

Antibacterial Bilayered Skin Patches Made of HPMA and Quaternary Poly(4-vinyl pyridine)

İ. Alper İsoğlu¹, Cemre Demirkan², Mine Gül Şeker³, Kadriye Tuzlakoğlu⁴, and Sevil Dinçer İsoğlu^{1*}

¹Bioengineering Department, Abdullah Gül University, Kayseri 38080, Turkey

²Bioengineering Department, Yıldız Technical University, İstanbul 34220, Turkey

³Molecular Biology and Genetics, Gebze Technical University, İzmit 41400, Turkey

⁴Polymer Engineering, Yalova University, Yalova 77000, Turkey

(Received June 8, 2018; Revised August 30, 2018; Accepted September 3, 2018)

Abstract: This study aimed to produce poly(4-vinyl pyridine) and hydroxypropyl methacrylamide (HPMA)-based bilayer wound dressings materials enhancing healing mechanism for the wounds which have self-healing problem and high infection risk. These materials were designed to protect wound from secondary traumas caused microorganism invasion and do not have toxic substance release problem. Synthesis of quaternary poly(4-vinyl pyridine) (poly(Q4-VP)) which is the antibacterial layer of wound dressing material was carried out in two stages. At first stage, poly(4-vinyl pyridine) polymer was synthesized from 4-vinyl pyridine monomer by free radical polymerization. Then, poly(Q4-VP) was synthesized from poly(4-VP) by alkylation reaction with 6-bromocaproic acid. Resulted polymer was structurally characterized by FT-IR. The macroporous spongy structure, as the lower layer of wound dressing material, was prepared by cryogelation of HPMA. Then, the antibacterial polymer was electrospun onto the cryogel structure and bilayered material was obtained. Cryogel structure, fiber morphology and layer integration was examined by SEM. In order to enhance wound healing process, ascorbic acid (vitamin C) was loaded to cryogel layer and release was followed by spectrophotometrically. The antimicrobial properties of the materials were examined against *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*, respectively. According to the results, bilayered, antibacterial and antifungal against *Staphylococcus aureus* and *Candida albicans*, temporary wound dressings which can stimulate wound healing and have high swelling capacity were obtained successfully.

Keywords: Antibacterial wound dressings, Quaternary poly(4-vinyl pyridine), Electrospinning, Cryogel

Introduction

Wound healing is a process consisting of different phases occurs in an ordered fashion. These phases involve coagulation and haemostasis, inflammation, proliferation and maturation. Achieving complete healing largely depends on wound dressing material as well as wound type and its pathological condition [1]. Dressing materials can be designed as permanent or temporary materials depending on the type of the wound. Permanent wound dressing materials are created based on tissue engineering so that material is going to be integrated to the tissue while healing occurs. For some cases, temporary and disposable dressing materials (as skin patches) can be enough for the complete healing process. These skin patches should have similar characteristics with the classical dressing materials in a way that they should be antibacterial, absorb exudate, soft, biocompatible, etc. [2]. Mimicking skin structure can also help to obtain a material that can be effective as a good skin patch or temporary dressing material.

Hydrogels are ideal materials for dry chronic wounds and burns. They retain high amount of water so that they can help granulation tissue and epithelium by providing a moisture environment [1]. They provide permeability to metabolites and ease of application and removal due to soft and elastic property. Some examples of hydrogel-based dressings are IntrasiteTM, Nu-gelTM and AquaformTM.

Cryogels that are produced by the polymerization in frozen state, where ice crystals behave as porogens, represent an important type of hydrogels. They provide highly macroporous structure, high water absorption capacity and good mechanical strength [3]. Therefore, they can be very useful for dressing material applications.

Due to susceptibility of wounded area to bacterial attacks, there is high possibility for an infection, which may cause a delay in wound healing process. In order to reduce this, several antimicrobial compounds or substances can be incorporated to the dressing material. Loading silver ions to the dressing material is a widely applied strategy. However, risk of toxic metal ion release has diminished its use for the antibacterial material preparation [4]. The antimicrobial polymers containing quaternary ammonium salts are probably the most widely used and studied ones as discussed in Xue *et al.*'s study [5]. Using these polymers may be a good option to obtain antimicrobial materials with no toxic release. As proposed in Xue *et al.*'s study, these polymers show their activity by penetration into the cell wall and then cause destructive interaction with the cytoplasmic membrane. It is followed by the leakage of intracellular components and consequent cell death. One way to prepare polymers with pendant quaternary ammonium groups is to prepare polymerizable monomers which are subsequently polymerized or copolymerized with other monomers [5]. Hence, poly(4-vinyl pyridine) (poly(4-VP)) can be chemically modified [6] and gains antimicrobial property. Accordingly, many studies

*Corresponding author: sevil.dincer@agu.edu.tr

have been reported the antimicrobial effects of quaternized poly(4-vinyl pyridine) by modifying with different agents [7].

