Afyon Kocatepe Üniversitesi Fen ve Mühendislik Bilimleri Dergisi
Afyon Kocatepe University – Journal of Science and Engineering
https://dergipark.org.tr/tr/pub/akufemubid

Araştırma Makalesi / Research Article
e-ISSN: 2149-3367
DOI: https://doi.org/10.35414/akufemubid.1673131
AKÜ FEMÜBİD **25** (2025) 067202 (1511-1520)
AKU J. Sci. Eng. **25** (2025) 067202 (1511-1520)

Antimicrobial Peptide Conjugated PEGDA Based Hydrogel for Wound Dressing Applications

Makale Bilgisi / Article Info
Alındı/Received: 10.04.2025
Kabul/Accepted: 14.06.2025
Yayımlandı/Published: 03.12.2025

Yara Örtüsü Uygulamaları için Antimikrobiyal Peptid Konjuge PEGDA Tabanlı Hidrojeller

Buse SEZER [1], Eda BİLGİÇ[2], Günnur PULAT[2*]

[1]*Department of Biomedical Technologies, İzmir Katip Çelebi University, İzmir, Türkiye*
[2] *Department of Biomedical Engineering, İzmir Katip Çelebi University, İzmir, Türkiye*

© 2025 The Authors | Creative Commons Attribution-Noncommercial 4.0 (CC BY-NC) International License

Abstract

Currently, management of chronic wounds poses a significant challenge. In the treatment of chronic wounds, hydrogel-based wound dressings considered an effective approach. Poly(ethylene glycol) diacrylate (PEGDA) hydrogel is an advantageous hydrogel due to its properties such as tunable mechanical properties and biocompatibility. However, PEGDA hydrogels lack antimicrobial properties. Therefore, the development of new antibacterial strategies combined with PEGDA hydrogel can be effective in overcoming the problems associated with chronic infected wounds. Herein, we developed an antimicrobial wound dressing by conjugating antimicrobial peptide KSL-W (KKVVFWVKFK) to PEGDA hydrogel via EDC/NHS chemistry. The antimicrobial activity was evaluated against MRSA and multi-drug resistant (MDR) *P. aeruginosa* in their planktonic form. Furthermore, cytotoxicity and wound healing were assessed using L929 mouse fibroblast cells. Developed KSL-W peptide conjugated PEGDA hydrogels showed strong antimicrobial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) and multidrug-resistant (MDR) *Pseudomonas aeruginosa* with a bacterial reduction of 2.44 and 1.15 log, respectively. Furthermore, developed hydrogels showed no undesired toxic effect against healthy mammalian cells and supported wound healing. Our findings provide insight into the development of antimicrobial hydrogels for biomedical applications to develop more effective antimicrobial wound dressing solutions.

Keywords: Biomaterials; Wound healing dressings; Hydrogels; Poly(ethylene glycol) diacrylate; Antimicrobial peptide.

Öz

Günümüzde, kronik yaraların bakımı önemli bir zorluk oluşturmaktadır. Kronik yaraların tedavisinde hidrojel bazlı yara örtüleri etkili bir yaklaşım olarak kabul edilmektedir. Poli(etilen glikol) diakrilat (PEGDA) hidrojeller, ayarlanabilir mekanik özellikler ve biyouyumluluk gibi özellikleri nedeniyle avantajlı bir hidrojel olarak kabul görmektedir. Ancak PEGDA hidrojelleri antimikrobiyal özelliklerden yoksundur. Bu nedenle, PEGDA hidrojellere antimikrobiyal özellikler kazanadırılması amacıyla yeni stratejilerin geliştirilmesi kronik enfekte yaralarla ilişkili sorunların üstesinden gelmek için kritik bir öneme sahiptir. Bu çalışmada, EDC/NHS kimyası kullanılarak antimikrobiyal peptit KSL-W'yi (KKVVFWVKFK) PEGDA hidrojeline konjuge ederek antimikrobiyal bir yara örtüsü geliştirilmiştir. Antimikrobiyal aktivite, planktonik formlarında MRSA ve çoklu ilaca dirençli (MDR) *P. Aeruginosa* üzerinde değerlendirilmiştir. Ayrıca, geliştirilen hidrojellerin sitotoksisitesi ve yara iyileşmesi üzerindeki etkinliği L929 fare fibroblast hücreleri kullanılarak değerlendirilmiştir. Geliştirilen KSL-W peptit konjuge PEGDA hidrojelleri, metisilin dirençli *Staphylococcus aureus* (MRSA) ve çoklu ilaç dirençli (MDR) *Pseudomonas aeruginosa*'ya karşı sırasıyla 2,44 ve 1,15 log'luk inhibisyon ile güçlü antimikrobiyal aktivite göstermiştir. Ayrıca, geliştirilen hidrojeller sağlıklı memeli hücrelerine karşı istenmeyen toksik etki göstermemiş olup yara iyileşmesini desteklemiştir. Bulgularımız, biyomedikal uygulamalarda daha etkili fonksiyonel yara örtüleri geliştirmesi amacıyla antimikrobiyal hidrojellerin tasarımı açısından yol gösterici niteliktedir.

Anahtar Kelimeler: Biyomalzemeler; yara örtüleri; hidrojeller; poli(etilen glikol) diakrilat; antimikrobiyal peptid.

1. Introduction

Chronic wounds, which affect millions of patients worldwide, are considered a significant challenge in modern medicine. The main causes of chronic wounds are known as diabetes, infections, and weakened immune responses. Chronic wounds may lead to serious life-threatening complications such as amputation (Sen et al. 2009). One of the most effective approaches in wound care is the usage of hydrogel-based dressings, which provide a moist healing environment, enhance cell proliferation, and can be engineered to deliver bioactive molecules (Firlar et al. 2022). Among the hydrogels, poly (ethylene glycol) diacrylate (PEGDA) has gained attention due to its tunable properties, biocompatibility, and flexibility. However, PEGDA hydrogels lack antimicrobial activity, which makes them susceptible to bacterial colonization and limits their effectiveness in chronic infected wound treatment (Lensen et al. 2011). To overcome this problem, researchers have developed various strategies to enhance the functionality of PEGDA

*Sorumlu Yazar/Corresponding Author: Günnur Pulat e-posta/e-mail: gunnur.onak@ikc.edu.tr

hydrogels with the incorporation of antimicrobial agents (Gao et al. 2021, Nurlidar et al. 2023, Nguyen et al. 2024). However, the effectiveness of functionalized PEGDA hydrogels still does not meet the therapeutic standards for the eradication of infections due to their bactericidal range or relatively slow bactericidal activity. Therefore, the development of novel antimicrobial strategies is considered crucial.

