicht die Darstellung eines biobasierten, *Inkjet*-druckbaren und photo-vernetzbaren Hydrogelsystems als Biomaterial für das *Bioprinting* von zellhaltigen Gewebemimetika: Die unvernetzten Lösungen der modifizierten Gelatine weisen niedrige Viskositäten auf und können mit einem *Inkjet*-Drucker verarbeitet werden. Gleichzeitig können die mechanischen Eigenschaften der resultierenden Gelatinehydrogele an die Eigenschaften natürlicher Gewebe angepasst werden.

Einleitung

In der vorliegenden Arbeit sollte die Anwendung des *Inkjet*-Druckverfahrens für das *Tissue Engineering* weiterentwickelt und die Erzeugung zellularisierter Gewebemimetika realisiert werden. Als Modellgewebe zur Methodenetablierung diente Gelenkknorpel. Als Trägerstruktur für die Zellen sollten mittels UVA-initiierter radikalischer Polymerisation erzeugte Hydrogele eingesetzt werden, da diese, wie natürliche Weichgewebe, eine besonders hohe Quellbarkeit in wässrigem Medium besitzen. Für die Nachbildung der natürlichen extrazellulären Matrix von Knorpelgewebe eignen sich besonders Biopolymere. Da Biopolymere in der Regel nicht per se polymerisierbar sind, muss zunächst eine chemische Modifizierung zur Einführung polymerisierbarer Gruppen erfolgen, beispielsweise eine Methacrylierung.
Die Herausforderung besteht dann darin, die Hydrogelprecursoren in eine Formulierung zu bringen, welche mittels *Inkjet*-Druck verarbeitbar ist und gleichzeitig die Zusammensetzung so zu wählen, dass sie die darin suspendierten Zellen schützt. Das Druckresultat soll eine möglichst naturidentische Umgebung bieten, das heißt sowohl die mechanischen als auch die biologischen Eigenschaften sollen an die natürliche Matrix eines Gewebes anpassbar sein.

Vorgehen und Ergebnisse

1. Synthese von vernetzbarer Gelatine mit *Inkjet*-druckbaren Lösungen

Nativer Gelenkknorpel besteht zu 50 % bis 80 % seiner Trockenmasse aus Kollagen.[1] Um die strukturelle und funktionelle Beschaffenheit natürlichen Knorpelgewebes möglichst gut nachzubilden, wurde Gelatine (denaturiertes Kollagen) als Ausgangsmaterial für das Hydrogelsystem gewählt. Zur Einführung vernetzbarer Gruppen wurden nukleophile Gruppen der Gelatine (insbesondere Aminogruppen) mit Methacrylsäureanhydrid (MAAnh) umgesetzt (siehe Abbildung 1).[2] Der Nachweis und die Quantifizierung der Methacrylierung erfolgten mittels ^{1}H-NMR-spektroskopischer Analysen und mittels eines kolorimetrischen *Assays* mit Trinitrobenzensulfonsäure.[3]
Durch Variation des eingesetzten molaren Überschusses an Methacrylsäureanhydrid wurde methacrylierte Gelatine (GM) mit Methacrylierungsgraden der Aminogruppen von ca. 70 % bis ca. 100 % gewonnen (siehe Tabelle 1).[3] Die NMR-spektroskopischen Analysen zeigten, dass ab einem zehnfachen molaren Überschuss des Methacrylsäureanhydrides gegenüber freier Aminogruppen der Gelatine nahezu 100 % der Aminogruppen und weitere nukleophile Gruppen der Gelatine (z.B. Hydroxylgruppen) derivatisiert wurden. Die Bereitstellung von Gelatine mit unterschiedlichen Methacrylierungsgraden ist eine wichtige

Voraussetzung für die Herstellung von Gelatinehydrogelen mit unterschiedlichen Materialeigenschaften aufgrund unterschiedlicher Vernetzungsgrade.
Eine zusätzliche Acetylierung von freien Amino- und Hydroxylgruppen, die bei der Erzeugung von Gelatine mit niedrigem Methacrylierungsgrad nicht umgesetzt worden waren, ermöglichte die Einstellung der Viskosität dieser Gelatinelösungen auf *Inkjet*-druckbare Werte (siehe Abschnitt 2. Viskosität der Gelatinelösungen), ohne Einfluss auf das eingestellte Vernetzungspotenzial.

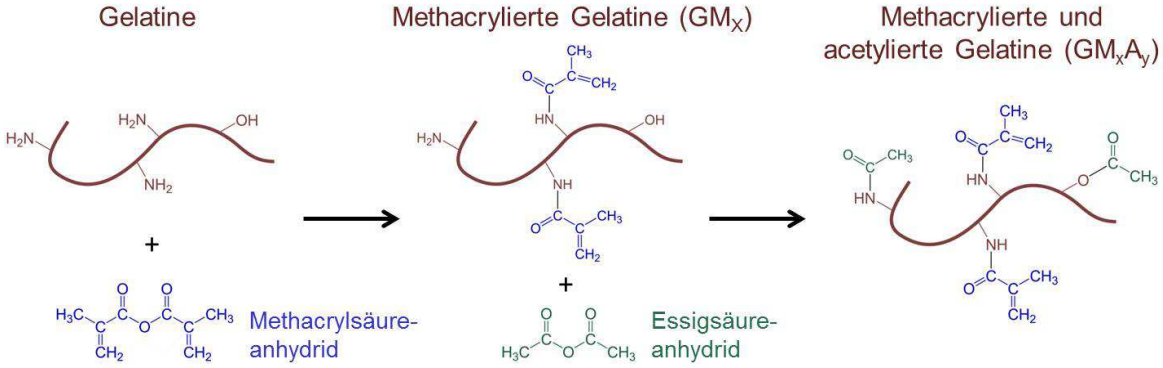

Abbildung 1 Derivatisierung von Gelatine mit Methacryl- und Acetylgruppen zur Erzeugung chemisch vernetzbarer Gelatinemakromonomere mit *Inkjet*-druckbarer Viskosität ihrer Lösungen. Die Indizes x und y geben den bei der Synthese eingesetzten molaren Überschuss an Methacrylsäureanhydrid bzw. Essigsäureanhydrid in Bezug auf freie Aminogruppen von unmodifizierter Gelatine an.

Tabelle 1: Eingesetzter molarer Überschuss an Methacrylsäureanhydrid (MAAnh) in Bezug auf freie Aminogruppen der Gelatine und dadurch erzeugte Methacrylierungsgrade (DM) der Gelatine. Der DM ist dabei als der prozentuale Anteil an methacrylierten Aminogruppen definiert. Die Quantifizierung des DM erfolgte mittels ^{1}H-NMR-Spektroskopie und eines kolorimetrischen *Assays* mit Trinitrobenzensulfonsäure (TNBS). Eine quantitative Umsetzung der Aminogruppen entspricht einem DM von 100 %.

Probe	molarer Überschuss MAAnh	DM mittels TNBS [%]	DM mittels ^{1}H-NMR [%]
Gelatine	-	0	0
GM_2	2	80,3 ± 5,4 (n = 2)	85,1 ± 10,9 (n = 4)
GM_2A_8	2	Nicht bestimmt	86,4 ± 1,0 (n = 3)
GM_{10}	10	97,7 ± 0,8 (n = 4)	quantitative Umsetzung (n = 3)

2. Viskosität der Gelatinelösungen

Die Viskosität stellt eine der wichtigsten Eigenschaften für die Verarbeitbarkeit einer Lösung mittels *Inkjet*-Druckverfahren dar. Für kommerzielle *Inkjet*-Druckköpfe werden dabei im Allgemeinen Viskositäten von ca. 1 mPa s bis 10 mPa s als optimal angesehen.[4, 5] In der vorliegenden Arbeit wurde die Viskosität von Lösungen der dargestellten Gelatinederivate mit unterschiedlichen Massenanteilen bei 25 °C und 37 °C untersucht (siehe Abbildung 2).

Sowohl die Methacrylierung als auch die Acetylierung bewirkte eine Abnahme der Viskosität der Lösungen. Dies ist auf die „Maskierung“ der Amino- und Hydroxylgruppen mit Methacryl- bzw. Acetylgruppen und die dadurch verhinderte Ausbildung von Wasserstoffbrücken unter den Gelatinemakromonomeren zurückzuführen. Während Lösungen der niedrig methacrylierten Gelatine (GM_2) bei 25 °C geliert vorlagen, besaßen Lösungen der hoch methacrylierten Gelatine (GM_{10}) sowie der zweifach funktionalisierten Gelatine (GM_2A_8) mit Massenanteilen bis zu 15 Gew.-% bei 25 °C und 37 °C *Inkjet*-druckbare Viskositäten < 10 mPa s. Die Doppelfunktionalisierung der Gelatine mit Acetyl- und Methacrylgruppen ermöglichte somit die simultane Kontrolle der Viskosität der Lösungen und des Gehaltes an verfügbaren vernetzbaren Methacrylgruppen, der die Eigenschaften der resultierenden Hydrogele bestimmt (siehe Abschnitt 3. Materialeigenschaften der resultierenden Hydrogele).

