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Slow Drug Release of Encapsulated Ibuprofen in Cross-linked Hydrogel for Tissue Engineering Application

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ABSTRACT

Dication cross-linked gellan gum hydrogel loaded with Ibuprofen with excellent mechanical properties had been synthesized as potential candidate for non-toxic biocompatible polymer material in tissue engineering. The gellan gum hydrogel with 5% Ibuprofen had produced a slow release profile with total drug release time of 25 hours as a resulting low swelling value recorded at 22 ± 0.5%. Its compressive strength, 200 ± 21 kPa was highest of all other hydrogel ratio of 0.5% and 1.0% Ibuprofen incorporation. Young's Modulus of the hydrogel with 5% Ibuprofen was recorded at 1800 ± 10 kPa, indicating good gel strength in which it is capable of withstanding a fair amount of subjected force during topical wound dressing application. Excellent mechanical properties, together with slow release profile, make the ibuprofen-loaded hydrogel a prospect candidate as biocompatible extracellular matrices in wound management.

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INTRODUCTION

Gellan gum, a substitute for agar which is produced by *Pseudomonas elodea* via fermentation, is currently getting attention among scientists as it is studied as precursor for biomaterial in many fields. Gellan gum has been studied for biomedical application as it has the potential to be used as matrices to repair and regenerate a wide variety of tissue and organ. This is due to the fact that gellan gum hydrogel pose lack toxicity and the fabrication requires mild conditions. In addition, this biomaterial exhibits intrinsic properties which are analogous to those of living tissues. For instance, its structure is similar to that of native cartilage glycosaminoglycans (Ruoslahti, 1989; Jen *et al.*, 1996) which functions as lubricants in human body. Furthermore, the breathability of hydrogel, a property which is akin to human skin, allows this material to function as an effective extracellular matrix especially in wound dressing application.

Ibuprofen is a drug classified as non-steroidal anti-inflammatory drugs (NSAIDs) and marketed under the brand name of Nurofen™ and Ibufen™. Together with acetaminophen and some other over-the-counter NSAIDs drugs, Ibuprofen is used as a medication to reduce pain and treat fever. Its mechanism of action is still ambiguous and no exclusive principle had been laid out by researchers hitherto regarding this. However, like other NSAIDs, Ibuprofen is believed to be giving the pain-reducing effect by blocking the cyclooxygenase (COX) through the inhibition of prostaglandin, a type of lipid which mediates inflammatory response (Ricciotti and FitzGerald, 2011) hence leading to reduction in pain and fever (Rao and Knaus, 2008).

NSAIDs applied as topical wound dressing is more preferred compared to oral administration as it can minimize gastrointestinal effects which usually involves stomach ulcer and inflammations. Ibuprofen has short half-life, can be absorbed in short amount of time and has good penetration into synovial fluid (Jorge *et al*., 2011). A qualitative study suggested that 5% Ibuprofen has the analogous efficacy to 400 mg oral intake of ibuprofen thrice daily for the treatment of acute pain following musculoskeletal injuries (Whitefield *et al*., 2002).

In this study, Ibuprofen used as the active drug for topical wound dressing is dispersed in hydrophilic polymer i.e. gellan gum as controlled release systems. This system can offer great promise due to the underlying advantages it can offer. Controlled release usually encompasses the delaying of the action of the active drug over time by allowing

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continual release from a polymeric dosage form (Robinson and Lee, 1987). This delay in process of drug release can be beneficial in avoiding frequent dressing change (Lisa, 1997) and become a favourable factor of compliance among patients.

Experimental:

Material:

Low-acyl gellan gum (Gelzan™ CM, $M_w \approx$ 2-3 x 10^5 Da, product number-G1910, lot number SLBB0374V), glycerin (product number-G2289, lot number SHBC2650V), and anhydrous calcium chloride, $CaCl_2$ (product number-C5670, lot number SHBC2650V) were obtained from Sigma Aldrich, Malaysia. Ibuprofen (Nurofen™, D598856102) was purchased from Farmasi Fajar Sdn. Bhd., Kuala Terengganu, Malaysia. All materials were used as received without further purification.

