Composite alginate and gelatin based bio-polymeric wafers containing silver sulfadiazine for wound healing

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ABSTRACT

Lyophilized wafers comprising sodium alginate (SA) and gelatin (GE) (0/100, 75/25, 50/50, 25/75, 0/100 SA/GE respectively) with silver sulfadiazine (SSD, 0.1% w/w) have been developed for potential application on infected chronic wounds. Polymer-drug interactions and physical form were characterized by Fourier transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD) respectively, while morphological structure was examined using scanning electron microscopy (SEM). Functional characteristics [(mechanical hardness and adhesion using texture analyzer, and swelling capacity)] of blank wafers were determined as performed in order to select the optimal formulations for drug loading. Finally, the *in vitro* drug dissolution properties of two selected drug loaded wafers were investigated. There was an increase in hardness and a decrease in mucoadhesion with increasing GE content. FTIR showed hydrogen bonding and electrostatic interaction between carboxyl of SA and amide of GE but no interaction between the polymers and drug was observed, with XRD showing that SSD remained crystalline during gel formulation and freeze-drying. The results suggest that 75/25 SA/GE formulations are the ideal formulations due to their uniformity and optimal mucoadhesivity and hydration. The drug loaded wafers showed controlled release of SSD over a 7 hour period which is expected to reduce bacterial load within infected wounds.

Keywords: Bio-polymeric; Wafers; Wound healing,

1. Introduction

In recent years, natural biopolymers such as alginate, collagen and chitosan have been studied because of their importance in formulation of different dressings for healing of burns and other types of wounds. This is due to several favorable characteristics including biocompatibility, biodegradability and some structural similarities with human tissues, as well as their implication in the repair of damaged tissues and consequently skin and tissue regeneration [1,2,3].

Alginate is an anionic polysaccharide, extracted from brown algae (Phaeophyceae) or obtained by bacterial biosynthesis from *Azotobacter* and *Pseudomonas spp*. It is composed of (1,4)-linked β-D-mannuronate (M) and α-L-guluronate (G) residues linked in homopolymeric blocks (---MMM--- or ---GGG---) or random blocks (---MGMG---). Depending on the block content, length and distribution in the polymeric chain, alginates possess different physical, chemical and gelling properties [4]. Alginate dressings are characterized by the formation of a gel due to the exchange between the ions present in the dressing and wound exudate [5]. This gel creates a moist environment that promotes healing and facilities easy removal [6]. This together with its high tissue compatibility, low toxicity and good mucoadhesive properties allow alginates to be used as biomaterials for wound dressings [7]. The impact of cross-linker cations such as Na^{+}, Ca^{2+}, Cu^{2+} or Zn^{2+} in modifying dressings' functional wound healing characteristics such as tensile strength and hydration has been reported [8]. However, with time, hydrated alginate can lose the cation cross-linkers, resulting in gel degradation. Therefore, it has been recommended to combine alginates with other biopolymers such as gelatin or chitosan in a single formulation [9].

Gelatin is a denatured protein from the triple helix of collagen. In solution, the chains are converted from random spirals at moderate temperature to helices once the temperature decreases below ambient, thus behaving as a gel [10]. Ideal characteristics such as biodegradability, ease of processing and its antigenic activity in physiological environments have resulted in the wide use of gelatin in biomedical applications. It also provides hemostasis and facilitates cell adhesion and proliferation during wound healing [11]. However, poor mechanical properties and low thermal stability have been described as some of the disadvantages of this biomaterial which can be improved by cross-linking and / or combining with other polymers [12]. Balakrishna and co-workers developed a hydrogel dressing based on the beneficial properties of oxidized alginate, gelatin and borax with the purpose of making a potential dressing that maintains a moist wound environment [13]. It has also been reported that oxidized alginate could be successfully utilized to stabilize gelatin films and therefore improve their mechanical properties [14].

Metal antimicrobials have been used over the years to combat bacterial infection with silver being the most common metal based antimicrobial in medicated wound dressings. At an appropriate concentration, silver shows broad spectrum activity against fungal and bacteria cells including methicillin resistant *Staphylococcus aureus* (MRSA) and vancomycin resistant *Enterococci* (VRE) [15]. Different products have been developed with silver, such as foams (Contreet F®), hydrocolloids (Contreet H®), alginates (Anticoat absorbent®) and films (Arglaes®) with indications for burns and heavily colonized wounds [16,17]. However, these products are all single polymer matrix systems which do not always control drug release appropriately as well as exhibit optimal functional properties such as adhesion, swelling and mechanical strength. The use of composite

dressings, combining more than one polymer with enhanced physical-mechanical characteristics has gained recent interest [2,3].

