Maastricht University

Photo-enzymatic dityrosine crosslinking for bioprinting

Citation for published version (APA):

Perin, F., Mota, C., Mancini, I., Motta, A., & Maniglio, D. (2022). Photo-enzymatic dityrosine crosslinking for bioprinting. *Polymer*, *252*, Article 124941. https://doi.org/10.1016/j.polymer.2022.124941

Document status and date:
Published: 14/06/2022

DOI:
10.1016/j.polymer.2022.124941

Document Version:
Publisher's PDF, also known as Version of record

Document license:
Taverne

Please check the document version of this publication:

• A submitted manuscript is the version of the article upon submission and before peer-review. There can be important differences between the submitted version and the official published version of record. People interested in the research are advised to contact the author for the final version of the publication, or visit the DOI to the publisher's website.
• The final author version and the galley proof are versions of the publication after peer review.
• The final published version features the final layout of the paper including the volume, issue and page numbers.

Link to publication

General rights
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

• Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
• You may not further distribute the material or use it for any profit-making activity or commercial gain
• You may freely distribute the URL identifying the publication in the public portal.

If the publication is distributed under the terms of Article 25fa of the Dutch Copyright Act, indicated by the “Taverne” license above, please follow below link for the End User Agreement:

www.umlib.nl/taverne-license

Take down policy
If you believe that this document breaches copyright please contact us at:

repository@maastrichtuniversity.nl

providing details and we will investigate your claim.

Download date: 16 May. 2024

Polymer 252 (2022) 124941

Contents lists available at ScienceDirect

Polymer

journal homepage: www.elsevier.com/locate/polymer

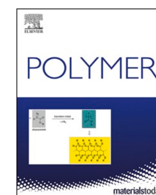

Photo-enzymatic dityrosine crosslinking for bioprinting

Francesca Perin [a,b,**], Carlos Mota [c], Ines Mancini [d], Antonella Motta [a,b,*], Devid Maniglio [a,b]

[a] BIOTech Research Center, European Institute of Excellence on Tissue Engineering and Regenerative Medicine, University of Trento, via Delle Regole 101, Trento, 38123, Italy

[b] Department of Industrial Engineering, University of Trento, Via Sommarive 9, 38123, Trento, Italy

[c] Department of Complex Tissue Regeneration, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Maastricht, 6211 LK, the Netherlands

[d] Laboratory of Bioorganic Chemistry, Department of Physics, University of Trento, Via Sommarive 14, 38123, Trento, Italy

A R T I C L E I N F O

Keywords:
Photocrosslinking
Riboflavin
Horseradish peroxidase
Enzymatic crosslinking
Bioprinting
Alginate
Dityrosine bonding

A B S T R A C T

In this research work, we demonstrate photo-triggering of the horseradish peroxidase (HRP) dityrosine crosslinking. The photo-enzymatic crosslinking of tyramine modified alginate was obtained by introducing riboflavin as a photosensitizer, and by exposing the polymer solution to light radiation at 365 nm. The activation of HRP by light was confirmed by photo-rheology. This crosslinking method proved to be superior to UV crosslinking by riboflavin alone both in terms of kinetics and obtainable storage modulus. Furthermore, activation of HRP by UV light offers the possibility to trigger the onset of the reaction in a precise manner, which is not available with the traditional use of hydrogen peroxide as the activator. The photo-enzymatic crosslinking was applied to bioprinting, to verify its potential in biofabrication techniques requiring precise on-demand crosslinking of hydrogels.

