Fabrication of mechanically robust PEG-based hydrogels for 3d printing and injection

Chapter 8

Valorization

8.1 Volumetric printing, a promising alternative to conventional fabrication technologies

The high annual mortality rate among people waiting for transplants worldwide has catalyzed significant progress in 3D biofabrication to develop patient-customized biological constructs that can lead to the regeneration or repair of impaired tissues.[1] Figures 8.1a and 8.1b illustrate the number of annual publications (research and review papers) and patents from 2000 to 2020 on 3D bioprinting.[2] These numbers highlight the growing interest and the efforts of academic communities and industries in this area to develop solutions to save lives.[2]

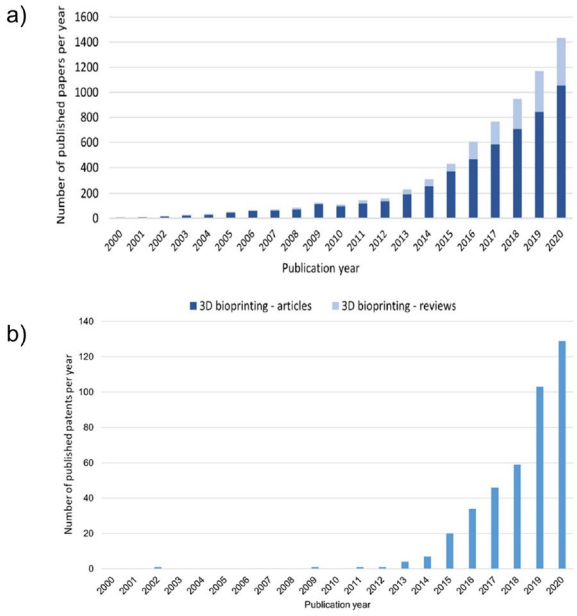

Figure 8.1: a) The number of papers published yearly from 2000 to 2020 on 3D bioprinting. Research papers are shown in dark blue, and review papers in light blue. b) The total number of patents published yearly from 2000 to 2020 on 3D bioprinting. Figures are reprinted from ref [2] with permission from Springer Nature 2021.

Advances in bioinks and biomaterial inks, and additive manufacturing (AM) technologies are necessary for pushing 3D bioprinting forward. Volumetric printing (VP) and volumetric bioprinting (VBP) are AM technologies that can revolutionize the field of 3D bioprinting by producing complex geometries in seconds.[3, 4] Figure 8.2 illustrates the increasing number of VP-related publications from 2017 to June 2023, with a notable increase anticipated by the end of 2024.[5]

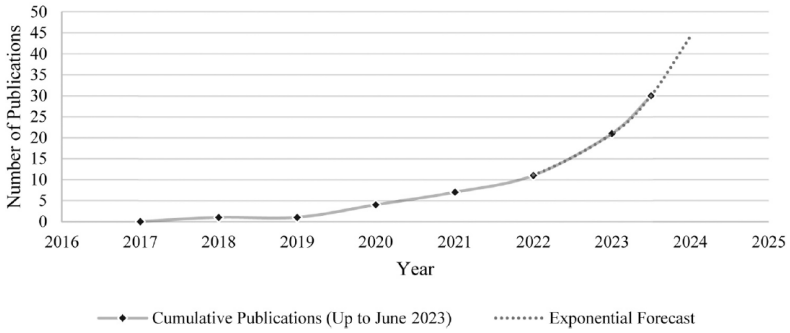

Figure 8.2: The number of published papers on VP from 2017 to June 2023, with a sharp increase expected by the end of 2024. The figure is reprinted from ref [5] with permission from Elsevier 2024.

8.2 The synthetic PEG-based Thiol-Norbornene VP resin

Chapter 5 demonstrated the volumetric printability of my fully synthetic PEG-based hydrogel resins made from linear PEG-Norbornene$_{10k}$ and a 4-arm PEG-Thiol$_{2k}$ crosslinker. Various robust and complex geometries were fabricated without supporting elements using these photosensible resins in less than 35 seconds with no post-printing curing.

The spectrum of VP-processable hydrogel bioinks and biomaterial inks is currently limited, and the majority of research published in this field has been on gelatin-based resins or the use of gelatin as a sacrificial bath to facilitate processing. As far as I know, my work presented one of the first fully synthetic hydrogel system

that crosslinks via step-growth thiol-norbornene chemistry for processing through VP.

