STUDY OF CYTOTOXICITY & ANTIMICROBIAL OF SODIUM ALGINATE / CARBOXYMETHYL CELLULOSE BASED HYDROGEL LOADED WITH ANTISEPTIC (OCTENIDINE DIHYDROCHLORIDE)

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Abstract: Objective of this study was to determine cytotoxicity and antimicrobial effects of SA-PEG, SA-PEG-OCT, CMC-PEG, CMC-PEG-OCT, SA-CMC-PEG and SA-CMC-PEG-OCT hydrogels for wound healing application. In this study, the cytotoxicity effect were observed using human foreskin fibroblast (HS-27) cell that is treated with concentration of hydrogels' extract of 25%, 50% and 100% for 24 hours. The cytotoxic activity was determined via CellTiter®-Blue cell viability assay. Meanwhile, disk diffusion method was used using *Staphylococcus aureus* to observe the Zone of Inhibition (ZOI) of hydrogels for antimicrobial study. Cell viability assay revealed that the results of mean cell viability for 100% hydrogel concentration according to decreasing order are as follow: SA-PEG-OCT > SA-CMC-PEG-OCT > SA-PEG > SA-CMC-PEG > CMC-PEG > CMC-PEG-OCT. All hydrogels loaded with OCT showed ZOIs that manifested antimicrobial effects against S. aureus. However, there is no ZOIs observed for hydrogels without OCT. The observed SA-PEG-OCT hydrogel, CMC-PEG-OCT hydrogel and SA-CMC-PEG-OCT hydrogel that contain approximately 0.5% OCT each demonstrated antimicrobial activity against *S. aureus* without undesirable cytotoxicity on fibroblast cell. Thus, SA/CMC based hydrogel loaded with OCT may be suitable for the use in wound healing management.

Keywords: hydrogel, cytotoxicity, antimicrobial, sodium alginate, carboxymethyl cellulose, octenidine dihydrochloride, antiseptic

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INTRODUCTION

Hydrogels are three-dimensional polymeric gels composed of cross-linked water insoluble polymers with a strong tendency for aqueous media. They have a porous and hydrophilic structure that allows a tremendous degree of water absorption, multiple times the initial dry weight. Hydrogels give distinctive characteristics or large water content (up to 99.5%), non-adhesive nature, malleability and biocompatible to human tissues; all combine to make them a perfect dressing candidate [1]. Hydrogels can be truly interactive dressings by formulating them to act responsively in such a way that they can regulate diffusion and release when loaded with a drug or active biomolecule. Hydrogels dressings are suitable for use in multitude number of wounds such as, but not restricted to, burn wounds, dry wounds with necrotic tissue, pressure ulcers, diabetic foot ulcers, chronic leg ulcers and low to moderately exuding wound [1,2].

Carboxymethycellulose (CMC) is an essential water-soluble cellulose ether derived from chloroacetic acid react with anhydroglucose units (AGUs) of cellulose. It has wide range of applications due to their low price. CMC, when cross linked with appropriate polymer or monomer have tendencies to absorb large amounts of water and swell to create polymeric networks with desirable properties [1,3,4].

Alginate, a natural polysaccharide obtained from marine brown algae and some soil bacteria, has attracted significant biomedical applications including wound treatment management. Alginate has the ability to form hydrogels by introducing divalent cations such as Ca2+ that binds to guluronate blocks of alginate chains allowing ionic cross-linking between the guluronate block of adjacent alginate chains in the egg-box cross-linking model that leads to gel formation [5]. In addition to that, sodium alginate has showed positive outcome on wound management, proved when rate of re-epithelialization, in vivo (rat model), was considerably increase in wound treated with alginate-based hydrogel dressings in contrast to gauge dressings [1].

Most common preventable challenge to wound healing is possible infection because an open wound is a desirable for microbial colonization [6]. Gram-positive bacteria such as *Staphylococcus aureus* (*S. aureus*) and *Streptococcus pyogenes*

(*S. pyogenes*) are the dominant organisms involved in the initial stage of the infectious period, while gram-negative organisms like *Escherichia coli* (*E. coli*) and *Pseudomonas aeruginosa* (*P. aeruginosa*) are only found later in the process when a chronic wound is formed [7].