1. Introduction

Horseradish Peroxidase (HRP) is a heme-containing enzyme produced by the horseradish *Armoracia rusticana*, a type of perennial herb commonly cultivated for culinary purposes. HRP role in plant metabolic cycles is to use hydrogen peroxide (H_2O_2) as a medium to oxidize numerous metabolic compounds, including phenols. This feature has been applied in the bioengineering field to form covalent dityrosine bonding in phenol containing hydrogels [1,2]. Dityrosine bonding is common in assorted proteins in nature (resilins, silks, elastin), all sharing high elasticity and toughness [1], and can be reproduced by enzymatically crosslinking phenol-containing polymers with HRP [3–10]. Commonly, hydrogels are crosslinked by mixing HRP in the prepolymer solution and adding H_2O_2 to trigger the crosslinking reaction after casting the prepolymer solution into molds. The peroxidase reaction kinetics are strongly dependent on the activity of the enzyme, on the concentration of H_2O_2 in the aqueous solution and the phenolic substrate oxidized as extensively studied by Nielsen et al. [11]. Analogously, studies on the enzymatic gelation kinetics of hydrogels containing phenolic-moieties report the variation of HRP and H_2O_2 as the main parameters to tailor the gelation kinetics [4,7]. Hasturk et al. [7] reported the production of enzymatic crosslinked hydrogels by using a co-flow microfluidic approach to incorporate H_2O_2 in the prepolymer solution right before deposition. This is indeed promising in opening up the possibility of applying this enzymatic crosslinking to fabricate constructs with high geometric precision by bioprinting. On the other hand, achieving a precise on-demand triggering of the enzymatic crosslinking reaction would allow to extend its use to a wider range of bioprinting techniques.

Here, we propose the use of a photoinitiator to introduce an on demand trigger to the enzymatic reaction. The activation of the HRP cycle by light and methylene blue as a photosensitizer has been demonstrated by Soares et al. [12], who hypotesized its use in nanotechnology to exploit solar energy. In this research work, the same principle was applied to crosslinking hydrogels and riboflavin (Rb) was selected as a photoinitiator. Rb, commonly known as vitamin B_2, is a known photosensitizer producing reactive oxygen species (ROS) when exposed to light radiation in the UV–Vis range, at 375 nm and 445 nm [13–15]. Rb

* Corresponding author. BIOTech Research Center, European Institute of Excellence on Tissue Engineering and Regenerative Medicine, University of Trento, via Delle Regole 101, Trento, 38123, Italy.

** Corresponding author. BIOTech Research Center, European Institute of Excellence on Tissue Engineering and Regenerative Medicine, University of Trento, via Delle Regole 101, Trento, 38123, Italy.

E-mail addresses: francesca.perin@unitn.it (F. Perin), antonella.motta@unitn.it (A. Motta).

https://doi.org/10.1016/j.polymer.2022.124941
Received 20 January 2022; Received in revised form 21 April 2022; Accepted 4 May 2022
Available online 16 May 2022
0032-3861/© 2022 Elsevier Ltd. All rights reserved.

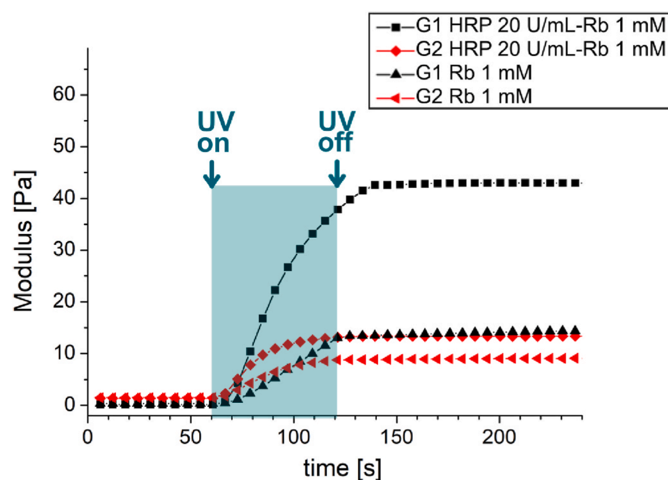

Fig. 1. Photorheology results on samples with 1 mM Rb, with and without HRP.