8.3 Challenges and future

The VP era, in my opinion, is just getting started. Despite the growing interest and promising potential, all VP-related research has been limited to a few research groups worldwide due to the high cost and limited accessibility of VP technology. Only two companies (Xolo and Readily3D) currently offer commercial volumetric 3D printers. I believe that as the technology continues to mature and more advancements—such as my current work—are published, the biofabrication community will become more aware of the capabilities of this technology. Consequently, more affordable commercial options will emerge.

Some companies, such as Bioinx (READYGEL INX) and CELLINK, produce commercial inks for VP. My PEG-based fully synthetic resin has properties such as tunable mechanics with minimal system modification, readily accessible and affordable polymers to make the resin's building blocks, straightforward synthesis protocols to create PEG-Norbornene and PEG-Thiol building blocks, biocompatibility, and biodegradability. Consequently, it has the potential to be commercialized by these companies following further optimizations to expand the range of processable hydrogel resins via VP.

8.4 References

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(2) Santoni, S.; Gugliandolo, S. G.; Sponchioni, M.; Moscatelli, D.; Colosimo, B. M. 3D Bioprinting: Current Status and Trends—a Guide to the Literature and Industrial Practice. *Bio-Des. Manuf.* **2022**, *5*, 14-42.

(3) Kelly, B. E.; Bhattacharya, I.; Heidari, H.; Shusteff, M.; Spadaccini, C. M.; Taylor, H. K. Volumetric Additive Manufacturing Via Tomographic Reconstruction. *Science* **2019**, *363*, 1075-1079.

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(5) Whyte, D. J.; Doeven, E. H.; Sutti, A.; Kouzani, A. Z.; Adams, S. D. Volumetric Additive Manufacturing: A New Frontier in Layer-Less 3D Printing. *Addit. Manuf* **2024**, 104094.

Summary

Hydrogels, due to their unique characteristics, are ideal candidates for tissue engineering and regenerative medicine applications. However, their inherent softness and brittleness, resulting from their high water content and the non-uniform structure formed during the crosslinking process, can limit their applications in load-bearing tissues such as cartilage, tendons, and ligaments. The overall aim of this work was to enhance the mechanical performance of poly(ethylene glycol) (PEG)-based hydrogels using two distinct and straightforward strengthening strategies compatible with injection and various light-based 3D printing technologies.

In Chapter 3, the strategy of creating relatively uniform networks using nucleophilic thiol-yne chemistry was applied to improve the mechanical performance of hydrogels by minimizing structural defects, which act as stress concentration zones during deformation. It was demonstrated that the mechanical properties could be tuned by varying parameters such as polymer content, molecular weight, and the architecture of the building blocks. To evaluate the effect of network uniformity on hydrogel mechanics, relatively non-uniform hydrogels were also prepared using identical building blocks but crosslinked via UV light. The results showed that the relatively uniform PBS-crosslinked gels were mechanically more robust than their UV-crosslinked counterparts, despite using the same building blocks. Furthermore, the mechanical properties correlated well with the crosslink and entanglement densities predicted by the Rubinstein–Panyukov model.

Continuing with the strategy of fabricating relatively uniform hydrogels, Chapter 4 explored thiol-norbornene chemistry to create PEG-based gels. A comprehensive

library of hydrogels was developed by varying the polymer content, the molecular weight of PEG-Norbornene and PEG-Thiol crosslinkers, and the molar ratio of reactive end groups. The structure-property relationships in these hydrogels were systematically analyzed, demonstrating that robust formulations suitable for load-bearing applications could be achieved. Additionally, it was shown that, regardless of formulation, these hydrogels exhibited rapid gelation kinetics, solidifying within seconds of light illumination.

In Chapter 5, the photocurable hydrogel resins developed in Chapter 4 were utilized for injection and processing via two advanced light-based 3D printing technologies: digital light processing (DLP) and volumetric printing (VP). These resins were proved highly suitable for these applications due to their fast gelation kinetics upon light exposure. However, modifications were necessary to meet the specific requirements of each printing technology. The fast fabrication of complex structures (<32 seconds) without any supporting elements was demonstrated via VP, outperforming conventional layer-based fabrication methods. Additionally, the printed structures were mechanically robust immediately after fabrication, requiring no post-printing curing.

In Chapter 6, an interpenetrating network (IPN) strategy was employed to create robust hydrogels by combining the thiol-yne PEG networks developed in Chapter 3 with sodium alginate networks using a straightforward one-pot preparation method. The effects of various parameters—such as the uniformity of the PEG network, the order of network formation, the characteristics of alginate, and the building blocks used—on the reinforcing effects were systematically studied. While the addition of alginate enhanced the elastic modulus and maximum stress in some formulations, its most pronounced effect was on the fracture energy of the

gels. The findings of this chapter provide valuable insights into designing hydrogel formulations tailored to specific application requirements.