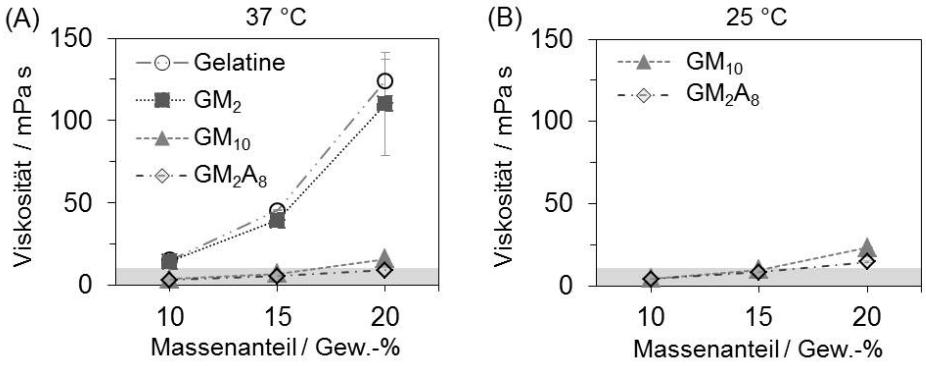

Abbildung 2 Viskosität von Lösungen unmodifizierter Gelatine, niedrig methacrylierter Gelatine (GM_2), hoch methacrylierter Gelatine (GM_{10}) sowie niedrig methacrylierter und acetylierter Gelatine (GM_2A_8). GM_2 und GM_2A_8 besaßen vergleichbare Methacrylierungsgrade. $1 \leq n \leq 5$, n gibt die Anzahl an durchgeführten unabhängigen Experimenten an.

3. Materialeigenschaften der resultierenden Hydrogele

Die Vernetzung der methacrylierten Gelatinederivate zu chemisch stabilen Hydrogelen (siehe Abbildung 3) erfolgte durch UVA-Strahlung in Anwesenheit des Photoinitiators Irgacure® 2959. Zur Charakterisierung der Materialeigenschaften der erzeugten Hydrogele zählten unter anderem die Analyse der Quellbarkeit und der viskoelastischen Eigenschaften in Abhängigkeit des Polymergehaltes, der Derivatisierungsart und des Derivatisierungsgrades.

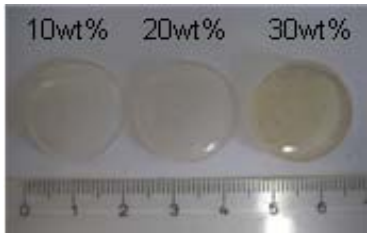

Abbildung 3 GM_{10}-Hydrogele mit unterschiedlichen Massenanteilen.

Für die GM-Hydrogele war eine Abnahme der Quellbarkeit und eine Zunahme der Festigkeit (Speichermodul G‘) mit steigendem Polymergehalt und/oder Methacrylierungsgrad zu

beobachten. Dies ist auf die Zunahme des Vernetzungsgrades der Hydrogele mit steigendem Polymergehalt und/oder Methacrylierungsgrad zurückzuführen. Die erreichten Quellbarkeiten und Festigkeiten lagen im Bereich verschiedener nativer Weichgewebe.[3]
Für GM_2 und GM_2A_8 wurde ein nahezu identischer Methacrylierungsgrad bestimmt. Dennoch wiesen GM_2A_8-Hydrogele eine höhere Quellbarkeit und niedrigere Festigkeit als vergleichbare GM_2-Hydrogele auf. Dies könnte auf eine geringere Vernetzungseffizienz der GM_2A_8-Makromonomere aufgrund sterischer Effekte der Acetylgruppen zurückzuführen sein.

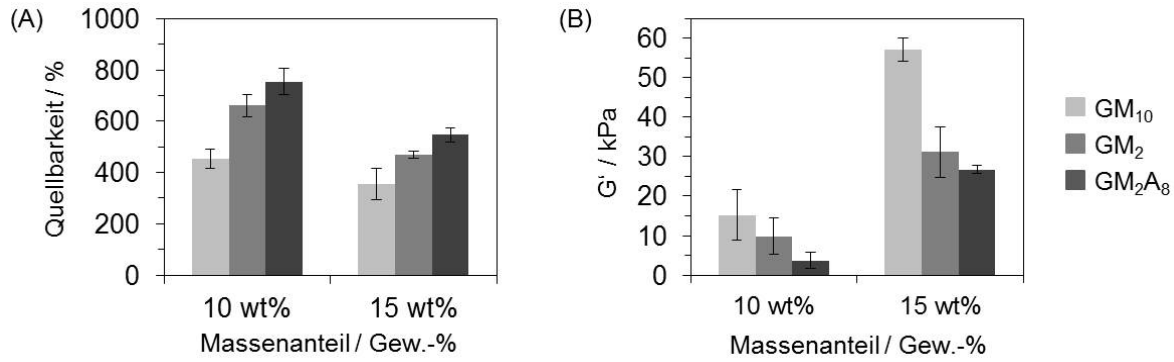

Abbildung 4 Quellbarkeit und mechanische Festigkeit (Speichermodul G') von Hydrogelen aus hoch methacrylierter Gelatine (GM_{10}), niedrig methacrylierter Gelatine (GM_2) sowie niedrig methacrylierter und acetylierter Gelatine (GM_2A_8). GM_2 und GM_2A_8 besaßen vergleichbare Methacrylierungsgrade. $n \geq 3$, n gibt die Anzahl an durchgeführten unabhängigen Experimenten an.

4. Zell-Material-Wechselwirkungen

Nach der Bestimmung der zytokompatiblen Konzentration des Photoinitiators Irgacure® 2959 (bis 2 mg mL^{-1}) sowie der UVA-Bestrahlungsdauer und -intensität (bis 10 min bei 8,5 mW cm^{-2}) wurden porcine Chondrozyten in dreidimensionale GM- und GMA-Hydrogele verkapselt.
Abbildung 5 zeigt die prozentuale Lebendzellzahl von porcinen Chondrozyten 24 h nach der Verkapselung in GM_2-, GM_{10}- und GM_2A_8-Hydrogele in Bezug zur eingesetzten Lebendzellzahl. Die Ergebnisse bestätigten die Zellverträglichkeit der gewählten Verkapselungsbedingungen (4 min UVA-Bestrahlung bei 8,5 mW cm^{-2}, 0,5 Gew.-% Irgacure® 2959 bezogen auf GM(A)-Masse) und zeigten, dass die entwickelten Gelatine-basierten Hydrogelsysteme potenziell als Matrices für den Aufbau zellhaltiger Gewebemimetika, insbesondere für den Aufbau von Knorpelmimetika geeignet sind.
Die im Vergleich zu den restlichen Proben niedrigere Lebendzellzahl in den 15 Gew.-%-igen GM_{10}-Hydrogelen könnte darauf zurückzuführen sein, dass aufgrund des hohen Vernetzungsgrades dieser sehr festen Hydrogele die Versorgung der Zellen mit Nährstoffen aufgrund einer eingeschränkten Diffusion unter den gewählten statischen Kultivierungsbedingungen nicht ausreichend war. In weiterführenden Arbeiten wird daher eine dynamische Kultivierung der zellhaltigen Hydrogele angestrebt.

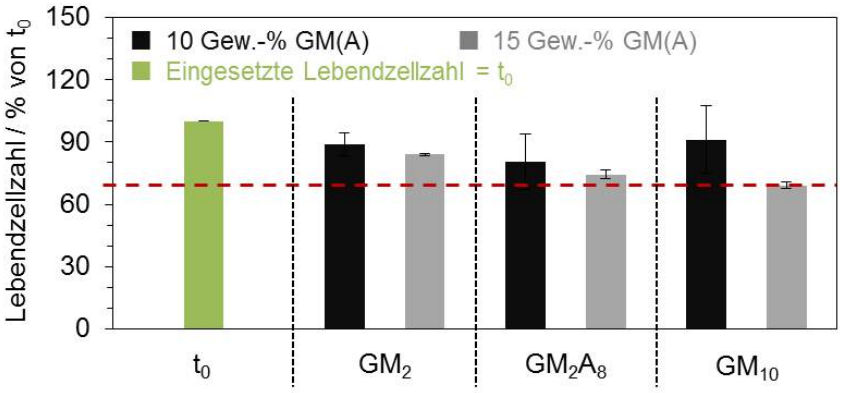

Abbildung 5 Prozentuale Lebendzellzahl von porcinen Chondrozyten 24 h nach der Verkapselung in GM- und GMA-Hydrogele verschiedener Massenanteile bezogen auf die eingesetzte Lebendzellzahl (t_0). GM_{10} = hoch methacrylierter Gelatine, GM_2 = niedrig methacrylierter Gelatine, GM_2A_8 = niedrig metharylierter und acetylierter Gelatine. GM_2 und GM_2A_8 besaßen vergleichbare Methacrylierungsgrade. n = 3, n gibt die Anzahl an durchgeführten unabhängigen Experimenten an.