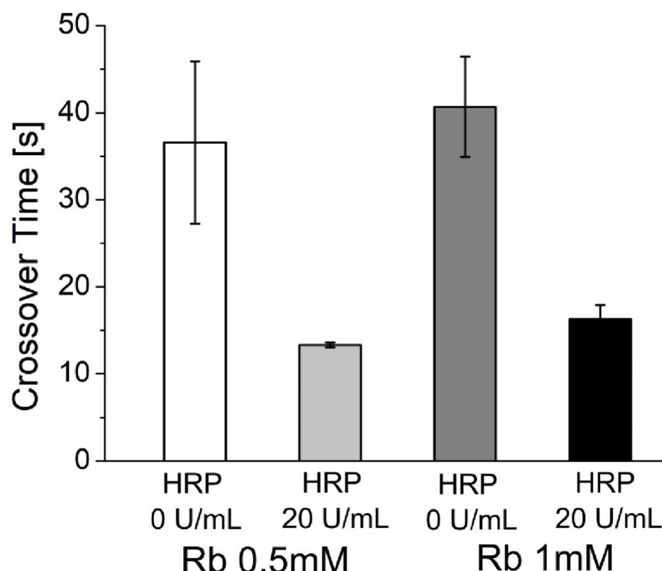

Fig. 2. Gelation times determined by photorheology for hydrogels with Rb content 0.5 mM and 1 mM, with and without HRP.

can induce dityrosine crosslinking after photosensitization, thanks to the production of ROS triggering oxidation of phenolic groups into tyrosil radicals and the consequent formation of dityrosine bonds from their self-oxidation. Rb and UV–Vis irradiation have been used to produce assorted natural origin polymers hydrogels [14,16–21], and to crosslink proteins in tissues for clinical applications [22]. A major drawback of this photo-crosslinking technique is the long exposure time required for gelation, ranging from minutes to hours as reported in the review by Liu et al. [23], making it not competitive for advanced biofabrication techniques such as bioprinting or digital light processing (DLP), considering that acrylates require just a few seconds exposure for UV curing [24,25]. This issue can be partially overcome by using persulfate salts as electron acceptors to enhance the kinetic of ROS production [14,19,26]. However as proved by Hong et al. [26] the UV irradiation and the radicals can be detrimental for polymers due to chain degradation, resulting in unstable hydrogels with limited lifetime. Furthermore, this technique raises cytocompatibility concerns related to the long exposure periods and to residual persulfate salts as demonstrated by Elvin et al. [27]. We assumed that by combining HRP and Rb in solution, the enzymatic crosslinking reaction could be triggered by the hydroxyl radicals produced by photosensitized Rb [28–30], achieving stable enzymatic crosslinked hydrogels with the convenience of the on demand triggering of the reaction by UV exposure. This hypothesis was tested and confirmed on tyramine functionalized alginate, producing hydrogels with higher moduli and shorter crosslinking times compared with the photocrosslinking with Rb alone, while avoiding the use of persulfates, excessively long UV exposures and their resultant cytotoxicity. Moreover, the possibility of activating and de-activating the crosslinking reaction on demand was confirmed and its potential was demonstrated on bioprinting.

2. Results and discussion

All the experiments described in this work were carried out with tyramine-modified-alginate at a concentration of 2% w/v, with a degree of substitution (DS) of 36 ± 2% as quantified by NMR analysis. The modification of alginate was obtained through carbodiimide chemistry following procedures already reported in literature [6,31,32]. Further information on the modification procedure and on the NMR analysis are available in the supplementary data (S1). As a preliminary study, to screen the validity of this crosslinking approach, hydrogels were prepared at different concentrations of Rb (0.1–0.5 mM) and HRP (0–20 U/mL), and their swelling and degradation behaviours were monitored during one week, in physiological conditions. Description and results of this preliminary experiment are available in the Supplementary data (S2). This experiment allowed to preliminarily corroborate the validity of the method, as in all the Rb concentrations tested, the hydrogels without HRP dissolved in less than 24 h while the ones with the enzyme lasted longer and gelled faster. Moreover, from this first evaluation it was clear that Rb below 0.5 mM could not guarantee a satisfying gelation kinetics. Photorheology was used to better analyze the gelation kinetics. Tests were performed on a Discovery HR2 Rheometer from TA instruments, equipped with a cone-plate geometry (diameter 50 mm, 2°, 100 μm) which base was modified to host a LED as the UV radiation source (*LZ4-44-UV00* from *LED ENGIN*, emitting at 365 nm, 2.9 W). AL-TY hydrogels (2% w/v) containing Rb 0.5 mM or 1 mM, with or without the enzyme, were studied by rheological measurements at constant strain (3%) and constant frequency (1Hz). All solutions were kept at room temperature overnight and then tested. Rheological measurements were all carried out without UV stimuli for 60 s and then by irradiating the samples for 60 s, and monitoring the evolution of the storage (G1) and loss modulus (G2) for 3 more minutes. Fig. 1 shows the rheological curve of the Rb 1 mM samples, with and without enzyme. A similar trend was observed for all the curves obtained, as evident in the whole set of curve available as Fig. S3.

