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Hypericum perforatum Releasing From Silver Sulphadiozine Loaded Sodium Alginate Membranes

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Abstract

This study presents that the controlled release of *Hypericum perforatum* (HP) from biofilm formation of sodium alginate (NaAlg). Its oil was incorporated in sodium alginate biofilm as a potential therapeutic agent. HP belongs from the family of Hypericaceae. HP is traditionally used for wound healing in Turkey and has medical importance. In addition, bacterial resistance of HP was investigated for against *Staphylococcus aureus*. Silver Sulfadiazine (SSD) was added in different concentration (0.5-2.0 w/v) to prepare the antibacterial biofilms. The addition of antibiotics and HP into the biofilm will ensure the delivery of all the necessary substances locally in the wound healing process. Biofilms were prepared by solvent casting method in different oil concentration (0.25-2.0 % v/v). The surface properties of biofilms were investigated by using Scanning Electron Microscopy (SEM), drug release profiles were studied at pH 5.5 and bacterial resistance of HP was investigated for against *Staphylococcus aureus*. As a result of our study, the new generation hydrogel wound dressing will, heal wounds effectively and quickly especially protecting the burns from microorganisms.

1. Introduction

Hydrogels, which are used more and more in recent years, are used as ideal dressing material. Hydrogels are hydrophilic polymers with a three-dimensional network structure containing 90-95% water. The most important advantage of hydrogel dressings is that they have high absorption capacity, do not stick to the wound surface, can be easily cleaned from the surface and take shape easily. Today, drug diffusion can be easily achieved on the wound surface with hydrogel dressings. However, hydrogels tend to poorly absorb biological components. Therefore; they are not good barriers to bacteria, so a second cover may be required for protection. Fibroblastic activity decreases in infected wounds. In order to get rid of these disadvantages, antibiotics have been added in the newly developed wound dressing materials, along with the materials that protect the wound from microorganisms during healing. A wound dressing material have adequate mechanical properties and remove toxic components from wound surface (Thu,, Zulfakar, Ng 2012; Paul, Sharma 2004; Pawar, Tetteh, Boateng, 2013; Jayakumar, Prabaharan, Kumar Sudheesh, Nair, Tamura, 2011).

In recent years, antibiotic use studies are also available in combination with hydrogels. In the study of Aoyagi et al (Aoyagi, Onishi, Machida 2007) Minocycline antibiotic was added to chitosan-polyurethane film dressings and showed beneficial effects in some burn wounds. In the study of Kim and coauthor (Kim, Choi, Park, Kim, Jin, Chang, Li, Hwang, Woo, Kim, Lyoo, Yong, Choi 2008) Clindamycin was loaded on hydrogels consisting of polyvinyl alcohol and sodium alginate and successful results were obtained. In our study; SSD antibiotic was used in combination with the hydrogel cover. SSD is one of the most commonly used prophylactic topical antibiotics in the treatment of burns and wounds. Silver ions create structural changes in the cell membrane of many microorganisms, disrupt the structure and prevent contamination.

Hypericum perforatum plant, which is abundant in our country, is a plant with proven effectiveness and safety and has been used for the treatment of injuries and burns for years. (Güneş, Tıhmınoğlu, 2017; Saddiqe, Naeem, Maimoona, 2010). It is widely studied both its antidepresant activity and its antioxidant, anticancer and anti-inflammatory activities (Avato, Raffo,Guglielmi, Vitali & Rosato, 2004; Raziq, Saeed, Shahis, Muhammad, Khan & Gul, 2016; Khan, Khan, Subhan, Gilani, 2010).

NaAlg has a biocompatible, nontoxic, biodegradable, non-immunogenic structure due to gluronic acid and manuronic acid (Adoor, Prathab, Manjeshwar, Aminabhavi, 2007; Rassu, Salis, Porcu, Giunchedi, Roldo, Gavini, 2016; Noppakundilograt, Piboon, Graisuwan, Nuisin, Kiatkamjornwong, 2015). The prepared hydrogel films; It will provide benefits such as protecting the wound from physical effects, allowing oxygen inlet and outlet, and creating a moist healing environment that needs the wound, which is the most important feature of hydrogels. In addition, SSD, an antibiotic, was used in biofilms prepared.

