

## Gellan Gum/Ibuprofen Hydrogel for Dressing Application: Mechanical Properties, Release Activity and Biocompatibility Studies

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### Abstract

In this study, gellan gum (GG) hydrogel incorporated with ibuprofen was successfully developed. Their mechanical and physical properties, drug release behavior, biocompatibility and antibacterial activities were investigated. Results show that GG hydrogel incorporated with 5.0% ibuprofen (GG10-IB5.0), showed good mechanical properties with the highest compressive strength and elastic modulus at  $200 \pm 21$  kPa and  $1820 \pm 10$  kPa, respectively. This ratio also displayed slow drug release profile with the total time required for the drug to be released was 15 h due to the low swelling percentage at  $22 \pm 1$  %. Water vapor transmission rates (WVTRs) value of GG10-IB5.0 was recorded at  $867.5 \pm 154$  g m<sup>-2</sup> d<sup>-1</sup>, which comparable with commercial wound dressing products. The hydrogel exhibited a slight antibacterial property towards *Staphylococcus aureus* with inhibition zone measured at  $9.7 \pm 1.15$  mm whereas *in vitro* cell study on normal human dermal fibroblast cells (CRL2522) indicated that the hydrogel formulation was biocompatible with the human cell line. The formulation of gellan gum hydrogel enhanced with an addition of ibuprofen can be a potential material for excellent wound dressing material.

**Keywords:** Gellan gum, ibuprofen, drug release, biocompatibility, antibacterial, hydrogel

## 1. INTRODUCTION

Gellan gum (GG) is an exopolysaccharide produced by a non-pathogenic Gram-negative bacterium of *Pseudomonas elodea*. It has been proven by the United States Food and Drug Administration (US FDA) and the European Union (EU) to be safely utilized in food industry [1]. Due to its multifunctional properties of GG as gelling, stabilizing and suspending agents in food industries, it has attracted many researchers to use this latter in pharmaceutical and biomedical applications. For instance, it has been studied as matrices to repair and regenerate a wide variety of tissue and organ [2]. Not limited to that, it has been used as scaffold materials for the application of tissue engineering[3], in development of wound dressing materials [4], as a vehicle for ophthalmic drug [5] and in eye drops [6]. GG also has shown a good compatibility against various live cells such as mouse fibroblast (L929 cell line)[4], human dermal fibroblast (HDFs)[7], human fetal osteoblast (hFOBs 1.19) [7], human skin fibroblast (CRL2522)[8] and human nasal cartilage[9].

Ibuprofen is a derivative of propanoic acid and has ever since been used as the common analgesic and antipyretic drug. Various studies have been incorporated ibuprofen and examine the release behavior by using different source of biopolymers. Some studies reported the release profile of ibuprofen within a short period, i.e. 20 min and up to 50 h. Waters and co-workers have reported the release of ibuprofen from stearic acid microspheres in 20 min to reach equilibrium [10]. Other study reported the release of ibuprofen from alginate bilayer hydrocolloid film for 8 h [11]. Longer duration of ibuprofen release was reported by Carafa and group from their polymeric system consisting locus bean/xanthan gum with addition of non-ionic surfactant. The matrix took 48 h to release 60% of the ibuprofen used [12]. From these studies, it shows that different release profile obtained by using different biopolymer. In our review, no study has utilized GG incorporated ibuprofen and studies the release behavior and biocompatibility of the hydrogel.

This study highlighted the use of GG hydrogels consist of ibuprofen for the transdermal application. Ibuprofen was dispersed in GG hydrogel as a drug model to treat pain and fever. The physical properties such as swelling capability, degree of crosslinking and water vapor transmission rates of the GG hydrogels were examined to optimize the properties of the systems. The release profile was conducted to determine the amount of ibuprofen dispersed in the system. Not limited to that, the cell viability and cell proliferation tests were also carried out to study the biocompatibility of the GG hydrogels containing ibuprofen involved human skin fibroblast cell (CRL2522, American Type Tissue Collection), while the antibacterial activities of the samples were assessed via *in-vitro* qualitative study against Gram-positive and Gram-negative bacteria.

## **2. MATERIALS AND METHOD**

### **2.1. Materials**

Low-acyl gellan gum (Gelzan™ cm,  $mw \approx 2-3 \times 10^5$  da, product number-G1910, lot number SLBB0374V), glycerin (product number-G2289, lot number SHBC2650V), anhydrous calcium chloride,  $CaCl_2$  (product number-C5670, lot number SHBC2650V) and ibuprofen (product number-14883, lot number MKBQ4505) were obtained from Sigma Aldrich, St. Louis, Missouri, USA. All materials were used as received without further purification. Penicillin G-P10 (product number-CT0043B, lot number-523987) was obtained from Oxoid, England. Norfloxacin (product number-N9890) was obtained from Fluka, Malaysia.