5. Inkjet-Druck von viablen Chondrozyten in den formulierten Gelatine-Biotinten

Für die Eignungsprüfung der dargestellten Gelatine-Biotinten für das *Inkjet-Bioprinting* mit lebenden Säugerzellen wurden Chondrozyten-haltige Gelatine-Biotinten mit einem piezoelektrischen Mikrodosiersystem der Firma GeSiM Gesellschaft für Silizium-Mikrosysteme mbH (Deutschland) verarbeitet und die Vitalität und Funktionalität der gedruckten Zellen untersucht.

Die *Inkjet*-Verarbeitung von GM_{10}- und GM_2A_8-Biotinten, die *Inkjet*-druckbare Viskositäten aufwiesen, war unter Bildung geeigneter Tropfen möglich (siehe Abbildung 6 A). Auf Substraten, auf die mit porcinen Chondrozyten beladene GM_{10}- bzw. GM_2A_8-Biotinten gedruckt worden waren, konnten 3 h nach erfolgtem Druck zahlreiche vitale Zellen nachgewiesen werden (siehe Abbildung 6 B). In der nachfolgenden Kultivierung zeigte diese außerdem eine für die zweidimensionale Kultivierung typische Morphologie und proliferative Aktivität (siehe Abbildung 6 C und D). Der *Inkjet*-Druck der Zellen in den dargestellten Gelatine-Biotinten wurde daher als zytokompatibel betrachtet.

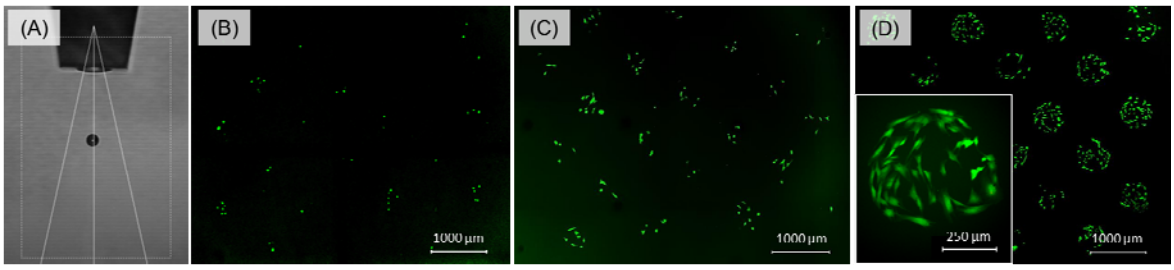

Abbildung 6 Eignungsprüfung der dargestellten Gelatine-Biotinten für das *Inkjet-Bioprinting* mit viablen Säugerzellen. (A) Optimale Tropfenbildung. (B-D) Porcine Chondrozyten 3 h, 24 h und 72 h nach erfolgtem *Inkjet*-Druck in einer 10 Gew.-%-igen GM_{10}-Biotinte. Die gedruckten Zellen wurden mithilfe einer Lebend/Tot-Färbung sichtbar gemacht: grüne Zellen = vital, rote Zellen = tot. GM_{10} = hoch methacrylierter Gelatine.

Schlussfolgerung

Die etablierte Doppelfunktionalisierung von Gelatine mit Methacryl- und Acetylgruppen ermöglicht die simultane Kontrolle der Viskosität der Gelatinelösungen und der Eigenschaften der resultierenden photo-vernetzten Hydrogele. Dabei können über den Methacrylierungsgrad Hydrogeleigenschaften wie die Quellbarkeit oder Festigkeit kontrolliert und an verschiedene native Weichgewebe angepasst werden. Die zusätzliche Acetylierung ermöglicht das Einstellen der Viskosität der Gelatinelösungen auf *Inkjet*-druckbare Werte. Der *Inkjet*-Druck von viablen Säugerzellen in den formulierten Gelatine-Biotinten ist zytokompatibel. Das dargestellte Gelatine-Hydrogelsystem stellt damit ein vielversprechendes Material für das *Inkjet-Bioprinting* von Gewebemimetika, beispielsweise von künstlichem Knorpel dar.

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Stiff gelatin hydrogels can be photo-chemically synthesized from low viscous gelatin solutions using molecularly functionalized gelatin with a high degree of methacrylation

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Abstract Gelatin is a very promising matrix material for in vitro cell culture and tissue engineering, e.g. due to its native RGD content. For the generation of medical soft tissue implants chemical modification of gelatin improves the mechanical properties of gelatin hydrogels and the viscous behavior of gelatin solutions for liquid handling. We present a systematic study on the influence of high degrees of methacrylation on the properties of gelatin solutions and photo-chemically crosslinked hydrogels. Changes from shear thinning to shear thickening behavior of gelatin solutions were observed depending on mass fraction and degree of methacrylation. Degrees of swelling of crosslinked hydrogels ranged from 194 to 770 % and storage moduli G′ from 368 to 5 kPa, comparable to various natural tissues including several types of cartilage. Crosslinked gels proofed to be cytocompatible according to extract testings based on DIN ISO 10933-5 and in contact with porcine chondrocytes.

1 Introduction

Biomaterials based on biopolymers can be designed to mimic features of the extracellular matrix (ECM) and thus open an attractive way to design artificial scaffolds for 3D tissue engineering and regenerative technologies in medicine. The ECM of native mammalian tissues is a collagen-based hydrogel with a quite complex supramolecular structure. Recent and promising approaches to ECM-mimics are based on gelatin, which is gained from native collagen by denaturation and partial hydrolysis: Gelatin displays the arginine–glycine–aspartic acid (RGD) sequence of collagen which is crucial for the interaction of cells with their surrounding ECM. Unlike collagen, gelatin is soluble at a physiological pH, making it an ideal precursor for hydrogel preparation [1–3]. Moreover, gelatin like collagen has the ability to form triple helices, yet the resulting gelatin gels are thermo-sensitive and melt at sol–gel transition temperatures in between 25 and 30 °C [4].

Various methods have been applied to achieve chemically crosslinked gelatin gels, e.g. by addition of crosslinkers such as glutardialdehyde [5], carbodiimide [6], eosin [7], genipin [8], hexamethylene diisocyanate or ethyl lysine diisocyanate [9], or by enzymatic crosslinking using transglutaminase [10, 11]. Furthermore, molecular modification of the gelatin side groups can be achieved by using e.g. methacrylates in order to prepare covalently crosslinkable gelatin molecules by photo-initiated radical polymerization. The gelatin side groups readily available for this modification are hydroxyl groups, carboxyl groups and amino groups. Different methods for their methacrylation are known, using e.g. glycidyl methacrylate [12, 13], methacryloyl chloride [14], or methacrylic anhydride (MAAnh) [4]. Glycidyl methacrylate already contains an ester group in itself which reduces the chemical stability of any crosslinked biopolymer network due to possible hydrolysis of the ester groups [13]. Methacrylation via formation of the chemically more stable amide group on the other hand, can be realized by addressing the amino groups of gelatin using methacryloyl chloride or MAAnh.

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Additionally, it has been reported that gelatin was less efficiently methacrylated by glycidyl methacrylate than by MAAnh [15].

Overall, hydrogels prepared from covalently crosslinked gelatin show higher thermal and mechanical stability than physically gelled gelatin. However, until now the reported mechanical strength of the gels is still limited. Nickerson et al. [8] prepared genipin-crosslinked gelatin gels with storage moduli of $G' \leq 10$ kPa. Van den Bulcke et al. [4] who originally established the preparation of methacrylated gelatin (Gel-MAAm) using MAAnh obtained hydrogels with storage moduli G' in the range $10\ \text{kPa} < G' \ll 100\ \text{kPa}$. Hu et al. [16] presented Gel-MAAm hydrogels holding storage moduli $G' \leq 10$ kPa. The storage moduli of various natural tissues are at least in the range of $G' = 10$ kPa, e.g. canine kidney cortex and canine medulla with $G' \approx 10$ kPa [17], or larger such as human nasal cartilage with $G' = 234$ kPa [18, 19]. Hyaline cartilage, one of the mechanically most stable native hydrogel materials, provides a storage modulus $G' \approx 800$ kPa [20]. Therefore the preparation of hydrogels with high storage moduli still remains an important issue in regard to the generation of matrices for tissue engineering and medical implants at the "stiffer end" of soft tissues.