Designing wound dressing materials with two layers is a very common strategy to mimic skin structure for achieving more efficient materials. Several bilayered skin substitutes have been prepared which are already commercially available. For example, Biobrane is composed of an silicone membrane and a knitted nylon mesh and used as epidermal substitute. Integra contains a porous collagen matrix with chondroitin-6-sulphate and a silicone sheet designed as an outermost layer [2,8]. However, there is still a need for more efficient dressing materials with sophisticated structures. In order to obtain such structures, different fabrication techniques such as cryogelation, freeze drying and electrospinning can be combined [9-12].

Electrospinning is a simple technique leading to produce nanofibrous membranes with a controlled porosity, diameter and thickness. Several polymers such as polycaprolactone (PCL), polylactic acid (PLA), polyglycolic acid (PGA), PLGA, and their copolymers, and natural polymers such as collagen, gelatin, chitosan have been successfully fabricated by electrospinning to produce fibers with diameters ranging from 3 nm to 5 nm [11-13].

This study aims to produce a temporary wound dressing material consisting of an upper antibacterial layer and a spongy basement part. HPMA-based spongy cryogel was first prepared and then chemically modified poly(4-VP) was electrospun onto the sponge basement layer. Then, vitamin C was also loaded to the cryogel part for the enhancement of wound healing. After all characterizations, final materials showed promising effectiveness as a temporary dressing material.

Experimental

Materials

Sodium carbonate (99.5 %, Sigma-Aldrich), amino-2-propanol (93 %, Aldrich), dichloromethane (Carlo Erba), sodium sulphate anhydrous (Carlo Erba), methacryloyl chloride (Fluka), acetone (Riedel-de-Haën), methanol (Fluka), ammonium persulfate (APS), (N,N,N',N'-tetramethylethylenediamine) TEMED, methylenebisacrylamide (MBA), vitamin C (Sigma-Aldrich), 4-vinyl pyridine (95 %, Aldrich), diethylether (99.5 %, Sigma-Aldrich), dimethylformamide (DMF, 99.8 %, Carlo Erba) were used as received. The Gram-positive bacteria *Staphylococcus aureus* ATCC 25923, Gram-negative bacteria *Escherichia coli* ATCC 8739 and fungi *Candida albicans* ATCC 10231 were used for the antibacterial assay and antifungal assay, respectively. All other reagents and solvents are of analytical grade and used as received.

Preparation of HPMA Monomer

HPMA monomer was prepared according to the procedure

given in literature [14]. Briefly, 0.34 mol anhydrous sodium carbonate was added to 0.30 mol amino-2-propanol in 85 ml dichloromethane and the temperature was brought to 0 °C. Then, 0.29 mol methacryloyl chloride in 40 ml dichloromethane was added to the mixture in dropwise manner. After that, the mixture was stirred for 30 min at 15 °C and anhydrous sodium sulphate was added. The mixture was filtrated and HPMA was obtained by crystallization from dichloromethane at -20 °C and purified by recrystallization from acetone.

Preparation of Wound Dressing Material

Base Layer: HPMA Cryogel Sponges

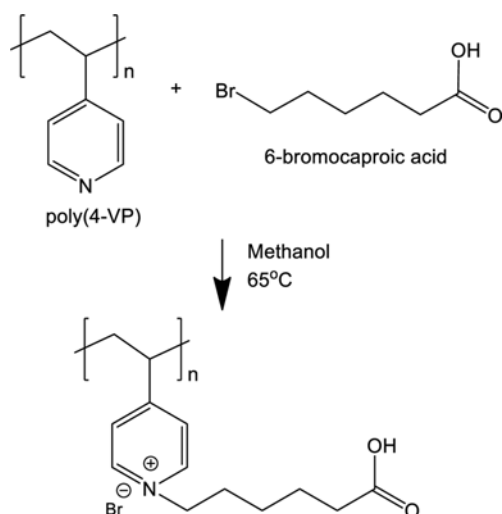
The first layer of dressing materials will be made of macroporous HPMA cryogel. First, MBA and HPMA were dissolved in distilled water by sonication and then HPMA solution was poured into MBA solution. After addition of APS and TEMED in certain quantities, mixtures were mixed thoroughly by vortex. A typical recipe is given as: 0.6 g HPMA, 0.1 g MBA, 0.024 g APS, 10 µl TEMED. In order to obtain cylindrical-shape cryogels, 2 ml-volume plastic moulds were used. Mixtures were transferred into these moulds and then immersed into liquid nitrogen immediately. Following this, they were placed to freezer at -20 °C overnight. After 2 days, samples were washed in distilled water for overnight. Then, cylindrical cryogels were cut into disc-shape pieces and dried. These cryogels were analysed by FTIR in structural characteristics.

Preparation of Upper Layer: Antimicrobial Poly(4-vinyl pyridine)

As indicated in introduction part, a wound dressing should act as a barrier towards bacterial attacks. We have designed an antibacterial top layer composed of a quaternized and electrospun poly(4-vinyl pyridine). By this way, material can be able to prevent bacterial attack and growth with the antibacterial effect of polymer as well as its nanofibrous network structure.