The aim of this study therefore, was to formulate freeze-dried wafers from gels combining different ratios of sodium alginate (SA) and gelatin (GE) and loaded with silver sulphadiazine (SSD) for potential application to infected wounds. Different analytical techniques have been used to characterize the functional physico-chemical properties of the starting polymers and wafer formulations, including scanning electron microscopy (SEM), X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), as well as texture analysis ('hardness', and adhesion), swelling and *in vitro* drug dissolution studies.

2. Experimental

2.1. Materials

Silver sulfadiazine [SSD, (batch number: 48118156)], Pluronic [F68, (batch number: 020M0029)], calcium chloride (batch number: 1291383) and trismethylamine (batch number: 87203010) were obtained from Sigma-Aldrich (Steinnheim, Germany). Sodium alginate [SA, (batch number: 0804532)] and sodium chloride (batch number: 1095753), were purchased from Fisher Scientific (Leicestershire, UK). Gelatin [GE, (batch number: 54008P03)] was obtained from Fluka Analytical (Steinheim, Germany).

2.2. Preparation of gels and freeze-dried wafers

Blank (BL) and SSD loaded (DL) gels were prepared with varying concentrations of SA and GE while keeping the amount of the other components (SSD and pluronic acid - F68) constant (Table 1).

The gels of SA and GE were prepared by dispersing the polymers and surfactant (F-68) in hot distilled water (50ºC) with continuous stirring until they were completely dissolved. For gels containing SSD, the drug was first dispersed into the vortex of hot surfactant solution (50ºC) before adding the different polymers (SA and GE). 7 g each of gel was transferred into 6 well plates (diameter 35 mm) and lyophilized using a Virtis Advantage XL 70 freeze dryer (Biopharma Process System, Winchester, UK) in automatic mode. The lyophilization procedure involved freezing the gels in a series of thermal ramps to -50°C over 7 hours (freezing phase), then heating during the primary drying phase to sublimate the ice under vacuum at -15°C (24 hours), followed by secondary heating at 20°C for 7 hours.

2.3. Visual evaluation and scanning electron microscopy (SEM)

The wafers were visually evaluated by capturing digital images of the different formulations. Further, the wafers were examined microscopically under low vacuum by a Jeol JSM-5310LV scanning microscope to obtain high-resolution surface information of their morphological structure. The samples were cut into small, thin pieces and placed on double-sided carbon tape on 15 mm aluminum stubs. Sample images were acquired at magnifications of ranging from x50 to x200.

2.4. X-ray diffraction (XRD)

A D8 Advance X-ray diffractometer (Bruker, Coventry, UK) equipped with Lynx Eye detector was employed to determine the crystalline or amorphous nature of the different pure starting materials and within the formulated wafers. The freeze-dried wafers were compressed using a pair of cover glasses to a size of 0.3 mm and introduced into the sample holder. All the samples were scanned between 2 theta of 5º and 45º with a step

size of 0.02 and a scan speed of 0.4 s. The same process was repeated for SSD, SA, GE and F-68.

2.5. Attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy

FTIR spectra of wafers and the different starting materials were acquired on a FTIR spectrophotometer (Thermo Nicolet, Thermoscientific, UK) combined with ZnSe attenuated total reflectance (ATR) crystal accessory based on a previously reported method [18]. After the crystal area had been cleaned, the material was placed on the ATR crystal and pressed by a pressure clamp positioned over the crystal/sample area to allow optimal contact between the material and the ATR crystal. The spectra were collected at a resolution of 4 cm^{-1} over a range of 650 to 4000 cm^{-1}.

2.6. Swelling study

This test determined the maximum hydration capacity of the wafers in simulated wound fluid (SWF). The SWF contained 0.02 M calcium chloride, 0.4 M sodium chloride and 0.08 M tris methylamine in deionized water. To adjust the pH of the solution, 2 M of hydrochloric acid (HCl) was added until a pH of 7.5 was attained [19]. Samples (n=4) were initially weighed and immersed into 20 ml of SWF at 37ºC. The change in weight of the wafers was measured every 15 minutes up to 120 minutes to observe the swelling behavior. At each time point, the hydrated wafers were carefully removed, blotted and then reweighed. Calculation of the percentage swelling index I_s (%) was determined using the following equation:

I_s (%) = (W_s – W_d / W_d) x 100

Where W_d is weight of the wafer before hydration and W_s indicates weight of the wafers after hydration.

174 *2.7. Texture analysis*

175 *2.7.1. Mechanical hardness*

176 A TA HD plus Texture analyzer (Stable Micro Systems Ltd., Surrey, UK) was employed
177 to select which formulations possessed optimal flexibility and determine how the drug
178 content can affect mechanical 'hardness' (resistance to compressive deformation) and
179 ease of recovery, compared with BL wafers [20]. Before compression, the probe height
180 was properly calibrated. Four different samples of each wafer formulation were
181 compressed with a 6 mm probe at five different locations, on both sides of the wafer to a
182 penetration depth of 2 mm, at a speed of 1 mm/s, with a 10 mm return distance.

