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"Die ultimative Antwort auf die Frage nach dem Leben, dem Universum und allem lautet: 42."

Deep Thought aus Per Anhalter durch die Galaxis

Für Opa

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Abkürzungsverzeichnis

3D	dreidimensional
APS	Ammoniumperoxodisulfat
ATR-FTIR	Abgeschwächte Totalreflexion Fourier-Transformations-Infrarotspektroskopie
<i>B. subtilis</i>	<i>Bacillus subtilis</i>
CalB	<i>Candida antarctica</i> Lipase B
<i>C. auris</i>	<i>Candida auris</i>
CHILS	Carbohydrate-based Ionic Liquids and Salts, Kohlenhydrat-basierte ionische Flüssigkeiten und Salze
CTB	Cell Titer Blue
DHEBA	<i>N,N'</i> -(1,2-Dihydroxyethylen) bis(acrylamid)
DMEM	Dulbecco's Modified Eagle's Medium, Zellkulturmedium
DMF	Dimethylformamid
DNA	Deoxyribonucleic acid, Desoxyribonukleinsäure
<i>E. coli</i>	<i>Escherichia coli</i>
EGDA	Ethylenglycoldiacrylat
EGDMA	Ethylenglycoldimethacrylat
EHEC	Enterohämorrhagische <i>Escherichia coli</i>
GelMA	Gelatine-Methacrylat
GMIM-I	1-(Methyl- α -D-glucopyranosid-6-yl)-3-methylimidazoliumiodid
GVIM-I	1-(Methyl- α -D-glucopyranosid-6-yl)-3-vinylimidazoliumiodid
HEMA	Hydroxyethylmethacrylat
IL(s)	ionic liquid(s), ionische Flüssigkeit(en)
IPN	Interpenetrierendes Polymernetzwerk
LAP	Lithium-Phenyl-2,4,6-trimethylbenzoylphosphinat
LPS	Lipopolysaccharid
LVE	Linear viskoelastisch
MBAA	<i>N,N'</i> -Methylenbisacrylamid
MeGlu	Methyl- α -D-glucopyranosid
MeGlul	Methyl-6-iodo- α -D-glucopyranosid
MNP	Metallnanopartikel
MONP	Metalloxidnanopartikel
MRE	Multiresistenter Erreger
NMR	Nuclear Magnetic Resonance, Kernspinresonanzspektroskopie
Omp	Outer membrane protein, Protein der äußeren Membran
PAAm	Polyacrylamid
PBS	Phosphate buffered saline, Phosphat-gepufferte Salzlösung
PEG	Polyethylenglycol
PEGDA	Polyethylenglycoldiacrylat
PLA	Poly(lactid) acid, Poly(lactid)
poly(GVIM-I)	Hydrogel aus 1-(Methyl- α -D-glucopyranosid-6-yl)-3-vinylimidazoliumiodid
polyIL(s)	Polymerisierte ionische Flüssigkeit(en)

PVA	Polyvinylalkohol
PVP	Polyvinylpyrrolidon
REM	Rasterelektronenmikroskopie
RNA	Ribonucleic acid, Ribonukleinsäure
SIRC	Statens Seruminstitut Rabbit Cornea, Hornhautepithelzelllinie des Kaninchens
TEM	Transmissionselektronenmikroskopie
TEMED	<i>N,N,N',N'</i> -Tetramethylethylendiamin
UV	Ultraviolett
ZOI	Zone of Inhibition, Inhibierungszone

Vom SI-System abweichende Einheiten und Definitionen

Die in dieser Arbeit verwendeten Maßeinheiten entsprechen den Einheiten im Internationalen Maßeinheitensystem (SI) gültigen Maßeinheiten. Aufgeführt sind alle abweichenden Einheiten und deren Umrechnungen.

°C	Grad Celsius, $\{\vartheta\}_{\text{C}} = \{T\}_{\text{K}} - 273,15 \vartheta$
E_a	Aktivierungsenergie
h	Stunde, $1 \text{ h} = 60 \text{ min} = 3600 \text{ s}$
kJ	Joule, $1 \text{ J} = 1 \text{ kg m}^2 \text{ s}^{-2}$
L	Liter, $1 \text{ L} = 0,001 \text{ m}^3$
min	Minute, $1 \text{ min} = 60 \text{ s}$
mm	Millimeter, $1 \text{ mm} = 0,001 \text{ m}$
wt%	Massenanteil, $w_i = m_i / (m_i + m_j)$
G^*	komplexer Schubmodul
G'	Speichermodul
G''	Verlustmodul
m_0	Ausgangsmasse
m_t	Masse zum Zeitpunkt t
$\text{p}K_s$	negativer dekadischer Logarithmus der Säurekonstante K_s
Q	Quellungsgrad
SR	Swelling Ratio, Quellungsverhältnis
γ	oszillatorische Scherdehnung
τ	oszillatorische Scherspannung

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Kurzfassung

Hydrogele sind dreidimensionale polymere Netzwerke, die große Mengen an Wasser oder biologischen Flüssigkeiten reversibel aufnehmen können, ohne dabei ihre Form zu verlieren oder sich aufzulösen. Ihre Eigenschaften hängen von Faktoren wie dem Ursprung der Monomere und Vernetzern, der Vernetzungsmethode, ihrer elektrischen Ladung und der Herstellungsweise ab. Je nach Anwendung können diese Eigenschaften variiert und angepasst werden. Das resultiert in einer Vielzahl an möglichen Anwendungsgebieten und großem Interesse in der Forschung. Für den bestmöglichen Einsatz, die vollständige Nutzung ihres Potenzials und eine gezielte Anpassung an spezifische Anwendungen ist ein tiefgreifendes und umfassendes Verständnis der Eigenschaften der Hydrogele essentiell.

Im Rahmen dieser Arbeit wurden zunächst neuartige, semi-synthetische Hydrogele auf der Basis von Methyl- α -D-glucopyranosid in drei Stufen hergestellt. Dabei wurde ein Screening von unterschiedlichen Arten und Konzentrationen an Vernetzern vorgenommen, um den jeweils optimalen Arbeitsbereich der Vernetzer zu finden.

In einem zweiten Schritt erfolgte die Charakterisierung dieser Hydrogele hinsichtlich verschiedener Eigenschaften. Die Gelierung wurde mittels rheologischer und spektroskopischer Untersuchungen verfolgt. Dabei wurde eine *in situ* Rheologie-Raman-Spektroskopie als eine neue Methode zur Hydrogelcharakterisierung etabliert. Die Aufnahme von wässrigen Medien wurde in einer Quellungsstudie untersucht. Für zukünftige Anwendungen im biomedizinischen Bereich wurden die antimikrobiellen Eigenschaften gegen die Krankenhauskeime *B. subtilis*, *E. coli* und *C. auris* getestet. Dabei zeigte sich, dass die Hydrogele eine starke wachstumshemmende Aktivität gegenüber *C. auris* aufweisen. Die Biokompatibilität der Hydrogele wurde mit L929-Mausfibroblasten mittels Eluattests ermittelt. Hydrogele mit PEGDA 757 als Vernetzer und LAP als Initiator zeigten vielversprechende Ergebnisse. Diese wurden im nächsten Schritt unterschiedlich behandelt und durch Direktkontakttests mit den Zellen weiter untersucht. Durch Waschen mit einem Zellkulturmedium konnten biokompatible Hydrogele erhalten werden.

Abstract

Hydrogels are three-dimensional polymer networks that can reversibly absorb large amounts of water and biological fluids without losing their shape or dissolving. Their properties depend on factors such as the origin of the monomers and crosslinkers, the crosslinking method, their electrical charge and the production method. Depending on the application, these properties can be varied and adjusted. This results in a wide range of possible applications and great interest in research. A deep and comprehensive understanding of the properties of hydrogels is essential for the best possible use, full utilization of their potential and targeted adaptation to specific applications.

As part of this work, novel, semi-synthetic hydrogels based on methyl- α -D-glucopyranoside were initially produced in three-step synthesis. Different types and concentrations of crosslinkers were screened in order to find the optimum working range for each crosslinker.

In a second step, these hydrogels were characterized with regard to various properties. Gelation was tracked by using rheological and spectroscopic investigations. *In situ* rheological Raman spectroscopy was established as a new method for hydrogel characterization. The absorption of aqueous media was analyzed in a swelling study. For future applications in the biomedical field, the antimicrobial properties against the hospital germs *B. subtilis*, *E. coli* and *C. auris* were tested. It was shown that the hydrogels exhibit strong growth-inhibition activity against *C. auris*. The biocompatibility of the hydrogels was determined with L929 mouse fibroblasts using eluate tests. Hydrogels with PEGDA 575 and LAP as initiator showed promising results. These were treated differently and further analyzed by direct contact tests with the cells. Biocompatible hydrogels were obtained by washing with cell culture medium.

1. Einleitung

1.1 Klassifizierung von Hydrogelen

Hydrogele sind von sehr großer Bedeutung und Interesse in einer Vielzahl unterschiedlicher Forschungsdisziplinen, wie z.B. in der Biomedizin, der Biotechnologie, in der Pharmazie, bei der Abwasseraufarbeitung, in der Landwirtschaft, der Lebensmittelverpackungsindustrie oder in der Kosmetik.¹ Sie sind definiert als dreidimensionale Netzwerke, die durch ihre hydrophile Polymerstruktur in der Lage sind, signifikante Mengen an Wasser aufzunehmen, ohne dabei ihre Form zu verlieren.²⁻⁵ Das erste Hydrogel, basierend auf Polyvinylalkohol verlinkt mit Formaldehyd, wurde bereits 1949 erstmalig beschrieben und wurde in biomedizinischen Implantaten eingesetzt.^{6,7} Die Veröffentlichung von WICHTERLE und LÍM 1960 über die Synthese von Polyhydroxyethylmethacrylat (Poly(HEMA)) und dessen Anwendung in dauerhaftem Kontakt zu menschlichem Gewebe markierte den Start der breitgefächerten Forschung zu Hydrogelen.⁸



Abbildung 1 Möglichkeiten zur Klassifizierung von Hydrogelen. Adaptiert nach FARASATI *et al.*⁹

Die Herstellungsweise, der Ursprung der Hydrogele, die Sensitivität, die Ladung des Polymernetzwerkes und die Art der Vernetzung (physikalisch oder chemisch) teilt Hydrogele in unterschiedliche Kategorien ein (Abbildung 1).⁹

Auf Stimuli empfindliche Hydrogele reagieren auf äußere Anreize und erfahren dadurch Veränderungen in ihrem Wachstum, der Quellung, der Netzwerkstruktur oder ihrer

mechanischen Festigkeit. Physikalisch sensitive Hydrogele reagieren auf Faktoren wie Licht, Druck, Temperatur, elektrische und magnetische Felder oder mechanische Belastungen. Ein chemischer Stimulus umfasst Reaktionen auf den pH-Wert, chemische Stoffe und ionische Verbindungen. Diese Eigenschaft wird beispielsweise für eine pH-Wert abhängige Insulinabgabe im menschlichen Körper eingesetzt. Dabei fungieren Glucose- und pH-Wert-sensitive Hydrogele als Wirkstofffreisetzungssystem.¹⁰ Hydrogele mit ionischen Seitengruppen können als Reaktion auf eine pH-Wertänderung Protonen abgeben oder aufnehmen. Anionische Hydrogele besitzen Gruppen wie z.B. Carbon- oder Sulfonsäuren, bei denen eine Deprotonierung stattfindet, wenn der pH-Wert der Umgebung über dem pK_s -Wert (neg. dekadischer Logarithmus der Säurekonstante) liegt. Dies führt zur Ionisierung der funktionalen Gruppen, welches die Quellung des Hydrogels im wässrigen Medium verstärkt. Kationische Hydrogele besitzen z.B. Aminogruppen, die protonieren, wenn der pH-Wert unterhalb des pK_s -wertes liegt. Dadurch erhöht sich die elektrostatische Abstoßung und die Quellung wird ebenfalls erhöht.^{1,10,11} Biochemisch stimulierte Hydrogele reagieren auf Liganden, Enzyme, Antigene und andere biochemische Stoffe.

Hydrogele, die auf Stimuli reagieren sind attraktive Biomaterialien für biomedizinische und biotechnologische Anwendungen, z.B. als Wirkstofffreisetzungssystem oder zur Überwachung von epidermalen Wunden (mittels pH-sensitivem Farbstoff im Hydrogel) und werden auch als "Smart Materials" bezeichnet.^{1,12-15}

Hydrogele werden auf Basis der Ladung ihres Polymernetzwerkes in drei Hauptkategorien eingeteilt: neutrale, ionische und zwitterionische Hydrogele. Ionische Hydrogele werden weiter in kationische und anionische eingeteilt.¹⁶ Neutrale Hydrogele enthalten in ihrer Polymerstruktur oder in der Seitenkette keine Ladung. Sie quellen nur durch Wasser-Polymer-Wechselwirkungen. Beispiele für neutrale Hydrogele sind Polyethylenglycol (PEG), PolyHEMA, Polyacrylamid (PAAm) oder Polyvinylalkohol (PVA).

Ionische Hydrogele enthalten entweder eine positive oder eine negative Ladung in ihrer Polymerstruktur. Sie sind pH-abhängig, da die Ionenketten bei unterschiedlichen Wasserstoffionenkonzentrationen in unterschiedlichem Maße dissoziieren. Kationische Hydrogele quellen bei niedrigen pH-Werten, während anionische Hydrogele bei hohen pH-Werten quellen. Kationische Hydrogele basieren auf kationischen Monomeren wie Vinylpyridin, Aminoethylmethacrylat oder Vinylimidazolen und anionische Hydrogele bestehen z.B. aus Polystyrolsulfonat oder basieren z.B. auf Kaliumsulfopropylacrylat oder -methacrylat.^{17,18} Zwitterionische Hydrogele besitzen sowohl eine kationische als auch eine anionische Ladung in ihrer Polymerstruktur und reagieren sehr sensitiv auf pH-Änderungen.¹⁶

Je nach Art der Vernetzungsmethode können Hydrogele in physikalisch und chemisch vernetzt eingeteilt werden. Bei den physikalisch vernetzten Hydrogelen kann das Polymernetzwerk durch ionische Interaktionen, Wasserstoffbrückenbindungen, nicht-kovalente Interaktionen usw. ausgebildet werden. Bei der Bildung von Wasserstoffbrücken zwischen den Polymerketten wird die Löslichkeit herabgesenkt, was zur Gelierung führt. Auch bei der ionischen Wechselwirkung zwischen Polyelektrolytlösungen und deren Gegenionen kann Gelbildung auftreten. Beispiele dafür sind Natrium-Alginat

mit Calcium (Ca^{2+}) oder Magnesium (Mg^{2+}) und Chitosan mit Calcium oder Glycerophosphat-Dinatriumsalz.^{19,20} Physikalisch vernetzte Hydrogele sind aufgrund von Konformationsänderungen in der Struktur reversibel, wohingegen chemisch vernetzte Hydrogele durch Veränderungen ihrer Konfiguration dauerhaft und irreversibel sind.¹

Chemisch vernetzte Hydrogele können mittels radikalischer Polymerisation, Kondensationsreaktion, enzymatischer Reaktion, durch Hochenergiestrahlung oder mittels Pfropfung hergestellt werden. Die radikalische Polymerisation, eine Kettenwachstumspolymerisation, wird oft zur Hydrogelsynthese unter Verwendung von Vinylmonomeren verwendet. Sie umfasst vier Schritte: i) Initiierung, ii) Kettenstart, iii) Kettenwachstum und iv) Kettenabbruch. Die Radikalbildung bei der Initiierung erfolgt durch die homolytische Spaltung einer labilen Bindung unter Zufuhr von Energie z.B. thermisch oder photochemisch. Das Initiatorradikal wird beim Kettenstart an den Vernetzer (z.B. Diacrylate) addiert. Im nächsten Schritt, dem Kettenwachstum, reagieren diese dann mit den Vinylmonomeren und mit weiteren Vernetzermolekülen. Dabei bildet sich das dreidimensionale Netzwerk aus. Der Kettenabbruch geschieht durch Rekombinationsreaktionen oder Disproportionierung.²¹ Eine etablierte Methode bei der radikalischen Polymerisation ist die Verwendung des Redoxsystems aus Ammoniumperoxodisulfat (APS) als Initiator und *N,N,N',N'*-Tetramethylethylendiamin (TEMED) als Aktivator, welcher durch herabsenken der Aktivierungsenergie E_a von 62 kJ/mol auf 22 kJ/mol die Zersetzung von APS in freie Radikale bei Raumtemperatur ermöglicht (Abbildung 2).²²

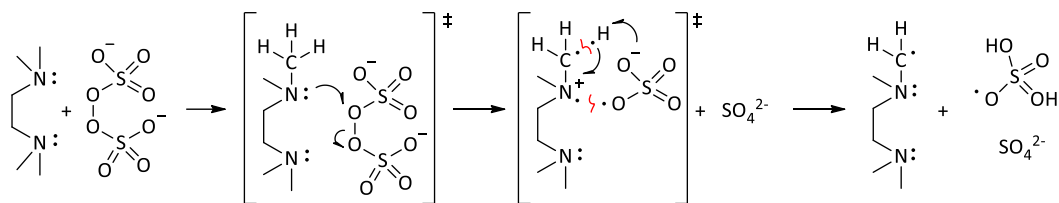


Abbildung 2 Mechanismus der primären Radikalbildung bei der Redox-Initiierung durch APS und TEMED.^{22,23}

Neben der Verwendung von APS und TEMED können auch Photoinitiatoren wie 2-Hydroxy-4'-(2-hydroxyethoxy)-2-methylpropiophenon (Irgacure 2959) oder Lithiumphenyl-2,4,6-trimethylbenzoylphosphinat (LAP) verwendet werden. Der Einsatz von Photoinitiatoren kann gegenüber dem System aus APS und TEMED einige Vorteile haben. Zunächst wird die Gelierung durch das An- bzw. Ausschalten der Lichtquelle (Ultraviolette (UV)-Strahlung oder sichtbares Licht) gezielt gesteuert. Weiterhin findet eine Photopolymerisation unter milden Bedingungen statt und verläuft sehr schnell.^{24,25} Beim Einsatz von Hydrogelen in Kontakt zu lebendem Gewebe ist es wichtig, dass die Materialien eine sehr gute Zellverträglichkeit aufweisen. Auch hier wird der Einsatz von Photoinitiatoren bevorzugt. LAP und Irgacure zeigten in Studien gute bis sehr gute Biokompatibilitäten bei Gelatine-Methacrylat (GelMA)- und Polyethylenglycoldiacrylat (PEGDA)-Hydrogelen.²⁶⁻²⁹

Besteht ein Hydrogel aus einer großen Anzahl von sich wiederholenden Einheiten einer Art, wird dieses Homopolymer genannt. Homopolymere können je nach Art der Einheiten und der verwendeten Polymerisationstechnik eine skelettartige Struktur ausbilden.

Copolymere sind Hydrogele, die aus zwei oder mehr unterschiedlichen Monomerarten bestehen und mindestens eine davon hydrophil ist. Hierzu gehören auch Hydrogele, die mithilfe von Vernetzern wie PEGDA oder *N,N'*-Methylenbisacrylamid (MBAA) hergestellt werden.³⁰ Copolymere können je nach Ausrichtung der Monomere Block-, Pfropf-, alternierende und zufällige Netzwerkstrukturen aufweisen.¹⁶ Bei interpenetrierenden Hydrogelen bzw. interpenetrierenden Polymernetzwerken (IPN) wird zunächst ein Polymernetzwerk hergestellt, welches dann in einer polymerisierbaren wässrigen Monomerlösung gequollen wird. Reagieren die Monomere zu physikalisch assoziierten Netzwerken, handelt es sich um ein IPN. Wenn ein Polymernetzwerk quervernetzt ist, während das andere Polymer physikalisch mit dem vernetzten Polymer verbunden ist, ohne dabei chemisch daran zu binden, handelt es sich um ein semi-IPN.³¹ Wenn das zweite Polymer ebenfalls mit dem ersten Polymernetzwerk vernetzt ist, wird das gebildete Netzwerk als voll-interpenetrierend (voll-IPN) bezeichnet.³²

Je nach Ursprung der Hydrogele können sie in natürliche, synthetische und semi-synthetische eingeteilt werden. Synthetische Hydrogele können aus synthetischen Monomeren wie PVA, Polyvinylpyrrolidon (PVP), Polylactid (PLA) oder PEG hergestellt werden.³³ Zu den wichtigsten Vorteilen von synthetischen Hydrogelen zählen die hohe Produktreinheit, die Reproduzierbarkeit und die Unbedenklichkeit der Immunogenität.¹⁶ Ihre mechanischen Eigenschaften können an die entsprechende Anwendung angepasst werden. Der Großteil der synthetischen Hydrogele ist chemisch und biologisch inert und somit nicht biodegradierbar und limitiert im Einsatz als Biomaterialien. Ein weiterer Nachteil stellt die Entsorgung dieser Hydrogele dar, da diese zeitaufwendig und teuer sein kann.^{34,35}

Natürliche Hydrogele werden aus Biopolymeren, die von lebenden Organismen wie Pflanzen und Tiere produziert werden, hergestellt. Die drei wichtigsten natürlichen Polymere sind Proteine bzw. Peptide (z.B. Kollagen, Fibrin, Gelatine, Seide, Myosin oder Keratin), Polynucleotide (Desoxyribonukleinsäure (DNA), lineare Plasmid-DNA oder Ribonukleinsäure (RNA)) und Polysaccharide (z.B. Cellulose, Chitosan, Alginat, Hyaluronsäure oder Chitin).³⁴ Natürliche Hydrogele sind gut zugänglich und kostengünstig, da sie reichlich vorhanden, ungiftig und biologisch abbaubar sind. Außerdem weisen sie eine außerordentlich gute Biokompatibilität auf, was sie für den Einsatz in der biomedizinischen Technik attraktiv macht, z.B. für die gezielte Freisetzung von Wirkstoffen im Körper, in der Gewebezüchtung oder der Wundheilung.³⁶⁻³⁸

Polysaccharide als Baustein zur Hydrogelsynthese bestehen aus verschiedenen Einheiten von Monosaccharid- und Disaccharidketten. Dadurch ergibt sich eine große Anzahl strukturell unterschiedlicher Polysaccharide. Die Verteilung, Verzweigung und Reihenfolge der verschiedenen Monomereinheiten sind nicht einheitlich, wodurch eine Reproduzierbarkeit bei der Hydrogelsynthese nicht gewährleistet werden kann.³⁴ Die größten Nachteile bei der Verwendung von natürlichen Polymeren als Grundgerüst für Hydrogele ist ihre geringe mechanische und thermische Stabilität und der "Burst-Effekt" eines Wirkstoffes beim Einsatz als Wirkstofffreisetzungssystem.¹⁶ Um diese Hindernisse zu umgehen, werden semi-synthetische Hydrogele aus natürlichen und synthetischen Materialien hergestellt. Sie kombinieren die Vorteile beider Materialtypen und weisen sehr gute chemische, physikalische und biologische Eigenschaften (Reproduzierbarkeit,

gute Biokompatibilität, mechanische Festigkeit, etc.) auf.¹⁶ Ein Beispiel für ein semi-synthetisches Biopolymer kann aus Chitosan, PVA und Methylcellulose bestehen. Durch ihre außerordentliche Biokompatibilität werden sie als Wirkstofffreisetzungssystem oder zur Hautgewebezüchtung eingesetzt.³⁹

Die im Rahmen dieser Arbeit verwendeten Hydrogele basieren auf 1-(Methyl- α -D-glucopyranosid-6-yl)-3-vinylimidazoliumiodid (GVIM-I). GVIM-I ist ein Monomer, welches der Klasse der carbohydrate-based ionic liquids and salts (CHILS) zugeordnet werden kann. CHILS sind eine Untergruppe der ionischen Flüssigkeiten (ILs), welche der Definition nach Salze mit einem Schmelzpunkt von unter 100 °C sind und sich aus einem Kation, oft Imidazol oder Pyridin, und einem Anion, z.B. Triflate, zusammensetzen. Sie weisen einen kaum messbaren Dampfdruck und einen weiten Flüssigkeitsbereich auf. ILs sind in der Regel wenig entzündlich und haben gute Lösungseigenschaften. Durch die Polymerisation von IL-Monomeren können polymerisierte ILs (polyILs) erhalten werden. Diese vereinen die positiven Eigenschaften von ILs mit den vorteilhaften Eigenschaften von Polymeren. Anwendung finden sie z.B. als Bausteine für die Herstellung von ionenleitenden Membranen.⁴⁰ Neben den positiven Eigenschaften von klassischen ILs sind diese Vertreter jedoch häufig toxisch, schlecht abbaubar und bringen ökologische Risiken bei der Entsorgung mit sich. Diese Nachteile können durch die Verwendung von biobasierten ILs umgangen werden.⁴¹ Wenn die IL eine intakte Kohlenhydrat-Einheit in der Struktur beinhaltet, egal ob im Kation oder Anion, zählt die IL zu den CHILS. In dieser Arbeit wird Methyl- α -D-glucopyranosid (MeGlu), ein Glucose-Derivat, als Kohlenhydrat-Einheit verwendet. Glucose-basierte CHILS sind in der Literatur bereits gut beschrieben.⁴²⁻⁴⁴ Aber auch andere Monosaccharide werden als Baustein bei der Synthese von CHILS eingesetzt, z.B. Galactose^{45,46}, Ribose^{47,48} oder Xylose.^{49,50} Anwendung finden CHILS in der Biokatalyse als Beschichtungsmaterial für Novozym 435, die immobilisierte Lipase der *Candida antarctica*, zur Erhöhung der Langzeitaktivität des Enzyms.^{51,52} CHILS sind von Natur aus diastereomere Moleküle, weshalb sie auch in der asymmetrischen Synthese als Lösungsmittel, Additive oder Katalysatoren von Bedeutung sind.^{45,50}

1.2 Charakterisierung von Hydrogelen

Hydrogele können, wie bereits erwähnt, sehr diverse Eigenschaften aufweisen. Um neuartige Hydrogele verstehen zu können, ist es von großer Bedeutung, diese zu charakterisieren.

Spektroskopische Charakterisierung

In den letzten Jahren haben sich viele spektroskopische Methoden zur Charakterisierung von Hydrogelen etabliert. Zu diesen Methoden gehören die Mikroskopie (Rasterelektronenmikroskopie (REM), Transmissionselektronenmikroskopie (TEM) und Varianten davon) zur Bestimmung der lokalen Hydrogelstruktur, die (Festkörper-) Kernspinresonanz (NMR) -spektroskopie als analytische Technik zur Bestimmung der chemischen Struktur, sowie die Raman-Spektroskopie als Technik zur Messung der molekularen Wechselwirkungen. Des Weiteren können die Fourier-Transformations-Infrarot-Spektroskopie (FT-IR) und die abgeschwächte Transmissionsreflexion (ATR-FTIR)

aufgrund ihrer Einfachheit, Zugänglichkeit und Schnelligkeit die chemische Zusammensetzung durch Messung von Schwingungen bestimmen.^{53,54}

REM und dessen Varianten bieten die Möglichkeit, Informationen über die Oberflächenbeschaffenheit, Additive und Poren im Hydrogel zu liefern. Sehr wichtig bei der Probenvorbereitung ist die Trocknung der Hydrogelprobe, da Wasser das Vakuum erheblich stört und des Abbildungsprozess während der Messung behindert. Luft- und Gefriertrocknung, sowie Trocknung mit überkritischem CO₂ sind gängige Methoden zur Probenvorbereitung. Bei der Luft- und Gefriertrocknung kann es zu Artefaktbildung kommen und die Morphologie des Hydrogels beeinträchtigen.⁵⁵⁻⁵⁷ Die Trocknung mit überkritischem CO₂ liefert den Vorteil, dass die Struktur und die Poren beim Trocknen geschützt werden und es nicht zu einem Porenkollaps kommt.⁵⁸

Bei der NMR-Spektroskopie wird die Probe im flüssigen bzw. gelöstem Zustand vermessen, was bei Hydrogelen aufgrund ihrer polymeren und unlöslichen Natur nicht möglich ist. Dafür kann NMR-Spektroskopie zur Strukturaufklärung von Monomeren und Vernetzern dienen, die schlussendlich zum Hydrogel verknüpft werden. Festkörper-NMR bietet die Möglichkeit, die chemische Struktur getrockneter Hydrogele vermessen zu können, was wir auch zeigen konnten.⁵⁹

Eine weitverbreitete Methode zur Hydrogelcharakterisierung ist die IR-Spektroskopie und ihre Variationen. Ein großer Vorteil dieser Methode ist die geringe Probenvorbereitung. Dennoch hat die IR-Spektroskopie Limitierungen. Das im Hydrogel enthaltene Wasser führt zu starken Banden im IR-Spektrum, die eventuelle Banden von Interesse überdecken können.^{54,60-62} Komplementär zur IR-Spektroskopie und oft in Kombination eingesetzt wird die Raman-Spektroskopie. Die Wasserbanden sind viel weniger dominant als bei der IR-Spektroskopie und stören somit die Raman-Banden kaum.^{63,64}

Rheologische Eigenschaften

Rheologische Messungen sind ein wesentlicher Bestandteil bei der Charakterisierung von Hydrogelen und das Verständnis für das rheologische Verhalten ist unerlässlich. Die rheologischen Eigenschaften, also das Verformungsverhalten, sind durch verschiedene Zusammensetzungen des Hydrogels einstellbar. So kann durch die Auswahl des Vernetzers, des Monomers und dessen Konzentration die Festigkeit bzw. Flexibilität beeinflusst werden.⁶⁵ Parameter wie Viskosität, Thixotropie und das viskoelastische Verhalten haben einen erheblichen Einfluss auf eine erfolgreiche Anwendung von Hydrogelen bzw. deren *in situ* Gelierung z.B. beim 3D-Druck oder beim Tissue Engineering.⁶⁶⁻⁷¹

Um Informationen über die viskoelastischen Eigenschaften eines Materials zu erhalten, werden oszillatorische Scherspannung und Scherdehnung mit kleiner Amplitude auf die Probe aufgebracht. Es wird eine oszillatorische Scherdehnung (γ) mit einer bestimmten Amplitude in einer bekannten Frequenz angelegt. Daraus resultiert eine oszillatorische Scherspannung (τ), die jedoch um δ phasenverschoben ist (Abbildung 3 A). Für Proben mit einem ideal elastischen Deformationsverhalten ist $\delta = 0^\circ$ und für ideal viskoses Fließverhalten gilt $\delta = 90^\circ$. Für reale Proben liegt δ zwischen 0° und 90° und sie zeigen ein viskoelastisches Verhalten.⁷²

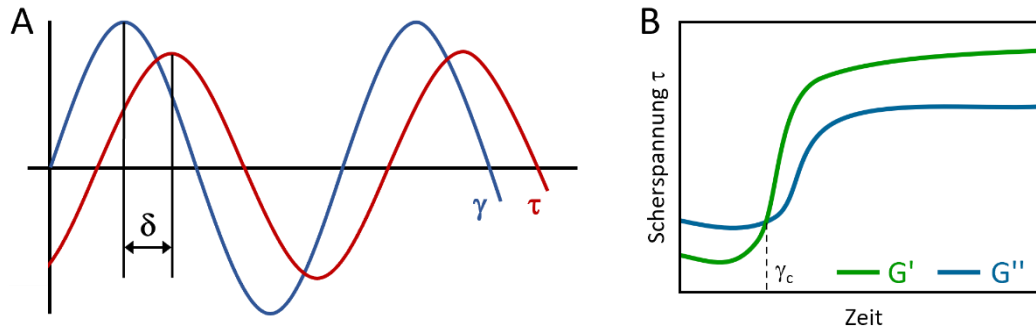


Abbildung 3 Oszillationstests für viskoelastisches Verhalten mit (A) vorgegebener Scherdehnung γ und resultierender Schubspannung τ und (B) schematische Darstellung einer Gelierungsverfolgung eines Hydrogels.⁷²

Der komplexe Schubmodul G^* beschreibt das gesamte viskoelastische Verhalten einer Messprobe. Wie in Formel 1 ersichtlich, setzt sich G^* aus dem Speichermodul G' und dem Verlustmodul G'' zusammen.

$$G^* = G' + iG''$$

Formel 1 Zusammenhang von komplexem Schubmodul, Speicher- und Verlustmodul.

G' repräsentiert den elastischen Anteil des viskoelastischen Verhaltens ("Festkörperverhalten") und G'' charakterisiert den viskosen Teil des viskoelastischen Verhaltens ("Flüssigkeitsverhalten"). Mithilfe eines Oszillationsversuches kann die Gelierung von Hydrogelen verfolgt werden. Die flüssige Probe wird mit konstanter Dehnungsamplitude und Frequenz mit der Zeit gemessen. Bis zum Zeitpunkt der Gelierung ist G'' größer als G' , da das flüssigkeitsähnliche Verhalten überwiegt. Am Gelierungspunkt (γ_c) gilt $G' = G''$. Nach dem Gelierungspunkt ist das Hydrogel gebildet und das feststoffähnliche Verhalten überwiegt (Abbildung 3 B).

Informationen über die Steifigkeit und den linear viskoelastische (LVE) Bereich lassen sich durch eine Amplituden-Sweep-Messung erhalten. Dabei wird die Auslenkung auf eine Probe von Messpunkt zu Messpunkt erhöht. Die Frequenz wird jedoch konstant gehalten. Der Bereich, in dem G' einen konstanten Wert behält, also unabhängig von der angewendeten Scherdehnung ist, wird als LVE Bereich bezeichnet. In diesem kann die Probe zerstörungsfrei verformt werden. Je größer der LVE Bereich ist, desto stabiler ist die Probe gegen Scherstress.^{72,73} Ist G' am Anfang dieser Messung größer als G'' handelt es sich um eine Probe mit viskoelastischem Festkörperverhalten. Ist G'' größer als G' liegt eine Probe mit viskoelastischem Flüssigkeitsverhalten vor.

Quellungseigenschaften von Hydrogelen

Eine Schlüsseleigenschaft von Hydrogelen ist ihre Fähigkeit in Wasser oder wässrigen Medien zu quellen, d.h. Wasser aufzunehmen, ohne dabei ihre dreidimensionale Form, bei der Zunahme der Größe zu verlieren. Im Polymernetzwerk existieren hydrophile funktionelle Gruppen, welche Wasserstoffbrückenbindungen mit dem wässrigen Medium ausbilden und so zur Quellung führen. Der Quellungsprozess besteht aus zwei separaten Transportprozessen. Beim ersten Prozess strömt das Medium von außen durch die Hydrogelporen in das Polymernetzwerk. Beim zweiten Prozess diffundiert das Medium

zwischen den Verstrebnungen des Polymernetzwerks.^{74,75} Die Quellung von Hydrogelen wird durch mehrere Parameter beeinflusst. Dazu gehören die Wechselwirkungen des Mediums mit dem Polymernetzwerk und dessen Bewegungen, die Art, Konzentration und Kettenlänge des Vernetzters, sowie der Vernetzungsgrad.⁷⁶

Um die Quellungseigenschaften unterschiedlicher Hydrogele miteinander zu vergleichen, kann der Quellungsgrad Q gravimetrisch bestimmt werden. Dazu kann folgende Formel verwendet werden:

$$Q = \frac{m_t - m_0}{m_0}$$

Formel 2 Formel zur Berechnung des Quellungsgrades von Hydrogelen.

mit m_t der Masse des Hydrogels zum Zeitpunkt t und m_0 der Masse des Hydrogels vor der Messung.^{18,59} In manchen Publikationen wird anstelle des Quellungsgrades auch das Quellverhältnis (Swelling ratio, SR) angegeben:

$$SR = \frac{m_t - m_0}{m_0} \cdot 100 \%$$

Formel 3 Formel zur Berechnung des Quellungsverhältnisses von Hydrogelen.

mit m_t der Masse des Hydrogels zum Zeitpunkt t und m_0 der Masse des Hydrogels vor der Messung.^{77,78}

Es wird bei der Quellung in drei verschiedene Arten von Hydrogelen unterschieden: i) quellende Hydrogele mit einem SR von $>150 \%$, ii) nicht quellende Hydrogele mit einem SR zwischen 0 und 150% und iii) schrumpfende Hydrogele, dessen SR unter 0% liegt.⁷⁸ Hydrogele, dessen SR weit über 150% (1.000 bis 100.000%) liegt und das bis zu 1.000 -fache ihres Eigengewichts an Wasser aufnehmen können, werden als Superabsorber bezeichnet.^{79,80} Die Kenntnis des Quellungsgrades von Hydrogelen ermöglicht die Vorhersage des Verhaltens der Gele in potentiellen Anwendungen. Sehr stark quellende Hydrogele eignen sich sehr gut für Wundverbände, da sie so die Wunde mit viel Feuchtigkeit versorgen können. Schwach oder gar nicht quellende Hydrogele sind für den Einsatz als Gewebeersatz oder als Wirkstofffreisetzungssystem geeignet.⁷⁸

Biokompatible Eigenschaften

Wenn Hydrogele im menschlichen Körper angewendet werden sollen und sie in direktem Kontakt mit Gewebe kommen, ist es essenziell, die Hydrogele zuvor auf ihre Biokompatibilität zu testen. Biokompatible Materialien zeichnen sich dadurch aus, dass sie entsprechend ihrer Bestimmung funktionieren und am anliegenden Gewebe bzw. im menschlichen Körper nur im zulässigen Maße unerwünschte Reaktionen, bzw. im besten Fall gar keine Reaktionen, hervorrufen. Hydrogele werden als Biomaterialien in den unterschiedlichsten biomedizinischen Disziplinen eingesetzt. Dazu gehören zum Beispiel die Anwendung als Wirkstofffreisetzungssystem, Implantat, Kontaktlinsen, Stentbeschichtung und bei der Gewebezüchtung oder Geweberekonstruktion (tissue engineering).⁸¹⁻⁸⁶

Hydrogele weisen im Allgemeinen eine hohe Biokompatibilität auf, was vor allem an ihren hydrophilen Gruppen im Polymergerüst liegt. Sie sorgen für die hohe Wasseraufnahmefähigkeit der Gele, welche die Eigenschaften von weichem menschlichen Gewebe imitieren kann.⁸⁷

Bekannt und weit verbreitete Hydrogele mit herausragender Biokompatibilität sind Hydrogele natürlichen Ursprungs. Natürlich vorkommende Polymere wie Cellulose, Chitosan oder Alginat werden häufig zur Herstellung von Biomaterialien verwendet. Der Vorteil von natürlichen Polymeren ist neben der Biokompatibilität auch ihre biologische Abbaubarkeit. Leider weisen viele dieser Materialien eine schlechte mechanische Stabilität auf und schränken die Anwendungsmöglichkeiten stark ein.⁸⁷ Um die mechanischen Eigenschaften zu verbessern, aber trotzdem die Biokompatibilität zu gewährleisten, werden semi-synthetische Hydrogele verwendet. Diese können, wie in dieser Arbeit dargestellt, aus einem natürlich vorkommenden Monomer bestehen und dann mit synthetischen Vernetzern zu Hydrogelen polymerisiert werden.

Der erste Schritt nach der physikalischen und chemischen Charakterisierung ist die Untersuchung der *in vitro*-Biokompatibilität. Dabei werden die zu untersuchenden Hydrogele in Kontakt mit unterschiedlichsten Zellen gebracht. Eine Möglichkeit, die *in vitro*-Biokompatibilität zu bestimmen, ist der Eluat-Test. Dabei werden die Zellen in Kontakt mit Eluaten der Hydrogele gebracht und zusammen inkubiert. Eine andere Möglichkeit ist, die Hydrogele in direkten Kontakt mit den Zellen zu bringen.

Biomaterialien werden nach ISO 10993-5 als zytotoxisch eingestuft, wenn die relative Zellviabilität unter 70 % liegt.⁸⁸ Häufig werden Fibroblasten, also Zellen des Bindegewebes, für *in vitro*-Tests verwendet. Bei spezifischen Anwendungen werden jedoch entsprechende Zellen genutzt. Zur Entwicklung von Methylcellulose-basierten Hydrogelen zur topischen Verabreichung von Arzneimitteln im Auge wurden bei den *in vitro*-Untersuchungen SIRC (Statens Seruminstitut Rabbit Cornea)-Zellen, also Zellen aus der Hornhaut von Kaninchen verwendet.⁸⁹

Wenn die *in vitro*-Biokompatibilität von Biomaterialien festgestellt wurde, ist der nächste Schritt bei der Charakterisierung die Untersuchung der *in vivo*-Biokompatibilität. Dabei werden die Materialien in Kontakt zu lebendem Gewebe (z.B. von Mäusen und Kaninchen) gebracht.