From the photorheology graphs it is evident that before UV irradiation both G1 and G2 are constant, and they both increase only when irradiated with light. The moduli stop increasing briefly after turning off the UV source. Gelation was determined at the modulus crossover point, when G1 overcomes G2, and was observed in all the conditions. The hydrogels without HRP are much weaker than the enzymatically crosslinked ones. The increased modulus achieved can be explained by the activation of the enzymatic reaction, triggered by the free radicals produced by photosensitized Rb. The crossover point for each curve was computed with the software *TRIOS* using the *"crossover modulus"* analysis function, and used to determine the average gelation time for each group, shown in Fig. 2.

It is evident that the enzyme containing solution gelled faster than its enzyme-free counterpart. We assumed that the faster gelation kinetic could be attributed to the activation of the enzymatic reaction. On the other hand, increasing the Rb content from 0.5 mM to 1 mM does not have a significant effect on the crossover time. This might indicate that at 0.5 mM, a plateau is reached in the efficiency of the reaction in function of the photoinitiator content, as described by Donnelly et al. [16] who studied the crosslinking of hyaluronan-tyramine with Rb. These preliminary experiments confirmed the hypothesis that Rb can be

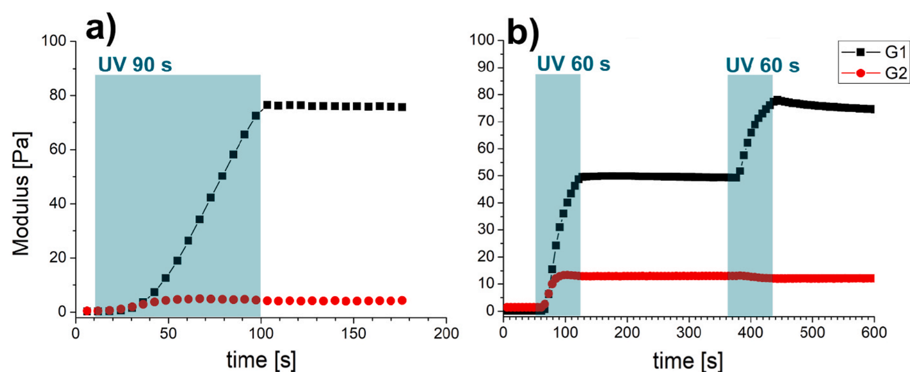

Fig. 3. Comparison of the effect of a single UV exposure of 90 s and two step crosslinking with separate UV exposures of 60 s. These photorheology experiments were carried out on samples with the following composition: AL-TY 2% w/v HRP 20 U/mL Rb 0.5 mM.

