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Research Article

Development of Biocompatible Polymer-Based Synthetic Wound Dressing

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Abstract

Biocompatible hydrogel wound dressings have garnered interest for their capacity to promote the regeneration of dermal and epidermal tissues. This study generated a biocompatible hydrogel wound dressing with Pluronic F127 and chitosan functionalized with Ala-Leu dipeptide nanotubes. The biocompatible structure was studied using FTIR, SEM-EDX, and TGA-DTA analyses. The analysis results validated the synthesized structure by instrumental approaches. The synthesized material's biological effects were assessed via antibacterial activity against *E. coli, P. aeruginosa, S. aureus*, and *S. pyogenes*, cytotoxicity tests with HS2, and wound healing evaluations using HS2. No notable antibacterial, cytotoxic, or wound-healing properties of the synthesized substance were observed. Hydrogels synthesized from chitosan functionalized with Ala-Leu dipeptide nanotubes can be enhanced with antimicrobial agents and wound healing promoters as therapeutic carrier wound dressings or in injectable gel form.

Keywords: wound dressing, dipeptide, biocompatibility, cytotoxicity, antimicrobial

Biyouyumlu Polimer Esaslı Sentetik Yara Örtüsü Geliştirilmesi

Öz

Biyouyumlu hidroejel yara örtüleri, dermal ve epidermal dokuların rejenerasyonunu artırdıkları için ilgi çekmiştir. Bu çalışmada Ala-Leu dipeptit nanotüp ile fonksiyonalize edilmiş kitosan kullanılarak Pluronic F127 tabanlı biyouyumlu hidrojel yara örtüsü geliştirilmiştir. Sentezlenen biyouyumlu yapı; FTIR, SEM-EDX ve TGA-DTA ile karaketerize edilmiştir. Analiz sonuçlarına göre, sentezlenen yapı enstrümental tekniklerle doğrulanmıştır. Sentezlenen materyalin biyolojik etkileri antimikrobiyal (*E.coli*, *P.aeroginosa*, *S.aureus*, *S.pyogenes*), sitotoksiste (HS2) aktivite ve yara iyileşme (HS2) testleri ile değerlendirilmiştir. Sentez materyalinin anlamlı antimikrobiyal, sitotoksik ve yara iyileştirici etkisi tespit edilmemiştir. Ala-Leu dipeptit nanotüp ile fonksiyonalize edilmiş kitosan kullanılarak hazırlanan Pluronic F127 tabanlı hidrojeller, antimikrobiyal ajanlar ve yara iyileşmesini teşvik edici ajanlar ile modifiye edilerek terapötik taşıyıcı yara örtüsü ve enjekte edilebilir jel formunda kullanılabilir.

Anahtar Kelimeler: Yara örtüsü, dipeptit, biyouyumluluk, sitotoksisite, antimikrobiyal.

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1. Introduction

The skin, the largest organ of the human body, is essential for safeguarding interior organs against physical contact, toxins, infections, heat, and dehydration while preserving the body's integrity [1]. The epidermis, the outermost layer, is composed of keratinocytes; the dermis, the middle layer, consists of collagen and fibroblasts; and the hypodermis, the innermost layer, is made up of lipocytes and collagen. Any impairment in these layers might result in adverse skin conditions, including rashes, erythema, dermatitis, cellulitis, and melanoma [2]. Wound dressings are employed in the healing of skin tissue injuries to promote the regeneration of dermal and epidermal layers. The principal objective of wound care is to attain optimal functional and aesthetic results. An optimal wound dressing must safeguard the wound against germs while exhibiting attributes such as flexibility, biodegradability, and permeability to oxygen and moisture [3, 4]. Bacterial infections at the wound site might impede the healing process. If this process is inadequately handled, chronic infections may result in life-threatening sepsis. Management of this process can be accomplished by administering topical antibiotics to the wound via wound dressings. The principal drawbacks of conventional formulations (creams, ointments, gels, etc.) include brief drug retention durations and inadequate patient adherence resulting from leakage, messiness, and expense. Moreover, dry dressings such as gauze and cotton wool possess limitations, including an inability to sustain the moist environment crucial for wound healing [5, 6]. These constraints can be mitigated by including antibiotics polymeric wound dressings [6]. Natural, synthetic, and semi-synthetic polymer-based wound dressings significantly enhance cell proliferation, migration, and differentiation owing to their biocompatibility, biodegradability, distinctive architectures, and mechanical properties [7]. Film dressings are favored for their ease of use, semi-permeability to water vapor and oxygen, and adaptability for administration on body regions including joints [8].