Antimikrobielle Eigenschaften

Bakterielle Infektionen stellen gegenwärtig für die menschliche Gesundheit ein großes Problem dar. Hinzu kommt, dass die Anzahl an antibiotikaresistenten Bakterien ständig zunimmt. Infektionen mit multiresistenten Erregern (MRE) sind mit erhöhter Mortalität und Kosten verbunden. Nicht nur in Krankenhäusern (z.B. bei Wundbehandlungen und Implantaten) sind MRE zu finden. Auch bei der Abwasserreinigung stellen sie ein Problem dar. Es ist also notwendig, neue antibakterielle Mittel zur Behandlung multiresistenter bakterieller Infektionen zu finden.^{90,91}

Hydrogele, die von Natur aus antibakteriell wirken, können hier eine gute Strategie sein. Im Gegensatz zu Antibiotika hängt die antibakterielle Wirkung von diesen spezifischen Hydrogelen hauptsächlich von ihrem internen Wirkmechanismus ab. Sie wirken eher auf

die mikrobielle Membran als auf intrazelluläre Stellen. Hydrogele haben, wie zuvor beschrieben, durch ihre gute Biokompatibilität ein hohes Potential zur Anwendung in der Biomedizin.⁹² Effiziente Biomaterialien basieren häufig auf natürlich vorkommenden Polymeren, wie Collagen oder Chitosan.⁹³ Chitosan ist eines der am häufigsten verwendeten Materialien. Die daraus resultierenden Hydrogelen werden z.B. für Wundverbände, zur Abwasserreinigung oder bei Lebensmittelverpackungen genutzt.^{94–96} Chitosan und dessen Derivate zeigen eine außerordentlich hohe antibakterielle Wirkung. Trimethyliertes Chitosan beispielsweise inhibiert das Wachstum von *Escherichia coli* (*E. coli*) und *Staphylococcus aureus* recht stark.^{97–99}

Auch (semi-)synthetische Hydrogele können sehr gute antibakterielle Eigenschaften aufweisen, wenn sie z.B. quartäre Ammoniumgruppen^{100,101} oder Imidazolderivate¹⁰² im Polymernetzwerk beinhalten. Die Immobilisierung von Metallnanopartikeln (MNP) wie Silber (AgNP)¹⁰³, Gold (AuNP)¹⁰⁴ oder Kupfer (CuNP)¹⁰⁵, als auch von Metalloxidnanopartikeln (MONP) wie Zinkoxid (ZnONP)¹⁰⁶ in eine Hydrogelmatrix sorgt für antibakterielle Eigenschaften.¹⁰⁷

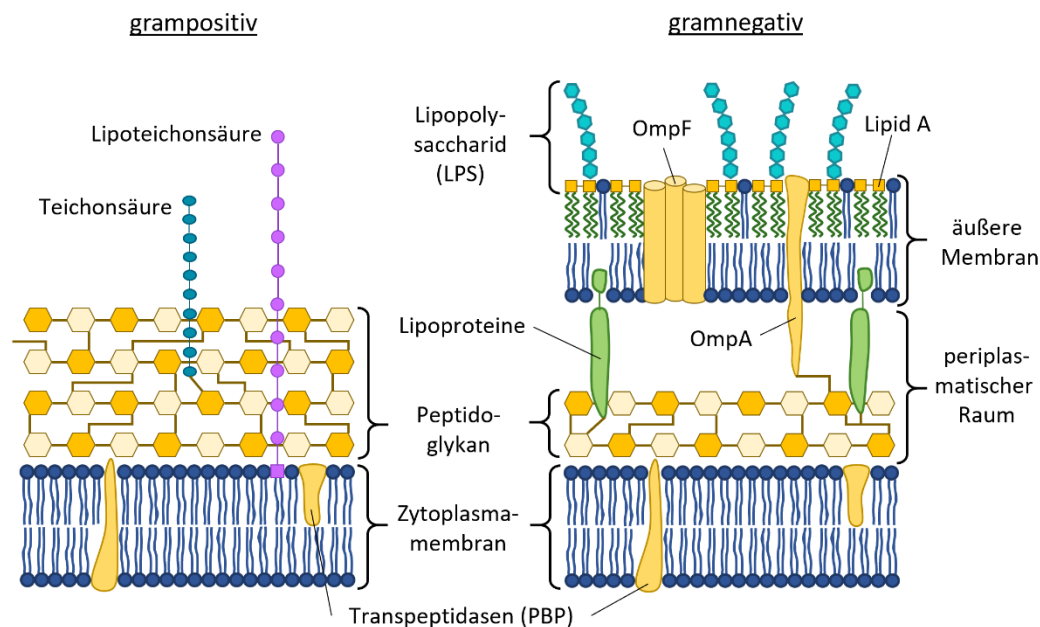


Abbildung 4 Darstellung einer grampositiven und gramnegativen Bakterienzellhülle (Omp = outer membrane proteins, auch Porine). Adaptiert nach H. Hof, R. Dörries, Duale Reihe Medizinische Mikrobiologie, Thieme, 2017.¹⁰⁸

Die Zellhülle von Bakterien kann in zwei Arten unterschieden werden: grampositive und gramnegative Bakterien. Grampositive Bakterien besitzen eine dicke Zellwand, bestehend aus vielen Schichten Peptidoglykan. Diese werden durch Lipoteichonsäure durchzogen und von Teichonsäure verstärkt (siehe Abbildung 4). Gramnegative Bakterien haben nur eine dünne Peptidoglykanschicht, welche durch Lipoproteine mit einer äußeren Membran verbunden ist. Wie in Abbildung 4 zu erkennen, sind in dieser u.a. Lipopolysaccharide (LPS) (z.B. Lipid A) und Porine (z.B. OmpF) verankert. Diese unterschiedlichen Strukturmerkmale können mithilfe der Gramfärbung sichtbar gemacht werden. Zunächst werden die Bakterien mit Kristallviolett angefärbt und dann mit Lugol-Lösung behandelt. Im

Anschluss werden die Bakterien mit einem Alkohol wieder entfärbt. Bei grampositiven Bakterien verhindert die dicke Peptidoglykanschicht, dass sich der gebildete Farbkomplex durch den Alkohol wieder aus der Zelle herauslöst, wodurch die Bakterien unter dem Mikroskop blau erscheinen. Durch die viel dünnere Zellwand der gramnegativen Bakterien kann der Farbkomplex entweichen und die Bakterien erscheinen unter dem Mikroskop rot.¹⁰⁸

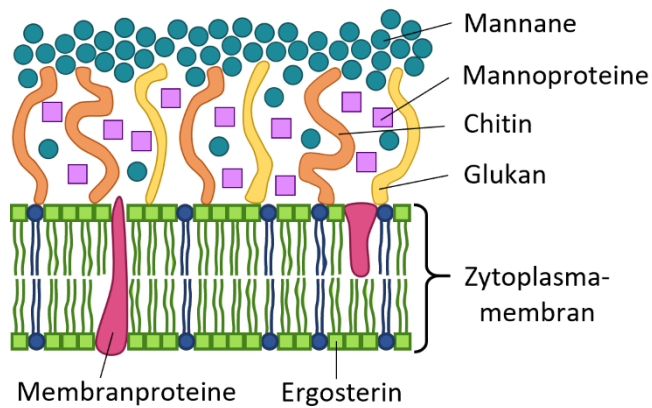


Abbildung 5 Darstellung einer Pilzzellhülle. Adaptiert nach H. Hof, R. Dörries, Duale Reihe Medizinische Mikrobiologie, Thieme, 2017.¹⁰⁸

Neben Bakterien gelten auch Hefepilze als weitverbreitete Pathogene. Sie besitzen im Gegensatz zu Bakterienzellen jedoch eine, das Erbmateriale eingrenzende Zellkernmembran, wodurch sie zu den Eukaryoten zählen. Hefepilzzellen sind bis zu 50 mal größer als Bakterienzellen.¹⁰⁹ Ihre Zellwand besteht unter anderem aus Chitin und Glukan. Bei der zytoplasmatischen Membran, einer Doppellipidschicht, ist hauptsächlich das Steroid Ergosterin als Lipidkörper vorhanden (Abbildung 5). Häufig verwendete Antimykotika sind Polyene (z.B. Amphotericin B). Sie binden sich an das Ergosterin und bilden so Oligomere, welche sich in der Membran einlagern und zu Porenbildung führen, welche für die Zelle tödlich sein können. Die in Europa am häufigsten auftretende Pilzinfektion wird durch den Hefepilz *Candida* verursacht. Viele *Candida*-Arten sind in der Lage, durch Bildung von Biofilmen Kunststoffoberflächen wie Prothesen oder Katheteroberflächen zu besiedeln und so eine Infektion zu verursachen. Die 2009 erstmals nachgewiesene Art *Candida auris* (*C. auris*) kann im Gegensatz zu allen anderen *Candida* Arten nosokomial von Patient zu Patient übertragen werden. Außerdem hat *C. auris* bereits Resistenzen gegen die gängigen Antimykotika Fluconazol und Amphotericin B entwickelt.¹¹⁰⁻¹¹⁴ Auch hier ist es notwendig, Alternativen zur Eindämmung bzw. der Behandlung dieser Infektion zu finden.

2. Ziel dieser Arbeit

Der Klimawandel und die Ausbeutung der fossilen Rohstoffe der Erde der letzten Jahrzehnte machen es erforderlich, neue Strategien und Methoden zu entwickeln, die nachhaltig und zukunftssicher sind.

Kohlenhydrate sind eine nachwachsende und nachhaltige Quelle für Ausgangsstoffe für chemische Verbindungen. Kohlenhydrate werden in Pflanzen und Phytoplankton photosynthetisch hergestellt. Sie können vielfältig chemisch modifiziert und funktionalisiert werden, sodass sie als Plattformchemikalien, ionische Flüssigkeiten und als Polymere weitere Anwendungen finden. Glucose ist das am häufigsten in der Natur vorkommende Monosaccharid und Baustein in Cellulose, dem am häufigsten vorkommenden Polysaccharid. Das Glucose-Derivat Methyl- α -D-glucopyranosid dient in dieser Arbeit als Ausgangsstoff zur Herstellung von semi-synthetischen Hydrogelen.

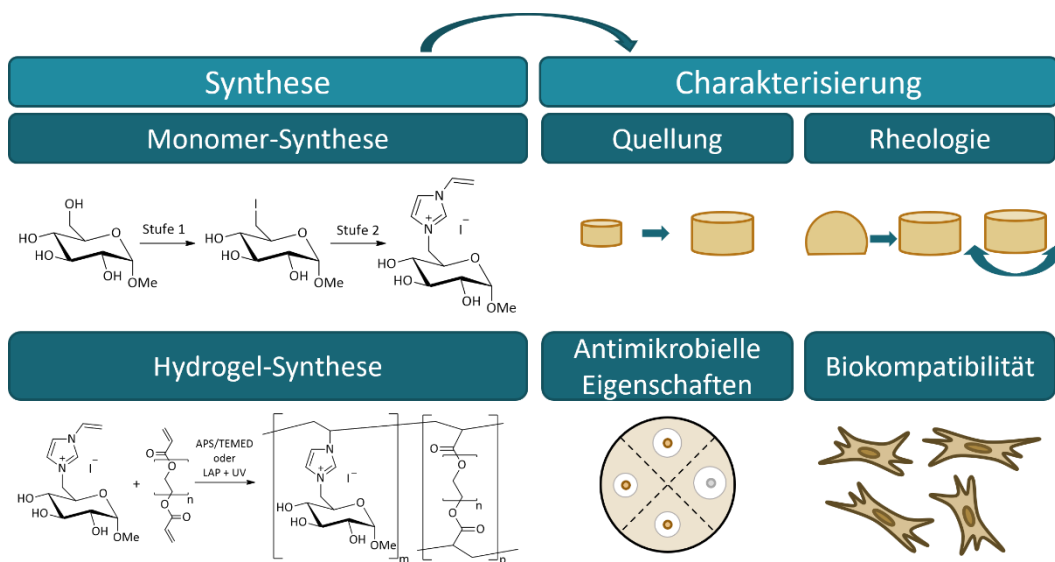


Abbildung 6 Schematische Darstellung der Zielstellung dieser Arbeit.

Im ersten Schritt wird in einer Zwei-Stufen-Synthese aus Methyl- α -D-glucopyranosid zunächst das iodierter Glucopyranosid hergestellt und im Anschluss wird das Iod durch ein Vinylimidazol substituiert.

Nach dieser Zwei-Stufen-Synthese wird im zweiten Schritt das Monomer zu einem Hydrogel umgesetzt. Dabei werden unterschiedliche Arten und Konzentrationen von Vernetzern ausgetestet, um den optimalen Arbeitsbereich für das Monomer zu untersuchen.

Im dritten Teil der Arbeit werden die hergestellten Hydrogele auf ihre Quellungseigenschaften, ihre rheologischen und antimikrobiellen Eigenschaften, sowie auf ihre Biokompatibilität untersucht. Dabei werden auch die unterschiedlichen Arten von Vernetzern, sowie deren Vernetzerkonzentrationen miteinander verglichen, um eine vollständige Übersicht über die Eigenschaften der neuen semi-synthetischen Hydrogele zu erhalten.

3. Synthese von Kohlenhydrat-basierten ionischen Hydrogelen

Hydrogele als auch polyILs haben in den letzten Jahren bzw. Jahrzehnten in der Forschung eine große Bedeutung erlangt. Sie finden in vielen Bereichen, wie der Medizin, Biotechnologie, aber auch bei der Abwasserbehandlung Anwendung. Seit mehreren Jahren wird in der chemischen Industrie viel Wert auf die Einhaltung der "12 Prinzipien der grünen Chemie" gelegt. Im Sinne dessen werden in dieser Arbeit einige Aspekte dieser Prinzipien umgesetzt. Die semi-synthetischen Hydrogele besitzen eine intakte Kohlenhydratkomponente, welche fossile Monomerquellen durch eine nachwachsende Alternative ersetzt.¹¹⁵ Die in dieser Arbeit hergestellten Hydrogele basieren alle auf dem Monomer GVIM-I. Dieses Monomer wird in einer Zwei-Stufen-Synthese aus MeGlu (Abbildung 7, 1) hergestellt und mit unterschiedlichen Vernetzern und Vernetzerkonzentrationen zu Hydrogelen umgewandelt. MeGlu, ein kommerziell erhältlich Glucose-Derivat, wird mithilfe einer angepassten Appel-Reaktion mit einer Ausbeute von 85 % in das Produkt Methyl-6-iodo- α -D-glucopyranosid (MeGlul, Abbildung 7, 2) umgesetzt.^{116,117} Die Methylgruppe an C-1 ist zur Blockierung des anomeren Zentrums notwendig, um die Folgereaktionen nicht zu behindern.¹¹⁶ Im zweiten Schritt wird mithilfe von 1-Vinylimidazol in einer S_N2 -Reaktion das MeGlul mit einer Ausbeute von 83 % in GVIM-I (Abbildung 7, 3) umgesetzt. Dieses Produkt wird nach der Synthese als weiße Kristalle erhalten und die Kristallstruktur wird in **Publikation I**¹¹⁸ dargestellt.

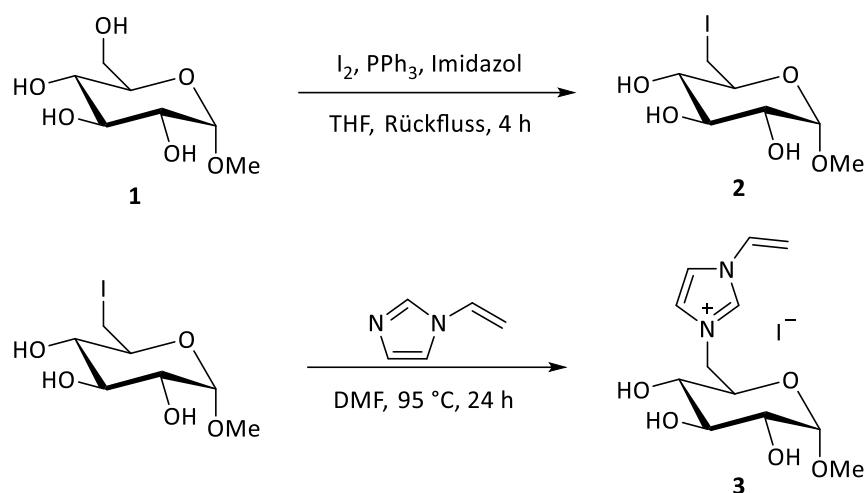


Abbildung 7 Zwei-Stufen-Synthese von GVIM-I.

Diese zwei Stufen zur Synthese von GVIM-I, einem Produkt, das in die Klasse der CHILS zugeordnet werden kann, ist im Vergleich zu anderen IL oder CHILS-Synthesen kurz mit hohen Ausbeuten. Viele CHILS-Synthesen umfassen mehrere Reaktionsschritte, einschließlich die Einführung und Abspaltung von Schutzgruppen. Aufgrund von vielen Synthesestufen werden oft nur geringe Gesamtausbeuten erzielt. In einigen Fällen liegt die Gesamtausbeute bei einer dreistufigen Synthese bei unter 40-50%.¹¹⁹ Die Reduzierung von Zwischenstufen in der Synthese ist ebenfalls ein Aspekt der "12 Prinzipien der grünen Chemie".¹¹⁵ Die in dieser Arbeit verwendete Zwei-Stufen-Synthese erzielt ohne Ein- und Ausbau von Schutzgruppen Gesamtausbeuten von bis zu 71 %.

Neben der Synthese von GVIM-I wurden in **Publikation II** auch GVIM-Br und GVIM-Cl beschrieben. Dabei wurde in Stufe 1 anstatt des Iods Brom (Ausbeute = 70 %), bzw. *N*-Chlorsuccinimid (Ausbeute = 31 %) als Halogenquelle verwendet. In Stufe 2 wurde, wie bei der GVIM-I-Synthese, Vinylimidazol zum halogenierten Glucopyranosid gegeben. Die Reaktionstemperatur der zweiten Stufe musste für Brom und Chlor angepasst werden. Während das GVIM-I bei einer Temperatur von 95 °C mit einer Ausbeute von 83 % hergestellt werden konnte, ergab sich bei der Synthese von GVIM-Br bei 110 °C Reaktionstemperatur eine Ausbeute von 79 % und bei GVIM-Cl bei 150 °C eine Ausbeute von 58 %. Die Produktausbeuten sowohl von Stufe 1 als auch Stufe 2 spiegeln mit $I > Br \gg Cl$ den generellen Trend der Reaktivität der Halogene wider. Durch die milderen Bedingungen und höhere Gesamtausbeute bei der Verwendung von Iod, wurde der Schwerpunkt der Arbeit auf die Herstellung von Hydrogelen mit GVIM-I gelegt.

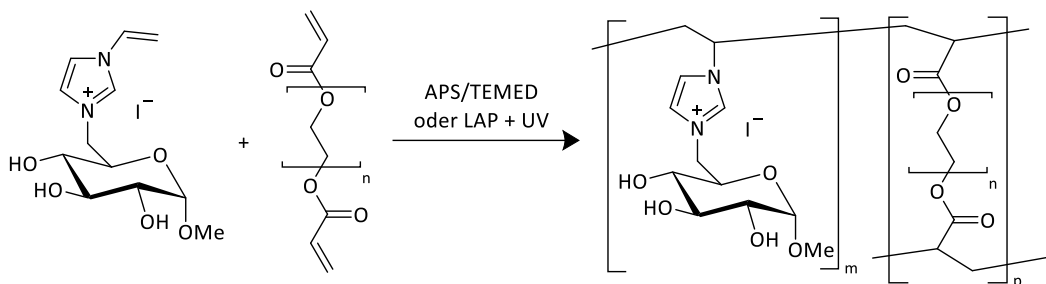


Abbildung 8 Synthese von poly(GVIM-I) mit PEGDA als Vernetzer.

Zur Synthese der Hydrogele (polymerisiertes GVIM-I, poly(GVIM-I)) wurden verschiedene Arten und Konzentrationen von Vernetzern untersucht, um den optimalen Arbeitsbereich für das verwendete GVIM-I Monomer zu finden. Die Hydrogele wurden mittels radikalischer Polymerisation unter Verwendung von APS und TEMED als Initiatorsystem (siehe Abbildung 2) oder LAP als Photoinitiator mit anschließender UV-Bestrahlung hergestellt (Abbildung 8). Es wurden folgende Vernetzer untersucht: Ethylenglycoldiacrylat (EGDA), Polyethylenglycoldiacrylat (PEGDA) mit M_n von 250 g/mol, 575 g/mol und 700 g/mol, *N,N'*-Methylenbisacrylamid (MBAA), Ethylenglycoldimethacrylat (EGDMA) und *N,N'*-(1,2-Dihydroxyethylen)bisacrylamid (DHEBA) (Abbildung 9).

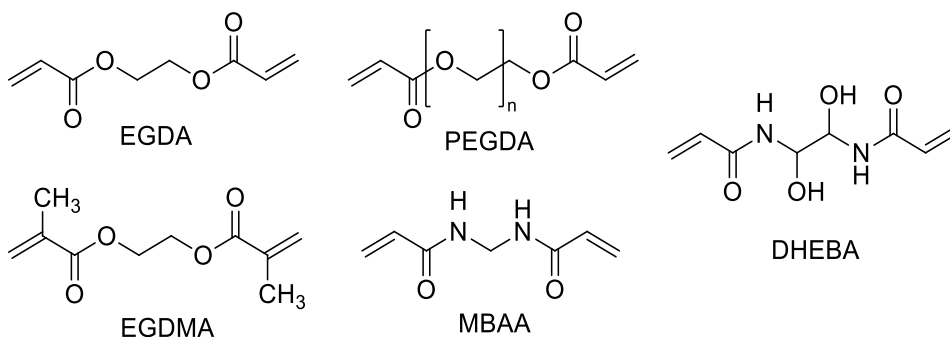


Abbildung 9 Strukturen der verwendeten Vernetzer. Ethylenglycoldiacrylat (EGDA); Poly(ethylenglycoldiacrylat) (PEGDA); Ethylenglycoldimethacrylat (EGDMA); *N,N'*-Methylenbisacrylamid (MBAA); *N,N'*-(1,2-Dihydroxyethylen)bisacrylamid (DHEBA).

EGDA und PEGDA besitzen die gleiche Grundstruktur, unterschieden sich jedoch in ihrer molaren Masse. EGDA ist ein kurzkettiger Vernetzer mit einer molaren Masse von 170,16 g/mol. PEGDA kann, je nach Anzahl sich wiederholender Einheiten von n , molare Massen von 250 bis >20.000 g/mol haben. PEGDA zeigt im vernetzten Zustand eine sehr gute Zellverträglichkeit und Biokompatibilität. Es wird daher oft eingesetzt, wenn Hydrogele im Kontakt zu menschlichem Gewebe stehen (z.B. Gewebezüchtung).^{120,121} In dieser Arbeit wurden drei unterschiedliche PEGDAs miteinander verglichen: PEGDA 250 ($M_n = 250$ g/mol), PEGDA 575 ($M_n = 575$ g/mol) und PEGDA 700 ($M_n = 700$ g/mol). Bei der Verwendung der unterschiedlich langen Vernetzern musste in der Synthese der Hydrogele die Konzentration der Vernetzer jeweils angepasst werden. So wurde EGDA in Konzentrationen von 10 wt% bis 30 wt% in Bezug auf die GVIM-I-Menge untersucht. Bei Konzentrationen unter 10 wt% fand keine Gelierung statt und bei über 30 wt% wurden die Hydrogele zu brüchig für weitere Untersuchungen. PEGDA 250 Hydrogele wurden ebenfalls im Bereich von 10 wt% bis 30 wt% hergestellt. Bei PEGDA 575 war 12,5 wt% die unterste mögliche Konzentration, bei der eine Gelierung stattgefunden hat. Die oberste Grenze liegt hier bei 40 wt%. Bei der Verwendung von EGDMA zeigte sich während der Hydrogelsynthese, dass sich inhomogene, nicht formstabilen Gele bildeten. Dieser Vernetzer wurde daher nicht weiter untersucht.

Zusätzlich zu Diacrylat-Vernetzern wurden die Acrylamid-Vernetzer MBAA und DHEBA untersucht. Bei der Verwendung von MBAA wurden mit 4,5 wt% bis 7,0 wt% deutlich geringere Konzentrationen benötigt. DHEBA, ein strukturell sehr ähnlicher Vernetzer zu MBAA erwies sich als nicht optimal für die Hydrogel-Synthese mit GVIM-I. Die Hydrogelsynthese war nur mit 7,5 wt% und 10 wt% DHEBA möglich und nur schwer zu reproduzieren. Dieses erste Screening unterschiedlichster Vernetzer zeigt, dass nicht alle Vernetzer für das GVIM-I geeignet sind, die (P)EGDAs und MBAA erweisen sich jedoch im ersten Schritt als vielversprechend. Hydrogele mit diesen Vernetzern werden im nächsten Kapitel charakterisiert.

4. Charakterisierung von Hydrogelen

4.1 Spektroskopische Untersuchungen

IR-Spektroskopie ist, wie bereits unter 1.2 beschrieben, eine einfache Möglichkeit um einen ersten Eindruck über die Struktur der Ausgangsstoffe und der Hydrogele zu erhalten. Das Zwischenprodukt MeGlul, das Monomer GVIM-I als auch daraus hergestellte Hydrogele, die nach der Synthese gefriergetrocknet wurden, wurden mit ATR-FTIR-Spektroskopie untersucht. Generell können die wichtigsten Banden des MeGlul (z.B. OH-Schwingung) und des GVIM-I (OH-Schwingung, C-N-Schwingung, C=C-Schwingung) beobachtet werden. Bei der Untersuchung einer Reaktionslösung von GVIM-I gelöst in wässrigem Medium mit Vernetzer und Initiator lassen sich mittels ATR-FTIR die Banden nur schwer zuweisen, da das Wasser relevante Banden überdeckt. Bei der *in situ* Polymerisationsverfolgung der Hydrogelbildung konnte die Abnahme der Intensität der C=C-Doppelbindung in den ersten 7 Minuten des Experimentes aufgezeichnet werden. Es zeigten sich jedoch auch einige Nachteile dieser Methode: Zunächst stören die breiten Banden der Wasser-Schwingungen im Spektrum, da Banden von Interesse überlagert werden. Außerdem veränderten sich während der Messung nicht nur die Intensitäten der C=C-Doppel- und C-C-Einfachschwingungen, sondern auch Banden, die eigentlich eine konstante Intensität haben sollten. Weiterhin zeigte sich in den aufgenommenen Spektren ein starkes Rauschen bei Banden über 2000 cm^{-1} , was der verwendeten Diamantsonde geschuldet ist.

Alternativ bzw. zusätzlich zur IR-Spektroskopie wurde Raman-Spektroskopie zur Charakterisierung des GVIM-I-Monomers und der Hydrogele angewendet. Von dem GVIM-I-Monomer, einer Reaktionslösung zur Herstellung von poly(GVIM-I) mit PEGDA 575 als Vernetzer und von einem gefriergetrockneten und gemörserten Hydrogel wurden Raman-Spektren aufgenommen. Die Unterschiede zwischen GVIM-I und dem poly(GVIM-I)-Hydrogel sind deutlicher zu erkennen als bei den aufgenommenen IR-Spektren. Das Wasser in der Reaktionslösung stört nicht im Spektrum, da Wasser nicht Raman-aktiv ist. Diese Ergebnisse wurden ausführlich in **Publikation III**¹²² und werden im folgenden Kapitel 4.2 diskutiert.

4.2 Rheologie und *in situ* Rheologie-Raman

Ein umfassendes Verständnis der Beziehung zwischen der chemischen Struktur und dem physikalischen Verhalten von Hydrogelen ist notwendig für die Entscheidung der zukünftigen Anwendungen, z.B. zur Nutzung von Hydrogelen im 3D-Druck in der Lebensmittelindustrie oder in der 3D-Zellkultur.^{123,124}

In **Publikation III**¹²² wurde das rheologische Verhalten von poly(GVIM-I)-Hydrogelen mit verschiedenen Vernetzern (siehe Abbildung 9) und mit LAP als Photoinitiator untersucht, indem die Gelierung verfolgt wurde und anschließend Amplituden-Sweep-Messungen durchgeführt wurden. In Abbildung 10 A ist ein typischer Verlauf einer Gelierungsverfolgung zu erkennen. Bei Beginn der Messung ist die UV-Lampe ausgeschaltet (grauer Bereich) und der Wert von G'' ist größer als von G' , da die Reaktionslösung als Flüssigkeit vorliegt. Nach Einschalten der UV-Lampe startet die Gelierung. Dieses ist am Anstieg von

G' zu erkennen. Der Punkt, bei dem $G' = G''$ gilt (Kreuzpunkt, t_c im Diagramm), kennzeichnet den Übergang eines flüssigkeitsähnlichem in ein feststoffähnliches Verhalten. Der Wert von G' erreicht nach ca. 100 s einen Plateauwert, welcher zeigt, dass die Gelierung abgeschlossen ist und wurde mit G'_∞ im Diagramm gekennzeichnet. Die Gelierzeit, der Kreuzpunkt von G' und G'' und der Wert von G'_∞ sind abhängig von der Polymerisationsrate, dem Vernetzungsgrad, sowie der Art und Konzentration des Vernetzers.¹²⁵ Die Abhängigkeit der Vernetzerkonzentration ist in Abbildung 10 C zu erkennen. Je höher die Konzentration des Vernetzers, desto größer ist der Wert von G' und desto steifer ist das Hydrogel. Die Kettenlänge des Vernetzers beeinflusst die Geschwindigkeit der Gelierung und die Steifheit der Hydrogele (Abbildung 10 D). Kurze Ketten wie bei EGDA und PEGDA 250 führen zu weichen Hydrogelen, wohingegen die Verwendung von Vernetzern mit höheren Kettenlängen (PEGDA 575 und PEGDA 700) zu steiferen Gelen führt. Bei der Untersuchung von MBAA fällt auf, dass diese Hydrogele eine längere Gelierungsdauer haben und ihren Plateauwert erst nach ca. 150 s erreichen, und nicht schon nach 100 s wie bei den Diacrylat-Vernetzern.

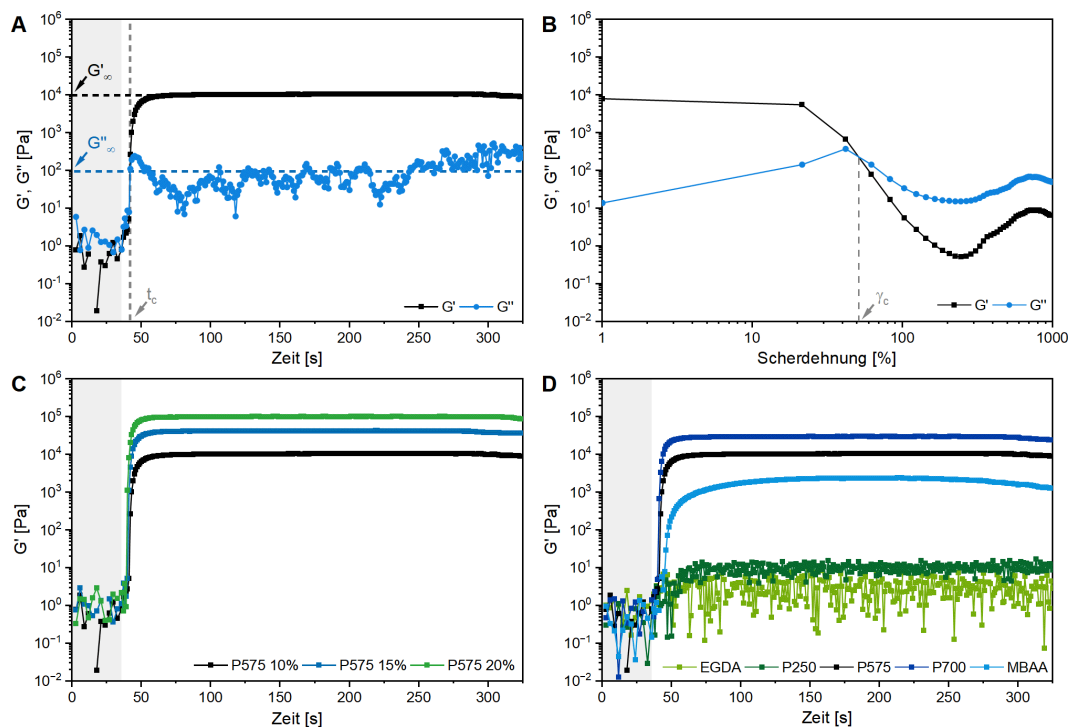


Abbildung 10 Rheologische Untersuchung von poly(GVIM-I) mit (A) Gelierungsverfolgung eines Hydrogels mit PEGDA 575 (10 mol%) als Vernetzer, (B) Amplituden-Sweep-Messung, (C) Gelierungsverfolgung in Abhängigkeit der Vernetzerkonzentration und (D) Gelierungsverfolgung in Abhängigkeit des Vernetzers ($c = 10$ mol%). Bei Beginn der Messung bei A, C und D war die UV-Lampe ausgeschaltet (grau hinterlegter Bereich). Die Messung wurde bei einer Oszillationsfrequenz von 0,1 Hz und bei 37 °C durchgeführt. PEGDA wurde in den Diagrammen aus Platzmangel mit P abgekürzt, $c_{GVIM-I} = 1,25$ mol/L.

Neben der Gelierungsverfolgungen wurden auch Amplituden-Sweep-Experimente durchgeführt. Ein typischer Verlauf von G' und G'' ist in Abbildung 10 B dargestellt. Amplituden-Sweep-Messungen liefern Information zur Steifheit und Formstabilität gegenüber einer angelegten Scherdehnung. Im LVE Bereich verläuft die Verformung der Hydrogele zerstörungsfrei. Wird das Limit dieses Bereiches erreicht, bilden sich Mikrorisse

im Hydrogel, welche bei weiterer Erhöhung der Scherdehnung zu Makrorissen werden, wodurch das Hydrogel sein festkörperähnliches Verhalten verliert. Indiziert ist das bei der Messung durch den Kreuzpunkt von G' und G'' (γ_c im Diagramm), nach welchem das Gel ein flüssigkeitsähnliches Verhalten annimmt. Hydrogele mit einem großen LVE Bereich gelten als stabil gegenüber Scherdehnung und Deformation. Alle untersuchten Hydrogele zeigten einen sehr kleinen LVE Bereich von 1 %. Ausnahme bilden hier die Hydrogele mit EGDA und PEGDA 250 als Vernetzer. Sie erreichen Werte von $48,7 \pm 67,4$ % bzw. $21,4 \pm 16,7$ % und sind somit deutlich stabiler gegenüber Scherstress als die Hydrogele mit PEGDA 575, 700 und MBAA als Vernetzer. Diese Varianz an LVE Bereichen ist auch in der Literatur beschrieben. ZHANG *et al.* stellten selbstheilende Xanthan-basierte Hydrogele her, dessen LVE Bereiche zwischen 24,2 % und 29,8 % lagen.¹²⁶ JASTRAM *et al.* synthetisierte Hydrogele, die ebenfalls eine Vinylimidazol-Einheit beinhalteten und mit MBAA als Vernetzer synthetisiert wurden. Diese Hydrogele wiesen LVE Bereiche zwischen 91 ± 2 % und 92 ± 7 % auf, was deutlich über den Werten der poly(GVIM-I)-Hydrogele liegt.¹²⁷

In dieser Arbeit wurde in Zusammenarbeit mit der Firma Anton Paar eine neue Methode zur Hydrogel-Charakterisierung etabliert. Die Kombination einer *in situ*-Raman-Messung mit der Gelierungsverfolgung am Rheometer wurde bereits zur Charakterisierung von Polymerschmelzen und des Aushärteverhaltens von Epoxidharzen von der Gruppe um VÖLKER-POP erfolgreich angewendet. KIDA *et al.* untersuchten die fließinduzierte Kristallisation von Polyethylen mit Hilfe dieser Methode.^{128,129} Für Hydrogele wurde die *in situ*-Rheologie-Raman-Spektroskopie jedoch von uns in **Publikation III** eingeführt.

Es wurde die Ausbildung eines poly(GVIM-I)-Hydrogels mit PEGDA 575 als Vernetzer und APS/TEMED als Initiatorsystem verfolgt. Mittels Raman-Messung wurde die strukturelle Veränderung, also die Polymerisation, gemessen. Durch die gleichzeitige rheologische Verfolgung konnte die Gelierung, also der Übergang von einer Flüssigkeit in ein Hydrogel, beobachtet werden. In Abbildung 11 A sind die *in situ* Raman-Spektren der Polymerisation über 8 Minuten aufgetragen. Die normalisierte Intensität der Banden bei 1656 cm^{-1} (C=C) nahmen mit der Zeit ab, während die Intensität der Banden bei 980 cm^{-1} (C-C) zunahm. Zur besseren Visualisierung der Intensitätsänderung werden in Abbildung 11 B die Intensitäten der beiden Banden gegen die Zeit dargestellt. Es ist deutlich zu erkennen, dass die Intensität der Bande der C=C-Doppelbindung (schwarz) innerhalb der ersten 200 s stark abnimmt und danach einen stationären Zustand annimmt. Die Intensität der C-C-Einfachbindung (blau) steigt im gleichen Zeitraum erst an und nimmt ebenfalls einen stationären Zustand an. Dies zeigt an, dass das Aufbrechen der C=C-Doppelbindungen zeitgleich mit der Ausbildung der C-C-Einfachbindungen im Polymerrückgrat stattfindet.

Die rheologische Messung, die während der Ausbildung des Hydrogels aufgenommen wurde, ist in Abbildung 11 C dargestellt. G' und G'' wurden gegen die Zeit aufgetragen. Zu Beginn der Aufzeichnung ist G' kleiner als G'' , was bedeutet, dass sich die Reaktionslösung wie eine viskoelastische Flüssigkeit verhält. Der Initiator TEMED wurde direkt vor Beginn der Datenaufnahme zu der Reaktionslösung gegeben. Der Kreuzpunkt von G' und G'' liegt bei 30 s, was für eine sehr schnelle Gelierung spricht. Nach dem Kreuzpunkt steigt der Wert von G' rapide an bis er nach etwa 450 s ein Plateau bei etwa 9700 Pa erreicht. Das Plateau zeigt an, dass die Ausbildung des dreidimensionalen Netzwerks abgeschlossen ist. In

Abbildung 11 D wird der Verlauf von G' der Gelierungsverfolgung mit der Intensität der Schwingung der C-C-Einfachbindung der *in situ*-Raman-Messung verglichen. Beim Übereinanderlegen der beiden Kurven können Schlüsse über die Gelierung und die Polymerisation gezogen werden. Die Gelierung, also der Übergang von einem flüssigkeitsähnlichen zu einem feststoffähnlichen Verhalten, findet demnach bei ca. 30 s statt. An diesem Punkt ist eine Erhöhung der Intensität der C-C-Bande von 0,15 auf 0,25 zu erkennen, was mit einem Umsatz von etwa 33 % der C=C-Doppelbindungen gleichzusetzen ist, wenn die normalisierte Intensität von 0,45 = 100 % gesetzt wird. Das Erreichen eines Plateaus bei der Polymerisation, also der strukturellen Änderung der Probe, liegt bei etwa 300 s. Die Verwendung der *in situ*-Rheologie-Raman-Spektroskopie ermöglicht also eine simultane Gelierungs- und Polymerisationsverfolgung über die Zeit und kann die Unterschiede sichtbar machen.

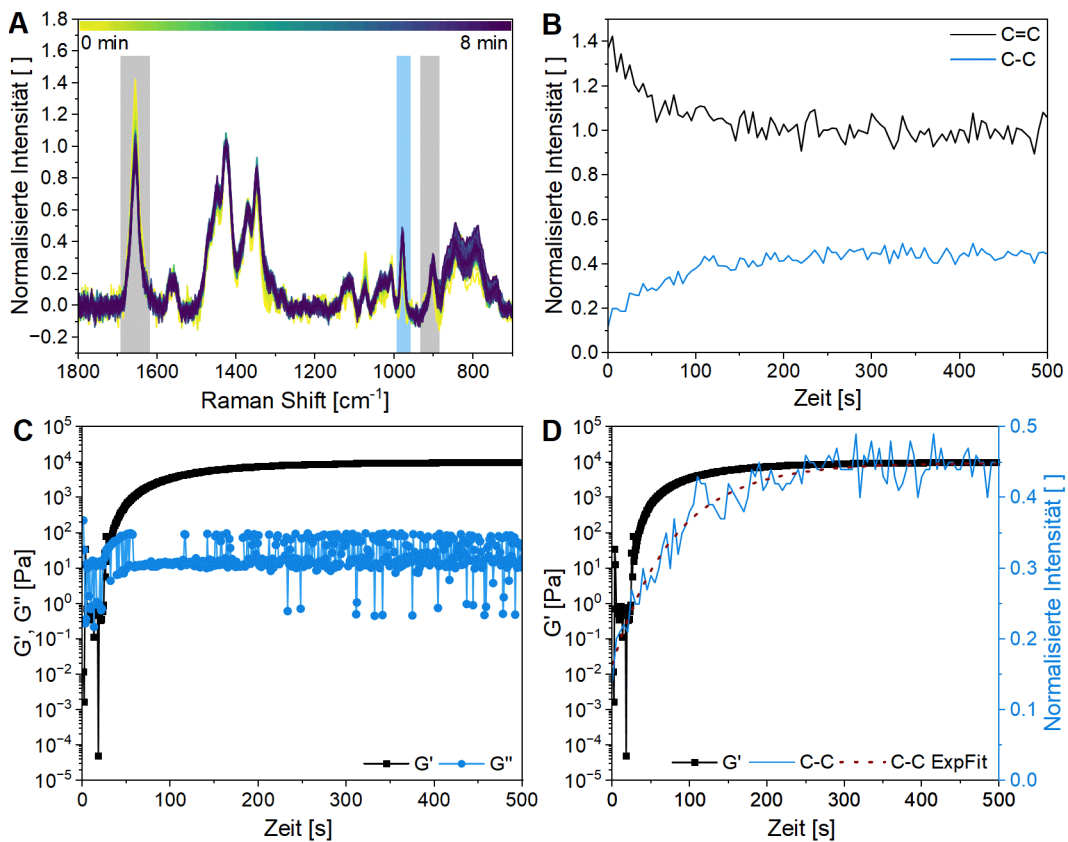


Abbildung 11 *In situ* Rheologie-Raman Untersuchungen mit (A) *in situ* Raman-Spektren bei $\lambda = 785$ nm zur Polymerisationsverfolgung über 8 min mit PEGDA 575 als Vernetzer und APS/TEMED als Initiatorsystem. Grau hinterlegt sind die C=C Streckschwingung (1656 cm⁻¹) und C=C Biegeschwingung (900 cm⁻¹) und blau hinterlegt sind die C-C-Streckschwingung (980 cm⁻¹). Die Spektren wurden auf die 1428 cm⁻¹ Bande normiert. (B) Zeitliche Verfolgung des C=C-Verbrauchs (Höhe der Bande bei 1656 cm⁻¹, schwarz) und der C-C-Bildung (Höhe der Bande bei 980 cm⁻¹, blau) während der Polymerisation, entsprechend den *in situ* Raman-Spektren von A, (C) Gelierungsverfolgung von Poly(GVIM-I) mit PEGDA 575 als Vernetzer und APS/TEMED als Initiatorsystem und (D) Überlagerung von G' während der Gelierung und der C-C-Bildung während der Polymerisation (approximiert mit einem exponentiellen Fit in rot) der *in situ* Rheologie-Raman-Spektroskopie.