183

184 *2.7.2. In-vitro adhesion studies*

185 *In vitro* wound adhesion studies were performed on four wafers of each sample with a
186 TA HD plus Texture analyzer (Stable Micro Systems, Surrey, UK) fitted with a 5 kg load
187 cell. The wafer was attached to the upper arm of a 75 mm diameter probe on the texture
188 analyzer machine using double sided adhesive tape. A 90 mm diameter Petri dish
189 containing 20 g of gelatin solution (6.67% w/w) allowed to set; was equilibrated with 0.5
190 ml of SWF (prepared above) to represent the wound surface as previously reported [3].
191 The experiment was performed by lowering the probe until the wafer made contact with
192 the set gelatin gel surface for 60 seconds to provide optimal contact. The probe was set at
193 a pre-test and test speeds of 0.5 mm/s and post-test speed of 1 mm/s applying a force of
194 1 N. The peak adhesive force (PAF) required to break the adhesive bond between the
195 wafer and the simulated wound surface was determined by the maximum force, the total
196 work of adhesion (WOA) was obtained from the area under the force-distance curve
197 (AUC), while cohesiveness which describes the flexibility of formulation was estimated

by the total distance (in mm) travelled by the probe before complete detachment of the wafer from the gelatin gel surface.

2.8. In vitro drug dissolution studies

In vitro drug dissolution studies were performed with a Franz diffusion cell across a wire mesh with SWF (pH 7.5) as dissolution media in the receptor compartment. The DL wafers (SA/GE 75/25 and 25/ 75) containing SSD was placed on the wire mesh. The temperature of the diffusion cell was maintained at 37±0.5°C by a circulating water jacket and the dissolution medium was constantly stirred throughout the experiments using magnetic beads on a magnetic stirrer. At predetermined time intervals, 0.5 mL aliquots of dissolution media were withdrawn and analyzed by HPLC (section 2.9 below) and replaced with the same amount of SWF to maintain a constant volume throughout. The release of SSD (µg) from the wafers was calculated and plotted against time.

2.9 HPLC analysis

This was performed using an Agilent 1200 HPLC equipped with an auto sampler (Agilent Technologies, Cheshire, UK,) and a Chemstation® software program. The stationary phase consisted of a C18 (250 x 4.6 mm, 10µm) column (HPLC Technology, UK). The mobile phase consisted of water: acetonitrile: phosphoric acid (90: 9.9: 0.1 % v/v), the flow rate of the mobile phase was maintained at 1.0 ml/min and detector wavelength at 254 nm and 20 µl volumes injected during each run. Standards from 1-10µg/ml were used to plot a calibration curve for SSD (r^2 = 0.998) and used to determine the drug loading efficiency (%) of SSD within the optimized formulations used for drug dissolution studies.

2.10. Statistical analysis

To compare BL and DL wafers statistical data evaluation was performed using two tailed student t-test at 95% confidence interval (p-value < 0.05) as the minimal level of significance for 'hardness', swelling and adhesion data.

3. Results

3.1. Morphology of freeze-dried wafers

Digital photographs of the different wafers formulated are shown in Fig. 1. It can be observed that wafers with higher ratios of SA/GE 100/0 (A and F) and 75/25 (B and G) possessed a smooth surface with a uniform texture as well as optimal balance between flexibility and toughness, making them suitable for easy application to wound sites. On the contrary, SA/GE 50/50 (C and H), 25/75 (D and I) and 0/100 (E and J) formulations were very sticky, making them difficult to remove without damaging the wafers. In addition, 0/100 SA/GE gels resulted in rigid wafers with a non-porous texture, which caused dramatic decrease in the thickness of the wafers, making them unsuitable for wound application. A rough appearance was observed for DL wafers with high concentration of GE (H and J) due to the formation of crystallized particles on the top the wafers. In addition to their rough appearance, there was an increase in brittleness with a consequent loss of flexibility which was deemed non-ideal.

3.2 Scanning electron microscopy (SEM)

The SEM results showed that all the formulations possessed a porous morphology, except in the case of 0/100 SA/GE formulations which were completely non-porous at the bottom and showed low porosity at the top, as is shown in Fig. 2A-B. Wafers with higher concentration of SA formed interconnecting polymeric networks with small, elongated,

and uniform pores (Fig. 2C) whilst wafers containing higher concentrations of GE also formed a polymeric network but with larger, non-uniform and circular shaped pores (Fig. 2D). These results seem to confirm the visual observations made from the digital photographs in Fig. 1. Addition of the drug resulted in wafers with a crystalline appearance due to the deposition of the silver metal among the strands of the polymeric network (Fig. 2E-F). It can also be observed that while, BL 25/75 SA/GE (Fig. 2D) wafers possessed regular and circular shaped pores, the addition of the drug resulted in larger, irregular and hexagonal shaped pores with thinner strands. Such changes in microstructure are known to impact on other physical properties such as 'hardness' and hydration [3].