Previous study has design three formulation of sodium alginate/ carboxymethyl cellulose (SA/CMC) based hydrogel loaded with octenidine dihydrochloride (OCT). The objective of this study was to determine cytotoxicity and antimicrobial effects of SA-PEG, SA-PEG-OCT, CMC-PEG, CMC-PEG-OCT, SA-CMC-PEG and SA-CMC-PEG-OCT hydrogels for wound healing application.

METHODS

Materials

All chemical were obtained from the following sources: sodium alginate (SA) (Chemiz, Malaysia), carboxymethyl cellulose (CMC) (Sigma-Aldrich, Germany), polyethylene glycol (PEG) (R&M Chemicals, UK), octenidine dihydrochloride (OCT) (Toronto Research Chemicals Inc., Canada), Staphylococcus aureus ATCC# BAA-977 (Kwik-Stik Microbiologics, USA), Mueller-Hinton agar powder (Condalab, Spain), Difco™ nutrient agar powder (Becton, Dickinson and Company, France), Dulbecco's minimal essential medium F-12 (Gibco, NY, USA), 1% penicillin-streptomycin (Gibco, NY, USA), 10% fetal bovine serum (Gibco, NY, USA), 0.4% tryptan blue. Doxorubicin® 50mg/25mL (Pfizer, USA), CellTiter-Blue® reagent (Promega, USA).

Preparation of Drug Loaded SA-PEG Based Hydrogel Film

Method of synthesis of hydrogel was adapted and modified from Capanema et al. [8]. SA-PEG was prepared by adding 10 g SA powder and 10 g PEG to 80 mL of distilled water and stirring at room temperature until complete solubilisation occurred. OCT-loaded hydrogels were formulated by dispersing 0.5% OCT slowly in an aqueous-based solution containing sodium alginate and PEG. The resulting mixture was spread onto petri dish and hot-air-dried at 40°C in oven for 7 days.

Preparation of Drug Loaded CMC-PEG Based Hydrogel Film

Method of synthesis of hydrogel was adapted and modified from Capanema et al. [8]. CMC-PEG solution was prepared by adding 20 g CMC powder and 10 g PEG to 80 mL of distilled water and stirring at room temperature until complete solubilisation occurred. OCT-loaded hydrogels were formulated by dispersing 0.5% OCT slowly in an aqueous-based solution containing CMC and PEG. The resulting mixture was spread onto petri dish and hot-air dried at 40°C in oven for 7 days

Preparation of Drug Loaded SA-CMC-PEG Based Hydrogel Film

Method of synthesis of hydrogel was adapted and modified from Capanema et al. [8]. SA-CMC-PEG solution was prepared by adding 6 g CMC powder, 8 g SA powder and 6 g PEG to 80 mL of distilled water and stirring at room temperature until complete solubilisation occurred. OCT-loaded hydrogels were formulated by dispersing 0.5% OCT slowly in an aqueous-based solution containing CMC and PEG. The resulting mixture was spread onto petri dish and hot-air dried at 40°C in oven for 7 days

Cell Cytotoxicity Test

Preparation of hydrogels' extract

Individual hydrogel discs were weighed (0.2 g in total) and immersed in 5 mL of Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 2 mM stable glutamine and 1% penicillin-streptomycin solution (complete DMEM). Extraction of hydrogels was performed under sterile conditions in a water bath at 37°C for 3 days. Extracts were then discarded and further examined on cell cultures in vitro [9].

Cell culture

Human foreskin fibroblasts (HS-27) cell line were routinely cultured in DMEM complete medium. The cells were incubated at 37°C with 5% CO^2. After washing the cell twice with sterile PBS, human fibroblasts were harvested using 0.05% trypsin in Ca^{2+}/Mg^{2+}-free Hanks' balanced salt solution (HBSS). Subsequently, the cells were centrifuged and re-suspended in fresh DMEM complete medium using a centrifuge tube [10].