4.3 Quellungseigenschaften

Der Quellungsgrad von Hydrogelen ist eine Kerneigenschaft und kann gravimetrisch mit Formel 2 ermittelt werden. In dieser Arbeit wurde der Quellungsgrad der Hydrogele in wässrigen Medien untersucht, wobei hauptsächlich phosphatgepufferte Salzlösung (phosphate buffered saline, PBS) bei einem pH-Wert von 7,4 verwendet wurde. Die Pufferwirkung von PBS garantiert einen stabilen pH-Wert und ist durch die Ionen vergleichbar mit der Osmolarität des menschlichen Gewebes, was für spätere Anwendungen im menschlichen Körper relevant ist.¹³⁰

Die Quellung von Hydrogelen besteht aus zwei verschiedenen Transportprozessen. Zunächst strömt das Medium, in dem die Quellung stattfindet, durch die äußeren Poren des Hydrogels. Anschließend diffundiert das Medium durch die Verstrebungen des Polymernetzwerkes. Die Quellung von Hydrogelen wird neben Parametern wie der Bewegung des Mediums und dessen Wechselwirkung mit dem Polymernetzwerk, aber vor allem durch die Art des Monomers, dessen Konzentration und der Struktur, Kettenlänge und Menge des Vernetzers beeinflusst.^{74,75}

Zur Bestimmung des Quellungsgrades wurden die Hydrogele nach der Synthese getrocknet und anschließend wurde ihre Massenzunahme in temperierter PBS-Lösung zeitlich und gravimetrisch verfolgt. Die Quellung der Hydrogele verläuft in den ersten 60 Minuten schnell. Das Hydrogel nimmt in dieser Zeit den Hauptanteil an Wasser auf. Die Quellung wird insgesamt 225 min verfolgt. Nach insgesamt 19 h werden nochmals zwei Messwerte aufgenommen, um die Gleichgewichtseinstellung zu überprüfen (Abbildung 12). Der Quellungsgrad wurde nach Formel 2 berechnet.

Der Quellungsgrad ist von der Vernetzer-Konzentration abhängig. In Abbildung 12 A-E ist zu erkennen, dass der Quellungsgrad in der Regel mit steigender Vernetzer-Konzentration abnimmt. Je höher die Vernetzer-Konzentration ist, desto kleiner sind die Lücken im Polymernetzwerk. Daher kann weniger Medium aufgenommen werden.¹³¹ Die Kettenlänge der Diacrylat-Vernetzer hat nur einen geringen Einfluss auf den Quellungsgrad. Das EGDA-Hydrogel mit 25 wt% hat einen Quellungsgrad im Gleichgewicht von $0,81 \pm 0,07$, während der höchste Quellungsgrad bei PEGDA 250 mit 10 wt% bei $2,97 \pm 0,35$ liegt. Die Quellungsgrade der MBAA-Hydrogele sind von allen untersuchten Vernetzern am niedrigsten und erreichen Werte zwischen $0,40 \pm 0,08$ bei 6,5 wt% im Gleichgewicht und $0,67 \pm 0,08$ bzw. $0,67 \pm 0,03$ bei 4,5 wt% bzw. 5,0 wt% im Gleichgewicht (Abbildung 12 E). Bei den MBAA-Hydrogelen verlaufen die Quellungskurven deutlich anders als bei den Diacrylat-Hydrogelen. Zunächst erreichen die Hydrogele eine maximale Quellung und verlieren anschließend wieder an Gewicht. Dieses Phänomen kann mit dem Ionenaustausch des Iodid-Anions aus der Polymerstruktur mit den Chlorid-Ionen aus dem PBS erklärt und mithilfe von Ionenchromatographie bewiesen werden. Mit Quellungsgraden zwischen 0,5 und 3 gelten diese Hydrogele nach der Einteilung nach FENG und WANG als nicht quellende Hydrogele (mit $Q = 0,5$ bis $1,5$) und quellende Hydrogele (mit $Q > 1,5$).⁷⁸

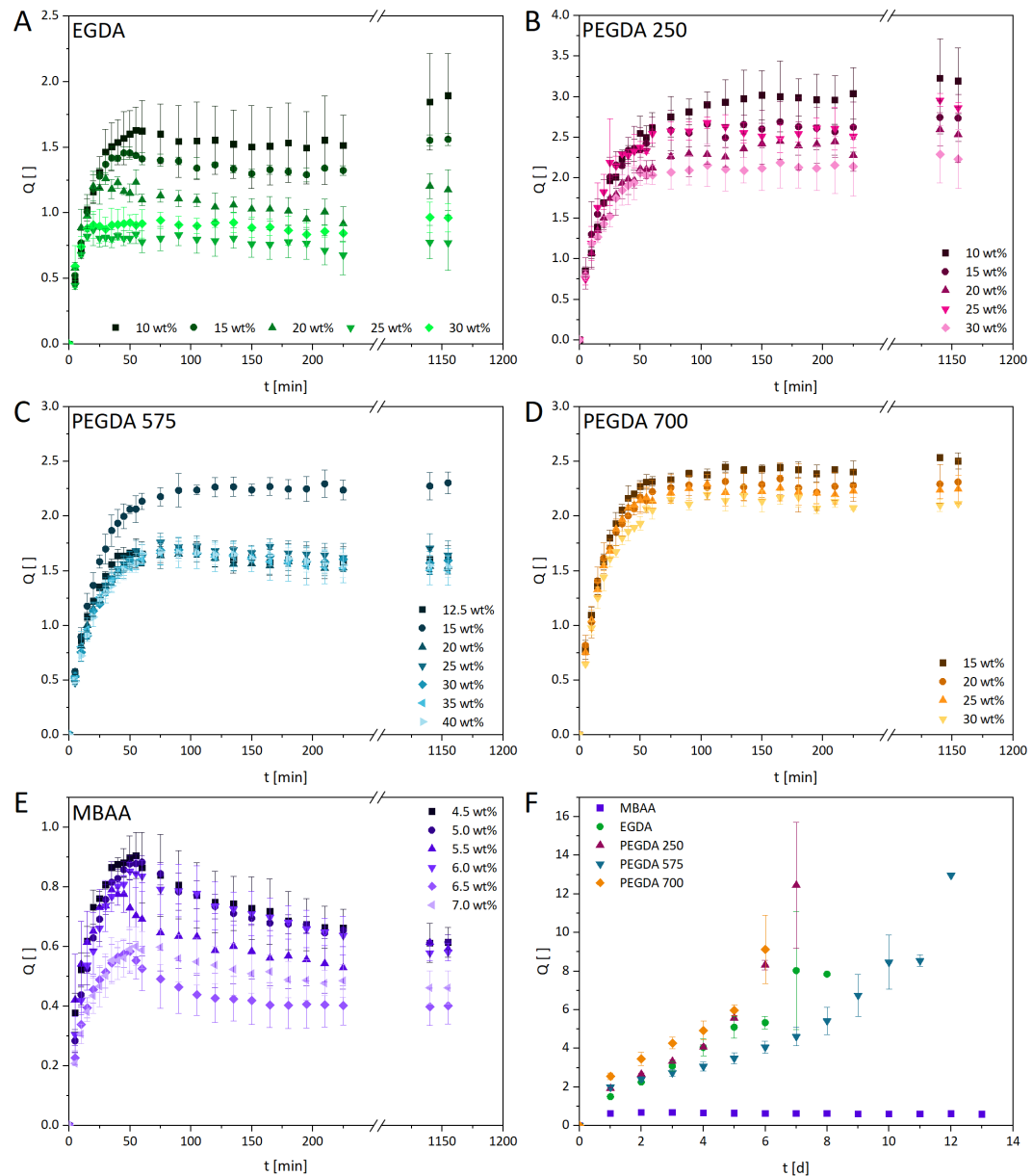


Abbildung 12 Quellung von poly(GVIM-I)-Hydrogelen in PBS (pH 7,4) bei $37\text{ °C} \pm 2\text{ °C}$ mit den Vernetzern (A) EGDA, (B) PEGDA 250, (C) PEGDA 575, (D) PEGDA 700 und (E) MBAA mit jeweils unterschiedlichen Konzentrationen in Gewichtsprozent im Bezug zur Monomermasse. (F) Langzeitquellung von poly(GVIM-I)-Hydrogelen mit unterschiedlichen Vernetzern mit den Konzentrationen von 5 wt% bei MBAA und 15 wt% bei EGDA und PEGDA.

Für die Verwendung von Hydrogelen ist nicht nur die Quellung über einen kurzen Zeitraum, sondern auch ihr Verhalten über mehrere Tage bis Wochen interessant. Deshalb wurde von jedem Vernetzer eine Konzentration gewählt (5 wt% bei MBAA und 15 wt% bei den Diacrylat-Vernetzern) und Quellungskurven in PBS (pH = 7,4, $37\text{ °C} \pm 1\text{ °C}$) über 13 Tage aufgenommen (Abbildung 12 F). Dabei ist interessant, dass die Hydrogele mit MBAA als Vernetzer als einzige über den gesamten Zeitraum beständig sind, ihre Form behalten und nicht degradieren. Sie erreichten ihren maximalen Quellungsgrad nach 24 h mit $0,63 \pm 0,05$. Die Hydrogele mit PEGDA 700 degradierten als erste nach 7 Tagen. Nach 8 bzw. 9 Tagen folgten die Hydrogele mit PEGDA 250 und EGDA und nach insgesamt 13 Tagen waren die Hydrogele mit PEGDA 575 vollständig degradiert. Vor der vollständigen

Degradation stieg der Quellungsgrad immer weiter an und die Hydrogele verloren ihre Form und Formstabilität. Diese Ergebnisse konnten in **Publikation II**⁵⁹ erfolgreich dargestellt und diskutiert werden.

4.4 Antimikrobielle Eigenschaften

In **Publikation IV**¹³² wurden verschiedene poly(GVIM-I)-Hydrogele auf ihre antimikrobiellen Eigenschaften gegenüber den Bakterien *Bacillus subtilis* (*B. subtilis*) und *E. coli*, sowie gegenüber dem Hefepilz *C. auris* untersucht und es wurde die *in vitro* Biokompatibilität der Hydrogele an L929-Mausfibroblasten getestet.

Eine antimikrobielle Aktivität von Biomaterialien bedeutet, dass sie das Wachstum von Mikroorganismen wie Bakterien und Hefepilzen hindern oder diese sogar abtöten können. Diese Eigenschaft ist von großer Bedeutung, da viele pathogene Bakterien bereits Resistenzen gegen die gängigen Antibiotika entwickelt haben.⁹¹ In dieser Arbeit wurden zwei typische Vertreter für Infektionen verursachende Bakterien verwendet: *B. subtilis* (grampositiv) und *E. coli* (gramnegativ, siehe Abbildung 4). *B. subtilis* ist ein stäbchenförmiges, begeißeltes Bodenbakterium, das fakultativ anaerob wächst und Sporen bildet.¹³³ *B. subtilis* wurde 2023 von der "Vereinigung für Allgemeine und Angewandte Mikrobiologie" zur Mikrobe des Jahres gekürt, da es viele positive Eigenschaften für den Menschen haben kann. Es wird als Antibiotika-Alternative in der Tierhaltung eingesetzt, da es eine probiotische Wirkung zeigt oder in der Bauwirtschaft zur Selbstheilung von Beton.^{134,135} Es kann aber auch, bei bereits immungeschwächten Menschen, für schwere Infektionen im Auge sorgen und zur Blindheit führen. *B. subtilis* bildet außerdem das Toxin Amyloisin, welches zu Lebensmittelvergiftungen führen oder bei wiederholter Aussetzung zu Allergien führen kann.^{136,137} *B. subtilis* gilt als am besten erforschtes grampositives Bakterium und ist ein weit verbreiteter Modellorganismus.

E. coli ist ein stäbchenförmiges, peritrich begeißeltes Bakterium, das im menschlichen Darm vorkommt und fakultativ anaerob ist. Es ist ein Modellorganismus und wird in der Gentechnik zur Klonierung von DNA-Fragmenten verwendet. Es existiert ein Spektrum von Darmerkrankungen bei Menschen und Tieren, die von pathogenen *E. coli* verursacht werden. Dazu gehört das enterohämorrhagische *E. coli*, das besser als "EHEC" bekannt ist und für Durchfall, Übelkeit und Erbrechen sorgt, teils mit schweren Verläufen, und in Einzelfällen auch zum Tod führen kann.¹³⁸

Nicht nur Bakterien können Infektionskrankheiten verursachen, sondern auch Hefepilze. Der in dieser Arbeit verwendete Hefepilz ist *C. auris* ist eine hefeartig wachsende Pilzart, die bei immungeschwächten Menschen Candidiasis verursachen kann, welche sogar tödlich verlaufen kann. Infektionen mit *C. auris* sind meldepflichtig und werden vor allem in Krankenhäuser von Patient zu Patient übertragen.¹³⁹

Die vermehrt auftretenden Resistenzen der Mikroorganismen gegen die gängigen Antibiotika und Antimykotika machen die Entwicklung von Alternativen notwendig. Neue Materialien, wie polyILs, hergestellt mit Vinylbutylimidazoliumchlorid und –bromid als Monomer und mit MBAA vernetzt, zeigten bereits vielversprechende antibakterielle Aktivitäten.¹⁴⁰

Die poly(GVIM-I)-Hydrogele dieser Arbeit zeigten im Plattendiffusionstest nach KIRBY und BAUER keine Wachstumsinhibierung der Bakterien *B. subtilis* und *E. coli* (Abbildung 13 A). Es ist lediglich ein weißer Kreis um die Hydrogelproben erkennbar. Der hohe Wasseranteil der Hydrogele erweicht vermutlich das Agar-Medium und lässt es aufquellen (Abbildung 13 B). Den einzigen Inhibierungseffekt zeigten die Hydrogele gegen den Hefepilz *C. auris*. Eine mögliche Erklärung ist der unterschiedliche Aufbau von Bakterien- und Hefepilzzellen. Hefepilze (Eukaryoten) besitzen im Vergleich zu Bakterien (Prokaryoten) kein zusätzliches Peptidoglykan in der Zellwand (siehe Abbildung 4 und Abbildung 5).¹¹⁰ Viele antibakterielle Wirkstoffe hemmen die Bildung von Peptidoglykan, dem wesentlichen Bestandteil der bakteriellen Zellwand. Die meisten Antimykotika wirken jedoch auf die Funktion oder Bildung von Ergosterol, welches ein wichtiger Bestandteil der Pilzzellmembran ist.¹⁴¹ Die Wechselwirkung des positiv geladenen Imidazoliums im poly(GVIM-I) mit der negativ geladenen Pilzmembran könnte diese beschädigen und so das Wachstum inhibieren.^{142,143}

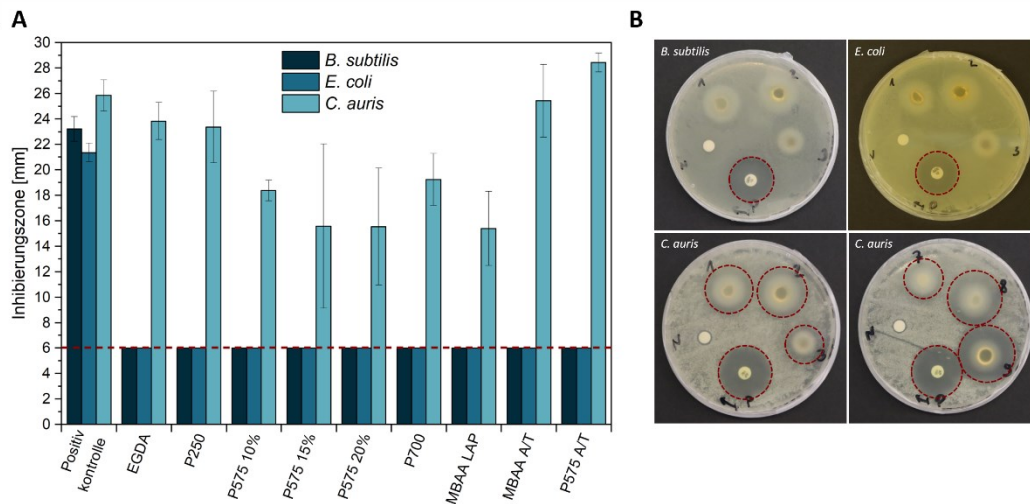


Abbildung 13 Antibakterielle Untersuchung von Hydrogelen gegen *B. subtilis*, *E. coli* und *C. auris* (WT) mittels Plattendiffusionstest mit (A) Inhibierungszonen (ZOI) (in mm, einschließlich der 6 mm Durchmesser der Proben). Alle Proben wurden im Triplikat angefertigt. Die rot gestrichelte Linie zeigt die Probengröße an, sodass nur eine ZOI über 6 mm eine antimikrobielle Aktivität indiziert. (B) Repräsentative Agarplatten, welche die durch Hydrogele und Antibiotika gebildete ZOI zeigt (rot gestrichelte Kreise) mit (1) EGDA, (2) PEGDA 250, (3) PEGDA 575 (10 mol%), (7) MBAA LAP, (8) MBAA APS/TEMED, (9) P575 APS/TEMED, (P) positive Kontrolle (Gentamicin oder Amphotericin B) und (N) negative Kontrolle (LB Medium).

Die Inhibierungszone (Zone of Inhibition, ZOI) von *C. auris* war bei den Hydrogelen mit den Vernetzern EGDA, MBAA APS/TEMED und PEGDA APS/TEMED mit $23,83 \pm 1,45$ mm, $25,43 \pm 2,85$ mm bzw. $28,45 \pm 0,74$ mm am größten. Die Proben PEGDA 575 15 %, PEGDA 575 20 % und PEGDA 700 zeigten die geringste Wachstumshemmung mit einem ZOI von $15,59 \pm 6,46$ mm, $15,54 \pm 4,60$ mm bzw. $15,40 \pm 2,90$ mm. Bei den Hydrogelen, welche mit LAP als Photoinitiator hergestellt wurden, zeigte die Verwendung der kurzkettigen Vernetzer EGDA und PEGDA 250 eine größere Wachstumsinhibierung als die Verwendung von langkettigen Vernetzern. Eine Erklärung wäre ein unvollständiger Umsatz von Monomer und Vernetzer, wodurch es bei der Inkubation der Gele mit den Bakterien zum

Herauslösen nicht reagierter Edukte kommt. Durch ihre toxischen Eigenschaften könnten diese das Wachstum von *C. auris* inhibieren.¹⁴⁴ Die beiden letzten Proben in Abbildung 13 A wurden mit APS und TEMED als Initiatorsystem hergestellt. Ein Vergleich der beiden Proben mit ihren Äquivalenten, die mit dem Photoinitiator LAP (P575 10 % und MBAA LAP) hergestellt wurden, zeigt, dass die LAP-Proben einen deutlich geringeren ZOI verursachen ($18,38 \pm 0,81$ mm und $15,40 \pm 2,90$ mm). Die Wahl des Vernetzers und Initiatorsystems beeinflusst demnach die Stärke der Wachstumsinhibierung.

4.5 *In vitro* Biokompatibilität

Eine gängige Methode zur Untersuchung der *in vitro* Biokompatibilität ist der bereits in Kapitel 1.2 erwähnte Eluattest. Hierbei wurden poly(GVIM-I)-Hydrogele nach DIN EN ISO 10993-12 in Zellkulturmedium für 72 h inkubiert. Das Eluat wurde dann in vier unterschiedlichen Konzentrationen (100 %, 10 %, 1 % und 0,1 %) in Kontakt zu L929-Mausfibroblasten gebracht und für 48 h inkubiert. Die potentielle Zytotoxizität wurde durch die Verwendung des Cell Titer Blue (CTB) Zellviabilität-Assays ermittelt (Abbildung 14). Das Assay ergab, dass alle getesteten Proben bei einer 100 %igen Eluatkonzentration zytotoxisch sind. Die 100 %ige Konzentration entspricht der unverdünnten Stammlösung der Hydrogeleluate. Es kann generell gesagt werden, dass die relative Zellviabilität mit abnehmender Eluatkonzentration steigt. Beim Vergleich der vier unterschiedlichen molaren Massen von (P)EGDA-Vernetzern (Abbildung 14 A) wird deutlich, dass EGDA und PEGDA 250 bei 10 %iger Eluatkonzentration ebenso starke zytotoxische Effekte zeigen wie bei 100 %. In Gegensatz dazu liegt die relative Zellviabilität von PEGDA 575 bei 10 % Eluatkonzentration bei über 70 %, weshalb es als biokompatibel beschrieben werden kann. Bei 1 % Eluatkonzentration zeigt PEGDA 700 eine ähnlich hohe Zellviabilität wie PEGDA 575. Die zytotoxische Wirkung der EGDA- und PEGDA 250-Eluate kann von dem unvollständigen Umsatz der Edukte bei der Hydrogelsynthese stammen. Nicht abreagierter Vernetzer, Vernetzer- oder Initiatorradikale wurden durch Inkubieren in Zellkulturmedium (Dulbecco's Modified Eagle's Medium, DMEM) aus den Hydrogelen gewaschen und können Zellschäden verursachen, was sich negativ auf die Zellviabilität auswirken kann.^{26,145,146}

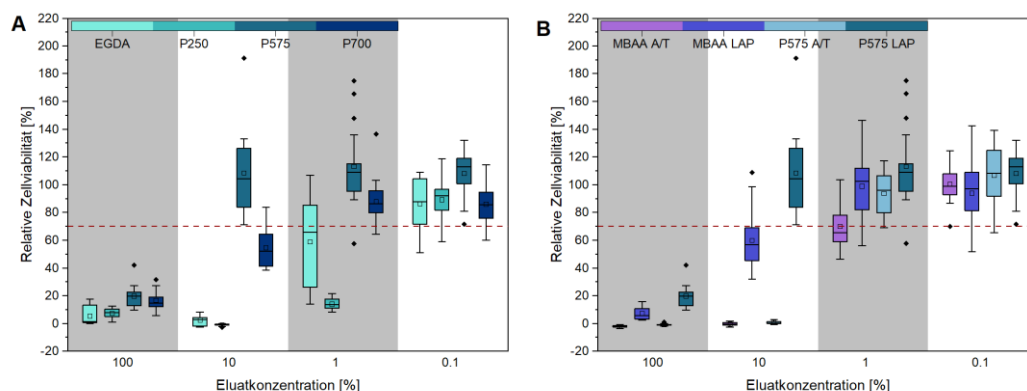


Abbildung 14 Eluattest mit relativen Zellviabilitäten von L929-Zellen nach Kultivierung für 48 h in verschiedenen konzentrierten (100 %, 10 %, 1 % und 0,1 %) Hydrogeleluaten. (A) Vergleich unterschiedlicher molaren Massen von (P)EGDAs ($M_{EGDA} = 170$ g/mol, $M_{n(P250)} = 250$ g/mol, $M_{n(P575)} = 575$ g/mol, $M_{n(P700)} = 700$ g/mol) und (B) Vergleich der Vernetzer MBAA und PEGDA 575

mit LAP bzw. APS/TEMED als Initiator. Es wurden drei biologische mit jeweils 6 technischen Replikaten angefertigt (außer bei P575 (10 %), hier wurden nur 2 biologische Replikate dargestellt). Die rot gestrichelte Linie zeigt die Grenze der Biokompatibilität an.

Der Einfluss von zwei verschiedenen Initiatorsystemen (LAP und APS/TEMED) wird in Abbildung 14 B dargestellt. Schon bei 100 %iger Eluatkonzentration zeigen die Proben mit LAP eine etwas höhere relative Zellviabilität (MBAA = $7,30 \pm 4,35$ %, PEGDA 575 = $20,00 \pm 7,69$ %) als die Proben mit APS/TEMED (MBAA = $1,90 \pm 0,86$ %, PEGDA 575 = $-0,71 \pm 0,83$ %). Dieser Effekt wird bei 10 %iger Eluatkonzentration noch deutlicher. Die MBAA-Hydrogeleluate mit LAP resultieren in einer relativen Zellviabilität von $60,09 \pm 21,23$ %, wohingegen die Werte für APS/TEMED bei $-0,15 \pm 1,08$ % liegen. Der gleiche Effekt ist bei den PEGDA 575-Proben zu sehen. Die LAP-Proben haben eine relative Zellviabilität von $108,60 \pm 33,31$ % und die APS/TEMED-Proben lediglich eine von $0,80 \pm 0,93$ %. Je geringer die Eluatkonzentration ist, desto geringer ist auch der Unterschied zwischen den verwendeten Initiatorsystemen. Aus den Ergebnissen des CTB-Assays kann geschlossen werden, dass die Verwendung von LAP der von APS/TEMED bei Zellkontakt-Anwendungen zu bevorzugen ist. Außerdem kann aus den Ergebnissen geschlossen werden, dass sowohl die Wahl des Vernetzers als auch des Initiatorsystems einen signifikanten Einfluss auf die relativen Zellviabilitäten hat.

Im Vergleich der antibakteriellen Untersuchungen und des Eluattests zeigen sich zwei Trends. Die Verwendung von APS/TEMED führt im Allgemeinen zu zytotoxischen Effekten gegenüber *C. auris* und den L929-Zellen und dass die Verwendung von langkettigen Vernetzern (PEGDA 575 und PEGDA 700) zu generell weniger zytotoxischen Hydrogelen führt als die Verwendung von kurzkettigen (EGDA und PEGDA 250).

Der Eluattest gibt einen ersten Eindruck über die Zytotoxizität der untersuchten Hydrogele. Es zeigt den Einfluss der aus den Hydrogelen herausgetretenen Komponenten während der Inkubation auf L929-Zellen. Um herauszufinden, wie sich diese Zellen im direkten Kontakt mit den Hydrogelen verhalten, wurden mit der vielversprechendsten Probe, PEGDA 575 mit LAP, Direktkontakttests durchgeführt.

Tabelle 1 Behandlung von PEGDA 575 Hydrogelproben für den Direktkontakttest.

Nummer	Behandlung
1	Keine Behandlung
2	Waschen in DMEM für 72 h
3	Waschen in DMEM für 3x24 h
4	Waschen in EtOH (70 %) für 24 h + waschen in DMEM für 2x24 h
5	UV-Bestrahlung für 1 h
6	UV-Bestrahlung für 1 h + waschen in DMEM für 3x24 h
7	UV-Bestrahlung für 1 h + waschen in EtOH für 24 h + waschen in DMEM für 3x24 h

Die Hydrogele wurden hergestellt und anschließend unterschiedlich behandelt (Tabelle 1), bevor sie auf die Zellen appliziert und zusammen für 48 h inkubiert wurden. Anschließend wurde ein CTB-Assay durchgeführt (Abbildung 15).

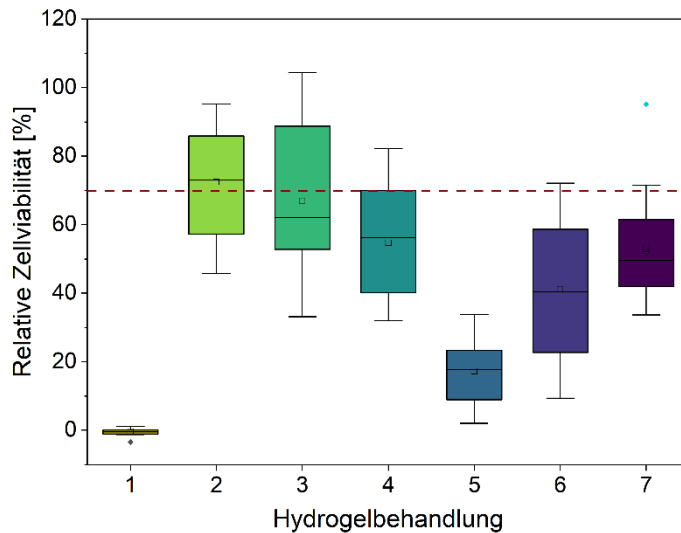


Abbildung 15 Direktkontakttest mit relativen Zellviabilitäten von L929-Zellen nach Kultivierung in direktem Kontakt zu unterschiedlich behandelten Hydrogelen mit (1) unbehandelt, (2) waschen in DMEM 72 h, (3) waschen in DMEM 3x24 h, (4) waschen in Ethanol (70 %) 24 h und in DMEM 2x24 h, (5) UV-Bestrahlung für 1 h, (6) UV-Bestrahlung für 1 h und waschen in DMEM 3x24 h und (7) UV-Bestrahlung für 1 h, waschen in Ethanol (70 %) 24 h und in DMEM 3x24 h. Es wurden drei biologische mit jeweils 6 technischen Replikaten angefertigt. Die rot gestrichelte Linie zeigt die Grenze der Biokompatibilität an.

In Abbildung 15 wird deutlich, dass unbehandelte Hydrogele (Nummer 1) bei L929-Zellen den Zelltod verursachen, da hier die relative Zellviabilität bei $-0,46 \pm 0,99 \%$ liegt. Jede Behandlung resultierte in einer höheren relativen Zellviabilität als keine Behandlung. Am effektivsten erwies sich das Waschen in DMEM. Bei einmaligem Waschen für 72 h (Nummer 2) lag die Viabilität bei $72,38 \pm 15,93 \%$. Wurde alle 24 h das Medium gewechselt (Nummer 3), lag sie bei $66,87 \pm 20,86 \%$. Der zusätzliche Schritt, die Proben für 24 h in 70 %igem Ethanol zu waschen (Nummer 4) führte nicht zu höheren Zellviabilitäten, sondern reduzierte sie auf $54,74 \pm 15,97 \%$. Das alleinige Bestrahlen der Proben mit UV-Licht für eine Stunde (Nummer 5) erwies sich als am ineffektivsten. Die relative Zellviabilität lag hier bei $17,20 \pm 9,50 \%$. Diese Behandlung wurde gewählt, um eine möglichst hohe Umwandlung von Monomer und Vernetzer zu erzielen, so dass möglichst wenig nicht reagierte Edukte zurückbleiben, da diese je nach Konzentration herausleachen könnten und zytotoxische Effekte zeigen.⁴⁴ Bei der Kombination von UV-Bestrahlung mit Waschen in DMEM (Nummer 6) bzw. mit Waschen in Ethanol und DMEM (Nummer 7) wurden höhere Viabilitäten erzielt, jedoch lagen diese unterhalb der Biokompatibilitätsgrenze (rot gestrichelte Linie in Abbildung 15).

5. Zusammenfassung und Ausblick

In dieser Dissertation wurde zunächst in einer Zwei-Stufen-Synthese ein neuartiges Kohlenhydrat-basiertes ionisches Monomer hergestellt, welches in die Klasse der CHLS eingeordnet werden kann. Daraus wurden im Anschluss semi-synthetische, ionische Hydrogele mit unterschiedlichen Arten und Konzentrationen an Vernetzern synthetisiert. Die Eigenschaften der Hydrogele wurden im dritten Schritt untersucht, wobei die Quellung in PBS, die rheologischen und antimikrobiellen Eigenschaften und die Biokompatibilität zur Charakterisierung genutzt wurden. Die Ergebnisse der verschiedenen Schritte wurden erfolgreich im Rahmen mehrerer wissenschaftlichen Publikationen veröffentlicht.

Publikation I zeigt die Kristallstruktur von GVIM-I mit Dimethylformamid (DMF). Dieses Produkt war der Ausgangsstoff für die Hydrogele, welche in den nächsten Publikationen charakterisiert wurden. Die Kristallstruktur von GVIM-I beweist die typische Sesselstruktur des Glucopyranosids und die Anbindung des positiv geladenen Imidazolrings an den Zucker in Position 6, sowie das erwartete Iodid-Anion.

In **Publikation II** wurde zunächst herausgefunden, dass Iodid durch seine höhere Reaktivität zu höheren Ausbeuten bei der MeGlu-X-Synthese und der anschließenden GVIM-X-Synthese führt, während Bromid und Chlorid geringere Ausbeuten bei härteren Bedingungen liefern. Im Hauptteil von **Publikation II** wurden mit GVIM-I neuartige, semi-synthetische, ionische Hydrogele durch radikalische Polymerisation hergestellt und auf ihre Quellungseigenschaften untersucht. Dabei wurden sowohl Diacrylat- als auch Acrylamid-Vernetzer in unterschiedlichen Konzentrationen zur Synthese verwendet, um den optimalen Arbeitsbereich für das GVIM-I-Monomer zu finden. Die Charakterisierung erfolgte durch die gravimetrische Aufnahme von Quellungskurven. Dabei konnte ein Zusammenhang zwischen der Konzentration des Vernetzers und dem Quellungsgrad beobachtet werden. Je höher die Konzentration ist, desto geringer ist der Quellungsgrad. Der Quellungsgrad von MBAA-Hydrogelen konnte von $0,47 \pm 0,05$ auf $0,67 \pm 0,08$ erhöht werden, indem die Vernetzerkonzentration von 7,0 wt% auf 4,5 wt% herabgesenkt wurde. Das Vernetzer-Screening konnte außerdem zeigen, dass die Verwendung von DHEBA und EGDMA zur Hydrogelsynthese mit dem GVIM-I-Monomer nicht geeignet ist. In einer Langzeituntersuchung zeigte sich, dass die MBAA-Hydrogele im Vergleich mit allen anderen getesteten Vernetzern als einzige über 13 Tage ihre Form behalten und nicht degradieren. Die Untersuchung der Quellungseigenschaften zeigte, dass die beiden verschiedenen Typen an Vernetzern unterschiedlich optimale Arbeitsbereiche bei der Hydrogelsynthese aufweisen. Bei MBAA liegt der Arbeitsbereich zwischen 4,5 wt% und 7,0 wt%, bei den Diacrylaten zwischen 10 wt% und 30 wt% in Bezug auf die Monomermasse.

Die Hydrogelsynthese selbst wurde in **Publikation III** genauer untersucht. Dazu wurde der Polymerisationsprozess mit *in situ* IR- und Raman-Spektroskopie und der Gelierungsprozess mittels Rheometer verfolgt. Die Verwendung von *in situ* IR-Spektroskopie erwies sich als nicht geeignet für wässrige Proben. Die Raman-Messungen waren allerdings erfolgreich, da Banden von Interesse nicht von Raman-inaktiven Wasserbanden

überlagert wurden. Bei der Gelierungsverfolgung mittels Rheometer wurden die Proben mit dem Photoinitiator LAP angesetzt. Dieser ermöglicht einen gesteuerten Start der Gelierung indem die UV-Quelle eingeschaltet wird. Durch die zeitliche Verfolgung von G' und G'' konnte gezeigt werden, dass die Hydrogele eine relativ kurze Gelierungszeit ($37,8 \pm 1,8$ s bei PEGDA 700 bis $47,2 \pm 5,9$ s bei PEGDA 250) haben und die Steifheit der Hydrogele mit der Kettenlänge der Vernetzer korreliert. Je länger die Ketten (je höher die molare Masse), desto steifer sind die Hydrogele. PEGDA 250 hat Werte von G' von $13,1 \pm 1,9$ Pa, während PEGDA 700 Werte von $29.2567 \pm 125,0$ Pa erreicht. Außerdem wurde deutlich, dass die Steifigkeit der Hydrogele bei steigender Vernetzerkonzentration zunimmt. Hydrogele mit einer Konzentration von 10 mol% PEGDA 575 hatten G' -Werte von $10.116,7 \pm 88,1$ Pa, während bei 20 mol% die Werte bei $97.766,7 \pm 436,5$ Pa lagen. Die LVE Bereiche aller Hydrogele (außer EGDA und PEGDA 250) lag lediglich bei 1 %, weshalb es für diese Gele für eine höhere mechanische Stabilität weiterer Optimierung bedarf.

Durch die Zusammenarbeit mit der Firma ANTON PAAR OPTOTEC GmbH gelang es, eine neue Methode zur Hydrogel-Charakterisierung zu entwickeln und in **Publikation III** zu veröffentlichen. Die *in situ* Rheologie-Raman-Spektroskopie bietet Vorteile für eine effektive Hydrogelcharakterisierung. Die Polymerisation wurde mit einer *in situ* Raman-Messung durch zeitliche Verfolgung der Intensität der C=C-Doppelbindung und der C-C-Einfachbindung untersucht. Gleichzeitig konnte durch die Gelierungsverfolgung mittels Rheometer die zeitliche Änderung von G' und G'' getrackt werden. Dies bietet eine Zeitersparnis und kann Unterschiede zwischen Gelierung und Polymerisation deutlich machen.

Eine mögliche Anwendung für die GVIM-I-Hydrogele wäre der Einsatz als Wirkstofffreisetzungssystem. Die ionische Komponente durch die positive Ladung am Imidazol kann hier zu einer vielversprechenden Bindung von negativ geladenen Wirkstoffen führen. Damit das realisierbar ist, wurden die antimikrobiellen Eigenschaften und die Biokompatibilität der Hydrogele in **Publikation IV** untersucht. Die GVIM-I-Hydrogele wurden mittels Plattendiffusionstests, dem allgemein akzeptierten Standard dieser Methoden, auf ihre antimikrobiellen Eigenschaften gegen *B. subtilis*, *E. coli* und *C. auris* untersucht. Die Hydrogele zeigten keine wachstumshemmenden Effekte gegen *B. subtilis* und *E. coli.*, jedoch eine starke Hemmung des Wachstums von *C. auris*. Die Stärke der Hemmung war von der Wahl des Vernetzers und des Initiatorsystems abhängig. Die Verwendung von APS/TEMED und die Nutzung von kurzkettigen Vernetzern wie EGDA und PEGDA 250 ergab eine stärkere Hemmung als die Verwendung von LAP und langkettigen Vernetzern.

Für die Untersuchung der *in vitro* Biokompatibilität wurden zunächst Eluattests durchgeführt. Diese zeigten, dass auch hier die Wahl des Vernetzers und des Initiatorsystems einen großen Einfluss auf die Zellviabilität haben. Kurzkettige Vernetzer wie EGDA und PEGDA 250 führen zu sehr geringen Viabilitäten bei 10 %iger Eluatkonzentration von $2,59 \pm 3,42$ % bzw. $-0,76 \pm 0,83$ %. Längerkettige Vernetzer wie PEGDA 575 und PEGDA 700 weisen bei gleicher Eluatkonzentration Viabilitäten von $108,60 \pm 33,31$ % bzw. $54,77 \pm 12,96$ % auf. Die Eluate von PEGDA 575 erwiesen sich bei Konzentrationen <10 % als biokompatibel. Die Verwendung von LAP ist der von

APS/TEMED klar zu bevorzugen. Die relativen Zellviabilitäten konnten durch die Verwendung von LAP bei 10 %iger Eluatkonzentration um 60,2 % bei MBAA und um 32,5 % bei PEGDA 575 erhöht werden. Hydrogele mit LAP und PEGDA 575 erwiesen sich bei den Eluattests als am vielversprechendsten und wurden für einen Direktkontakttest ausgewählt.

Das Ziel der Direktkontaktversuche war eine Hydrogel-Behandlung zu finden, welche zu einer höheren relativen Zellviabilität führt und im besten Fall biokompatible Hydrogele liefert. Die poly(GVIM-I)-Hydrogele hergestellt mit PEGDA 575 und LAP wurden unterschiedlich behandelt, bevor sie in den direkten Kontakt zu L929-Zellen gebracht wurden. Das Waschen in DMEM für 1x72 h und 3x24 h erwies sich mit relativen Zellviabilitäten von $72,38 \pm 15,93$ % bzw. $66,87 \pm 20,86$ % am effektivsten. Es kann geschlussfolgert werden, dass die Behandlung mit DMEM zu biokompatiblen Hydrogelen führt.

Das Wissen, das durch die Quellungs-, Rheologie- und Biokompatibilitätsstudien erlangt wurde, hilft dabei mögliche Anwendungen für die GVIM-I-Hydrogele zu finden. Die Quellungsstudien ergaben Quellungsgrade von 0,5 bis 3, und in DMEM gewaschene Hydrogele zeigten gute Zellviabilitäten im direkten Kontakt zu L929-Zellen. Dies kann der Ausgangspunkt zur Entwicklung der Hydrogele für eine Anwendung beim Tissue Engineering oder als Wirkstofffreisetzungssystem sein.⁷⁸ Die ionische Ladung im poly(GVIM-I) ist vorteilhaft für eine Vermeidung eines anfänglichen "Burst-Release", da negativ geladene Wirkstoffe (z.B. Ibuprofen-Natrium) ionisch mit dem Hydrogel wechselwirken.⁸¹ Die ionische Ladung im Hydrogel sorgt außerdem dafür, dass das Hydrogel pH-sensitiv ist. Hierzu sollte in zukünftigen Arbeiten geforscht werden, da auch eine pH-abhängige Wirkstofffreisetzung von großem Interesse in der Biomedizin ist.^{147,148}

Eine andere Möglichkeit, die poly(GVIM-I)-Hydrogele zu verwenden, könnte die Anwendung in biokatalytischen Reaktionen sein. Frühere Studien zeigten, wie vielversprechend die Unterstützung von Novozym 435, einer immobilisierten Lipase B von *Candida antarctica* (CalB), mit CHILS wie 1-(Methyl- α -D-glucopyranosid-6-yl)-3-methylimidazoliumiodid (GMIM-I) ist, welches strukturell sehr ähnlich zu GVIM-I ist.⁵² Die CHILS-gestützten Novozym 435-Biokatalysatoren wurden in einer Modellreaktion (Veresterung von Acrylsäure mit *n*-Butanol) mit nicht gestütztem Novozym 435 verglichen. Ohne die CHILS-Unterstützung wurde eine *n*-Butylacrylat-Ausbeute von 55 % erreicht, wohingegen die Ausbeute mit GMIM-I-Unterstützung zu einer Ausbeute von 67 % geführt hat. Darüber hinaus erreichten die GMIM-I-gestützten Novozym 435-Biokatalysatoren eine höhere Langlebigkeit und Rezyklierbarkeit als die ungestützten. Es wäre daher interessant, die Vorzüge des poly(GVIM-I) als Immobilisierungsmatrix für CalB zu testen. Die Immobilisierung von CalB wurde bereits erfolgreich mit poly(ILs) durchgeführt, welche ebenfalls eine Vinylimidazol-Einheit besaßen. Hier wurde die Enzymaktivität von CalB durch die Immobilisierung erhöht.¹⁴⁹ Dieses Verfahren könnte auf die neuen poly(GVIM-I)-Hydrogele übertragen werden.

