Preparation and characterization of vancomycin-loaded chitosan/PVA/PEG hydrogels for wound dressing

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Abstract
This study describes a drug-loaded porous hydrogel for delivery of vancomycin. Hydrogels based on chitosan (CS), Polyvinyl alcohol (PVA) and Polyethylene glycol (PEG) were prepared by lyophilization. Scanning electron microscopy (SEM), differential scanning calorimetry (DSC), and fourier transform infrared (FTIR) spectroscopy were used to characterize the structures. Water uptake percentage and vancomycin release were also measured. The antibacterial activity against Staphylococcus aureus was investigated. According to the results, mean pore diameter (MPD) was decreased by addition of PEG and reached to 1.3 ± 0.5 μm. On the other hand, 43% decrease in water content of the hydrogels showed along with the incorporation of PEG. The inhibition zone confirmed antibacterial effect of the vancomycin-loaded hydrogels. The porous CS/PVA/PEG hydrogels containing vancomycin could be good candidates to potentially be used as wound dressing.

Introduction
One of the major long-term problems in burn care is the formation of hypertrophic scar (HS), which is a skin condition characterized via excessive fibrosis with disordered collagen fibers. HS usually develops during the wound healing process subsequent burn injury and its formation could be a result of the imbalance between ECM synthesis and degradation [1–4]. Scar scales include several variables such as color, extent and may even contain subjective factors such as pain and itching. Scars can cause severe itching, tenderness, sleep disturbance, anxiety, depression, and disruption of daily activities [2, 5].

The most important goals of wound dressing are improving the rate of epithelization, preventing the scar formation and infection. It has been observed that decreasing the rate of epithelization may lead to scar formation. An ideal wound dressing should act as a barrier against bacteria, absorbs and prevents of body fluid loss, permeable to oxygen, have no antigenicity, no toxicity, good handling and also the ability to drug release [6–8]. Wound dressing also acts as a shield to protect wound from mechanical trauma [9, 10]. Several investigations have done on synthesis and modification of biomaterials to develop potential wound dressings, which can be employed in the management of burns [4, 11, 12]. It is widely accepted that moist environments are superior in advance for increasing the re-epithelialization rate of wound. In this context, special attentions have been received on hydrogels. Hydrogels are 3D hydrophilic polymeric networks, which may be suitable for absorbing wound exudates and serve as temporary wound covers [13–16].

Chitosan is a linear polysaccharide which consists of 2-acetamido-D-glucose and 2-amino-D-glucose units linked with glycosidic linkages. Chitosan is a hydrophilic polymer, obtained industrially from chitin by alkali deacetylation. According to past research, chitosan possesses antibacterial, antifungal and hemostatic activity. Chitosan has high degradability rate in an acidic environment that is often present in wound beds. Freeze drying has been broadly used to prepare porous hydrogels from various biomaterials [17–19].

Polyvinyl alcohol (PVA) is a hydrophilic synthetic polymer which is formed by the polymerization of vinyl acetate. PVA is endowed with high biocompatibility, good chemical stability and long-term pH and temperature
stability [6]. Moreover, PVA is non-toxic to living tissues, and exhibits minimal cell adhesion and protein adsorption characteristics [20, 21]. In order to improve stability and form hydrogel, PVA can be either physically crosslinked by repeated freeze-thawing, or chemically crosslinked by crosslinking agents such as glutaraldehyde or radiation such as electron beams or gamma radiation. However, pure PVA hydrogels are known to be quite fragile in some applications and this is a weakness point. To overcome this limitation, PVA could incorporate with other polymers [22, 23]. In the mentioned strategy, hydrogels with diverse characteristics and behaviors could be synthesized [10]. Polyethylene glycol (PEG) is one of the most widely used synthetic hydrophilic polymer. PEG is generally biocompatible, water soluble, and relatively inexpensive. According to the extreme hydrophilicity, PEG is commonly used in the form of hydrogels and is able to absorb and retain water inside [24–26]. The purpose of wide range of literatures are development of antibacterial delivery systems for antibiotics such as, vancomycin, with potential application in the prevention or treatment of wound infections [27, 28].