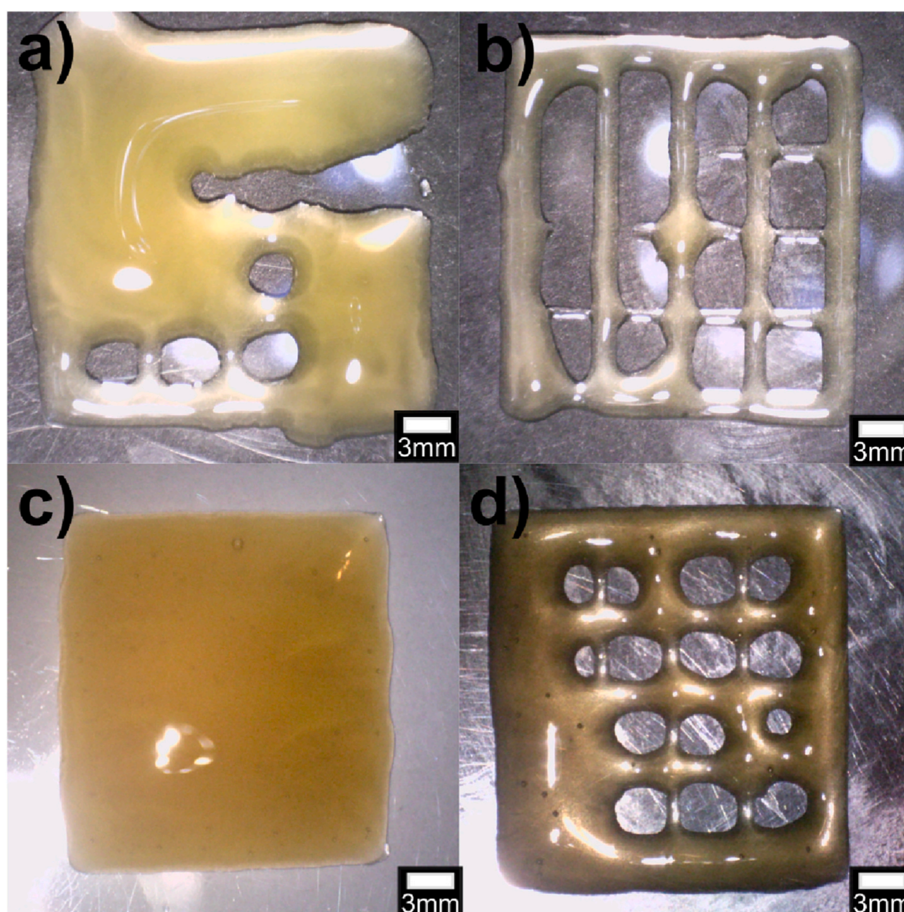

Fig. 4. Printed 1 layer grids of AL-TY 3% w/v, HRP 20 U/mL and Rb 1 mM: a) without UV crosslinking, pore closure is evident; b) irradiating the printing bed (365 nm) helps preserving geometrical control. Printed 3 layers grids of AL-TY 2% w/v HA-TY 4% w/v, HRP 20 U/mL and Rb 1 mM: c) without UV crosslinking, the grid ink collapses into a square; d) 10s UV crosslinking performed after each layer hinders pore closure and improves shape fidelity.

used to photo-trigger the activation of the peroxidase enzymatic crosslinking. However, this reaction should be further studied to deeply understand its kinetics and to be able to control the final properties of the hydrogels by varying the process parameters, such as relative concentration of reagents, light exposure time, wavelength used. For now, we could not recreate hydrogels as strong as the ones produced by activating HRP with H_2O_2 (G1 around 1000 Pa), therefore optimization is still needed for this crosslinking technique. Attempts were made to increase the modulus of the photo-enzymatic-crosslinked hydrogels by increasing the UV exposure time and with re-irradiation of the hydrogels. Increasing the exposure time from 60 s to 90 s increased the storage modulus from around 40 Pa to around 76 Pa (Fig. 3 a). A similar modulus increase can be achieved by re-exposing the hydrogel to UV radiation, as shown in Fig. 3 b in which one of the solutions from the condition Rb 0.5 mM and HRP 20 U/mL has been irradiated for 60 s, in two different steps during photorheology, for a total of 120s of light exposure time. When subjecting the polymer to multiple irradiation steps, longer exposure is needed to reach the same storage modulus because HRP is activated specifically by hydroxyl radicals, which are not produced instantaneously after irradiation but are the result of

decomposition of radical species produced by photo-sensitized riboflavin. Moreover, once the polymer is partially crosslinked, HRP has less phenol substrate to react since part of the tyramine groups already reacted into di-tyrosine bonds, which will slow down the crosslinking reaction. This result is promising for bioprinting, as it introduces the possibility to use a brief exposure to precrosslink the bioinks to ensure a sufficient viscosity enhancing the shape retention after extrusion, and subsequently re-irradiate to consolidate the printed construct.