To achieve this, we first synthesized poly(4-vinylpyridine) by radical polymerization in presence of AIBN as initiator and DMF as solvent. Monomer/initiator ratio was held as 500:1 and mixture was purged with nitrogen for 30 min before polymerization. The reaction was conducted at 65 °C for about 24 hours. Following that, product was purified by precipitation from toluene 3 times, and kept in vacuum oven for the modification step.

Following the synthesis of poly(4-vinyl pyridine), quaternization was performed using 6-boromo caproic acid as given in literature by various studies [15,16]. To do this, poly(4-VP) and alkylation agent (1:2) were dissolved in methanol and mixture was purged with nitrogen gas for 30 min (Scheme 1). Reaction mixture was refluxed at 65 °C for 4 and 8 days. After that the product was purified by precipitation from diethylether and quaternized polymer (poly(Q4-VP)) was obtained successfully. FTIR was used for the confirmation of quaternization process. Quaternization



Scheme 1. Quaternization reaction of poly(4-VP).

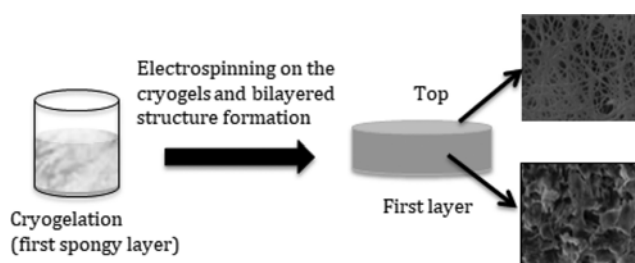
degree was determined by FTIR spectrum and the comparison of the transmittance values of the 4th and 8th day samples with the unquaternized poly(4-vinyl pyridine).

Preparation of Bilayered Patches

With the aim of mimicking epidermal layer, HPMA sponges were coated with antibacterial poly(Q4-VP) by electrospinning. To do this, poly(Q4-VP) was dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) in about 20% concentration. This solution was transferred into a 1.0 ml syringe mounted in a syringe pump (New Era Pump Systems, USA). Then, the syringe was capped with a needle (22 gauge end). The distance between needle tip and collector was 12 cm, and the flow rate was 3 ml/h. HPMA cryogel discs were mounted on a grounded target where poly(Q4-VP) nanofibers were collected on. A positive voltage of 10 kV was applied. Only 8 days quaternized polymer was used as antibacterial part here because polymer did not reveal enough antibacterial characteristics after 4 days modification. Scheme 2 illustrates the preparation and structure of bilayered patch.

Characterization of Bilayered Patches

All materials were characterized morphologically by SEM (Zeiss Leo440, Germany). Fiber size and intensity, cryogel



Scheme 2. Illustration of bilayered material formation.

structure, porosity and integration of two membranes were evaluated. Moreover, water uptake capacity of the cryogel was evaluated by swelling ratio.

For swelling test, disc-shaped, skin patches were weighed and soaked into the phosphate buffer solution for 15 min at 37 °C. The excess solution on the wet materials was removed by filter paper and the samples were weighed again. The measurements were repeated in every 15 min until reach at the equilibrium. The swelling ratio of the patches was calculated by following equation;

$$\text{Swelling ratio (\%)} = \frac{m(w) - m(i)}{m(i)} \times 100$$

where $m(w)$: weight of swollen gel, $m(i)$: weight of dry gel.

Vitamin C Loading

Due to wound healing enhancing effect of vitamin C, it was loaded to the base part of the skin patches, which has intimate contact with the skin. For the loading process, absorption method was applied. Briefly, 50 mg vitamin C was dissolved in 500 μ l PBS and then, 100 μ l of this solution were added to the patches for the absorption, dried at room temperature for overnight. For the release study, the patches were immersed into phosphate-buffered saline (PBS, 0.01 M, pH 7.2-7.4) and were then shaken in an incubator for 3 days at 37 °C. To evaluate the cumulative release of vitamin C, 1 ml samples were extracted at different time intervals, and fresh buffer solution was added to the medium. The amount of vitamin C in the samples was analysed by UV-spectrophotometer (Schimadzu, Japan) at 266 nm.

Antimicrobial Activity Assays

The antimicrobial properties of the bilayered scaffolds were screened by zone inhibition tests by agar well method on Mueller Hinton Agar (MHA) [17]. For antimicrobial screening *S. aureus*, *E. coli* and *C. albicans* were used as control strains. As described above, skin patches had an electrospun upper layer, which will be contact to the air so that it is designed as a barrier to microorganisms.