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2. Material and Method

2.1. Materials

NaAlg (medium viscosity), SSD and Tween-80 were supplied from Sigma Chemical Co (Louis, USA). $CaCl_2$ was supplied by Merck (Darmstadt, Germany). HP was provided from local pharmacy in Turkey (Zade Vital).

2.2. Methods

2.2.1. Preparation of NaAlg based membranes

Membranes were prepared by casting method. Briefly, 1.5 g of NaAlg was dissolved in 100 mL deionized water. HP and SSD were dispersed into the NaAlg solution in different ratios and then placed onto petri dishes. HP and SSD were added in different ratio as given in the Table 1. Tween-80 was added as the dispersing phase. Solvent was evaporated at 40 ^{0}C to form the membrane. The dried membrane was crosslinked with calcium chloride (5% w/v) for 24 h.

Table 1: Formulation codes of the prepared NaAlg membranes (crosslinking concentration: 5 % w/v)

Formulation	HP	SSD
F0	-	-
F1	% 0.25 (v/v)	-
F2	% 1.0 (v/v)	-
F3	% 1.5 (v/v)	-
F4	-	%0.5 (w/v)
F5	-	%1.0 (w/v)
F6	-	%2.0 (w/v)

The morphology and surface structure of the both NaAlg and SSD and HP added NaAlg membranes were investigated using the Quanta 4000 F Field Emission SEM.

2.2.2. In vitro release study

In vitro drug release from the membranes was studied in a phosphate buffer solution (PBS) with pH 5.5 and incubated in a shaking water bath at 37 ˚C. At specific time intervals (1 h), samples precipitated and supernatants were withdrawn and replaced by fresh PBS to maintain a constant volume. The study was continued 24 h until the completion of the dissolution study. The amount of the HP released at each time interval was analyzed spectrophotometrically at 584 nm. From the absorbance values, the cumulative percentage of the amount of release was determined.

2.2.3. Antibacterial activity

The antibacterial study was carried out depending on the well diffusion method and prepared materials were tested against to Staphylococcus aureus Gram-positive microorganism that act as the predominant pathogenic bacteria for severe skin wound infection (Peng, Li, 2014; Abdollahzadeh, Rezaei, Hosseini, 2014; Burt, 2004). Bacterium suspension was seeded on Luria-Bertani (LB) solid media and 7 mm diameter wells were opened on agar. 50 µl of samples were added and incubated at 37 °C for 24 h. The diameter of the inhibition zone (mm) was measured to determine the antibacterial activity of the samples. After incubating at 37 °C for 24 h, an

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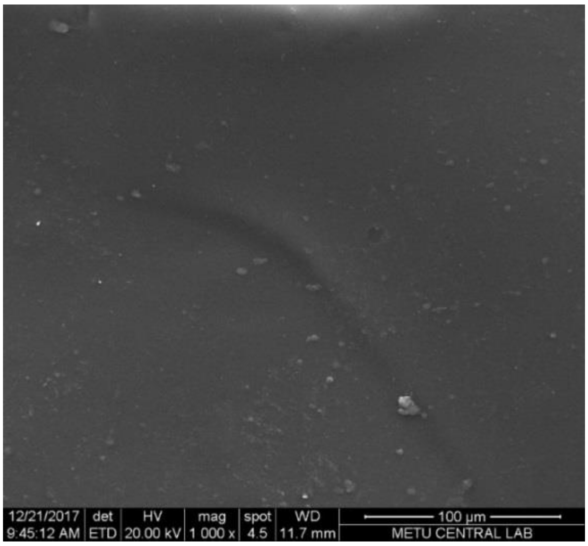
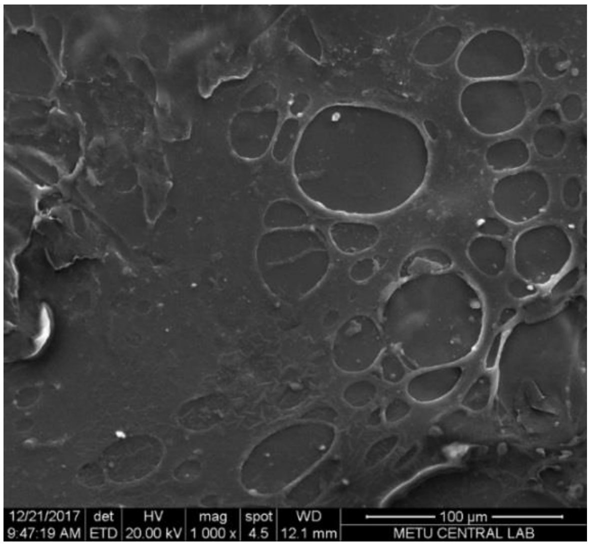
Figure 1: SEM images of a) NaAlg, b) SSD and HP added NaAlg membranes