6. Literaturverzeichnis

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A Anhang: Originalpublikationen

Originalpublikationen:

I Publikation I, Full Paper:

S. Lambrecht, A. Villinger, S. Jopp

1-(Methyl- α -D-glucopyranosid-6-yl)-3-vinylimidazolium iodide dimethylformamide monosolvate

IUCrData **2022**, 7, x220265.

DOI: <https://doi.org/10.1107/S2414314622002656>

Das Konzept der Synthese wurde von S. Jopp erarbeitet. Die Synthesen wurden von S. Lambrecht durchgeführt. Die Kristallstruktur wurde von A. Villinger durchgeführt. Der Manuskriptentwurf wurde von S. Jopp verfasst und anschließend von S. Lambrecht und A. Villinger überarbeitet.

II Publikation II, Full Paper:

S. Lambrecht, H. Schröter, H. Pohle, S. Jopp

Swelling Behavior of Novel Hydrogels Produced from Glucose-Based Ionic Monomers with Varying Cross-Linkers

ACS Omega **2024**, 9, 5418-5428.

DOI: <https://doi.org/10.1021/acsomega.3c06804>

Das Konzept der Studie wurde von S. Lambrecht erarbeitet. Sämtliche experimentelle Arbeiten wurden von S. Lambrecht, H. Schröter und H. Pohle durchgeführt. Die Ergebnisse wurden von S. Lambrecht ausgewertet. Der Manuskriptentwurf wurde von S. Lambrecht verfasst, von H. Schröter und H. Pohle kommentiert und anschließend von S. Jopp überarbeitet.

III Publikation III, Full Paper:

S. Lambrecht, M. Biermann, S. Kara, S. Jopp, J. Meyer

A novel characterisation technique for hydrogels – in situ Rheology-Raman spectroscopy for gelation and polymerization tracking

RSC Materials Advances **2024**, 5, 6957-6966.

DOI: [10.1039/d4ma00543k](https://doi.org/10.1039/d4ma00543k)

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IV Publikation IV, Full Paper:

S. Lambrecht, A. Gazizova, S. Kara, S. Jopp, J. Meyer

Antimicrobial properties and biocompatibility of semi-synthetic carbohydrate-based ionic hydrogels

RSC Advances **2024**, 14, 30719-30731.

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I. Publikation I

Full Paper *IUCRData* **2022**, 7, x220265.

**1-(Methyl- α -D-glucofuranosid-6-yl)-3-vinylimidazolium iodide dimethylformamide
monosolvate**

S. Lambrecht, A. Villinger, S. Jopp

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1-(Methyl- α -D-glucopyranosid-6-yl)-3-vinylimidazolium iodide dimethylformamide monosolvate

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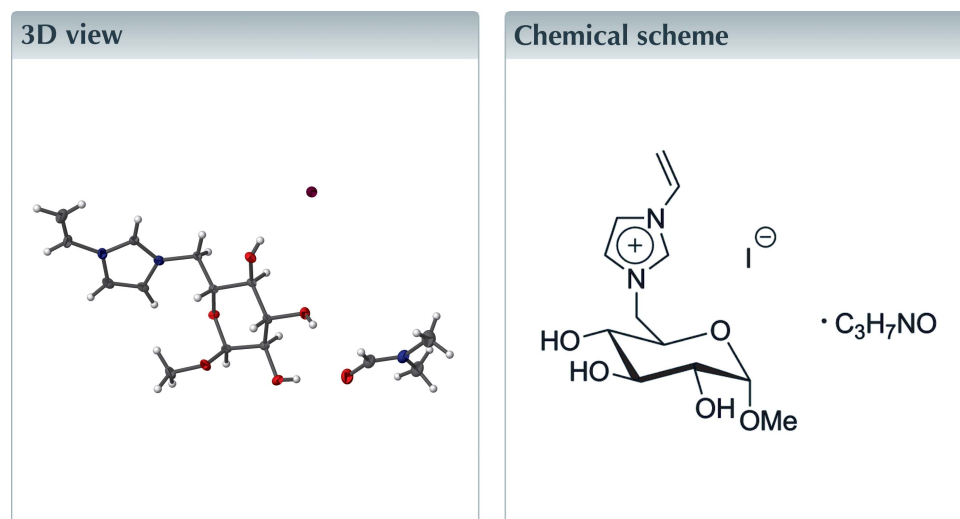
Edited by L. Van Meervelt, Katholieke Universiteit Leuven, Belgium

Keywords: crystal structure; carbohydrate; imidazolium.

CCDC reference: 2157239

Structural data: full structural data are available from iucrdata.iucr.org

The title solvated molecular salt, [MeGluVIm]I (MeGluVIm = 1-(methyl- α -D-glucopyranosid-6-yl)-3-vinylimidazolium), or $C_{12}H_{19}N_2O_5^+ \cdot I^- \cdot C_3H_7NO$, was synthesized from methyl- α -D-6-iodoglucopyranoside and vinylimidazole in DMF. It crystallizes through precipitation from ethyl acetate solution directly after the reaction procedure. The crystal structure consists of an iodide anion and a [MeGluVIm] cation. Furthermore, the crystal structure contains one molecule of DMF, which accepts two O—H...H hydrogen bonds from the OH groups of the glucopyranoside.



Structure description

[MeGluVIm]I is part of a sub-category of ionic liquids, called carbohydrate-based ionic liquids (CHILs; Jopp, 2020). These molecules are defined as ionic organic compounds in which either the cation or the anion consists of an intact carbohydrate moiety. Our group has recently discovered a straightforward synthetic strategy for CHILs, in which methyl- α -D-glucopyranoside is transformed into methyl- α -D-6-iodoglucopyranoside in the first step (Skaanderup *et al.*, 2002) and then in the second step quarternized with an *N*-substituted imidazole of choice to achieve a carbohydrate-based ionic liquid (Schnegas & Jopp, 2021). The title compound [MeGluVIm]I contains a vinylimidazolium ring bound to atom C6 of the glucopyranoside. Fig. 1 shows the asymmetric unit, including one molecule of dimethylformamide, which was used as the reaction solvent. The title compound crystallizes in a monoclinic unit cell. The crystal structure contains three classical hydrogen bonds and additional C—H...O/I interactions (Table 1). One hydrogen bond is formed between O3—H3A of the glucopyranoside and O7 of DMF

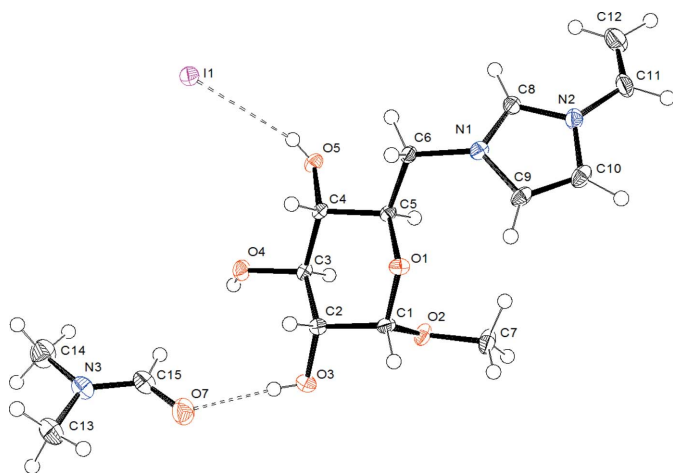


Figure 1
Molecular structure of the title compound. Displacement ellipsoids correspond to 50% probability.

with an H···H length of 2.09 (4) Å. Two additional hydrogen bonds exists between the [MeGluVIm] cation and the iodide anion, which are O4—H4A···I1 with 2.71 (5) Å and O5—H5A···I1 with 2.75 (5) Å. Fig. 2 gives an alternative view of the cation, indicating the distinctive chair conformation of the glucopyranoside as well as the overall stereochemistry of the compound. The configurations of the stereogenic centres in the chosen cation are *S* (C1), *R* (C2), *S* (C3), *S* (C4) and *R* (C5).

Synthesis and crystallization

Methyl-6-iodo- α -D-glucopyranoside (1.824 g; 6 mmol) and 1-vinylimidazole (0.821 g; 10 mmol) were dissolved in DMF (10 ml) and stirred at 95°C for 24 h. After cooling down, ethyl acetate (80 ml) was added and the flask was stored in a fridge

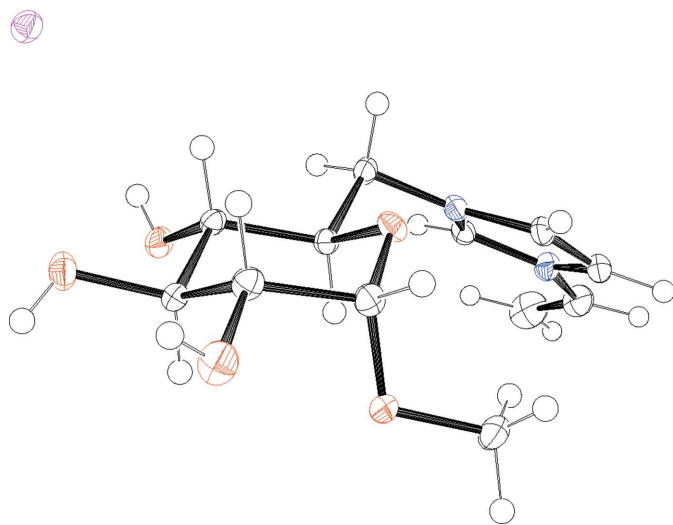


Figure 2
Molecular structure of the title compound. Displacement ellipsoids correspond to 50% probability. The DMF was removed for a clear view of the chair conformation.

Table 1
Hydrogen-bond geometry (Å, °).

<i>D</i> —H··· <i>A</i>	<i>D</i> —H	H··· <i>A</i>	<i>D</i> ··· <i>A</i>	<i>D</i> —H··· <i>A</i>
O3—H3A···O7	0.72 (4)	2.09 (4)	2.797 (4)	167 (5)
O4—H4A···I1 ⁱ	0.78 (5)	2.71 (5)	3.482 (3)	171 (4)
O5—H5A···I1	0.74 (5)	2.75 (5)	3.474 (3)	165 (4)
C6—H6A···O5 ⁱⁱ	0.99	2.46	3.332 (4)	147
C8—H8···O4 ⁱⁱ	0.95	2.44	3.252 (4)	143
C8—H8···O5 ⁱⁱ	0.95	2.53	3.285 (4)	136
C9—H9···O3 ⁱⁱⁱ	0.95	2.51	3.404 (5)	156
C10—H10···O7 ⁱⁱⁱ	0.95	2.40	3.159 (5)	137
C11—H11···I1 ^{iv}	0.95	3.02	3.925 (3)	161
C15—H15···O4	0.95	2.58	3.297 (5)	132

Symmetry codes: (i) $x, y - 1, z$; (ii) $-x, y + \frac{1}{2}, -z + 1$; (iii) $-x + 1, y + \frac{1}{2}, -z + 1$; (iv) $x, y, z - 1$.

Table 2
Experimental details.

Crystal data	
Chemical formula	C ₁₂ H ₁₉ N ₂ O ₅ ⁺ ·I ⁻ ·C ₃ H ₇ NO
<i>M_r</i>	471.29
Crystal system, space group	Monoclinic, <i>P</i> 2 ₁
Temperature (K)	123
<i>a</i> , <i>b</i> , <i>c</i> (Å)	10.816 (2), 7.0106 (15), 13.169 (3)
β (°)	106.833 (4)
<i>V</i> (Å ³)	955.7 (3)
<i>Z</i>	2
Radiation type	Mo <i>K</i> α
μ (mm ⁻¹)	1.71
Crystal size (mm)	0.29 × 0.08 × 0.03
Data collection	
Diffractometer	Bruker Kappa APEXII CCD
Absorption correction	Multi-scan (<i>SADABS</i> ; Bruker, 2003)
<i>T_{min}</i> , <i>T_{max}</i>	0.629, 0.746
No. of measured, independent and observed [<i>I</i> > 2 σ (<i>I</i>)] reflections	17430, 6072, 5626
<i>R_{int}</i>	0.038
(<i>sin</i> θ / λ) _{max} (Å ⁻¹)	0.725
Refinement	
$R[F^2 > 2\sigma(F^2)]$, $wR(F^2)$, <i>S</i>	0.028, 0.060, 1.03
No. of reflections	6072
No. of parameters	242
No. of restraints	1
H-atom treatment	H atoms treated by a mixture of independent and constrained refinement
$\Delta\rho_{\max}$, $\Delta\rho_{\min}$ (e Å ⁻³)	1.42, -0.44
Absolute structure	Refined as an inversion twin, 2815 Friedel pairs.
Absolute structure parameter	0.006 (19)

Computer programs: *APEX2* and *SAINT* (Bruker, 2003), *SHELXTL* (Sheldrick, 2008), *SHELXL2014/7* (Sheldrick, 2015) and *ORTEP-3 for Windows* (Farrugia, 2012).

overnight. The solvent was decanted and the precipitated solid was washed with ethyl acetate (3 × 40 ml) and dried under high vacuum to achieve the product as a beige solid (1.752 g; yield 73%). Single crystals of the compound were formed during the precipitation (m.p.: 448–453 K; *T_d*: 509 K).

¹H NMR (300 MHz, D₂O): δ = 3.21–3.30 (*m*, 3H, OCH₃); 3.58 (*dd*, 1H, ³*J* = 9.77, ³*J* = 3.77, H-2); 3.66–3.75 (*m*, 1H); 3.95 (*dd*, 1H, ³*J* = 6.3, ³*J* = 3.72); 4.50 (*dd*, 1H, ³*J* = 14.55, ³*J* = 7.38, H-6a); 4.70 (*dd*, 1H, ³*J* = 14.55, ³*J* = 2.55, H-6 b); 4.85 (*d*, 1H, ³*J* = 3.77, H-1); 5.49 (*dd*, 1H, ³*J* = 8.68, ³*J* = 2.84, vinyl-CH);

5.86 (*dd*, 1H, $^3J = 15.58$, $^3J = 2.85$, vinyl-CH₂ - *a*); 7.2 (*dd*, 1H, $^3J = 15.58$, $^3J = 8.70$, vinyl-CH₂ - *b*); 7.70 (*d*, 1H, $^3J = 2.0$, H_{Ar}); 7.86 (*d*, 1H, $^3J = 2.0$, H_{Ar}); 9.16 (*s*, 1H).

¹³C NMR (300 MHz, D₂O): $\delta_{\text{m}} = 36.9$ (NCH); 50.2 (C-6); 55.1 (OCH₃); 69.2, 40.5, 71.0, 72.8 (C-2, C-3, C-4, C-5); 99.3 (C-1); 109.8 (CH₂); 119.4, 123.8, 128.1 (CH_{Ar}).

HRMS (ESI, *m/z*): calculated for C₁₂H₁₉N₂O₅⁺, 271.1299; measured 271.1306. Calculated for I⁻, 126.9040; measured 126.9045.

Refinement

Crystal data, data collection and structure refinement details are summarized in Table 2. The crystal studied was refined as a two-component inversion twin.

Funding information

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full crystallographic data

IUCrData (2022). 7, x220265 [https://doi.org/10.1107/S2414314622002656]

1-(Methyl- α -D-glucopyranosid-6-yl)-3-vinylimidazolium iodide dimethylformamide monosolvate

Sina Lambrecht, Alexander Villinger and Stefan Jopp

3-Ethenyl-1-(methyl- α -D-glucopyranosid-6-yl)imidazolium iodide dimethylformamide monosolvate

Crystal data

$C_{12}H_{19}N_2O_5^+ \cdot I^- \cdot C_3H_7NO$

$M_r = 471.29$

Monoclinic, $P2_1$

$a = 10.816$ (2) Å

$b = 7.0106$ (15) Å

$c = 13.169$ (3) Å

$\beta = 106.833$ (4)°

$V = 955.7$ (3) Å³

$Z = 2$

$F(000) = 476$

$D_x = 1.638$ Mg m⁻³

Mo $K\alpha$ radiation, $\lambda = 0.71073$ Å

Cell parameters from 7185 reflections

$\theta = 3.2$ – 31.1 °

$\mu = 1.71$ mm⁻¹

$T = 123$ K

Needle, colourless

$0.29 \times 0.08 \times 0.03$ mm

Data collection

Bruker Kappa APEXII CCD
diffractometer

Radiation source: sealed tube

Detector resolution: 10.4167 pixels mm⁻¹

phi and ω scans

Absorption correction: multi-scan
(SADABS; Bruker, 2003)

$T_{\min} = 0.629$, $T_{\max} = 0.746$

17430 measured reflections

6072 independent reflections

5626 reflections with $I > 2\sigma(I)$

$R_{\text{int}} = 0.038$

$\theta_{\max} = 31.0$ °, $\theta_{\min} = 3.8$ °

$h = -15 \rightarrow 15$

$k = -10 \rightarrow 10$

$l = -19 \rightarrow 18$

Refinement

Refinement on F^2

Least-squares matrix: full

$R[F^2 > 2\sigma(F^2)] = 0.028$

$wR(F^2) = 0.060$

$S = 1.03$

6072 reflections

242 parameters

1 restraint

Hydrogen site location: mixed

H atoms treated by a mixture of independent
and constrained refinement

$w = 1/[\sigma^2(F_o^2) + (0.0231P)^2]$

where $P = (F_o^2 + 2F_c^2)/3$

$(\Delta/\sigma)_{\max} = 0.001$

$\Delta\rho_{\max} = 1.42$ e Å⁻³

$\Delta\rho_{\min} = -0.44$ e Å⁻³

Absolute structure: Refined as an inversion
twin, 2815 Friedel pairs.

Absolute structure parameter: 0.006 (19)

Special details

Geometry. All esds (except the esd in the dihedral angle between two l.s. planes) are estimated using the full covariance matrix. The cell esds are taken into account individually in the estimation of esds in distances, angles and torsion angles; correlations between esds in cell parameters are only used when they are defined by crystal symmetry. An approximate (isotropic) treatment of cell esds is used for estimating esds involving l.s. planes.

Refinement. All H atoms were positioned geometrically and refined using a riding model, with C—H = 0.98 (methyl groups), 0.99 Å (methylene groups), 1.00 Å (methine groups) or 0.95 Å (aryl CH) and with $U_{\text{iso}}(\text{H}) = 1.5$ times $U_{\text{eq}}(\text{C})$ (methyl groups) or with $U_{\text{iso}}(\text{H}) = 1.2$ times $U_{\text{eq}}(\text{C})$ (methylene groups, aryl CH, methine groups). Torsion angles of all methyl groups were allowed to refine.

Refinement of F^2 against ALL reflections. The weighted R-factor wR and goodness of fit S are based on F^2 , conventional R-factors R are based on F, with F set to zero for negative F^2 . The threshold expression of $F^2 > 2\sigma(F^2)$ is used only for calculating R-factors(gt) etc. and is not relevant to the choice of reflections for refinement. R-factors based on F^2 are statistically about twice as large as those based on F, and R-factors based on ALL data will be even larger.

Refined as a two-component inversion twin.

Fractional atomic coordinates and isotropic or equivalent isotropic displacement parameters (\AA^2)

	<i>x</i>	<i>y</i>	<i>z</i>	$U_{\text{iso}}^*/U_{\text{eq}}$
N1	0.1863 (3)	1.0859 (4)	0.3364 (2)	0.0151 (5)
N2	0.1065 (3)	1.1296 (4)	0.1676 (2)	0.0179 (6)
O1	0.3511 (2)	0.8245 (4)	0.4934 (2)	0.0146 (5)
O2	0.3182 (3)	0.5032 (4)	0.4445 (2)	0.0184 (5)
O3	0.4060 (3)	0.3939 (4)	0.6541 (3)	0.0222 (6)
O4	0.2120 (3)	0.5948 (4)	0.7282 (2)	0.0197 (5)
O5	0.0572 (2)	0.8579 (4)	0.5786 (2)	0.0169 (5)
C1	0.3882 (3)	0.6322 (5)	0.5202 (3)	0.0150 (7)
H1	0.4821	0.6180	0.5263	0.018*
C2	0.3673 (3)	0.5847 (5)	0.6269 (3)	0.0156 (6)
H2	0.4240	0.6703	0.6816	0.019*
C3	0.2266 (3)	0.6217 (5)	0.6246 (3)	0.0137 (6)
H3	0.1679	0.5327	0.5733	0.016*
C4	0.1910 (3)	0.8273 (5)	0.5914 (3)	0.0129 (6)
H4	0.2438	0.9163	0.6464	0.015*
C5	0.2174 (3)	0.8639 (4)	0.4853 (3)	0.0123 (6)
H5	0.1607	0.7793	0.4299	0.015*
C6	0.1951 (3)	1.0688 (4)	0.4498 (3)	0.0139 (6)
H6A	0.1141	1.1156	0.4618	0.017*
H6B	0.2671	1.1487	0.4921	0.017*
C7	0.3439 (4)	0.5229 (5)	0.3446 (3)	0.0238 (8)
H7A	0.3086	0.4128	0.2997	0.036*
H7B	0.4374	0.5295	0.3558	0.036*
H7C	0.3033	0.6400	0.3097	0.036*
C8	0.0831 (3)	1.1454 (4)	0.2617 (3)	0.0153 (7)
H8	0.0055	1.1915	0.2729	0.018*
C9	0.2788 (3)	1.0274 (5)	0.2893 (3)	0.0174 (7)
H9	0.3618	0.9775	0.3244	0.021*
C10	0.2286 (4)	1.0547 (5)	0.1843 (3)	0.0200 (7)
H10	0.2698	1.0273	0.1313	0.024*
C11	0.0197 (3)	1.1684 (9)	0.0656 (3)	0.0238 (7)
H11	0.0524	1.1636	0.0060	0.029*
C12	−0.1014 (4)	1.2099 (7)	0.0494 (3)	0.0322 (11)
H12A	−0.1368	1.2158	0.1075	0.039*
H12B	−0.1547	1.2344	−0.0205	0.039*
H3A	0.426 (4)	0.391 (7)	0.711 (3)	0.014 (12)*

H4A	0.186 (4)	0.495 (7)	0.740 (4)	0.025 (12)*
H5A	0.051 (4)	0.940 (7)	0.612 (4)	0.018 (12)*
I1	0.06251 (2)	1.18116 (3)	0.77975 (2)	0.02003 (6)
N3	0.4772 (3)	0.4326 (5)	1.0319 (3)	0.0235 (7)
O7	0.5214 (3)	0.4185 (5)	0.8731 (2)	0.0306 (7)
C13	0.6115 (5)	0.4439 (8)	1.0952 (4)	0.0328 (10)
H13A	0.6663	0.4637	1.0485	0.049*
H13B	0.6363	0.3249	1.1349	0.049*
H13C	0.6225	0.5508	1.1450	0.049*
C14	0.3817 (4)	0.4304 (7)	1.0890 (4)	0.0341 (10)
H14A	0.3828	0.5529	1.1250	0.051*
H14B	0.4016	0.3275	1.1416	0.051*
H14C	0.2960	0.4092	1.0391	0.051*
C15	0.4445 (5)	0.4205 (6)	0.9273 (4)	0.0241 (8)
H15	0.3550	0.4126	0.8906	0.029*

Atomic displacement parameters (\AA^2)

	U^{11}	U^{22}	U^{33}	U^{12}	U^{13}	U^{23}
N1	0.0161 (13)	0.0129 (12)	0.0185 (14)	-0.0016 (10)	0.0086 (11)	-0.0002 (11)
N2	0.0226 (14)	0.0170 (14)	0.0156 (13)	-0.0010 (10)	0.0079 (11)	0.0006 (10)
O1	0.0115 (12)	0.0128 (12)	0.0203 (13)	-0.0008 (9)	0.0061 (10)	0.0012 (10)
O2	0.0274 (14)	0.0139 (12)	0.0178 (13)	-0.0039 (10)	0.0125 (11)	-0.0033 (11)
O3	0.0272 (15)	0.0191 (12)	0.0205 (14)	0.0079 (10)	0.0072 (12)	0.0051 (11)
O4	0.0252 (13)	0.0200 (12)	0.0170 (12)	0.0001 (10)	0.0109 (10)	0.0032 (10)
O5	0.0146 (12)	0.0199 (12)	0.0185 (12)	0.0001 (9)	0.0081 (10)	-0.0027 (10)
C1	0.0138 (14)	0.0141 (17)	0.0181 (15)	0.0027 (10)	0.0064 (12)	0.0005 (11)
C2	0.0163 (15)	0.0147 (15)	0.0158 (15)	0.0024 (11)	0.0049 (12)	0.0002 (12)
C3	0.0156 (15)	0.0140 (13)	0.0132 (14)	-0.0010 (11)	0.0065 (12)	-0.0021 (11)
C4	0.0130 (14)	0.0130 (14)	0.0135 (15)	0.0004 (11)	0.0052 (12)	-0.0024 (13)
C5	0.0108 (14)	0.0131 (13)	0.0137 (15)	-0.0002 (10)	0.0047 (12)	-0.0014 (12)
C6	0.0170 (15)	0.0132 (13)	0.0135 (15)	0.0008 (11)	0.0075 (12)	-0.0004 (12)
C7	0.038 (2)	0.0196 (17)	0.0184 (17)	-0.0027 (15)	0.0156 (16)	-0.0031 (14)
C8	0.0205 (14)	0.011 (2)	0.0169 (14)	-0.0008 (10)	0.0085 (11)	0.0021 (11)
C9	0.0175 (16)	0.0153 (15)	0.0237 (18)	-0.0017 (12)	0.0129 (14)	-0.0021 (13)
C10	0.0234 (17)	0.0175 (15)	0.0241 (18)	-0.0043 (13)	0.0149 (15)	-0.0032 (14)
C11	0.0362 (17)	0.0203 (18)	0.0148 (13)	0.001 (2)	0.0072 (12)	0.005 (2)
C12	0.042 (2)	0.030 (3)	0.0215 (16)	0.0067 (18)	0.0031 (15)	0.0061 (18)
I1	0.02599 (10)	0.01685 (9)	0.01752 (9)	0.00241 (13)	0.00673 (7)	0.00025 (13)
N3	0.0222 (16)	0.0241 (16)	0.0226 (17)	-0.0012 (13)	0.0040 (13)	0.0042 (14)
O7	0.0305 (16)	0.0392 (17)	0.0239 (15)	0.0009 (13)	0.0108 (13)	0.0044 (13)
C13	0.028 (2)	0.041 (2)	0.024 (2)	0.003 (2)	-0.0027 (18)	0.0025 (19)
C14	0.033 (2)	0.042 (2)	0.031 (2)	-0.0034 (19)	0.0139 (19)	0.005 (2)
C15	0.022 (2)	0.0260 (18)	0.022 (2)	-0.0009 (17)	0.0021 (18)	0.0034 (17)

Geometric parameters (Å, °)

N1—C8	1.324 (4)	C5—H5	1.0000
N1—C9	1.383 (4)	C6—H6A	0.9900
N1—C6	1.472 (4)	C6—H6B	0.9900
N2—C8	1.339 (4)	C7—H7A	0.9800
N2—C10	1.379 (5)	C7—H7B	0.9800
N2—C11	1.424 (4)	C7—H7C	0.9800
O1—C1	1.421 (4)	C8—H8	0.9500
O1—C5	1.446 (4)	C9—C10	1.345 (5)
O2—C1	1.396 (4)	C9—H9	0.9500
O2—C7	1.428 (5)	C10—H10	0.9500
O3—C2	1.416 (4)	C11—C12	1.298 (6)
O3—H3A	0.72 (4)	C11—H11	0.9500
O4—C3	1.430 (4)	C12—H12A	0.9500
O4—H4A	0.78 (5)	C12—H12B	0.9500
O5—C4	1.424 (4)	N3—C15	1.322 (6)
O5—H5A	0.74 (5)	N3—C14	1.442 (6)
C1—C2	1.523 (5)	N3—C13	1.453 (5)
C1—H1	1.0000	O7—C15	1.243 (5)
C2—C3	1.536 (5)	C13—H13A	0.9800
C2—H2	1.0000	C13—H13B	0.9800
C3—C4	1.523 (5)	C13—H13C	0.9800
C3—H3	1.0000	C14—H14A	0.9800
C4—C5	1.527 (5)	C14—H14B	0.9800
C4—H4	1.0000	C14—H14C	0.9800
C5—C6	1.509 (4)	C15—H15	0.9500
C8—N1—C9	108.9 (3)	C5—C6—H6A	109.6
C8—N1—C6	124.9 (3)	N1—C6—H6B	109.6
C9—N1—C6	126.0 (3)	C5—C6—H6B	109.6
C8—N2—C10	108.2 (3)	H6A—C6—H6B	108.1
C8—N2—C11	127.4 (3)	O2—C7—H7A	109.5
C10—N2—C11	124.2 (3)	O2—C7—H7B	109.5
C1—O1—C5	113.8 (3)	H7A—C7—H7B	109.5
C1—O2—C7	112.6 (3)	O2—C7—H7C	109.5
C2—O3—H3A	105 (4)	H7A—C7—H7C	109.5
C3—O4—H4A	117 (4)	H7B—C7—H7C	109.5
C4—O5—H5A	108 (3)	N1—C8—N2	108.5 (3)
O2—C1—O1	112.4 (3)	N1—C8—H8	125.8
O2—C1—C2	108.8 (3)	N2—C8—H8	125.8
O1—C1—C2	109.3 (3)	C10—C9—N1	106.9 (3)
O2—C1—H1	108.8	C10—C9—H9	126.6
O1—C1—H1	108.8	N1—C9—H9	126.6
C2—C1—H1	108.8	C9—C10—N2	107.5 (3)
O3—C2—C1	109.2 (3)	C9—C10—H10	126.2
O3—C2—C3	112.5 (3)	N2—C10—H10	126.2
C1—C2—C3	110.9 (3)	C12—C11—N2	123.8 (3)

O3—C2—H2	108.0	C12—C11—H11	118.1
C1—C2—H2	108.0	N2—C11—H11	118.1
C3—C2—H2	108.0	C11—C12—H12A	120.0
O4—C3—C4	108.1 (3)	C11—C12—H12B	120.0
O4—C3—C2	110.0 (3)	H12A—C12—H12B	120.0
C4—C3—C2	109.4 (3)	C15—N3—C14	121.8 (4)
O4—C3—H3	109.8	C15—N3—C13	121.5 (4)
C4—C3—H3	109.8	C14—N3—C13	116.7 (4)
C2—C3—H3	109.8	N3—C13—H13A	109.5
O5—C4—C3	109.9 (3)	N3—C13—H13B	109.5
O5—C4—C5	108.6 (3)	H13A—C13—H13B	109.5
C3—C4—C5	108.9 (3)	N3—C13—H13C	109.5
O5—C4—H4	109.8	H13A—C13—H13C	109.5
C3—C4—H4	109.8	H13B—C13—H13C	109.5
C5—C4—H4	109.8	N3—C14—H14A	109.5
O1—C5—C6	105.7 (3)	N3—C14—H14B	109.5
O1—C5—C4	110.4 (3)	H14A—C14—H14B	109.5
C6—C5—C4	112.8 (3)	N3—C14—H14C	109.5
O1—C5—H5	109.3	H14A—C14—H14C	109.5
C6—C5—H5	109.3	H14B—C14—H14C	109.5
C4—C5—H5	109.3	O7—C15—N3	125.3 (5)
N1—C6—C5	110.4 (3)	O7—C15—H15	117.4
N1—C6—H6A	109.6	N3—C15—H15	117.4
C7—O2—C1—O1	65.7 (4)	O5—C4—C5—C6	64.9 (3)
C7—O2—C1—C2	-173.1 (3)	C3—C4—C5—C6	-175.4 (3)
C5—O1—C1—O2	61.2 (4)	C8—N1—C6—C5	117.5 (3)
C5—O1—C1—C2	-59.7 (3)	C9—N1—C6—C5	-56.3 (4)
O2—C1—C2—O3	57.7 (3)	O1—C5—C6—N1	74.9 (3)
O1—C1—C2—O3	-179.2 (3)	C4—C5—C6—N1	-164.3 (3)
O2—C1—C2—C3	-66.8 (3)	C9—N1—C8—N2	-0.8 (4)
O1—C1—C2—C3	56.3 (3)	C6—N1—C8—N2	-175.6 (3)
O3—C2—C3—O4	63.2 (4)	C10—N2—C8—N1	0.9 (4)
C1—C2—C3—O4	-174.2 (3)	C11—N2—C8—N1	176.6 (4)
O3—C2—C3—C4	-178.2 (3)	C8—N1—C9—C10	0.5 (4)
C1—C2—C3—C4	-55.6 (3)	C6—N1—C9—C10	175.1 (3)
O4—C3—C4—O5	-66.2 (3)	N1—C9—C10—N2	0.1 (4)
C2—C3—C4—O5	174.1 (3)	C8—N2—C10—C9	-0.6 (4)
O4—C3—C4—C5	175.0 (3)	C11—N2—C10—C9	-176.5 (4)
C2—C3—C4—C5	55.3 (3)	C8—N2—C11—C12	-6.4 (8)
C1—O1—C5—C6	-176.5 (3)	C10—N2—C11—C12	168.6 (5)
C1—O1—C5—C4	61.2 (3)	C14—N3—C15—O7	-178.8 (4)
O5—C4—C5—O1	-177.0 (3)	C13—N3—C15—O7	-0.4 (7)
C3—C4—C5—O1	-57.3 (3)		

Hydrogen-bond geometry (Å, °)

<i>D—H···A</i>	<i>D—H</i>	<i>H···A</i>	<i>D···A</i>	<i>D—H···A</i>
O3—H3A···O7	0.72 (4)	2.09 (4)	2.797 (4)	167 (5)
O4—H4A···I1 ⁱ	0.78 (5)	2.71 (5)	3.482 (3)	171 (4)
O5—H5A···I1	0.74 (5)	2.75 (5)	3.474 (3)	165 (4)
C6—H6A···O5 ⁱⁱ	0.99	2.46	3.332 (4)	147
C8—H8···O4 ⁱⁱ	0.95	2.44	3.252 (4)	143
C8—H8···O5 ⁱⁱ	0.95	2.53	3.285 (4)	136
C9—H9···O3 ⁱⁱⁱ	0.95	2.51	3.404 (5)	156
C10—H10···O7 ⁱⁱⁱ	0.95	2.40	3.159 (5)	137
C11—H11···I1 ^{iv}	0.95	3.02	3.925 (3)	161
C15—H15···O4	0.95	2.58	3.297 (5)	132

Symmetry codes: (i) $x, y-1, z$; (ii) $-x, y+1/2, -z+1$; (iii) $-x+1, y+1/2, -z+1$; (iv) $x, y, z-1$.

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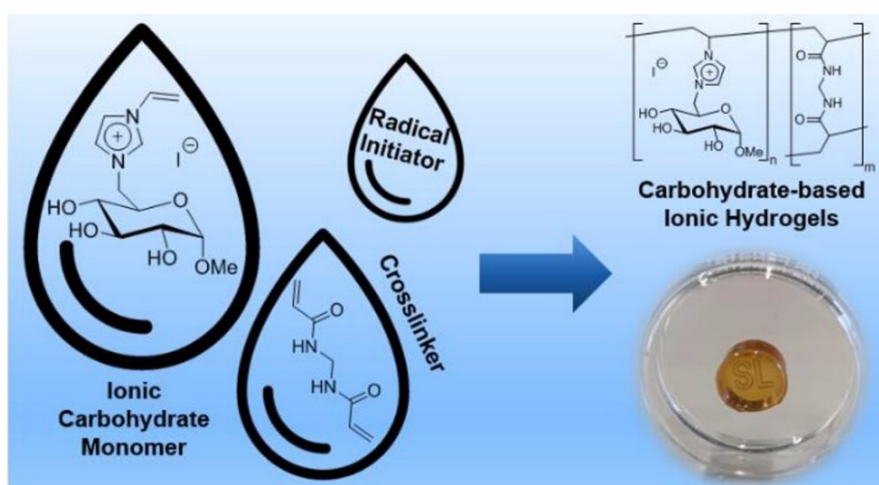
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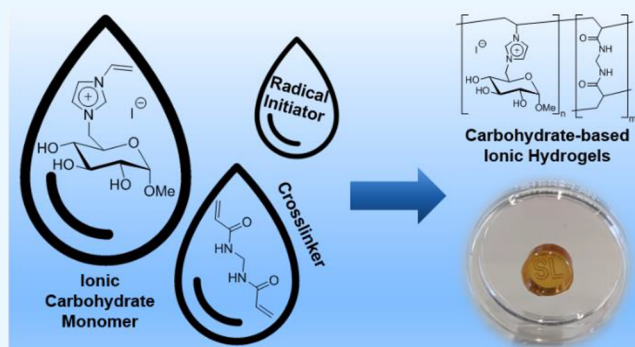


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Supporting Information

ABSTRACT: This work presents the synthesis of glucose-based vinyl imidazolium monomers and the hydrogels produced thereof. These novel semisynthetic, cationic hydrogels were prepared by radical polymerization with different types of commercial cross-linkers such as *N,N'*-methylenebis(acrylamide) or poly(ethylene glycol) diacrylate. Both the type and the amount of cross-linker were investigated as influencing factors with respect to the swelling degree. It was found that the cross-linker type majorly influences the swelling degree and long-term stability of the hydrogels. Last, the influence of different anions (e.g., halogens, acetate, and triflate) in the monomer molecule on the swelling properties of the hydrogels was investigated.



INTRODUCTION

Hydrogels are hydrophilic, three-dimensional cross-linked polymers that can, depending on their type of monomer and cross-linking degree, reversibly absorb large amounts of water due to the hydrogen bond formation while retaining their shape.¹ Hydrogels have very diverse properties depending on their origin (natural, synthetic, or semisynthetic), their polymer composition (homo-, co-, or multipolymeric), their charge (ionic, nonionic, zwitterionic, or amphoteric), their physical appearance (matrix, film, or microsphere), their preparation (free radical polymerization or radiation), their cross-linking method (physical or chemical), and their configuration (amorphous, crystalline, or semicrystalline).² Hydrogels are often attributed with properties such as biocompatibility and biodegradability,^{3,4} either stiffness^{5,6} or flexibility,⁷ depending on the used material, and antibacterial⁸ and stimuli-responsive³ properties. Therefore, they are suitable for application in medicine (e.g., as a drug delivery system),⁹ in biotechnology,¹⁰ as an immobilization matrix for biocatalysts (e.g., *Candida antarctica* lipase B),¹¹ or in agricultural industry as water storage.¹²

Carbohydrate-based hydrogels, as one subgroup of the abovementioned natural hydrogels, are usually produced from polysaccharides such as cellulose, hyaluronic acid, or alginate. These polysaccharides are usually cross-linked using chemical methods by forming ether or ester bonds.¹³ Carbohydrate-based hydrogels are already well-known in many applications in the medical field (e.g., wound dressing and drug delivery) or for enzyme encapsulation.¹⁴

In contrast to the usage of (bio)polymers as starting materials, the direct hydrogel production from a mixture of

monomer, cross-linker, and polymerization initiators in water allows for a more controlled approach, enabling the user to adjust the ratios between the monomer and cross-linker more freely. This method has recently been performed with commercially available ionic liquids such as vinyl imidazolium-based ILs.⁸ Using polymerizable ionic liquids as starting materials furthermore leads to either anionic, cationic, or zwitterionic hydrogels, which bear interesting possibilities in the medical field due to their ion exchange-controlled drug release.⁹ In the case of carbohydrates, notable work has been done on glucose- and mannose-based acryl esters,^{15,16} on carbohydrate vinyl ethers,¹⁷ and even on cationic glucose-based monomers,¹⁸ all of which bear isopropylidene or acetyl protecting groups and have been used for copolymerizations. However, hydrogels produced from carbohydrate-based monomers have, to the best of our knowledge, not been produced and investigated before.