S. aureus, *E. coli* and *C. albicans* were inoculated in Mueller Hinton Broth (MHB) from stock microorganism cultures. They were adjusted to 0.5 McFarland [about 10⁸ colony forming unit (cfu)/ml for *E. coli* and *S. aureus*, 10⁵ cfu/ml for *C. albicans*] in sterile phosphate buffer saline (PBS 0,85%) by using nephelometer (Crystal Spec™, Becton Dickinson, USA). They were inoculated on MHA with sterile swab. Incubation period was kept for bacteria at 30 °C for 24 h and for fungi at 37 °C for 24-48 h. Inhibition zones was determined by measuring (mm) clear area around the wells on each dish. Non-quaternized poly(4-VP) was used as negative control. Each microorganism was studied double. As positive controls, Chloramphenicol disk (30 mg) (HIMEDIA) Nystatin (10000 u/ml) were used against

bacteria and fungi, respectively.

Results and Discussion

Structural Analysis

As described before, skin patch has two layers; one is HPMA sponge prepared by cryogelation. According to FTIR spectrum (Figure 1), O-H and C=O stretching were appeared at 3293 cm^{-1} and 1738 cm^{-1} , respectively [18]. And the peak at 1100 cm^{-1} is attributed to C-O-C asymmetric stretching.

Electrospun upper layer of skin patch is prepared by quaternized poly(4-vinyl pyridine). Before material preparation, first poly(4-VP) was synthesized by random radical polymerization of 4-VP which was characterized by FTIR spectrum (Figure 2). Pyridine ring stretching is appeared at 1597 cm^{-1} , while quaternization is attributed to the peaks observed at 3400 cm^{-1} , 1639 cm^{-1} ve 1716 cm^{-1} [19].

The intensity of the peak at 1597 cm^{-1} was decreased by quaternization time as seen in Figure 2. The peak at 3400 cm^{-1} confirmed that caproic acid was incorporated to the structure of polymer resulting quaternization of pyridine unit. The changes in these two peaks approved the effect of

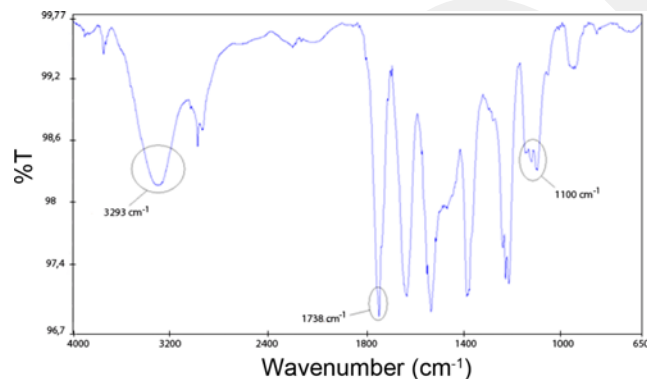


Figure 1. FTIR spectrum of HPMA-based cryogel sponge.

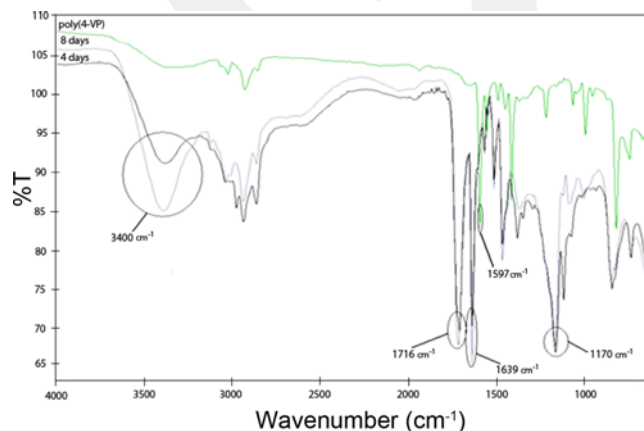


Figure 2. FTIR spectra of poly(4-VP) and 4 and 8 days quaternized polymers.

quaternization time on the reaction.

Based on the transmittance/absorbance values for the peaks at 1597 and 1639 cm^{-1} (for pyridine ring stretching and quaternized one, respectively), we calculated the quaternization degree. According to Figure 2, we compared the transmittance values of the 4th and 8th day samples with the unquaternized poly(4-vinyl pyridine) and estimated 55 % and 62 % degree of quaternization for 4th and 8th days.

Morphological Analysis

An ideal wound dressing material should have a porous structure which has interconnectivity throughout the materials in order to reach high exudate absorption. In addition, the outer layer of the material should possess less porosity compared to lower layer so that it can be a barrier towards bacterial attack. This is the motivation of our study in which a double-layered skin patch was designed to fulfil the abovementioned requirements.