Preparation of Gellan Gum Hydrogel:

Gellan gum hydrogel was synthesized via *in-situ* drug loading in which the powdered Ibuprofen (Nurofen™) was first dissolved in deionized water (18 MΩ cm^{-1}) and mixed with the dissolved gellan gum before establishing the physical crosslinking protocol, which in this case was carried out using 10 mM $CaCl_2$. The solution was stirred at 500 rpm using hot plate set at temperature of 80 °C for a total mixing of 2 hours. Glycerin, 40% weight relative to gellan gum was added as plasticizer. Gellan gum hydrogel incorporated with Ibuprofen with percentage of 0.5 %, 1.0% and 5.0% synthesized in this study are hereon referred to as GG0.5, GG1.0 and GG5.0, respectively. The solutions were left to cure for 24 hours before storing in desiccator for further characterizations.

Characterizations:

Compression measurements were obtained using an Instron Universal Testing machine (model 3366) with ± 10 kN grips and cross-speed set at 10 mm/min. All hydrogels were cut to 20 mm x 20 mm dimension. A minimum of three independent measurements were obtained per sample of a defined ratio.

The gellan gum hydrogel with and without Ibuprofen were tested for functional groups using Perkin Elmer Spectrum 100 FTIR Spectrometer using Attenuated Total Reflectance (ATR) technique with wave number region of 600-4000 cm^{-1}. The resulting characteristic peaks were recorded.

For swelling test, dried sample of dimensions 2 cm x 2 cm were immersed in a sealed beaker (100 mL) containing 20 mL amount of PBS solution (pH 7) at 37 °C in water bath for duration of 24 hours. The weight of wet sample was measured after sample was lightly dried using tissue paper. The test was made triplicates for each ratio. The calculation was done as follows;

Swelling Degree, SD (%) = (Mw- Md)/Md

where Mw = Weight of swollen sample

Md = Weight of dry sample

In-vitro release study was performed using UV Vis Spectrophotometer Shimadzu UV-1800. The hydrogel sample was cut in 7 x 30 mm dimension and immersed in quartz cuvette containing 2.5 mL of 50 mM phosphate buffer solution of pH 7. The fingerprint peak for Ibuprofen was recorded at wavelength of 264 nm. The resulting absorbance value was related with the concentration of Ibuprofen released via constructed standard curve.

RESULTS AND DISCUSSION

Compressive Strength:

Good mechanical properties of a hydrogel for wound dressing application are very crucial for it to be able to cope with the exerted stresses from varying contours of different body parts especially knees and elbows (Sakchai et., al., 2006; Khan *et al*., 2000). Compressive strength, as per outlined by Hooke's Law, is defined as the amount of force (N) exerted on a square meter of a sample (m^2) and expressed in Nm^{-2} (Pa). Strong hydrogel can withstand higher amount of force exerted on it. Our results show that the addition of more Ibuprofen in gellan gum (GG) hydrogel had resulted in stronger but more brittle hydrogel. From Table 1, it is observed that upon addition of Ibuprofen, the compressive strength had increased from 103 ± 3 kPa for pure GG hydrogel to 200 ± 21 kPa for 5% ibuprofen.

Table 1: Results for Compression Test and Swelling Percentages of Ibuprofen Loaded Gellan Gum Hydrogel.

Hydrogel	Compressive strength (kPa)	Young's Modulus (kPa)	Compressive Strain, ε (%)	Swelling (%)
GG	103 ± 2	816 ± 10	3.7 + 0.1	103 ± 3
GG0.5	147 ± 9	1440 ± 100	4.5 ± 0.5	35 ± 6
GG1.0	183 ± 0.5	2100 ± 230	3.8 ± 0.6	34 ± 3
GG5.0	200 ± 21	1800 ± 10	2.5 ± 0.2	22 ± 0.5

However, the increase of compressive strength at higher contents of Ibuprofen come at a cost of decreased compressive strain-at-break (ε) which probably indicating the increased of brittleness (Fig. 1). The ε of pure GG hydrogel was recorded at 3.7±0.1% and decreased to 2.5 ± 0.2% for 5% Ibuprofen. On the other hand, the Young's Modulus (E) shows the similar trend to compressive strength, which significantly increases upon addition of Ibuprofen. The E of 1% Ibuprofen increased to 2100

± 230 kPa than 816 ± 10 kPa for pure hydrogel and slightly decreased at 5% Ibuprofen to 1800 ± 10 kPa. The E values from our study are within the range of the compressive modulus of human articular cartilages which was recorded at 1000-18000 kPa (Stammen *et al*., 2001). Hence, the hydrogel formulated is suitable for the use of wound dressing since the E value falls within the range of that of human cartilages, making the mechanical property of the hydrogel analogous to human cartilages.