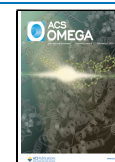
Our group works in the field of carbohydrate-based ionic liquids.^{19–21} We previously found that glucosyl imidazolium-based ionic liquids can be produced in high total yields up to 90%²² and that they exhibit a remarkably high biocompatibility in comparison to commercial imidazolium ILs.²³ In this work, we synthesized a series of novel glucosyl vinyl imidazolium salts and used these as monomers to produce novel glucose-

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based ionic hydrogels, cross-linked by several commercially available cross-linkers (Figure 1). These hydrogels combine

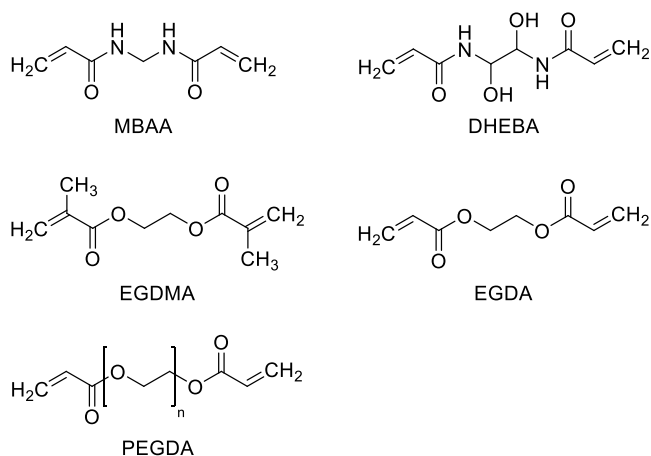


Figure 1. Cross-linkers used in this work (MBAA = N,N' -methylenebis(acrylamide), DHEBA = N,N' -(1,2-dihydroxyethylene)-bis(acrylamide), EGDMA = ethylene glycol dimethacrylate, EGDA = ethylene glycol diacrylate, and PEGDA = poly(ethylene glycol) diacrylate).

several properties and techniques previously not found in this combination: They are semisynthetic hydrogels (produced from a synthetically modified natural carbohydrate source) bearing a unique cationic charge not found in natural carbohydrate hydrogels and are synthesized directly from a monomer–cross-linker mixture. To characterize our novel hydrogels, we investigated the swelling behavior. Knowledge of the swelling degree of hydrogels as a function of different cross-linkers enables prediction of the behavior of these gels in potential applications. High-swelling hydrogels for example are more suited for wound dressing, while low- or nonswelling hydrogels are used in tissue engineering or drug delivery.²⁴ The swelling behavior of our hydrogels has been investigated in different media, with different cross-linkers and at different cross-linker concentrations in each case. Furthermore, the influence of different anions on the swelling behavior was examined.

Thus, the overall goal of this work is to establish the synthesis of the abovementioned novel monomers and hydrogels and to investigate their swelling behavior, laying the groundwork for future applications.

MATERIALS AND METHODS

General Information. All reagents and solvents were purchased from commercial sources and used as received without further purification, if not stated otherwise. The NMR spectra were recorded on a Bruker AVANCE 250 II, Bruker AVANCE 300 III, or 500 NEO. CDCl_3 was calibrated as 7.27 (^1H) and 77.00 (^{13}C). $\text{DMSO-}d_6$ was calibrated as 2.49 (^1H) and 39.50 (^{13}C). D_2O was calibrated as 4.80 (^1H). ATR IR spectra were obtained using a Thermo Fisher Scientific Nicolet 380 FT-IR at room temperature. ESI-MS was measured in an Agilent 1200/6210 Time-of-Flight LC-MS or a Thermo Scientific Exactive ESI/DART FTMS. The specific rotations were measured with a Dr. Kernchen Gyromat-HP Digital Automatic Polarimeter with concentrations given in mg per mL. The measurements of thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) were performed

on a Setaram Labsys TGA-DSC 1600 with a heating program from 25 to 500 °C and a heating rate of 10 °C/min under an argon flow of 100 mL/min. Ion chromatograms were measured on a two-channel ion chromatography system “ProfIC 940 vario” with conductivity detection from the company Metrohm. The solid-state ^{13}C NMR experiment was carried out on a Bruker AVANCE III HD spectrometer with an operating field of 400.5 MHz proton frequency with a Bruker ASCEND DNP 9.4 T widebore (89 mm) magnet while using a 1.3 mm probe.

Experimental Section. *Methyl 6-Iodo- α -D-glucopyranoside 2a.* 2a was prepared according to a procedure previously published by our group.²²

Methyl α -D-glucopyranoside (4.855 g, 25.0 mmol), triphenylphosphine (9.836 g, 37.5 mmol), iodine (9.518 g, 37.5 mmol), and imidazole (3.404 g, 50.0 mmol) were refluxed in THF (150 mL) for 4 h. The resulting solid was filtered off, the solvent was removed, and the product was obtained as a white solid (6.486 g, 85%) after column chromatography (chloroform/methanol 12:1).

$T_m = 148\text{--}149$ °C. $[\alpha]_D^{25} = +94.2$ ($c = 1.0$, H_2O). ^1H NMR (300 MHz, $\text{DMSO-}d_6$): $\delta = 2.87\text{--}2.95$ (m, 1H); 3.16–3.27 (m, 3H); 3.31 (s, 3H, OCH_3); 3.34–3.42 (m, 1H); 3.50–3.57 (m, 1H); 4.54 (d, 1H, $^3J = 3.65$ Hz, H-1); 4.78 (d, 1H, $^3J = 6.43$ Hz, OH); 4.86 (d, 1H, $^3J = 4.99$ Hz, OH); 5.17 (d, 1H, $^3J = 5.83$ Hz, OH). ^{13}C NMR (75 MHz, $\text{DMSO-}d_6$): $\delta = 9.5$ (C-6); 54.6 (OCH_3); 70.9, 71.9, 72.7, 74.1 (C-2, C-3, C-4, C-5); 99.8 (C-1). ATR-IR 3430, 3285, 2911, 2880, 2840, 1455, 1032 cm^{-1} .

Methyl 6-Bromo- α -D-glucopyranoside 2b. 2b was synthesized following the same procedure as 2a, using bromine (5.993 g, 1.92 mL, 37.5 mmol) instead of iodine. The bromine was slowly added to the THF solution at room temperature; afterward, the reaction was refluxed. The product was obtained as a white solid (4.493 g, 70%).

$T_m = 127\text{--}129$ °C. $[\alpha]_D^{25} = +105.6$ ($c = 1.2$, H_2O). ^1H NMR (300 MHz, $\text{DMSO-}d_6$): $\delta = 2.98\text{--}3.06$ (m, 1H); 3.16–3.23 (m, 1H); 3.29 (s, 3H, OCH_3); 3.34–3.41 (m, 1H); 3.45–3.56 (m, 2H); 3.72–3.76 (m, 1H); 4.55 (d, 1H, $^3J = 3.67$ Hz, H-1); 4.79 (d, 1H, $^3J = 6.42$ Hz, OH); 4.87 (d, 1H, $^3J = 5.05$ Hz, OH); 5.19 (d, 1H, $^3J = 5.86$ Hz, OH). ^{13}C NMR (75 MHz, $\text{DMSO-}d_6$): $\delta = 35.2$ (C-6); 54.5 (OCH_3); 70.9, 71.8, 72.3, 73.0 (C-2, C-3, C-4, C-5); 99.8 (C-1). The NMR data is according to the literature.²⁵

Methyl 6-Chloro- α -D-glucopyranoside 2c. 2c was synthesized following the same procedure as 2a, using N -chlorosuccinimide (5.007 g, 37.5 mmol) instead of iodine. The product was obtained as a white solid (1.637 g, 31%).

$T_m = 110\text{--}112$ °C. $[\alpha]_D^{25} = +137.8$ ($c = 1.2$, H_2O). ^1H NMR (300 MHz, $\text{DMSO-}d_6$): $\delta = 3.06$ (ddd, 1H, $^3J = 9.68$ Hz, $^3J = 8.62$ Hz, $^3J = 5.83$ Hz, H-3); 3.19 (ddd, 1H, $^3J = 9.75$ Hz, $^3J = 6.38$ Hz, $^3J = 3.69$ Hz, H-2); 3.27 (s, 3H, OCH_3); 3.37 (ddd, 1H, $^3J = 9.43$ Hz, $^3J = 8.76$ Hz, $^3J = 4.96$ Hz, H-4); 3.49–3.55 (m, 1H, H-5); 3.67 (dd, 1H, $^3J = 11.58$ Hz, $^3J = 6.39$ Hz, H-6a); 3.84 (dd, 1H, $^3J = 11.57$ Hz, $^3J = 2.17$ Hz, H-6b); 4.56 (d, 1H, $^3J = 3.65$ Hz, H-1); 4.79 (d, 1H, $^3J = 6.41$ Hz, OH-2); 4.86 (d, 1H, $^3J = 5.04$ Hz, OH-4); 5.17 (d, 1H, $^3J = 5.85$ Hz, OH-3). ^{13}C NMR (75 MHz, $\text{DMSO-}d_6$): $\delta = 45.6$ (C-6); 54.4 (OCH_3); 71.2, 71.2, 71.8, 73.1 (C-2, C-3, C-4, C-5); 99.8 (C-1). The NMR data is according to the literature.²⁶

1-(Methyl- α -D-glucopyranosid-6-yl)-3-vinylimidazolium iodide **3a** (GVIM-I). **3a** was prepared according to a procedure previously published by our group.²⁷

2a (3.649 g, 12.0 mmol) and *N*-vinylimidazole (1.882 g, 20.0 mmol) were dissolved in DMF (20 mL) and stirred at 95 °C for 24 h. After cooling down, ethyl acetate (160 mL) was added and the flask was stored in a fridge overnight. The solvent was decanted, and the precipitated solid was washed with ethyl acetate (3 × 80 mL) and dried under high vacuum to achieve the product as a light-brown solid (3.959 g, 83%).

$T_m = 185$ °C. $T_d = 236$ °C. $[\alpha]_D^{24} = +30.3$ ($c = 2.6$, H₂O). ¹H NMR (500 MHz, D₂O): $\delta = 3.27$ (s, 3H, OCH₃); 3.24–3.28 (m, 1H, H-4); 3.58 (dd, 1H, ³ $J = 9.79$ Hz, ³ $J = 3.82$ Hz, H-2); 3.69–3.72 (m, 1H, H-3); 3.96 (ddd, 1H, ³ $J = 9.96$ Hz, ³ $J = 7.47$ Hz, ³ $J = 2.46$ Hz, H-5); 4.50 (dd, 1H, ² $J = 14.57$ Hz, ³ $J = 7.46$ Hz, H-6a); 4.70 (dd, 1H, ² $J = 14.56$ Hz, ³ $J = 2.49$, H-6b); 4.85 (d, 1H, ³ $J = 3.80$ Hz, H-1); 5.49 (dd, 1H, ³ $J = 8.67$ Hz, ² $J = 2.87$ Hz, Vinyl-CH₂); 5.86 (dd, 1H, ³ $J = 15.58$ Hz, ² $J = 2.86$ Hz, Vinyl-CH₂); 7.20 (dd, 1H, ³ $J = 15.58$ Hz, ³ $J = 8.68$ Hz, Vinyl-CH); 7.70 (d, 1H, ³ $J = 2.09$ Hz, H_{Ar}); 7.86 (d, 1H, ³ $J = 2.11$ Hz, H_{Ar}). ¹³C NMR (125 MHz, D₂O): $\delta = 50.2$ (C-6); 55.1 (OCH₃); 69.2 (C-5); 70.5 (C-4); 71.0 (C-2); 72.8 (C-3); 99.3 (C-1); 109.8 (Vinyl-CH₂); 119.4, 123.8 (CH_{Ar}); 128.1 (Vinyl-CH); 135.2 (CH_{Ar}). ATR-IR 3396, 3088, 2998, 2910, 1657, 1566, 1549, 1048, 1015 cm⁻¹. HRMS (ESI, m/z): Calculated for C₁₂H₁₉N₂O₅⁺, 271.1299; measured 271.1306. Calculated for I⁻, 126.9040; measured 126.9045.

1-(Methyl- α -D-glucopyranosid-6-yl)-3-vinylimidazolium Bromide **3b** (GVIM-Br). **3b** was synthesized following the same procedure as **3a** but instead using **2b** (1.536 g, 6.0 mmol) as a starting material and a reaction temperature of 110 °C. The product was obtained as a light-brown solid (1.659 g, 79%).

$T_m = 178$ °C. $T_d = 225$ °C. $[\alpha]_D^{25} = +83.4$ ($c = 1.2$, H₂O). ¹H NMR (300 MHz, D₂O): $\delta = 3.28$ (s, 3H, OCH₃); 3.23–3.33 (m, 1H, H-4); 3.58 (dd, 1H, ³ $J = 9.79$ Hz, ³ $J = 3.79$ Hz, H-2); 3.68–3.75 (m, 1H, H-3); 3.96 (ddd, 1H, ³ $J = 10.01$ Hz, ³ $J = 7.47$ Hz, ³ $J = 2.58$ Hz, H-5); 4.51 (dd, 1H, ² $J = 14.54$ Hz, ³ $J = 7.40$ Hz, H-6a); 4.71 (dd, 1H, ² $J = 14.56$ Hz, ³ $J = 2.56$, H-6b); 4.86 (d, 1H, ³ $J = 3.75$ Hz, H-1); 5.50 (dd, 1H, ³ $J = 8.68$ Hz, ² $J = 2.84$ Hz, Vinyl-CH₂); 5.87 (dd, 1H, ³ $J = 15.58$ Hz, ² $J = 2.85$ Hz, Vinyl-CH₂); 7.21 (dd, 1H, ³ $J = 15.61$ Hz, ³ $J = 8.67$ Hz, Vinyl-CH); 7.71 (d, 1H, ³ $J = 2.10$ Hz, H_{Ar}); 7.87 (d, 1H, ³ $J = 2.12$ Hz, H_{Ar}); 9.17 (s, 1H, H_{Ar}). ¹³C NMR (75 MHz, D₂O): $\delta = 50.2$ (C-6); 55.0 (OCH₃); 69.3 (C-5); 70.5 (C-4); 71.0 (C-2); 72.8 (C-3); 99.3 (C-1); 109.8 (Vinyl-CH₂); 119.4, 123.8 (CH_{Ar}); 128.1 (Vinyl-CH); 135.5 (CH_{Ar}). HRMS (ESI, m/z): Calculated for C₁₂H₁₉N₂O₅⁺, 271.1299; measured 271.1296. Calculated for ⁷⁹Br⁻, 78.9183; measured 78.9183. Calculated for ⁸¹Br⁻, 80.9163; measured 80.9162.

1-(Methyl- α -D-glucopyranosid-6-yl)-3-vinylimidazolium Chloride **3c** (GVIM-Cl). **3c** was synthesized following the same procedure as **3a** but instead using **2c** (0.638 g, 3.0 mmol) as a starting material and a reaction temperature of 150 °C. The product was obtained as a brown solid (0.535 g, 58%).

$T_d = 201$ °C. $[\alpha]_D^{24} = +89.6$ ($c = 3.2$, MeOH). ¹H NMR (300 MHz, D₂O): $\delta = 3.27$ (s, 3H, OCH₃); 3.23–3.29 (m, 1H, H-4); 3.57 (dd, 1H, ³ $J = 9.79$ Hz, ³ $J = 3.78$ Hz, H-2); 3.68–3.74 (m, 1H, H-3); 3.96 (ddd, 1H, ³ $J = 9.80$ Hz, ³ $J = 7.41$ Hz, ³ $J = 2.60$ Hz, H-5); 4.50 (dd, 1H, ² $J = 14.57$ Hz, ³ $J = 7.38$ Hz, H-6a); 4.70 (dd, 1H, ² $J = 14.55$ Hz, ³ $J = 2.59$, H-6b); 4.85 (d, 1H, ³ $J = 3.76$ Hz, H-1); 5.49 (dd, 1H, ³ $J = 8.68$ Hz, ² $J = 2.85$ Hz, Vinyl-CH₂); 5.87 (dd, 1H, ³ $J = 15.58$ Hz, ² $J = 2.85$ Hz,

Vinyl-CH₂); 7.21 (dd, 1H, ³ $J = 15.59$ Hz, ³ $J = 8.69$ Hz, Vinyl-CH); 7.71 (d, 1H, ³ $J = 2.12$ Hz, H_{Ar}); 7.86 (d, 1H, ³ $J = 2.15$ Hz, H_{Ar}). ¹³C NMR (75 MHz, D₂O): $\delta = 50.2$ (C-6); 55.0 (OCH₃); 69.3 (C-5); 70.5 (C-4); 71.0 (C-2); 72.8 (C-3); 99.4 (C-1); 109.7 (Vinyl-CH₂); 119.4, 123.8 (CH_{Ar}); 128.1 (Vinyl-CH). HRMS (ESI, m/z): Calculated for C₁₂H₁₉N₂O₅⁺, 271.1299; measured 271.1291.

1-(Methyl- α -D-glucopyranosid-6-yl)-3-vinylimidazolium Acetate **4a** (GVIM-OAc). **3a** (5.03 mmol, 2.002 g) and silver acetate (5.03 mmol, 838 mg) were suspended in water (20 mL) and stirred for 30 min under the absence of light. The yellow precipitate was removed via filtration. Activated charcoal (~500 mg) was added to the filtrate, and the mixture was stirred for 2 h. The product **4a** was achieved as a white solid (1.320 g, 79%) after filtration and removal of water.

$T_d = 287$ °C. $[\alpha]_D^{23} = +91.6$ ($c = 1.3$, MeOH). ¹H NMR (500 MHz, D₂O): $\delta = 1.92$ (s, 3H, CH₃); 3.25 (s, 3H, OCH₃); 3.21–3.25 (m, 1H, H-4); 3.55 (dd, 1H, ³ $J = 9.79$ Hz, ³ $J = 3.82$ Hz, H-2); 3.67–3.71 (m, 1H, H-3); 3.93 (ddd, 1H, ³ $J = 9.97$ Hz, ³ $J = 7.47$ Hz, ³ $J = 2.49$ Hz, H-5); 4.48 (dd, 1H, ² $J = 14.57$ Hz, ³ $J = 7.46$ Hz, H-6a); 4.68 (dd, 1H, ² $J = 14.56$ Hz, ³ $J = 2.51$ Hz, H-6b); 4.83 (d, 1H, ³ $J = 3.81$ Hz, H-1); 5.47 (dd, 1H, ³ $J = 8.68$ Hz, ² $J = 2.87$ Hz, Vinyl-CH₂); 5.84 (dd, 1H, ³ $J = 15.58$ Hz, ² $J = 2.87$ Hz, Vinyl-CH₂); 7.18 (dd, 1H, ³ $J = 15.59$ Hz, ³ $J = 8.68$ Hz, Vinyl-CH); 7.68 (d, 1H, ³ $J = 2.11$ Hz, H_{Ar}); 7.84 (d, 1H, ³ $J = 2.13$ Hz, H_{Ar}). ¹³C NMR (125 MHz, D₂O): $\delta = 23.2$ (CH₃); 50.2 (C-6); 55.0 (OCH₃); 69.2 (C-5); 70.4 (C-4); 71.0 (C-2); 72.8 (C-3); 99.3 (C-1); 109.7 (Vinyl-CH₂); 119.4, 123.8 (CH_{Ar}); 128.1 (Vinyl-CH); 135.2 (CH_{Ar}); 181.3 (C=O). HRMS (ESI, m/z): Calculated for C₁₂H₁₉N₂O₅⁺, 271.1299; measured 271.1303.

1-(Methyl- α -D-glucopyranosid-6-yl)-3-vinylimidazolium Methanesulfonate **4b** (GVIM-OMs). **3a** (5.03 mmol, 2.002 g) and silver methanesulfonate (5.03 mmol, 1.023 g) were suspended in water (80 mL) and stirred for 30 min under the absence of light. The yellow precipitate was removed via filtration. Activated charcoal (~500 mg) was added to the filtrate, and the mixture was stirred for 24 h. After filtration and removal of water, the remaining solid was purified via column chromatography (methanol) to achieve the product **4b** as colorless wax (1.198 g, 65%).

$T_d = 226$ °C. $[\alpha]_D^{21} = +68.2$ ($c = 2.5$, MeOH). ¹H NMR (500 MHz, D₂O): $\delta = 2.82$ (s, 3H, CH₃); 3.25 (s, 3H, OCH₃); 3.22–3.26 (m, 1H, H-4); 3.56 (dd, 1H, ³ $J = 9.79$ Hz, ³ $J = 3.83$ Hz, H-2); 3.67–3.71 (m, 1H, H-3); 3.94 (ddd, 1H, ³ $J = 9.97$ Hz, ³ $J = 7.48$ Hz, ³ $J = 2.47$ Hz, H-5); 4.49 (dd, 1H, ² $J = 14.57$ Hz, ³ $J = 7.47$ Hz, H-6a); 4.68 (dd, 1H, ² $J = 14.56$ Hz, ³ $J = 2.48$ Hz, H-6b); 4.83 (d, 1H, ³ $J = 3.81$ Hz, H-1); 5.48 (dd, 1H, ³ $J = 8.67$ Hz, ² $J = 2.87$ Hz, Vinyl-CH₂); 5.85 (dd, 1H, ³ $J = 15.58$ Hz, ² $J = 2.88$ Hz, Vinyl-CH₂); 7.19 (dd, 1H, ³ $J = 15.59$ Hz, ³ $J = 8.69$ Hz, Vinyl-CH); 7.69 (d, 1H, ³ $J = 1.78$ Hz, H_{Ar}); 7.85 (d, 1H, ³ $J = 1.86$ Hz, H_{Ar}); 9.14 (s, 1H, H_{Ar}). ¹³C NMR (125 MHz, D₂O): $\delta = 38.5$ (CH₃); 50.2 (C-6); 55.0 (OCH₃); 69.3 (C-5); 70.5 (C-4); 71.0 (C-2); 72.8 (C-3); 99.3 (C-1); 109.7 (Vinyl-CH₂); 119.4, 123.8 (CH_{Ar}); 128.1 (Vinyl-CH); 135.5 (CH_{Ar}). HRMS (ESI, m/z): Calculated for C₁₂H₁₉N₂O₅⁺, 271.1299; measured 271.1301. Calculated for CH₃O₃S⁻, 94.9798; measured 94.9797.

1-(Methyl- α -D-glucopyranosid-6-yl)-3-vinylimidazolium Trifluoromethanesulfonate **4c** (GVIM-OTf). **3a** (5.03 mmol, 2.002 g) and silver trifluoromethanesulfonate (5.03 mmol, 1.295 g) were suspended in water (80 mL) and stirred for 2 h

under the absence of light. The yellow precipitate was removed via filtration. Activated charcoal (~500 mg) was added to the filtrate, and the mixture was stirred for 2 h. The product **4c** was achieved as a colorless viscous liquid (1.850 g, 88%) after filtration and removal of water. If the black silver particles still remain in the product, a third filtration from water is necessary.

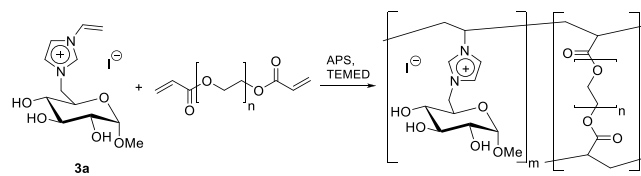
$T_d = 190\text{ }^\circ\text{C}$. $[\alpha]_D^{23} = +54.1$ ($c = 1.3$, MeOH). $^1\text{H NMR}$ (300 MHz, D_2O): $\delta = 3.27$ (s, 3H, OCH_3); 3.23–3.29 (m, 1H, H-4); 3.58 (dd, 1H, $^3J = 9.78$ Hz, $^3J = 3.80$ Hz, H-2); 3.68–3.74 (m, 1H, H-3); 3.96 (ddd, 1H, $^3J = 9.91$ Hz, $^3J = 7.42$ Hz, $^3J = 2.50$ Hz, H-5); 4.50 (dd, 1H, $^2J = 14.54$ Hz, $^3J = 7.43$ Hz, H-6a); 4.70 (dd, 1H, $^2J = 14.53$ Hz, $^3J = 2.56$ Hz, H-6b); 4.85 (d, 1H, $^3J = 3.78$ Hz, H-1); 5.50 (dd, 1H, $^3J = 8.68$ Hz, $^2J = 2.86$ Hz, Vinyl- CH_2); 5.86 (dd, 1H, $^3J = 15.58$ Hz, $^2J = 2.86$ Hz, Vinyl- CH_2); 7.20 (dd, 1H, $^3J = 15.59$ Hz, $^3J = 8.70$ Hz, Vinyl-CH); 7.71 (d, 1H, $^3J = 1.82$ Hz, H_{Ar}); 7.86 (d, 1H, $^3J = 1.89$ Hz, H_{Ar}); 9.15 (s, 1H, H_{Ar}). $^{13}\text{C NMR}$ (75 MHz, D_2O): $\delta = 50.2$ (C-6); 55.0 (OCH_3); 69.3 (C-5); 70.5 (C-4); 71.0 (C-2); 72.8 (C-3); 99.3 (C-1); 109.7 (Vinyl- CH_2); 119.4 (CH_{Ar}); 119.6 (q, $^1J = 317.2$ Hz, CF_3); 123.8 (CH_{Ar}); 128.1 (Vinyl-CH); 135.4 (CH_{Ar}). $^{19}\text{F-NMR}$ (282 MHz, D_2O): $\delta = -79.8$. HRMS (ESI, m/z): Calculated for $\text{C}_{12}\text{H}_{19}\text{N}_2\text{O}_5^+$, 271.1299; measured 271.1295. Calculated for $\text{CF}_3\text{O}_3\text{S}^-$, 148.9515; measured 148.9519.

1-(Methyl- α -D-glucopyranosid-6-yl)-3-vinylimidazolium Bis(trifluoromethanesulfonyl)-imide **4d** (GVIM-NTf₂). **3a** (5.03 mmol, 2.002 g) and silver bis-(trifluoromethanesulfonyl)-imide (5.03 mmol, 1.948 g) were suspended in water (30 mL) and stirred for 20 h under the absence of light. The yellow precipitate was removed via filtration. Activated charcoal (~500 mg) was added to the filtrate, and the mixture was stirred for 20 h. The product **4d** was achieved as a yellow liquid (2.014 g, 73%) after filtration and removal of water. If the black silver particles still remain in the product, a third filtration from water is necessary.

$T_d = 286\text{ }^\circ\text{C}$. $[\alpha]_D^{22} = +46.7$ ($c = 1.1$, MeOH). $^1\text{H NMR}$ (300 MHz, D_2O): $\delta = 3.27$ (s, 3H, OCH_3); 3.23–3.29 (m, 1H, H-4); 3.58 (dd, 1H, $^3J = 9.78$ Hz, $^3J = 3.80$ Hz, H-2); 3.68–3.74 (m, 1H, H-3); 3.95 (ddd, 1H, $^3J = 9.98$ Hz, $^3J = 7.47$ Hz, $^3J = 2.56$ Hz, H-5); 4.50 (dd, 1H, $^2J = 14.56$ Hz, $^3J = 7.43$ Hz, H-6a); 4.70 (dd, 1H, $^2J = 14.57$ Hz, $^3J = 2.54$ Hz, H-6b); 4.85 (d, 1H, $^3J = 3.77$ Hz, H-1); 5.50 (dd, 1H, $^3J = 8.68$ Hz, $^2J = 2.86$ Hz, Vinyl- CH_2); 5.87 (dd, 1H, $^3J = 15.58$ Hz, $^2J = 2.87$ Hz, Vinyl- CH_2); 7.20 (dd, 1H, $^3J = 15.59$ Hz, $^3J = 8.69$ Hz, Vinyl-CH); 7.71 (d, 1H, $^3J = 1.82$ Hz, H_{Ar}); 7.86 (d, 1H, $^3J = 1.89$ Hz, H_{Ar}); 9.15 (s, 1H, H_{Ar}). $^{13}\text{C NMR}$ (75 MHz, D_2O): $\delta = 50.2$ (C-6); 55.0 (OCH_3); 69.3 (C-5); 70.5 (C-4); 71.0 (C-2); 72.8 (C-3); 99.3 (C-1); 109.7 (Vinyl- CH_2); 119.2 (q, $^1J = 319.7$ Hz, CF_3); 119.4, 123.8 (CH_{Ar}); 128.1 (Vinyl-CH); 135.4 (CH_{Ar}). $^{19}\text{F-NMR}$ (282 MHz, D_2O): $\delta = -79.2$. HRMS (ESI, m/z): Calculated for $\text{C}_{12}\text{H}_{19}\text{N}_2\text{O}_5^+$, 271.1299; measured 271.1301. Calculated for $\text{C}_2\text{F}_6\text{NO}_4\text{S}_2^-$, 279.9168; measured 279.9173.

Hydrogel Synthesis. **3a–3c** or **4a–4d** were dissolved in pure water and an appropriate weight-percent of cross-linker was added. Radical polymerization (Scheme 1) was initiated by adding APS solution followed by TEMED and then immediately mixing the solution thoroughly for 10 s. The used amounts of monomer, cross-linker, water, APS, and TEMED for each hydrogel composition can be found in the Supporting Information (Tables S1–S12). After transferring the solution in cylindrical-shaped molds (10 mm diameter, 10

Scheme 1. Synthesis of Carbohydrate-Based Ionic Hydrogels via Radical Polymerization with PEGDA as a Cross-Linker



mm height), the gelation took place within seconds or a few minutes. After removal of the gels, they were stored in a compartment dryer at $40\text{ }^\circ\text{C}$ for 3 days.

After the initial synthesis and drying, the hydrogels can be washed with pure water (3×10 mL, 10 min each) to remove the unreacted monomers and TEMED, as performed for the NMR studies of the hydrogels (see Results and Discussion Section).

Gravimetric Swelling Experiments. The solvent uptake was measured gravimetrically by weighing the mass of the hydrogel as a function of time. After determining the dry mass of the samples, the gels were placed in a sieve, which was in a beaker filled with PBS, water, or isotonic NaCl solution (see Supporting Information Figures S29 and S30 for the setup) and allowed to soak at $37 \pm 1\text{ }^\circ\text{C}$. The sieves were removed at monitored time intervals. Both the sieves and the gels were carefully blotted with paper towel to remove the surface-bound solvent. It was then weighed and returned to solution. These swelling studies were performed in triplicate to investigate the swelling behavior.

Calculations from Gravimetric Swelling Experiments. The experimental equilibrium swelling (q_t) of the hydrogels was calculated from the data by the following term

$$q_t = \frac{w_t - w_0}{w_0}$$

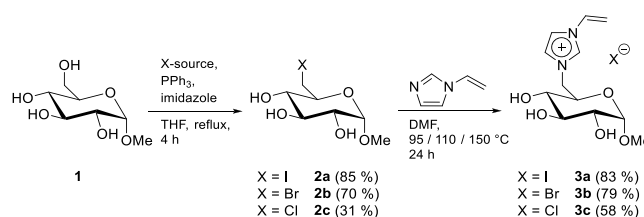
with w_t being the weight of the swollen gel after time t and w_0 being the weight of the initial dry gel at the time $t = 0$.

RESULTS AND DISCUSSION

Monomer Synthesis. The first step of this work was to produce the envisioned starting material **3** needed for the hydrogel synthesis. We adopted the reaction conditions from our previous works on glucosyl imidazolium salts bearing different alkyl chain lengths on the imidazole,^{22,23} this time however focusing on vinyl imidazole (Scheme 2). Here, the vinyl group is crucial for the three-dimensional cross-linking of the resulting monomer.

The synthesis starts with commercially available methyl α -D-glucopyranoside **1**. The methyl glycoside is necessary to block the anomeric center of the carbohydrate, which would

Scheme 2. Synthesis of Glucosyl Vinyl Imidazolium Salts



otherwise hinder the following two reaction steps. **1** was chemoselectively converted into the 6-halogenated glucopyranosides **2a–2c** by using Appel reaction conditions. Previously, our group only worked with the 6-iodo product **2a**, which is synthesized in a high yield of 85%.²² This time we also produced the 6-bromo product **2b** (70% yield) and the 6-chloro product **2c** (31% yield) by using bromine or *N*-chlorosuccinimide as halogen sources.

As the next step, vinyl imidazole was added to the 6-halogenated glucopyranosides **2a–2c**. Here, the reaction temperature needed to be adjusted for each material. While a reaction temperature of 110 °C leads to 99% yield in the case of the glucosyl vinyl imidazolium iodide (GVIM-I) **3a**, this product also turned to a dark brown color at this temperature. Though the NMR analysis remains seemingly pure, this dark color would negatively impact the later hydrogel studies. Lowering the reaction temperature to 95 °C leads to a visibly cleaner product with only a slight drop in yield to 83%. GVIM-Br **3b** was produced at 110 °C in 79% yield without any dark coloration and GVIM-Cl **3c** needs a high temperature of 150 °C to even reach a mediocre yield of 58% and also exhibits the aforementioned dark color. Furthermore, product **3a** has also been individually confirmed by X-ray analysis (Figure 2).

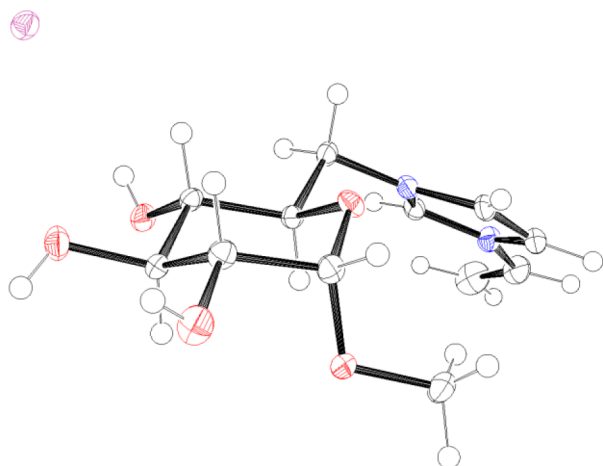


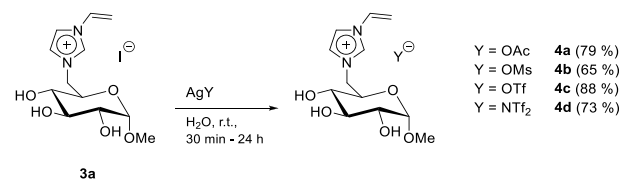
Figure 2. ORTEP of GVIM-I **3a**. Reproduced with permission from ref 22. Copyright 2022 IUCrData.

As expected, the product yield for both the 6-halogenated glucopyranosides **2a–2c** as well as for the glucosyl vinyl imidazolium halogenides **3a–3c** is in the order of $I > Br \gg Cl$, mirroring the general reactivities of the halogens.

Since our glucosyl vinyl imidazolium products are salts with a cationic carbohydrate core, which will also turn into a cationic polymer network with freely movable anions in the hydrogels, we were also interested in the impact of different anions onto the swelling behavior of the hydrogels. Thus, besides the already produced salts **3a–3c** with halogenide anions, we also synthesized the products **4a–4d** in good yields from 65 to 88% by using anion exchange reactions with silver salts (Scheme 3). It should be noted that the anion exchange itself is achieved in full conversion; however, the several filtration steps necessary to remove all silver particles from the products lead to lower yields.

These products contain anions that are similar to typical, commercially available imidazolium-based ionic liquids, and thus, unsurprisingly, all four products GVIM-OAc **4a**, GVIM-

Scheme 3. Anion Exchange Reactions



OMs **4b**, GVIM-OTf **4c**, and GVIM-NTf₂ **4d** are viscous liquids or waxes at room temperature.

Hydrogel Synthesis. Initially, GVIM-I **3a** as a monomer and 5.0 wt % MBAA (Figure 1), relative to the monomer weight, as a cross-linker were investigated for hydrogel synthesis. For the cross-linking polymerization between **3a** and MBAA, the radical initiators ammonium persulfate (APS) and tetramethyl ethylenediamine (TEMED) were used as this system has previously also been used for dialkyl substituted vinyl imidazolium ionic liquids.²⁸ The appropriate amounts of APS and TEMED in relation to the monomer or cross-linker usually differ for each monomer/cross-linker system,^{29–31} and in our case, a molar ratio (cross-linker:APS:TEMED) of 1:1.5:5.6 was found to lead to a successful gelation for **3a** and 5.0 wt % MBAA. This molar ratio was used as a starting point for all of the different monomers, cross-linkers, and cross-linker concentrations investigated in this work; however, individual optimization toward higher or lower amounts of APS/TEMED was still necessary in some cases. The used amounts of monomer, cross-linker, water, APS, and TEMED for each hydrogel composition can be found in the Supporting Information (Tables S1–S12).

A first characterization of the hydrogels was performed via ATR-IR. The IR spectra of the GVIM-MBAA and GVIM-PEGDA575 hydrogels are mostly a direct combination of the spectrum of GVIM-I **3a** and the corresponding cross-linker. The fingerprint area of the carbohydrate monomer is also present in the spectra of the hydrogels (see Supporting Information Figures S25–S28 for the IR spectra).

As a second characterization method, we also performed a solid-state ¹³C NMR of a GVIM-MBAA hydrogel. Solid-state NMR is necessary due to the insolubility of the hydrogels in any common solvent. For sample preparation, a dried hydrogel was milled into a powder and the said hydrogel powder was washed (three times with pure water, 10 min each) and dried again. The washing water was analyzed by NMR, and a mixture of unreacted GVIM-I and TEMED was found (see Supporting Information Figure S23). The solid-state ¹³C NMR of the GVIM-MBAA hydrogel shows broad signals as expected for polymers. In comparison to GVIM-I, a new broad signal of the polymeric backbone in the aliphatic region (~25 to 50 ppm) and a new signal at ~175 ppm for the amide group of MBAA can be seen, while the characteristic vinyl-CH₂ signal at ~110 ppm disappeared, thus proving the formation of the expected polymeric structure (see Supporting Information Figure S24).

Hydrogels: Swelling Behavior. A key property of hydrogels is their ability to absorb large amounts of water reversibly, increasing in mass and volume without losing their structure or shape. Hydrogel swelling consists of two separate transport processes. First, the solvent converges through the pores of the gel. Then, the solvent diffuses between the struts of the polymer network.^{28,32} Several parameters affect the swelling of the hydrogels. In addition to solvent motion and interaction with the polymeric network, these include

thermodynamic compatibility, the nature of the cross-linker, its chain length, and the degree of swelling.²⁸

Our initial hydrogel of **3a** with 5.0 wt % MBAA was first investigated in ultrapure water, isotonic sodium chloride solution, and phosphate buffered saline (PBS, pH = 7.4). Figure 3 shows that, independent of the medium, the mass of

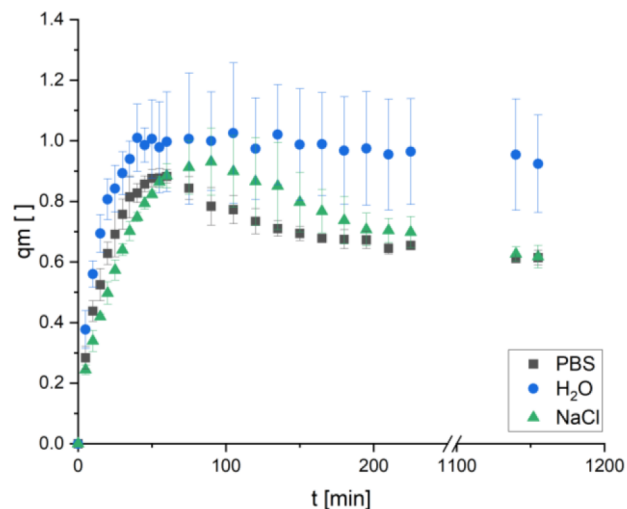


Figure 3. Swelling tests of GVIM-I hydrogels with 5.0 wt % MBAA in water, isotonic NaCl, and PBS (pH = 7.4) (37 ± 1 °C; $n = 3$).

the hydrogels increased continuously over time until a maximum was reached. The hydrogels swollen in ultrapure water reach a plateau in their weight, while the mass of the hydrogels swollen in isotonic sodium chloride or PBS solutions decreased after their maximum. After some time, these gels also reached an equilibrium. The reason for this is an ion exchange of the hydrogels (I^-) with the medium (Cl^- or phosphate). The anion exchange could be proven by performing ion chromatography of the medium before and after swelling (see Supporting Information Figures S31–33 for the ion chromatograms).

After this initial test, we next tested the influence of different anions present in **3a–3c** (I^- = iodide, Br^- = bromide, Cl^- = chloride) and **4a–4d** (OAc^- = acetate, OMs^- = mesylate, OTf^- = triflate, and NTf_2^- = bistriflimide) on the swelling behavior (Figure 4). While **3a–3c** were all suitable monomers and easily lead to hydrogels under our radical polymerization conditions, the production of hydrogels from **4a–4d** proved to be more difficult, as even small amounts of remaining silver from the anion exchange step (Scheme 3) will prematurely catalyze the radical polymerization.³³ This can be avoided by thoroughly purifying the products **4a–4d** from any remaining silver with activated charcoal.

To directly compare the influence of the different anions on the swelling behavior without factors like anion exchange, these swelling curves were measured in ultrapure water. It can be discerned from Figure 4 that the overall chemical behavior of each anion directly correlates with the swelling degrees of the hydrogels. More hydrophobic anions, like the fluorinated OTf^- and NTf_2^- anions, lead to comparatively lower swelling degrees, while the nonfluorinated counterpart of OTf^- , the mesylate OMs^- , reaches a higher swelling degree. Interestingly, the chemically similar halogenides exhibit notably different swelling degrees, with around 2.2 for chloride, 1.7 for bromide, and 1.0 for iodide. This may be explained with the increasing

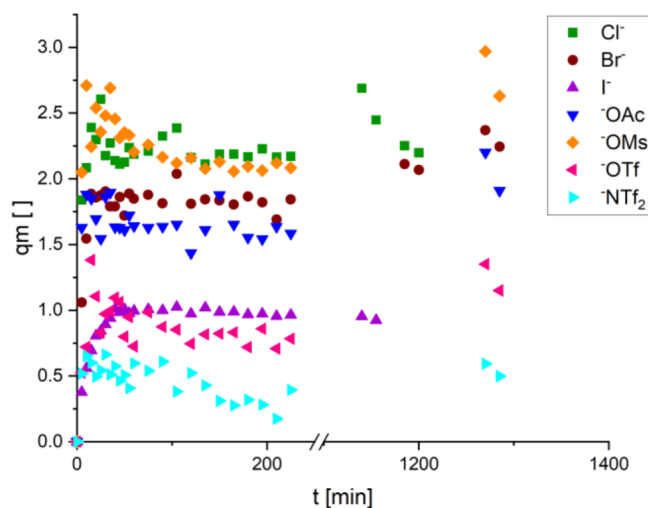


Figure 4. Swelling tests of GVIM hydrogels with varying anions with 5.0 wt % MBAA in water (37 ± 1 °C; $n = 3$; the standard deviations were omitted for more clarity, see Supporting Information Figure S34 including standard deviations).

atom size from chloride to iodide, which then leads to less space for the water to occupy in the polymeric hydrogel network. Alternatively, the presence of the different anions during radical polymerization could potentially affect the polymerization process itself, thus leading the structurally changed networks.