As mentioned above, one of the layers is prepared by cryogelation in order to obtain a sponge-like material that mimic the dermal part of the skin. The morphological structure of this material was examined by SEM as given in Figure 3. Here, it was confirmed that these spongy material has a highly porous structure, which are connected to each other and separated by thin polymeric walls. Pore diameters are measured between $4\text{--}35\text{ }\mu\text{m}$. Initial polymer concentration

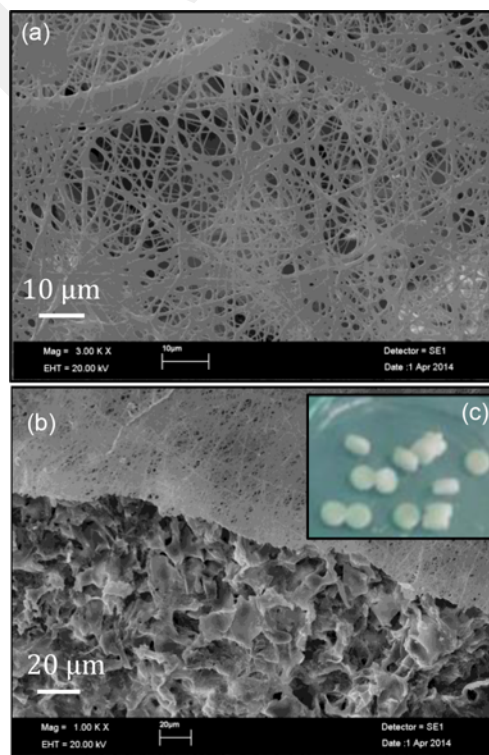


Figure 3. SEM image of (a) electrospun poly(Q4-VP) membrane, (b) PHPMA cryogel/electrospun poly(Q4-VP) bilayered structure, and (c) shows HPMA discs.

and temperature are two main criteria in the porosity and pore dimensions. Lower temperatures cause formation of smaller solvent crystals and hence, smaller pores are formed [20]. Uygun and colleagues [21] produced PHEMA cryogels using MBA and TEMED in the presence of APS as initiator. These materials had pores between 10-20 μm in diameter. They reported that poly(2-hydroxy methacrylate-co-1,5-naphthalene bismaleimide) [poly(HEMA-co-NBMI)] cryogels have porosity between 10-100 μm . In Alkan *et al.*'s study, supermacroporous cryogels having pore sizes between 10-200 μm were produced [22]. Based on these findings from literature, cryogels produced in our study are in a good consistency with the ones prepared similar technique.

Nanofibrous membranes produced by electrospinning allow an easy exudate remove from the wound area by the courtesy of high surface area, porosity and good filtration capacity. Besides, they can provide a moistened environment as well as enhanced healing because of good oxygen exchange and controlled water loss.

Porosity and surface wettability are directly proportional to fiber diameter that lower porosity and wettability are achieved once fiber diameter gets smaller [23]. Khil and [24] colleagues prepared polyurethane-based nanofiber membranes as wound dressing material. They obtained fibers in 250-300 nm diameter from 25 % polymer solution in THF:DMF mixture (70:30) by applying 18 kV voltage and 18 cm distance from collector. Chen and colleagues [25] used chitosan and collagen mixture to get composite membranes by applying 30 kV voltage, 25 cm distance, 0,5 ml/h flow rate to the solution prepared in 0.5 M acetic acid (1:3), and achieved fibers with 134-398 nm in diameter. Rujitanaroj and colleagues [26] prepared gelatine dressing material using acetic acid (70 %) as solvent with a 22 % polymer concentration and obtained 230-280 nm fibers. Then, these membranes were cross-linked by glutaraldehyde and this process caused higher fiber diameter (380-410 nm).

In our study, we have obtained double-layered dressing material consisting PHPMA cryogel and poly(Q4-VP) membrane on top of it, as seen in Figure 3. No bead formation was occurred during fiber production proving optimal experimental conditions were achieved. Fiber thickness ranged between 260 nm-6 μm with an abundance of 0,8-1 μm diameter fibers. These characteristics are in consistence with the requirements for an ideal wound dressing material. In a similar study, Dong and colleagues prepared 290 nm in diameter fibers of poly(4-VP) in DMF with a 350 mg/ml concentration [27]. As an example study for a double-layered material, Shemesh ve Zilberman [28] reported the production of PDLGA/collagen materials by freeze drying. Uzunalan *et al.* developed a collagen-based double-layered material by mimicking dermal and epidermal parts of the skin using freeze drying and electrospinning for the preparation of first and upper layers, respectively. In this study, they added silver nanoparticles to the upper layer for

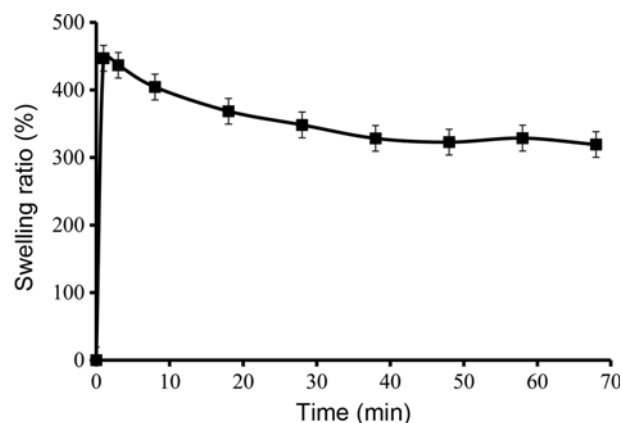


Figure 4. Change in swelling ratio of the cryogel part by time.

antibacterial activity while the spongy substitute consisting fibrinogen for the enhancing of wound healing [12].