Besides the adjustment of the mechanical properties of the resulting hydrogels, the control of the viscous behavior of the precursor solutions has become increasingly interesting. Future medical technology based on regenerative medicine needs flexible and effective technologies, e.g. liquid handling techniques, which can lead to easily controllable and automatable processes. However, due to their high viscosity and gelling at room temperature, gelatin solutions are prone to clogging pipets and dispensers [21]. The properties of the precursor solutions depend on the chemical structure of the deployed macromonomers. Therefore, the chemical modifications of gelatin on a molecular level might ease the liquid handling of gelatin solutions.

In this study we prepared photo-crosslinkable gelatin by methacrylation. We used MAAnh in order to achieve hydrolytically stable and effective substitution of nucleophilic groups in aqueous media. We generated a broad range of degrees of methacrylation (DM). Special attention was paid to the generation of very high DM, as there is no systematic investigation of stiff hydrogels with high storage moduli available yet. We characterized the crosslinked gelatin methacrylamide (Gel-MAAm) by their gel yield, degree of swelling, and rheological properties. For the first time we also investigated the influence of the DM on the viscous properties of the gelatin solutions. Finally, we tested the cytocompatibility of crosslinked hydrogels according to extract testings based on DIN ISO 10993-5 and by direct cultivation of porcine articular chondrocytes on Gel-MAAm in order to preliminarily proof the material's potential to act as tailored matrix for the preparation of various artificial soft tissues including artificial cartilage.

2 Experimentals

2.1 Materials

MAAnh, sodium hydroxide, gelatin (bovine skin, type B, ~225 bloom), fluorescein diacetate (FDA) and propidium iodide (PI) were purchased from Sigma Aldrich (Germany). The radical photoinitiator 1-[4-(2-hydroxyethoxy)phenyl]-2-hydroxy-2-methyl-1-propan-1-one (Irgacure 2959) was a kind gift from Bodo Möller Chemie GmbH (Germany). Dialysis was conducted using dialysis membranes (MWCO 12–14 kDa) from Medicell International Ltd (United Kingdom).

Porcine chondrocytes were cultured in Dulbecco's modified Eagle's medium (DMEM)/Ham's F12 (PAA Laboratories, Austria) containing 10 % (v/v) fetal calf serum (FCS; Invitrogen, Germany), 1 % (v/v) gentamicin (Invitrogen, Germany), and 50 µg/mL ascorbate-2-phosphate (Sigma Aldrich, Germany). Collagenase (standard grade from *clostridium histolyticum*) and trypsin–EDTA were purchased from Invitrogen (Germany) and Serva Electrophoresis (Germany), respectively. The Cell Proliferation Reagent WST-1 was purchased from Roche (Germany). For the present study one type of gelatin (bovine skin, type B, ~225 bloom) from one single batch was used. It has to be kept in mind that the results may vary when using other types of gelatin. In order to compare different types and batches the average molecular weight (Bloom number) should be taken into account and the amount of free amino functions should be determined anew.

2.2 Methacrylation of gelatin

Gel-MAAm was synthesized according to a procedure previously described [4]. Gelatin (25 g) was dissolved in 200 mL phosphate buffer (pH 7.4) at 40–50 °C. The aqueous solution was then adjusted to pH 7.4 using 4 M NaOH solution. MAAnh was added dropwise after the gelatin was completely dissoluted. To prepare Gel-MAAm with a range of DM the amount of MAAnh was varied between 2, 2.6, 13 and 26 mL to give a 1:1.5, 1:2, 1:10 or 1:20 molar excess of MAAnh with respect to free amino groups of gelatin based on a content of 0.335 mmol amino groups per gram gelatin (bovine skin, type B) reported by Van den Bulcke et al. [4]. The mixture was stirred vigorously. During the methacrylation reaction the pH of the solution was kept constant between 7.0 and 7.4. After 2 h of reaction time, the reaction mixture was dialyzed for 4 day against distilled water at room temperature (batches

1:10 and 1:20) or 40 °C (batches 1:1.5 and 1:2). The reaction product was then freeze-dried and stored at room temperature until use.

2.3 Quantification of the degree of methacrylation (DM) of gelatin

The methacrylation of gelatin was proved using ^{1}H-NMR spectroscopy. Samples of 5 mg Gel-MAAm dissolved in 650 μL deuterium oxide were analyzed. High-resolution 500 MHz proton NMR spectra were taken on a Bruker Avance 500 spectrometer. Sodium 3-tri-methylsilyl-propionate-2,2,3,3 served as an internal standard.

The DM was defined as the percentage of ε-amino groups of gelatin (lysine, hydroxylysine) that are modified in Gel-MAAm. It was determined by two methods: ^{1}H-NMR and TNBS assay.

For the quantification of the DM by ^{1}H-NMR the spectra were normalized to the phenylalanine signal (6.9–7.5 ppm), which represents the concentration of gelatin. Subsequently, the lysine methylene signals (2.8–2.95 ppm) of gelatin spectra and Gel-MAAm spectra were integrated to obtain the areas [A(lysine methylene of non-modified gelatin) and A(lysine methylene of Gel-MAAm)]. The DM of the different Gel-MAAm batches were calculated as

$$\mathrm{DM_{NMR}}(\%) = \left(1 - \frac{\mathrm{A(lysine\ methylene\ of\ Gel\text{-}MAAm)}}{\mathrm{A(lysine\ methylene\ of\ non\text{-}modified\ gelatin)}}\right) \times 100 \quad (1)$$

The TNBS assay is a colorimetric method developed by Habeeb using 2,4,6-trinitrobenzene-sulfonic acid (TNBS) for the labeling of free amino groups [22]. Quantification of free amino groups was done in comparison with standard solutions of known glycine concentrations.

2.4 Viscosity measurements

The viscosity of Gel-MAAm solutions with protein concentrations of 10, 15, 20 and 30 wt % of Gel-MAAm and different DM was determined by rotary concentric cylinder rheometry using a Physica Modular Compact MCR301 Rheometer (Anton Paar, Germany) at shear rates from 1 to 4,000 s^{-1} at 37 °C. Gel-MAAm solutions were prepared by dissolving Gel-MAAm in physiological phosphate buffer at 40 °C.

2.5 Hydrogel preparation

Hydrogels with initial protein concentrations of 10, 15, 20 and 30 wt% of Gel-MAAm and different DM were prepared by photo-initiated radical crosslinking in the presence of the water soluble photoinitiator Irgacure 2959 (I2959). I2959 is the most commonly used photoinitiator for tissue engineering applications due to its good toxicological profile and water solubility [23, 24]. Different studies proved the good cytocompatibility of I2959 in comparison to other photoinitiators [25–27].

For hydrogel preparation Gel-MAAm was dissolved in physiological phosphate buffer at 40 °C containing 0.05 % Irgacure 2959 (w/w to protein concentration). The warm mixture was poured into a cylindrical cast (1 × 30 mm^2) and the cast was covered by a quartz glass pane. The samples were cured by the exposition to UVA light (15 mW cm^{-2}) for 10 min. After irradiation the pane was removed. The crosslinked hydrogels were taken out of the cast and cut into the desired shape and used for further experiments.

2.6 Gel yield and degree of swelling

To investigate the efficiency of the crosslinking process and the degree of crosslinking, the gel yield and swelling ratio of the hydrogels were determined in dependence on the Gel-MAAm mass fraction and DM. Immediately after crosslinking and cutting the hydrogels, they were dried at 80 °C under vacuum overnight and weighed [weight(Gel-MAAm)]. Then gels were washed in distilled water at 37 °C for at least 5 h. Water was changed five times. The washed gels were vacuum-dried and weighed again [weight(crosslinked Gel-MAAm)]. Afterwards the gels were swollen in physiological phosphate buffer at 37 °C and weighed [weight (crosslinked Gel-MAAm$_{\mathrm{PBS\ swollen}}$)].

The gel yield (%) was calculated as

$$\text{Gel yield}\ (\%) = \frac{\mathrm{weight(crosslinked\ Gel\text{-}MAAm)}}{\mathrm{Weight(Gel\text{-}MAAm)}} \times 100 \quad (2)$$

The degree of swelling/% was calculated as

$$\text{Degree of swelling}\ (\%) = \frac{\mathrm{weight(crosslinked\ Gel\text{-}MAAm_{PBS\ swollen})} - \mathrm{weight(crosslinked\ Gel\text{-}MAAm)}}{\mathrm{Weight(crosslinked\ Gel\text{-}MAAm)}} \times 100 \quad (3)$$

2.7 Rheological measurements

Dynamic rheological tests were used to characterize the viscoelastic properties of the crosslinked gelatin hydrogels in dependence on the Gel-MAAm mass fraction and DM. The oscillatory dynamic measurements were performed with a Physica Modular Compact MCR301 Rheometer from Anton Paar (Germany) using a parallel plate model. All hydrogels were examined in a swollen state at 37 °C and a load of 2 N. First, oscillatory strain amplitude sweeps ($0.01\ \% \leq \gamma \leq 100\ \%$) at fixed frequency (1.5 Hz) were performed to establish the range of linear viscoelasticity. Then, mechanical spectra were recorded in a constant strain mode with low deformations γ maintained over the frequency range of 0.01–100 Hz (gels with gelatin mass fractions of 10 and 20 wt%: $\gamma = 0.05\ \%$; gels with gelatin mass fraction of 30 wt%: $\gamma = 0.02\ \%$).