3.2. X-ray diffraction (XRD)

Fig. 3a shows the diffractograms of all the different components of the wafers. Both GE and SA possessed a completely amorphous structure, as shown by the low count numbers. The diffractograms of F-68 and SSD revealed typical crystalline peaks due to their ordered structure. XRD diffractograms of all the wafers (Fig. 3b and Fig. 3c) showed low intensity peaks at 19º and 23.5º that were attributed to the pluronic F-68. In addition, the small peaks at 13.5º and 21.5º for SA disappeared, and intensified at 19º and 23.5º with the addition of GE. In the case of DL wafers, the characteristic peaks of the silver at 8º, 10º were observed confirming the crystallinity as observed from the SEM results. Further, the intensities of these peaks were more intense in DL loaded 50/50 and 0/100 SA/GE wafers suggesting that GE contributes more to wafer crystallinity and therefore modifies the physical properties of SA wafers.

3.3. Fourier transform infrared (FTIR) spectroscopy

The FTIR spectrum (Fig. 4a) of SA showed peaks around 1652 cm^{-1} and 1456 cm^{-1} representing the asymmetric and symmetric stretching vibration respectively of the carboxylic acid. Other peaks were observed at 3270 cm^{-1} and 1116 cm^{-1}, due to OH and C-O-C stretching vibration. On the other hand, GE spectrum revealed the presence of an amide I and II band for primary amide at 1648 cm^{-1} and 1540 cm^{-1}, respectively. The amide I band is due to C=O stretching while the amide II band is attributed to NH bending vibration. It also exhibited C-O-C stretching at 1116 cm^{-1}. The spectrum of SSD showed an intense and well defined peak at 1224 cm^{-1} characteristic of SO_2 asymmetric stretching. Other peaks were observed for the SSD at 3384 cm^{-1}, 1594 cm^{-1} and 1548 cm^{-1} which were assigned to NH stretching of phenyl and pyrimidine skeletons respectively.

As shown in Fig. 4b, 100/0 SA/GE wafers showed an absorption band of the carboxyl group at 1600 cm^{-1} while this absorption band for 75/25, 50/50 and 25/75 SA/GE was observed at 1596 cm^{-1}, 1644 cm^{-1} and 1646 cm^{-1}, respectively. It can be also seen that the C-O-C stretching still remained with a peak of 1031 cm^{-1} approximately for all the formulations. The presence of the SO_2 asymmetric stretching at 1232 $cm^{-1}$from SSD was retained in all DL formulations without any shifts (Fig. 4c).

3.4. Swelling studies

Fig. 5 shows the percentage swelling index of BL and DL wafers. The results show maximum hydration within 90 minutes for all the BL formulations. BL formulations containing only SA possessed a relatively higher swelling capacity with a maximum of 2299.79 ± 151.29%. On the other hand, increase in the content of GE caused a decrease in the swelling of the wafers, except for 75/25 SA/GE which showed a maximum value of 2210 ± 231.32% with statistically non-significant difference (p = 0.594). This change

298 in swelling with GE confirms the observations from XRD where GE appears to increase
299 the crystalline nature of the wafers. No statistically significant differences were observed
300 between BL and DL loaded wafers ($p > 0.05$) at the maximum swelling value, except for
301 25/75 SA/GE. It also is interesting to note that the addition of SSD in this formulation
302 (25/75 SA/GE) improved the hydration capacity with a maximum value in 15 minutes of
303 1842.53 ± 295.57%, compared to 934.30 ± 114.33% for the BL wafers, which was a
304 significant difference (p value = 0.02).
305
306 *3.5. Texture Analysis*
307 *3.5.1. Mechanical characteristics*
308 Fig. 6 shows the differences in 'hardness' (resistance to compressive deformation) values
309 between top and bottom of the BL and DL wafers when compressed at five different
310 locations on both sides ($n = 4$). This difference could be due to the freeze-drying process
311 where the polymer density might be higher at the bottom of the container, than at the top.
312 This is possible because the shelf-type freeze-dryer used caused freezing to start from the
313 bottom of the gel upwards. This could also explain the differences observed in
314 morphology between the top and bottom of the wafers. However, this may require further
315 investigation. In addition, the data suggests that the increase of GE resulted in an increase
316 in the 'hardness' and hence decreased the flexibility of the wafers, which could affect
317 swelling and mucoadhesion performance. Addition of SSD resulted in an increase in the
318 peak resistance to compression due to the concentration of crystals, except in 25/75
319 SA/GE where the silver appears to be lodged among the thinner walls of the polymeric
320 network, resulting in a reduction of the hardness. Statistically significant differences ($p <$
321 0.05) in 'hardness' between top and bottom were observed for all formulations except for
322 75/25 SA/GE SSD ($p = 0.78$).

