Chapter 30

Methacrylated Gelatin as an On-Demand Injectable Vehicle for Drug Delivery in Dentistry

W. Benton Swanson, Abdel Hameed Mahmoud, Seth Woodbury, and Marco C. Bottino

Abstract

Gelatin methacrylate (GelMA) is a biodegradable and biocompatible engineered material with significant promise for its applications in tissue engineering, drug delivery, and 3D bioprinting applications. Gelatin is functionalized with terminal methacrylate groups which allow for its photoinducible crosslinking, and thereby tunable properties. Photocrosslinking of GelMA solution in situ allows for fabrication of hydrogels to fit patient-specific defects. Given its favorable biologic properties, GelMA may be used as a carrier for bioactive substances necessary to induce regenerative phenotypes or augment healing, such as growth factors and biotherapeutics. Gelatin is cleaved by cell-secreted enzymes such that its degradation, and subsequently release of bioactive substances, is well-matched to tissue regeneration processes. GelMA may be mixed with a wide array of additives to enhance and improve the specificity of its biologic activity. Here, we present two protocols for novel fabrications and their uses as clinically relevant drug delivery systems. GelMA hydrogels provides a versatile platform for the development of injectable drug delivery therapeutics for broad applications in regenerative dental medicine.

Key words Drug delivery, Tissue engineering, Regenerative medicine, 3D bioprinting, Scaffolds, Biofabrication

1 Introduction

Tissue engineering aims to restore, maintain, improve, or replace various types of biological tissues in the human body by combining cells, biomaterial matrices, and appropriate biochemical and physicochemical factors [1–3]. Biomaterial matrices are an important factor in the tissue engineering triad, providing mechanical support and organization to engineered tissues throughout the regenerative process [4, 5]. Significant advancements have been made in identifying favorable architectural features critical to their success in vivo, including extracellular matrix (ECM) mimicking nanofibers and porosity [6]. The highly porous nature of hydrogels and biomaterial matrices fabricated for tissue engineering applications facilitates

a significantly increased surface area within its internal structure [7]. This high surface area provides attachment for cells as well as protein adsorption [8, 9], and moreover is capable of facilitating controlled release of inductive cues or biomolecules to augment tissue regeneration within the defect site [10, 11].

Gelatin methacrylate (GelMA) is a biodegradable and biocompatible engineered gelatin-based material that has proven to be versatile for tissue engineering, drug delivery, and 3D bioprinting applications. Gelatin is isolated from porcine skin through denaturation of collagen, a major ECM component, and functionalized with terminal methacrylate groups by nucleophilic addition, which are subsequently capable of photoinduced polymerization, to form GelMA [12–14]. Its utility has been demonstrated in a variety of tissue engineering applications including skin, tendon, bone, cartilage, and vascular regeneration, reviewed by Piao et al. [15]. Traditionally, gelatin-based materials have demonstrated poor mechanical properties and unpredictable degradation [16]. Their crosslinking results in significantly increased mechanical stability and predictable biodegradation profiles [17]. The stiffness and density of hydrogels may be adjusted by altering the polymer dry mass, degree of functionalization, photo-initiator concentration, UV intensity and exposure duration [13]. To further tailor its properties, GelMA may be mixed with other materials to create scaffolds that are suited for certain purposes, such as in bone regeneration [18]. Other modifications may include pH and temperature responsive properties, and inclusion of functional groups could enhance properties.

Hydrogels have a high water content, tissue-like elastic properties and possess unique tissue engineering advantages [17]. Hydrogels from GelMA are synthesized by radical polymerization, catalyzed by a photo-inducible radical agent, such as lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP, 405 nm excitation wavelength) [19–21]. A solution of GelMA and LAP may be injected into a mold or directly into a tissue defect and polymerized in situ using an LED light [19–22]. Scaffolds that can release growth factors or drugs are excellent prospects for tissue engineering [3, 23]. Modifications including the incorporations of nanomaterials increase the predictability of tissue-specific regenerative outcomes [24]. A GelMA macromonomer precursor solution may also be combined with a variety of other additives such as nanofibers, nanoparticles or other carriers, proteins, and/or small molecules. After crosslinking, these components become a part of the matrix and are released as the GelMA matrix degrades.