### **2.2. Preparation of gellan gum hydrogel with ibuprofen**

The gellan gum (GG) hydrogel was prepared via evaporative casting method. The GG solution was prepared by dissolving 1.5% (w/v) of GG in 100 mL deionized water (18 M $\Omega$ ), followed by glycerin at 50% w/w with continuous stirring for 2 h at 80 °C. Gellan gum-ibuprofen solution was synthesized via in-situ drug loading. For in-situ drug loading, the ibuprofen powder was dissolved in deionized water and mixed with the GG solution. Calcium chloride ( $CaCl_2$ ) (10 mM) was added into the mixture and stirred for 2 h at 80 °C. These gellan gum solution containing ibuprofen at 0.5%, 1.0% and 5.0% (w/w) hereafter were known as GG-IB0.5, GG-IB1.0 and GG-IB5.0, respectively. 50 mL of each solutions were deposited onto petri dishes (90 mm x 15 mm) and dried in room temperature (24 °C) for 24 h to enable the GG solution to transform into a stable 3D-matrix (gel form). Prior to any characterization, the hydrogels were pre-conditioned and kept in a desiccator containing silica gel for 48 hrs.

### **2.3. Characterization of the Hydrogel**

#### **2.3.1. Compression of Hydrogel**

Stress-strain measurements were obtained using Instron Universal Testing machine (model 3366) with  $\pm 10$  kN grips and cross-speed set at 10 mm/min. All hydrogels were cut to 20 mm x 20mm for compression determination. Compressive stress and strain at break were calculated from the slope of the linear part of the stress-strain curve. Compressive strain at maximum compressive extension and Young's modulus were also recorded. A minimum of three independent measurements were obtained per sample of a defined ratio.

#### **2.3.2. FTIR Characterization**

ATR-FTIR spectra were collected using a Perkin Elmer Spectrum 100 FTIR spectrophotometer with PIKE Miracle ATR accessory (single-bounce beam path, 45 ° incident angle, 16 scans, and 4 cm<sup>-1</sup> resolution). All spectra were corrected by the Perkin Elmer Spectrum 100 software.

### 2.3.3. Swelling Test

The swelling test was carried out by immersing hydrogel (20 mm x 20 mm) in phosphate buffer solution (pH 7) in water bath ( $37 \pm 0.5$  °C). The samples were then removed after 24 h, lightly blotted with a wet filter paper to expel surface solution prior to measuring the weight. The test was repeated triplicates for each ratio. Finally, the swelling degree was then determined from the equilibrium-swelling ratio:

$$\text{Swelling Degree, SD (\%)} = (M_w - M_d)/M_d$$

where  $M_w$  = Weight of swollen sample and  $M_d$  = Weight of dry sample

### 2.3.4. Gel Fraction

The hydrogel sample was cut to 20 mm x 20 mm and dried for 24 hours. After drying, the gellan gum hydrogel was weighed ( $W_1$ ) and then swelled in 10 mL deionized water at room temperature for 24 h. After removing the wet hydrogel from the solution, it was dried in an oven for another 24 h at 50 °C and then weighed again ( $W_2$ ). The content of the hydrogel was calculated using the following equation:

$$\text{Gel content (\%)} = (W_2 / W_1) \times 100$$

### 2.3.5. Water Vapor Transmission Rates

The water vapor transmission rate (WVTR) test was carried out using the method adapted from the previous study [13]. Briefly, gellan gum hydrogel with dimension of 30 mm x 30 mm was cut and put as a cap of a vial with a diameter 16 mm containing 10 mL distilled water, followed by the system being weighed and recorded. Each ratio of gellan gum samples were made into triplicates, led to having the vials with hydrogels as the caps put in humidity chamber for 24 h at  $T = 37.5$  °C and relative humidity of  $60\% \pm 5$ . The value for WVTR ( $\text{g m}^{-2} \text{day}^{-1}$ ) was calculated as follows;

$$\text{WVTR} = (W_i - W_f)/(A)$$

where  $A$  = area of the vial opening,  $W_i$  = initial weight of the system and  $W_f$  = final weight of the system.

### 2.3.6. Ibuprofen Release Study

The ibuprofen's release study was performed using UV-Vis Spectrophotometer Shimadzu UV-1800 with the mode menu set to kinetics method. UV-Probe software (version 2.43) was used to analyze the results. In this method, the UV-1800 measured the drug release of the fingerprint peak of ibuprofen at wavelength of  $\lambda = 264$  nm at pre-determined duration for every samples. The hydrogel samples (cylinder form, diameter = 3.3 mm, length = 30 mm) were immersed in quartz cuvette containing 3 mL of 50 mM phosphate buffer solution of pH 7. The resulting absorbance values were compared to the concentration of ibuprofen released via constructed standard curve and used to calculate the profile release of the drug into the system.

### 2.3.7. Cell Study

#### Routine Cell Culture

The cultivation of normal human skin fibroblast cells (CRL-2522, ATCC) was prepared by using the Eagle's Minimum Essential Medium (EMEM, ATCC, USA) with 10 % (v/v) fetal bovine serum (FBS, Sigma Aldrich, USA) and 1% (v/v) antibiotic (Penicillin/ Streptomycin, Science Cell, USA). In addition, the cells were cultured at 37 °C in a humidified 5 % CO<sub>2</sub> atmosphere and were sub-cultured every three days as established protocols and harvested at 60-80 % confluence.