Antimicrobial peptides (AMPs) play a crucial role in the innate immune defense of living organisms. These peptides exhibit potent and broad-spectrum antimicrobial properties to effectively eradicate bacteria, fungi, and viruses. AMPs have a unique mechanism of action, which disrupts bacterial membrane integrity and lead to rapid cell lysis. Consequently, AMPs eliminate the pathogens without causing bacterial resistance (Lei et al. 2019). Among the various AMPs, KSL-W (KKVVFWVKFK) is a short AMP that has a broad-spectrum antibacterial activity, particularly against drug-resistant strains. Its mechanism of action is based on disrupting bacterial membranes. Due to its potent antimicrobial nature, KSL-W holds great promise as an alternative to conventional antibiotics, particularly for treating infected wounds (Camó et al. 2019). Despite their powerful antimicrobial activity, high concentrations of AMPs may show a toxic effect on mammalian cells. To address this problem, researchers have designed combined therapies to enhance antimicrobial efficacy while minimizing toxicity (Gera et al. 2022).

Herein, we developed a novel antimicrobial PEGDA-based wound dressing by functionalizing PEGDA with AMP. In this study, KSL-W was conjugated to the surface of the PEGDA hydrogel via EDC/NHS chemistry, which increased antimicrobial activity against MRSA and multidrug-resistant (MDR) *P. aeruginosa* in their planktonic form. The cytotoxicity of PEGDA hydrogels conjugated with KSL-W was assessed on L929 cells, and no toxic effects were observed. The designed dressing combines the desirable properties of an "ideal" wound dressing, such as broad-spectrum antimicrobial activity and biocompatibility. Our findings contribute to the optimization of PEGDA hydrogels for biomedical applications to develop more effective antimicrobial wound dressing solutions.

2. Materials and Methods

2.1 KSL-W AMP Synthesis

Reagents required for AMP synthesis were sourced from AAPPTEC (Louisville, KY, USA). KSL-W (KKVVFWVKFK) was synthesized following the Fmoc solid-phase peptide synthesis (SPPS) strategy using a 4-Methylbenzhydrylamine (MBHA) resin with a substitution of 0.67 mmol/g. An automated peptide synthesizer (AAPPTEC Focus Xi, Louisville, KY, USA) was utilized for this process, in accordance with previously established protocols (Onak et al. 2020). To initiate the synthesis, 400 mg of MBHA resin was swollen in dimethylformamide (DMF) for 30 minutes (min), followed by rinsing with DMF to ensure proper conditioning. The peptide chain was assembled by sequentially coupling Fmoc-protected amino acids (2 equivalents) in the presence of 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU; 1.95 equivalents) and N,N-diisopropylethylamine (DIEA; 3 equivalents). Each amino acid was reacted with resin for 1 hour (h). After each coupling, Fmoc deprotection was done by treating the resin with 20% piperidine in DMF, with stirring at room temperature (RT) for 30 min. This cycle of coupling and deprotection continued until the full peptide sequence was completed. Once the sequence was complete, the peptide was cleaved from the resin using a trifluoroacetic acid (TFA), triisopropylsilane (TIPS), and water mixture (95:2.5:2.5%, v/v/v). The resulting peptide-containing solution was precipitated in cold ether and centrifuged to separate the solid peptide from the supernatant. The peptide was then frozen at -80°C and subsequently lyophilized using a Biobase Biodustry Bk-FD10P freeze dryer (Shandong, China). Final peptide was obtained in powdered form.

2.2 Fabrication of PEGDA/AMP Hydrogels

For the functionalization of PEGDA hydrogels with KSL-W AMP, a previously described protocol was used (Castaño 2017). Briefly, PEGDA solution was prepared by dissolving it in phosphate-buffered saline (PBS) with a final concentration of 10% (w/v). As a photoinitiator 2-hydroxy-4'-(2-hydroxyethoxy)-2-methylpropiophenone (Irgacure 2959) was added to the solution to a final concentration of 0.1% (w/v). To functionalize PEGDA with carboxyl groups, acrylic acid was added into PEGDA/Irgacure solution with a final concentration of 0.3% or 1.2% (w/v), and referred to as PEGDA/AA-0.3 and PEGDA/AA-1.2, respectively. Final PEGDA solution was transferred into a teflon mold, and hydrogels were illuminated with UV light (light intensity of 686 mW/cm2) for 200 seconds from 5.5 cm distance. Following the crosslinking process, hydrogel samples were placed in a 96 well-plate and left to swell in PBS for 3 h.

For peptide conjugation, PEGDA hydrogels were left for incubation with EDC/NHS (50mM/30mM) solution in 0.1M MES buffer (pH 4.7) at 37°C for 30 min. After the incubation, the solution was removed from the samples

and the hydrogels were rinsed with PBS 3 times. KSL-W AMP solution with a final concentration of 1 mM was prepared in PBS, and hydrogels were incubated with AMP solution at 4°C for 24 h. Then, the AMP solution was removed, and samples were washed with PBS for 3 times. AMP functionalized hydrogels were referred to as PEGDA/AMP. Before each experiment, samples were exposed to UV light for 1 h for the sterilization process.

2.3 Characterization of Hydrogels

The density of the carboxylic groups in PEGDA was characterized with the toluidine blue O (TBO) assay. PEGDA solution containing 10% PEGDA and 1% Irgacure 2959 was prepared in PBS. Acrylic acid with 0.3% and 1.2% concentrations was added to hydrogel solutions. PEGDA without acrylic acid (control), 0.3% acrylic acid functionalized PEGDA (PEGDA/AA-0.3), and 1.2% acrylic acid functionalized PEGDA (PEGDA/AA-1.2) hydrogels were analyzed with the TBO assay. After the UV crosslinking, samples were left to swell in PBS for 3h. Each sample was left in contact with 15 mM, pH 10, TBO solution overnight at RT on an orbital shaker. After incubation, TBO was removed from samples, and unbound dye was removed by rinsing samples with PBS for 3 days. Then, 50 vol.% acetic acid was added to the hydrogels and incubated for 2 h at RT. Acetic acid solutions were transferred to another well plate, and absorbance values were measured at 633 nm with a multi-plate reader (Biotek Synergy HTX, USA). Evaluation of the carboxylic groups on the samples was quantified by using the TBO calibration curve prepared by obtaining absorbances of known concentrations at 633 nm.

Successful copolymerization of PEGDA with acrylic acid, followed by peptide conjugation on hydrogels, was confirmed with ATR-FTIR spectroscopy. The FTIR spectra of PEGDA, 0.3% acrylic acid functionalized PEGDA (PEGDA/AA-0.3), 1.2% acrylic acid functionalized PEGDA (PEGDA/AA-1.2), and KSL-W peptide conjugated PEGDA (PEGDA/AMP) hydrogels were obtained using a Perkin Elmer Spectrum Two equipped with an attenuated total reflection (ATR) accessory over the range of 400-4000cm^{-1}.