CellTiter-Blue® Cell Viability Assay

CellTiter-Blue® reagent stored in the freezer was thawed in the 37°C water bath before adding to the cells. The staining process was done in a dark condition since the dyes in the reagent are light sensitive. Briefly, the assay was carried out according to manufacturer's protocol. The HS-27 were seeded in a 96-well tissue-culture plate, a final volume of 100 µL medium containing 10 x 10^4 cells. The cells were then allowed to attach overnight in a 5% CO^2 incubator at 37°C. After 24 hours, the cells were treated with different concentrations of hydrogel extracts which were 25%, 50% and 100% respectively. The control used in this assay positive was doxorubicin 1.5 µg/mL (reference) while the negative control was the untreated cell control. The cells then incubated for 24 hours in a 5% CO^2 incubator at 37°C. After the incubation, 20 µL of CellTiter-Blue® reagent was added to each well. The plate then gently swirled to ensure even distribution of the reagent before incubating it for another 4 hours at 37°C in a humidified 5% CO^2 incubator [11]. Next, the absorbance reading was measured using microplate ELISA reader at 570 nm filters. The percentage of viable cells can be calculated by the following formula [9]:

Cell Viability (%) = (Absorbance of Sample)/(Absorbance of control) x 100%

Antimicrobial test

Preparation of bacteria suspension

S. aureus was streaked onto Mueller-Hinton agar plates to obtain single colonies. Plates were incubated for 24 h at 37°C. Three morphologically similar colonies from fresh agar plate of previous step was chosen and the top of each selected colony was touched using a sterile loop. The single colony was transferred into a sterile capped glass tube containing sterile saline solution and mixed using vortex mixer. Turbidity was assessed by comparing the test and McFarland Standard containing 0.5 $BaSO^4$. Turbidity was verified by measuring the absorbance of the suspension spectrophotometrically. Absorbance must be in the same range as McFarland Standard 0.5 (0.08-0.13 in OD 625 nm). Suspension's turbidity was adjusted by adding sterile saline if the turbidity was too high or adding more bacteria if it was too low [12].

Disk diffusion method

The disk diffusion method was used for the assessment of the anti-bacterial activity of the hydrogel film. Solutions (1.5×10^5 CFU/mL) of *S. aureus* strain were prepared and 0.1 mL of each

strain spread separately on set nutrient agar media. The inoculated microorganisms were incubated at 37°C for 4 h to initiate growth of *S. aureus* on the inoculated culture medium before placing the hydrogel dressings. The hydrogel films were cut into 1 cm diameter circular shape. Further, circular paper discs (1 cm diameter), wetted with reference solutions (80 µL) of 0.5% OCT was used as positive controls. Negative controls were paper disc wetted by distilled water. The plates were then incubated at 37°C for 24 h and the zones of inhibition (ZOI) in millimetres were measure (Pawar et al., 2019).

Statistical Analysis

The results were analysed using One-Way analysis of variance (ANOVA) followed by Tukey's post-hoc test via Statistical Package for the Social Sciences (SPSS) version 25. Microsoft Excel was also used to generate bar charts. The results were considered significant when $p<0.05$.

RESULTS

Cell cytotoxicity study

As shown in Figure 1, the results of mean cell viability for 100% hydrogel concentration according to decreasing order are as follow: SA-PEG-OCT > SA-CMC-PEG-OCT > SA-PEG > SA-CMC-PEG > CMC-PEG > CMC-PEG-OCT. All hydrogels' extract concentration showed no cytotoxic effect as the samples did not exhibit any decrease in viability of the cells below the threshold of 70% according to PN-EN ISO 10993-5:2009 standard (in relation to the control cells).