#### Cell Proliferation

Hydrogel samples with diameter ~ 6 mm were directly placed into the 96-well culture plates (Nunc, Germany) containing EMEM medium. Specimens were placed into 96-well culture plates and sterilized in a laminar airflow chamber using UV radiation for 20 min. Cell proliferations was quantified by MTS assay, Cell Titer 96 Promega (3-(4,5-dimethylthiazol-2-y-l)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H tetrazolium)). Tissue culture polystyrene plates, TCP, were used as a positive control. Prior to addition of the assay solution (20 µL in each well), the media in all wells that contained samples except for TCP were replaced with fresh media and later incubated for 4 h at 37 °C in an atmosphere containing 5 % CO<sub>2</sub>. After 4 h, the 96-well plate was taken for quantification by Elisa (Ascent Software). The absorbance values are directly proportional to the cell count (cell numbers/well) from the standard curve constructed beforehand. The number of cells for the duration of 24, 48 and 72 h were determined by correlating the absorbance value from Elisa reader to the corresponding cell number from standard curve.

#### Cell Viability

The hydrogel samples were dried in the oven at 40 °C for 24 h until GG films are formed with approximate thickness of 100 µm. Then the GG films (diameter ~ 6 mm) were placed into the 96-well culture plates containing EMEM medium and left overnight (24 h) in order for the GG films to transform into hydrogel. GG hydrogels in the 96-well culture plates were sterilized in a laminar airflow chamber under UV radiation for 20 min. The next step of cell seeding was followed by three replicates for each type of samples. The CRL 2522 cells (5000 cells/ well) were seeded into wells containing samples and cultured at 37 °C in 5 % CO<sub>2</sub> atmosphere. After 24, 48, and 72 h of incubation, the cell viability was observed by using an Olympus TH4-200 microscope equipped with Olympus U-RFL-T UV pack stained with Calcein-AM.

### 2.3.8. Antibacterial Study

Gram-positive *Staphylococcus aureus* (*S. aureus*) and Gram-negative *Escherichia coli* (*E. coli*) bacterial suspensions were used for the antibacterial assay. Mueller-Hinton (MH, Difco, Malaysia) agar was used for the growth of both bacterial types. Each Gram-positive and Gram-negative bacteria suspension was evenly spread on the solid MH agar and dried in a laminar flow air chamber. The wells were designed on each solid MH agar so that the hydrogel samples (diameter ~ 6mm) can be placed into

them. The MH agar with the hydrogel samples was incubated at 37°C for 24 h. The presence of any clear zone around the hydrogels on the MH agar was recorded as an indication of inhibition against the *S. aureus* and *E. coli*.

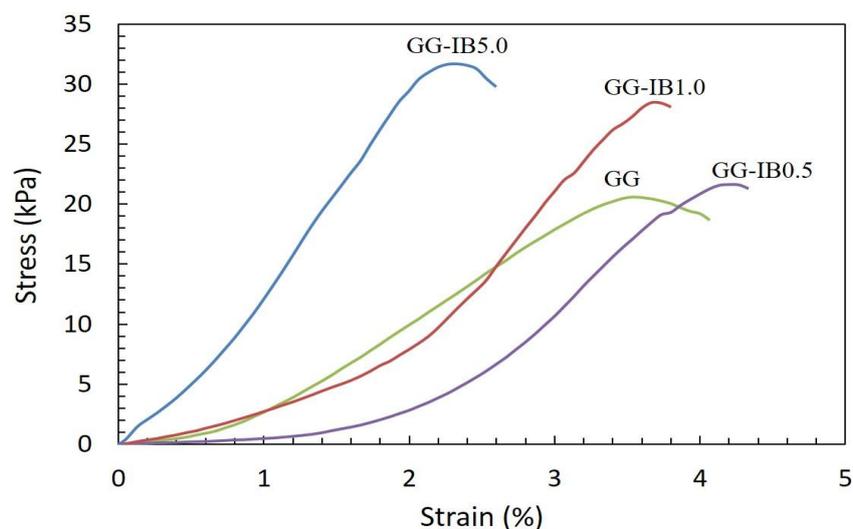
### 3. RESULT AND DISCUSSION

#### 3.1. Compression of Hydrogel

Strong gel is required for the use of material as wound dressings in order to be able to withstand the different contours of human parts [14]. Figure 1 shows the stress-strain curves of GG hydrogel incorporated ibuprofen. The inclusion of ibuprofen into GG hydrogels improved the compressive strength ( $\sigma$ ) of the hydrogel at a cost of decreased compressive strain ( $\varepsilon$ ) (Table 1). For example, the  $\sigma$  value of GG-IB0.5 hydrogels was recorded at  $147 \pm 9$  kPa and improved to  $200 \pm 21$  kPa for GG-IB5.0 hydrogel. In contrast, the  $\varepsilon$  of GG-IB5.0 hydrogels reduced to almost 2-fold than GG-IB0.5 hydrogels. The increase of compressive strength may be due to the fact that the addition of more solute transformed the material more rigid. Hence, they could withstand greater force imposed on them, at the same time caused the hydrogel more brittle and decreased the compressive strain.