Poloxamers, marketed as Pluronic®, are triblock copolymers composed of polyethylene oxide and polypropylene oxide (PEO–PPO–PEO), with several kinds exhibiting distinct molecular weights. Within the Pluronic group, Pluronic® F127 (PF127) is widely used for controlled drug release due to properties such as reversible thermal gelation, solubilization of hydrophobic solutes, micelle formation, extended drug release, and non-toxicity [9-11]. PF127 demonstrates good solubility in water and forms micelles and a 3D structure in response to temperature changes through interactions of the hydrophobic components of PPO [12]. PF127 gels have demonstrated enhancements in wound healing [13]. Notwithstanding these benefits, PF127 exhibits a propensity to dissolve readily due to its low mechanical strength and diminished stability in formulations [14]. In several studies, the mechanical strength of PF127 has been improved by physically mixing it with various polymers. For example, improvements have been observed in PF127 gels prepared with alginate/PF127 [15], chitosan/PF127 [16], and hyaluronic acid/PF127 [17].

In recent years, chitosan has been utilized in various fields such as controlled drug release, scaffolds, surgical sutures, and wound dressings [18]. Chitosan is a polymer commonly investigated in tissue engineering research owing to its biocompatibility, biodegradability, hemostatic capabilities, and antibacterial characteristics [19]. Chitosan is non-toxic and

exhibits superior bioadhesive characteristics [20]. As a natural polymer found in the outer shells of arthropods, chitosan is second only to cellulose in abundance. Depending on its source and production process, chitosan varies in terms of average molecular weight and degree of N-acetylation [21]. Chitosan is a linear copolymer comprising β-(1→4)-N-acetyl-d-glucosamine (degree of acetylation, DA) and β-(1→4)-D-glucosamine fractions [22]. The poor solubility of chitosan in water limits its effective applicability. This can be overcome through chemical modification of its functional groups [23]. Chitosan derivatives, which can be modified thanks to reactive functional groups in their structure, can be endowed with desired properties while preserving their biocompatibility and biodegradability [24].

Peptides play crucial roles in the body in processes such as signaling, homeostasis, growth, development, wound healing, and wound repair. Many studies have shown significant effects of antibacterial peptides, neuropeptides, collagen peptides, etc., in wound repair [25]. Peptide-modified hydrogel dressings are promising and attractive for biomedical applications due to their versatility and multifunctionality, such as biocompatibility, biodegradability, adaptability to surface morphology, enhancement of therapeutic efficacy of drugs, low immunogenicity, and suitability for modification. However, they require further development due to their need for secondary coatings, low tensile strengths, and mechanical instability [26].

Despite excellent drug delivery and wound healing properties, PF127 has been investigated for its use as a component of temporary wound dressings due to its poor mechanical properties. Although various studies have aimed to enhance PF127's mechanical strength and biocompatibility, the use of PF127-based hydrogels crosslinked with chitosan functionalized with Ala-Leu dipeptide nanotubes as wound dressing has not been explored. Within this scope, crosslinked composites of PF127-based hydrogels prepared using chitosan functionalized with Ala-Leu dipeptide nanotubes were developed, and their chemical structure and surface properties were examined, along with their antimicrobial, cytotoxicity, and wound healing effectiveness.

2. Materials and Methods

The following components were utilized for the manufacture of wound dressings: Chitosan (C3646, Sigma, USA), Pluronic® F-127 (P2443, Sigma, USA), acetic acid (27225, Sigma, USA), Ala-Leu (A1878, Sigma, USA), 1,1,1,3,3,3-Hexafluoro-2-propanol (42060, Sigma, USA), and Glyoxal solution (128465, Sigma, USA). Dulbecco's Modified Eagle Medium (DMEM) (REF 41966-029, Gibco, UK), Penicillin/Streptomycin antibiotic solution (P4333, Sigma, USA), Fetal Bovine Serum (FBS) (REF:10500-064, Gibco, UK), Trypsin-EDTA (REF:25200-056, UK), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (CAS:298-93-1, ACROS ORGANICS, USA), and DMSO (41640, Sigma, USA) were employed for cell culture studies. Nutrient Broth (05443, Merck, Germany) and Nutrient Agar (05450, Merck, Germany) were utilized for antibacterial investigations.