This protocol includes recent advances in GelMA-based therapeutics for drug delivery and highlights its uses. We provide a step-by-step method for GelMA synthesis, purification, and lyophilization. This method facilitates the encapsulation, delivery, and sustained release of biologically active substances from the

hydrogel. We also describe methods to fabricate and formulate hydrogel-based biomaterials for drug delivery applications. Finally, we highlight two clinically relevant applications to demonstrate the versatility and functionality of GelMA-based materials for drug delivery.

2 Materials

2.1 Gelatin Methacrylation

1. Gelatin derived from porcine skin (Sigma, SLBM9945V), 10 g
2. Phosphate buffered saline (PBS, sterile), 100 mL
3. Methacrylic anhydride (Sigma, 276685), 8 mL
4. Dulbecco's phosphate buffered saline (DPBS, sterile), 100 mL
5. Magnetic stir plate with heating element
6. Erlenmeyer flask (250 mL)
7. Magnetic stir bar
8. P1000 pipette

2.2 GelMA Purification

1. Dialysis membrane (Spectro/Por molecular porous membrane tubing, MWCO 12–14,000, Fisher Scientific), $2\times$ 20–30 cm length
2. Dialysis membrane clips
3. Funnel
4. Large plastic beaker (5 L)
5. Magnetic stir bar
6. Magnetic stir plate
7. 40 °C oven

2.3 Lyophilization

1. 50 mL Falcon tubes
2. 0.22 μm filtration cup (Millipore)
3. Lyophilizer/freeze dryer

2.4 Hydrogel Fabrication

1. Lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP, Sigma: 900899)
2. Silicone mold
3. LED dental curing light (405 nm wavelength)
4. Syringe and 25G needle
5. Bioactive substances
   (a) Example 3.4.1: Halloysite aluminosilicate clay nanotubes (HNT) loaded with chlorohexidine (CHX)
   (b) Example 3.4.2: Ciprofloxacin inclusion complexes (CIP-IP) and polydioxanone (PDS)-based electrospun fibers

3 Methods

3.1 Gelatin Methacrylation

1. Combine 10 g Gelatin Porcine Skin with 100 mL PBS (sterile) in a cleaned Erlenmeyer flask with a magnetic stir bar.

2. Set undissolved mixture on heating plate with gentle stirring (240 rpm) at 50 °C until Gelatin is completely dissolved. Cover Erlenmeyer flask with aluminum foil (ca. 1 h). Check temperature using a thermometer (*see* **Note 1**).

3. When Gelatin is melted, add 8 mL Methacrylic Anhydride dropwise and allow emulsion to rotate (240 rpm) at 50 °C for 2 h, covered (Fig. 1).

4. Preheat 100 mL of sterile PBS in Erlenmeyer flask to 50 °C. Use this preheated DPBS to dilute Gel-MA solution (total final volume of the solution should be 200 mL). After mixing of DPBS with concentrated solution, stir gently for 10 min at 50 °C.

3.2 GelMA Purification

1. Prepare dialysis membrane by cutting into appropriate sizes (45 mm flat diameter membrane, 2× 20–30 cm) and immerse them into distilled water to soften them. Close one end by twisting the membrane and making a knot (*see* **Note 2**) (Fig. 2).

2. Transfer diluted GelMA using a funnel into the dialysis membranes (*see* **Note 3**). Close the second end of the membrane using the same approach. Place dialysis tubing into distilled water in 5 L plastic beaker.
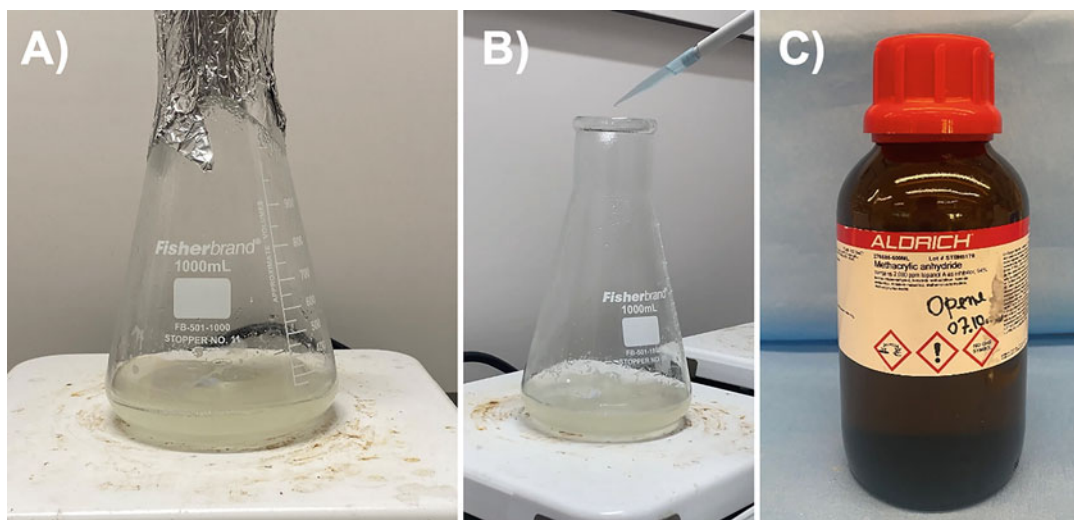