3.5.2. In-vitro adhesion studies

Fig. 7 shows the peak adhesive force (PAF), the work of adhesion (WOA) and the cohesiveness that was required to detach the BL and DL wafers from the simulated wound surface. Results for the BL wafers showed that an increase in GE decreased the PAF and WOA which could be explained by the SA-GE interactions. DL wafers showed a decrease in the PAF and an increase in the WOA and cohesiveness compared with BL wafers. Although the difference in PAF and WOA was not statically significant ($p > 0.05$), all the different formulations exhibited significant differences for the cohesiveness ($p < 0.05$). It can be seen that DL loaded 25/75 SA/GE wafers possessed the highest cohesiveness (5.62 ± 0.25mm) which could be attributed to the increase of the apparent pore size with the addition of the drug which is expected to result in rapid initial hydration and entanglement which are important for mucoadhesion.

3.6 In vitro drug dissolution studies

HPLC was used to assay the drug loading within the wafers and to determine the amounts of drug released with time during prior to dissolution study. The drug loading efficiency for 25/75 SA/GE and 75/25 SA/GE was 80% and 93% respectively and the difference here is largely due to the latter being more flexible and easier to remove from the mould with smaller loss of material. The drug dissolution profiles for optimized DL wafers (n = 3) are shown in Fig. 8 showing controlled drug release over a 7 hour period. The formulations containing higher percentage of GE appear to release the drug more rapidly in the initial stages compared to the wafers containing higher proportions of SA though the differences do not appear marked based on the error bars.

4. Discussion

SA is a common excipient that has been used in pharmaceutical formulations such as hydrogels and wafers, due to its structure. On the contrary, GE possesses certain disadvantages such as thermal instability and poor mechanical strength. However, some studies have reported that these limitations can be improved by combining with alginates [21,22]. The differences in pore size (SEM) between the BL and DL wafers have been reported to affect mechanical, hydration and adhesive properties of the different wafer formulations [20]. The changes in XRD patterns with increasing ratios of GE can be attributed to the interaction between GE and SA, where the GE alters the molecular packing of SA and produces an ordered structure with the formation of crystallites [21]. It appears from the FTIR data that the addition of GE caused a shift of the carboxyl peak towards a higher wave number, which indicated an interaction between the carboxyl group of SA and the amide group of GE. This is specifically a reaction between the positive charges from the amino groups of GE and the negative charges from the carboxyl group of SA [23]. Furthermore, these changes could also be related to the molecular interaction between SA and GE chains via hydrogen bonding and electrostatic attractions [24]. Such interactions can affect swelling and adhesion behavior of the wafers. The presence of the SO_2 asymmetric stretching at 1232 $cm^{-1}$from SSD was retained in all DL formulations without any shifts which was in agreement with the XRD results, where the crystalline form of the SSD was present in DL wafers, and confirms that there was no interaction between the drug and the polymers and that the drug maintained its original structure within the wafers. It has been reported that the effective bactericidal properties of SSD is caused by the slow interaction of silver ions with negatively charged proteins, RNA and DNA present in the pathogen cell wall [25].

373 Alginate is a weak polyacid, with pK_a values of 4.0 and 3.5 for 1,4 linked β-D-mannuronic
374 acid units and 1,4 linked α-L-guluronic acid units, respectively. GE is a polymeric
375 ampholyte with carboxyl (COOH) and amido (NH) groups consistent with its protein
376 nature with an isoelectric point of pH 4.9. Under the SWF conditions (pH 7.5), both SA
377 and GE exist as polyanions owing to the ionization of the carboxyl groups while the
378 amido of GE remains un-ionized [26]. The higher swelling index for formulations
379 containing higher amounts of SA can be attributed to its anionic nature, which can
380 produce anion-anion repulsive forces among the chains. This transfers mobility and
381 therefore an extension in the polymeric network, which results in an increase in the degree
382 of hydration [27,28]. In combination with FTIR results, we suggest that this reduction in
383 swelling capacity could be attributed to higher interaction between SA and GE with the
384 consequent reduction in availability of COO^- ions to form H bonding and electrostatic
385 interaction with the SWF. Wafers with no SA (0/100 SA/GE) showed the minimum
386 hydration capacity due to their poor flexibility and possibly smaller pore sizes but the
387 latter will, require confirmation with porositometry data.
388
389 The increase in swelling capacity for DL 25/75 SA/GE wafers could be associated with
390 the formation of thinner and large pore strands showing deposition of silver on the walls,
391 allowing a faster ingress of water and therefore increasing its hydration capacity. After
392 15 minutes, this formulation formed a gelatinous mass which affected its stability and
393 therefore started to disintegrate with resultant flattening of the swelling values. These
394 results demonstrate that the SA/GE wafers have a high holding capacity for wound
395 exudate and can subsequently be used for moderate to highly exuding wounds [19,29].
396

Hardness and mucoadhesion studies were performed in order to select the optimal formulation. However, wafers containing GE without SA (0/100 SA/GE), were not included in the hardness and mucoadhesion experiments owing to their poor flexibility which made them difficult to handle. The hardness results along with SEM, confirmed that 75/25 SA/GE wafers possessed the most favorable properties for wound healing due to the integrity, flexibility, uniformity and easy handling properties. This is important as there is always the need to avoid hard and brittle dressings which can cause trauma and damage to newly formed skin cells on a healing wound surface [6].