AMP conjugation to PEGDA hydrogels was confirmed using Fluorescein isothiocyanate (FITC) labeling by using an established method (Onak et al. 2020). Briefly, once the synthesis of the sequence of KSL-W peptide was completed, Fmoc-Lys(MTT)-OH was added as the next amino acid. For the deprotection of the Mtt side chain, a solution containing 2.85 mL TFA, 0.075 mL TIPS, 0.075 mL distilled water, and 57 mL DCM was prepared. The resin was left on the orbital shaker to react with the solution for 30 min and rinsed with DCM. Later, the resins were rinsed thoroughly with DCM containing 5% (v/v) DIEA. FITC labeling was performed between the amine groups of the Lys and the succinimidyl ester group of the FITC molecule. The FITC coupling solution was prepared by dissolving 389.40 mg of FITC (Sigma Aldrich, St. Louis, MO, USA) and 256.8 µL of DIEA in 3.0 mL of DMF. This solution was added to the resin, and the reaction took place for 24 h. Following the FITC labelling, the peptide was cleaved from the resin and obtained in powder form. Afterwards, AMP conjugation to PEGDA hydrogel was performed, and the resulting hydrogel was named PEGDA/FITC-AMP. To confirm peptide conjugation, PEGDA and PEGDA/FITC-AMP samples were observed under a fluorescence microscope (Olympus CKX41, Tokyo, Japan).

2.4 In Vitro Antimicrobial Evaluation

MRSA and MDR *P. aeruginosa* were chosen as gram (+) and gram (-) bacteria, respectively. 1 mL of frozen stocks of each organism was added to 9 mL of tryptic soy broth (TSB; Sigma Aldrich; St. Louis, MO, USA) medium and left for incubation at 37 °C in a thermal shaker incubator. Organisms were cultured on tryptic soy agar (TSA; Sigma Aldrich; St. Louis, MO, USA) plates. Before each experiment, single colony was selected and inoculated in TSB medium, followed by incubation at 37 °C at 200 rpm overnight. The optical density was observed at 600 nm to arrange the bacterial concentration using a spectrophotometer (PG Instruments Limited, UK). The effective concentration of KSL-W peptide on MDR *P. aeruginosa* and MRSA was determined by kinetic growth analysis over 18 h. Peptide solutions were prepared with concentrations of 0, 5, 10, 20, 40, 150, 250, 300, 400, 500, 600, 700, 800, 900, and 1000 µm in TSB with 10^5 CFU ml^{-1} MRSA and MDR *P. aeruginosa*. The multi-plate reader was used to measure optical densities of bacterial strains with different peptide concentrations at 600 nm every 30 min for 18 h. For the investigation of the antimicrobial activity of functionalized hydrogels, the suspended cultures of planktonic forms of MDR *P. aeruginosa* and MRSA strains were placed on PEGDA and PEGDA/KSL-W hydrogel and left for 24 h in contact with the hydrogels. Afterwards, serial dilutions were performed and 100 µl of each dilution was spread on agar plates. After the incubation of agar plates at 37 °C for 24 h, colony counting was conducted to assess the antibacterial activity of the functionalized hydrogel.

2.5 Cytotoxicity Analysis

Cell cytotoxicity analysis was conducted with Mouse Fibroblast Cells, L929 cell lines. For cell culture, Dulbecco's Modified Eagle's Medium (DMEM; Sigma Aldrich, USA)

supplemented by 10% fetal bovine serum (FBS; Sigma-Aldrich, Germany), 1% L-glutamine (Genaxxon BioScience, Germany), and 0.1% penicillin/streptomycin (Genaxxon BioScience, Germany) was utilized as culture media. Cells were incubated at 37°C and 5% CO_2 with the described culture media.

The cytotoxicity analysis of the control, PEGDA and PEGDA/AMP groups was conducted considering ISO 10993-5 standards. 0.2g of hydrogel was left in contact with 1 ml serum free basal media at 37°C for 24 hours in an incubator. L929 cells were seeded on 24-well plates at a density of 5 x 10^4 cell/well and left for incubation for 24 h with 10% FBS and 1% Penicillin/Streptomycin containing DMEM medium. Later, PEGDA and PEGDA/AMP hydrogels were incubated with culture media at 5% CO_2, 37°C for 24 h. PEGDA and PEGDA/AMP extracts were added to the seeded L929 cells. For the control group, only serum-free culture media was added to the cells. Evaluation of the cell cytotoxicity was determined using 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide (MTT; Invitrogen, USA) assay. The MTT solution was prepared by 10% MTT and 90% DMEM and added on each well, left for incubation at 5% CO_2, 37°C for 2 h. After the incubation, the MTT solution was removed, and DMSO was added on each well to dissolve formazan crystals. Absorbance of resulting solutions was determined by reading at 570nm by using a Synergy™ HTX Multi-Mode Microplate Reader (BioTek, USA).

2.6 Wound Healing Analysis

To assess the wound closure impact of produced hydrogels, the scratch assay was conducted. Briefly, L929 cells with a density of 2.5 x 10^5 cells/well were seeded on a 24-well plate and incubated at 5% CO_2, 37°C for 24 h. After 24 h of incubation, 200 µL pipette tips were used to create a scratch by gently scraping the cells. Each well was washed with sterile PBS to remove cellular debris caused by scraping. To obtain hydrogel extracts, PEGDA and PEGDA/AMP hydrogels were immersed in serum-free culture media (100 mg PEGDA hydrogel in 1 mL of culture medium), considering ISO 10993-5 standards. The hydrogel extracts were added to the wound model. For the control group, serum-free DMEM was used as culture medium. Wound closure was observed at 0, 24, 48, and 72 h with fluorescence-enhanced phase contrast microscopy (CKX41, Olympus, Germany). The percentage of wound area was analyzed using the Wound Healing Tool in ImageJ software.

2.7 Statistical Analysis

Each experimental group was assessed with a minimum of three replicates. The mean values and standard deviations were calculated for the obtained data. Group comparisons were performed using one-way and two-way Analysis of Variance (ANOVA), followed by Tukey's multiple comparison test or student's t test for multiple comparisons. Statistical significance was defined as a p-value of less than 0.05.

3. Results

3.1 Characterization of PEGDA/AMP Hydrogels

PEGDA hydrogels were treated with different concentrations of acrylic acid to functionalize PEGDA with carboxyl groups and carboxyl groups were quantified by TBO assay. For PEGDA, PEGDA/AA-0.3, and PEGDA/AA-1.2, the carboxyl group amount was determined as 0.05±0.01, 0.23±0.05, and 0.67±0.01 nmol/cm^3, respectively (Figure 1). The PEGDA hydrogels were successfully functionalized with carboxyl groups. Furthermore, with the increasing concentration of acrylic acid, the number of carboxyl groups was also increased. Thus, PEGDA/AA-1.2 was selected for further experiments.