In order to demonstrate the possibility to apply this technique in bioprinting, some grids were printed, with and without photo-crosslinking, as shown in Fig. 4. 1-layer grids of AL-TY 3% w/v, with HRP 20 U/mL and 1 mM Rb, were printed on a *BioX* ^{TM} from Cellink, using an extrusion pressure of 25 kPa, a 20G needle and a printing speed of 15 mm/s. Without the aid of a pre-crosslinking technique (i.e. calcium), a support printing bath or a calcium bed, printing alginate at this concentration results in very poor shape retention and quick pore closure. This is evident in Fig. 4 a), in which the polymer solution was printed without the aid of UV crosslinking. On the other hand, when irradiating the printing bed with a UV source, pore closure was hindered and the geometry of the grid was better preserved, as shown in Fig. 4 b).

Additionally, 3 layer grids were printed with a blend of alginate-tyramine and hyaluronan-tyramine (HA-TY). The final composition of the blend was 2% w/v AL-TY, 4% w/v HA-TY, 20 U/mL HRP, and 1 mM Rb. The blend was printed using an extrusion pressure of 40 kPa, a 25G conical needle and a printing speed of 6 mm/s. The blend chosen produces quite well defined grids when extruded at low pressures, however it quickly collapses and undergoes pore closure, especially when printing several layers, as shown in Fig. 4 c). When using brief UV crosslinking after each layer (10 s, 365 nm), pore closure is hindered and the geometry of the grid is better preserved, as shown in Fig. 4 d). Moreover, after printing, the mechanical properties of the constructs can be improved by reactivating the enzymatic reaction by briefly placing them in a bath containing hydrogen peroxide. A preliminary evaluation of cytocompatibility of this crosslinking method showed a good cell viability up to 48h (Fig. S4). Further studies will aim at investigating longer culture periods and differentiation of hMSCs towards bone constructs.

3. Conclusions

In conclusion, we have demonstrated how the photo-activation of the peroxidase enzymatic reaction is suitable for the production of dityrosine crosslinked hydrogels. By photorheology, the use on HRP combined with Rb was proven to produce stronger hydrogels with shorter UV exposures, which is generally less detrimental for the polymers and for encapsulated cells. Further research is still needed to gain full knowledge of the reactions involved in this crosslinking technique and to unravel its full potential in terms of mechanical properties of the produced hydrogels. Nonetheless, we have proved that this method is suitable for those bioprinting techniques requiring precise on-demand photochemical triggered crosslinking, enabling realistic translation of HRP to biofabrication.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This project was realized with the aid of the funding from Fondazione VRT. This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 101008041.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.polymer.2022.124941.

References

[1] B.P. Partlow, M.B. Applegate, F.G. Omenetto, D.L. Kaplan, Dityrosine cross-linking in designing biomaterials, ACS Biomater. Sci. Eng. (2) (2016) 2108–2121.

[2] W. Song, J. Ko, Y.H. Choi, N.S. Hwang, Recent advancements in enzyme-mediated crosslinkable hydrogels: in vivo-mimicking strategies, APL Bioeng (5) (2021).

[3] S. Sakai, K. Hirose, K. Moriyama, K. Kawakami, Control of cellular adhesiveness in an alginate-based hydrogel by varying peroxidase and h2o2 concentrations during gelation, Acta Biomater. (6) (2010) 1446–1452.

[4] S. Sakai, T. Matsuyama, K. Hirose, K. Kawakami, In situ simultaneous protein-polysaccharide bioconjugation and hydrogelation using horseradish peroxidase, Biomacromolecules (11) (2010) 1370–1375.