2.1 Synthesis and Characterization Studies

2.1.1 Synthesis Studies

Temperature-sensitive chitosan-Pluronic F127 wound dressings were prepared using a physical mixture of chitosan (CS) solution and a specified proportion of PF127. Chitosan solutions (CS) were dissolved at a concentration of 1% w/v in acetic acid solution (1% v/v) using a mechanical stirrer (Scilogex MS-HS, Washington, USA) for 24 hours, and a certain amount was stored for structural characterization. To functionalize chitosan, Ala-Leu dipeptide was prepared at a concentration of 100 mg/ml, dissolved in 4 ml of distilled water and 1 ml of HFIP, and left at room temperature for 24 hours to allow the solvents to evaporate and for peptide nanotube structures to naturally form. The prepared peptide nanotube structure was added to chitosan solutions and mixed using a mechanical stirrer for 24 hours to ensure chemical bonding. At the end of the process, a certain amount of the chitosan-dipeptide (CS-PN) solution was stored for structural characterization (CS-PN; CS-Ala-Leu). Subsequently, PF127 was weighed at 20% w/v and gradually added to the CS-PN solutions. After achieving uniform distribution, the solution was placed in a cooler set to +4°C and stirred mechanically for 24 hours. At the end of the process, a certain amount of the CS-PN-PF127 solution was stored for structural characterization. Following this, 10 µl of cross-linking agent glyoxal (Gx) was added to the CS-PN-PF127 solution. The CS-PN-PF127-Glx solution was stirred again using a magnetic stirrer in a cooler set to +4°C for 24 hours. After the specified time, samples were taken from the solution for structural characterization and stored. CS-PN-PF127-Glx wound dressing solutions were prepared using the "cold method" [27], and all stocks were preserved at +4°C. Temperature-sensitive chitosan-Pluronic F127 wound dressings were created using a physical amalgamation of chitosan (CS) solution and a designated ratio of PF127. Chitosan solutions (CS) were prepared at a concentration of 1% w/v in a 1% v/v acetic acid solution using a mechanical stirrer (Scilogex MS-HS, Washington, USA) for 24 hours, with a portion reserved for structural characterisation. Ala-Leu dipeptide was synthesized at a concentration of 100 mg/ml, diluted in 4 ml of distilled water and 1 ml of HFIP, and allowed to stand at room temperature for 24 hours to facilitate solvent evaporation and the natural formation of peptide nanotube structures. The synthesized peptide nanotube structure was incorporated into chitosan solutions and agitated with a mechanical stirrer for 24 hours to facilitate chemical bonding. At the conclusion of the process, a specific quantity of the chitosan-dipeptide (CS-PN) solution was preserved for structural characterisation (CS-PN; CS-Ala-Leu). Subsequently, PF127 was measured at 20% w/v and incrementally incorporated into the CS-PN solutions. Subsequent to attaining uniform dispersion, the solution was positioned in a cooler maintained at +4°C and subjected to mechanical stirring for 24 hours. Upon completion of the process, a certain quantity of the CS-PN-PF127 solution was preserved for structural characterization. Subsequently, 10 µl of the cross-linking agent glyoxal (Gx) was added to the CS-PN-PF127 solution. The CS-PN-PF127-Glx solution was agitated with a magnetic stirrer in a cooler maintained at +4°C for 24 hours. Subsequent to the designated duration, samples were extracted from the solution for structural analysis and preserved. CS-PN-PF127-Glx wound dressing solutions were formulated utilizing the "cold method" [27], and all stocks were maintained at +4°C.

2.1.2 Characterization Studies

The structural characterisation of biocompatible polymeric wound dressings was conducted using Fourier Transform Infrared (FTIR) spectroscopy, Scanning Electron Microscopy with Energy Dispersive X-ray Spectroscopy (SEM-EDX), and Thermogravimetric and Differential Thermal Analysis (TGA/DTA). The chemical structure investigations of CS, CS-PN, CS-PN-PF127, and CS-PN-PF127-Glx materials were conducted at room temperature using an Agilent Technologies Cary 630 FTIR instrument, covering the range of 4000–400 cm^{-1}. The spectra acquired in this range were utilized to analyze the distinctive signals of CS, CS-PN, CS-PN-PF127, and CS-PN-PF127-Glx [20]. The surface microstructural morphology and chemical composition of Pluronic F127-based hydrogels, produced with chitosan functionalized by Ala-Leu dipeptide nanotubes, were analyzed by SEM-EDX. The thermal properties and stability of CS-PN-PF127-Glx samples were examined using TGA/TDA [9].