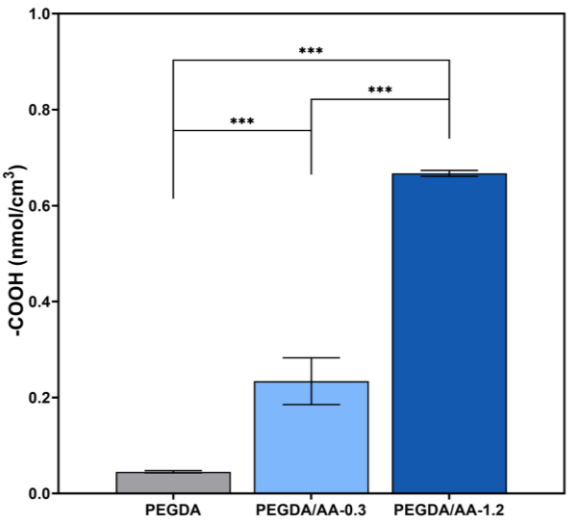


Figure 1. Density of -COOH on PEGDA, 0.3% acrylic acid functionalized PEGDA (PEGDA/AA-0.3) and 1.2% acrylic acid functionalized PEGDA (PEGDA/AA-1.2) obtained by toluidine blue staining. Error bars represent mean ±SE (n = 3). (Significant differences compared to control was determined by one-way ANOVA (Tukey's multiple comparison test (∗p\0.05, ∗∗p\0.01, ∗∗∗p\0.001))).

PEGDA hydrogels were further characterized with ATR-FT-IR. The specific peaks of PEGDA are successfully represented in the FT-IR spectrum (Figure 2). The peaks associated with C=C stretching appear around 1630 cm^{-1}, while the peaks of carbonyl groups are observed around 1720 cm^{-1} (Imani et al. 2007). Additionally, the asymmetric and symmetric bending of alkane C-H is represented by a peak around 2860 cm^{-1}. After the treatment of PEGDA hydrogel with acrylic acid, in the

spectrum of PEGDA/AA-0.3%, no significant change was observed in the FT-IR spectrum. However, in the spectrum of PEGDA/AA-1.2%, a new peak appeared around 3500 cm^{-1}, representing the presence of hydroxyl groups. Additionally, a new peak associated with the carboxyl group of acrylic acid was observed around 2980 cm^{-1}. Also, the peak around 1720 cm^{-1}, which represents the carbonyl group, is elongated compared to PEGDA (Myat et al. 2022). This elongation can be attributed to the additional incorporation of the carbonyl group from acrylic acid. In the PEGDA-AMP spectrum, a new peak was observed around 3250 cm^{-1} which corresponds to N-H stretching. These results confirmed successful functionalization of PEGDA hydrogel with carboxyl groups, with the incorporation of acrylic acid and peptide conjugation on functionalized PEGDA hydrogels.

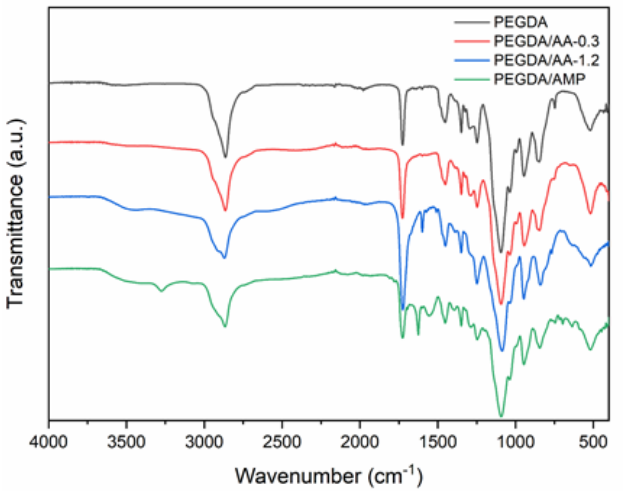


Figure 2. FT-IR spectra for PEGDA, 0.3% acrylic acid functionalized PEGDA (PEGDA/AA-0.3), 1.2% acrylic acid functionalized PEGDA (PEGDA/AA-1.2) and KSL-W peptide conjugated PEGDA (PEGDA/AMP) hydrogels.

To further confirm peptide conjugation to PEGDA, FITC-labelled AMP was conjugated to PEGDA, and fluorescence images were obtained (Figure 3). As expected, no fluorescence signal was observed from PEGDA hydrogel. However, after peptide conjugation, in PEGDA/AMP hydrogel, a distinct and significant green signal was observed due to FITC-labeled AMP. This result further confirmed the successful incorporation of the peptide into the hydrogel structure.

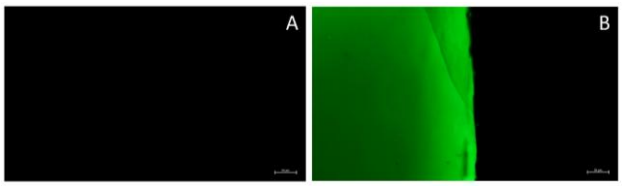


Figure 3. Fluorescent microscopy images of A) PEGDA hydrogel and B) FITC labeled KSL-W peptide conjugated PEGDA hydrogels (Scale bar represents 50 µm).

3.2 Antimicrobial Evaluation of PEGDA/AMP Hydrogels

Antimicrobial studies were conducted with planktonic forms of clinic isolate MRSA and MDR *P. aeruginosa*. Kinetic growth curves of bacteria for 18 h were obtained with 10^5 CFU mL^{-1} MRSA and MDR *P. aeruginosa* to determine the effective inhibition concentration of KSL-W peptide (Figure 4). KSL-W peptide downregulates key genes involved in bacterial growth and transition, which results in the inhibition of bacterial growth (Theberge et al. 2013). For the kinetic readings, 0, 5, 10, 20, 40, 150, 250, 300, 400, 500, 600, 700, 800, 900, and 1000 µM KSL-W concentrations were used for both of the bacteria strains. Considering the effect of KSL-W on MRSA, increasing peptide concentration resulted in lower bacterial growth compared to the control group. Concentrations between 40 – 700 µM suppressed bacteria growth for 2 to 10 h, however did not totally inhibit the bacteria growth. Nevertheless, 800, 900, and 1000 µM of KSL-W concentration resulted in a bacteriostatic effect with a total bacterial growth inhibition. According to kinetic readings, 800 µM peptide concentration was found to be the minimum inhibitory concentration of KSL-W on clinic isolate MRSA. Correspondingly, same peptide concentrations were used for MDR *P. aeruginosa,* and at each concentration of KSL-W peptide provided bacteriostatic activity. 5 µM of KSL-W peptide was sufficient for the total inhibition of the bacteria.