[5] S. Bagheri, Z. Bagher, S. Hassanzadeh, S. Simorgh, S.K. Kamrava, V.T. Nooshabadi, R. Shabani, M. Jalessi, M. Khanmohammadi, Control of cellular adhesiveness in hyaluronic acid-based hydrogel through varying degrees of phenol moiety cross-linking, J. Biomed. Mater. Res. (109) (2021) 649–658.

[6] A. Darr, A. Calabro, Synthesis and characterization of tyramine-based hyaluronan hydrogels, J. Mater. Sci. Mater. Med. 20 (2009) 33–44.

[7] O. Hasturk, K.E. Jordan, J. Choi, D.L. Kaplan, Enzymatically crosslinked silk and silk-gelatin hydrogels with tunable gelation kinetics, mechanical properties and bioactivity for cell culture and encapsulation, Biomaterials 232 (2020), 119720.

[8] Y. Ogushi, S. Sakai, K. Kawakami, Synthesis of enzymatically-gellable carboxymethylcellulose for biomedical applications, J. Biosci. Bioeng. (1) (2007) 30–33.

[9] R. Jin, L.S.M. Teixeira, P.J. Dijkstra, Z. Zhong, C.A. van Blitterswijk, M. Karperien, J. Feijen, Enzymatically crosslinked dextran-tyramine hydrogels as injectable scaffolds for cartilage tissue engineering, Tissue Eng. Part A 16 (8) (2010) 2429–2440.

[10] L.S.M. Teixeira, S. Bijl, V.V. Pully, C. Otto, R. Jin, J. Feijen, C.A. van Blitterswijk, P. J. Dijkstra, M. Karperien, Self-attaching and cell-attracting in-situ forming dextran-tyramine conjugates hydrogels for arthroscopic cartilage repair, Biomaterials 33 (2012) 3164–3174.

[11] K.L. Nielsen, C. Indiani, A. Henriksen, A. Feis, M. Becucci, M. Gajhede, G. Smulevich, K.G. Welinder, Differential activity and structure of highly similar peroxidases. spectroscopic, crystallographic, and enzymatic analyses of lignifying arabidopsis thaliana peroxidase a2 and horseradish peroxidase a2, Biochemistry 40 (2001) 11013–11021.

[12] V. Soares, D. Severino, H. Junqueira, I. Tersariol, C. Shida, M. Baptista, O. Nascimento, I. Nantes, Light-driven horseradish peroxidase cycle by using photo-activated methylene blue as the reducing agent, Photochem. Photobiol. 83 (2007) 1254–1262.

[13] H.-F. Ji, L. Shen, A dft study on deactivation of triplet excited state riboflavin by polyphenols, Int. J. Mol. Sci. 9 (2008) 1908–1914.

[14] E. Kim, M.H. Kim, J.H. Song, C. Kang, W.H. Park, Dual crosslinked alginate hydrogels by riboflavin as photoinitiator, Int. J. Biol. Macromol. 154 (2020) 989–998.

[15] M.A. Sheraz, S.H. Kazi, S. Ahmed, Z. Anwar, I. Ahmad, Photo, thermal and chemical degradation of riboflavin, Beilstein J. Org. Chem. (10) (2014) 1999–2012.

[16] P.E. Donnelly, T. Chen, A. Finch, C. Brial, S.A. Maher, P.A. Torzilli, Photocrosslinked tyramine-substituted hyaluronate hydrogels with tunable mechanical properties improve immediate tissue-hydrogel interfacial strength in articular cartilage, J. Biomater. Sci. Polym. Ed. 28 (6) (2017) 582–600.

[17] S. Piluso, D.F. Gomez, I. Dokter, L.M. Texeira, Y. Li, J. Leijten, R. van Weeren, T. Vermonden, M. Karperienc, J. Malda, Rapid and cytocompatible cell-laden silk hydrogel formation via riboflavin-mediated crosslinking, J. Mater. Chem. B 8 (2020) 9566.

[18] A. Tirella, T. Liberto, A. Ahluwalia, Riboflavin and collagen: new crosslinking methods to tailor the stiffness of hydrogels, Mater. Lett. 74 (2012) 58–61.