In this study, in order to overcome this limitation and improve the stability of chitosan, chitosan was successfully blended with PVA and PEG and crosslinked with glutaraldehyde.

Materials

Chitosan (medium molecular weight) was purchased from Sigma-Aldrich. PVA (72000 Da), polyethylene glycol (10000 Da), acetic acid, and glutaraldehyde were from Merck.

Methods

Porous hydrogel preparation

Chitosan solution (5% v/v) was prepared in dilute acetic acid. PVA and PEG was dissolved in distilled water and added to the chitosan solution with different ratios. After completely mixing, vancomycin was added to the polymeric solution and stirred for 5 min. The solutions were placed in freezer at $-80\,^\circ\text{C}$ for 24 h. Finally, the samples were freeze-dried at $-50\,^\circ\text{C}$ for 48 h (Dena, FD-5010-BT). The prepared porous hydrogels were immersed in glutaraldehyde solution (1%/v/v) and freeze dried again for 24 h.

Morphology study

To evaluate the morphology of the hydrogels scanning electron microscopy (SEM) was used Hitachi S4160, Cold Field Emission. The freeze-dried samples were fixed on stubs, coated with a thin film of gold for about 10 min, and finally, studied by SEM.

Water uptake of the hydrogels

A known weight of hydrogel was placed in PBS for 24 h. The wet weight of the scaffold was recorded after blotting the hydrogel surface with filter paper, to remove excess surface water. The percentage of water uptake by the hydrogel was calculated according to the following equation:

$$\text{Water Uptake(\%)} = \frac{W_w - W_d}{W_d}$$

where $W_w$ represent the wet weight of hydrogels immersed in the PBS, $W_d$ is the dry wet of hydrogels.

Attenuated total reflectance-fourier transform infrared (ATR-FTIR) spectroscopy

The chemical structure of the prepared hydrogels was characterized using ATR-FTIR spectrophotometer (Bruker EQUINOX 55). Each spectrum was recorded at wave number range of 4000–400 cm$^{-1}$.

Differential scanning calorimetry (DSC)

Thermal analysis was carried out using a differential scanning calorimeter (PL-DSC) for pure Chitosan, CP, and CPP samples. The samples were placed in an aluminum-sealed pan and then heated from 0 to 300 $^\circ\text{C}$ at a scanning rate of 10 $^\circ\text{C}$ min$^{-1}$ under nitrogen flux. The data were processed on a personal computer.

Drug release study

In order to study the in vitro release, the vancomycin loaded samples were immersed in 10 ml of PBS at 37 $^\circ\text{C}$ for 120 h. At scheduled times sampling was performed and the amount of drug released was determined using an ultraviolet spectrophotometer at 280 nm (Shimadzu UV-1800). All release experiments were performed in triplicate.
Antibacterial study
The Kirby-Bauer test, is a valuable standard tool for measuring the effectiveness of antimicrobics against pathogenic microorganisms. The antibacterial assay of the dressings was evaluated qualitatively using disk diffusion method against *Staphylococcus aureus*. 0.5 McFarland standard in a sterile test tube was prepared according the protocol [29, 30]. Briefly, 5–6 bacterial isolate colonies were emulsified in sterile distilled water and the turbidity was adjusted to $1.5 \times 10^8$ CFU/mL (equivalent to 0.5 McFarland standard). This was followed by dipping a sterile cotton swab into the standardized bacterial suspension and inoculated on the corresponding Mueller–Hinton agar plates evenly. Then plates were left to dry for 5 min [29, 31]. The hydrogel disks, 1 cm in diameter, was sterilized under UV light for 20 min, and then placed on *Staphylococcus aureus* agar plates and kept in an incubator overnight at 37 °C for 24 h. Finally, the zone of inhibition formed around the sample was measured.