2.2 In Vitro Experiments

2.2.1 Cell Culture Conditions

Cytotoxicity and wound healing assessments for CS-PN-PF127-Glx wound dressings were conducted utilizing the human keratinocyte cell line HS2, established by Prof. Dr. İsmet Deliloğlu Gürhan. The cell lines were acquired from the Biomaterials and 3D Biointerfaces Laboratory at Ege University. Dulbecco's Modified Eagle's Medium (DMEM) was utilized for the cell line cultures. The DMEM medium was formulated by including Penicillin/Streptomycin antibiotic solution (P4333) at 1% (v/v) and Fetal Bovine Serum (FBS, Gibco-REF: 10500-064) at 10% (v/v). The cell lines were cultivated in T25 and T75 filtered cell culture flasks, along with 6-well and 96-well culture plates, under a humidified incubator at 37°C with 5% CO_2 [28]. Cytotoxicity and wound healing assessments for CS-PN-PF127-Glx wound dressings were conducted utilizing the human keratinocyte cell line HS2, established by Prof. Dr. İsmet Deliloğlu Gürhan. The cell lines were acquired from the Biomaterials and 3D Biointerfaces Laboratory at Ege University. Dulbecco's Modified Eagle's Medium (DMEM) was utilized for the cell line cultures. The DMEM medium was formulated by including Penicillin/Streptomycin antibiotic solution (P4333) at 1% (v/v) and Fetal Bovine Serum (FBS, Gibco-REF: 10500-064) at 10% (v/v). The cell lines were cultivated in T25 and T75 filtered cell culture flasks, along with 6-well and 96-well culture plates, under a humidified incubator at 37°C with 5% CO_2 [28].

2.2.2 Cytotoxicity Studies

The cytotoxic effects of the synthesized CS-PN-PF127-Glx wound dressings were assessed utilizing the MTT test technique. Cells were inoculated in 96-well microplates at a density of 5×10^3 cells per well and incubated at 37°C with 5% CO_2 for 24 hours. Upon conclusion of the incubation phase, the cells were subjected to CS-PN-PF127-Glx wound dressing solutions at

varying final concentrations (0.0625, 0.125, 0.25, 0.5, 1, and 2 mg/mL) for intervals of 0, 24, 48, and 72 hours. Subsequent to the designated time intervals, an MTT solution (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was introduced to the microplates, which were then incubated at 37°C in a 5% CO_2 environment for 3 hours. The solution medium in the wells was subsequently eliminated, and 100 µL of DMSO was introduced to each well to solubilize the formazan crystals. The plates underwent a second incubation for 20 minutes. Upon completion of the incubation, the amounts of formazan crystals in the microplates were quantified, and their optical densities were documented at 570 nm utilizing a microplate reader (Multi Scan Go, Thermo) [29]. Prior to application, the stock solutions of CS-PN-PF127-Glx wound dressings were disinfected using UV radiation for 30 minutes in a chilled atmosphere [30]. Cell viability was quantified as the proportion of viable cells compared to the negative control (cells cultured in DMEM media treated with PBS, designated as 100%). All experiments were performed in duplicate.

2.2.3 The scratch wound studies

Cells were inoculated in 6-well plates at a density of 6×10^5 cells per well and incubated in a humidified environment with 5% CO_2 at 37°C until achieving 90% confluence. Once the desired confluence was attained, scratches were made in the wells utilizing a 200 µL pipette tip. The medium was discarded, and the wells were rinsed thrice with sterile new PBS, which was thereafter eliminated. Fresh DMEM media with CS-PN-PF127-Glx wound dressing solutions at designated final concentrations (0.5, 1, and 2 mg/mL) were administered, and the cells were cultured in a humidified incubator with 5% CO_2 at 37°C. The initiation time of the scratches was designated as 0 hours. Images were captured bi-hourly for a duration of 72 hours. The migration distances were quantified utilizing Image J software, and the wound closure distances were determined using the following equation:

$$Migration\ distance\%\ (\%) = \frac{\text{Scratch distance at initial time} - \text{Scratch distance at the end of the experiment}}{\text{Scratch distance at initial time}} x100$$