Samenvatting

Door hun unieke eigenschappen zijn hydrogels ideale kandidaten voor toepassingen in weefselengineering en regeneratieve geneeskunde. Hun inherente zachtheid en brosheid, voortvloeiend uit hun hoge watergehalte, en de niet-uniforme structuur die ontstaat tijdens het crosslinkproces, kunnen echter hun toepassing in dragende weefsels zoals kraakbeen, pezen en ligamenten beperken. Het algemene doel van dit werk was het verbeteren van de mechanische prestaties van poly(ethyleenglycol) (PEG)-gebaseerde hydrogels door twee verschillende, eenvoudige versterkingsstrategieën toe te passen die compatibel zijn met toediening via injectie en diverse lichtgebaseerde 3D-printtechnologieën.

In Hoofdstuk 3 werd nucleofiele thiol-yne-chemie toegepast als strategie om relatief uniforme en mechanisch robuustere netwerken te creëren door structurele defecten, die fungeren als spanningsconcentratiezones tijdens vervorming, te minimaliseren. Het werd aangetoond dat de mechanische eigenschappen konden worden afgestemd door parameters, zoals het polymeergehalte, het molecuulgewicht en de architectuur van de bouwstenen, te variëren. Om het effect van netwerkuniformiteit op de mechanische sterkte van hydrogels te evalueren, werden ook minder uniforme hydrogels bereid door diezelfde bouwstenen te crosslinken via UV-licht. De resultaten toonden aan dat de relatief uniforme, met PBS gecrosslinkte gels mechanisch robuuster waren dan hun met UV gecrosslinkte tegenhangers, ondanks het gebruik van dezelfde bouwstenen. Bovendien correleerden de mechanische eigenschappen goed met de crosslink- en verstrengelingsdichtheden die werden voorspeld door het Rubinstein–Panyukov-model.

Voortbouwend op de strategie toegepast in Hoofdstuk 3, werd in Hoofdstuk 4 thiol-norborneen-chemie onderzocht om uniforme PEG-gebaseerde gels te creëren. Een uitgebreide bibliotheek van hydrogels werd ontwikkeld door het variëren van het polymeergehalte, het molecuulgewicht van PEG-norborneen en PEG-thiol crosslinkers, en de molaire verhouding van reactieve eindgroepen. De structuur-eigenschap-relaties in deze hydrogels werden systematisch geanalyseerd, waaruit bleek dat robuuste formuleringen geschikt voor dragende toepassingen konden worden bereikt. Daarnaast werd aangetoond dat deze hydrogels, ongeacht de formulering, snelle geleringstijden vertoonden en binnen enkele seconden na blootstelling aan licht uitharden.

In Hoofdstuk 5 werden de fotohardende hydrogelprecursoren die in Hoofdstuk 4 zijn ontwikkeld, gebruikt voor injectie en verwerking via twee geavanceerde lichtgebaseerde 3D-printtechnologieën: digital light processing (DLP) en volumetric printing (VP). Deze precursoren bleken zeer geschikt voor deze toepassingen vanwege hun snelle geleringstijden bij blootstelling aan licht. Aanpassingen waren echter nodig om te voldoen aan de specifieke eisen van elke printtechnologie. Het snelle fabriceren van complexe structuren (<32 seconden) zonder ondersteunende elementen werd gedemonstreerd via VP, dat beter presteerde dan conventionele op lagen gebaseerde fabricagemethoden. Bovendien waren de geprinte structuren direct na fabricage mechanisch robuust, zonder dat nabehandeling vereist was.

In Hoofdstuk 6 werd een interpenetrerend netwerk (IPN)-strategie toegepast om robuuste hydrogels te creëren door de thiol-yne PEG-netwerken, ontwikkeld in Hoofdstuk 3, te combineren met natriumalginaatnetwerken via een eenvoudige one-pot-bereidingsmethode. De effecten van verschillende parameters

(uniformiteit van het PEG-netwerk, volgorde van netvorming, de eigenschappen van alginaat en de gebruikte bouwstenen) op de mechanische sterkte van de hydrogel werden systematisch bestudeerd. Hoewel de toevoeging van alginaat de elastische modulus en de maximale spanning in sommige formuleringen verbeterde, was het meest uitgesproken effect te zien op de breukenergie van de gels. De bevindingen van dit hoofdstuk bieden waardevolle inzichten voor het ontwerpen van hydrogelformuleringen die zijn afgestemd op specifieke toepassingsvereisten.