Cytotoxicity evaluation
Extraction of the samples after 48 h and cell culture was done according to the previous study [32]. Briefly, L929 fibroblasts were cultured into a 96-well plate at $1 \times 10^4$ cells/well. After 24 h, the culture medium of each well was replaced with 90 μl extract of the samples plus 10 μl of fetal bovine serum. After 24 h, the medium of each well were removed and 100 μl of MTT solution was added. The plate was then incubated for 4 h at 37 °C in a CO$_2$ incubator. Finally, 100 μl isopropanol was added and left the plate for 15 min at 37 °C. The optical density (OD) of each well was recorded at 545 nm using a microplate reader (STAT FAX-2100) and normalized to the control OD.

Results

Morphology of the hydrogels
Morphology of the hydrogels was studied by SEM and the micrographs are presented in figure 1. Pore size distribution histograms of the hydrogels are shown in figure 2. As evident, PVA/CS hydrogels possesses highly porous structures. The mean pore size of PVA hydrogel was 17.8 ± 4.4 μm (figure 1(a)), whereas that of PVA/CS (2:1) (figure 1(b)) and PVA/CS (1:1) (figure 1(c)) hydrogels were 33.6 ± 8.9 μm and 35.8 ± 13.1 μm, respectively. According to the results, addition of chitosan causes an increase in pore size of the hydrogel. The porosity of the hydrogels decreased with incorporation of PEG and matrix density increased. This could be attributed to enhanced entanglements in the polymeric chains. As a result, MPD decreased and reached to 1.3 ± 0.5 μm in the case of PEG containing hydrogel (figures 1(d), (e)).

Water uptake
To provide a moist environment over wound bed, fluid uptake efficiency of the hydrogel plays a key role. On the other hand, water uptake of the hydrogel is an important parameter to determine drug release property. The prepared hydrogels absorbed water and reached to equilibrium within 90 min. The equilibrium water uptake profiles of the hydrogels were studied and the data are displayed in figure 3. A gradual decrease in the water uptake percentage was observed by the addition of PEG. This can be explained by considering the morphological dependence of the hydrogels. As SEM results showed, the PVA/CS hydrogels are porous, therefore can reserve more water in their larger pores, resulting in higher water uptake ability. The decrease in water uptake percentage in PVA/CS/PEG could be due to the smaller pores and increased matrix density. Therefore, water content could be restricted.

Based on the hydrophilic functional groups of PVA backbone, hydrophilic nature of the chitosan and PEG and also crosslinking density, water uptake of the hydrogels varies [33].

FTIR spectra
ATR-FTIR spectroscopy was used to confirm the structure of pure chitosan, CP and CPP hydrogels (figure 4). The broad peak at 3219 cm$^{-1}$ is due to the super-imposition of O–H and N–H vibrations [34, 35]. The strong peak at 2872 cm$^{-1}$ is related to the C–H stretching vibration of the polysaccharide backbone. The sharp peak at 1721 cm$^{-1}$ could be attributed to the C–O stretching vibration. The band at 1572 cm$^{-1}$ could be attributed to the bending vibration of the free primary amino group on the chitosan backbone [35]. In the FTIR spectra of blend hydrogels, absorption peaks belonging to the components can be confirmed.

DSC thermograms
Figure 5 and table 1 show the DSC results obtained for pure chitosan and blend samples. The glass transition temperature of chitosan has appeared at 55.3 °C. In other literatures we have found that $T_g$ of chitosan had appeared at 118 °C in dry state [36]. The results show the effect of water in chitosan on $T_g$. Researchers reported
that $T_g$ could be in the range of $-23^\circ C$ to $67^\circ C$ according to the water content [36–38]. The variations in $T_g$ values confirms importance of role of water as plasticizer in chitosan, i.e., hydrogen bonding between water molecules and amine and hydroxyl groups of chitosan may cause molecular rearrangement which improve chain mobility and lead to decrease in $T_g$. The DSC analysis also shows an exothermic peak at about $300^\circ C$ which is assigned to $T_m$. The thermogram of pure chitosan, exhibits a broad endothermic peak at $120^\circ C$ which is attributed to the loss of water.