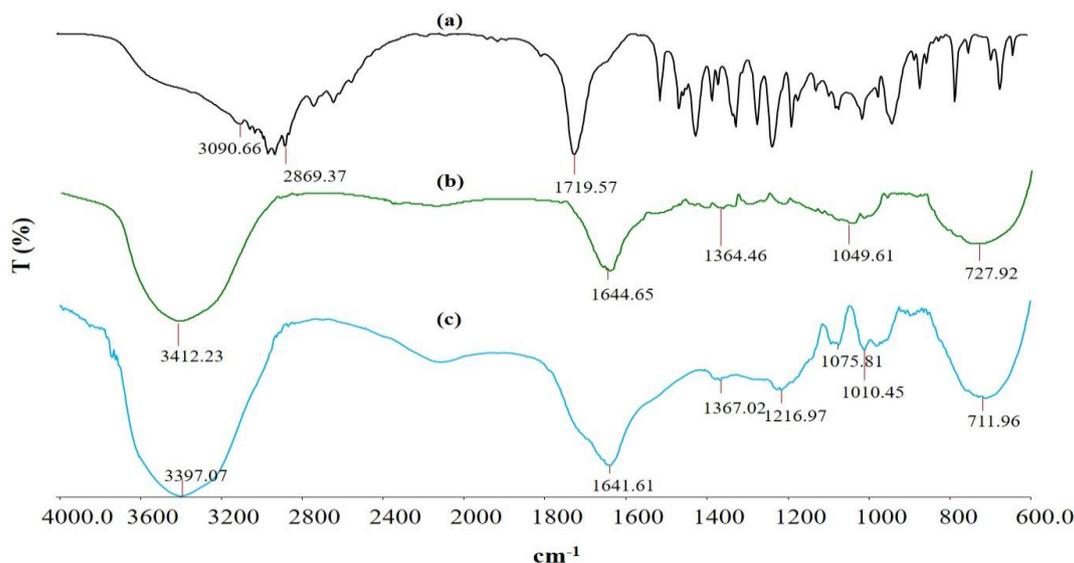
**Table 1:** Summary of the compressive strength ( $\sigma$ ), Young's modulus (YM), compressive strain ( $\varepsilon$ ), swelling (%) and WVTR ( $\text{g m}^{-2} \text{day}^{-1}$ ) of gellan gum-ibuprofen hydrogels.

Sample	$\sigma$ (kPa)	YM (kPa)	$\varepsilon$ (%)	Swelling (%)	Gel fraction (%)	WVTR ( $\text{g m}^{-2} \text{day}^{-1}$ )
Blank	-	-	-	-	-	$2536 \pm 108$
GG	$103 \pm 2$	$816 \pm 10$	$3.7 \pm 0.1$	$103 \pm 3$	$63 \pm 2$	$2210 \pm 139$
GG-IB0.5	$147 \pm 9$	$1440 \pm 100$	$4.5 \pm 0.5$	$35 \pm 6$	$64 \pm 9$	$2202 \pm 157$
GG-IB1.0	$183 \pm 10$	$2100 \pm 230$	$3.8 \pm 0.6$	$34 \pm 3$	$66 \pm 5$	$1971 \pm 112$
GG-IB5.0	$200 \pm 21$	$1820 \pm 10$	$2.5 \pm 0.2$	$22 \pm 1$	$67 \pm 1$	$867 \pm 154$



**Figure 1.** Stress-strain curves of gellan gum (GG) hydrogel, GG hydrogel incorporated with 0.5% w/w (GG-IB0.5), 1.0% w/w (GG-IB1.0) and 5.0% w/w (GG-IB5.0) of ibuprofen.

To investigate the possible interaction between GG and ibuprofen, ATR-FTIR was carried out. Figure 2 shows the shifting and disappearances of a few functional peaks in representative spectrum of GG-IB5.0 hydrogel as compared to GG hydrogel. GG hydrogel shows a few main peaks which contribute to the hydrophilicity of GG10 materials, i.e. hydroxyl (O-H) and carbonyl groups (C=O) at  $\lambda = 3412 \text{ cm}^{-1}$  and  $\lambda = 1644 \text{ cm}^{-1}$ , respectively. Moreover, the inclusion of ibuprofen to gellan gum hydrogel (GG-ibuprofen) shows the shifting of stretching vibration of O-H group in gellan gum hydrogel at  $3412.23 \text{ cm}^{-1}$  to  $3397.07 \text{ cm}^{-1}$  of GG-IB5.0, indicating the hydroxyl O-H vibration of H-bonded of gellan gum and ibuprofen [15].