2.3. Antimicrobial Tests

The antimicrobial efficacy of the synthesized CS-PN-PF127-Glx materials was evaluated against the microorganisms *Escherichia coli (E. coli,* ATCC25922), *Pseudomonas aeruginosa* (*P. aeruginosa*, ATCC27853), *Staphylococcus aureus* (*S. aureus*, ATCC25923), and *Streptococcus pyogenes* (*S. pyogenes*, ATCC19615). The disk diffusion method was employed to assess the antibacterial properties of the synthesized compounds. Bacterial strains were cultivated in Nutrient Broth (NB) medium at 37°C and 150 rpm in an incubator until achieving a density corresponding to the 0.5 McFarland standard (1.5×10^8 CFU/mL). The density was validated by measuring absorbance at 600 nm with a spectrophotometer, confirming values between 0.8 and 1. From the test culture of microorganisms, 100 µL was extracted and disseminated onto the surface of solid Nutrient Agar (NA) medium. Thereafter, sterile disks were positioned on the agar medium. Suspensions of the synthesized substance at several concentrations (0.5, 1, 2, and 4 mg/disk) were produced in PBS, with 10 µL applied to

each disk. Following the inoculation of the bacteria *E. coli* (ATCC25922), *P. aeruginosa* (ATCC27853), *S. aureus* (ATCC25923), and *S. pyogenes* (ATCC19615) onto the petri dishes, the plates were incubated at 37°C for a duration of 24 hours. After incubation, the sizes of the inhibitory zones were determined in millimeters. Imipenem (10 µg/disk) and erythromycin (15 µg/disk) served as positive controls, whereas PBS functioned as the negative control [31]. All tests were performed in duplicate.

Statistical Analysis

The statistical significance was assessed with the Student's t-test in the Microsoft Office Excel program. Values are presented as mean ± standard deviation, with all trials performed in triplicate ($p \leq 0.05$ vs control; $^{**}p \leq 0.01$ vs control; $^{***}p \leq 0.001$ vs control; $^{****}p \leq 0.0001$).

3. Discussion

FTIR Analysis

The FTIR spectrum of chitosan (CS) displays wide aliphatic N-H and O-H stretching vibrations at 3300 cm^{-1}, with C-H stretching vibrations detected at 2850 and 2910 cm^{-1} (Figure 1). The identified distinctive peaks attributed to residual N-acetyl groups—1630 cm^{-1} (C=O stretching of amide I) and 1310 cm^{-1} (C-N stretching of amide III)—align with the literature [32-34]. The bending peak of primary amine N-H is observed at 1490 cm^{-1}. The C-H and C-O stretching vibrations of Pluronic F127 are observed at 2880 cm^{-1} and 1100 cm^{-1}, respectively, with the C-H stretching vibrations occurring below the N-H and O-H stretching vibration bands of chitosan (Figure 1). The C-O stretching at 1100 cm^{-1} is identified after the functionalization of chitosan with the Ala-Leu peptide nanotube structure and its subsequent conversion into gel form utilizing Pluronic F127 [34, 35]. Following the functionalization of chitosan with Ala-Leu dipeptide nanotubes and cross-linking with 10 µL of glyoxal, the resultant biocompatible structure was converted into gel form with PF127. The FTIR spectrum indicates that glyoxal is undetectable at quantifiable levels, signifying that minimal quantity of glyoxal was utilized in cross-linking (Figure 1).

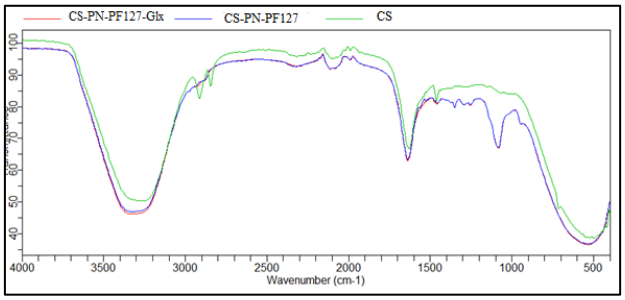


Figure 1. FTIR Spectra of CS, CS-PN-PF127, and CS-PN-PF127-Glx

The FTIR spectra of Ala-Leu dipeptide, before and during its conversion into a nanotube structure in HFIP, revealed peaks indicative of nanotube production in the 1100–1300 cm^{-1}

range (Figure 2). It may be deduced that these distinctive structures were maintained after the functionalization of chitosan with the dipeptide nanotube.