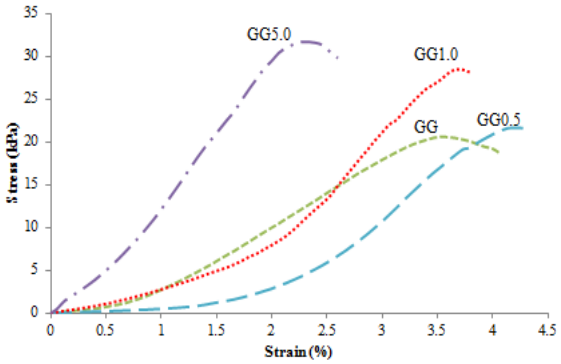

Fig. 1: Stress vs Strain of hydrogel with Ibuprofen

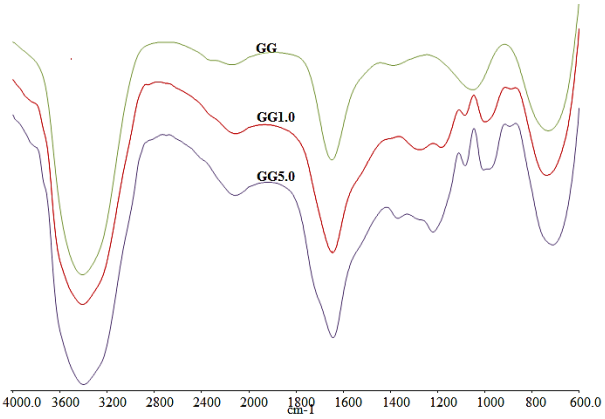

Fig. 2: FTIR spectra of gellan gum with ibuprofen.

FTIR (Attenuated Total Reflectance, ATR):

Shifting or disappearances of the frequency of functional characteristic peaks indicate the interaction between polymer and drug (Prakash *et al*., 2014). Hydrophilicity, which was reported to be the main factor to hydrogel's ability to swell, is contributed by the presence of hydroxyl, carboxyl, sulphonic, amidic and primary amidic functional groups (Ganji *et al*., 2010). The IR peaks of free standing gellan gum hydrogel and that with Ibuprofen (Fig. 2) are compared.

From the peaks obtained via FTIR (ATR) characterization of gellan gum, it was shown that there was the assignment of transmittance band at 3411 cm^{-1} for gellan gum which indicates the stretching vibration of –OH group in gellan gum hydrogel (Ray *et al*., 2010). For gellan gum hydrogel with Ibuprofen, there was shifting to 3386.71 cm^{-1} and 3397.07 cm^{-1} for GG1.0 and GG5.0 respectively, indicating the hydroxyl O-H vibration of H-bonded (Pavia *et al*., 2009). The shifting of carbonyl functional groups, i.e. C=O stretching from the glycosidic link in gellan to lower wave number was also observed in all Ibuprofen loaded hydrogel to 1641 cm^{-1} compared to free standing gellan gum hydrogel at 1648 cm^{-1} (Shah *et al*., 2009; Countinho *et al*., 2010).

The peak at 1051.11 cm^{-1} in free standing gellan gum is assigned for C-O stretching vibration. The values shifted to higher wave numbers of 1075.22 cm^{-1} and 1075.81 cm^{-1} for GG1.0 and GG5.0 respectively, suggesting the interaction between gellan gum and ibuprofen. Band at ~1380 cm^{-1} for methyl C-H bending (Sudhamani *et al*., 2003) observed in gellan gum with 5% ibuprofen also indicated the interaction of gellan gum with the drug since the peak is absence in gellan gum free standing.