**Figure 2.:** ATR-FTIR spectra of (a) ibuprofen, (b) gellan gum hydrogel (GG), and (c) GG hydrogel with 5% (w/w) ibuprofen (GG-IB5.0)

The shifting of carbonyl functional groups, i.e. C=O stretching from the glycosidic link in gellan to lower wave number was also observed GG-IB5.0 hydrogel to  $1641\text{ cm}^{-1}$  as compared to free standing GG hydrogel at  $1644\text{ cm}^{-1}$ . Band at  $\sim 1367\text{ cm}^{-1}$  for methyl C-H bending observed in GG-IB5.0 also indicated the interaction of gellan gum with the drug since the peak was absent in gellan gum free standing [16]. Moreover, the peak at  $1049\text{ cm}^{-1}$  in free standing GG was assigned for C-O stretching vibration shifted to higher wave numbers of  $1075\text{ cm}^{-1}$  also suggested the interaction between gellan gum and ibuprofen [17]. The shifting of a few functional groups in GG-IB5.0 hydrogel could support the strong bonding which might occur between both materials (GG and ibuprofen) in which consequently resulting in improved compressive strength of the hydrogels.

### 3.2. Swelling, Gel Fraction and WVTR values

The swelling properties, gel fraction and water vapor transmission rates (WVTRs) of the GG hydrogel with addition of ibuprofen is summarized in Table 1. The swelling behavior of the hydrogels was decreased upon addition of more ibuprofen. GG hydrogels absorb  $103 \pm 3\%$  of PBS (pH 7.2) and decreased to  $22 \pm 1\%$  for GG-IB5.0 hydrogels. Similar observation was reported in previous study by incorporate ketoprofen in GG hydrogel [18].

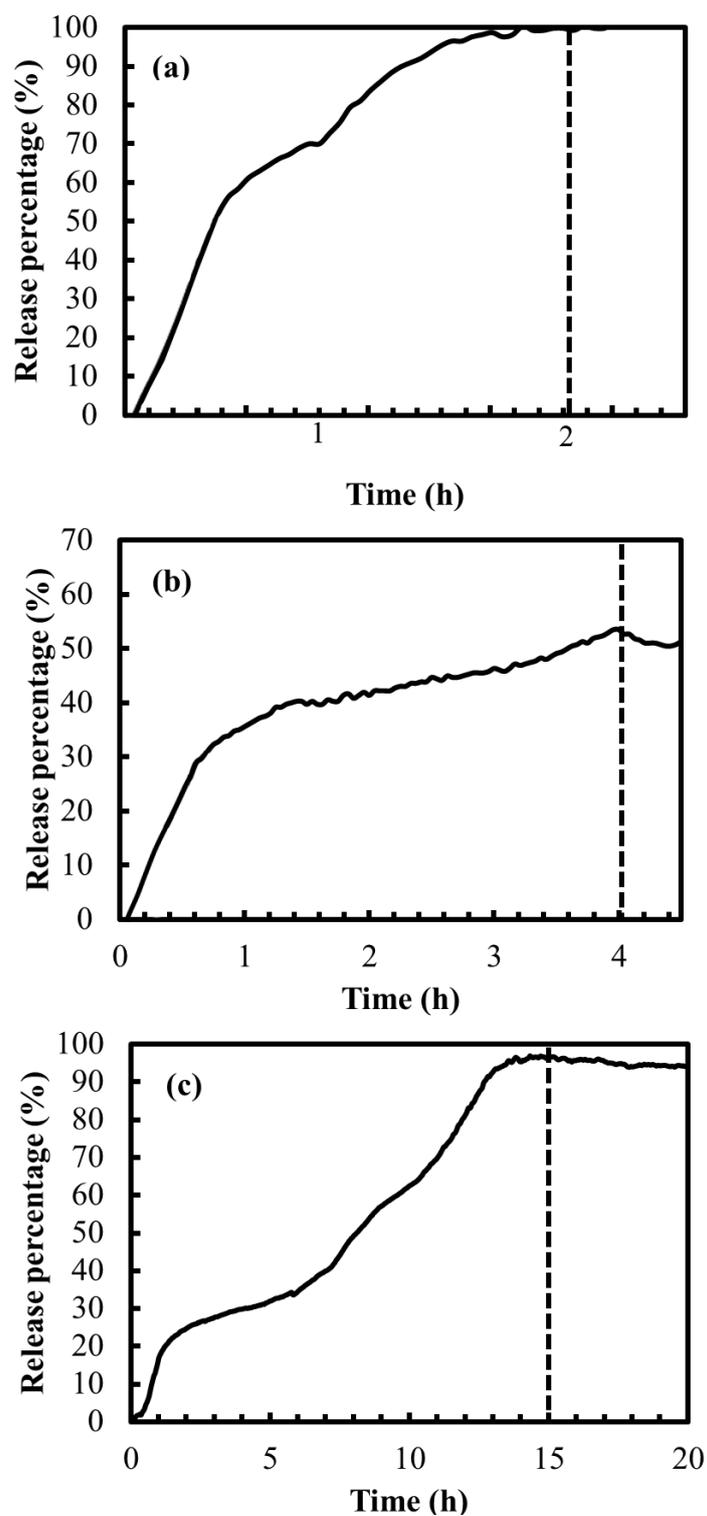
In contrast to swelling behavior, gel fraction was increased with the addition of more ibuprofen. Gel fraction is used as an indication of crosslinking between GG hydrogels and ibuprofen. Higher gel fraction with the addition of more ibuprofen resulted in more hampered swelling capability. This is due to the increase of compactness of the GG structure with the addition of more ibuprofen. The rigidity of GG structure

containing ibuprofen has increased its stability and resistance towards swelling media, thus decreased the swelling properties of GG-IB5.0 hydrogel. The crosslinking effects also diminished the mobility of polymer chains which subsequently lowers the swelling ratio [19].