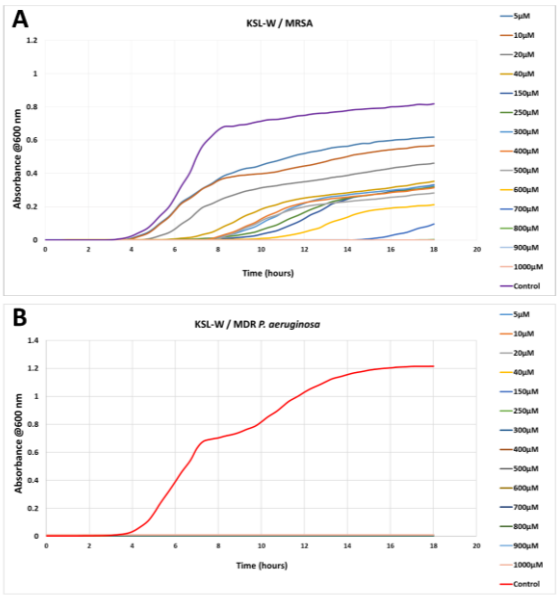


Figure 4. Growth kinetics of 10^5 CFU mL^{-1} A) MRSA, B) MDR P. aeruginosa in different concentrations of KSL-W antimicrobial peptide.

Following the evaluation of the antimicrobial activity of KSL-W peptide against a planktonic form of drug-resistant

bacteria, the antimicrobial effect of KSL-W AMP-conjugated PEGDA hydrogels was also investigated (Figure 5). In MRSA, bacterial viability was determined as 5.42±0.08 and 2.98±0.14 log for control and PEGDA/AMP, respectively. Similarly, for control and PEGDA/AMP groups, MDR *P. aeruginosa* viability was determined as 5.40±0.17 and 4.25±0.05 log, respectively. These results revealed that, AMP conjugation to PEGDA hydrogels resulted in a strong antibacterial activity against both gram-positive and gram-negative bacteria with a 2.44 and 1.15 log bacterial death for MRSA and MDR *P. aeruginosa*, respectively.

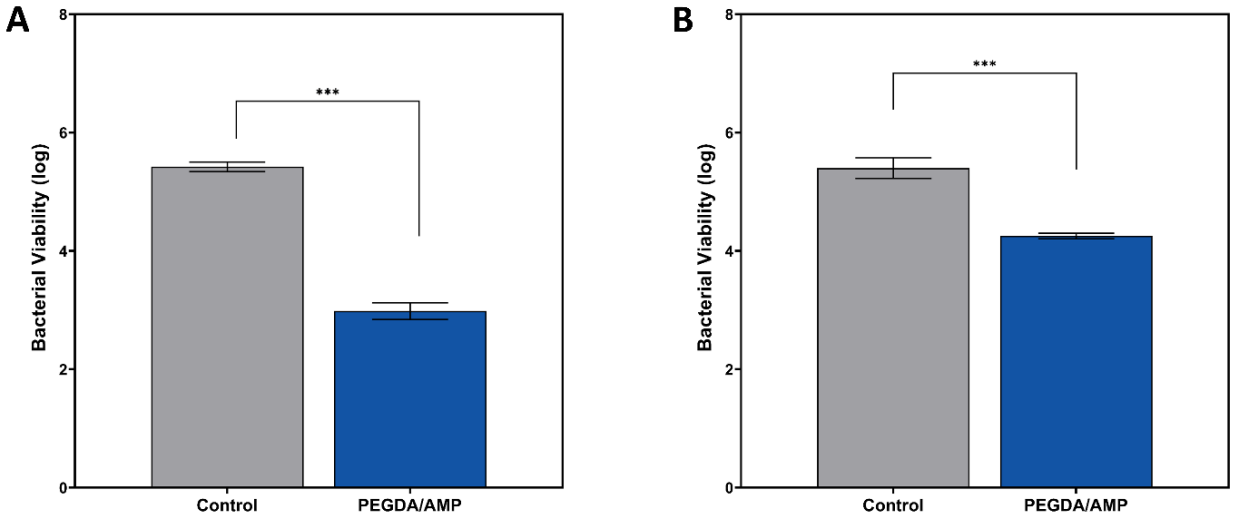


Figure 5. Bacterial viability for A) MRSA and B) MDR *P. aeruginosa* after the contact time of 24 h with PEGDA (control) and KSL-W peptide conjugated PEGDA (PEGDA/AMP) hydrogels. Error bars represent mean ± SE (n = 3). (Significant differences compared to control were determined by student's t test (∗p\0.05, ∗∗p\0.01, ∗∗∗p\0.001)).

3.3 Cell Viability and Wound Healing Analysis

The effect of AMP-conjugated PEGDA hydrogels on L929 viability was investigated by using MTT analysis at day 1, 4, and 7 (Figure 6). After 24 h of incubation, L929 viability was determined as 100.0±2.5%, 109.7±10.2, and 107.2±9.8% for control, PEGDA, and PEGDA/AMP, respectively. Although the cell viability was slightly increased for PEGDA and PEGDA/AMP compared to the control, there was no significant difference ($p > 0.1$). Cell viability remained above 100% at every time point for all groups. After 7 d of incubation, L929 viability was reached up to 149.5±10.8%, 126.8±24.9 and 133.8±16.5% for control, PEGDA, and PEGDA/AMP, respectively. Consequently, developed PEGDA/AMP hydrogels did not show any undesired toxic effects and were found to be compatible with healthy mammalian cells.

Furthermore, the effect of PEGDA/AMP hydrogels on wound healing was determined with the scratch assay (Figure 7). After 24 h of incubation, the wound area for control, PEGDA, and PEGDA/AMP was determined as 33.15±2.76, 65.60±1.52, and 68.66±6.19%, respectively. During the first 24 h, the created wound healed more rapidly in the control group compared to PEGDA and PEGDA/AMP groups ($p < 0.001$). However, after 48 h of incubation, there was no significant difference between the control and PEGDA/AMP group ($p > 0.1$). Although PEGDA slightly delayed wound closure, the PEGDA/AMP group supported faster wound healing compared to PEGDA. Furthermore, by the 72nd h, the wound area was 2.70±0.74, 6.47±0.99 and 1.56±0.08% for control, PEGDA and PEGDA/AMP groups, respectively. These results showed that the developed PEGDA/AMP hydrogel further supports wound healing.

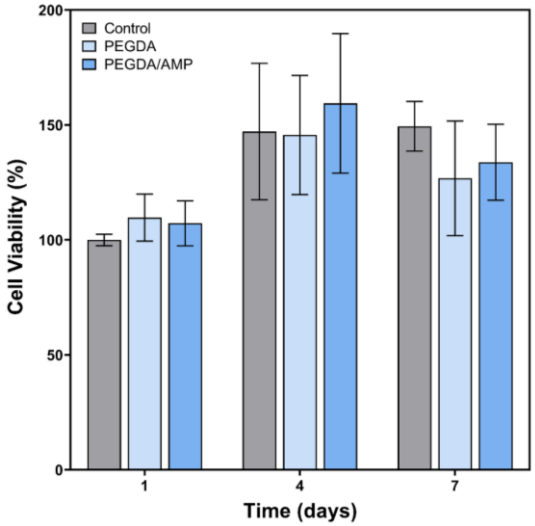


Figure 6. The effect of PEGDA and KSL-W peptide conjugated PEGDA (PEGDA/AMP) hydrogels on L929 cell viability at days 1, 4 and 7. Error bars represent mean ± SE (n = 3). (Significant differences compared to control were determined by one-way ANOVA (Tukey's multiple comparison test (∗p\0.05, ∗∗p\0.01, ∗∗∗p\0.001))).