Adhesion plays an important role in determining an ideal wound dressing as it improves the bioavailability of the drug by increasing the retention time at the wound site as well as reducing the need for frequent dressing change which can result in patient non-compliance. Flexibility, presence of chemical groups, charge and hydration of the polymers, have been reported as factors that can affect the adhesion of any delivery system [30,31,32]. If polymer-polymer interactions are greater than the polymer-fluid interaction, there will be fewer possibilities for interaction between the free groups of the polymers and the ions present in SWF [33]. Moreover, as was observed from the swelling studies, the increase in GE, resulted in reduced availability of ionized groups (COO^-) at alkaline pH, resulting in weaker H-bonding and ionic interactions with the SWF.

Although 25/75 SA/GE wafer did not possess the highest swelling capacity, its larger pore sizes (SEM results) allowed a higher initial hydration at the beginning of the adhesion process between the surface of the wafer and the SWF. This makes the wafer sticky and more difficult to be fully separated from the wound surface, resulting in a higher distance of travel (cohesiveness) in mm. On the other hand, 75/25 SA/GE wafers exhibited the most optimal adhesion results possibly due to their high flexibility which

enhanced the possibilities of diffusion of the salts of the SWF within the polymer and therefore an improvement of the H-bonding and ionic interactions and optimal entanglement with the model wound surface.

To ensure effective antibacterial action, it is important to ensure adequate initial release of loaded drug as well as sustained release over a reasonable period of time, ultimately resulting in rapid wound healing. The increased initial drug release in formulations containing higher amounts of GE, could be explained by the higher initial hydration and swelling, which therefore enhanced ultimate drug diffusion from the swollen gels. However, as observed in the swelling profiles, this was reversed with time, and the formulations with higher amounts of SA eventually released drug faster than the wafers containing higher amounts of GE. Overall, however, the total amount of drug released within 7 hours is higher than the reported MIC values of SSD [34] against common infection causative bacteria and expected to significantly reduce bacterial bio-burden as well as prevent re-infection during the period of application. This should in turn result in more rapid healing of infected wounds. However, this will need to be confirmed in an *in vitro* antibacterial study for both Gram positive and negative bacteria commonly found in infected wounds such as *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* [3].

5. Conclusions

Composite bio-polymeric lyophilized wafers comprising different ratios of SA and GE and loaded with SSD have been formulated and functionally characterized for potential wound healing application. XRD and FTIR results revealed polymer-polymer interaction between SA and GE, which affect swelling and mucoadhesion properties but there was

no interaction between the polymer and SSD, which maintained its crystalline structure within the wafers. The results show that BL 75/25 SA/GE wafers were uniform, flexible and stable with optimal hydration (swelling) and adhesivity which can be used for wound healing. However, 25/75 SA/GE formulations increased in swelling capacity with addition of SSD which implies a potential use in moderate to highly exuding wounds. The DL wafers (75/25 and 25/75 SA/GE) showed controlled release of SSD over a 7 hour period which is expected to reduce bacterial bio-burden in infected wounds.

References

[1] G.D. Mogoşanua, A.M. Grumezescu, Natural and synthetic polymers for wounds and burns dressing, Int. J. Pharm. 463 (2014) 127– 136.

[2] H.V. Pawar, J. Tetteh, JS Boateng, Preparation, optimization and characterization of novel wound healing film dressings loaded with streptomycin and diclofenac, Coll. Surf. B: Biointerf. 102 (2013) 102–110.

[3] H.V. Pawar, J. Tetteh, J.S. Boateng, Multi functional medicated lyophilized wafer dressing for effective chronic wound healing, J. Pharm. Sci. 103(6) (2014) 1720 - 1733.

[4] R. Pereira, A. Carvalho, D.C. Vaz, M.H. Gil, A. Mendes, P. Bártolo, Development of novel alginate based hydrogel films for wound healing applications, Int. J. Biol. Macromol. 52 (2013) 221-230.

[5] A. Thomas, K.G. Harding, K. Moore. Alginates from wound dressings activate human macrophages to secrete tumor necrosis factor-α. Biomater. 21 (2000) 1797-1802.

[6] J.S. Boateng, K.H. Matthews, H.N.E. Stevens, G.M. Eccleston. Wound Healing Dressings and Drug Delivery Systems: A Review. J. Pharm. Sci. 97(8) (2008) 2892-2923.