Fig. 1 Images (**a–c**) show components and addition of methacrylic anhydride to the gelatin solution in the flask
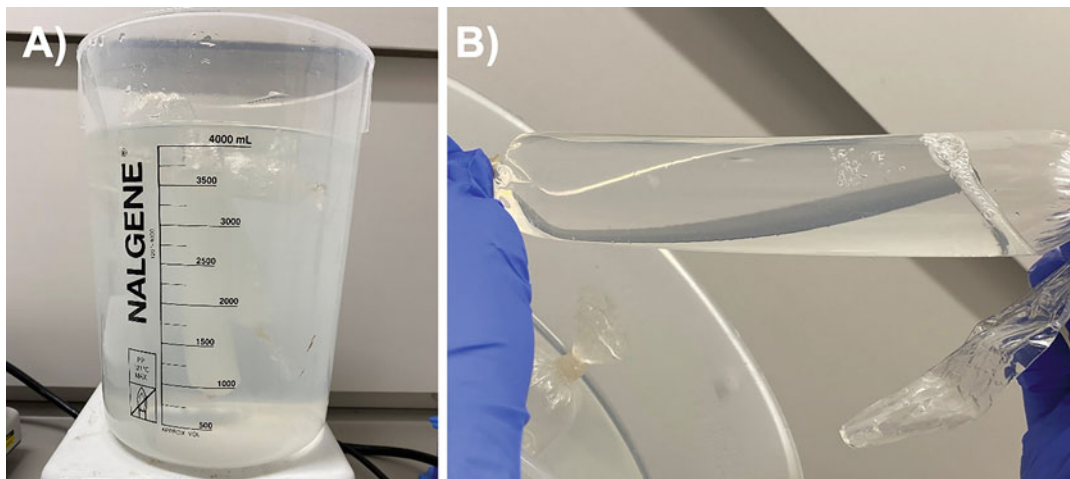
Fig. 2 Dialysis purification of GelMA to remove unreacted methacrylic anhydride. Images (**a**) and (**b**) show dialysis tubing within the distilled water of the 5 L plastic beaker which is stirred

3. Continue dialysis at 40 °C for at least 5 days with magnetic stirrer (ca. 500 rpm) and covered with aluminum foil. Change water twice daily (*see* **Note 4**).

3.3 Lyophilization

1. Add 200 mL of ultrapure water (same amount as the gel within the dialysis membranes) in an empty conical flask and add GelMA. Heat the solution on a hot plate for 15 min covered at 40 °C (*see* **Note 5**).

2. Prepare 50 mL Falcon tubes, each containing 25 mL of the solution.

3. Use sterile vacuum 0.22 μm filtration cup to filter the liquid rapidly (Fig. 3),

4. Transfer sterilized polymer into 50 mL Falcon tubes (no more than 25 mL in each). Store tubes at −80 °C for at least 2 days. Store Falcon tubes horizontally when freezing (*see* **Note 6**).

5. The frozen GelMA is freeze-dried for 5 days. Remove caps and cover the Falcon tubes' opening with Kimwipes secured with rubber band. Verify that the pressure is reduced to ~100–200 × 10^{-3} Mbar after ~30 min to ensure that the vessel containing the Falcon tubes is appropriately sealed (Fig. 4).

3.4 Hydrogel Fabrication

GelMA, a bifunctional polymer, may be used directly through radical-initiated crosslinking to fabricate hydrogels. These gels can be loaded with various nanoparticles or drug delivery modalities by dispersion in the matrix [25, 26]. A general protocol is presented along with two specific examples from the recent literature.