In our study, prepared material is designed as a temporary skin patch. Electrospun poly(Q4-VP) layer possessed antibacterial characteristics because of quaternary ammonium groups through the polymer chain as well as nanofibrous structure. Besides, lower spongy part contains vitamin C in order to induce wound healing process in that particular wound area.

Swelling Behavior

One of the important characteristics of wound dressing materials is the capacity to absorb exudate in wounded area. Absorption capacity of the material depends on the composition, porosity as well as external effects. Swelling behavior was measured in PBS represented in Figure 4. As seen here, double-layered spongy material showed very high and rapid swelling (400 %) reaching to the equilibrium (320 %) after about 1 hour, due to its cryogel structure. Mu *et al.* obtained a rapid equilibrium swelling (318 %) reaching in 10 minutes with a collagen-based cryogel crosslinked by dialdehyde starch [29]. Bereli *et al.* produced anti-hIgG immobilized PHEMA-based cryogels showing 750 % swelling [30]. In Uygun *et al.*'s study, poly(HEMA-co-GMA) cryogels with a swelling degree 554 % were obtained [21]. Cryogels, which are the materials with a very high swelling degree, can have ability to absorb exudate and maintain a moistened environment. Due to this fact, spongy patches in our study show a great promise as a temporary wound dressing material.

Ascorbic Acid Release

It has been reported that ascorbic acid (vitamin C) has an important role in wound healing process. In addition, lack of ascorbic acid in tissues may cause a reduction in tissue regeneration capacity in a way that an inadequate collagen synthesis and fibroblast proliferation resulting lower healing activity [31]. By this motivation, ascorbic acid was loaded to the base layer, which will be in contact to the wound of the

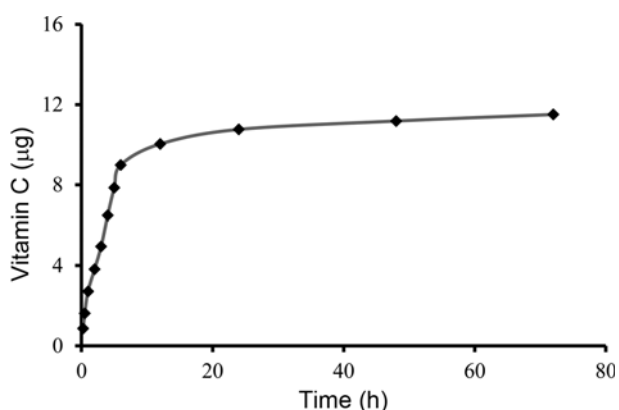


Figure 5. Cumulative release of ascorbic acid (vitamin C) by time.

patch. The release of ascorbic acid was followed by spectrophotometrically. Aliquots of samples were taken in every one hour and analyzed for ascorbic acid content, using a UV-Vis spectrophotometer at 520 nm. After each sampling, an equal volume of phosphate buffer solution was added to the medium in order to keep sink conditions. The experiment was done in triplicate and mean value was calculated. According to the Figure 5, an initial burst effect was observed and then the release was slower in further time intervals. Ascorbic acid release was slower after 6th hours reaching to the constant release after 48 hours. Ascorbic acid loading was calculated about 13 µg to the selected disc-shaped cryogel sample. Approximately 94 % release was achieved at the end of the 24th hours. The release kinetics of ascorbic acid exhibited burst release in first 10 hours, followed by a drug elution rate that decreased with time and lasted for at least 3 days. We expect the released ascorbic acid within this time interval will enhance fibroblast activation and collagen synthesis.

Yang *et al.* obtained similar release behaviour with ascorbic acid [32].

Antibacterial and Antifungal Characterizations

The antimicrobial property of the skin patches is necessary to be proved in order to be used for wound healing

applications as the presence of pathogenic microbes like *S. aureus* and *E. coli* and fungi like *C. albicans* that are usually seen in wound infections or burns. Table 1 show antibacterial and antifungal effects of quaternary 4-VP layer of the skin patches on *E. coli*, *S. aureus* and *C. albicans*, respectively. First, no antibacterial or antifungal activity was observed for poly(4-VP) which is the original form of the polymer before quaternization process, as expected (number 1). And all polymers have no any antimicrobial effect against to *E. coli*. Poly(Q4-VP) with 8-hours quaternization exhibited both antibacterial and antifungal activity with 22 and 23 mm zone inhibition (number 2), respectively. Reduced quaternization time (4 hours) revealed no zone inhibition meaning no effect on all control microorganisms. Number 4 shows our control which contains distilled water only (Figure 6 and 7).