For the following experiments with different cross-linker types and concentrations, we decided to continue with only GVIM-I **3a** as a monomer since this monomer has the most yield-efficient synthesis of all products (Scheme 2) and it leads to the most form-stable, flexible, and reproducible hydrogels from all of the tested GVIM monomers **3a–3c** and **4a–4d**. We furthermore decided to continue all further swelling experiments in PBS (pH = 7.4) since we aim to study the use of our hydrogels as drug delivery systems in the future. PBS provides a constant pH value and is comparable to the osmolarity and ion concentration of the human organism.³⁴

The swelling behavior of MBAA was next tested in cross-linker concentrations between 4.5 and 7.0 wt % in relation to the weight of monomer (Figure 5). These concentrations are the upper and lower limit for MBAA in the hydrogel synthesis as no gelation takes place below 4.5 wt % and the gelation process becomes too fast for handling above 7.0 wt %. Independent of the cross-linker content, the mass of the hydrogels initially increased continuously over time until they reached a maximum weight value. After 35 min, the gels with 5.5 wt % MBAA were the first to reach their maximum swelling degree of 0.79. All other gels reached the maximum value between 50 and 60 min. The gel with 4.5 wt % MBAA swelled the most, and the maximum degree of swelling was 0.90. It was expected that the gels with the lowest amount of cross-linker would swell the most and that the degree of swelling would decrease with an increase in amount of cross-linker since a higher degree of cross-linking leads to smaller meshes in the polymer network for the water to occupy.³⁵ In principle, this trend can also be seen in Figure 5 with the gels with 4.5 wt % MBAA, the lowest cross-linker concentration, exhibiting the highest swelling degree. However, the swelling degree of 6.5 wt % is unexpectedly the lowest, and not 7.0 wt %.

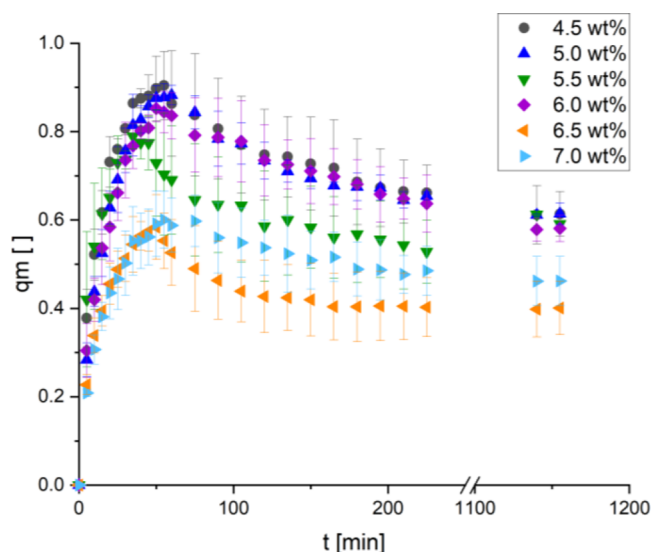


Figure 5. Swelling tests of GVIM-I hydrogels with different MBAA amounts in PBS, pH = 7.4 (37 ± 1 °C; $n = 3$).

In addition to MBAA, DHEBA (Figure 1) was also investigated as a structurally similar acrylamide-based cross-linker (Figure 6). The use of DHEBA as a cross-linker led to a

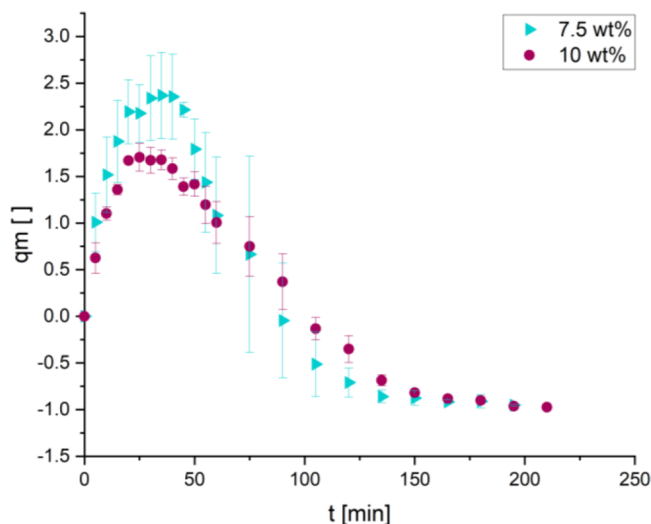


Figure 6. Swelling tests of GVIM-I hydrogels with two different DHEBA amounts in PBS, pH = 7.4 (37 ± 1 °C; $n \geq 2$).

2.65-fold increase in the degree of swelling compared to the swelling degree of MBAA-gels. This was expected due to the additional hydroxy groups present in this cross-linker. Furthermore, Figure 6 shows clearly that at 7.5 wt % DHEBA, the gels absorbed more water at their maximum swelling degree than the gels with 10 wt %, as expected. Interestingly, the gels degraded completely within a few hours. Just like the MBAA-gels, the DHEBA gels initially increased until they reached their maximum degree of swelling. After the maximum, the masses decreased steadily until they reached their dry weight and subsequently lost even more mass until they finally completely degraded. It is assumed that the degradation is caused by splitting of the polymer network. Since the cross-linking between GVIM-I 3a and the acrylamide groups of MBAA leads to stable hydrogels, it can be assumed

that the dihydroxy ethyl functionality of DHEBA, its only difference from MBAA, is the cause of this degradation. No further DHEBA concentrations were investigated due to their instability and degradation.

Besides acrylamides, the second type of cross-linker studied were ethylene glycol diacrylates with different chain lengths. PEGDA and EGDMA (Figure 1) are well-known in the literature as cross-linking agents for a number of hydrogels of different origins and show high biocompatibility in the 3D cross-linked state.^{36–38} This is of major importance for subsequent applications in the fields of medicine or biotechnology.^{39–42}

Since EGDMA was known and successfully used in the literature, this dimethacrylate cross-linker was started with. However, it was found that this cross-linker did not form reasonable hydrogels with GVIM-I 3a. They either gelled inhomogeneously or were not stable in shape. On the other hand, EGDA (Figure 1), which has the same structure as EGDMA, but without the additional methyl groups on the acrylate, was suitable for hydrogel synthesis. The EGDA hydrogels were firm and retained their shape, which made it possible to investigate their swelling behavior (Figure 7).

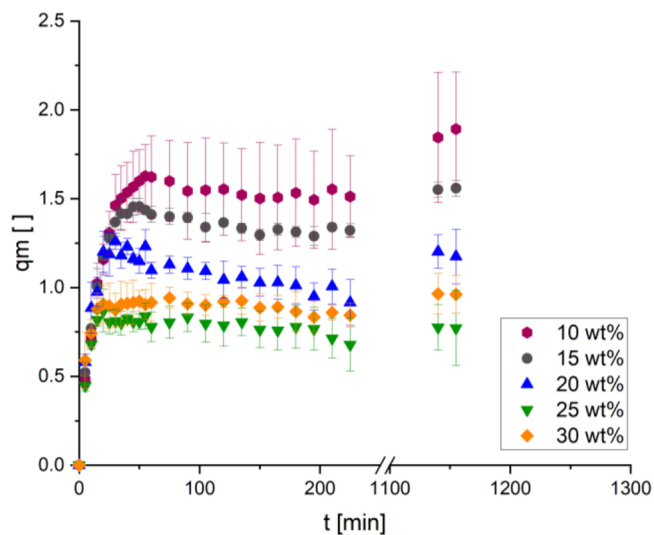


Figure 7. Swelling tests of GVIM-I hydrogels with different EGDA amounts in PBS, pH = 7.4 (37 ± 1 °C; $n = 3$).

The EGDA hydrogels were investigated with cross-linker concentrations between 10 wt %, which was the lowest possible concentration for gelation to take place, and 30 wt %. Gelation above 30 wt % was possible but led to more brittle hydrogels. On average, the EGDA cross-linked gels swelled more than the MBAA gels, although the cross-linker concentration was significantly higher. The highest swelling degree reached by the MBAA gels was 0.9 for 4.5 wt % MBAA, while the highest swelling degree of the EGDA gels was around 1.6 with 10 wt % EGDA. Interestingly, while the MBAA gels show a weight loss after their maximum, which was attributed to anion exchange processes, this effect cannot, or only slightly, be seen for the EGDA gels. Thus, the hydrogel composition of GVIM-I 3a and EDGA seems to lead to a decreased anion exchange in comparison to MBAA as a cross-linker. Last, the EDGA gels show a notable increase in their mass after 20 h of swelling. This indicates that the gels did not reach their

equilibrium yet (see Figure 13 for long-term swelling experiments).

Since PEGDA is a widely used cross-linker in addition to EGDA, PEGDA with three different chain lengths ($M_n = 250$, 575, and $700 \text{ g}\cdot\text{mol}^{-1}$) was used in this work (Figure 1).

Figure 8 shows the swelling behavior of different PEGDA250 concentrations. Like EDGA, the investigated

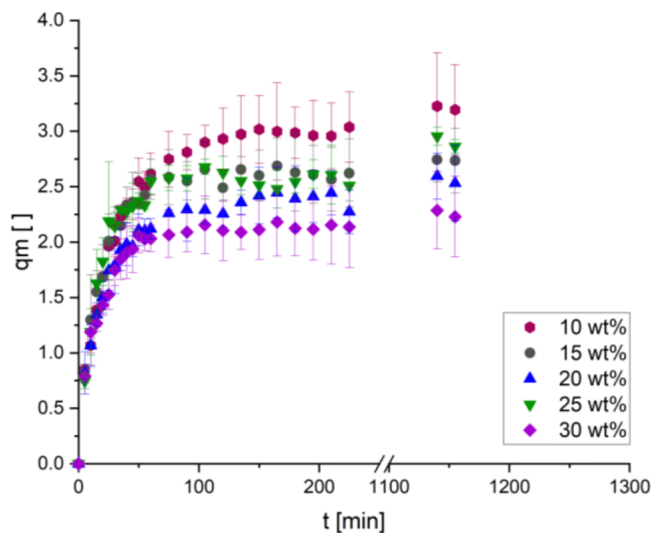


Figure 8. Swelling tests of GVIM-I hydrogels with different PEGDA250 amounts in PBS, pH = 7.4 ($37 \pm 1 \text{ }^\circ\text{C}$; $n = 3$).

cross-linker concentrations were between 10 and 30 wt %, which was found to be the optimal range for gelation. As with all gels considered so far, the mass of the PEGDA250 gels initially increased quickly, regardless of their cross-linker concentration. After the initial exponential swelling, they reached a plateau around their maximum weight. The hydrogel with the lowest cross-linker concentration (10 wt %) swelled the most and reached a degree of swelling of 3.04, while the gel with the highest PEGDA250 concentration (30 wt %) reached the lowest degree of swelling with 2.18, thus confirming again that lower cross-linker concentrations lead to higher swelling degrees. Compared to the previously used cross-linkers, the PEGDA250 hydrogels swelled the most.

After PEGDA250, PEGDA575 was used as the next cross-linker. Figure 9 shows examples of the freshly synthesized gel (I), which has a diameter of 10 mm due to the mold used for synthesis, the dried gel (II), which shrinks in size due to the removal of water, and the swollen gel (III), which increases in size by 1.5 to 15 mm.

The swelling behavior of PEGDA 575 hydrogels is shown in Figure 10. In difference to every other cross-linker investigated in this work, all PEGDA575 hydrogels, independent of their cross-linker concentration, swelled at the same rate and reached the same maximum degrees of swelling of 1.68. The only outlier here is the hydrogel produced with 15 wt % PEGDA575, which achieved a degree of swelling of 2.29. To rule out any mistakes during measurement, this particular concentration was produced and measured six times instead of the usual three times.

As the third and final candidate in the PEGDA series, PEGDA700 was also used as a cross-linker (Figure 11).

Similar to PEGDA575, the different concentrations of PEGDA700 show only a very small influence on the swelling

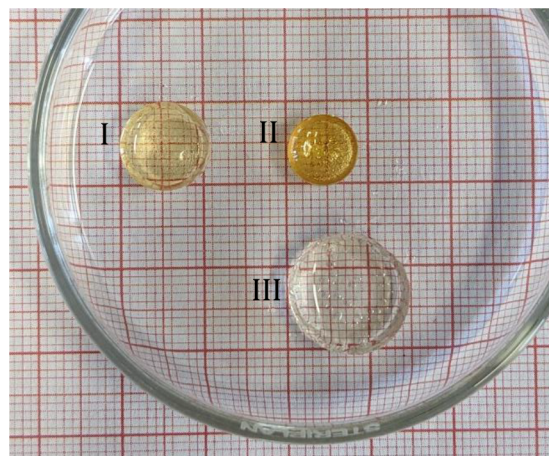


Figure 9. PEGDA575 gels in three different stadiums. (I = fresh synthesized, II = dried for 3 days at room temperature, III = swollen gel).

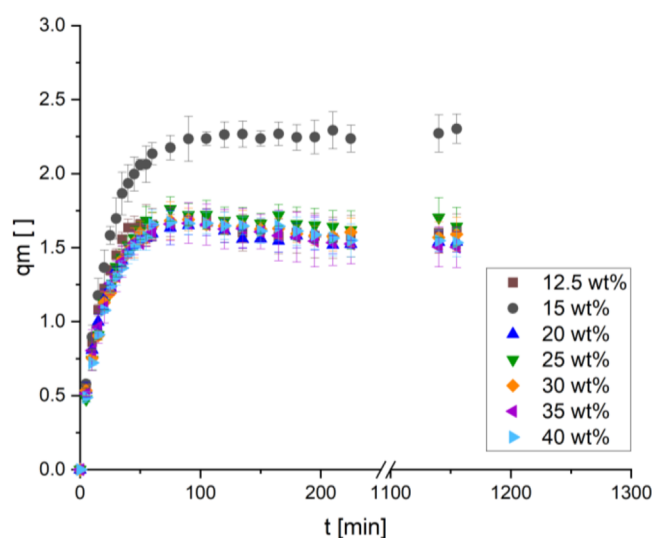


Figure 10. Swelling tests of GVIM-I hydrogels with different PEGDA575 amounts in PBS, pH = 7.4 ($37 \pm 1 \text{ }^\circ\text{C}$; $n = 3$).

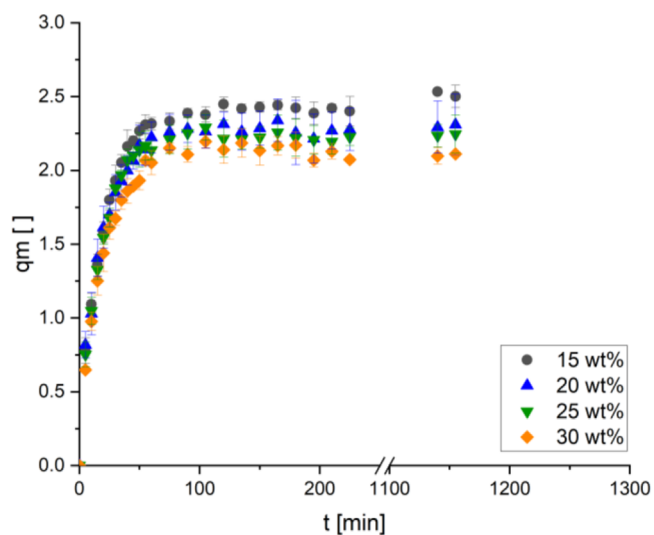


Figure 11. Swelling tests of GVIM-I hydrogels with different PEGDA700 amounts in PBS, pH = 7.4 ($37 \pm 1 \text{ }^\circ\text{C}$; $n = 3$).

degree, though the already discussed trend of lower cross-linker concentrations leading to higher swelling degrees can also be seen with PEGDA700. The swelling degrees here are between 2.20 and 2.45.

To achieve a direct comparison between the three similar PEGDA cross-linkers, 10 wt % PEGDA250, 25 wt % PEGDA575, and 30 wt % PEGDA700 are plotted together in Figure 12, as these three swelling curves correlate to roughly

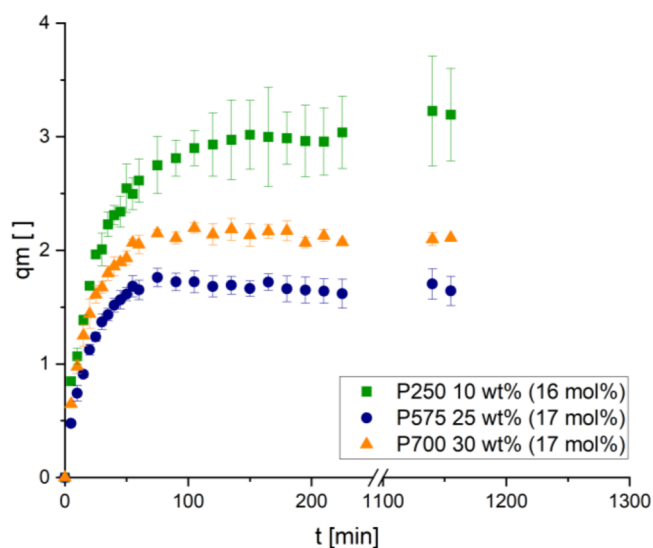


Figure 12. Swelling diagram of all three PEGDA cross-linkers with 16 to 17 mol % of PEGDA.

the same mol % of cross-linker (16–17 mol %). In this comparison, PEGDA700 as the longest of the three cross-linkers would be expected to lead to the highest swelling degrees (due to bigger pore sizes available for water to occupy) followed by PEGDA575 and PEGDA250. This trend however cannot be seen in Figure 12, as in our case, PEGDA250 leads to the highest swelling degree in the direct comparison followed by PEGDA700 and then PEGDA 575. It is possible that other effects, like additional van der Waals forces, influence this unsuspected trend.

Overall, the tested cross-linkers MBAA, EGDA, PEGDA250, PEGDA575, and PEGDA700 all exhibited the same swelling behavior of reaching a clear plateau, an equilibrium, after an initial exponential swelling phase. However, some hydrogels, most clearly the ones produced with EDGA and PEGDA250, continued to gain weight after a total of 19 to 20 h in PBS. To assess the long-term behavior of the gels, a long-term swelling was carried out. It was decided to use 5 wt % MBAA and 15 wt % cross-linker concentrations for all diacrylate cross-linkers. The hydrogels were prepared and evaluated in the same way as the other swelling tests. The hydrogels were placed in PBS (37 ± 1 °C, pH = 7.4), and the mass was taken every 24 h (Figure 13).

The MBAA gels already reached their maximum degree of swelling after 24 h and then remained in equilibrium. Their shape was stable over the entire time, and there was no visible degradation. In contrast, the degree of swelling of all (poly)ethylene glycol diacrylate gels increased with each measurement. During the increase in mass, the diacrylate gels gradually lost their shape until a complete degradation took place. The PEGDA700 gels were the first to degrade after 7 days, the PEGDA250 and EGDA gels followed on days 8 and

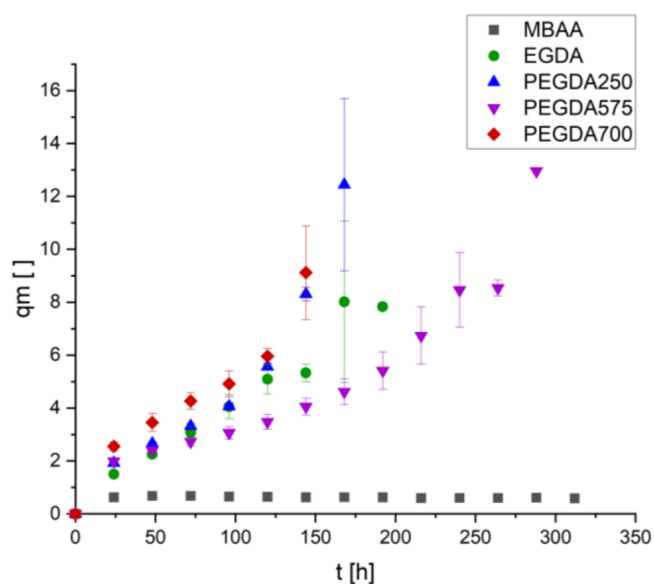


Figure 13. Long-term swelling of the hydrogels with 5 wt % MBAA and 15 wt % diacrylate cross-linkers in PBS, pH = 7.4 (37 ± 1 °C; $n = 3$).

9, respectively, and after a total of 14 days, the PEGDA575 gels were also completely degraded. The maximum degrees of swelling achieved by the gels in this long-term experiment ranged from 0.68 (5 wt % MBAA) to 12.96 (15 wt % PEGDA575).

CONCLUSIONS

In this work, we synthesized seven novel glucose-based vinyl imidazolium (GVIM) monomers with varying anions. These GVIM monomers have been used for the production of novel semisynthetic hydrogels with a unique cationic carbohydrate-based polymeric network, which cannot be found in previously known natural carbohydrate-based hydrogels, which are either neutral (e.g., cellulose and chitosan) or anionic (e.g., alginate).

We overall evaluated the influence of the counteranion as well as the type and concentration of each tested commercially available cross-linker (MBAA, DHEBA, EGDMA, EGDA, and PEGDA) on the swelling behavior of the GVIM hydrogels. In the case of the anions, both the anion size and their hydrophilicity or hydrophobicity have been found to influence the swelling behavior. GVIM iodide, which can be produced in a simple two-step process with a high total yield of 71% and which was recently evaluated by our group as remarkably biocompatible in comparison to the dialkyl imidazolium ionic liquids, was found to be the most promising of the GVIM monomers.

The bis(acrylamide) cross-linker MBAA leads to overall lower swelling degrees than the (poly)ethylene glycol cross-linkers EGDA and PEGDA; however, the GVIM-MBAA hydrogels exhibit a high long-term stability, while the GVIM-(P)EDGA hydrogels all degrade over time. This opens up unique applications of each hydrogel depending on the cross-linker. In the case of the different cross-linker concentrations, we found that the swelling degree of the hydrogel decreases with increasing cross-linker amounts. Both DHEBA and EGDMA were found to be unsuitable as cross-linkers for our monomer.

As the next steps, we aim to measure the biocompatibility and antimicrobial properties of our hydrogels and, assuming a

high biocompatibility, to evaluate our GVIM hydrogels as drug delivery systems, where we expect interesting ion controlled properties.

■ ASSOCIATED CONTENT

SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsomega.3c06804>.

All ^1H , ^{13}C , and ^{19}F NMR spectra of the products described in this work, experimental setup of the hydrogel synthesis and swelling degree measurements, ion chromatograms of the PBS buffer before and after swelling, IR spectra of selected starting materials and hydrogels, and composition of the hydrogels (PDF)

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Author Contributions

All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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III. Publikation III

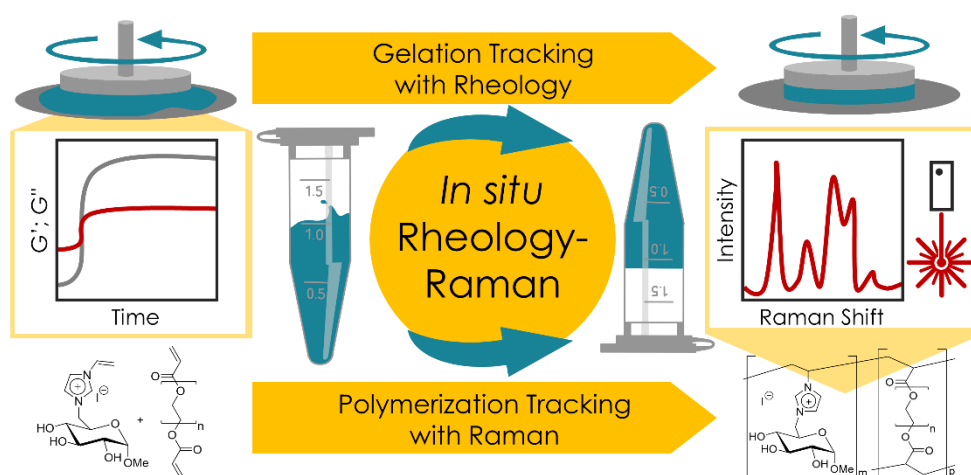
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A novel characterisation technique for hydrogels – in situ Rheology-Raman spectroscopy for gelation and polymerization tracking

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A novel characterization technique for hydrogels – *in situ* rheology-Raman spectroscopy for gelation and polymerization tracking†

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The interest in hydrogels has grown considerably across a number of disciplines, including but not limited to the immobilization of (bio)catalysts in matrices and in the medical sector, for example, in drug delivery systems, contact lenses, biosensors, electrodes, and tissue engineering. Consequently, the characterization of these materials is frequently the subject of cutting-edge research. However, hydrogels are often insoluble, which precludes the use of many analytical methods, such as nuclear magnetic resonance (NMR). Consequently, other established analytical techniques, such as attenuated total reflection (ATR), infrared spectroscopy (IR), Raman spectroscopy, or rheological measurements, are frequently employed. These methods are generally straightforward to use and can be completed rapidly. However, IR spectroscopy, for instance, is inherently limited by the interference of water's vibrational bands. In this study, we present a method for the characterization of hydrogels that can simultaneously observe the gelation and polymerization of hydrogels.

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Introduction

Hydrogels are three-dimensional (3D) networks, consisting of hydrophilic polymer structures that do not dissolve in water but, conversely, absorb large amounts of water or aqueous media. While swelling, hydrogels retain their 3D shape, while increasing their volume.^{1–4} These polymers are well established in various disciplines, such as biotechnology (stent-coating),^{5–7} biomedicine (drug-delivery, tissue engineering),^{8,9} agriculture (water storage),¹⁰ and catalysis (immobilization matrix).^{11,12} The versatility of hydrogels in terms of their chemical structure and properties allows them to be applied in this wide range of possible applications.¹³ The specific type of hydrogel employed in this study was a semi-synthetic carbohydrate-based ionic hydrogel.

These hydrogels were synthesized *via* free radical polymerization and with different types and amounts of crosslinkers, including polyethylene glycol diacrylate (PEGDA) (Scheme 1).

Various methods have already been established to analyze the structure of hydrogels and their precursors, such as monomers or polymer chains that need to be crosslinked in 3D. These methods include microscopy (such as scanning electron microscopy (SEM), transmission electron microscopy (TEM) and related techniques) to determine the local hydrogel structure, (solid-state) nuclear magnetic resonance (NMR) as an analytical technique to analyze the chemical structure, as well as Raman spectroscopy as a technique to measure the molecular interaction. In addition, Fourier transform infra-red (FT-IR) spectroscopy and attenuated transmission reflectance Fourier transform infra-red (FT-IR) spectroscopy (ATR-FTIR) can be employed to quantify the chemical composition of a given sample by measuring the vibrations that occur as a result of the sample's composition. These techniques are effective, simple, and rapid. The thermal stability of hydrogels can be investigated through the use of thermal gravimetric analysis (TGA) and differential scanning calorimetry (DSC). The characterization of the mechanical properties of hydrogels is best achieved through the utilization of rheological measurements. The synthesis and characterization of hydrogels is a broad research field. As this article covers only provides a brief overview of a few methods, we would like to direct interested readers to a comprehensive review article by Raghuwanshi and Garnier, presenting a good overview of the mentioned methods for characterizing hydrogels with the respective advantages and drawbacks, and the expansion of this article by Patel and Thareja.^{14,15}

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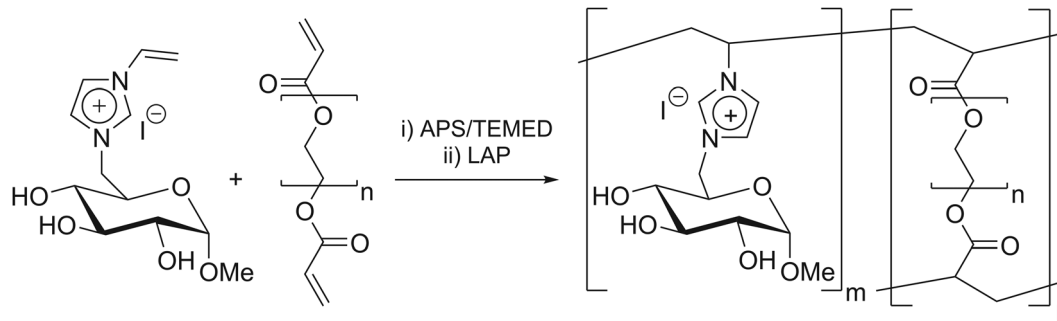
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Scheme 1 Synthesis of polyGVIM-I via radical polymerization with PEGDA as crosslinker.

On the one hand, NMR spectroscopy is usually performed in solution and is therefore generally not applicable to insoluble hydrogels. It can be used for the characterization of the monomers and crosslinkers prior to hydrogel formation. On the other hand, solid state NMR can be used to determine the chemical structure of hydrogels, as recently shown by our group.¹⁶

SEM and associated variance techniques provide information about the surface morphology, additives in the gel, and inter-connecting pores. It is, however, essential that the sample must be prepared by drying, as the presence of water significantly disturbs the vacuum and impedes the imaging process during the measurement. Two further options are available for sample preparation: air drying and freeze drying. However, these can lead to the formation of artifacts and impair the morphology of the sample.^{17–19} An alternative is supercritical drying with liquid CO₂. This protects a gel from pore collapse and the structure remains the same in the dried state as in the wet state.²⁰

IR spectroscopy is a simple, non-destructive, and sensitive characterization technique that requires minimal sample preparation. Das *et al.* employed the FTIR technique to analyze the functional groups of their cellulose materials, thereby confirming the reaction between the hydroxyl and carboxyl groups during the crosslinking process due to the diminution of the hydroxyl group's stretching vibration.²¹ Mao *et al.* and Azam *et al.* have recently used FTIR to identify the chemical composition and structural characteristics of their alginate hydrogels, to cite a few examples.^{22,23} Nevertheless, this technique is not without significant limitations. The strong bands of water present in the hydrogel may overlap with the bands from the polymer phase, thereby dominating them due to their intense nature.^{15,24–26}

Raman spectroscopy, which is complementary in nature to IR spectroscopy, can be used in addition to it, but also be employed as its own characterization method.^{27–29} The water bands are much less dominant than in IR spectroscopy and do hardly interfere with the Raman bands. Nie *et al.* employed IR and Raman spectroscopy to investigate the effect of calcium ions on the structure of their cellulose-based hydrogel beads.³⁰ Affatato *et al.* demonstrated, using Raman spectroscopy, that C=C double bonds were not fully converted and determined the degree of crosslinking. Additionally, it was observed that the washing of the hydrogels in an aqueous medium resulted in the leaching of the unreacted crosslinker.³¹

Rheological measurements are crucial for characterizing hydrogels. One important parameter derived from rheological investigations is the viscoelastic response.³² The measurement of the shear strain provides insight into the stiffness and stability of the hydrogel against shear stress, enabling the monitoring of its formation. An understanding of the rheological behavior of hydrogels is essential. The rheological parameters, such as viscosity, thixotropy, and viscoelastic behavior have a significant impact on the success of 3D-printing, as demonstrated by recent studies.^{33–38} These parameters are also relevant in other fields such as the food industry,^{39,40} cell culture, or tissue engineering.^{41,42}

One of the mentioned methods alone cannot fully characterize the chemical structure and the properties of a sample. It is always necessary to combine several methods.^{14,29} One option for using two methods simultaneously is the rheology-Raman combination, which enables the recording of Raman spectra and simultaneously investigate the rheological properties of a sample. Völker-Pop's group has used the rheology-Raman combination to characterize polymer melts and the curing behavior of epoxy resins. The transition to the solid phase, as observed by rheological measurements, was found to occur at a higher temperature than the crystallization indicated by the Raman data.⁴³ Kida *et al.* investigated the flow-induced crystallization (FIC) of high-density polyethylene. The effect of shear rate, shear-flow time, and molecular weight on the time dependencies of the different conformer mass fractions of the molecular chains during crystallization was analyzed, and it was found that the *trans* chains formed during isothermal crystallization were strongly dependent on the FIC conditions.⁴⁴ In this work, the rheology-Raman combination is introduced as a powerful tool for characterizing hydrogels, and a comparison is made between this method and established methods.

Experimental

Materials

The chemicals methyl- α -D-glucopyranoside (99%), triphenylphosphine (99%), imidazole (99%), N-vinylimidazole (99%), ethyleneglycol diacrylate (EGDA; >90%) and ammonium persulfate (APS; 98%) have been supplied by Thermo Fisher Scientific and were used as received. Polyethylene glycol diacrylate (PEGDA; $M_n = 250, 575$ and 700 g mol^{-1}), N,N'-methylene



bisacrylamide (MBA; 99%) and *N,N,N',N'*-tetramethylethylenediamine (TEMED; >99%) were supplied by Sigma-Aldrich (Germany) and were used as received. Iodine (>99.5%) was supplied by Carbolution. Lithium phenyl(2,4,6-trimethylbenzoyl) phosphinate (LAP; >98%) was supplied by TCI and was used as received. The solvents THF (99.9%) and ethyl acetate (99.7%) were supplied by Honeywell Riedel-de-Haën and the solvents chloroform (>99.8%), methanol (99.8%) and DMF (99.5%) were supplied by Thermo Fisher Scientific and were used as received. Column chromatography was performed with silica gel (230–400 mesh particle size) supplied by Supelco (Germany). Additionally, phosphate-buffered saline (PBS, Thermo Fisher Scientific Inc., Waltham, USA) was used as received.

Procedure for the syntheses of the 1-(methyl- α -D-glucopyranosid-6-yl)-3-vinylimidazolium iodide hydrogels (polyGVIM-I)

The method for synthesizing the monomer 1-(methyl- α -D-glucopyranosid-6-yl)-3-vinylimidazolium iodide (GVIM-I) has been previously described by our group.¹⁶ Furthermore, the method and analytics are outlined in the ESI.† The hydrogels in this study were produced using two different methods: (i) with APS and TEMED as the initiator system and (ii) with LAP as the photoinitiator. In the initial approach, GVIM-I (1.25 mol L⁻¹) was dissolved in PBS and the appropriate weight-percent of the crosslinker was added. Radical polymerization (Scheme 1) was initiated by adding the APS solution and TEMED. Once the solution had been thoroughly mixed for 10 s, the gelation took place within seconds or a few minutes. In the second method, GVIM-I (1.25 mol L⁻¹), crosslinker (10, 15 or 20 mol%) and the appropriate weight-percent of LAP were dissolved in PBS (pH = 7.4). The monomer solution was photopolymerized with an UV intensity ($\lambda = 365$ nm, Biolinker, VILBER, Collégien, France) of 1.2 J cm² and 2.4 J cm², respectively.

Characterization of the monomers and hydrogels

NMR spectra. The NMR spectra were recorded on a Bruker AVANCE 250 II, 300 III or 500. DMSO-*d*₆ was calibrated as 2.49 (¹H) and 39.50 (¹³C). D₂O was calibrated as 4.80 (¹H).

ATR-FTIR spectra. ATR-FTIR spectra were obtained using a Specac Single reflection diamond ATR-Quest at room temperature. The hydrogel sample was prepared according to (ii) with 10.4 mol% of the crosslinker PEGDA 575 and then freeze-dried overnight.

Raman spectra. Raman spectra of the samples were recorded on a Cora 5001 (Anton Paar GmbH, Graz, Austria) at a wavelength of 785 nm and 1064 nm.

In situ IR spectra. *In situ* IR spectra were obtained using a Mettler Toledo React-IR 15 with a diamond probe at room temperature. The sample was prepared in accordance with the first method (i), but without the addition TEMED. The probe was introduced into the vial containing the sample solution. After recording a spectrum, the vial was removed, TEMED was added, mixed thoroughly for 5 s and the probe was immersed again.

Rheological characterization. The gelation of hydrogels prepared after step (ii) was monitored *via in situ* crosslinking using UV irradiation (Delolux 80, Delo, Germany), with a light intensity of 20 mW cm⁻² at 365 nm and a UV irradiation duration of 5 min. The storage and loss moduli were determined *via* a time sweep oscillatory test, with a constant strain amplitude of 0.1% and at a constant frequency of 1 Hz. The mechanical stiffness of the photopolymerized hydrogels was determined at 37 °C by using oscillatory rheology (amplitude sweep). The MCR 302 modular compact rheometer (Anton Paar GmbH, Graz, Austria) equipped with a plate–plate geometry (20 mm diameter plate), was utilized, with the oscillation was varying from 0.1 to 1000%.

In situ rheology-Raman spectroscopy. The gelation tracking of hydrogels produced after (i) were determined at 37 °C by using MCR 302e modular compact rheometer (Anton Paar GmbH, Graz, Austria) equipped with plate–plate geometry (25 mm diameter plate). Storage and loss moduli were measured in a time sweep oscillatory test with a constant strain amplitude of 0.1% and at a constant frequency of 1 Hz. The mechanical stiffness of the hydrogels was determined at 37 °C by means of oscillatory rheology, in which the oscillation was varied from 0.1 to 1000%. A Cora 5001 (Anton Paar GmbH, Graz, Austria) was used for the simultaneous recording of the Raman spectra at a wavelength of 785 nm.

Results and discussion

Hydrogel characterization with ATR-FTIR spectroscopy

The ATR-FTIR spectra of GVIM-I (red), the reaction solution with GVIM-I solved in PBS containing crosslinker and APS (blue) and a freeze-dried polyGVIM-I with PEGDA 575 as crosslinker and APS/TEMED as initiator system (black) are shown in Fig. 1A. The broad band observed in the range of 3150 to 3600 cm⁻¹ is attributed to –OH stretching vibrations. The various C–H vibrations are observed within the range of 3088 cm⁻¹ to 2833 cm⁻¹. The typical vibrations of the imidazole ring are present in the range of 1550–1570 cm⁻¹ (C=N vibration) and at 1450 cm⁻¹ (deformation vibration of the –CH backbone). In the fingerprint region, the C–O–C stretching vibration can be observed at 1133 cm⁻¹ and 896 cm⁻¹, while the stretching and deformation vibration of CH from the imidazole ring are found at 1092 cm⁻¹. The C=CH₂ vibrations are observed in the range from 920 to 956 cm⁻¹.^{45,46} The spectra of the GVIM-I and the hydrogel polyGVIM-I differ significantly in the visible vibrations of the C=C double bond in the monomer spectrum (highlighted in grey). The band at 1605 cm⁻¹ can be attributed to the stretching vibrations, while the band at 927 cm⁻¹ can be associated with the bending vibrations of the vinyl group.

Hydrogel characterization with *in situ* IR spectroscopy

In order to gain a deeper insight into the polymerization process of the hydrogel and to monitor the bands of interest, an *in situ* IR measurement was conducted. The polymerization was tracked for a total of 90 min. The initial 8 min of the measurement are presented in this manuscript, as no change



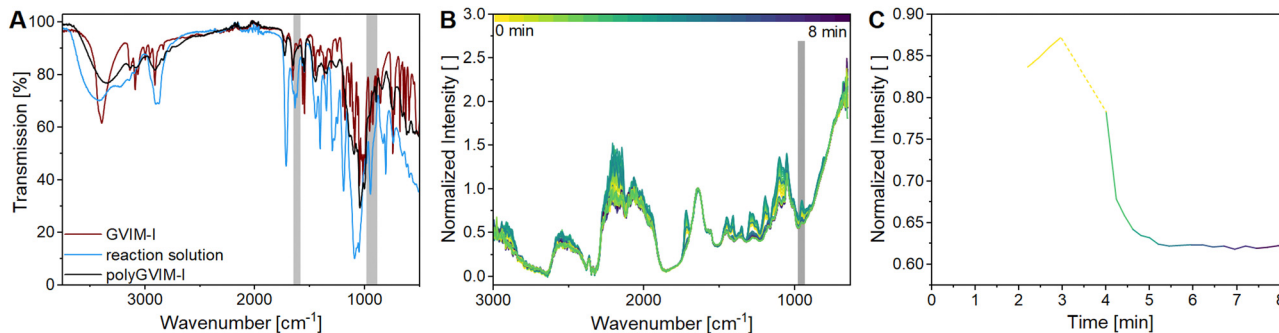


Fig. 1 IR spectra of the carbohydrate-based hydrogels *via* ATR-FTIR spectroscopy and *in situ* IR spectra: (A) ATR-FTIR spectra of GVIM-I, the reaction solution (containing GVIM-I, PEGDA 575 and APS solved in PBS) and polyGVIM-I with PEGDA 575 as crosslinker (freeze-dried). Grey highlighted are the significant vibration bands of C=C double bonds (1605 cm^{-1} and 927 cm^{-1}), showing only in the IR spectrum of GVIM-I and the reaction solution, but not in polyGVIM-I. (B) *In situ* IR spectra for polymerization tracking of polyGVIM-I with PEGDA 575 as crosslinker and APS/TEMED as an initiator system. Grey highlighted is one of the significant vibration bands of the C=C double bond (955 cm^{-1}), spectrum was normalized on the band at 1637 cm^{-1} . (C) Time trace of the 955 cm^{-1} band during polymerization tracking of the hydrogel (from B highlighted in grey from 969 to 917 cm^{-1}). The dashed line represents the progression of the intensity from initial intensity without TEMED until the addition of the radical starter TEMED and consequently the start of the radical polymerization ($C_{\text{GVIM-I}} = 1.25\text{ mol L}^{-1}$, $C_{\text{PEGDA575}} = 10.4\text{ mol}\%$).

occurred afterwards (Fig. 1B). In this experiment, APS and TEMED were used as the initiator system. Additionally, all bands above 2000 cm^{-1} are indicative of noise due to the resolution of the Mettler Toledo React-IR 15 used in this experiment, and the fact that the baseline is not flat, the spectra are difficult to interpret. Besides, several bands do not correspond to those depicted in Fig. 1A. This discrepancy may be due to the fact that the samples measured *in situ* are in an aqueous solution and consist of a mixture of the crosslinking components (GVIM-I, PEGDA, APS, and TEMED). Despite this, the bending vibration bands of the C=C double bond at 955 cm^{-1} could be detected and are highlighted in grey in Fig. 1B, whereas the tracing of the intensity decrease is shown in Fig. 1C. The initial intensity of the reaction solution and the intensity of the first measurement in the gelation process are connected by a dashed line. During this interval, the reaction solution was supplemented with TEMED, thereby initiating the polymerization of the monomer to form the hydrogel. The graph shows that the intensity of the band of C=C double bonds is high before the addition of TEMED and decreases rapidly as soon as it is added to the reaction solution. During polymerization, the C=C double bonds of GVIM-I and the crosslinker react, causing the intensity to decrease. After about 7 minutes, the intensity of the band reaches a steady state, indicating that the polymerization is complete.