The increase of gel fraction at higher amount of ibuprofen also affected the water vapor transmission rates (WVTRs) of the GG hydrogels (Table 1). WVTRs values of the GG-IB5.0 were significantly decreased to  $867 \pm 154 \text{ g m}^{-2} \text{ d}^{-1}$  than GG at  $2210 \pm 139 \text{ g m}^{-2} \text{ d}^{-1}$ . The addition of more ibuprofen caused the structure of GG hydrogel to be stronger (high gel fraction) which results in lowering the water vapor transmission rates. However, the WVTRs values obtained for the samples were still within the range reported for commercial wound dressing i.e. OpSite, Metalline, Biabrone and Omiderm at  $792 \text{ g m}^{-2} \text{ d}^{-1}$ ,  $1272 \text{ g m}^{-2} \text{ d}^{-1}$ ,  $3696 \text{ g m}^{-2} \text{ d}^{-1}$ , and  $4992 \text{ g m}^{-2} \text{ d}^{-1}$ , respectively [20].

### 3.3. Release Study

The release study has been carried out on all hydrogels samples, i.e. GG-IB0.5, GG-IB1.0 and GG-IB5.0 hydrogels (Figure 3). GG10-IB0.5 and GG10-IB1.0 hydrogels exhibit the total time needed for the drug to achieve equilibrium after 2 h and 4 h, respectively. On the other hand, GG-IB5.0 exhibits substantially good release profile in which a total of 15 h was needed for the hydrogel to reach the equilibrium of drug release. The slow drug release was possibly caused by the low swelling properties of the GG-IB5.0 hydrogel ( $22 \pm 1\%$ ). This low swelling property possesses the unique ability of the hydrogel to swell in a slow fashion over a set of time. It was observed that 96% of the total drug was released from polymeric hydrogel after 15 h. Meanwhile, GG10-IB0.5 and GG10-IB1.0 hydrogels have total percentages release of 99 % and 54 %, respectively. The release percentage of GG10-IB1.0 accounts for only half the value of its counterparts, i.e. GG-IB0.5 and GG-IB5.0, which could be due to the non-homogenous blend of gellan gum hydrogel. The amount of polymer and crosslinking agent in a formulation also can affect the release of drug into the said system [18]. Further study will be conducted to investigate the reason of such results obtained.



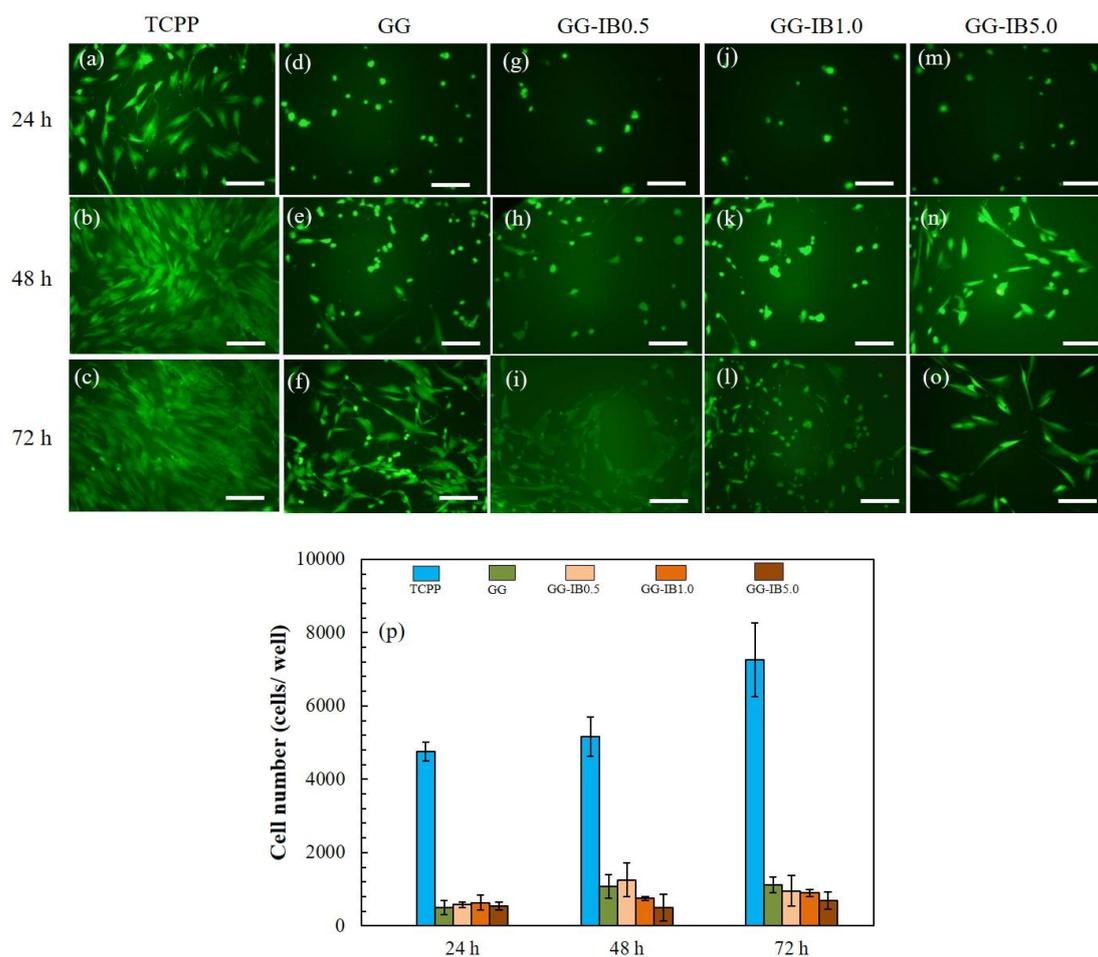
**Figure 3.** : Release percentage of ibuprofen from (a) GG-IB0.5), (b) GG-IB1.0 and (c) GG-IB5.0 hydrogels