2.8 Isolation of porcine chondrocyte

Primary chondrocytes were isolated from knee cartilage of 5–8-month-old German Landrace pigs (8–15 kg). Articular cartilage slices were detached from adherent connective tissues and cut into smaller pieces. Chondrocytes were isolated by enzymatic digestion with collagenase (0.3 U/mL in DMEM/Ham's F12 containing 10 % (v/v) fetal calf serum, 1 % (v/v) gentamicin, and 50 μg/mL ascorbate-2-phosphate) for 16–18 h at 37 °C. The isolated chondrocytes were centrifuged, washed, and seeded in 175 cm^2 T-flasks. They were cultured under standard environmental conditions (37 °C, humidified atmosphere, 5 % CO_2) using DMEM/Ham's F12 containing 10 % (v/v) fetal calf serum, 1 % (v/v) gentamicin, and 50 μg/mL ascorbate-2-phosphate. Culture medium was changed twice per week. At 80 % confluence the cell cultures were passaged by rinsing with PBS and subsequent trypsinization using trypsin–EDTA. For all experiments chondrocytes were used at passage 2.

2.9 In vitro cytocompatibility

The cytocompatibility of the gelatin hydrogels was preliminarily estimated by extract analysis based on the international standard DIN EN ISO 10993-5 and by direct cell-material contact testing using porcine chondrocytes.

For the extract testings porcine chondrocytes were harvested by trypsinization, seeded on cell culture 96-multiwell plates, and cultivated to subconfluency. Gelatin hydrogels were prepared using Gel-MAAm, which was sterilized by heating twice to 60 °C for 8 h. Hydrogels were crosslinked under a clean bench as described above using sterile filtered PBS and Irgacure 2959 solution. The hydrogel cast and the quartz glass pane were sterilized using 70 % ethanol. Immediately after UVA exposure discs with 1 cm in diameter were punched out of the crosslinked gelatin hydrogels. Gelatin hydrogels were extracted according to DIN EN ISO 10993-5 and the porcine chondrocytes were incubated with the resulting extracts for 24 h. Cell viability was analyzed by a WST-1 cell proliferation assay.

For the direct cell-material experiments hydrogels were prepared as described above. Immediately after UVA exposure round shaped discs 1 cm in diameter were punched out of the crosslinked gelatin hydrogels. After sterilization by incubating in 70 % ethanol for 60 min at room temperature the discs were washed with PBS several times to remove the alcohol.

Chondrocytes were harvested by trypsinization and seeded on the gelatin hydrogels by pipetting 30 μL cell suspension containing 6×10^3 chondrocytes onto the hydrogels. The seeded discs were kept for 4 h under standard cultivation conditions (37 °C and 5 % CO_2 in a humidified incubator) to allow cell adhesion. After the 4 h period the hydrogels were covered by medium. After 1 and 6 day cultivation cell adhesion, morphology, and cell confluency were examined by fluorescence-based live/dead staining using FDA and PI.

For the FDA/PI staining the hydrogels were put into a petri dish and carefully washed with PBS. 20 μL FDA/PI solution (10 μL 5 μg/mL FDA in acetone + 10 μL 0.5 μg/mL PI in PBS + 980 μL PBS) were pipetted onto each hydrogel and incubated for 3 min at 37 °C. Samples were viewed using a Zeiss Axiovert 200 M inverted microscope. To quantify cell proliferation during the 6 day period the cells were counted on representative pictures showing an area of about $1.7 \times 1.1\ mm^2$.

3 Results and discussion

3.1 Methacrylation of gelatin

Gel-MAAm was synthesized using MAAnh in various molar ratios with respect to the free amino functions of the gelatin. The successful derivatization was verified by ^{1}H-NMR (Fig. 1). Quantitative ^{1}H-NMR analysis and TNBS were used to determine the extent of conversion of free amine groups in gelatin samples (DM) and to quantify the differences between different batches.

Figure 1 shows ^{1}H-NMR spectra of unmodified gelatin and of gelatin samples that were reacted with various amounts of MAAnh (Gel-$MAAm_{1.5–20}$). The suffix denotes the molar excess of MAAnh added referring to the amount of free amino groups in the gelatin chain. Comparing the spectra of gelatin and Gel-MAAm new signals can be observed at $\delta = 5.3$ ppm and $\delta = 5.5$ ppm. These were assigned to the acrylic protons of methacrylic functions. The new signal at $\delta = 1.8$ ppm was assigned to the methyl

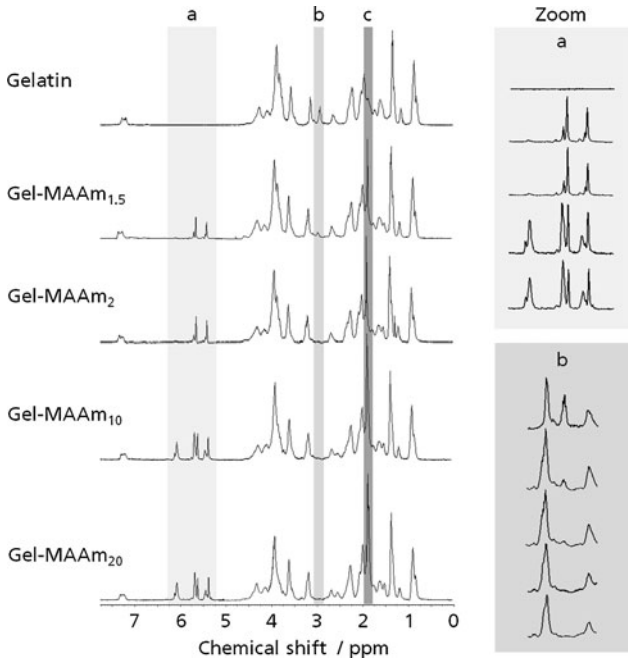

Fig. 1 ^{1}H-NMR spectra of gelatin and Gel-MAAm of different DM. The signals of the acrylic protons and methyl function of the introduced methacrylate are indicated as *a* and *c*, respectively, the lysine methylene signals of gelatin as *b*

function of the introduced methacrylate. Therefore we conclude that MAAnh has been successfully grafted to the gelatin molecules for all molar ratios applied. The continuous decrease of the lysine methylene signal at $\delta = 2.9$ ppm with increasing anhydride ratio in the reaction confirmed the modification of lysine residues. These findings are in good correlation with results reported by Hu et al. [16]. At a molar ratio of 1:10 (gelatin amino groups to MAAnh) the signal disappeared, indicating the complete conversion of the amino groups. Reactions with molar ratios of 1:10 or 1:20 resulted in spectra displaying additional signals at $\delta = 5.4$ ppm, $\delta = 5.7$ ppm and $\delta = 6.1$ ppm, which we assume to refer to additional methacrylation reactions of nucleophilic functions other than amino groups, e.g. hydroxyl functions.

The quantification of the lysine methylene signals ($\delta = 2.8$–2.95 ppm) of gelatin and Gel-MAAm yielded 68.5 %, 85.1 % and quantitative conversion of free amino functions at molar ratios of 1:1.5, 1:2 and 1:10 (1:20), respectively (Table 1).

The quantification of the DM by TNBS was achieved by comparison to glycine solutions with known concentrations (Table 1). The amount of free amino groups in unreacted gelatin was determined to be 0.396 ± 0.012 mmol g^{-1} by TNBS and thereby is in excellent correlation with data published earlier (0.355 mmol g^{-1}) [4]. After the methacrylation reaction at 1.5-fold molar excess the TNBS assay yielded 33 % of the free amino functions of unreacted

Table 1 Reaction conditions for the methacrylation of gelatin with MAAnh and resulting DM determined by TNBS assay and ^{1}H-NMR spectroscopy

Sample	*x* fold molar excess of MAAnh	DM by TNBS (%)	DM by ^{1}H-NMR (%)
Gelatin	–	0	0
Gel-MAAm$_{1.5}$	1.5	67.2 ($n = 1$)	68.5 ± 10.1 ($n = 3$)
Gel-MAAm$_{2}$	2	80.3 ± 5.4 ($n = 2$)	85.1 ± 10.9 ($n = 4$)
Gel-MAAm$_{10}$	10	97.7 ± 0.8 ($n = 4$)	Quantitative conversion ($n = 3$)
Gel-MAAm$_{20}$	20	98.2 ± 0.5 ($n = 3$)	Quantitative conversion ($n = 1$)

gelatin and thus we assume that 67 % of the free amino groups have been converted into amid bonds. This result is in good correlation with the DM determined by ^{1}H-NMR. The application of 10-fold molar excess of MAAnh resulted in nearly 100 % conversion of free amino groups. The same holds true for reaction at a molar ratio of 1:20.