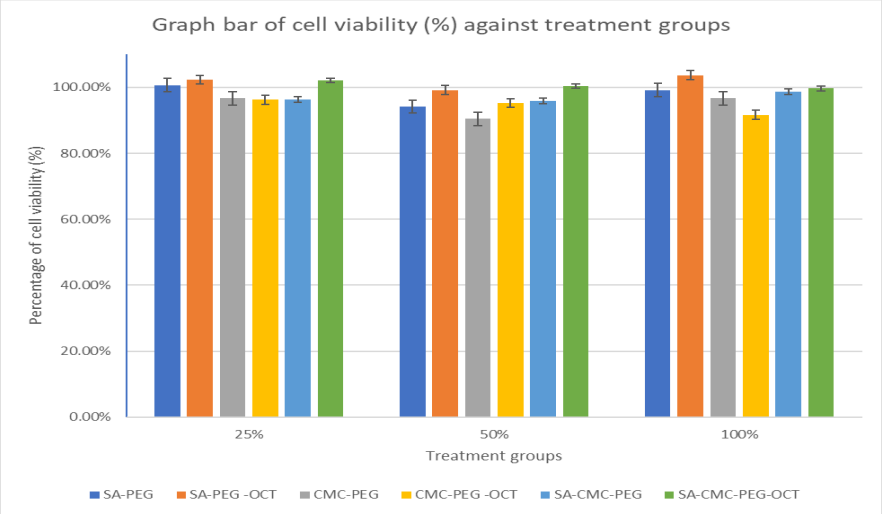

Figure 1 shows graph bar of cell viability (%) against treatment groups.

A one-way ANOVA was conducted to compare percentage of cell viability among six groups of hydrogels (SA-PEG, SA-PEG-OCT, CMC-PEG, CMC-PEG-OCT, SA-CMC-PEG, SA-CMC-PEG-OCT). For HS-27 cells that were treated with 100% concentration of hydrogels for 24 hours, One-Way ANOVA test revealed that there is no significant difference in the mean percentages of cell viability with the different hydrogels ($p=0.534$). Meanwhile, for HS-27 cells that were treated with 50% concentration of hydrogels for 24 hours, One-Way ANOVA test revealed that there is no significant difference in the mean percentages of cell viability with the different hydrogels ($p=0.737$). Next, for HS-27 cells that were treated with 25% concentration of hydrogels for 24 hours, One-Way ANOVA test revealed that there is no significant difference in the mean percentages of cell viability with the different hydrogels ($p=0.736$).

***In Vitro* Antimicrobial Study**

Six sample of hydrogels and two controls, which were distilled water (negative control) and circular paper discs (1 cm diameter) wetted with OCT at concentrations of 0.5 g /100 mL (positive control) were tested against *S. aureus*. After 24 hours of incubation, these plates were observed and measured for its zone of inhibition to assess the anti-bacterial properties of hydrogels. The result for inhibition zone is as shown in Table 1.

Table 1. Zone of inhibition of treatment groups in mm (Mean±SD)

Hydrogels and controls	n	Zone of Inhibition in mm (Mean±SD)	F	p
Positive control ^{bcdefg}	3	8.68±0.02	772.03	0.000
Negative control ^{adfh}	3	0.00±0.00		
SA-PEG ^{adfh}	3	0.00±0.00		
SA-PEG-OCT ^{abcefgh}	3	20.53±0.60		
CMC-PEG ^{adfh}	3	0.00±0.00		
CMC-PEG-OCT ^{abcdegh}	3	24.52±1.77		
SA-CMC-PEG ^{adfh}	3	0.00±0.00		
SA-CMC-PEG-OCT ^{abcdefg}	3	28.74±0.90		

Notes: Significance values were according to One-way ANOVA (Post-Hoc test)

[a.] statistically significant when compared to positive control ($p < 0.001$)
[b.] statistically significant when compared to negative control ($p < 0.001$)
[c.] statistically significant when compared to SA-PEG hydrogel ($p < 0.001$)
[d.] statistically significant when compared to SA-PEG-OCT hydrogel ($p < 0.001$)
[e.] statistically significant when compared to CMC-PEG hydrogel ($p < 0.001$)
[f.] statistically significant when compared to CMC-PEG-OCT hydrogel ($p < 0.001$)
[g.] statistically significant when compared to SA-CMC-PEG hydrogel ($p < 0.001$)
[h.] statistically significant when compared to SA-CMC-PEG-OCT hydrogel ($p < 0.001$)