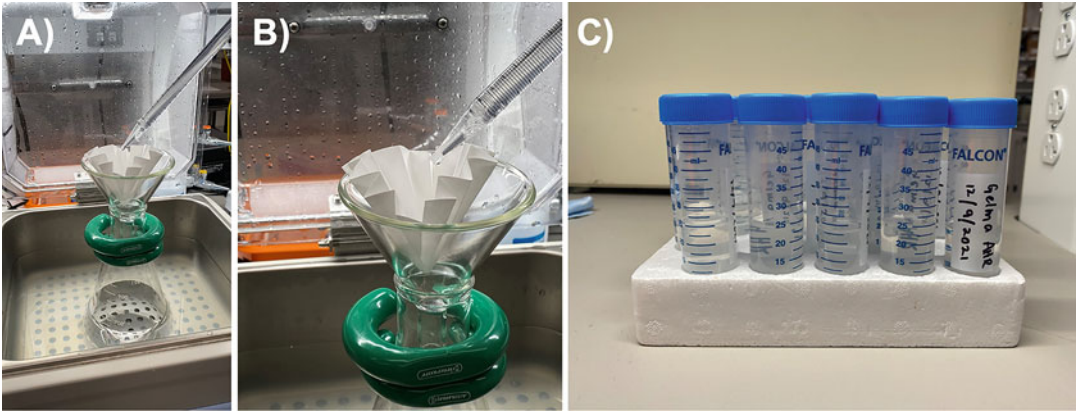1. GelMA is solubilized in PBS at 50 °C at a concentration of 15–20% w/v.

Fig. 3 Filtration of GelMA (**a** and **b**) in 50 mL conical tubes (**c**) used for the lyophilization procedure
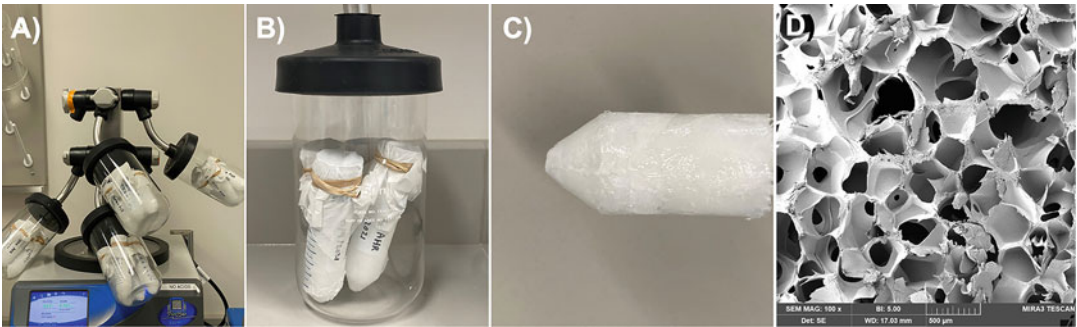

Fig. 4 Lyophilization of GelMA (**a** and **b**) resulting in the final product (**c**). Morphology is assessed by scanning electron microscopy (SEM, **d**)

2. Nanoparticles or other modalities of drug delivery are incorporated by mechanical stirring to disperse in the solution at a standardized weight-percent concentration.

3. LAP photo-initiator is added to give a final concentration of 0.05% w/v.

4. A small volume, for example, 100 μL, is pipetted into a silicone mold followed by UV crosslinking (405 nm wavelength) for 60 seconds using an LED device.

   • As an alternative, the solution may be injected into a defect and photopolymerized in situ for on-demand hydrogel synthesis.

3.4.1 Utilization of the Biodegradability of GelMA for Controlled Delivery of Chlorohexidine

1. Load halloysite aluminosilicate clay nanotubes (HNT) with chlorohexidine (CHX), an antimicrobial agent commonly used in clinical dentistry (*see* **Note 7**).