A relatively large and sharp endothermic peak is observed at about $190^\circ C$ and $183^\circ C$ (figures 5(b), (c)) and is assigned to the melting temperature of pure PVA. This peak is shifted to $163^\circ C$ with the addition of PEG (figure 5(d)). This is because the majority of the chains with lower molecular weights that cause in a non-crystalline state [39]. In conclusion, the DSC thermograms for the porous structures show clearly the melting transitions of the CPP blend, in which there are significant effects of the PVA and PEG content. A notable

![Figure 1. SEM micrographs of the hydrogels. (a): PVA, (b): PVA/CS (2/1), (c): PVA/CS (1/1), and (d): PVA/CS/PEG, (e): PVA/CS/PEG at higher magnification.](image-url)
decrease in $T_m$ value as the PVA and PEG content increased was found, which points to the good miscibility of the polymers that affect crystallization process of PVA.

Drug release
The release profiles of vancomycin from PVA/CS/PEG hydrogels are presented in figure 6. The release of free vancomycin was relatively fast (28% of total drug was released within 24 h). In the case of PVA/CS/PEG the release rate was meaningfully reduced, suggesting that the hydrogel had an important effect to extend the release time of vancomycin. In these hydrogels, drug release shows a complicated behavior which may affected by water uptake of the hydrogel and PEG release from the hydrogel with time.
In the current study, the disc diffusion method was used to evaluate the bacterial effect of vancomycin-loaded PVA/CS/PEG hydrogels. The antibacterial activity of the hydrogels is shown in figure 7, which indicates that the release of vancomycin has broad activity against *Staphylococcus aureus*. In figure 7(a), unloaded-hydrogel showed a zone of inhibition of 16 ± 0.5 mm, meaning that the hydrogel had antibacterial effect. Chitosan exhibited a wide-range of bioactivity as an antibacterial material. Therefore, the inhibition zone in figure 7(a) is

| Sample          | $T_g$ (°C) | $T_m$ (°C) | $\Delta H_m$ (J g$^{-1}$) |
|-----------------|------------|------------|---------------------------|
| CS              | 55.3       | 272.7      | -27.4                     |
| PVA/CS (1/1)    | 51         | 267.2      | -27.6                     |
| PVA/CS (2/1)    | 50.5       | 265.6      | -2.3                      |
| PVA/CS/PEG      | 52         | 261.6      | -60.3                     |

**Antibacterial study**

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Figure 6. Cumulative release profile of vancomycin from drug-loaded hydrogel.

Figure 7. Growth inhibition zone produced against *Staphylococcus aureus* using, (a): unloaded PVA/CS and (b): vancomycin-loaded PVA/CS, and (c): vancomycin-loaded PVA/CS/PEG hydrogels.

Figure 8. Viability of fibroblast L929 cells exposed to extracts of (a): unloaded PVA/CS and (b): vancomycin-loaded PVA/CS, and (c): vancomycin-loaded PVA/CS/PEG hydrogels. a: tissue culture polystyrene as control.
related to the CS content of the hydrogels. This trend of results is in agreement with other literatures, they investigated and confirmed the antibacterial properties of chitosan [40–42]. The drug-loaded PVA/CS and PVA/CS/PEG hydrogels produced an inhibition zone with diameters of 19 ± 3 mm and 22 ± 2 mm, respectively, as shown in figure 7(b). It confirmed that vancomycin formulated and released from the hydrogel, maintained its bioactivity and antibacterial effect against Staphylococcus aureus. It suggested that vancomycin structure did not degrade under hydrogel preparation steps. According to the SEM images, the pores of different size are observed by addition of PEG into the hydrogels. The drug release rate from PVA/CS/PEG hydrogel is directly influenced by pore size.

Cytotoxicity evaluation
The cytotoxic activity of the hydrogels with and without drug loading was evaluated in fibroblast L929 cell line using the MTT assay after 48 h in culture (figure 8). Cell viability was determined and compared with the control. Cell viability in the presence of drug loaded PVA/CS/PEG hydrogel extract was a little more than unloaded hydrogel extract and control (p > 0.05). Thus, according to the results, the extracts of the hydrogels had not shown cytotoxic effect.