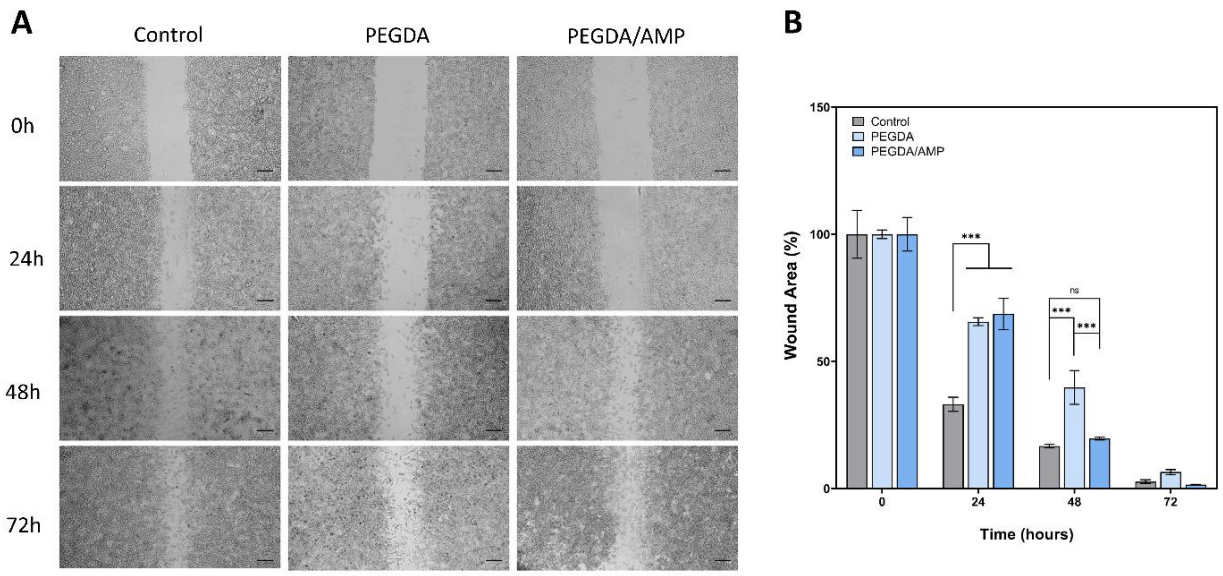


Figure 7. A) Microscopic images of created wounds by scratch assay and B) the percentage of the wound area at 0, 24, 48 and 72 h after incubation with PEGDA and KSL-W peptide conjugated PEGDA (PEGDA/AMP) hydrogels. The scale bar represents 100 µm. Error bars represent mean ±SE (n = 3) (significant differences compared to control was determined by two-way ANOVA (Tukey's multiple comparison test (∗p\0.05, ∗∗p\0.01, and ∗∗∗p\0.001))).

4. Discussion

Hydrogels can be functionalized with peptides by using different approaches including physical immobilization and covalent bonding such as photoconjugation, click chemistry and EDC/NHS chemistry (Lyu et al. 2021). Among these strategies, photoconjugation is commonly reported in the literature due to its procedural simplicity and compatibility with photo-crosslinkable polymers. In this method, peptides are mixed with a prepolymer solution and crosslinked under UV exposure (Prakash Parthiban et al. 2017). However, this method has several drawbacks. Certain peptide sequences are susceptible to UV-induced photochemical degradation or conformational changes, which may result in the loss of bioactivity or reduced antimicrobial efficacy (Maria Teresa et al. 2012). In contrast, EDC/NHS chemistry facilitates covalent attachment of peptides via amide bond formation between carboxyl and amine groups under mild, aqueous conditions (Prabhakar et al. 2025). This method preserves peptide functionality since it avoids potential denaturation of peptide due to UV exposure and allows for better control over conjugation concentration. Furthermore, click chemistry is another alternative strategy which is particularly known as azide-alkyne cycloaddition. This strategy offers high specificity and efficiency, although it often requires pre-functionalization of both peptide and polymer, which may increase system complexity (Lowe et al. 2010). Moreover, physical entrapment of peptides into hydrogels remains the simplest strategy but causes several difficulties such as rapid peptide release and lack of stability. Thus, EDC/NHS chemistry offers a stable immobilization which makes it a favorable choice for applications requiring sustained peptide performance. However, the molecular structure of PEGDA inherently lacks functional groups. Therefore, to achieve peptide conjugation with EDC/NHS chemistry, PEGDA functionalization with carboxyl groups is an essential step. Incorporation of acrylic acid into PEGDA hydrogel is a widely utilized strategy to introduce carboxyl groups (Browe et al. 2017, Castaño 2017). Acrylic acid contains a carboxyl group, which provides the necessary reactive site for peptide attachment. Furthermore, since acrylic acid has a vinyl group, the copolymerization of PEGDA and acrylic acid during photo-crosslinking is possible (Castaño 2017). Herein, we successfully functionalized and copolymerized PEGDA hydrogels with acrylic acid, followed by peptide conjugation to the carboxyl ends of PEGDA/AA hydrogels.

Furthermore, the effect of KLS-W peptide on soluble form against both gram-positive MRSA and gram-negative MDR *P. aeruginosa* was investigated. Compared to MRSA, the growth of MDR *P. aeruginosa* was inhibited with lower peptide concentrations. MDR *P. aeruginosa* has a cell membrane composed of lipopolysaccharide (LPS), which is negatively charged. LPS-rich negatively charged outer membrane of gram-negative bacteria is inclined to interact with cationic AMPs, thus, this interaction may result in a stronger antibacterial activity (Barreto-Santamaría et al. 2021). Since KSL-W peptide is also a cationic AMP, these results can be associated with the negatively charged outer membrane of MDR *P. aeruginosa.* Furthermore, the antimicrobial activity of the

hydrogels was evaluated by conjugating the KSL-W peptide to PEGDA hydrogels. Whereas the kinetic readings indicated that KSL-W peptide shows high activity against MDR *P. aeruginosa,* PEGDA/AMP hydrogels demonstrated higher antimicrobial efficacy against MRSA with an approximately 2.5 log bacterial reduction. Nevertheless, PEGDA/AMP hydrogels also caused more than 1 log bacterial reduction against MDR *P. aeruginosa.* These findings showed that the developed AMP functionalized PEGDA hydrogels exhibit moderate antimicrobial activity against MDR bacteria which is lower than expected. For future studies, the antimicrobial performance could potentially be enhanced by replacing or combining the initial peptide with alternative AMPs that exhibit stronger activity against gram-negative MDR bacteria. Moreover, increasing the initial peptide concentration may lead to a higher peptide concentration on the PEGDA hydrogel, thereby improving the overall antibacterial efficacy.