2. Combine CHX-HNT in suspension with 15% w/v GelMA and LAP photoinitiator, as described in the general protocol above,
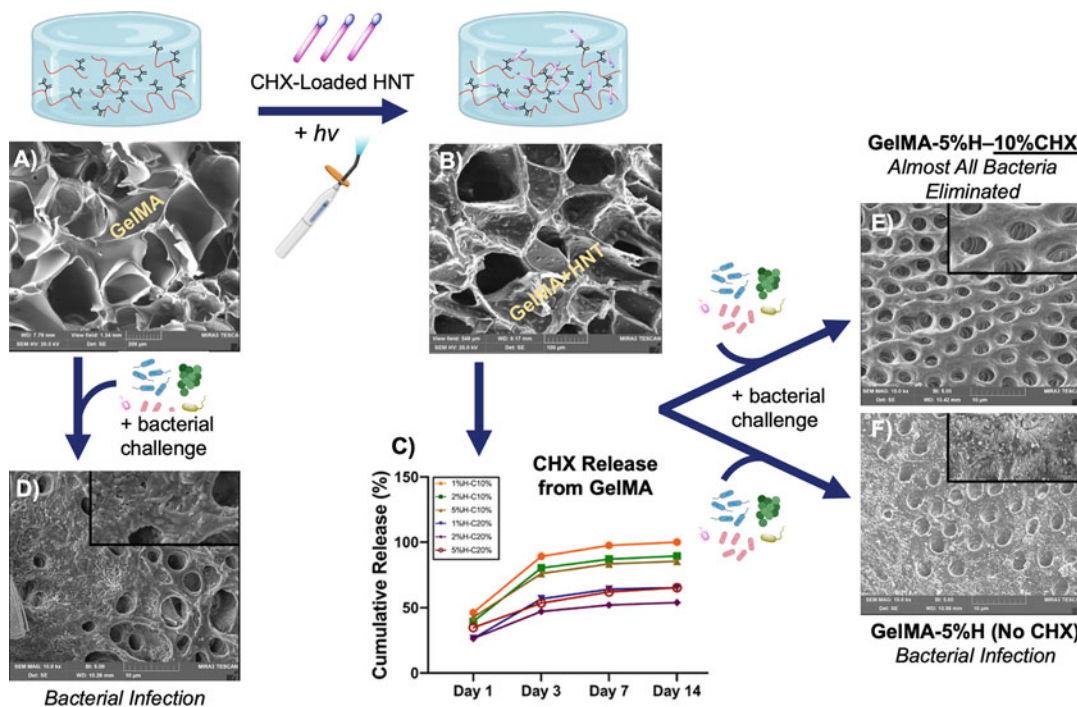
Fig. 5 Schematic of the formulated GelMA hydrogels containing CHX-loaded HNT. (**a** and **b**) SEM micrographs of a cross section in (**a**) a GelMA hydrogel and (**b**) a GelMA hydrogel modified with CHX-loaded HNT (5% H–10%CHX). (**c**) Cumulative release percentage of CHX as a function of time from GelMA hydrogels modified with distinct HNT (1, 2, and 5%) concentrations, loaded with unique CHX solution concentrations (10% and 20%). (**d**–**f**) SEM micrographs of the bacterial biofilms on the dentin surfaces of (**d**) Unmodified GelMA, (**e**) GelMA-5%H–10%CHX, and (**f**) GelMA-5%H with no loaded CHX

and light-cure to induce photo-crosslinking. When incubated in solution in vitro, CHX is released over the course of 14 days and the amount released correlates to the weight-percent incorporation of CHX-HNT incorporated into the gel at the time of fabrication.

3. The amount of CHX-HNT incorporated, in addition to the weight-percent of GelMA, influences mechanical properties and degradation rate; therefore, it is critical to consider these factors when optimizing a construct for a specific application.

4. A schematic overview is provided in Fig. 5.

3.4.2 An Antibiotic-Eluting Hydrogel for Oral Infection Ablation

1. Fabricate ciprofloxacin inclusion complexes (CIP-IP) to improve CIP solubility and bioavailability. The controlled release of small molecules is relatively straightforward for hydrophilic molecules; however, for hydrophobic molecules such as CIP, a carrier is necessary (*see* **Note 8**).