Based Table 3.1, SA-CMC-PEG-OCT has the highest ZOIs followed by CMC-PEG-OCT, SA-PEG-OCT and lastly, positive control. Positive control that contain 0.5% OCT together with hydrogels loaded with 0.5% OCT presented anti-bacterial activity against *S. aureus* strain. However, there are no zone of inhibition in hydrogel without OCT indicating that these two products do not have anti-bacterial properties. A post-hoc test suggested that the mean ZOIs are significantly different between SA-PEG-OCT and with all other hydrogels and controls (p=0.00). Also, a significant difference between CMC-PEG-OCT and with all other hydrogels and controls (p=0.00) together with significant difference between SA-CMC-PEG-OCT and with all other hydrogels and controls (p=0.00) is observed.

DISCUSSION

Cell cytotoxicity study

In the previous study, SA/CMC based hydrogels loaded with OCT were designed for wound healing applications. Thus, it is significant for hydrogel to be biocompatible and non-toxic in order to be utilised for wound management. The primary purpose of hydrogel biocompatibility assessment is to ensure the patient's safety is protected. Furthermore, the antiseptics' beneficial prophylactic use needs to be weighed against the risk of potential cell cytotoxicity to avoid a possible delay in wound healing. Thus, biocompatibility of hydrogel is evaluated by in vitro cytotoxicity of human foreskin fibroblast (HS-27) cell line that can demonstrate death of cell. The assessment of cell death can be based in the integrity of cell membrane, ascertained by uptake of foreign molecule such as neural red into the cell. CellTiter-Blue® assay was used as it provides a homogeneous, fluorometric method for estimating the number of viable cells present in multiwell plates in an effective and quick manner [11].

Human foreskin fibroblast (HS-27) was used to evaluate toxicity of hydrogel as it found in the dermis layer of skin [13]. Moreover, hydrogels were designed to manage burn and chronic wound that are mostly exposed dermis layer that mainly consist of fibroblast cell. In addition to that, fibroblast produce collagen, elastin and cytokines including growth factor that are essential for wound healing process [13].

It is clear that, all extracts, which were analysed after 24 h of exposure, did not show any toxic activity in relation to the cells of the fibroblast as all samples show high cell viability in all extract concentration. These samples did not exhibit any decrease in viability of the cells below the threshold of 70% according to PN-EN ISO 10993-5:2009 standard (in relation to the control cells). Pertaining to the potential cytotoxicity of OCT-based antiseptics, previously published data have revealed that there was no cytotoxicity when cell was incubated for 1 hour incubation with OCT [14]. One of the main disputed issues is the transferability of laboratory based in vitro data for OCT to clinical observations concerning the effect of OCT application in patients, and consequently to the tolerability of OCT in vivo. However this issue is resolved by Eisenbeiß et al. when a research was conducted in a human randomised, double-blind and controlled clinical trial showed that there was no evidence of significant cytotoxicity of OCT [15]. The results of this present study is in accordance with the result of another study done by Stahl et al. (2010) which was another in vivo study, which revealed no differences in wound epithelisation after treatment with OCT combined with phenoxyethanol as compared to povidone-iodine (an antiseptic)[16].

The results of mean cell viability for 100% hydrogel concentration according to decreasing order are as follow: SA-PEG-OCT > SA-CMC-PEG-OCT > SA-PEG > SA-CMC-PEG > CMC-PEG > CMC-PEG-OCT. From this result, it was observed that SA alone has more viable cells than CMC alone meanwhile combination of SA-CMC hydrogel has higher viable cells than CMC alone but lesser than SA alone. These results are supported by study by Peng et al. that showed the activity of mitochondrial dehydrogenase in the fibroblast cells increased from Day 1 to Day 14 when exposed to alginate hydrogel extract for 14 days [17]. The increase in dehydrogenase activity showed that the cells were viable, and the alginate hydrogel exhibited no cytotoxic effect on the cells. The cells were able to proliferate on the surface of the fibers and into the pores of alginate hydrogel. Hence, the cells form aggregates during proliferation, which still maintain viability and cellular activity [17]. Alginate hydrogel has been found to be a non-cytotoxic and biocompatible biomaterial that is suitable for wound care in many studies. On the other hand, a study done by Magnani et al. demonstrated that human hepatocytes (HepG2) has the ability to adhere and proliferate in 50% concentration of CMC and 100% concentration of CMC. Magnani et al. (2000) also mentioned that the hydrogels did not alter the cellular functionality as no changes in cell morphology was observed [18]. The lower viable cells observed in CMC in this study may not due to cytotoxic effect of the compound but may due to physical characteristics. The structures of CMC is not compact as compared to SA which is highly compact and cross-linked thus the cells do not find a suitable substrate for adhesion [18].This is supported by in vitro study done by Capanema et al. that demonstrate CMC has no cytotoxic effects on human embryonic kidney cells (HEK293T) [8].