Recently, hydrogel-based wound dressings have been frequently preferred in the treatment of skin defects due to their unique biochemical and mechanical properties. Hydrogels enhance the wound healing process by mimicking the natural extracellular matrix (ECM) and maintaining the moist environment, which are critical for effective tissue repair (Liang et al. 2021). Among the hydrogels, PEGDA is considered an excellent candidate material due to its biocompatibility, flexibility, and stability under numerous conditions (Levin et al. 2024). To further improve the properties of PEGDA hydrogels, peptide functionalization is a preferred strategy (Zhu et al. 2009, Clevenger et al. 2022). Zhu *et al.* reported a strategy for cyclic RGD (cRGD) peptide conjugation to PEGDA hydrogels to enhance their bioactivity via improving integrin-specific cell adhesion (Zhu et al. 2009). Briefly, they designed a tailed cRGD peptide, c[RGDFE(SSSKK-NH_2)], with a hydrophilic spacer (SSS) and a lysine linker (KK) for covalent attachment to acryloyl-PEG-NHS to create cRGD-PEGDA macromers. Developed biomimetic cRGD-PEGDA hydrogels showed enhanced cell adhesion and spreading on the hydrogel surface. Moreover, Clevenger *et al.* developed an immunocompatible PEGDA-based hydrogel wound dressing by covalently tethering growth factor (GF) binding peptides (Clevenger et al. 2022). Briefly, GF binding peptides, KGLPLGNSH (targeting human Transforming Growth Factor beta-1; hTGFβ1), DRVQRQTTTVVA (targeting human Vascular Endothelial Growth Factor; hVEGF), and cell adhesion peptide, RGDS, were separately conjugated to Acrylate-PEG-Succinimidyl Valerate (ACRL-PEG-SVA). To prepare PEGDA hydrogels, synthesized peptide conjugated ACRL-PEG-SVAs were dissolved with PEGDA (10% w/w) and crosslinked with light application in the presence of Lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) photoinitiator. Developed PEGDA hydrogels showed enhanced early wound healing responses with higher expression levels of collagen type I and TGFβ1. These studies showed the high potential of peptide functionalization of PEGDA-based hydrogels to enhance the material performance.

However, the antimicrobial properties of hydrogels are also considered essential to improve wound healing by preventing bacterial infection (Huang et al. 2019). Thus, there are several PEGDA-based hydrogels functionalized with antibiotics to provide antimicrobial properties (Nurlidar et al. 2023, Nguyen et al. 2024). However, antibiotic use has become a major concern since it can induce bacterial resistance. Therefore, Huang *et al.* developed a double-network hydrogel system composed of PEGDA and chitosan (CS)/thiolated chitosan (TCS) that is loaded with PSI (RRWPWWPWRR-NH_2) AMP to provide antibacterial properties (Huang et al. 2019). To functionalize the developed hydrogel with PSI AMP, PSI was directly mixed into PEGDA and TCS pre-gel solution and cross-linked by UV irradiation with the aid of Irgacure 2959. Hydrogels showed strong antibacterial activity against E. Coli and S. Aureus with an antimicrobial rate of 80% and above. In this study, the AMP was incorporated with hydrogel through photo-induced conjugation, and results demonstrated the potential of PEGDA hydrogels combined with AMPs. However, to the best of our knowledge, there has been no study which involves the chemical conjugation of PEGDA hydrogels using EDC/NHS chemistry. Herein, we successfully functionalized PEGDA hydrogels with KSL-W AMP by using EDC/NHS chemistry to develop an effective PEGDA-based antimicrobial wound dressing against multidrug-resistant bacteria. Moreover, the developed wound dressing showed no toxic effects on healthy mammalian cells and further supported wound healing in the developed 2D *in vitro* model.

In this study, we reported an alternative strategy for the functionalization of PEGDA hydrogels with peptides. This newly developed strategy offers a promising approach for an effective infected wound care, while also introduces a method for the functionalization of PEGDA hydrogels with various bioactive peptides.

Conclusions

Herein, we developed a strategy for peptide conjugation to PEGDA hydrogels by acrylic acid functionalization followed by peptide conjugation via EDC/NHS chemistry.

The developed methodology may be an alternative approach compared to the strategies in the literature. Developed PEGDA/AMP hydrogels showed strong antibacterial activity against drug-resistant bacteria. Furthermore, hydrogels did not show undesired effects on healthy mammalian cells and found to be effective on wound healing. This methodology may provide a novel approach for conjugating peptides on PEGDA hydrogels and allow the incorporation of not only antimicrobial peptides but also various bioactive peptides.

Credit Authorship Contribution Statement

Author-1: Methodology, Investigation, Software, Validation, Formal analysis, Data curation, Visualization and writing – original draft.

Author-2: Methodology, Investigation, Software, Validation, Formal analysis, Visualization and writing – original draft.

Author-3: Conceptualization, Methodology/Study design, Software, Validation, Investigation, Resources, Writing – review and editing, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors have no conflicts of interest to declare regarding the content of this article.

Data Availability Statement

All data generated or analyzed during this study are included in this published article.

Datasets are available on request. The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

5. References

Barreto-Santamaría, A., Arévalo-Pinzón, G., Patarroyo, M. A. and Patarroyo, M. E. (2021) "How to Combat Gram-Negative Bacteria Using Antimicrobial Peptides: A Challenge or an Unattainable Goal?" Antibiotics **10** https://doi.org/10.3390/antibiotics10121499

Browe, D. P., Wood, C., Sze, M. T., White, K. A., Scott, T., Olabisi, R. M. and Freeman, J. W. (2017). "Characterization and optimization of actuating poly(ethylene glycol) diacrylate/acrylic acid hydrogels as artificial muscles." Polymer **117**: 331-341. https://doi.org/10.1016/j.polymer.2017.04.044.

Camó, C., Bonaterra, A., Badosa, E., Baró, A., Montesinos, L., Montesinos, E., Planas, M. and Feliu, L. (2019). "Antimicrobial peptide KSL-W and analogues: Promising agents to control plant diseases." Peptides **112**: 85-95. https://doi.org/10.1016/j.peptides.2018.11.009.

Castaño, A. G. (2017). Engineering poly (ethylene glycol) diacrylate-based microstructures to develop an in vitro model of small intestinal epithelium. PhD, Universitat de Barcelona.

Clevenger, A. J., Jimenez-Vergara, A. C., Tsai, E. H., de Barros Righes, G., Díaz-Lasprilla, A. M., Ramírez-Caballero, G. E. and Munoz-Pinto, D. J. (2022). "Growth Factor Binding Peptides in Poly (Ethylene Glycol) Diacrylate (PEGDA)-Based Hydrogels for an Improved Healing Response of Human Dermal Fibroblasts." Gels **9**(1). https://doi.org/10.3390/gels9010028.