These results demonstrated the ability to control the acrylation reaction with the DM of lysine and hydroxylysine residues varying roughly from 68 % to quantitative conversion.

Hu et al. [16] constituted crosslinkable gelatin for the encapsulation of chondrocytes by immobilization of methacrylic acid via EDC-activated amidation and detected a DM of 49 % of the free amino groups. Khademhosseini and co-workers [28] reported on generation of methacrylated gelatin for generation of cell-laden hydrogels and achieved high, middle and low DM of 20, 54 and 80 %, respectively. Van den Bulcke et al. [4], who originally established the preparation of methacrylated gelatin, investigated samples with DM of 7, 21 and 40 %. Thus, to our knowledge the large number of potential crosslinking sites provided in this study has not been reported on before.

Three types of Gel-MAAm (Gel-MAAm$_{1.5}$, Gel-MAAm$_{2}$ and Gel-MAAm$_{10}$) with different DM (68, $\sim$80 %, and quantitative conversion, respectively) were used for the preparation and characterization of hydrogels.

3.2 Viscous behavior

The viscosity of protein solutions is affected by the weight and the structure of the protein, the protein–protein interactions, as well as the protein–solvent interactions [29]. For automated liquid handling, i.e. pipetting and dispensing techniques, the control of the solution viscosity is crucial. To investigate the influence of the methacrylation on the intermolecular forces of the gelatin molecules we analyzed the viscosity of Gel-MAAm solutions by rotary concentric cylinder rheology (Fig. 2).

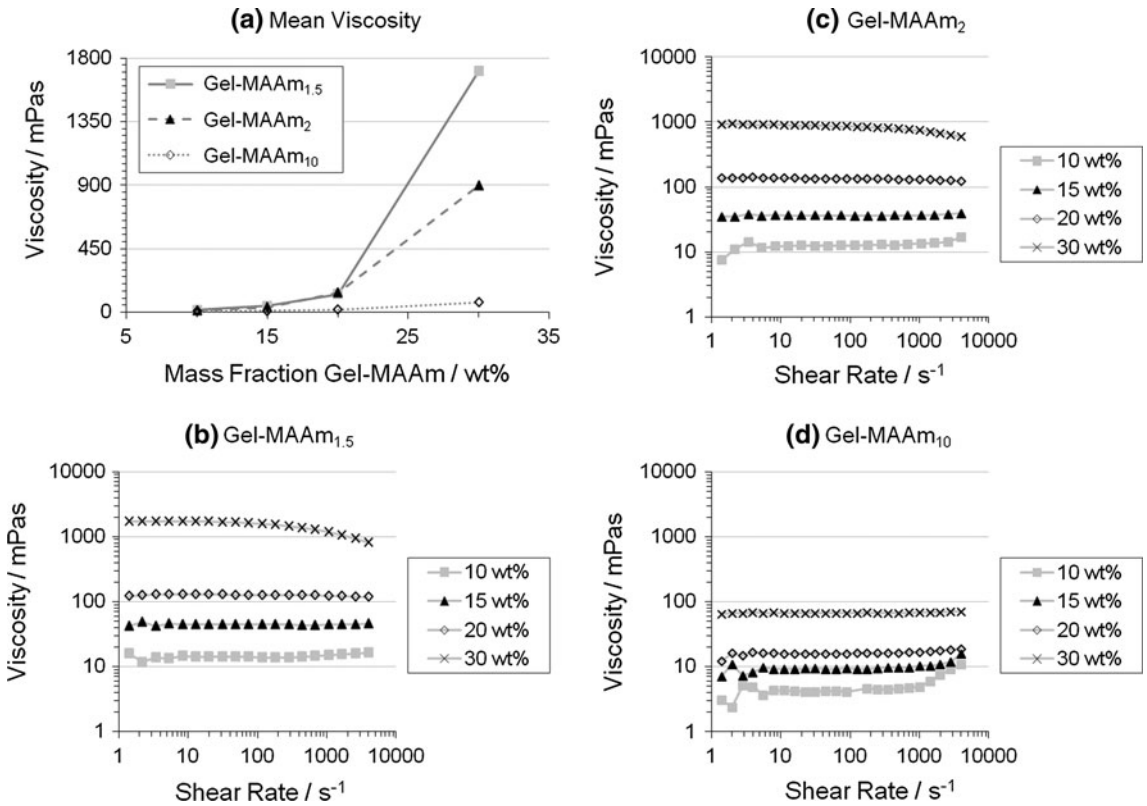

Fig. 2 Mean dynamic viscosity (**a**, $n \geq 3$) and shear-rate dependent viscous behavior of gelatin-solutions with various concentrations and DM of the gelatin molecules (**b**–**d**, representative curves out of $n \geq$ measurements). The *lines* are added for guidance of the eye only

Figure 2a represents the mean viscosity of Gel-MAAm solutions of different DM and mass fractions for shear rates from 1 to 100 s^{-1}. For all DM we detected an increase in viscosity with increasing mass fraction. This effect is well described in literature [30, 31] and caused by the rising protein–protein interactions due to the higher number of gelatin molecules per volume. For all mass fractions the viscosity of the solution was lowest at high DM. Obviously the methacrylation of nucleophilic groups weakened the interaction between neighboring molecules. The decline of the slope in Fig. 2 indicated the reduction of gelling ability [30]. This decrease in gelatinization of gelatin solutions was also observed in laboratory praxis. While solutions of Gel-$MAAm_{1.5}$ and Gel-$MAAm_2$ gelatinized at cooling down to room temperature, he higher the mass fraction, the faster the gelatinization occured, Gel-$MAAm_{10}$ solutions did not gelatinize at room temperature.

We also analyzed the influence of the shear rate on the viscous behavior of Gel-MAAm solutions. Figure 2a–c represents the viscosity of Gel-MAAm solutions for shear rates between 1 and 4,000 s^{-1}. For all Gel-MAAm solutions of low mass fractions (10 and 15 wt%) we observed shear thickening behavior. Due to the low concentration of the Gel-MAAm solutions the Gel-MAAm macromonomers predominantly build intramolecular forces compared to intermolecular forces. With increasing shear rates the macromonomers are assumed to become stretched and aligned and to form intermolecular forces which cause an increase in viscosity.

In contrast to this, we found shear thinning behavior for Gel-$MAAm_{1,5}$ and Gel-$MAAm_2$ solutions of high mass fractions (20 and 30 wt%). At high concentrations the Gel-MAAm macromonomers with low degrees of derivatization form a physical network stabilized by intermolecular forces. With increasing shear rates the macromonomers become disentangled, protein–protein as well as protein-solvent interactions are attenuated and the viscosity decreases.

For Gel-$MAAm_{10}$ the shear thickening of solutions with low mass fractions was most distinctive compared to smaller DM and shear thinning behavior was not observed. We ascribe this effect to weakened intermolecular and intramolecular interactions of the gelatin macromolecules due to the high number of introduced methacrylate functions. These investigations showed that the dynamic viscosity of gelatin solutions could be controlled by modification of nucleophilic groups such as lysine residues, thus without alteration of the RGD cell adhesion

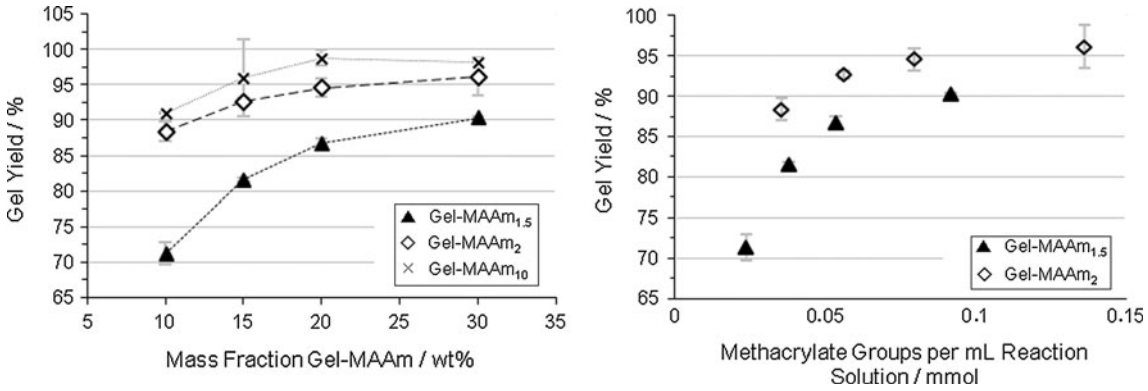

Fig. 3 *Left* gel yield of Gel-MAAm hydrogels of different DM plotted against the mass fraction. *Right* gel yield plotted against the number of methacrylate functions per volume. (The absolute number of double bounds can only be provided for Gel-MAAm1.5 and Gel-MAAm2. For higher DM the indirect quantification of double bonds by TNBS via quantification of free amino groups was not possible because all amino groups and further functions have been modified by MAAnh.) The *lines* are added for guidance of the eye only $n \geq 3$

sequences. This aspect may be of considerable interest in liquid handling applications which are sensitive to the viscosity of the processed fluids.