[19] J.Y. Shin, Y.H. Yeo, J.E. Jeong, S.A. Park, W.H. Park, Dual-crosslinked methylcellulose hydrogels for 3d bioprinting applications, Carbohydr. Polym. 238 (2020), 116192.

[20] M. Ahearne, A. Coyle, Application of uva-riboflavin crosslinking to enhance the mechanical properties of extracellular matrix derived hydrogels, J. Mech. Behav. Biomed. Mater. 54 (2016) 259–267.

[21] H.-Y. Liu, H.D. Nguyen, C.-C. Lin, Dynamic peg–peptide hydrogels via visible light and fmn-induced tyrosine dimerization, Adv. Healthcare Mater. 7 (2018), 1800954.

[22] R.W. Redmond, I.E. Kochevar, Medical applications of rose bengal- and riboflavin-photosensitized protein crosslinking, Photochem. Photobiol. 95 (2019) 1097–1115.

[23] C. Liu, J. Hua, P.F. Ng, B. Fei, Photochemistry of bioinspired dityrosine crosslinking, J. Mater. Sci. Technol. 63 (2021) 182–191.

[24] S.H. Kim, Y.K. Yeon, J.M. Lee, J.R. Chao, Y.J. Lee, Y.B. Seo, M.T. Sultan, O.J. Lee, J. S. Lee, S. il Yoon, I.-S. Hong, G. Khang, S.J. Lee, J.J. Yoo, C.H. Park, Precisely printable and biocompatible silk fibroin bioink for digital light processing 3d printing, Nat. Commun. 9 (2018) 1620.

[25] C.D. O'Connell, B. Zhang, C. Onofrillo, S. Duchi, R. Blanchard, A. Quigley, J. Bourke, S. Gambhir, R. Kapsa, C.D. Bella, P. Choong, G.G. Wallacea, Tailoring

the mechanical properties of gelatin methacryloyl hydrogels through manipulation of the photocrosslinking conditions, Soft Matter 14 (2018) 2142–2151.
[26] B.M. Hong, S.A. Park, W.H. Park, Effect of photoinitiator on chain degradation of hyaluronic acid, Biomater. Res. 21 (23) (2019).
[27] C.M. Elvin, T. Vuocolo, A.G. Brownlee, L. Sando, M.G. Huson, N.E. Liyou, P. R. Stockwell, R.E. Lyons, M. Kim, G.A. Edwards, G. Johnson, G.A. McFarland, J. A. Ramshaw, J.A. Werkmeister, A highly elastic tissue sealant based on photopolymerised gelatin, Biomaterials 31 (32) (2010) 8323–8331.
[28] M. Dequaire, B. Limoges, J. Moiroux, J.-M. Save', Mediated electrochemistry of horseradish peroxidase. catalysis and inhibition, J. Am. Chem. Soc. 9 (124) (2002) 240–253.
[29] J.N. Rodrı'guez-Lo'pez, D.J. Lowe, J. Herna'ndez-Ruiz, A.N.P. Hiner, F. Garcı'a-Ca'novas, R.N.F. Thorneley, Mechanism of reaction of hydrogen peroxide with horseradish peroxidase: identification of intermediates in the catalytic cycle, J. Am. Chem. Soc. 123 (2001) 11838–11847.
[30] B.R. Agnieszka Grzelak, G. Bartosz, Light-dependent generation of reactive ocygen species in cell culture media, Free Radic. Biol. Med. 30 (12) (2001) 1418–1425.
[31] A. Schulz, M.M. Gepp, F. Stracke, H. von Briesen, J.C. Neubauer, H. Zimmermann, Tyramine-conjugated alginate hydrogels as a platform for bioactive scaffolds, J. Biomed. Mater. Res. A 107A (2019) 114–121.
[32] J. Hou, C. Li, Y. Guan, Y. Zhang, X.X. Zhu, Enzymatically crosslinked alginate hydrogels with improved adhesion properties, Polym. Chem. 6 (2015) 2204–2213.