Firlar, I., Altunbek, M., McCarthy, C., Ramalingam, M. and Camci-Unal, G. (2022). "Functional Hydrogels for Treatment of Chronic Wounds." Gels **8**(2). https://doi.org/10.3390/gels8020127.

Gao, Y., Zhang, W., Cheng, Y. F., Cao, Y., Xu, Z., Xu, L. Q., Kang, Y. and Xue, P. (2021). "Intradermal administration of green synthesized nanosilver (NS) through film-coated PEGDA microneedles for potential antibacterial applications." Biomaterials Science **9**(6): 2244-2254. https://doi.org/10.1039/D0BM02136A.

Gera, S., Kankuri, E. and Kogermann, K. (2022). "Antimicrobial peptides – Unleashing their therapeutic potential using nanotechnology." Pharmacol. Ther. **232**: 107990. https://doi.org/10.1016/j.pharmthera.2021.107990

Huang, L., Zhu, Z., Wu, D., Gan, W., Zhu, S., Li, W., Tian, J., Li, L., Zhou, C. and Lu, L. (2019). "Antibacterial poly (ethylene glycol) diacrylate/chitosan hydrogels enhance mechanical adhesiveness and promote skin regeneration." Carbohydrate Polymers **225**: 115110. https://doi.org/10.1016/j.carbpol.2019.115110.

Imani, M., Sharifi, S. and Ziaee, F. (2007). "Monitoring of Polyethylene Glycol-diacrylate-based Hydrogel Formation by Real Time NMR Spectroscopy." Iran. Polym. J. **16**: 13-20.

Lei, J., Sun, L., Huang, S., Zhu, C., Li, P., He, J., Mackey, V., Coy, D. H. and He, Q. (2019). "The antimicrobial peptides and their potential clinical applications." Am J Transl Res **11**(7): 3919-3931.

Lensen, M. C., Schulte, V. A. and Diez, M. (2011). "Cell Adhesion and spreading on an intrinsically anti-adhesive PEG biomaterial." Biomaterials—Physics and Chemistry: 397-414.

Levin, M., Tang, Y., Eisenbach, C. D., Valentine, M. T. and Cohen, N. (2024). Understanding the Response of Poly(ethylene glycol) diacrylate (PEGDA) Hydrogel Networks: A Statistical Mechanics-Based Framework. Macromolecules **57**(15): 7074-7086. https://doi.org/10.1021/acs.macromol.3c02635.

Liang, Y., He, J. and Guo, B. (2021). "Functional Hydrogels as Wound Dressing to Enhance Wound Healing." ACS Nano **15**(8): 12687-12722. https://doi.org/10.1021/acsnano.1c04206.

Lowe, A. B., Hoyle, C. E. and Bowman, C. N. (2010). "Thiol-yne click chemistry: A powerful and versatile

methodology for materials synthesis." Journal of Materials Chemistry **20**(23): 4745-4750. https://doi.org/10.1039/B917102A.

Lyu, Y. and Azevedo, H. S. (2021). "Supramolecular Hydrogels for Protein Delivery in Tissue Engineering." Molecules **26**(4). https://doi.org/10.3390/molecules26040873.

Maria Teresa, N.-P., Steffen, P. and Gnana Prakash, G. (2012). UV Light Effects on Proteins: From Photochemistry to Nanomedicine. Molecular Photochemistry. S. Satyen. Rijeka, IntechOpen**:** Ch. 7.

Myat, Y. Y., Ngawhirunpat, T., Rojanarata, T., Opanasopit, P., Bradley, M., Patrojanasophon, P. and Pornpitchanarong, C. (2022) "Synthesis of Polyethylene Glycol Diacrylate/Acrylic Acid Nanoparticles as Nanocarriers for the Controlled Delivery of Doxorubicin to Colorectal Cancer Cells." Pharmaceutics **14** https://doi.org/10.3390/pharmaceutics14030479.

Nguyen, D.-V., Yuan, Y., Kukumberg, M., Wang, L., Lim, S. H., Hassanbhai, A. M., Chong, M., Kofidis, T., Tan, E. C. K., Seliktar, D., Kang, L. and Rufaihah, A. J. (2024). "Controlled release of vancomycin from PEGylated fibrinogen polyethylene glycol diacrylate hydrogel." Biomaterials Advances **161**: 213896. https://doi.org/10.1016/j.bioadv.2024.213896.

Nurlidar, F., Rahayu, D. P., Lasmawati, D., Yunus, A. L., Heryani, R. and Suryani, N. (2023). "A simple method for the simultaneous encapsulation of ciprofloxacin into PEGDA/alginate hydrogels using gamma irradiation." Arab. J. Chem. **16**(7): 104793. https://doi.org/10.1016/j.arabjc.2023.104793.

Onak, G., Ercan, U. K. and Karaman, O. (2020). "Antibacterial activity of antimicrobial peptide-conjugated nanofibrous membranes." Biomed Mater **16**(1): 015020. https://doi.org/10.1088/1748-605X/abb722.

Prabhakar, T., Giaretta, J., Zulli, R., Rath, R. J., Farajikhah, S., Talebian, S. and Dehghani, F. (2025). "Covalent immobilization: A review from an enzyme perspective." Chemical Engineering Journal **503**: 158054. https://doi.org/10.1016/j.cej.2024.158054.

Prakash Parthiban, S., Rana, D., Jabbari, E., Benkirane-Jessel, N. and Ramalingam, M. (2017). "Covalently immobilized VEGF-mimicking peptide with gelatin methacrylate enhances microvascularization of endothelial cells." Acta Biomaterialia **51**: 330-340. https://doi.org/10.1016/j.actbio.2017.01.046.

Sen, C. K., Gordillo, G. M., Roy, S., Kirsner, R., Lambert, L., Hunt, T. K., Gottrup, F., Gurtner, G. C. and Longaker, M. T. (2009). "Human skin wounds: a major and snowballing threat to public health and the economy." Wound Repair Regen **17**(6): 763-771. https://doi.org/10.1111/j.1524-475X.2009.00543.x.

Theberge, S., Semlali, A., Alamri, A., Leung, K. P. and Rouabhia, M. J. B. m. (2013). "C. albicans growth, transition, biofilm formation, and gene expression modulation by antimicrobial decapeptide KSL-W." BMC Microbiol. **13**: 1-14. https://doi.org/10.1186/1471-2180-13-246.

Zhu, J., Tang, C., Kottke-Marchant, K. and Marchant, R. E. (2009). "Design and synthesis of biomimetic hydrogel scaffolds with controlled organization of cyclic RGD peptides." Bioconjug Chem **20**(2): 333-339. https://doi.org/10.1021/bc800441v.