[7] A. Saarai, V. Kasparkova, T. Sedlacek, P.A. Saha, Comparative Study of Crosslinked Sodium Alginate/Gelatin Hydrogels for Wound Dressing. In: Recent Researches in Geography Geology, Energy, Environment and Biomedicine (eds: N. Mastorakis et al. WSEAS Press, Greece, (2011) 384-389.

[8] C.H. Goh, P.W.S. Heng, L.W. Chan, Cross-linker and non-gelling Na+ effects on multi-functional alginate dressings. Carbo. Polym. 87(2) (2012) 1796–1802.

[9] L.I.F. Moura, A.M.A. Dias, E. Carvalho, H.C. de Sousa, Recent advances on the development of wound dressings for diabetic foot ulcer treatment- A review. Acta Biomater. 9 (2013) 7093-7114.

[10] M. Panouille, V. Larreta-Garde, Gelation behavior of gelatin and alginate mixtures. Food Hydrocoll. 23 (2008) 1074-1080.

[11] A. Saarai, V. Kasparkova A, T. Sedlacek, P. Saha, On the development and characterization of crosslinked sodium alginate/gelatin hydrogels. J. Med. Behav. Bio. Med. Mater. 18 (2013) 152-166.

[12] A.J. Kuijpers, G.H.M. Engbers, J. Krijgsveld, S.A.J. Zaat, J. Dankert, J. Feijen, Cross-linking and characterization of gelatin matrices for biomedical applications. J. Biomater. Sci. Polym. 11(3) (2000) 225–243.

[13] B. Balakrishnam, M. Mohanty, P.R. Umaskankar, A. Jayakrishnan, Evaluation of an in situ forming hydrogel wound dressing based on oxidized alginate and gelatin. Biomater. 26 (2005) 6335-6342.

[14] E. Boanini, K. Rubini, S. Panzovolta, A. Bigi, Chemico-physical characterization of gelatin films modified with oxidized alginate. Acta Biomater.

6 (2010) 383-8.

[15] B.S. Atiyeh, M. Costagliola, S.N. Hayek, S.A. Dibo, Effect of silver on burn wound infection control and healing: Review of the literature. Burns 33 (2007) 139-148.

[16] M.J. Carter, K. Tingley-Kelley, R.A. Warriner, Silver treatments and silver-impregnated dressings for the healing of leg wounds and ulcers: A systematic review and meta-analysis. J. Amer. Acad. Dermatol. 63 (4) (2010) 668-679.

[17] N.F.S. Watson, W. Hodgkin, Wound dressings. Surg. 23(2) (2005) 52-55.

[18] I. Ayensu, J.C. Mitchell, J.S. Boateng, Effect of membrane dialysis on characteristics of lyophilized chitosan wafers for potential buccal delivery of proteins. Int. J. Biol. Macromol. 50 (2012) 905-909.

[19] J.S. Boateng, H.V. Pawar, J. Tetteh, Polyox and carrageenan based composite film dressing containing anti-microbial and anti-inflammatory drugs for effective wound healing. Int. J. Pharm. 441(1-2) (2013) 181-91.

[20] J.S. Boateng, A.D. Auffret, K.H. Matthews, M.J. Humphrey, H.N.E. Stevens, G.M. Eccleston, Characterization of freeze-dried wafers and solvent evaporated films as potential drug delivery systems to mucosal surfaces Int. J. Pharm. 389 (2010) 24–31.

[21] Z. Dong, Q. Wang, Y. Du, Alginate/gelatin blend films and their properties for drug controlled release. J. Membr. Sci. 280 (2006) 37–44.

[22] Y.S. Choi, S.R. Hong, Y.M. Lee, K.W. Song, M.H. Park, Y.S. Nam, Study on gelatin-containing artificial skin: I. Preparation and characteristics of novel gelatin-alginate sponge. Biomater. 20(5) (1999) 409 -417.