Kawabata and Nishiguchi [33] reported that poly(Q4-VP)s quaternized with benzyl bromide showed antibacterial activity towards Gram positive bacteria such as *A. atrocyaneus*, *B. subtilis*, *S. aureus* and some Gram negative bacteria such as *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Serratia marcescens*. They also indicated that the antibacterial effect depended on molecular weight of the polymer. Krishnan *et al.* quaternized polystyrene-b-poly(4-VP) with 6-perfluorooctyl-1-bromohexane and observed antibacterial effect to *S. aureus* comparing to their non-fluorinated analogs [34]. Tiller *et al.* [35]

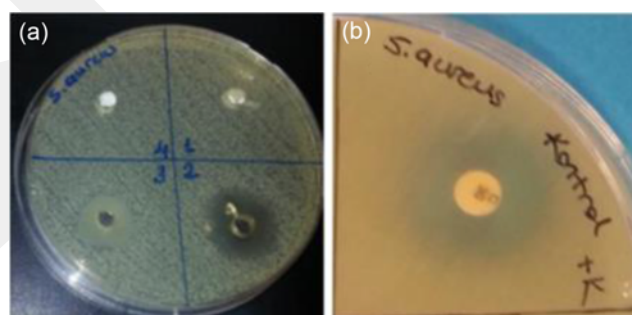


Figure 6. Antibacterial test of quaternary part (a) 1:poly (4-VP), 2:8 days quaternized poly (Q4-VP), 3:4 days poly (Q4-VP), 4:(-) control dH₂O and (b) positive control chloramphenicol 30 µg.

Table 1. Antibacterial and antifungal effects of quaternary 4-VP layer of the skin patches

Sample no.	Skin patches polymers	Inhibition zone diameter (mm)		
		<i>E. coli</i> ATCC 8739	<i>S. aureus</i> ATCC 29213	<i>C. albicans</i> ATCC 10231
1	Poly(4-VP)	-	-	-
2	Poly(Q4-VP) 8 days quaternized	-	22	23
3	Poly(Q4-VP) 4 days quaternized	-	-	-
4	Negative control dH ₂ O	-	-	-
5	Positive control chloramphenicol	27	30	-
6	Positive control nystatin	-	-	30

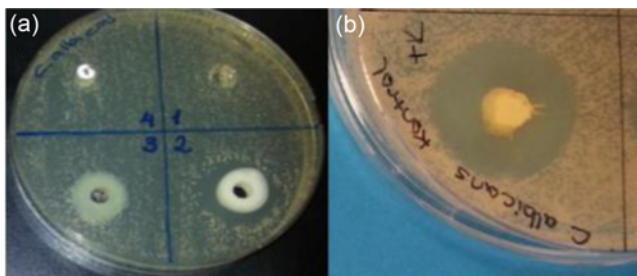


Figure 7. Antifungal test of quaternary part (a) 1: poly (4-VP), 2: 8 days quaternized poly (Q4-VP), 3: 4 days poly (Q4-VP), 4: (-) control dH₂O and (b) positive control Nystatin (10000 u/ml).

investigated the antibacterial effect of quaternized poly(Q4-VP)s by different linear alkyl bromides and observed 1-boromohexane as the most effective one. They also showed antibacterial effect towards *S. aureus*, *S. epidermidis*, *P. aeruginosa* and *E. coli*.

According to our study, 8-days long quaternization process provided antibacterial and antifungal characteristics to the polymer, which is going to be the upper layer of skin patch, while 4 days quaternization revealed almost no antibacterial and antifungal effect. By using poly(Q4-VP), the risk of any antibacterial substance such as silver ions will be prevented.

Conclusion

In this study, a novel double-layered wound dressing material containing vitamin C and having self-antimicrobial characteristics have been developed.

Temporary patches were prepared by using two methods together: electrospinning for the top layer, cryogelation for the base (lower) layer. Upper layer was electrospun using a chemically modified poly(4-VP) which is antimicrobial, whereas spongy and micron-size porous base part was prepared by crosslinking at cryogelation conditions. In order to enhance wound-healing characteristics of the lower part, vitamin C was loaded and 94 % release was achieved at the end of the 24th hours. Swelling ability of the patches were higher than 300 % with a very fast response. In addition, poly(Q4-VP) with 8-hours quaternization (62 % quaternization degree) exhibited both antibacterial and antifungal activity with 22 and 23 mm zone inhibition, respectively. According to the results, skin tissue patches with a great potential application as wound dressing material were obtained successfully.