Conclusion
Hydrogels are useful matrices for delivery of drugs, such as antibiotics, due to their high water content. In order to prepare porous PVA/CS and PVA/CS/PEG hydrogels for vancomycin release, freeze drying method was used. Pore size was decreased by incorporating PEG into the hydrogel. As the pore size was decreased, the water content of the scaffolds was decreased. The prepared PVA/CS/PEG hydrogels markedly inhibited the growth of bacteria tested with the respective diameters zone of inhibition.

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References
[1] Shirakami E, Yamakawa S and Hayashida K 2020 Strategies to prevent hypertrophic scar formation: a review of therapeutic interventions based on molecular evidence Burns & Trauma 8 10k003
[2] Brusselsers N et al 2010 Burn scar assessment: a systematic review of objective scar assessment tools Burns 36 1157–64
[3] Brewin M and Homer S 2018 The lived experience and quality of life with burn scarring—the results from a large-scale online survey Burns 64 1801–10
[4] Agarwal V et al 2017 Polymeric nanofibre scaffold for the delivery of a transforming growth factor β1 inhibitor Aust. J. Chem. 70 280–5
[5] Bayat A, McGrouther D A and Ferguson M W 2003 Skin scarring Burns Med. J. 32 68–92
[6] Kamoun E A, Kenawy E R S and Chen X 2017 A review on polymeric hydrogel membranes for wound dressing applications: PVA-based hydrogel dressings J. Adv. Res. 8 217–33
[7] Zhao X et al 2017 Antibacterial anti-oxidant electroactive injectable hydrogel as self-healing wound dressing with hemostasis and adhesiveness for cutaneous wound healing Biomaterials 122 34–47
[8] Qu J et al 2018 Antibacterial adhesive injectable hydrogels with rapid self-healing, extensibility and compressibility as wound dressing for joints skin wound healing Biomaterials 183 185–99
[9] Gupta A et al 2013 Nitrofurazone-loaded PVA–PEG semi-IPN for application as hydrogel dressing for normal and burn wounds J. Appl. Polym. Sci. 128 4031–9
[10] Das S and Subuddhi U 2019 Controlled delivery of ibuprofen from poly (vinyl alcohol)— poly (ethylene glycol) interpenetrating polymeric network hydrogels Journal of Pharmaceutical Analysis 9 108–16
[11] Capanema N S et al 2018 Superabsorbent crosslinked carboxymethyl cellulose-PEG hydrogels for potential wound dressing applications Int. J. Biol. Macromol. 106 1218–34
[12] Dhiya S, Padma V V and Santhini E 2015 Wound dressings—a review BioMedicine 5 24–8
[13] Dutta J 2012 Synthesis and characterization of γ-irradiated PVA/PEG/CaCl2 hydrogel for wound dressing Am. J. Chem. 2 6–11
[14] Fan L et al 2016 Preparation and characterization of chitosan/gelatin/PEG hydrogel for wound dressings Carbohydrate Polym. 146 427–34
[15] Mohamad N et al 2019 In vivo evaluation of bacterial cellulose/acyric acid wound dressing hydrogel containing keratinocytes and fibroblasts for burn wounds Drug Deliv. Transl. Res. 9 444–52
[16] Liu H et al 2018 A functional chitosan-based hydrogel as a wound dressing and drug delivery system in the treatment of wound healing RSC Adv. 8 7533–49
[17] Sautrot-Ba P et al 2019 Photoinduced chitosan–PEG hydrogels with long-term antibacterial properties J. Mater. Chem. B 7 6526–38
[18] Anjum S et al 2016 Development of antimicrobial and scar preventive chitosan hydrogel wound dressings Int. J. Pharm. 508 92–101
[19] Mozalewska W et al 2017 Chitosan-containing hydrogel wound dressings prepared by radiation technique Radiat. Phys. Chem. 134 1–7
[20] Nuryantini A Y, Munir M M, Rahma A, Suciaji T and Khairurrijal 2015 Poly (Vinyl Alcohol)/Chitosan nanofibrous membrane containing anredera cordifolia (Ten.) steenis Advanced Materials Research 1112 (Trans Tech Publ. Ltd.) 453–7