Hydrogel characterization with rheology

A comprehensive understanding of the relationship between the chemical structure and physical behavior of hydrogels is crucial for possible applications.^{13,47,48} For example, the migration, proliferation and differentiation of cells are closely linked to the mechanical properties of hydrogels. Various levels of stiffness lead to stem cells differentiating into different cell types.^{49,50} The stiffness and other relevant properties of a hydrogel can be determined using rheological measurements. Furthermore, in the production of ceramic inks for direct ink writing, an understanding of rheological properties is crucial

for the efficient design of ceramic inks with enhanced performance.⁵¹ In 3D printing, there are many different areas where understanding these properties is of great economic importance. For example, in the development of inks for extrusion-based 3D bioprinting to obtain bioinks with high structural fidelity or in 3D printing in the food industry for the production and improvement of bigels and edible gels.^{52–56}

In this work, the rheological behaviour of the hydrogels was determined *via* gelation tracking and amplitude sweep measurements. The gelation tracking method is of particular interest for the hydrogel characterization, to estimate an optimal gelation time.

Fig. 2A shows the formation of a hydrogel following the shear storage modulus G' and the loss modulus G'' as a function of time. G' represents the elastic part of the viscoelastic behaviour and stands for the stored deformation energy. In contrast G'' characterises the viscous part of the viscoelastic behaviour, representing the deformation energy that is lost due to internal friction during flow.^{57,58}

At the start of the measurement, the value of G'' is greater than G' , because the monomer solution is a liquid. As soon as the UV lamp is switched on, gelation begins. This is clearly shown by the increase in G' . The point where $G' = G''$ is referred to as the crossing point or gel point and is labelled t_c in the diagram. It indicates the transition from a liquid-like behaviour to a solid-like behavior.⁵⁷ The value of G' increases furthermore until it reaches a steady state plateau. This is marked as G'_∞ in the diagram. The gelation time, the crossing point of G' and G'' , and G'_∞ value depend on the polymerization rate, crosslinking density, crosslinker structure, and type of crosslinking.^{59,60}

Fig. 2C shows the results of testing three samples with the same monomer concentration (1.25 mol L^{-1}) but different crosslinker concentrations (10, 15 and 20 mol%). The values of G' for all three samples increased rapidly as soon as the UV lamp was switched on, with G'_∞ values being reached after approximately 100 s. The stiffness of the sample is directly proportional to its G' value. As expected, the hydrogel became



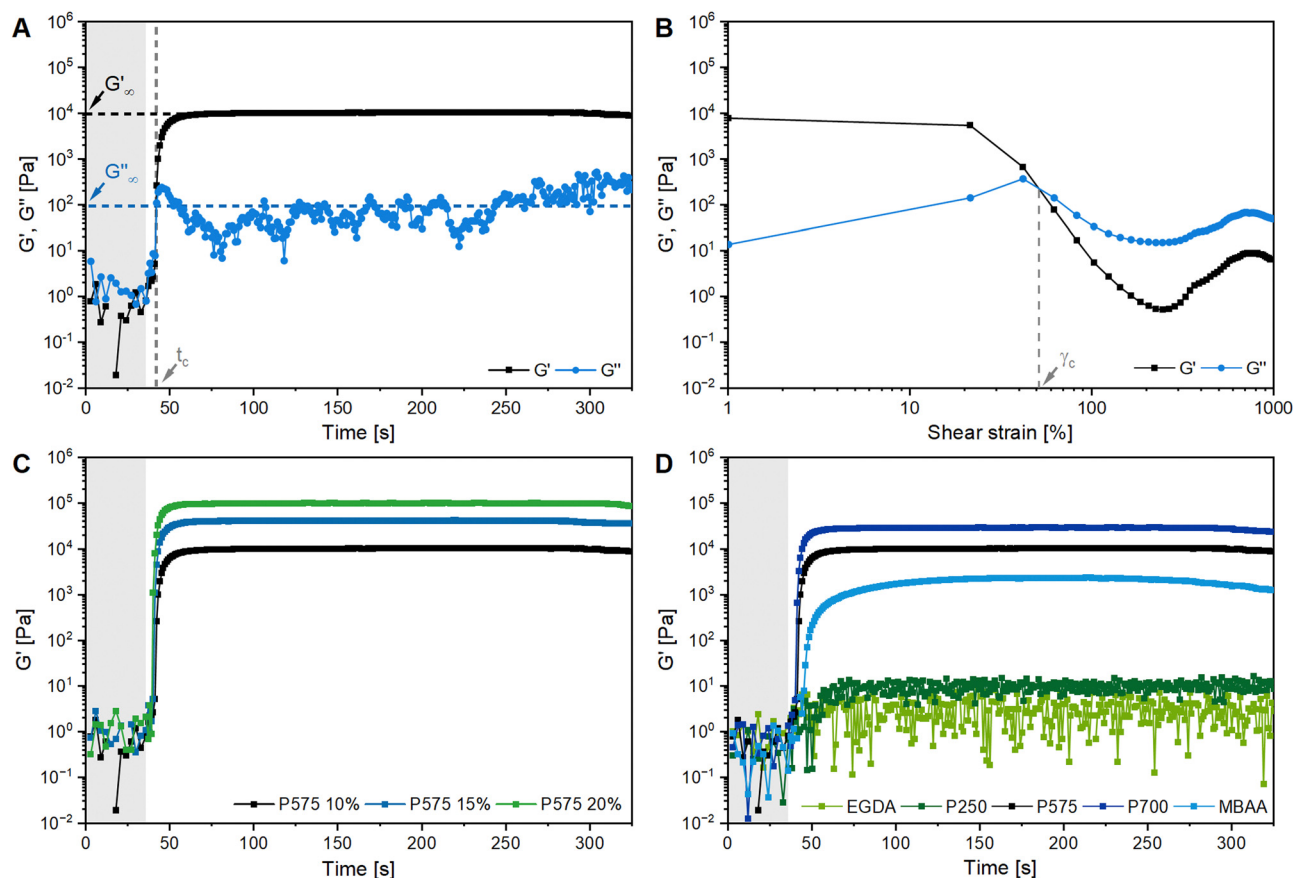


Fig. 2 Rheological characterization of polyGVIM-I hydrogels with LAP as photoinitiator: (A) gelation tracking of polyGVIM-I with PEGDA 575 (10 mol%) as crosslinker and (B) the following amplitude sweep measurement of the same hydrogel, with G' and G'' plotted against the shear strain. Gelation tracking of polyGVIM-I hydrogels with (C) PEGDA 575 in three different concentrations (10, 15, and 20 mol%) and (D) five different crosslinker structures ($C_{\text{crosslinker}} = 10 \text{ mol}\%$). The values of the storage modulus G' , the loss modulus G'' and the crossing point t_c were read of each diagram, according to A and listed in Table 1. At the beginning of the measurement, the UV lamp of the rheometer remains switched off (highlighted in grey). (The oscillating frequency was 0.1 Hz at 37 °C. PEGDA was abbreviated to P due to space constraints in the figures. $C_{\text{GVIM-I}} = 1.25 \text{ mol L}^{-1}$.)

stiffer with increasing crosslinker concentration, which was confirmed by this measurement (Table 1).

The gelation behaviour can be influenced by varying the molar mass and the resulting chain length of the crosslinkers (Fig. 2D). Samples with lower molar mass crosslinkers (PEGDA 250) only show an increase in G'_{∞} to 13.1 Pa, while the G'_{∞} for PEGDA 700 is 29 256.7 Pa. This indicates that the stiffness of the hydrogels increases with longer crosslinker chains. Additionally, an acrylamide crosslinker (MBAA) was also used in this work. MBAA crosslinked gels gel slower than PEGDA 575 and

only reach a G'_{∞} of 2243.3 Pa, which is significantly softer than the PEGDA 575 at 10 116.7 Pa (Table 1). This difference in stiffness is probably due to the structure of the crosslinker. MBAA, as an amide, is more electron-rich than the corresponding ester of PEGDA, leading to better stabilization of the formed radical during gelation. This increased stability of the radical in case of MBAA results in slower overall gelation due to its lower reactivity.⁶¹

In addition to gelation tracking, the amplitude sweep measurement provides useful information about the stiffness

Table 1 Overview of evaluated parameters in the gelation tracking and amplitude sweeps experiments (LAP as the photoinitiator; ND = not determinable)

Crosslinker	G'_{∞} [Pa]	G''_{∞} [Pa]	t_c [s]	γ_L [%]	γ_c [%]
MBAA	2243.3 ± 53.4	45.3 ± 24.3	46.2 ± 0	1.0 ± 0	62.2 ± 0
EGDA	ND	ND	40.5 ± 1.7	48.7 ± 67.4	660.3 ± 51.5
PEGDA 250	13.1 ± 1.9	4.6 ± 1.5	47.2 ± 5.9	21.4 ± 16.7	931.7 ± 38.7
PEGDA 575 10 mol%	10 116.7 ± 88.1	59.8 ± 69.9	42.2 ± 0	1.0 ± 0	69.2 ± 9.7
PEGDA 575 15 mol%	41 106.7 ± 411.0	309.4 ± 50.7	41.2 ± 0	1.0 ± 0	62.4 ± 0
PEGDA 575 20 mol%	97 766.7 ± 436.5	1217.7 ± 92.4	40.2 ± 0	1.0 ± 0	69.2 ± 9.7
PEGDA 700	29 256.7 ± 125.0	306.3 ± 61.7	37.8 ± 1.8	1.0 ± 0	28.3 ± 9.7



of a hydrogel and its stability to shear stress. This measurement shows the linear viscoelastic (LVE) range of a sample, where G' is independent of the shear strain (γ_L in Table 1). This indicates that the deformation behaviour of the hydrogel is in the non-destructive range ($\pm 5\%$ of the initial value of G'). If the LVE range limit is exceeded, the sample will begin to form microcracks and exhibit brittle fracturing behaviour. The value of G' is greater than G'' , indicating a gel-like structure and the behaviour of a viscoelastic solid. The microcracks develop into macrocracks within the hydrogel. After the crossing point ($G' = G''$, γ_c in Fig. 2B), G'' is significantly larger than G' , indicating the behaviour of a viscoelastic liquid.⁴ The hydrogel is more stable against shear stress and deformation with a longer LVE region.³²

All samples with a high G' value in gelation tracking have a very short LVE range (Table 1). Hydrogels containing MBAA and PEGDA, without PEGDA 250, become brittle after only 1% of shear strain, indicating instability to deformation.³² Hydrogels containing EGDA and PEGDA 250 as crosslinkers have a longer LVE of $48.7 \pm 67.4\%$ and $21.4 \pm 16.7\%$, respectively. The crossing point of these two samples ($660.3 \pm 51.5\%$ and $931.7 \pm 38.7\%$) occurs at a much higher shear strain than all other samples (28–69%).

A wide range of γ_L and γ_c values is also reported in the literature. For example, Zhang *et al.* prepared self-healing hydrogels based on xanthan gum. These hydrogels had critical strains ranging from 24.2 to 29.8% and showed G' recovery rates ranging from 91.36 to 93.69%.⁶² In another study, Jastram *et al.* synthesized hydrogels that also contained a vinylimidazole unit and were crosslinked with MBAA.⁴ These hydrogels showed γ_L values of 91 ± 2 to $92 \pm 7\%$, which is significantly higher than the values achieved in this work.

The hydrogels, with the exception of EGDA and PEGDA 250, exhibit high stiffness and low stability against shear stress, indicating their rigidity. Therefore, they need to be adapted for medical applications, such as stent coating or in tissue engineering. Copolymerisation with a long-chain monomer or polymer could increase their stability to deformation stress and generally improve the mechanical properties. The use of PEGDA with molar masses of 3000 to 20 000 g mol^{-1} would be a promising approach here.^{63,64}

Hydrogel characterization with Raman spectroscopy

Raman spectroscopy is another option for characterizing hydrogel structure. Fig. 3A shows the Raman-spectra of the monomer GVIM-I (red spectra), the reaction mixture without TEMED

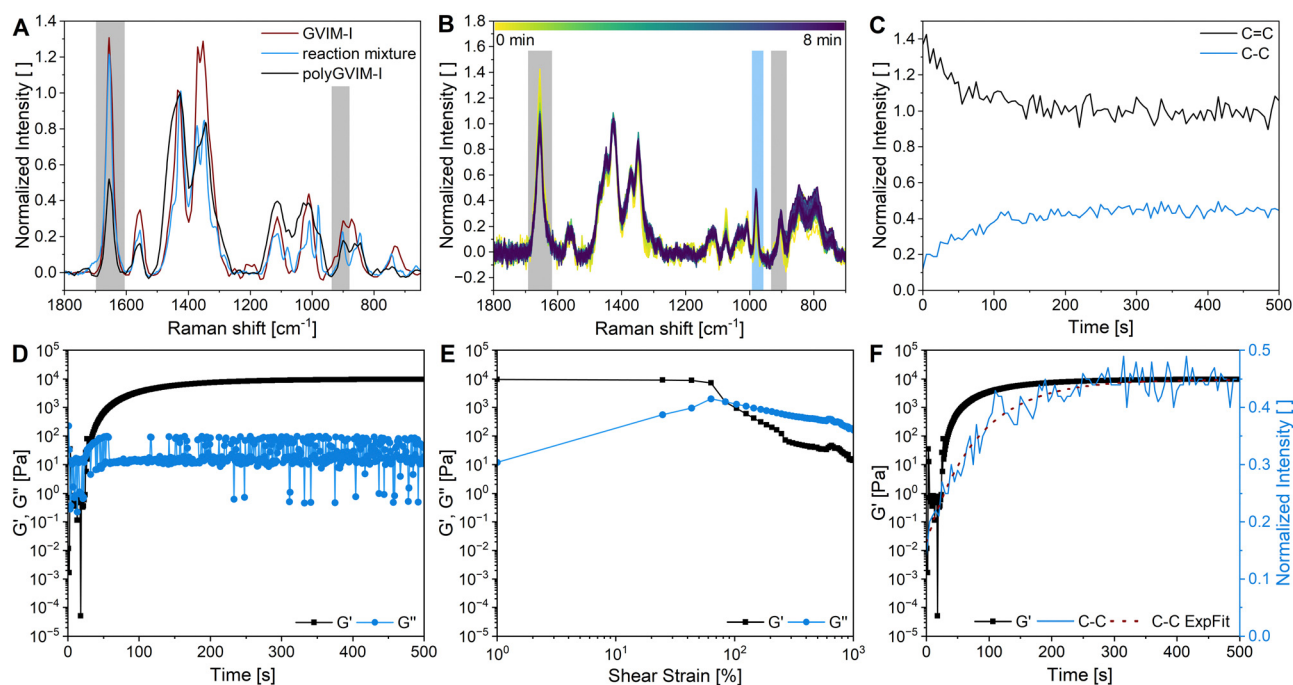


Fig. 3 Characterization of polyGVIM-I hydrogels via (A) Raman spectroscopy, as well as (B)–(F) *in situ* rheology-Raman spectroscopy: (A) Raman spectra at a 1064 nm wavelength of the monomer GVIM-I (red), the reaction solution in PBS with PEGDA 575 as crosslinker without TEMED at a wavelength of 785 nm (blue) and the dried corresponding polyGVIM-I hydrogel at a wavelength of 1064 nm (black). Grey highlighted are the significant bands for C=C stretching vibrations (1656 cm^{-1}) and C=C bending vibrations (900 cm^{-1}). The spectra were height normalized on the 1426 cm^{-1} band. (B) *In situ* Raman spectra at 785 nm wavelength for polymerization tracking over 8 min of polyGVIM-I with PEGDA 575 as a crosslinker and APS/TEMED as an initiator system. Grey highlighted are the significant bands for C=C stretching vibrations (1656 cm^{-1}) and C=C bending vibrations (900 cm^{-1}). Blue highlighted is the significant band for C–C stretching vibrations (980 cm^{-1}). The spectra were normalized on the 1428 cm^{-1} band. (C) Time trace of the C=C consumption (height of the band at 1656 cm^{-1}) (black) and C–C formation (height of the band at 980 cm^{-1}) (blue) during polymerization, corresponding to the *in situ* Raman spectra (B). (D) Gelation tracking of polyGVIM-I with PEGDA 575 as crosslinker and APS/TEMED as initiator system and (E) the corresponding amplitude sweep measurement. (F) Overlay of the G' during gelation and the C–C formation during polymerization (approximated with an exponential fit in red) of the *in situ* rheology-Raman spectroscopy.



(since polymerization occurs within seconds of adding the initiator) (blue spectra), and the dried polyGVIM-I hydrogel (black spectra). The Raman shift at 750 cm^{-1} indicates the band from C–O–C, which belongs to the GVIM-I monomer and the crosslinker PEGDA. The Raman shifts in the range from 850 to 1150 cm^{-1} are attributed to C–C stretching vibrations originating from the GVIM-I sugar backbone, but also from the backbone of the polymer chain. This explains why the Raman shifts in the hydrogel have a higher normalized intensity, as the backbone forms during gelation and the number of C–C bonds increases. The Raman shift at 1426 cm^{-1} indicates the deformation vibration of CH_2 at the C-6 position in the sugar. This band was used for normalization, as it does not change during polymerization. The vibrations of the C=C double bonds can be seen in the spectra at 1656 cm^{-1} . The normalized intensity of the band is significantly lower after the hydrogel formation than in the monomer solution. During the formation of the hydrogel, the C=C groups of the crosslinker are linked to the vinyl group of the monomer, which explains the decrease in intensity of the band.

Hydrogel characterization with *in situ* rheology-Raman spectroscopy

The stiffness and the LVE region of hydrogels as well as the gelation time of a reaction mixture to a hydrogel, can be determined by rheological measurements, as shown in two previous chapters. The formation of a polyGVIM-I hydrogel with PEGDA 575 as crosslinker and APS/TEMED as initiator system was followed with the rheometer-Raman combination, which means an *in situ* Raman measurement and simultaneous gelation tracking on the rheometer. Fig. 3B shows the Raman spectra of the *in situ* Raman measurement. The normalized intensity of the band at 1656 cm^{-1} decreases during the measurement and the band at 980 cm^{-1} increases. To better visualize the change in intensity of these two bands, the intensities were plotted against time (Fig. 3C). It can clearly be seen that the intensity of the vibration of the C=C double bond (black) decreases sharply within the first 200 s and then reaches a steady state at 300 s, while the intensity of the vibration of the C–C single bond (blue) increases and then also reaches a steady state at the same time. The graph shows that the breaking of the C=C double bonds occurs simultaneously with the formation of the single bonds of the polymer backbone. The rheological measurement taken during gel formation is shown in Fig. 3D. Here, as already shown in the previous chapter, G' and G'' have been plotted against time. At the start of the data recording, the value of G' is less than G'' , which means that the monomer solution behaves like a viscoelastic fluid, as expected. The initiator TEMED was added just before the start of the data recording. It can be seen, that the crossing point of G' and G'' occurred after 30 s, indicating a very fast gelation. After the crossing point, the value of G' rises rapidly, then flattens out until it reaches a plateau at about 200 s. The plateau indicates that the formation of the three-dimensional hydrogel structure is complete. Fig. 3E shows the subsequent amplitude sweep measurement of the same hydrogel. The LVE

range of this hydrogel is 24.7%, which is much higher than the same hydrogels with LAP as initiator instead of APS/TEMED (see Table 1). The value of γ_c with 83.7% is at a significantly higher shear strain, indicating an overall more stable hydrogel. Fig. 3F compares the progression of G' of the gelation tracking with the intensity of the C–C single bond vibration of the *in situ* Raman measurement. By overlaying the two data sets, conclusions can be drawn about the gelation and polymerization processes. Gelation *i.e.* the transformation of the sample from liquid-like to solid-like behavior, can be seen in the rheometer data after 30 s. The value of G' is greater than G'' from this point onwards. The Raman data shows in direct comparison that only a small proportion of C–C bonds have formed after 30 s. At the gelation point of the gel (crossing point of G' and G'') an increase in the intensity of the C–C bond from 0.15 to 0.25, which can be correlated with a conversion of the double bonds of 33%, if the normalized Raman intensity of 0.45 is attributed to a conversion of around 100%. The formation of C–C single bonds, *i.e.* the structural change of the sample, is the polymerization. It can be assumed that complete polymerization occurred approximately 300 seconds after the start of the experiment, as evidenced by a normalized intensity of the C–C vibration of 0.45 in the Raman spectrum (100% conversion of the C=C bond in the Raman spectrum). However, the value of G' continued to increase (from 7413.9 Pa to 9782.0 Pa at the end of the measurement). This could be caused not only by incomplete gelation but also by the drying effects of the hydrogel.

Conclusion

In the present study, we have successfully characterized carbohydrate-based hydrogels, before, after and during gelation or polymerization, with different formulations using established methods and have demonstrated a new alternative technique for hydrogel characterization.

We were able to show that ATR-FTIR is initially suitable for spectroscopic characterization to get a first impression of the structure of the monomer (GVIM-I) and the hydrogel. The advantage of ATR-FTIR is that it requires little sample preparation, and is often accessible and inexpensive. Nevertheless, the water present in the reaction solution can lead to the formation of broad bands which may mask signals of interest. This issue can be avoided by pre-drying the samples. The *in situ* IR measurement shows strong noise of signals above 1900 cm^{-1} which can be attributed to the device setup (quartz glass), resulting in superposition of the signals of interest. Furthermore, the results of the C=C double bond vibration tracking should be treated with caution. In addition to the intensity of the band at 955 cm^{-1} , numerous other band intensities that should remain constant, also decrease. This indicates that the polymerization monitoring by IR was unsuccessful in this setup.

These limitations do not apply to Raman spectroscopy. Water signals do not interfere with the measurements and do not overlay the bands of interest, giving a clearer resolution



than the IR spectra. The *in situ* Raman measurement allowed the monitoring of the intensity decrease of the C=C double bonds and the intensity increase of the C-C bonds.

In addition to spectroscopic methods for monitoring polymerization, rheological techniques can be employed to track the gelation. For the additional determination of gelation time, it is also possible to identify other characteristics, such as stiffness and LVE region of hydrogels with different crosslinker types and concentrations.

The *in situ* rheology-Raman spectroscopy, a novel methodology for the characterization of hydrogels, was presented for the first time in this study and compared with conventional methods. This method has so far been limited to radical polymerizing hydrogels, although initial experiments have already investigated the curing of epoxy resins using rheometer-Raman.⁴³ Furthermore, this technique could be applied in the future to hydrogels based on click chemistry, for example Diels-Alder crosslinking. However, it should be noted that this method is not suitable for hydrogel gelation based on ionic interaction.

The rheometer-Raman combination offers several advantages for hydrogels with already described polymerization forms, such as radical polymerization. These advantages include the ability to track the Raman bands corresponding to the C=C double bond and the C-C bond, as well as the direct comparison to the gelation of the rheological measurement and the aforementioned bands. All in all, the combination of rheology and Raman spectroscopy, has led to a significant expansion of knowledge in a relatively short period by running the methods simultaneously, enabling the detection of differences between polymerization and gelation.

Author contributions

Sina Lambrecht: formal analysis, investigation & writing – original draft; Marek Biermann: formal analysis, investigation & writing – review & editing; Selin Kara: funding acquisition, writing – review & editing; Stefan Jopp: writing – review & editing; Johanna Meyer: conceptualization, methodology, formal analysis, investigation & writing – original draft. All authors approved the final version of the manuscript.

Data availability

The authors confirm that the data supporting the findings of this study are available within the article and its supporting information. Raw data that support the findings of this study are available from the corresponding author.

Conflicts of interest

There are no conflicts to declare.

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IV. Publikation IV

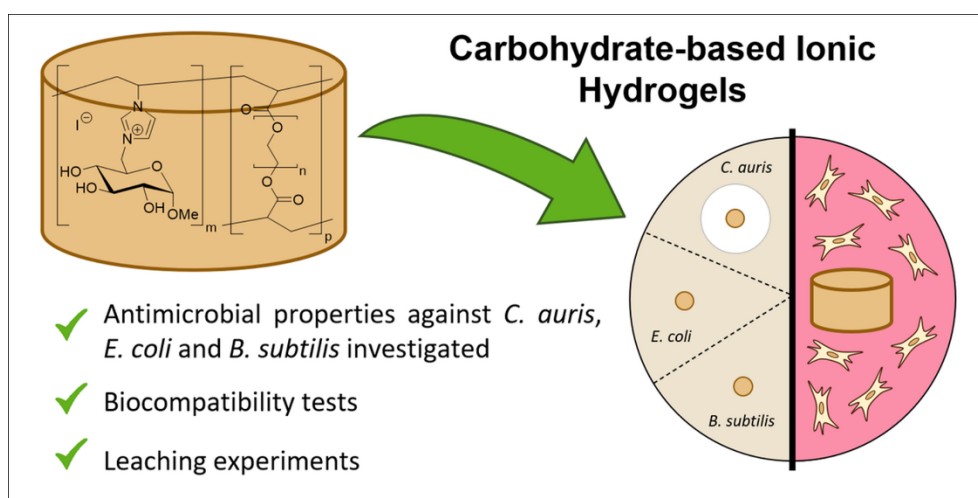
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Antimicrobial properties and biocompatibility of semi-synthetic carbohydrate-based ionic hydrogels

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Antimicrobial properties and biocompatibility of semi-synthetic carbohydrate-based ionic hydrogels†

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Hydrogels have gained significant interest in the last decades, especially in the medical sector, due to their versatile properties. While hydrogels from naturally occurring polysaccharides (e.g. cellulose) are well-known, those produced from polymerizable carbohydrate-based monomers remain underexplored. However, these semi-synthetic hydrogels offer the great advantage of having adjustable properties for customization depending on their application. The objective of this study was to characterize semi-synthetic carbohydrate-based ionic hydrogels produced from GVIM-I (glucosyl vinyl imidazolium iodide). The antimicrobial activity was evaluated using the disk diffusion method, which demonstrated that all samples exhibit inhibitory effects on the growth of *Candida auris*. *In vitro* biocompatibility was determined by cell viability studies with L929 mouse fibroblasts, and a correlation was observed between eluate concentration and cell viability. In particular, the type of initiator system employed for polymerization was found to affect cell viability. The direct contact assessments showed that specific pre-treatments of the hydrogels resulted in higher cell viability than non-treated hydrogels. The results also revealed the impact of crosslinker concentration and type and identified poly(ethylene glycol) diacrylate (PEGDA) 575 as a promising crosslinker for future medical applications. LC-MS analysis of the wash medium identified unreacted GVIM-I as the leached material, which is presumed to be the cause of the observed cytotoxicity. Overall, the study provides valuable insights into the characteristics of GVIM-I based hydrogels and sheds light on the factors that influence their cytotoxicity and potential for medical application.

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Introduction

Hydrogels, defined as hydrophilic, three-dimensional polymer networks, have been known since the 1960s¹ and are widely used in the medical and pharmaceutical industries, e.g. as drug delivery systems,² stent coatings,^{3,4} contact lens materials⁵ or for tissue engineering.⁶ Depending on their origin (natural or synthetic), polymer composition, charge, as well as method and degree of crosslinking *etc.*, hydrogels can possess promising properties such as tunable mechanical properties (from stiffness to flexibility),^{7,8} non-toxicity, biocompatibility and even self-healing capabilities.⁹ The aforementioned possible

characteristics render hydrogels as promising materials for use in medical applications. In this regard, especially natural hydrogels are of interest due to their high biocompatibility and biodegradability.¹⁰

Well-known representatives of hydrogels of natural origin are produced from polysaccharides like cellulose, alginate and chitosan, which consist of repeating monosaccharide units and thus also multiple hydroxyl groups.^{11–13} These hydrogels made from sustainable and renewable polysaccharide materials are of great importance in the medical field.^{13,14} Recently, Ding *et al.* showed that non-swelling, injectable chitosan hydrogels are biocompatible with human mesenchymal stem cells (hMSCs) and furthermore exhibit no adverse effects in *in vivo* studies with rats, making them potentially useful as smart biomaterials.¹⁵ As another example, Ren *et al.* synthesized an injectable hydrogel from quaternized chitosan, gelatin and dopamine for use as drug delivery system for the treatment of Parkinson's disease.¹⁶

In comparison to the natural polysaccharide-based hydrogels, the development and biocompatibility of semi-synthetic hydrogels with a carbohydrate component has barely been explored before. The only known previous work in this field, to

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the best of our knowledge, was published by Goel *et al.*, who recently demonstrated the successful synthesis of biocompatible microporous D-galactose-based hydrogels with a high water uptake of up to 526%. They applied these hydrogels as hydrophilic drug-carrier.^{17,18} Our group has recently studied the synthesis, structural analysis, swelling behavior and degradation of novel semi-synthetic carbohydrate-based ionic hydrogels produced from the cationic starting material GVIM-I (glucosyl vinyl imidazolium iodide) crosslinked with different commercial crosslinkers like polyethylene glycol diacrylate (PEGDA) and *N,N'*-methylenebis(acrylamide) (MBAA).¹⁹ These hydrogels have a unique combination of properties not found in natural polysaccharide-based hydrogels, as they firstly are polymerized in controllable ratios from a monomer–crosslinker mixture and secondly bear a cationic charge, whereas natural carbohydrates are always neutral or anionic.^{11–13} This cationic charge in the hydrogel potentially enables the binding of anionic drugs such as ibuprofen or naproxen, so that the hydrogel can be used as drug delivery system.^{2,20}

Our overall aim is to utilize our novel semi-synthetic carbohydrate-based ionic GVIM-I hydrogels in the biomedical field, *e.g.* in tissue engineering or drug delivery, as previously pointed out. Their low swelling degrees and cationic charge, as investigated in our previous article,¹⁹ make our GVIM-I hydrogels suitable for these kind of applications.^{2,21} To work towards this goal, we recently also investigated the rheological properties of our GVIM-I hydrogels.²²

In this work, we want to extend the characterization of our GVIM-I hydrogels in terms of their antimicrobial properties and biocompatibility, as well as investigate the components that leach out of the hydrogels in an aqueous medium.

To determine the antimicrobial activity, we used the gold standard, the disk diffusion method established by Bauer and Kirby *et al.*,²³ and used *Bacillus subtilis* as Gram-positive, *Escherichia coli* as Gram-negative bacteria strain, as well as *Candida auris* as a widespread yeast. These analyses were performed according to the Clinical Laboratory Standard Institute (CLSI) “Performance Standards for Antimicrobial Disk Susceptibility Tests”.²³ Furthermore, the *in vitro* biocompatibility was tested by measuring the viability of L929 cells after treatment with hydrogel eluates. Additionally, the viability of cells in direct contact with the GVIM-I hydrogels has also been investigated in this work. These two approaches, in which the cells both come into direct contact with the hydrogels and into contact with the eluates, are mandatory for subsequent applications in the medical field.

Experimental

Materials

The chemicals methyl- α -D-glucopyranoside (99%), triphenylphosphine (99%), imidazole (99%), *N*-vinylimidazole (99%), ethylene glycol diacrylate (EGDA) (>90%) and ammonium persulfate (APS) (98%) were obtained from Thermo Fisher Scientific (Darmstadt, Germany). Polyethylene glycol diacrylate (PEGDA) ($M_n = 250, 575$ and 700 g mol^{-1}), *N,N'*-methylene bisacrylamide (MBAA) (99%) and *N,N,N',N'*-

tetramethylethylenediamine (TEMED) (>99%) were supplied by Sigma-Aldrich Chemie GmbH (Taufkirchen, Germany). Iodine (>99.5%) was acquired from Carbolution Chemicals (St. Ingbert, Germany). Lithium phenyl(2,4,6-trimethylbenzoyl)phosphinate (LAP) (>98%) was supplied by TCI (Eschborn, Germany). The solvents THF (99.9%) and ethyl acetate (99.7%) were obtained from Honeywell (Seelze, Germany), the solvents chloroform (>99.8%), methanol (99.8%) and DMF (99.5%) were supplied by Thermo Fisher Scientific (Darmstadt, Germany) and ethanol (99%) was supplied by VWR Chemicals (Darmstadt, Germany) and then diluted to 70% with distilled water. Column chromatography was performed with silica gel (230–400 mesh particle size) obtained from Supelco (Darmstadt, Germany). Additionally, phosphate-buffered saline (PBS; Thermo Fisher Scientific Inc., Waltham, USA) was used.

The starting material 1-(methyl- α -D-glucopyranosid-6-yl)-3-vinylimidazolium iodide (GVIM-I) was synthesized in two steps from methyl- α -D-glucopyranoside as previously published by our group.¹⁹

General procedure hydrogel synthesis

The hydrogels were prepared using either the photoinitiator LAP (i) or an initiator system consisting of APS and TEMED (ii). For photopolymerization (i), GVIM-I and LAP were weighed into 1.5 mL Eppendorf reaction tubes, dissolved in PBS (pH = 7.4) and the corresponding amount of crosslinker was added (the exact weights can be found in Tables S1 and S2†). After thorough mixing, the solution was sterile-filtered (Filtropure S, PES, 0.2 μm , Sarstedt AG and Co. KG, Nümbrecht, Germany) using a syringe and poured directly into 6 mm diameter wells of a silicon mold. The samples were then photopolymerized with a UV intensity ($\lambda = 365 \text{ nm}$, Biolinker, VILBER, Collégien, France) of 1.2 J cm^{-2} and 2.4 J cm^{-2} , respectively. For radical polymerization (ii),¹⁹ GVIM-I was weighed into 1.5 mL microcentrifuge plastic tube, dissolved in PBS (pH = 7.4), APS solution and the corresponding amount of crosslinker were added. After thorough mixing, the solution was sterile-filtered and syringed in a sterile Eppendorf reaction tube. TEMED was added, the solution was mixed well and then poured into the silicon mold wells. The gelation took place within a few seconds. Sample names and corresponding components are listed in Table 1.

Table 1 List of used crosslinkers, their concentrations, and initiator system, $c_{\text{GVIM-I}} = 1.25 \text{ mol L}^{-1}$. All samples with PEGDA as a crosslinker are abbreviated with P, A/T means the initiator system APS and TEMED

Sample name	Crosslinker	$c_{\text{crosslinker}}$ [mol%]	Initiator
EGDA	EGDA	10	LAP
P250	PEGDA 250	10	LAP
P575 10%	PEGDA 575	10	LAP
P575 15%	PEGDA 575	15	LAP
P575 20%	PEGDA 575	20	LAP
P700	PEGDA 700	10	LAP
MBAA LAP	MBAA	10	LAP
MBAA A/T	MBAA	13	APS/TEMED
P575 A/T	PEGDA 575	10	APS/TEMED



Antimicrobial activity testing

The antimicrobial activity of the samples was tested using the disk diffusion method, which was established by Bauer and Kirby *et al.* and is considered the gold standard for testing antimicrobial susceptibility.²³ Tests were performed against some of the most common strains for infections, *Escherichia coli* K-12, *Bacillus subtilis*, and *Candida auris* (WT). These species were stored as glycerol cultures with 20% v/v glycerol at $-80\text{ }^{\circ}\text{C}$. For pre-culture, LB media prepared according to Miller (5 g yeast extract, 10 g peptone, and 10 g NaCl in 1 L ultrapure water) were adjusted to pH 7.0, sterilized by autoclaving, and 10 g per L glucose was added after autoclaving. Bacteria and yeast were cultivated in 150 mL baffled shake flasks at 150 rpm. After the inoculation, the strains were pre-cultured overnight at $35 \pm 2\text{ }^{\circ}\text{C}$ before use.

Examinations were performed on Mueller–Hinton agar (for fungal cultures 2% v/v glucose was added), prepared according to the manufacturer's instructions, and poured into 100 mm plates. Bacterial solutions were adjusted to a concentration of 1 to 2×10^7 CFU mL⁻¹ (0.5 McFarland Standard, OD₆₀₀ \approx 0.120) and spread evenly over the entire Mueller–Hinton agar plate, using a sterilized cotton swab soaked in bacteria solution. The samples (hydrogels with a diameter of 6 mm) were placed on the agar plates with sterile forceps. The negative controls were filter paper disks (6 mm in diameter) with 10 μ L LB medium, while the positive controls were gentamicin (Roti@Antibiotic Disks Gentamicin (GEN) 10 μ g, 6 mm, 50 Units, Carl Roth, Karlsruhe, Germany) for the bacterial strains and amphotericin B (Roti@Antibiotic Disks Amphotericin B (AP) 100 Units, Carl Roth, Karlsruhe, Germany) for the yeast. Bacteria agar plates were incubated for 18 h, and the yeast agar plates were incubated for 24 h at $35 \pm 2\text{ }^{\circ}\text{C}$. The zones of inhibition (ZOI, diameter) were measured in mm. Experiments were performed in triplicate.

In vitro biocompatibility

Cell line and culture conditions. The method was adapted from Jopp and Meyer *et al.*,²⁴ in short: L929 cells Murine (*Mus musculus*) fibroblasts (L-929, DMSZ No. ACC2) were purchased from Cell Line Service GmbH (Eppelheim, Germany) were routinely cultivated in 175 cm² cell culture flasks (Sarstedt AG and Co. KG, Nümbrecht, Germany) in Dulbecco's Modified Eagle's Medium (DMEM; Sigma-Aldrich Chemie GmbH, Steinheim, Germany), supplemented with 10% fetal calf serum

(Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) as well as 100 U mL^{-1} of penicillin and $100\text{ }\mu\text{g mL}^{-1}$ of streptomycin (penicillin–streptomycin antibiotic solution; Sigma-Aldrich Chemie GmbH, Steinheim, Germany) in a 5% CO₂ and humidified atmosphere at $37\text{ }^{\circ}\text{C}$ (Heracell 240 incubator, Thermo Fisher Scientific Inc., Waltham, USA). Cells were uncultivated at 70–85% confluency by trypsin/EDTA solution (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) treatment after a washing step with phosphate-buffered saline (PBS; Thermo Fisher Scientific Inc., Waltham, USA). Experiments were performed with cells of passage numbers below 34. 24 h prior to the start of an experiment, cells were seeded in 96 well plates (Sarstedt AG and Co. KG, Nümbrecht, Germany) at a density of 8000 cells per well in 200 μ L cell culture medium or in 24 well plates (Sarstedt AG and Co. KG, Nümbrecht, Germany) at a density of 50 000 cells per well in 1 mL cell culture medium.

Preparation of the eluate for biocompatibility studies. To examine the biocompatibility of the hydrogels, the eluate was prepared according to ISO 10993-12:2021(E) (Biological Evaluation of Medical Devices—Part 12: Sample Preparation and Reference Materials). To obtain the eluate, the hydrogels were incubated for $72 \pm 2\text{ h}$ at $37\text{ }^{\circ}\text{C}$ (with a surface area/volume ratio of $3\text{ cm}^2\text{ mL}^{-1}$) in the respective culture media. A control was established by incubating the culture medium for $72 \pm 2\text{ h}$ at $37\text{ }^{\circ}\text{C}$. For each of the samples under investigation, eluate was removed from the hydrogels and sterile filtration was employed to create a stock solution. The stock solutions were subsequently diluted into concentrations of 100%, 10%, 1%, and 0.1%.

Preparations of the hydrogels for direct contact biocompatibility studies. As previously stated, hydrogels were synthesized following the aforementioned methodology (i). For direct contact, only hydrogels with PEGDA 575 at a crosslinker concentration of 10% were used. After gelation, the hydrogels were treated in seven different ways, as detailed in Table 2. Each washing step was performed with a ratio of one milliliter of medium to one hydrogel.

CellTiter-Blue (CTB) viability assay

Cell viability of L929 cells was quantified using the CellTiter-Blue® cell viability assay (Promega GmbH, Mannheim, Germany). This involved the use of cell-free controls for background fluorescence correction and untreated cell controls, in accordance with the manufacturer's instructions. In

Table 2 Overview hydrogel treatments

Sample number	Treatment
1	No treatment
2	Washing in DMEM 72 h
3	Washing in DMEM $3 \times 24\text{ h}$
4	Washing in EtOH 24 h and in DMEM $2 \times 24\text{ h}$
5	UV irradiation 1 h
6	UV irradiation 1 h and washing in DMEM $3 \times 24\text{ h}$
7	UV irradiation 1 h, washing in EtOH 24 h and in DMEM $3 \times 24\text{ h}$



metabolically active cells, the reduction of blue resazurin to purple, fluorescent active resorufin occurs. The resulting fluorescence intensity is found to be correlated with the number of viable cells. Resorufin formation was monitored using a fluorescence plate reader (Fluoroskan Ascent, Thermo Fisher Scientific Inc., Waltham, USA) with an excitation wavelength of 544 nm and an emission wavelength of 590 nm. L929 cells were cultured in cell culture medium or cell culture medium with varying concentrations of hydrogel eluates for 48 h (approximately 27 500 cells per cm²). Afterward, the medium was carefully removed, and 100 μ L (96 well plate) or 300 μ L (24 well plates) cell culture medium containing 10% CTB stock solution was added to each well. The cells were then incubated until the control fluorescence, which was measured in a plate reader, reached a range of 100–400 relative fluorescence units (RFU). Three biological replicates, each comprising six technical replicates, were analyzed.