References

1. S. Dhivya, V. V. Padma, and E. Santhini, *ioMedicine*, **5**, 4 (2015).
2. S. P. Zhong, Y. Z. Zhang, and C. T. Lim, *Wiley*

3. V. I. Lozinsky, I. Y. Galayev, F. M. Pileva, I. N. Savina, H. Junqvist, and B. Mattiasson, *Trends in Biotechnol.*, **21**, 10 (2003).
4. I. Chopra, *J. Antimicrob. Chemother.*, **59**, 587 (2007).
5. Y. Xue, H. Xiao, and Y. Zhang, *Int. J. Mol. Sci.*, **16**, 3626 (2015).
6. P. Chowdhury, S. K. Saha, and S. P. Bayen, *J. Macromol. Sci., Part A*, **50**, 976 (2013).
7. M. Arslan, D. Saraydın, Y. Öztıp, and N. Şahiner, *Polym.-Plast. Technol. Eng.*, **56**, 12 (2017).
8. F. Groeber, M. Holeiter, M. Hampel, S. Hinderer, Schenke, and K. Layland, *Adv. Drug Deliv. Rev.*, **63**, 352 (2011).
9. H. Schoof, J. Apel, I. Heschel, and G. Rau, *J. Biomed. Mater. Res.*, **58**, 352 (2001).
10. B. Subia, J. Kundu, and S. C. Kundu in "Biomaterial Scaffold Fabrication Techniques for Potential Tissue Engineering Applications" (D. Eberli Ed.), Vol. 12, pp.141-157, InTech Open, 2010.
11. V. J. Reddy, S. Radhakrishnan, R. Ravichandra, S. Mukherje, R. Balamurugan, S. Sundarrajan, and S. Ramakrishna, *Wound Repair and Regeneration*, **21**, 1 (2013).
12. G. Uzunalan, M. T. Ozturk, S. Dincer, and K. Tuzlakoglu, *J. Compos. Biodegr. Polym.*, **1**, 8 (2013).
13. E. Pişkin, N. Bölgen, S. Eğri, and İ. A. İşoğlu, *Nanomedicine*, **2**, 441 (2007).
14. J. Kopeček and P. Kopečková, *Adv. Drug Delivery Rev.*, **62**, 122 (2010).
15. F. X. Hu, K. G. Neoh, L. Cen, and E. T. Kang, *Biotechnol. Bioeng.*, **89**, 474 (2004).
16. E. S. Park, H. S. Kim, M. N. Kim, and J. S. Yoon, *Eur. Polym. J.*, **40**, 2819 (2004).
17. C. Valgas, S. M. De Souza, E. F. Smania, and A. Smania, *Braz. J. Microbiol.*, **38**, 369 (2007).
18. A. M. Pana, G. Bandur, L. M. Rusnac, and R. Halmagean, *Chem. Bull.*, **55**, 1 (2010).
19. P. Mondal, S. K. Saha, and P. Chowdhury, *J. Appl. Polym. Sci.*, **127**, 5045 (2013).
20. V. Lozinsky, F. Plieva, I. Galayev, and B. Mattiasson, *Bioseparation*, **10**, 163 (2002).
21. M. Uygun, R. H. Şenay, N. Avcıbaşı, and S. Akgöl, *Appl. Biochem. Biotechnol.*, **172**, 1574 (2014).
22. H. Alkan, N. Bereli, Z. Baysal, and A. Denizli, *Biochem. Eng. J.*, **45**, 201 (2009).
23. S. G. Kumbar, P. S. Nukavarapu, R. James, L. S. Nair, and C. T. Laurencin, *Biomaterials*, **29**, 4100 (2008).
24. M. S. Khil, D. I. Cha, H. Y. Kim, I. S. Kim, and N. Bhattarai, *J. Biomed. Mater. Res. Pt. B Appl. Biomater.*, **67B**, 675 (2003).
25. J. P. Chen, G. Y. Chang, and J. K. Chen, *Colloids and Surfaces*, **313**, 183 (2008).
26. P. Rujitanaroj, N. Pimpha, and P. Supaphol, *Polymer*, **49**, 4723 (2008).

27. H. Dong, E. Fey, and A. Gandelman, and W. E. Jones Jr., *Chem. Mater.*, **18**, 2008 (2006).
28. M. Shemesh and M. Zilberman, *Acta Biomaterialia*, **10**, 1380 (2014).
29. C. Mu, F. Liu, Q. Cheng, H. Li, B. Wu, G. Zhan, and W. Lin, *Macromol. Mater. Eng.*, **295**, 100 (2010).
30. N. Bereli, G. Ertürk, M. A. Tümer, R. Say, and A. Denizli, *Biomed. Chromatogr.*, **27**, 599 (2012).
31. S. Guo and L. A. DiPietro, *J. Dent. Res.*, **89**, 219 (2010).
32. S. Yang, W. Liu, C. Liu, W. Liu, G. Tong, H. Zheng, and W. Zhou, *J. Dispersion Sci. Technol.*, **33**, 1608 (2012).
33. N. Kawabata and M. Nishiguchi, *Appl. Environ. Microbiol.*, **54**, 2532 (1988).
34. S. Krishnan, R. J. Ward, A. Hexemer, K. E. Sohn, K. L. Lee, E. R. Angert, D. A. Fischer, E. J. Kramer, and C. K. Ober, *Langmuir*, **22**, 11255 (2006).
35. J. C. Tiller, K. Lewis, and A. M. Klibanov, *Proc. Natl. Acad. Sci.*, **98**, 5981 (2001).