SA/CMC based hydrogel loaded with OCT showed good tissue compatibility together with antimicrobial effects. These findings are also in line with in vitro study for OCT done by Muller et al. which categorised OCT as a highly effective antiseptic with favourable cytotoxicity compared to other antiseptics such as benzalkonium chloride, chlorhexidine digluconate, triclosan, silver proteins and povidone-iodine [19]. It is known that it is impractical to extrapolate in vitro data directly to clinical outcomes. Nonetheless, in vitro models can be useful in improving our understanding of how topical

antimicrobial agents and wound dressing can promote wound treatment. Every attempt must be made to ensure that in vitro models are as comprehensive and practical as possible, as was the case in this research.

***In vitro* antimicrobial study**

Infection of wound occurs when there is an imbalance between wound bioburden and the immune system leading to proliferation of microorganisms at the site of wound. Currently, bacterial infection of skin wounds are accountable for the high rates of morbidity and mortality [20]. To address this health issue, various labs around the globe started to develop antimicrobial wound dressings to prevent wound infection. Therefore, previous study has developed SA/CMC based hydrogel loaded with antiseptic (OCT) as a prophylaxis for wound contamination and also to combat against antibiotic resistance pathogen.

The current strategy for treating burns and chronic wounds includes removing necrotic or infected tissue (debridement), ensuring sufficient blood circulation, preserving the moist wound environment, and prevention of wound infection. According to Guo & Dipietro. and Hess dry atmosphere triggers dehydration and death of cells thus affecting wound healing process [21,22]. Hydrogels facilitate wound healing through their moisture retaining ability that keeps the wound hydrated will improve epidermal cell migration and promoting epithelisation. Hydrogel dressings also provide a soothing, cooling impact and reduce the pain related with dressing removal attributed to their high content of moisture. Moreover, the restricted adhesion of hydrogels makes it easy to remove them from the wound without causing further damage to the healing tissue [1]. Thus, SA/CMC based hydrogels loaded with OCT are of great importance to maintain wound in moist environment and provide a prophylaxis against infection throughout exposure of the wound under occlusive conditions.

Due to high susceptibility of burns and chronic wounds to bacterial colonisation, the application of antimicrobial is essential to reduce risk of infection. Typically, infected wounds stop at the inflammatory point and therefore unable to continue the standard healing phases [1,13]. According to Guo & Dipietro and Hess common bacteria that infect the wound are *S. aureus*, *P. aeruginosa*, and *β-hemolytic streptococci* [21,22]. *Staphylococcus* was the most commonly isolated bacterial genus from wound, recovered from 65% of chronic wounds and 60% of acute wounds according to James et al. [23]. In this study, *S. aureus* were used as test microorganism as studies supported the use of the bacteria as the test microorganisms. Furthermore, using these knowledges, OCT was used as the antiseptics as it is a broad-spectrum antiseptic that widely used in modern wound care [14,24]. Octenidine binds on negatively charged surfaces of microbial cell envelopes and eukaryotic cell membrane, disrupts microcellular metabolism and thus inactivates Gram-positive and Gram-negative bacteria, yeasts, dermatophytes, enveloped viruses and echinococcal cysts [14]. In addition to that, OCT demonstrated excellent efficacy for treatment of *P. aeruginosa* and S. aureus infections within biofilms. This activity is significant for wound management as the biofilm will protect the bacteria from host defence and preventing interaction of bacteria with antibiotics thus inhibit their antimicrobial activity [6]. Additionally, no resistance has been observed against OCT in contrast to local therapy with antibiotics that often leads to microbial resistance[14]. These significant advantages over antibiotic supported the use of OCT as antimicrobial compound in this study.