Swelling Percentage:

From the result obtained, gellan gum hydrogel with 5% Ibuprofen had the lowest swelling percentage at 22±0.5%, which a significant decreases than free standing gellan hydrogel at 103±3% (Table I). Despite this low percentage of swelling of GG5.0, the hydrogel is a very good candidate for slow release of drug in wound dressing due to the fact that it can be administered topically over a sustained amount of time hence reducing the need for frequent change of dressing. For GG0.5 and GG1.0, the swelling percentage is 35±6% and 24±3%, respectively. Swelling decreases with the addition of more ibuprofen in formulation due to the formation of more rigid structure of gellan gum hydrogel. The rigidity of gellan gum structure had increased its stability and resistance towards swelling media and resulted in decreased swelling. This type of dressing is most suited to be applied in low to moderately exuding wounds.

In vitro drug release:

In controlled drug delivery system, researchers seek to achieve the formulation for the polymeric system which is capable of administering the desired drug via zero order release profile (Boateng *et al*., 2008).

This is due to the fact that the system enables the release of the targeted drug without the dependence on concentration of drug incorporated in the said system. In this study, *in-vitro* release study was carried out in neutral environment using 50 mM phosphate buffer solution of pH 7 to mimic human skin condition. Fig. 3 shows that for GG0.5 and GG1.0, the total time needed for the drug release to achieve equilibrium is 1½ hour and 2½ hour, respectively. The gellan gum hydrogel with 5% Ibuprofen, GG5.0 had shown substantially good release profile. A total of 25 hours were needed for

the hydrogel to reach the equilibrium drug release.

It is clearly observed that the release of drug is dependent on concentration of drug incorporated inside the polymeric matrix, indicating that the release profile does not follow the zero order kinetic release. This result is comparable to the release profile of loperamide encapsulated in liposomal gel for topical drug delivery system (Hua, 2014). Like ibuprofen which is hydrophobic in nature, loperamide had a total drug release of 25 hours. Another study on the release of Ibuprofen from a polymeric system had total drug release of 50 hours (Marianeccia *et al*., 2011). From the study which made use of a polymeric system locus bean/xanthan gum incorporated with Ibuprofen, 60% of the drug was released in 50 hours. Upon addition of a non-ionic surfactant, niosomes, into the system, the release of Ibuprofen had increased to 70% after 50 hours. Addition of niosomes had enhanced the release of Ibuprofen from the system.

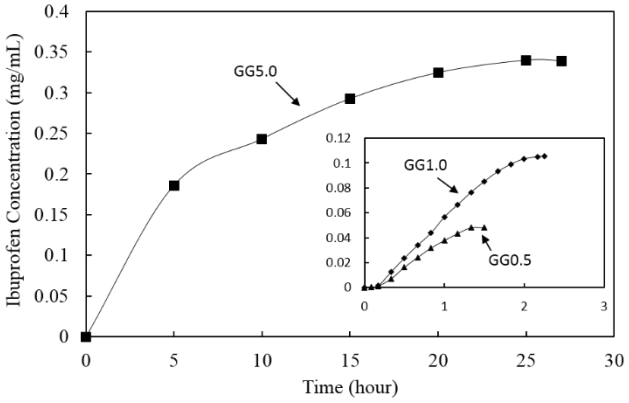

Fig. 3: Release profile of ibuprofen loaded in gellan gum hydrogel at different concentrations.

In wound dressing application, the longer the drug can be released continuously from a polymeric dose, the more favourable the system is. Swelling plays an important role in drug release mechanism. Although the swelling percentage of gellan gum hydrogel loaded with Ibuprofen was low, the hydrogel possess the unique ability to swell in a slow fashion over a set of time. Therefore, the slow swelling property of the formulation had rendered the matrix a potential system for sustained slow release. Different formulations will produce different release profile. There are several key factors that affect the drug release profile and among them are the nature of polymers, percentage of drugs loaded, the drug loading method and also the interaction between drug and polymer itself.

Conclusion:

Gellan gum hydrogel incorporated with Ibuprofen is a potential candidate for wound dressing material in tissue engineering. The release profile shows that the release of the drug is dependent on the concentration of drug incorporated in the gellan gum hydrogel. The slow release profile of gellan gum hydrogel, together with the excellent mechanical properties, allows the biomaterial to be applied in slow and moderately exuding wounds.

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