[21] Ahmed A et al 2018 PVA–PEG physically cross-linked hydrogel film as a wound dressing: experimental design and optimization Pharm. Dev. Technol. 23 751–60
[22] Agnihotri S, Mukherji S and Mukherji S 2012 Antimicrobial chitosan–PVA hydrogel as a nanoreactor and immobilizing matrix for silver nanoparticles Applied Nanoscience 2 179–88
[23] Sanchez-Alvarado D I et al 2018 Morphological study of Chitosan/Poly (vinyl alcohol) nanofibers prepared by electrospinning, collected on reticulated vitreous carbon Int. J. Mol. Sci. 19 1718
[24] Buranachai T, Praphairaksit N and Muangsin N 2010 Chitosan/polyethylene glycol beads crosslinked with tripolyphosphate and glutaraldehyde for gastrointestinal drug delivery Aaps PharmSciTech 11 1128–37
[25] Lusiana R A, Sangkota V D A and Santosa S J 2018 Chitosan succinate/PVA–PEG membrane: preparation, characterization and permeation ability test on creatinine J. Kim. Sains Apt 21 80–4
[26] Masood N et al 2019 Silver nanoparticle impregnated chitosan–PEG hydrogel enhances wound healing in diabetes induced rabbits Int. J. Pharm. 559 23–36
[27] Censi R et al 2019 Thermosensitive hybrid hydrogels for the controlled release of bioactive vancomycin in the treatment of orthopaedic implant infections Eur. J. Pharm. Biopharm. 142 322–33
[28] Gustafson C T et al 2016 Controlled delivery of vancomycin via charged hydrogels PLoS One 11
[29] El-Kased R F et al 2017 Honey–based hydrogel: in vitro and comparative in vivo evaluation for burn wound healing Sci. Rep. 7 1–11
[30] Leboffe M J and Pierce B E 2012 A photographic atlas for the microbiology laboratory (Medical, Environmental, and Food Microbiology) (Englewood, Colorado: Morton Publishing Company) 223–4
[31] Le Thi P et al 2018 Catechol–rich gelatin hydrogels in situ hybridizations with silver nanoparticle for enhanced antibacterial activity Materials Science and Engineering: C 92 52–60
[32] Naeimi M et al 2014 Silk fibroin-chondroitin sulfate-alginate porous scaffolds: Structural properties and in vitro studies J. Appl. Polym. Sci. 131 41048
[33] Ahmed E M 2015 Hydrogel: preparation, characterization, and applications: a review J. Adv. Res. 6 105–21
[34] Kim S J, Park S J and Kim S I 2003 Swelling behavior of interpenetrating polymer network hydrogels composed of poly (vinyl alcohol) and chitosan React. Funct. Polym. 55 53–9
[35] Chauhan D, Dwivedi J and Sarkararamakrishnan N 2014 Facile synthesis of smart biopolymeric nanofibers towards toxic ion removal and disinfection control RSC Adv. 4 54694–702
[36] Dhawade P P and Jagtap R N 2012 Characterization of the glass transition temperature of chitosan and its oligomers by temperature modulated differential scanning calorimetry Adv. Appl. Sci. Res. 3 1372–82
[37] Lazaridou A and Biliaderis C G 2002 Thermophysical properties of chitosan, chitosan–starch and chitosan–pullulan films near the glass transition Carbohydrate Polym. 48 179–90
[38] Ratto J, Hatakeyama T and Blumstein R B 1995 Differential scanning calorimetry investigation of phase transitions in water/chitosan systems Polymer 36 2915–9
[39] Karim M R and Islam M 2011 Thermal behavior with mechanical property of fluorinated silane functionalized superhydrophobic pullulan/poly (vinyl alcohol) blends by electrospinning method J. Nanomater. 2011 979458
[40] Raafat D and Sahl H G 2009 Chitosan and its antimicrobial potential—a critical literature survey Microb. Biotechnol. 2 186–201
[41] Verlee A, Mincke S and Stevens C V 2017 Recent developments in antibacterial and antifungal chitosan and its derivatives Carbohydrate Polym. 164 268–83
[42] Benhabiles M et al 2012 Antibacterial activity of chitin, chitosan and its oligomers prepared from shrimp shell waste Food Hydrocolloids 29 48–56