(a)



(b)

inhibition zone around the samples was recorded as an indication of antimicrobial activity. Well diffusion study was performed in 4 parallel, mean and standard deviation were calculated.

3. Results

3.1. Membranes characterization

The surface morphologies of membranes were investigated by SEM. The SEM micrographs of the surface of both NaAlg and HP and SSP loaded NaAlg membranes were shown in Figure 1. In the figure 1b, the presence of SSD and HP in the membrane was confirmed. The SEM micrographs were taken at magnifications of 1000.

3.2. In vitro release studies

The in-vitro release of HP from crosslinked NaAlg membranes was studied in skin pH conditions (pH 5.5) at 37 0C. The effect of the HP release on the release rate of HP was shown in Figure 2. The figure shows that the cumulative release of HP from the membranes increases with the increasing amount of HP in the membranes. In the figure 2, the highest HP release was obtained for a release period of 6h as nearly 15% by using the F3 formulated hydrogel. The effect of the SSD release on the release studies was shown in Figure 3. It was seen from the Figure 3, the highest SSD release was obtained as nearly 45% bu using the F6 formulated hydrogel.

Figure 2: Effect of the HP ratio on the release studies (♦: F1, ■: F2, ●: F3, crosslinking concentration: 5% w/v)

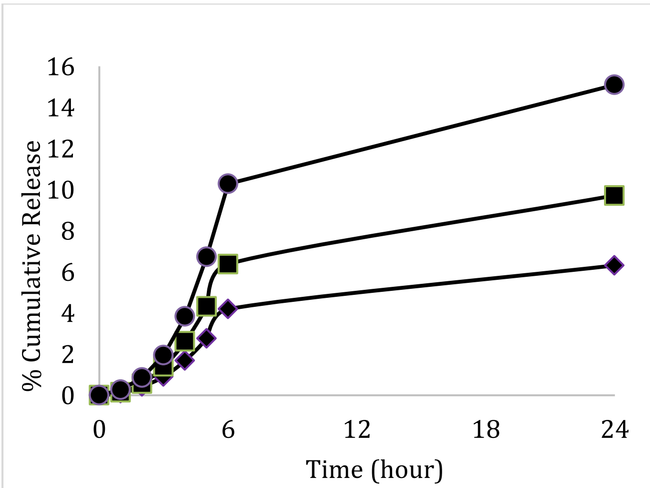


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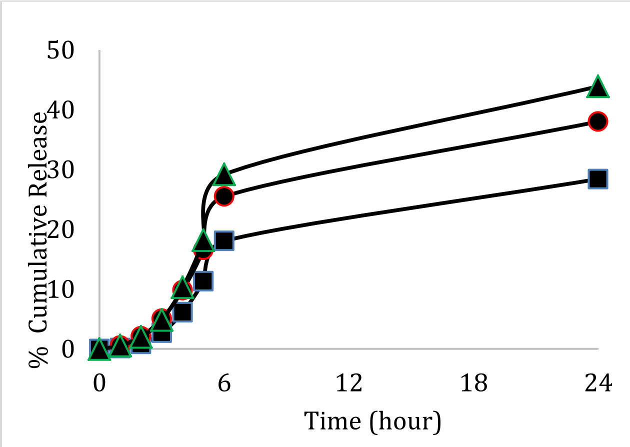
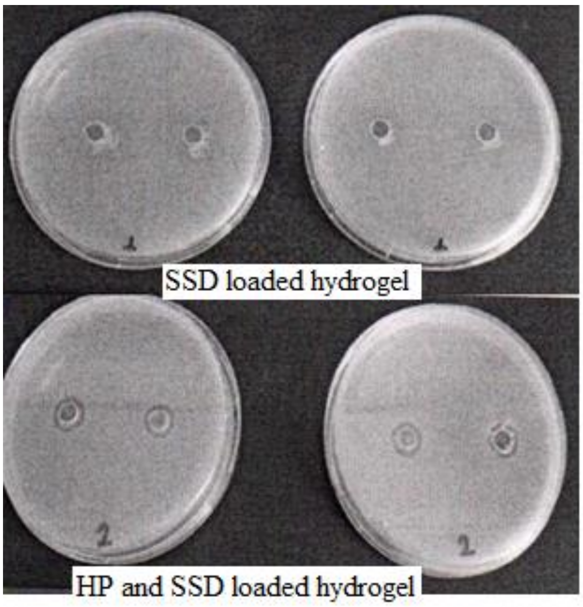
Figure 3: Effect of the SSD ratio on the release studies (▲: F6, ●: F5, ■: F4, crosslinking concentration: 5% w/v)



3.3. Antibacterial Activity

Antibacterial properties would benefit to characterize the wound dressing material. Thus, we performed a test for antibacterial assessment using Gram positive Staphylococcus aureus ATCC 25923 microorganism by the well diffusion method in this study. The results of antibacterial studies on SSD and F6 formulated membranes were shown in Figure 4.

Figure 4: Antibacterial performance of (a) SSD, (b) SSD and HP loaded membranes