In the realm of wound treatment involving the development of polymeric formulation using several drugs, the longer duration of drug can be released from a polymeric dose, the more favorable the system is. For instance, many studies have been reported with regard to the release profile of different active ingredients into the different systems such as tablets, films and spheres at longer duration. For example, Shoaib and co-workers reported that their tablet containing ibuprofen has been fully released to the system within 12 hours [21]. Meanwhile, another study reported that the alginate gel incorporating with ibuprofen took over 8 hours to be fully released into the system [22]. Other study reported the release behavior of ibuprofen loaded in a polymeric system consists of locus bean/xanthan gum released 60% of the total drug after 50 hours [12]. From these studies, it clearly shows that the different polymeric systems exhibit different release profiles and possibly to prolong the release of drugs into the media.

### **3.4. Cell Study**

Figure 4 shows the cell viability and cell proliferation results of GG hydrogel with ibuprofen against human dermal fibroblast cell (CRL2522, ATCC). Tissue culture polystyrene plate (TCPP) showed substantially good growth over 72 hours of incubation, where the cells were transformed to spindle and elongated shape and completely covered the bottom of the flask (Figure 4(c)). Moreover, GG hydrogels also showed a good cell growth, in which the cells were in rounded state after 24 hours and gradually elongated only after 48 hours of incubation (Figure 4(e)). In addition, this could be due to the fact that the cells were still adapting to adhering to the surface of the GG hydrogel substrate. The cells continued the differentiation process i.e. mitosis process and fully elongated after 72 hours.

GG hydrogel loaded with ibuprofen has been proven to be non-cytotoxic to CRL 2522. As can be seen from Figure 4(h) and (k), the cells cultured on GG-IB0.5 and GG-IB1.0 hydrogels elongated and were growing after 48 hours, indicating the surface of the hydrogel would be suitable and preferable for cells to grow. This situation also suggests that the release of ibuprofen from the hydrogel matrix is not toxic to the living cells. Although the population of fibroblast cells seemed to be limited after 72 hours for GG hydrogel with three ratios of ibuprofen compared to TCPP, the formulation was not toxic to cells and biocompatible to the fibroblast tested.



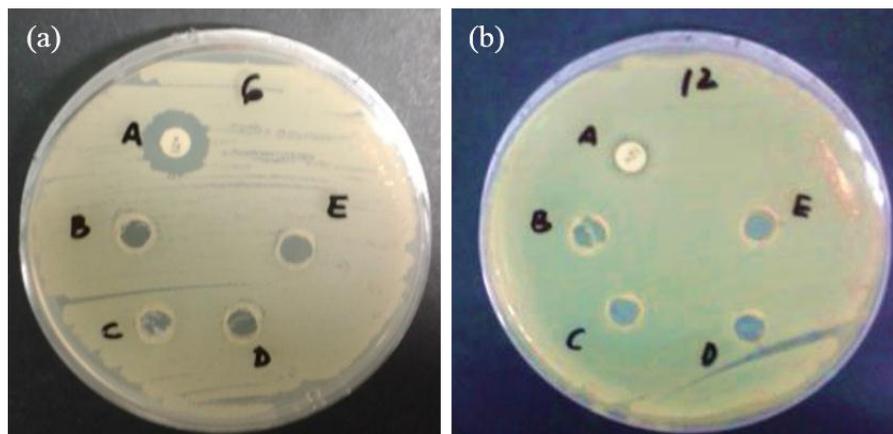
**Figure 4.:** Viability of human dermal fibroblast cells cultured on (a-c) tissue culture polystyrene plate (TCPP), (d-f) gellan gum hydrogel (GG), (g-i) GG hydrogel incorporated with 0.5% w/w ibuprofen (GG-IB0.5), (j-l) GG hydrogel incorporated with 1.0% w/w ibuprofen (GG-IB1.0), (m-o) GG hydrogel incorporated with 5.0% w/w ibuprofen (GG-IB5.0) and (p) cell proliferation of GG and GG-IB hydrogels cultured in the medium with human skin fibroblast cells (CRL2522) for 72 h. Error bars indicated standard deviation (n=3). Scale bars represent 100  $\mu$ m.