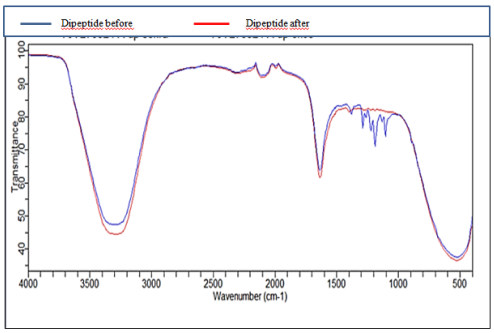
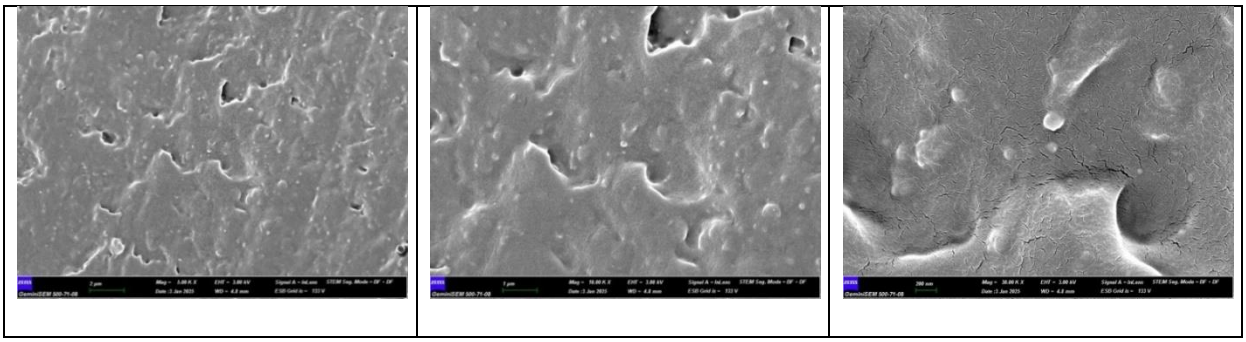


Figure 2. FTIR spectra of Ala-Leu dipeptide before and after its transformation into a nanotube structure in HFIP.

SEM-EDX Analysis

The surface microstructure of Pluronic F127-based hydrogels, formulated with chitosan functionalized by Ala-Leu dipeptide nanotubes, was analyzed via SEM (Figure 3), and their elemental composition was assessed using EDX. Prior to SEM measurement, the gel was deposited as a thin coating on the grid surface and subsequently dried before imaging. The produced functionalized structures exhibited particle sizes between 20 and 220 nm, as measured from pictures captured at various magnifications. The EDX spectrum data (Figure 3) indicates that the chemical composition of the nanostructured particles comprises 62.07% carbon and 37.93% oxygen.



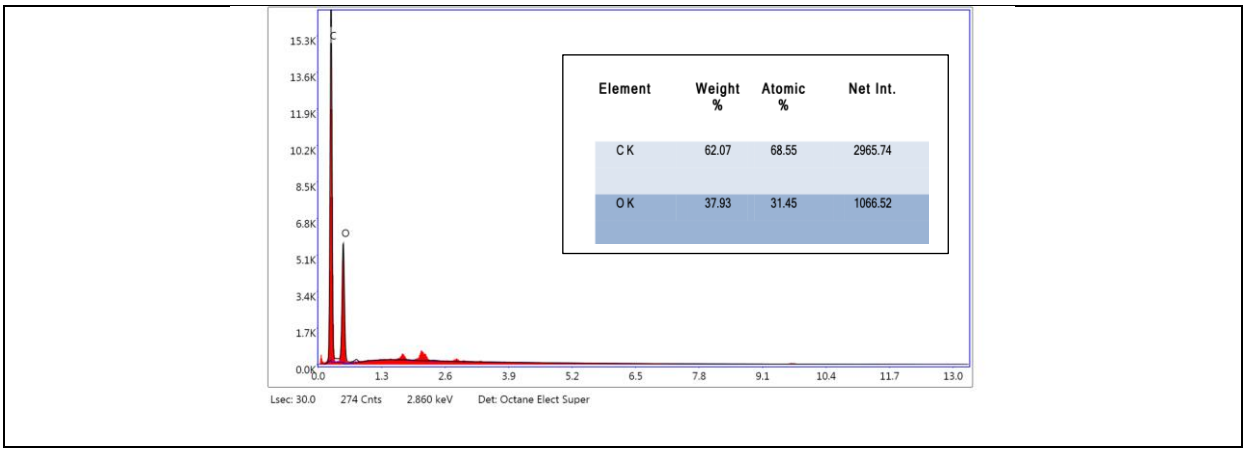


Figure 3. SEM images of the synthesized functionalized structures at different magnification levels and their EDX spectrum.