The microorganism to be cultivated in the medium was activated for 2 hours in the Tryptic Soy Broth liquid medium. After the density of the active cultures was adjusted to McFarland 2.5 turbidity, the bacteria were planted in the Luria-Bertani (LB) solid medium. Wells with a diameter of 7 mm were drilled with the help of sterile punches on the agar. 50 µl of the samples to be investigated for antimicrobial activity in the wells were added and left for 24 hours incubation at 37ºC. After the incubation, inhibition zones formed around the wells were measured with a caliper. Well diffusion study was carried out with 4 parallels, and average and standard deviations were calculated.

The bacterial growth inhibition sites from the figure were observed in diameters of 5.25 ± 0.50 mm for the F6 formulated membranes. These results showed that the SSD containing membranes had antibacterial properties in the absence of HP.

When the well diffusion test results were examined, no formation of zones was observed on the plate sown on the silver sulfadiazine antibiotic. However, the area in which the film-forming solution containing the silver sulfadiazine antibiotic + St. John's Wort oil acts on the bacterially planted plate is clearly visible. Due to the natural antimicrobial properties of St. John's wort oil, the polymer film became stronger and the silver sulfadiazine antibiotic adhered to the film more firmly.

The hydrogel form of HP, which is traditionally used for wound healing, was prepared and it was provided for easier use. In this way, the release of the active substance, which is also controlled, can continue for longer periods.

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5. Conclusion

HP incorporated NaAlg membranes were prepared for potential wound dressing material. In our study, the effects of HP oil into NaAlg membranes on antibacterial and in vitro release properties were investigated. The highest HP and SSD releases were obtained as nearly 15% and 45%, respectively. The antibacterial test results demonstrated that the prepared membranes have an antibacterial property on Staphylococcus aureus Gram-positive bacteria species in the study.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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