A few studies previously have reported the biocompatibility of ibuprofen. For example, a group of researchers has reported that the addition of ibuprofen as much as 100 mg/mL was able to support the survival of L929 mouse fibroblast cells [23]. In fact, as the concentration of ibuprofen declined, the protective effect of ibuprofen also decreased. However, different topical presentations of the same drug especially ibuprofen, which is intended for subcutaneous action, must not be expected to be equivalent or interchangeable with one another [24] due to different interaction between the base material and the drug itself that may vary in several key aspects, hence causing different effects on the cells tested.

To enumerate the cell growth on GG hydrogels with ibuprofen, cell proliferations were quantified by using Celltiter assay after being incubated for 24 h, 48 h and 72 h (Figure 4(p)). GG hydrogels show that the cell number increased up to 2-fold from 500 cell/well to 1100 cells/well after being incubated for 72 h. Meanwhile, for GG-IB0.5, GG-IB1.0 and GG-IB5.0 hydrogels, the cell growth slightly depleted after cultured for 72 h to 1260 cells/well, 900 cells/well and 700 cells/well, respectively. From these results, it shows that the higher loading of ibuprofen in GG hydrogel caused the decrease in cell growth (incubated for 72 h), i.e. GG-IB0.5 hydrogel exhibiting the highest cell number as compared to GG-IB5.0 hydrogels. Based on the previous study reported by Hockertz and co-workers, using 100 mg/mL ibuprofen and the fibroblast cells were able to survive in media. In our study the concentration of ibuprofen used for GG-IB5.0 hydrogel is 2.3 mg/mL, in which much smaller than the concentration used by Hockertz and co-workers. Because of that, we assume that the highest cell growth obtained for GG-IB0.5 hydrogels is due to the fact that GG-IB0.5 has the highest swelling percentage as compared to its counterparts. Also, the ability of substrates to support cell growth is closely related to its swelling capability and it was observed that wettable surfaces could increase adsorption of serum proteins which subsequently enhanced cell growth [25]. Despite the growth of cells on gellan gum hydrogel loaded with ibuprofen was observed relatively lower than that of gellan gum free- standing, cells were viable and therefore can be deduced that hydrogel loaded with ibuprofen is non-cytotoxic to CRL 2522.

### 3.5. Antibacterial Study

Qualitative in-vitro antibacterial results show that the GG-IB5.0 hydrogels were the only samples inhibited the Gram-positive bacteria, i.e. *S. aureus* with an inhibition of  $9.7 \pm 1.15$  mm as other samples did not inhibit the growth of *S. aureus* nor *E. coli* (Figure 5). As suggested by the previous study, although ibuprofen showed antibacterial properties, its antibacterial action was not clear for many species of pathogenic bacteria [26]. For instance, a study has reported the antibacterial property of ibuprofen against several bacterial isolates including *S. aureus* and *E. coli* cultured in Muller Hinton (MH) broth [26]. They noted that *S. aureus* is more susceptible to ibuprofen and acetaminophen at lower MIC of 1.25 mg/mL than other isolates. *E. coli* on the other hand, is susceptible to ibuprofen at the higher minimum inhibitory concentration (MIC) value i.e. 2.5 mg/ml. These data indicate that higher concentration of ibuprofen is needed to inhibit the isolates of *E. coli* compared to *S. aureus*. Therefore, we can conclude that higher concentration of ibuprofen in GG hydrogel is needed to successfully combat the pathogen especially *E. coli*.



**Figure 5.** Qualitative antibacterial property of gellan gum hydrogel incorporated ibuprofen against (a) *Staphylococcus aureus* and (b) *Escherichia coli*. The hydrogel samples were filled in 6 mm diameter on Muller Hinton agar and labeled as follow; A) Penicillin disc, B) GG, C) GG-IB0.5, D) GG-IB1.0, and E) GG-IB5.0.

#### 4. CONCLUSION

The gellan gum (GG) hydrogel incorporated ibuprofens were successfully prepared. The addition of ibuprofen improved the compressive stress of the hydrogels at a cost of decreased the compressive stain. Due to compact and rigid structure of GG hydrogel at higher amount of ibuprofen, the gel fraction values were increased. In contrast, the swelling properties and water vapor transmission rates of the hydrogels were decreased. The release profile of GG hydrogels with 5% (w/w) ibuprofen (GG-IB5.0) exhibits substantial duration of 15 h to be fully released due to low swelling capabilities. The cell studies show the GG incorporated ibuprofen were non-cytotoxic against human skin fibroblast cell (CRL2522). While, for antibacterial activity, the GG-IB5.0 hydrogel inhibits the growth of *S. aureus* but not against *E. coli* due to low concentration of ibuprofen used. The slow release of ibuprofen from the GG hydrogel, together with the excellent mechanical properties, antibacterial property and biocompatibility could render this hydrogel as the wound dressing material to treat slow and moderately exuding wounds.

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#### CONFLICT OF INTERESTS

The authors confirm that this article content has no conflict of interest.

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