TGA-DTA Analysis

The thermal properties of the Pluronic F127-based hydrogel, synthesized with chitosan functionalized by Ala-Leu dipeptide nanotubes, were analyzed using TGA-DTA (Figure 4). A mass reduction of approximately 91.1% was noted as a result of water extraction from the structure between 20°C and 112.2°C. After the deterioration of the organic structure between 347–453°C, 1.4% of the total mass persisted.

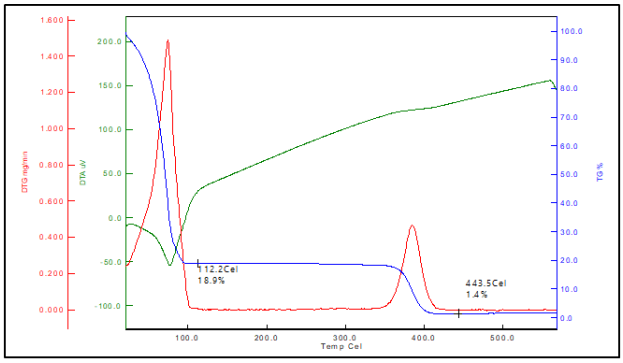


Figure 4. TGA-DTA results of the Pluronic F127-based hydrogel prepared using chitosan functionalized with Ala-Leu dipeptide nanotubes.

Cytotoxicity Studies

The cytotoxicity assays indicated that Pluronic F127-based hydrogels formulated with chitosan functionalized by Ala-Leu dipeptide nanotubes demonstrated no significant cytotoxic effects on the cell line, regardless of exposure to varying final concentrations (0.0625, 0.125, 0.25, 0.5, 1, and 2 mg/mL) over 0, 24, 48, and 72 hours (Figure 5). One significant drawback of peptide nanotubes is their cytotoxicity and processing challenges [36]. Our work demonstrated that the dose- and time-dependent administration of the synthesized material to the HS2 human keratinocyte cell line exhibited no significant cytotoxic effects. This study indicates that, if favorable findings are achieved in future testing phases, the material may be employed as a wound dressing.

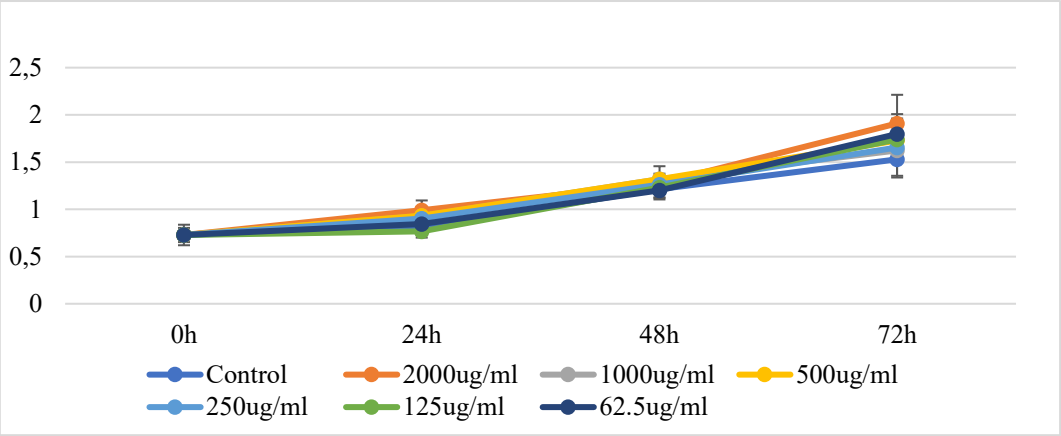


Figure 5. Cytotoxicity results of the Pluronic F127-based hydrogel prepared using chitosan functionalized with Ala-Leu dipeptide nanotubes.

The scratch wound studies

The wound healing test results indicated that Pluronic F127-based hydrogels, formulated with chitosan functionalized by Ala-Leu dipeptide nanotubes at varying final concentrations (2, 1, and 0.5 mg/mL), exhibited no significant enhancement in wound healing in the cell line subjected to 72 hours of exposure. No significant difference in wound healing rates was noted between the treated cell groups and the control group; however, the results indicate that chemical alterations may further augment the material's efficacy as a wound dressing. The lack of harmful effects at all doses underscores the potential of this material as a viable choice for wound dressing applications (Figure 5).