The focus in this study was to observe the effects of the developed hydrogels loaded with OCT by establishing its efficacy and safety. All observed hydrogel that loaded with 0.5% OCT showed zone of inhibition when tested on *S. aureus*. Inhibition zone formation confirms that SA-PEG-OCT, CMC-PEG-OCT and SA-CMC-PEG-OCT hydrogels has antimicrobial activity. This was in line with in vivo study conducted by Eisenbeiß et al. that demonstrated a significant antimicrobial efficacy using hydrogel containing 0.05% OCT [15]. Besides, a study by Koburger et al. showed that OCT and polyhexanide is the most effective antiseptics followed by chlorhexidine, triclosan and PVP-iodine with regard to their Minimum Inhibitory (MIC) and Minimum Bactericidal Concentration (MBC) [14]. However, there were no inhibition zone was observed for SA-PEG, CMC-PEG, and SA-CMC-PEG. These indicated that the SA and CMC had no antimicrobial activity. These results are supported by study by Tan et al. who demonstrated that sodium alginate hydrogel alone has no antimicrobial properties [25]. Besides that, study by Hassan et al. proved that CMC hydrogel alone doesn't exhibit antimicrobial properties as it lacks antimicrobial functional group[26].

The results denoted that SA-CMC-PEG-OCT showed the highest antimicrobial activity followed by CMC-PEG-OCT and SA-PEG-OCT. SA-CMC-PEG-OCT indicated 28.73±1.90 mm inhibition zones against *S. aureus* (ATCC# BAA-977) and this result is triple with the standard 0.5% OCT (positive control). Also, there is significant difference between SA-CMC-PEG-OCT, CMC-PEG-OCT, SA-PEG-OCT and the positive control. The significant difference may be due to two main aspects that may contribute to larger inhibition zone which is drug released and expansion of the hydrogel itself.

In the previous study, it was observed that the drug released for all three hydrogels are in a controlled manner in the first 3 hour. However, sudden increase in OCT released from the CMC-PEG-OCT hydrogel observed in the 6th to 8th hour due to breakdown of the hydrogel. This is due to dissolving of hydrogel at 5h during expansion study cause more OCT to be released as cellulose have high biodegradation rates and improved solubility[27]. Study by Gerayeli showed that influx of water dilutes the polymer below its critical gelation concentration and the matrix losses its gel like properties [28]. Therefore, in the presence of CMC, the network of hydrogel contain degradable crosslink and improved water solubility hence dissolving the hydrogel. Meanwhile, SA-PEG-OCT hydrogel continued to release OCT constantly over 8 hours due to great crosslink density of the hydrogel. These explain the greater ZOI for CMC-PEG-OCT than SA-PEG-OCT. The CMC-PEG-OCT hydrogel itself absorbs more water and expand thus loss its gel-like properties and dissolved hence, has bigger diameter than SA-PEG-OCT. SA-PEG-OCT hydrogel is better in maintaining its hydrogel integrity than CMC-PEG-OCT. On the other hand, SA-CMC-PEG-OCT hydrogel showed a constant release of hydrogel after 8 hours during the drug released study with the least cumulative amount of OCT released. The ZOI for SA-CMC-PEG-OCT was largest might be due to expansion of the hydrogel. Previous study has observed that SA-CMC-PEG-OCT can expand up to 60% of its original size after 7th hour and not expand further than that. This may due to their mechanical strength and higher

crosslinking density in SA-CMC-PEG-OCT compare to SA and CMC alone as Riyajan & Nuim has mentioned that SA-CMC have good mechanical and thermal properties [29].

CONCLUSION

The results from present study proved that SA/CMC based hydrogel loaded with OCT did not exhibit any cytotoxic effects against HS-27 cell line and has antimicrobial activity.

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