2. Combine CIP-IP with polydioxanone (PDS)-based electrospun fibers to disperse the CIP-IPs (*see* **Note 9**).
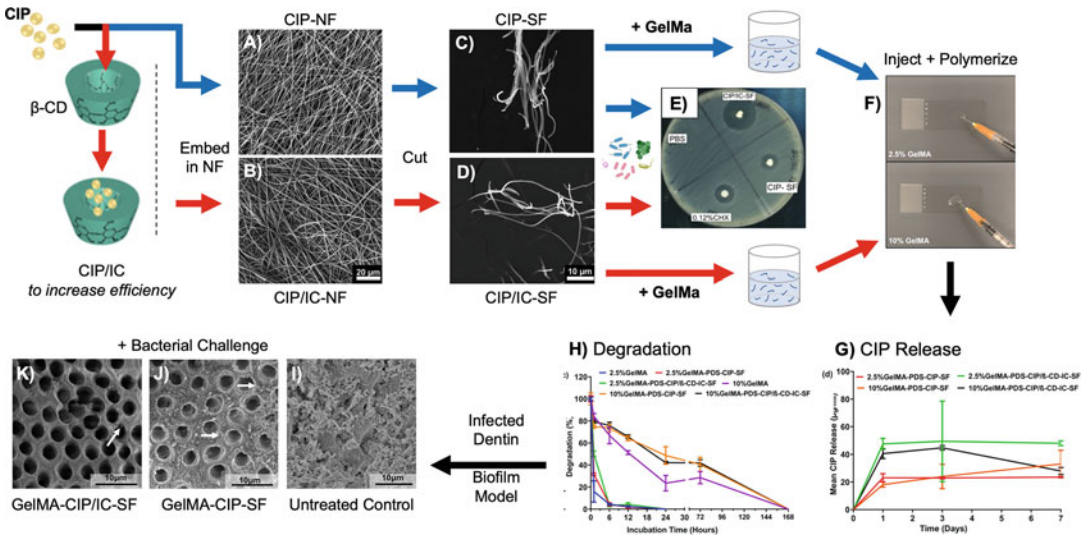
Fig. 6 Schematic of the procedural overview for synthesizing the GelMA-CIP-SF and GelMA-CIP/IC-SF along with important experiments characterizing the release of the different systems and their biologic efficacies. (**a–b**) Electrospun PDS nanofibers embedded with (**a**) CIP and (**b**) CIP/IC. (**c–d**) Cryo-cut short fibers from the previous nanofibers in (**a–b**) containing (**c**) CIP and (**d**) CIP/IC. (**e**) Agar diffusion assay against *E. faecalis* using the short fibers from (**c–d**) demonstrating the effectiveness of the CIP/IC. (**f**) 2.5% or 10% GelMA solution containing one type of short fibers from (**c–d**) being injected from a needle onto a microscope slide as a model for clinical translation. (**g**) Enzymatic degradation of the GelMA hydrogels (2.5% or 10%) unloaded or loaded (CIP-SF or CIP/IC-SF) quantifying the degradation of the 2.5% GelMA hydrogels to be significantly faster than that of the 10% GelMA hydrogel group. (**h**) Release kinetics of CIP from the different GelMA hydrogels (2.5% or 10%) loaded with CIP-SF or CIP/IC-SF. (**i–k**) SEM micrographs of the infected detin biofilm models evaluating the antimicrobial efficacy of treatment by (**i**) the control, (**j**) 10% GelMA-CIP-SF, and (**k**) 10% GelMA-CIP/IC-SF. Significantly fewer bacteria appear in (**j–k**) because of the CIP release

3. Cryo-cut PDS fibers to improve their dispersion and allow the solution to be injectable. These carriers add a degree of complexity to encapsulation and delivery strategies, which is well-accommodated by GelMA.

4. Suspend CIP-IC in a GelMA macromonomer precursor solution with LAP photoinitiator. Use Tween80 as an adjunctive surfactant to prevent CIP-IP aggregation.

5. Light-cure GelMA gels as described in the protocol above.

6. A schematic overview is provided in Fig. 6.

4   Notes

1. Gelatin melting is characterized by a uniform solution, i.e., no more visible granula.

2. It is important that the dialysis tubing is shorter than the dialysis vessel to avoid contacting the stir bar. It is similarly important to examine the membrane tubing *to ensure there are no holes or defects*.

3. When loading GelMA solution into the dialysis tubing, remember to leave extra space inside the membrane (i.e., do not squeeze GelMA; this will allow some water in and allow enhanced dialysis). Check again the tubing for leakage.

4. During each water change during dialysis, turn/flip the membranes upside down 5–6 times to homogenize the content! (This step removes the toxic unreacted MA, so if more GelMA is dialyzed, consider changing the water more often and/or let the step run longer).

5. This step must be handled rapidly to maintain the liquid at 40 °C.

6. The purpose of storing tubes horizontally is to ensure an optimal repartition in the tube when gel will be lyophilized.