3.3 Gel yield

The gel yield is defined as the percentage of crosslinked polymer mass as to the total polymer mass applied. It gives information about the effectiveness of the crosslinking reaction. The gel yield of photo crosslinked Gel-MAAm gels was investigated relative to the DM and the Gel-MAAm concentration (Fig. 3). The gel yield correlated positively with the increase in mass fraction as well as with the increase of the DM at a given mass fraction. The influence of the DM was more pronounced at lower mass fractions. When plotting the gel yield against the total number of double bonds per volume, it became obvious that the intermolecular crosslinking was not linearly correlated with the total number of reactive sites available in a certain volume. The crosslinking was significantly more effective when the available double bonds were concentrated on few molecules (low mass fraction) instead of being distributed on a larger amount of molecules (high mass faction) (Fig. 3, right).

We conclude that for a most quantitative integration of Gel-MAAm molecules at any polymer concentration the DM should be as high as possible.

3.4 Degree of swelling

The degree of swelling was defined as the ratio of water uptake of a gel in relation to its dry weight. In chemically crosslinked gels the degree of swelling is dependent on the mesh size of the polymer network and the interaction between the polymer and the solvent.

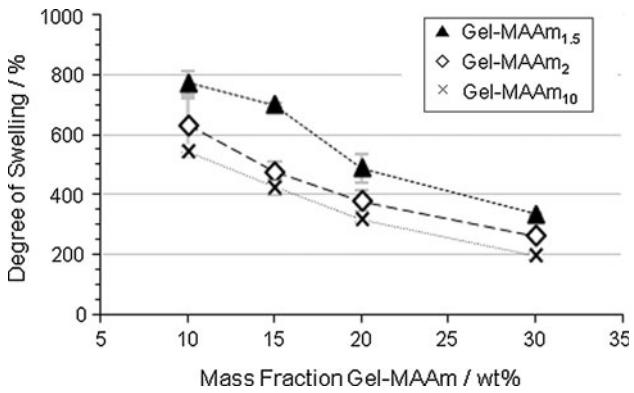

Fig. 4 Degree of swelling of gelatin methacrylamid (Gel-MAAm) hydrogels plotted against the mass fraction. The *lines* are added for guidance of the eye only $n \geq 3$

Figure 4 shows the degree of swelling of hydrogels with three different DM in relation to the gelatin mass fraction. For each DM the degree of swelling decreased as the gelatin mass fraction was increased. This was assigned to a reduction of the mesh size as the chemical nature of the gel can be assumed to stay reasonably unchanged. Increasing the polymer mass fraction resulted in more effective crosslinking as was deduced from the determination of gel yield. This limited the maximum volume increase of the gel. Additionally, the spaces between the crosslinked sites became more and more filled with polymer chains, therefore leaving less room for water uptake. Decreasing the DM resulted in an increase of the degree of swelling. This could be due to the lower crosslinking density and due to changes of the chemical nature of the Gel-MAAm precursors (lower DM).

The high degrees of swelling up to 772 ± 42 % generally reflected the high capability of gelatin to take up and hold back water. Furthermore, this study demonstrated that

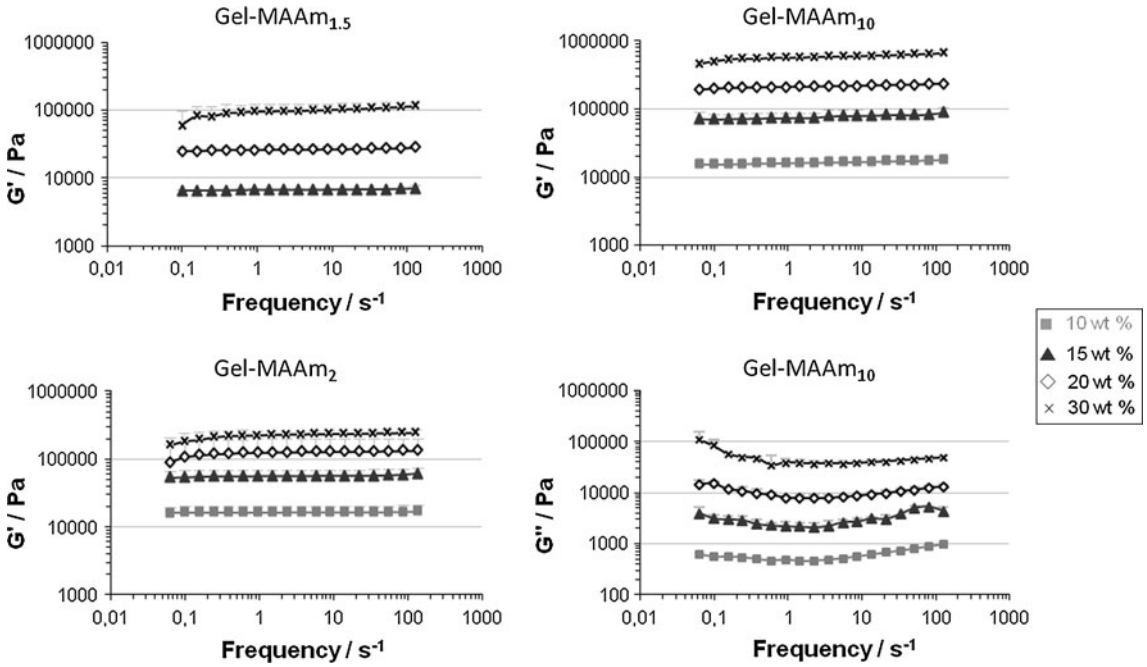

Fig. 5 Storage modulus (Gel-MAAm$_{1.5,\ 2,\ 10}$) and loss modulus (Gel-MAAm$_{10}$) of crosslinked Gel-MAAm hydrogels. Increasing the mass fraction as well as the DM provides an improvement in the storage modulus. The *lines* are added for guidance of the eye only $n \geq 2$

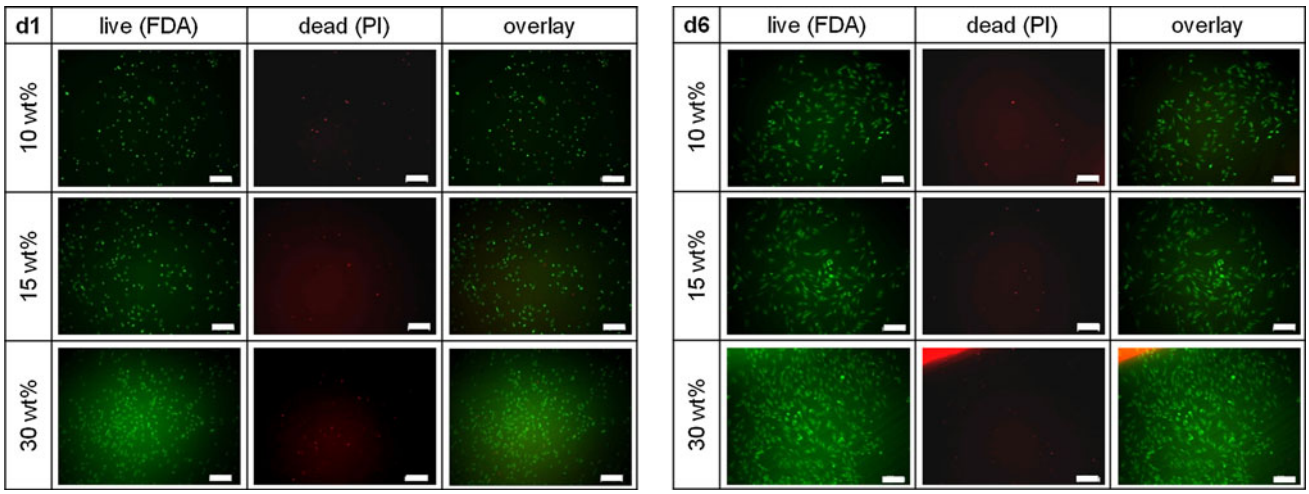

Fig. 6 Porcine chondrocytes with live cell stain (*green*) and dead cell stain (*red*) on Gel-MAAm$_2$ hydrogels of different mass fractions after 1 and 6 day cultivation. *Scale bar* 200 μm (Color figure online)

the swelling behavior of crosslinked gelatin gels could be adjusted over a broad range and could be reduced down to 194 ± 14 % by controlling the DM and the mass fraction. The swelling capacity of porcine hyaline cartilage (knee) was determined to be 517 ± 44 % (own measurements, $n = 3$). This is one example of natural tissue, which exhibits a swelling behavior within the range that was addressed in this study. Due to its origin from the ECM and especially because of its natural RGD content gelatin is assumed to be a versatile matrix for all kinds of in vitro tissue engineering. Therefore it seems desirable to further tune its mechanically and physiologically important properties.