Microscopic analysis

Microscopic imaging of the cells was performed using an IncuCyte S3 Live-Cell Analysis Instrument (Sartorius AG, Göttingen, Germany) prior to and after 24 h and 48 h of treatment with the eluate or hydrogel samples. Phase contrast imaging was conducted using intrinsic auto-exposure function of the IncuCyte software (Sartorius AG, Göttingen, Germany) with the 10 \times objective.

Live/dead staining of cells

For the purpose of live/dead staining, the cells were treated for a period of 48 h with the different hydrogel eluate concentrations previously described. Subsequently, the medium was carefully removed from the incubated cells, after which 100 μ L (96 well plate) or 300 μ L (24 well plate) of cell culture medium containing 5 μ M calcein-AM (Sigma-Aldrich Chemie GmbH, München, Deutschland) and 0.125 μ g per mL propidium iodide (PI) (Sigma-Aldrich Corporation, St. Louis, MO, USA) was added to each well. After the incubation at 37 $^{\circ}$ C for 15 min, the samples were analyzed with the BioTek Cytation 5 Cell Imaging Multi-Mode Reader (Agilent, Santa Clara, CA, USA). Imaging was performed in brightfield using the intrinsic auto-exposure function of the Gen5 imaging software (Version 3.10.06, Bio-Tek Instruments GmbH, Bad Friedrichshall, Germany) with a 4 \times objective. For the detection of dead cells, dyed with PI, in the red channel, the Texas Red filter cube (excitation: 586/15 nm; emission: 647/57 nm) was employed. Conversely, for the detection of calcein-dyed, viable cells, the GFP filter cube (excitation: 469/35 nm, emission: 525/39 nm) was utilized. The following parameters were employed for the detection of PI-stained cells: LED intensity was set to 10, integration time to 1.88 s, and gain to 11. And the following parameters were employed for the detection of calcein-stained cells: LED intensity was set to 5, integration time to 58 ms, and gain to 0.

Liquid chromatography-mass spectrometry (LC-MS)

A calibration curve was prepared by diluting a GVIM-I solution with a concentration of 1 mg mL⁻¹ into a series of standard

solutions, with concentrations of 0.2, 0.1, 0.05, 0.025, 0.01, 0.005 and 0.001 mg mL⁻¹. Calibration samples were measured on Dionex UltiMate™ 3000 (LC) coupled with LTQ XL™ (MS) with a Phenomenex® Kinetex® C18 column (150 \times 2.1 mm, 2.6 μ m) at a constant oven temperature of 35 $^{\circ}$ C with an isocratic eluent composition of 40 : 60 (v/v) MeOH : H₂O (+0.1% formic acid) (isocratic) and a flow rate of 0.15 mL min⁻¹. The detection was conducted *via* MS for the specific mass of the compound.

Hydrogel samples were prepared according to the hydrogel preparation method (i) with PEGDA 575 (10 mol%) as a cross-linker. Subsequently, the hydrogels were placed in 20 mL vials containing pure water (1 mL of water per hydrogel). The hydrogels were stored in water at 37 \pm 3 $^{\circ}$ C for either (A) 72 h or (B) the water was changed after 24 h, 48 h and 72 h. Eluate samples were analyzed using the aforementioned LC-MS system with the following eluent gradient: 40 : 60 (v/v) MeOH : H₂O (+0.1% formic acid) from 0 min to 3 min, 80 : 20 (v/v) from 10 min to 20 min and again 40 : 60 (v/v) from 30 min to 40 min with a flow rate of 0.15 mL min⁻¹. The detection was conducted *via* MS (positive scan mode) for the specific mass of the leached compounds and in parallel *via* MSMS (collision-induced dissociation with a normal collision energy value of 35) for fragments of 271 *m/z*.

Results and discussion

As the first step, carbohydrate-based ionic hydrogels with different compositions were prepared (Table 1). The initiator system of APS and TEMED was used to build on our previous work characterizing the hydrogels, which was focused on the degree of swelling.¹⁹ Besides the previously used APS and TEMED radical initiator system, we also applied LAP as photoinitiator in this work. It is known from literature that photoinitiators (*e.g.* Irgacure or LAP) exhibit good to very good results in biocompatibility studies with GelMA and PEGDA hydrogels.^{25–28} Xu *et al.* showed that LAP is less cytotoxic than Irgacure 2959 at higher concentrations during 3D printing.²⁹ Besides the favorable results of biocompatibility studies, the use of photoinitiators has further advantages. First of all, the polymerization process can be controlled by switching the light source (either UV and/or visible light) on or off. Second of all, photopolymerization takes place under mild conditions (room temperature) and performs very quickly.^{30–32}

Antimicrobial studies

First, the carbohydrate-based ionic hydrogels with different crosslinkers were tested for their antimicrobial activity towards the Gram-positive strain *B. subtilis*, the Gram-negative strain *E. coli* K-12 and *C. auris* (WT) as a yeast by carrying out disk diffusion assays (Fig. 1). All samples with a diameter of 6 mm were placed on agar plates with the three different microbes. Notably, no inhibitory effect was observed against *B. subtilis* and *E. coli* K-12 in any of the samples. Noteworthy is the whitish circle around the samples in Fig. 1B. This phenomenon could be attributed to the high-water content of the hydrogels, which softens and swells the agar, yet does not affect the microbial



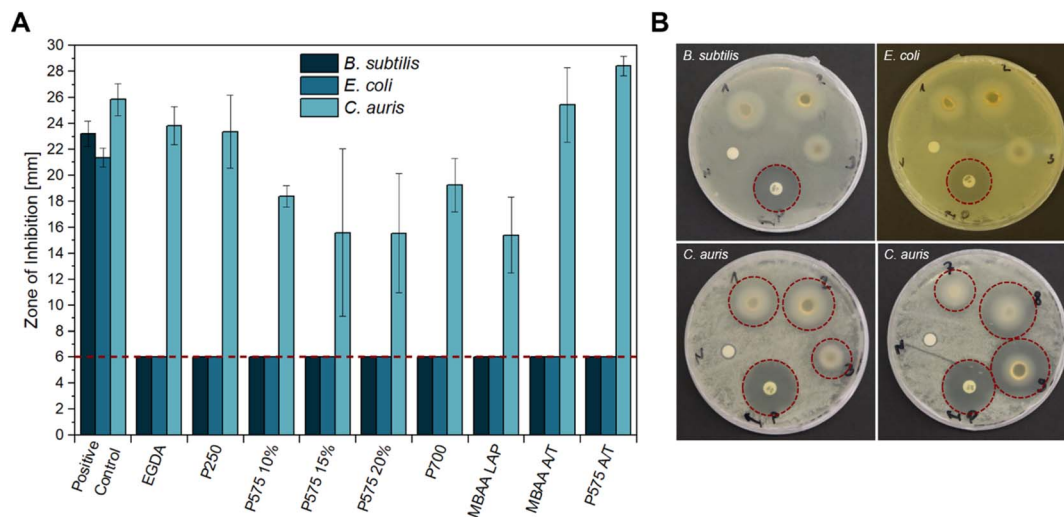


Fig. 1 Antimicrobial activity of different hydrogel samples against *B. subtilis*, *E. coli* K12, and *C. auris* (WT) obtained by disk diffusion method. (A) The mean diameter of the zone of inhibition (ZOI) (in mm, including the 6 mm diameter of the disk and sample) of all hydrogels performed in $n = 3$ experiments, error bars indicate \pm SD. The red dashed line indicates sample size, so that only a ZOI with a bigger size showed an antimicrobial effect. (B) Representative sample agar plates showing the ZOI formed by the hydrogels and the antibiotics (illustrated by the red dashed line), (1) EGDA, (2) P250, (3) P575 10%, (7) MBAA LAP, (8) MBAA APS/TEMED, (9) P575 APS/TEMED, (P) positive control (GEN or AP), (N) negative control (LB medium)). Complete overviews of the disk diffusion tests are given in Fig. S7–S9.†

growth. The hydrogels are based on the GVIM-I monomer, which has previously been investigated by Jopp and Meyer *et al.* regarding its antimicrobial activity. This previous analysis demonstrated that GVIM-I itself also does not affect bacteria or yeast growth. They demonstrated that GVIM-I, when used at a concentration of 0.1 mol L^{-1} , has no inhibitory effect on the growth of *B. subtilis*, *E. coli*, and *C. auris*.²⁴ The hydrogels used in this study were prepared with a GVIM-I concentration of 1.25 mol L^{-1} .

In the literature, well-known carbohydrate-based materials with antibacterial properties are chitosan-based hydrogels. Chitosan is the second most abundant natural polymer and has antibacterial and antifungal properties.^{33–35} Lahooti *et al.* showed in disk diffusion tests that chitosan–poly(vinyl alcohol) gelatin thyme honey hydrogel films are active against *Staphylococcus aureus* (Gram-positive) and *Pseudomonas aeruginosa* (Gram negative), whereby the higher the chitosan, PVA and honey concentration, the stronger the growth inhibition.³⁶ Fan *et al.* and Sajomsang *et al.* showed that quaternary ammonium chitosan hydrogels exhibit strong activity against *S. aureus* and *E. coli*.^{37,38} If silver sulfadiazine or silver nanoparticles are additionally incorporated into chitosan hydrogels, the antibacterial effect against *E. coli* and *S. aureus*, among others, can be further enhanced.^{39–41}

The only inhibitory effect of our GVIM-I hydrogel samples was against the yeast *C. auris*. One possible explanation for this could be the different structures of yeast and bacteria. Yeasts (eukaryotes) have no additional peptidoglycan in the cell wall compared to bacteria (prokaryotes).⁴² While many antibacterial agents inhibit the steps that are important for peptidoglycan formation, the essential component of the bacterial cell wall, most antifungal agents act on the function or formation of ergosterol, which is an important component of the fungal cell

membrane.⁴³ Enache and Cojocaru *et al.* showed that chitosan–nystatin hydrogels have an inhibitory effect against *Candida albicans*, *Candida dubliniensis*, *Candida glabrata* as well as *Candida auris*. They attribute this to the interaction of positively charged amino groups in the chitosan with the negatively charged fungal membrane. The electrostatic attraction of this mechanism causes damage to the cell membrane.^{44,45} Since a positively charged imidazolium is present in the GVIM-I monomer and therefore also in the hydrogel used in this work, this mechanism could also be effective here.

The zone of inhibition (ZOI) of *C. auris* was greatest for the samples of EGDA, MBAA A/T, and PEGDA 575 A/T with $23.83 \pm 1.45 \text{ mm}$, $25.43 \pm 2.85 \text{ mm}$ and $28.45 \pm 0.74 \text{ mm}$ respectively. PEGDA 575 A/T thus inhibits the growth of *C. auris* stronger than AP as a positive control (ZOI of $25.84 \pm 1.22 \text{ mm}$). In comparison to the hydrogels with PEGDA 575 and PEGDA 700, the EGDA and PEGDA 250 hydrogels contain a significantly shorter-chained crosslinker. This can lead to incomplete conversion of monomer and crosslinker and it is assumed that the crosslinker is leached out of the hydrogel during the incubation period. This could potentially inhibit yeast growth due to its toxic properties.⁴⁶ The samples PEGDA 575 15%, PEGDA 575 20%, and PEGDA 700 showed the lowest growth inhibition with a ZOI of $15.59 \pm 6.46 \text{ mm}$, $15.54 \pm 4.60 \text{ mm}$, and $15.40 \pm 2.90 \text{ mm}$ respectively.

The final two samples in Fig. 1A were prepared with APS and TEMED as polymerization initiator systems. A comparison of the two samples with their equivalents produced with photoinitiator LAP (P575 10% and MBAA LAP) reveals that the LAP samples cause a significantly smaller ZOI ($18.38 \pm 0.81 \text{ mm}$ and $15.40 \pm 2.90 \text{ mm}$). The composition of the reaction solution, which is gelled to form the hydrogel, should be noted here. In the LAP preparations, a concentration of 0.5 wt% to 1.3 wt% of



LAP is used in respect to the GVIM-I mass. In case of APS/TEMED, a total concentration of 6.8 wt% to 10.7 wt% is used for the preparations, which results from preliminary investigations.¹⁹ The exact concentrations for the respective samples can be found in Tables S1 and S2.† The amount of initiator system is therefore higher in APS/TEMED hydrogels, which means that more radicals are present in the system overall. Unreacted radicals (from the initiator, or through further reaction also OH[•] and monomer radicals) can have a negative effect on *C. auris*, whereby its growth is more strongly inhibited.^{47–49}

The inhibitory effect against *C. auris* is generally a very valuable property, as it is an invasive pathogenic fungus that is widespread worldwide and poses a threat to human health.⁵⁰ In addition, 93% of *C. auris* strains are resistant to fluconazole, 35% to amphotericin B, and 7% to echinocandins, the common antimycotics used to treat infections with *C. auris*.⁵¹

In vitro biocompatibility

Eluate tests. A standard protocol for biocompatibility testing, as established by the International Organization for Standardization (ISO), employs the use of L929 cells. This cell

line is a trustworthy choice for testing skin contact materials (e.g. implants). They are also recommended by several biocompatibility standards (e.g. DIN EN ISO 10993-1:2021-05).⁵² In this study, the potential cytotoxicity of hydrogels to L929 cells was investigated using CTB cell viability assay (Fig. 2) and live/dead staining with calcein-AM and PI (Fig. 5B).

As illustrated in Fig. 2, the CTB assay reveals that all samples exhibit cytotoxic properties at the highest concentration tested (100%), which corresponds to the undiluted stock solution of the hydrogel eluates. Fig. 2A compares samples prepared with crosslinkers of the same structure but different molar masses. It can be observed that the samples with EGDA ($M = 117.15 \text{ g mol}^{-1}$) and PEGDA 250 ($M_n = 250 \text{ g mol}^{-1}$) exhibit cytotoxic effects at a 10% eluate concentration. In contrast, the samples PEGDA 575 ($M_n = 575 \text{ g mol}^{-1}$) and PEGDA 700 ($M_n = 700 \text{ g mol}^{-1}$) exhibit significantly higher cell viability, and PEGDA 575 can even be described as biocompatible, as the relative cell viability is over 70%. A reduction in cell viability of more than 30% is considered cytotoxic.⁵³ At a 1% eluate concentration, PEGDA 700 also exhibits a similar high cell viability as PEGDA 575. One potential explanation for the high cytotoxicity of EGDA

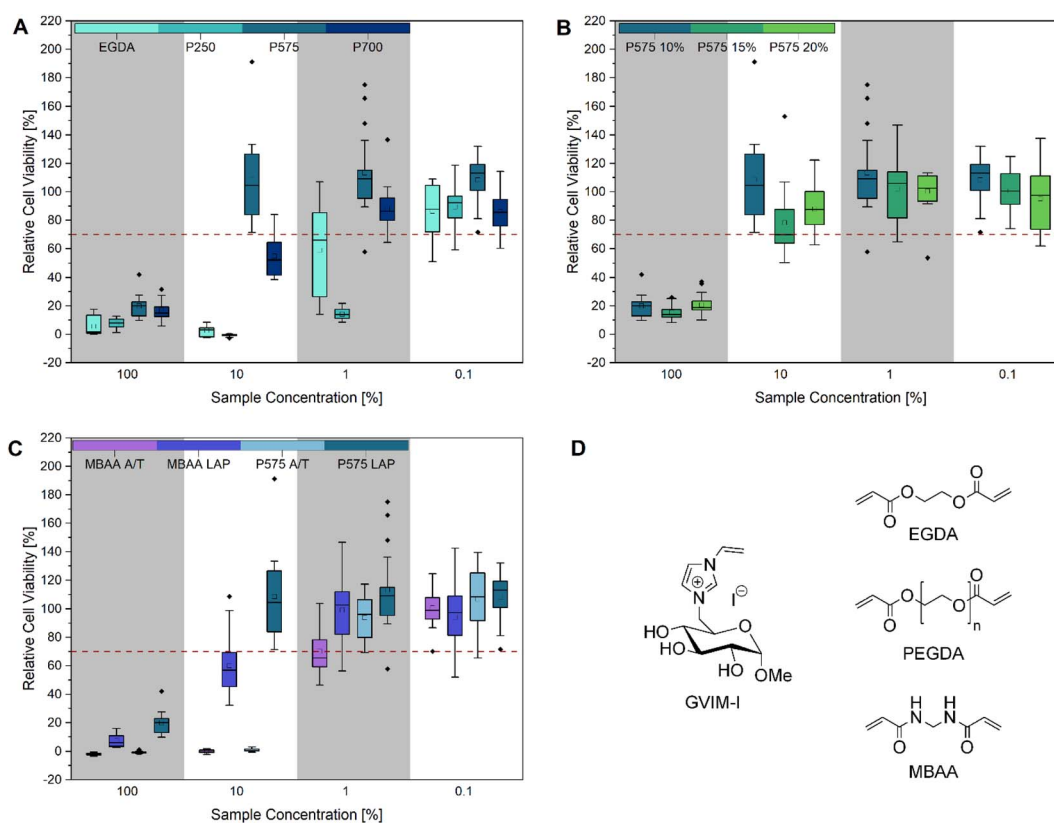


Fig. 2 Cell viability after cultivation for 48 h of L929 cells in different concentrated (100%, 10%, 1% and 0.1%) hydrogel eluates: (A) different molar masses of (P)EGDAs ($M_{(\text{EGDA})} = 170.16 \text{ g mol}^{-1}$, $M_{n(\text{P250})} = 250 \text{ g mol}^{-1}$, $M_{n(\text{P575})} = 575 \text{ g mol}^{-1}$, $M_{n(\text{P700})} = 700 \text{ g mol}^{-1}$), (B) different concentrations (10 mol%, 15 mol% and 20 mol%) of PEGDA 575, (C) comparison of MBAA and PEGDA 575 hydrogels with LAP and APS/TEMED as initiator and (D) structure of GVIM-I and the crosslinkers used in this work. The mean value of the wells without cells (background fluorescence) was subtracted from the fluorescence values of the rest of the wells, and the values of treated cultures were normalized to the mean fluorescence of the control cultures. Three biological replicates with six technical replicates each were analyzed, except for P575 10 mol% (10%) and P575 15 mol% (0.1%). These two samples had one biological replication significantly different from the other two and were defined as outliers. Every cell viability of each biological replicate is demonstrated in Fig. S10 and S11.†



and PEGDA 250 is an incomplete conversion during hydrogel synthesis, resulting in the presence of unreacted monomers, crosslinkers, crosslinker radicals, or initiator radicals in the eluate.⁵⁴ The crosslinker radicals are formed during irradiation with UV light and can cause cell damage due to their cytotoxicity, resulting in low cell viability.^{28,55–57} It can be generally stated that cell viability increases as the eluate concentration decreases. At a concentration of 0.1%, all samples exhibited high cell viability. In Fig. 2B, the influence of varying crosslinker concentrations of PEGDA 575 (10, 15 and 20 mol%) on cell viability was investigated. At 100% eluate concentration, small differences in relative cell viability can be seen with $20.00 \pm 7.69\%$, $15.49 \pm 5.28\%$ and $21.71 \pm 7.50\%$, respectively. At 10% eluate concentration, the differences are larger with $108.60 \pm 33.31\%$, $78.21 \pm 23.70\%$ and $87.99 \pm 14.76\%$ respectively, but not significant due to the high standard deviation. The crosslinker concentration can be used to adjust the stiffness of the hydrogel, as we were recently able to show using this particular example.²² Depending on the application, a variable stiffness can be advantageous.⁵⁸

Fig. 2C shows that the use of different initiator systems does have an influence on cell viability. At 100% eluate concentration, the hydrogels photopolymerized with LAP showed slightly higher cell viability (MBAA = $7.30 \pm 4.35\%$, PEGDA 575 = $20.00 \pm 7.69\%$) than the gels with the initiator system APS/TEMED (MBAA = $1.90 \pm 0.86\%$, PEGDA 575 = $-0.71 \pm 0.83\%$). This effect was even more pronounced at the eluate concentration of 10%. The MBAA hydrogel eluates with LAP result in a relative cell viability of $60.09 \pm 21.23\%$, while it is $-0.15 \pm 1.08\%$ for the samples with APS/TEMED. This can also be seen with PEGDA 575. The LAP hydrogel eluates have a relative cell viability of $108.60 \pm 33.31\%$ and the APS/TEMED samples only $0.80 \pm 0.93\%$. This trend is also confirmed by the brightfield microscopy images and the live/dead staining (Fig. 5). At the highest concentration of PEGDA 575 LAP and PEGDA 575 APS/TEMED eluate, cells exhibit a rounded morphology and reduced growth compared to the control in the brightfield microscopy pictures, indicating that both samples are cytotoxic at this concentration. However, the live/dead staining revealed a significant difference between the two samples. In contrast to the PEGDA 575 LAP sample, in which some cells were stained with calcein-AM and appeared rounded, the PEGDA 575 APS/TEMED sample exhibited a near absence of viable cells stained with calcein-AM. At a concentration of 10% eluate, the brightfield microscopy image of PEGDA 575 LAP demonstrates a significantly higher number of cells with a normal cell morphology than the PEGDA 575 APS/TEMED sample. The number of living cells stained with calcein-AM is also clearly higher. From the CTB cell viability assay and the live/dead staining results, it can be concluded that the use of LAP is preferable to the use of APS/TEMED as an initiation system for cell contact applications.⁵⁹ Fairbanks *et al.* showed that LAP is well suited for the photo encapsulation of living cells and has advantages (*e.g.* better water solubility and higher polymerization rates) over another well-known photoinitiator Irgacure 2959.²⁸ Wilems *et al.* were able to prove that the mESC (mouse embryonic stem cells) and hNSC (human-induced pluripotent

stem cell-derived neural stem cells) used exhibited low viabilities after contact with APS/TEMED, making them cytotoxic according to ISO 10993-5. If the initiator is not completely converted during gelation, this can have a negative effect on the cell viability.^{53,60}

Direct contact tests. The previous eluate tests demonstrate the impact of components leached from hydrogels on L929 cells. In this subsequent stage, it is necessary to investigate the growth and morphology of the L929 cells in direct contact with the hydrogels. Therefore, hydrogels were synthesized and treated in accordance with the experimental procedures outlined in Table 2. The treated hydrogels were applied on top of L929 cells that had been incubated for 24 h. After a further 48 h

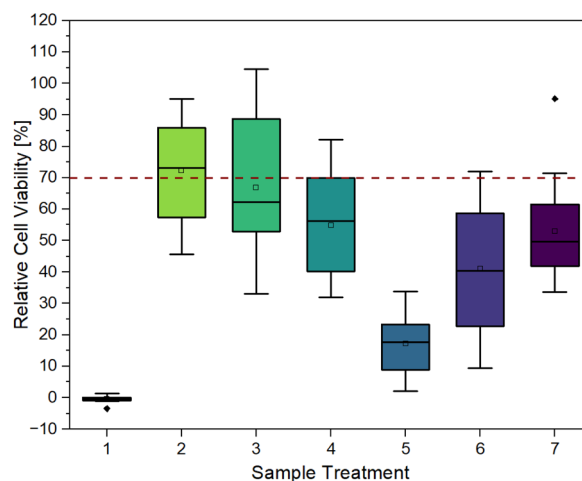


Fig. 3 Cell viability after cultivation for 48 h of L929 cells in direct contact with differently treated hydrogels (PEGDA 575, 10 mol%): (1) no treatment, (2) washing in DMEM 72 h, (3) washing in DMEM 3×24 h, (4) washing in EtOH (70%) 24 h and in DMEM 2×24 h, (5) UV irradiation 1 h, (6) UV irradiation 1 h and washing in DMEM 3×24 h, (7) UV irradiation 1 h, washing in EtOH (70%) and in DMEM 3×24 h (biological replicates $n = 3$, with 6 technical replicates each).

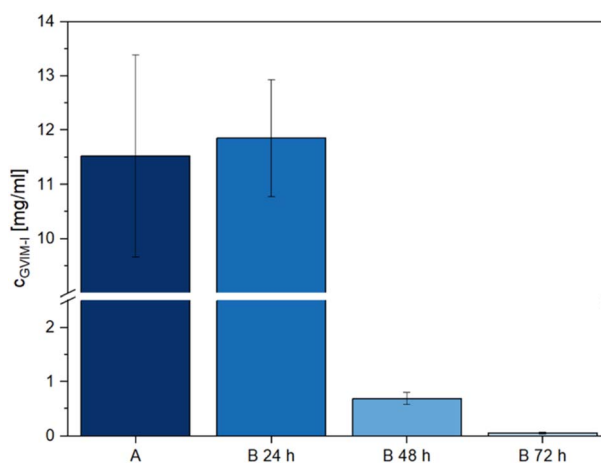


Fig. 4 The concentration of leached-out unreacted GVIM-I from hydrogel samples measured by LC-MS. (A) Washing in ultrapure water for 72 h, (B) washing in ultrapure water for 1×24 h (B 24 h) 3×24 h (B 48 h) 3×24 h (B 72 h) ($n = 3$, each sample was measured 3 times via LC-MS).



incubation, a CTB cell viability assay (Fig. 3) and live/dead staining with calcein-AM and PI (Fig. 6) were performed.

Fig. 3 provides clear evidence that contact with untreated hydrogels (sample 1) induces cell death, as indicated by relative cell viability of $-0.46 \pm 0.99\%$. This is also confirmed by the microscopy images in Fig. 6. After 48 h of contact, cells exhibited a spherical morphology, indicating cell death. In live/

dead staining, calcein stain shows a low number of viable cells, and a high number of dead cells, in comparison to the control.

It can be observed that each treatment leads to a higher relative cell viability than using the hydrogel untreated. The least positive effect was observed in the case of irradiation with UV light for one hour (sample 5) with a relative cell viability of $17.20 \pm 9.50\%$. This treatment was chosen to ensure the highest

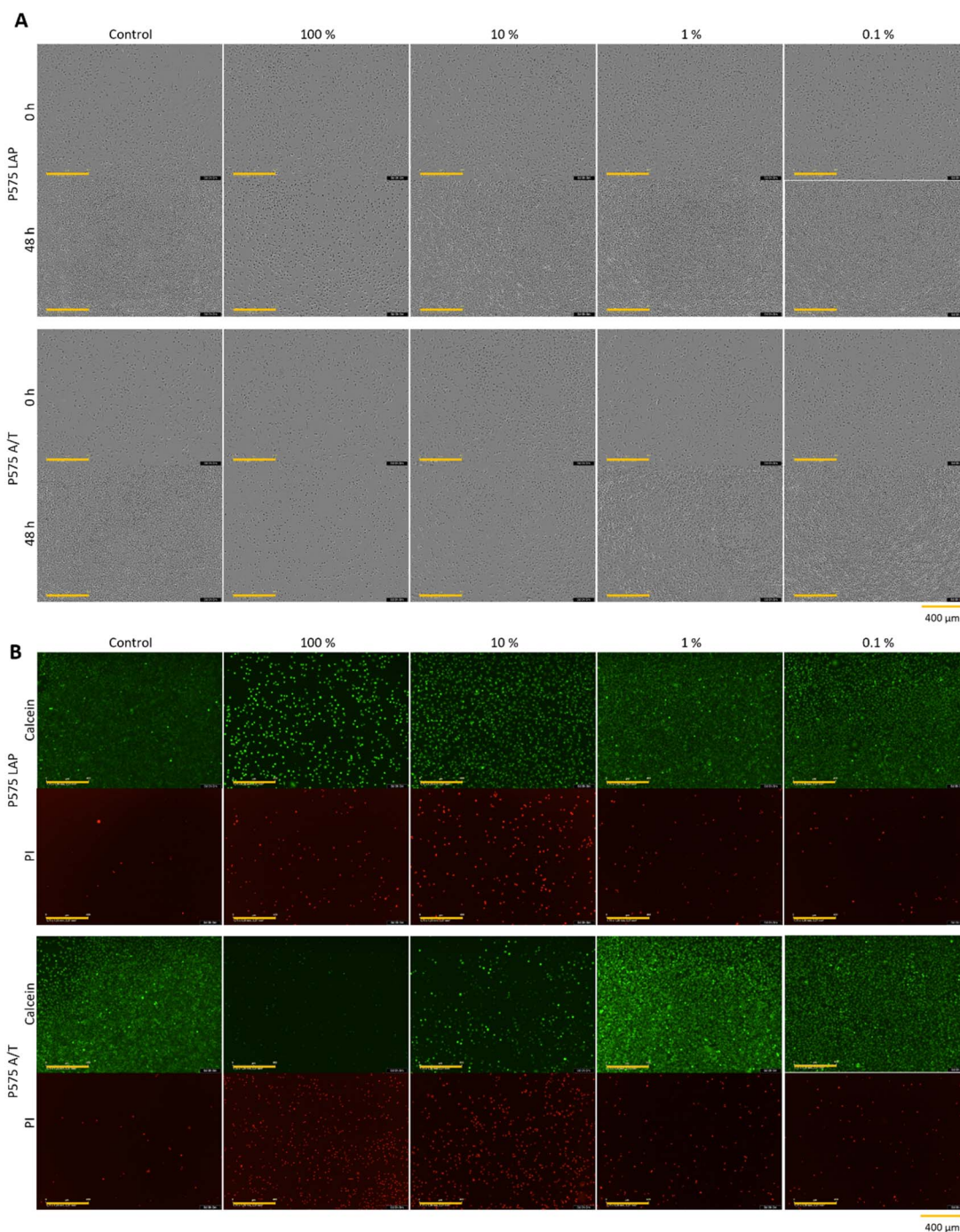


Fig. 5 (A) Microscopic images after 0 h and 48 h (yellow scale bar in each image = 400 μm) and (B) calcein-AM/PI staining (yellow scale bar in each image = 400 μm) of L929 cell cultivated for 48 h in different concentrated (100%, 10%, 1% and 0.1%) hydrogel eluates from the hydrogel samples P575 LAP and P575 A/T (which represent hydrogels produced with the 10% PEGDA 575 as crosslinker, polymerized with LAP or APS/TEMED). One representative of each concentration of all samples is demonstrated in Fig. S13, S14 and S16–S18 (see ESI).†



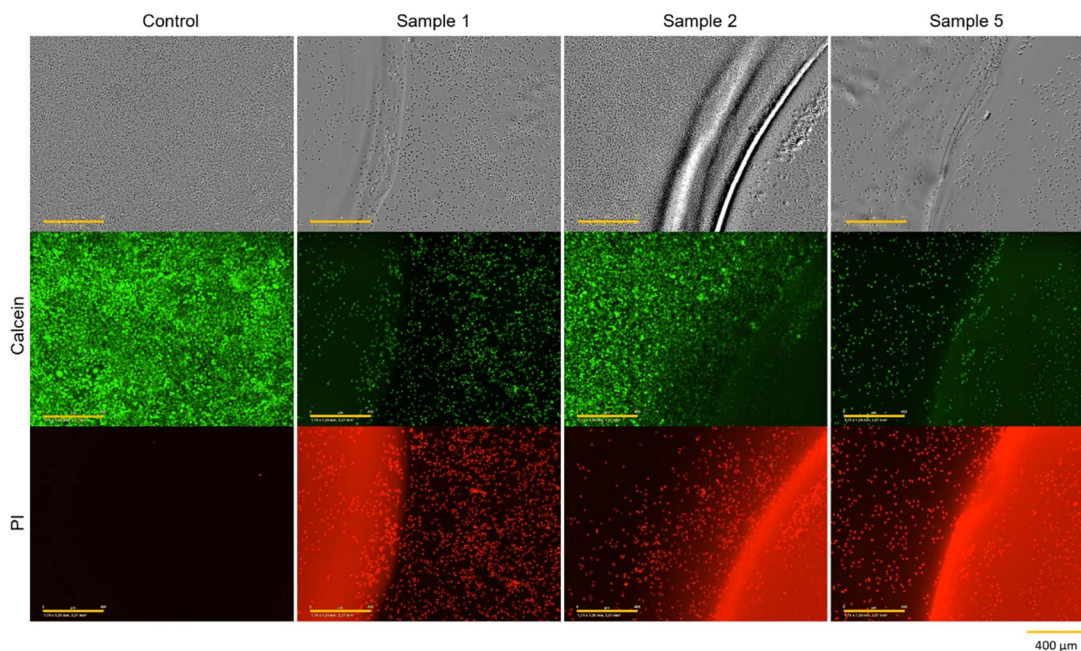


Fig. 6 Microscopic images and calcein-AM/PI staining of L929 cells cultivated for 48 h in direct contact with sample 1 (no treatment), sample 2 (washing in DMEM 3×72 h) and sample 5 (UV irradiation for 1 h) of PEGDA 575 10 mol% hydrogels (yellow scale bar in each image = $400 \mu\text{m}$). One representative of each sample treatment is demonstrated in Fig. S15 and S19 (see ESI)†

possible conversion of monomer and crosslinker so that little unreacted monomer and crosslinker as possible is left and can leach out, as these can have cytotoxic effects depending on their concentrations.²⁴ As observed in Fig. 6, the low cell viability is reflected in the cell morphology. The cells exhibited a spherical morphology, indicative of cell death. Which is also demonstrated in PI staining, as the majority of cells present are dead.

The combination of UV irradiation for one hour and washing the hydrogels for 3×24 h in DMEM at 37°C (sample 6) resulted in a relative cell viability improvement of approximately 24%. Washing the hydrogels for 3×24 h without prior UV irradiation (sample 3) showed a relative cell viability of $66.87 \pm 20.86\%$. When the gels were washed for 1×72 h (sample 2), *i.e.* without changing media, relative cell viability was $72.38 \pm 15.93\%$. This high viability is also evident in microscopy images. Fig. 6 shows that the cells have not only survived but have grown. After 48 h, a full lawn can be seen. Live/dead staining confirms this statement. Fig. 6 also indicates that there are more dead cells under and directly next to the hydrogel than further away from the edge of the gel and that cells further away are alive.

In addition to washing with DMEM, ethanol (70%) was also used as a washing medium for samples 4 and 7 (1×24 h). However, this did not result in an exceptionally positive effect. Without the ethanol washing step, relative cell viability is higher. In conclusion, different hydrogel treatments lead to an increase in relative cell viability, especially by washing in DMEM.

LC-MS analysis

The direct contact test results show that washing the hydrogels has a positive effect on relative cell viability. In light of this, it is crucial to ascertain the specific components that are removed

from the hydrogels during the washing process, as this process is likely responsible for the pronounced cytotoxicity observed in samples 1 and 5 in Fig. 3. To determine which components had leached out of the samples, hydrogels were prepared following the methodology employed for direct contact tests. They were then washed for 72 h in ultrapure water at $37 \pm 2^\circ\text{C}$ (Fig. 4, A), to simulate sample 2 of Fig. 3. The hydrogels were washed 3×24 h in ultrapure water at $37 \pm 2^\circ\text{C}$ (Fig. 4, B 24 h, B 48 h and B 72 h) to simulate sample 3 of Fig. 3. Subsequently, the washing water was then analyzed using LC-MS.

In preliminary investigations, the positive scan of the LC-MS method demonstrated a clear signal at m/z 271 (Fig. S21A and B†), which precisely corresponds to the mass of the GVIM⁺ monomer ion. In the negative scan mode, the counterion I^- was identified at m/z 126 (Fig. S21C and D†). In all further investigations, the positive scan mode was employed to determine to which extent unreacted GVIM-I is washed out of the hydrogels.

Fig. 4 illustrates that the majority of unreacted GVIM-I is washed out within the first 24 h, with only a very low concentration remaining after the third 24 h period. If the hydrogels are washed only once for 72 h, the total amount of GVIM-I washed out is smaller than with 3×24 h, as the concentration gradient is repeatedly increased here by changing the medium. One milliliter of wash medium contains 11.53 ± 1.86 mg of GVIM-I after 72 h of washing. After the first 24 h of washing 11.86 ± 1.08 mg mL^{-1} of GVIM-I was detected, after the second 24 h 0.69 ± 0.11 mg mL^{-1} , and after the third time washing 0.06 ± 0.01 mg mL^{-1} was washed out of the hydrogels. To prepare the hydrogels a GVIM-I solution of 500 mg in 1 mL PBS is employed for one batch. An amount of 11.53 ± 1.86 mg washed out from the hydrogels once for 72 h corresponds to



2.3% of the initial GVIM-I amount, whereas 12.61 ± 1.20 mg washed out after washing three times for 24 h and changing the medium in between corresponds to 2.5% GVIM-I of the initially GVIM-I amount. Assuming 2.5% GVIM-I was washed out implies that 97.5% GVIM-I was crosslinked and is involved in hydrogel formation. As a complete conversion of GVIM-I cannot be guaranteed, washing the hydrogels and changing the medium regularly to remove unreacted monomer is a reasonable strategy. This can improve the relative viability of cells *in vitro* biocompatibility studies.^{6†}

Conclusions

In the present study, we successfully investigated our novel carbohydrate-based ionic hydrogels for their antimicrobial properties and *in vitro* biocompatibility. We investigated the properties of the hydrogel eluates on mouse L929 cells as well as the influence of direct contact of the samples on the cells and their growth. In addition, we analyzed the eluates qualitatively and quantitatively by LC-MS.

The hydrogels tested did not affect the growth of the Gram-positive *B. subtilis* and the Gram-negative *E. coli* during disk diffusion tests. However, growth inhibition of *C. auris* was observed in all samples. The choice of crosslinker and initiator system influences the strength of growth inhibition. With the short-chain crosslinkers EGDA and PEGDA 250, the inhibition of *C. auris* growth stronger than with PEGDA 575. It was noticeable that especially the hydrogels with APS/TEMED as the initiator system showed a greater effect than the samples with LAP as initiator.

In our investigation of the eluate toxicity of different hydrogel compositions, we demonstrated that both the choice of crosslinker and initiator system exerts a significant influence on the relative cell viability. The hydrogels with short-chain crosslinkers EGDA and PEGDA 250 showed very low relative cell viabilities with $2.59 \pm 3.42\%$ and $-0.76 \pm 0.83\%$, respectively, at an eluate concentration of 10%. In contrast, relative cell viabilities of $108.60 \pm 33.31\%$ and $54.77 \pm 12.96\%$ were achieved for hydrogels with the longer-chain crosslinker PEGDA 575 and PEGDA 700 at the same eluate concentration. Different crosslinker concentrations (10, 15 or 20 mol%) of the same crosslinker (PEGDA 575) exhibited no significant difference in the relative cell viability. The comparison of the polymerization initiators APS/TEMED and LAP revealed that the hydrogel eluates with LAP achieved significantly higher cell viabilities. At 10% eluate concentration, the use of LAP improved cell viability by 60.2% for MBAA and by 32.5% for PEGDA 575.

The direct comparison of the antimicrobial study and the *in vitro* biocompatibility reveals two trends: first, APS/TEMED generally leads to cytotoxic effects and is thus less preferable over the more biocompatible LAP, and second, long-chain crosslinkers (PEGDA 575 and 700) lead to a generally lower cytotoxicity than short-chain crosslinkers (EGDA and PEGDA 250), with PEGDA 575 showing the overall most promising results.

The objective of the direct contact tests was to ascertain the most effective treatment method for enhancing the cell viability of the hydrogels with 10 mol% PEGDA 575 as crosslinker, which

are toxic if non-treated. Washing treatments with DMEM and EtOH as well as UV treatment, and combinations thereof, were compared. It was found that for our hydrogels washing treatments are generally preferable over a UV irradiation treatment. The best results were achieved by washing the hydrogels in DMEM for 72 h or 3×24 h, leading to relative cell viabilities of $72.38 \pm 15.93\%$ and $66.87 \pm 20.86\%$, respectively. Thus, the hydrogels pre-treated with DMEM are biocompatible in the direct contact *in vitro* biocompatibility tests.

LC-MS analysis of the wash water of hydrogels treated with ultrapure water identified unreacted GVIM⁺ in the positive scan mode and found the counter ion I⁻ in the negative scan mode. Quantification revealed that approximately 2.5% of the GVIM-I used in the hydrogel synthesis was found in the wash water, which could possibly be the cause the cytotoxicity in the eluate tests.

The knowledge accumulated in this work enables the development of a standardized production process for our new GVIM-I hydrogels. It is essential to identify synthesis techniques that minimize the quantity of residual starting material. Furthermore, the direct contact experiments showed how important the washing process is for the GVIM-I hydrogels to prepare them for future biomedical applications.

Data availability

The data supporting this article have been included as part of the ESI.†

Author contributions

Sina Lambrecht: formal analysis, investigation & writing – original draft; Alina Gazizova: investigation & writing – review; Selin Kara: funding acquisition, writing – review & editing; Stefan Jopp: writing – review & editing; Johanna Meyer: conceptualization, methodology, formal analysis, investigation & writing – original draft. All authors approved the final version of the manuscript.

Conflicts of interest

There are no conflicts to declare.

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B Anhang: Sonstiges

I Doktorandenerklärung

I. Doktorandenerklärung

Doktorandinnen/Doktoranden-Erklärung gemäß § 4 Absatz 1 Buchstaben g und h der Promotionsverordnung der Mathematisch-Naturwissenschaftlichen Fakultät der Universität Rostock

Name: Sina Lambrecht
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Ich habe meine Dissertation zum Thema:

Synthese und Charakterisierung von kohlenhydrat-basierten ionischen Hydrogelen

an der Mathematisch-Naturwissenschaftlichen Fakultät der Universität Rostock
angefertigt. Dabei wurde ich von Herrn Dr. rer. nat. Stefan Jopp betreut.

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