[23] N. Devi, D.K. Kakati, Smart porous microparticles based on

520 gelatin/sodium alginate polyelectrolyte complex. J. Food Eng. 117 (2013) 193–
521 204.
522 [24] Y. Li, H. Ji, Q. Cheng, F. Pan, Z. Jiang, Sodium alginate–gelatin
523 polyelectrolyte complex membranes with both high water vapor permeance and
524 high selectivity. J. Membr. Sci. 375(1–2) (2011) 304–312.
525 [25] Z. Aziz, S.F. Abu, N.J. Chong, A systematic review of silver-containing
526 dressings and topical silver agents (used with dressings) for burn wounds. Burns
527 38 (2012) 307-318.
528 [26] G. Liu, X. Zhao, Electroresponsive behavior of gelatin/alginate semi-
529 interpenetrating polymer network membranes under direct-current electric field.
530 J. Macromol. Sci. Part A: Pure App. Chem. 43 (2006) 345–354.
531 [27] A. Roy, J. Bajpai, A.K. Bajpai, Development of calcium alginate-gelatin
532 based microspheres for controlled release of endosulfan as a model pesticide. Ind
533 J. Chem. Technol. 16 (2009) 388-395.
534 [28] A.R. Fajardo, L.C. Lopes, A.O. Caleare, E.A. Britta, C.V. Nakamura, A.F.
535 Rubira, E.C. Muniz, Silver sulfadiazine loaded chitosan/chondroitin sulfate films
536 for a potential wound dressing application. Mater. Sci. Eng. C. 33 (2013) 588–
537 595.
538 [29] B. Singh, S. Sharmab, A. Dhimana, Design of antibiotic containing
539 hydrogel wound dressings: Biomedical properties and histological study of wound
540 healing. Int. J. Pharm. 457 (2013) 82–91.
541 [30] N. Salamat-Miller, M. Chittchang, T.P. Johnston, The use of
542 mucoadhesive polymers in buccal drug delivery. Adv. Drug Del. Rev. 57 (2005)
543 1666 – 1691.
544 [31] G.S. Asane, S.A. Nirmal, K.B. Rasal, A.A. Naik, M.S. Mahadik, Polymers

for Mucoadhesive Drug Delivery System: A Current Status. Drug Dev. Ind. Pharm. 34 (2008) 1246–1266.

[32] S. Roy, K. Pal, A. Anis, K. Pramanik, B. Prabhakar, Polymers in Mucoadhesive Drug-Delivery Systems: A Brief Note. Design Monom. Polym. 12 (2009) 483–495.

[33] L. Jiang, L. Gao, X. Wang, L. Tang, J. Ma, The application of mucoadhesive polymers in nasal drug delivery. Drug Dev Ind Pharm 36(3) (2010) 323–336.

[34] S.C. Howard, T.J. Wlodkowski, H.S. Rosenkranz, Silver sulfadiazine: in vitro antibacterial activity. Antimicro. Agents Chemother. 4 (5) (1973) 585 – 587.

570 Table 1: Composition of blank (BL) and drug loaded (DL) loaded gels with total polymer
571 (SA and GE) content of 3 % w/w in each case.
572

% ratio of SA/GE	SA (% w/w)	GE (% w/w)	F68 (% w/w)	Drug (% w/w)
BL 100/0	3.00	0.00	0.20	-
BL 75/25	2.25	0.75	0.20	-
BL 50/50	1.50	1.50	0.20	-
BL 25/75	0.75	2.25	0.20	-
BL 0/100	0.00	3.00	0.20	-
DL 100/0	3.00	0.00	0.20	0.10 SSD
DL 75/25	2.25	0.75	0.20	0.10SSD
DL 50/50	1.50	1.50	0.20	0.10 SSD
DL 25/75	0.75	2.25	0.20	0.10SSD
DL 0/100	0.00	3.00	0.20	0.10 SSD

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583 ## Figure Legends

584 **Figure 1** Digital photographs of BL SA/GE wafers (A) 100/0, (B) 75/25, (C) 50/50, (D)
585 25/75, (E) 0/100 and SSD loaded SA/GE wafers (F) 100/0, (G) 75/25, (H) 50/50, (I)
586 25/75, (J) 0/100.

587 **Figure 2** SEM images of wafers obtained by imaging (A) BL 0/100 SA/GE: bottom
588 section, (B) blank 0/100 SA/GE top section with magnification of x50, (C) BL 75/25
589 SA/GE: top section with magnification of x50 (D) BL 25/75 SA/GE top section, (E) DL
590 25/75 SA/GE: top section with magnification x100, (F) DL 25/75 SA/GE: top section
591 with magnification x200.

592 **Figure 3** XRD patterns of (a) the different starting material, (b) BL wafers and (c) DL
593 wafers (ratios SA/GE)

594 **Figure 4** FTIR spectra of (a) the different starting components (SA, GE, F68, SSD); (b)
595 BL wafers (ratios SA/GE) and (c) the DL wafers (ratios SA/GE).

596 **Figure 5** Swelling behavior of BL and SSD (DL) loaded wafers (ratios SA/GE).

597 **Figure 6** Hardness profiles for BL and DL (SSD) wafers ($n = 4$) compressed at five
598 different locations on both sides of the formulation (ratios SA/GE). Statistically
599 significant differences ($p < 0.05$) in 'hardness' between top and bottom were observed
600 for all formulations except for 75/25 SA/GE SSD ($p = 0.78$).

601 **Figure 7** Mucoadhesion profiles showing peak force of adhesion (PAF), work of
602 adhesion (WOA) and cohesiveness of BL and SSD loaded (DL) wafers containing
603 varying ratios of SA/GE. The difference in PAF and WOA was not statically significant
604 ($p > 0.05$), whilst all the different formulations exhibited significant differences for the
605 cohesiveness ($p < 0.05$).

606 **Figure 8** *In vitro* drug dissolution profiles for SSD released from optimized drug loaded
607 wafers containing different proportions of SA/GE.