7. CHX-HNT/GelMA constructs are assessed for their potential cytotoxicity; their degradation products showed no adverse effects on cellular proliferation [26]. The same constructs are implanted in an animal model to assess inflammation; GelMA constructs should demonstrate no signs of host inflammation. When tested for efficacy with oral pathogenic bacteria, significant inhibition is achieved by the controlled release of CHX. This method is not exclusive to the delivery of CHX, but may be adapted for other molecules including dexamethasone, as described by Bordini et al. [27] for the purpose of an injectable drug delivery system for hard tissue regeneration.

8. Of relevance to the clinical context, the authors have shown that a GelMA solution could be injected and cured in situ [28]. Both 2.5% w/v and 10% w/v solutions are injectable. 2.5% w/v gels are rapidly degraded within 24 h, while 10% gels degrade over the course of 1 week. As the gel degrades, CIP-IPs are released as the gel degrades, over the course of the first day of incubation, more quickly from a 2.5% w/v gel compared to 10% w/v gel. In an in vitro model of a dental pathogen, *E. faecalis*, CIP-IP-eluting GelMA hydrogels causes significant inhibition of bacterial growth in an agar diffusion assay and biofilm model.

9. Electrospinning should be undertaken in a vented fume hood due to the nature of the volatile organic solvents used.

Acknowledgments

References

1. Langer R, Vacanti JP (1993) Tissue engineering. Science 260:920–926. https://doi.org/10.1126/science.8493529

2. Langer R, Vacanti J (2016) Advances in tissue engineering. J Pediatr Surg 51:8–12. https://doi.org/10.1016/j.jpedsurg.2015.10.022

3. Ma PX (2008) Biomimetic materials for tissue engineering. Adv Drug Deliv Rev 60:184–198

4. Chan BP, Leong KW (2008) Scaffolding in tissue engineering: general approaches and tissue-specific considerations. Eur Spine J 17 (Suppl 4):467–479. https://doi.org/10.1007/s00586-008-0745-3

5. Kim Y, Ko H, Kwon IK, Shin K (2016) Extracellular matrix revisited: roles in tissue engineering. Int Neurourol J 20:S23–S29. https://doi.org/10.5213/inj.1632600.318

6. Swanson WB, Ma PX (2020) Nanofibrous and porous biomaterials. In: Zhang G (ed) *Biomaterials science, an introduction to materials in medicine*. Elsiever

7. Rasouli R, Barhoum A, Bechelany M, Dufresne A (2018) Nanofibers for biomedical and healthcare applications. Macromol Biosci 19. https://doi.org/10.1002/mabi.201800256

8. Zhang R, Ma PX (1999) Porous poly(L-lactic acid)/apatite composites created by biomimetic process. J Biomed Mater Res A 45: 285–293

9. Liu X, Ma PX (2010) The nanofibrous architecture of poly(L-lactic acid)-based functional copolymers. Biomaterials 31:259–269. https://doi.org/10.1016/j.biomaterials.2009.09.046

10. Swanson WB, Gong T, Zhang Z, Eberle M, Niemann D, Dong R, Rambhia KJ, Ma PX (2020) Controlled release of odontogenic exosomes from a biodegradable vehicle mediates dentinogenesis as a novel biomimetic pulp capping therapy. J Control Release 324:679–694. https://doi.org/10.1016/j.jconrel.2020.06.006

11. Swanson WB, Zhang Z, Xiu K, Gong T, Eberle M, Wang Z, Ma PX (2020) Scaffolds with controlled release of pro-mineralization exosomes to promote craniofacial bone healing without cell transplantation. Acta Biomater 118:215–232. https://doi.org/10.1016/j.actbio.2020.09.052

12. Bello AB, Kim D, Kim D, Park H, Lee SH (2020) Engineering and functionalization of gelatin biomaterials: from cell culture to medical applications. Tissue Eng Part B Rev 26: 164–180. https://doi.org/10.1089/ten.TEB.2019.0256

13. Aldana AA, Malatto L, Rehman MAU, Boccaccini AR, Abraham GA (2019) Fabrication of gelatin methacrylate (GelMA) scaffolds with nano- and micro-topographical and morphological features, vol 9, Nanomaterials (Basel). https://doi.org/10.3390/nano9010120

14. Yue K, Trujillo-de Santiago G, Alvarez MM, Tamayol A, Annabi N, Khademhosseini A (2015) Synthesis, properties, and biomedical applications of gelatin methacryloyl (GelMA) hydrogels. Biomaterials 73:254–271. https://doi.org/10.1016/j.biomaterials.2015.08.045