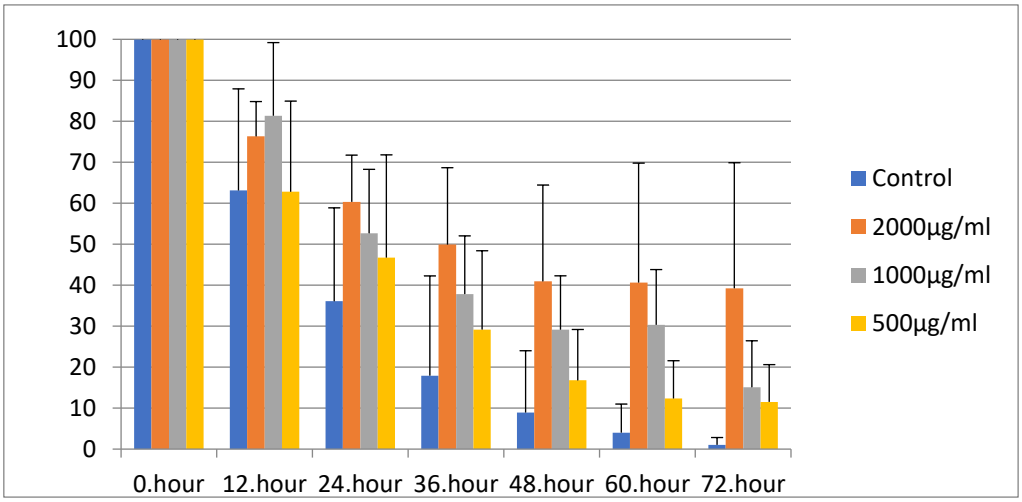


Figure 5 depicts the wound healing results of the synthesized material.

Antimicrobial Activity Studies

Antibacterial susceptibility tests were conducted with *E. coli*, *P. aeruginosa*, *S. aureus*, and *S. pyogenes* to assess the antibacterial efficacy of Pluronic F127-based hydrogels formulated with chitosan functionalized by Ala-Leu dipeptide nanotubes. The results indicated that the

synthesized material CS-PN-PF127-Glx did not yield zone diameters of sufficient significance across various concentration applications (Table 1). The widths of the zones derived from the positive control groups are likewise displayed in Table 1.

Table 1. The values of antimicrobial activities of the synthesized materials

	Microorganisms			
Dose (mg/disc)	***E. coli*[b]**	***P. aeruginosa*[b]**	***S. aureus*[b]**	***S. pyogenes*[c]**
4	N.A	N.A	N.A	N.A
2	N.A	N.A	N.A	N.A
1	N.A	N.A	N.A	N.A
0.5	N.A	N.A	N.A	N.A
P.C.	30±0.25	20±0.8	44±0	25±0,5
N.C.	N.A	N.A	N.A	N.A

a: The results provided are presented as the average and ± standard deviation of three repeated measurements. N.A: Not Activite. P.C: Imipenem and Erythromycin[c] used as Positive Control. N.C: PBS used as Negative Control.

Due to their exceptional biocompatibility and antibacterial properties, chitosan-based hydrogels are extensively utilized in numerous biomedical applications [37]. The antibacterial efficacy of chitosan is contingent upon its molecular weight; its capacity to permeate the cell membrane dictates its action within or outside the cell [38]. It has been proposed that cross-linked chitosan does not impede bacterial proliferation, suggesting that only soluble chitosan is effective in preventing such development [39, 40]. A study indicated that Fluronik F127, formulated at concentrations between 20% and 40%, exhibited no antimicrobial activity against certain gram-positive and gram-negative bacteria, and was recognized as an optimal neutral carrier hydrogel system for the encapsulation of antibiotics and other antimicrobial agents [41].

4. Conclusion

The objective of our research was to create cross-linked composite wound dressings utilizing Pluronic F127-based hydrogels formulated with chitosan functionalized with Ala-Leu dipeptide nanotubes. The findings from the characterization studies of the synthesized material were satisfactorily aligned with the literature in this study. The analysis of the cytotoxicity study data indicated no substantial cytotoxic effects of the substance. The analysis of the wound healing study data indicated that the structure had no impact on cell migration. Data from antimicrobial testing revealed that the substance had no antibacterial action against the microorganisms evaluated. Hydrogels based on Pluronic F127, formulated with chitosan functionalized by Ala-Leu dipeptide nanotubes, can be enhanced with

antimicrobial agents and wound healing promoters to create carrier wound dressings and injectable gels that facilitate wound healing.

Ethics in Publishing

There are no ethical issues regarding the publication of this study.

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