3.5 Rheological properties of crosslinked gels

Dynamic shear oscillation measurements were used to characterize the mechanical properties of the crosslinked hydrogels. The storage moduli G′ and the loss moduli G″ are presented in Fig. 5 over a frequency range of 0.1–100 s^{-1}. The storage (or elastic) modulus represents the elastic part, the loss (or viscous) modulus represents the viscous part of

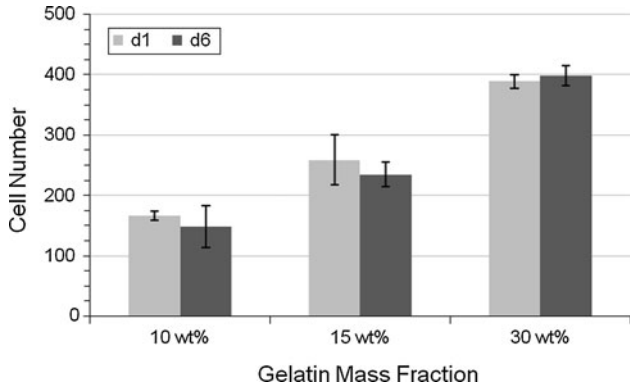

Fig. 7 Number of viable porcine chondrocytes cultivated on Gel-$MAAm_2$ hydrogels of different mass fractions after 1 and 6 day cultivation $n = 3$

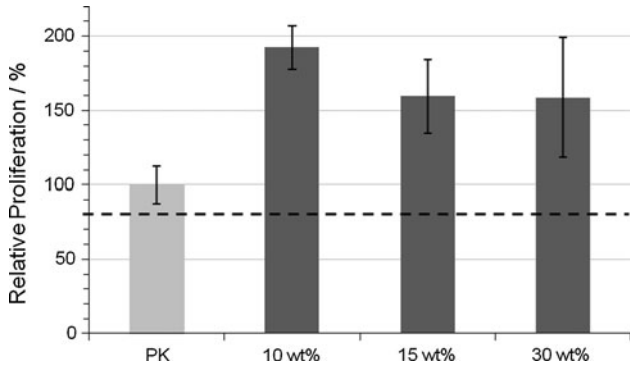

Fig. 8 Relative proliferation of porcine chondrocytes cultured in standard culture medium as positive control (PK) and in extracts of 10, 15, and 30 wt% Gel-$MAAm_2$ hydrogels, respectively. The high viability of cells incubated with the extracts show that the extracts do not exhibit cytotoxic effects on porcine chondrocytes $n = 3$

the material. For all gels the storage modulus was at least five times and up to thirty times higher than the loss modulus. This proved that all gels were elastomeric materials. G′ was reasonably constant with increasing frequency while the loss modulus showed some variations, yet without any sign of gel break. Frequency related variations of G″ were assigned to weakly crosslinked or non-crosslinked gelatin molecules within the network as these variations were more distinct for lower DM (data not shown). The absolute value of the storage modulus within the linear visco-elastic range also represents the strength of the material, i.e. the force that withstands deformation (G'_{LVER} = mean of G′ within the linear visco-elastic range). As shown in Fig. 5 the strength of the gels could be controlled by the DM of the precursor molecules and by the mass fraction applied. G′ correlated positively with increases in the DM and also with increases in polymer concentration.

In contrast to many hydrogels described in literature [32–34], the Gel-MAAm gels prepared in this study achieved G′ in the medium kPa range. G′ of the strongest gels prepared was 368 kPa. Moduli of various natural tissues also range in the kPa area, e.g. canine kidney cortex and medulla with ~10 kPa [17], or human nasal cartilage with 234 kPa [18, 19]. Hyaline cartilage, one of the strongest hydrogel materials, holds G′ = ~800 kPa [20]. Therefore, the highly crosslinked Gel-MAAm hydrogels described in this study are considered to be promising material systems with a view to the generation of tissue engineering matrices at the "stiffer end" of soft tissues.

3.6 In vitro cytocompatibility

With regard to applications in e.g. Advanced Therapy Medicinal Products (ATMPs) preliminary in vitro cytocompatibility testing was performed, investigating material extracts and direct cell-material interactions with porcine chondrocytes. For direct cell-material contact cells were seeded onto Gel-$MAAm_2$ gels with different gelatin mass fractions. Cell adhesion, cell viability and cell confluency were evaluated by performing live/dead staining on day one (d 1) and on day six (d 6). Figure 6 shows representative fluorescence images of the stained cells and Fig. 7 gives the results of the counting of viable cells on the hydrogels. Very few dead cells were detected on the gels independent of the mass fraction. Cytotoxicity assays according to extract testings based on DIN EN ISO 10993-5 were carried out and confirmed the favorable cytocompatibility of the Gel-MAAm hydrogels (Fig. 8).

Chondrocyte adhesion (d 1) was weakest on the 10 wt% hydrogels and increased with increasing gelatin content. Cell spreading was rarely observed on 10 wt% gels (round shaped cells) but increased with increasing gelatin content (flat cells). As gelatin provides RGD motifs, assumingly the number of RGD motifs was increased as the increase of the gelatin mass fraction was increased. This might have accounted for the improved adhesion of chondrocytes to the gels of higher mass fraction. Yet, various authors have shown that material properties such as the material strength also influenced cell behavior [35–37]. Therefore, the differences in material strength may have also played a role in the cells' adhesion preferences. Evaluation of the cells on day six (d 6) revealed that the cell spreading had increased while the cell number remained fairly unchanged on the hydrogels (Fig. 7). The non-proliferative behavior might be considered to confirm the cytocompatibility of the material for chondrocytes, which natively stay in a quiescent state. We therefore conclude that Gel-MAAm hydrogels are potential biomaterials for medical application and tissue engineered scaffolds, in particular for artificial cartilage.

4 Conclusion

Chemically crosslinkable gelatin is a promising matrix material for in vitro cell culture and tissue engineering because of its origin from the ECM, its natural RGD content, good availability, and relatively low cost. In this study we demonstrated that the controlled integration of chemically crosslinkable methacryl moieties allowed for tailoring soft tissue analogous hydrogels that resemble the stiffness and swellability of natural ECM of different tissue. In particular we prepared hydrogels with degrees of swelling in the range of 770–194 % and storage moduli in the range of 5–368 kPa, respectively, and thereby covered the properties of a broad range of natural tissues matrices including various types of cartilage. We also showed that chemical modification of nucleophilic groups by methacrylation systematically affected the viscosity of Gel-MAAm solutions. Decreasing the gelatin concentration and increasing the DM resulted in a decrease in solution viscosity from 1,100 to 3 mPas. Additionally, we observed a change from shear thickening to shear thinning viscous behavior of methacrylated gelatin solutions when increasing the concentration from 10 to 30 %. This effect occurred at shear rates $>1{,}000\ s^{-1}$ and may prove to be helpful for liquid handling applications which are sensitive to the viscosity of the processed fluids. The cytotoxicity assay of chemically crosslinked Gel-MAAm gels according to extract testings based on DIN ISO 10993-5 and the cultivation of porcine chondrocytes on Gel-MAAm gels proofed excellent cytocompatibility and confirmed good cell-material interaction.

Based on these results we conclude that chemically crosslinked gelatin gels have high potential to constitute excellent substitutes of native extracellular matrices. They may be applied for in vitro cell cultivation and tissue engineering or serve as biobased implants to replace soft tissue for example after tumor resections. Biomaterials with tailored elasticity and stiffness are particularly needed to maintain intrinsic tissue stability, e.g. for the replacement of parts of the trachea. Furthermore we anticipate the encapsulation of cells into crosslinked gelatin hydrogels to provide ATMPs for accelerated healing of soft tissue defects. It has been shown that matrix-assisted autologous chondrocyte transplantations currently are the most promising therapy for cartilage lesions with the aim to regenerate hyaline cartilage and prevent the formation of fibrous cartilage. Gel-MAAm hydrogels with tunable rheological properties in a wide range of elasticity are considered to be ideal for optimizing the matrix properties. Further material tuning and combining of two or more material components will be investigated in order to achieve high storage moduli and high degrees of swelling simultaneously.

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