15. Piao Y, You H, Xu T, Bei H-P, Piwko IZ, Kwan YY, Zhao X (2021) Biomedical applications of gelatin methacryloyl hydrogels. Engineered Regeneration 2:47–56. https://doi.org/10.1016/j.engreg.2021.03.002

16. Gorgieva S, Kokol V (2011) Collagen- vs. gelatine-based biomaterials and their biocompatibility: review and perspectives. In: *Biomaterials applications for nanomedicine*. InTech Rijeka

17. Zhu M, Wang Y, Ferracci G, Zheng J, Cho N-J, Lee BH (2019) Gelatin methacryloyl and its hydrogels with an exceptional degree of controllability and batch-to-batch consistency.

Sci Rep 9. https://doi.org/10.1038/s41598-019-42186-x

18. Lantigua D, Wu X, Suvarnapathaki S, Nguyen MA, Camci-Unal G (2021) Composite scaffolds from gelatin and bone meal powder for tissue engineering. Bioengineering 8. https://doi.org/10.3390/bioengineering8110169

19. Monteiro N, He W, Franca CM, Athirasala A, Bertassoni LE (2018) Engineering microvascular networks in LED light-cured cell-laden hydrogels. ACS Biomater Sci Eng 4: 2563–2570. https://doi.org/10.1021/acsbiomaterials.8b00502

20. Athirasala A, Lins F, Tahayeri A, Hinds M, Smith AJ, Sedgley C, Ferracane J, Bertassoni LE (2017) A novel strategy to engineer pre-vascularized full-length dental pulp-like tissue constructs. Sci Rep 7. https://doi.org/10.1038/s41598-017-02532-3

21. Monteiro N, Thrivikraman G, Athirasala A, Tahayeri A, França CM, Ferracane JL, Bertassoni LE (2018) Photopolymerization of cell-laden gelatin methacryloyl hydrogels using a dental curing light for regenerative dentistry. Dent Mater 34:389–399. https://doi.org/10.1016/j.dental.2017.11.020

22. Bertassoni LE, Cecconi M, Manoharan V, Nikkhah M, Hjortnaes J, Cristino AL, Barabaschi G, Demarchi D, Dokmeci MR, Yang Y et al (2014) Hydrogel bioprinted microchannel networks for vascularization of tissue engineering constructs. Lab Chip 14: 2202–2211. https://doi.org/10.1039/c4lc00030g

23. Ren X, Zhao M, Lash B, Martino MM, Julier Z (2020) Growth factor engineering strategies for regenerative medicine applications. Front

Bioeng Biotechnol 7. https://doi.org/10.3389/fbioe.2019.00469

24. Scantlebury T, Ambruster J (2012) The development of guided regeneration: making the impossible possible and the unpredictable predictable. Journal of Evidence Based Dental Practice 12:101–117. https://doi.org/10.1016/s1532-3382(12)70022-2

25. Bordini EAF, Ferreira JA, Dubey N, Ribeiro JS, de Souza Costa CA, Soares DG, Bottino MC (2021) Injectable multifunctional drug delivery system for hard tissue regeneration under inflammatory microenvironments. ACS Applied Bio Materials 4:6993–7006. https://doi.org/10.1021/acsabm.1c00620

26. Ribeiro JS, Bordini EAF, Ferreira JA, Mei L, Dubey N, Fenno JC, Piva E, Lund RG, Schwendeman A, Bottino MC (2020) Injectable MMP-responsive nanotube-modified gelatin hydrogel for dental infection ablation. ACS Appl Mater Interfaces 12:16006–16017. https://doi.org/10.1021/acsami.9b22964

27. Bordini EAF, Ferreira JA, Dubey N, Ribeiro JS, de Souza Costa CA, Soares DG, Bottino MC (2021) Injectable multifunctional drug delivery system for hard tissue regeneration under inflammatory microenvironments. ACS Appl Bio Mater 4:6993–7006. https://doi.org/10.1021/acsabm.1c00620

28. Ribeiro JS, Daghrery A, Dubey N, Li C, Mei L, Fenno JC, Schwendeman A, Aytac Z, Bottino MC (2020) Hybrid antimicrobial hydrogel as injectable therapeutics for oral infection ablation. Biomacromolecules 21:3945–3956. https://doi.org/10.1021/acs.biomac.0c01131