Preparation and Characterization of Low Cost Asymmetric Thin Film as Accelerating Wound Healing Material

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Abstract. Gelatin and sodium alginate bioblends of dissimilar ratio were prepared by casting. In order to prepare the asymmetric thin (AT) film, 2% $CaCl_2$ was incorporated in gelatin/sodium alginate blends. The physico-mechanical properties of the prepared films were investigated. AT film composed of gelatin and sodium alginate at a ratio of 70:30 with 2% of $CaCl_2$ showed higher tensile strength (25 MPa) and elongation at break (9%). The water and buffer uptake capacity of the gelatin/sodium alginate/$CaCl_2$ (70:30:2) blends was found to be higher than other blends. pH of this AT film was 7.82. Interaction among gelatin, sodium alginate and $CaCl_2$ in the films was tested and this film showed no cytotoxic effect.

Key words: Gelatin, Sodium alginate, Asymmetric thin film, Cytotoxicity.

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1 INTRODUCTION

In recent years, there has been a considerable interest in the bioacceptable polymeric film, often known as asymmetric thin film (AT film) as advanced dressing material so that the tissues can be designed to grow in such a way that they match specifically the requirements of the individual in terms of size, shape and immunological compatibility, minimizing further requirement of treatment. The clinical success of the formulation is largely dependent on the quality of the starting scaffold composition that would promote not only the initial cellular adhesion but also the subsequent cell proliferation. Compared to the constituent polymers, the AT film has advantages when applied as coating membranes and controlled-release delivery systems. It is more stable to pH changes (Cascone, 1997 and Rathna et al., 1996) and has been proven more effective in limiting the release of encapsulated materials compared with either polymer alone (Smith, 1994; Elquin, 1995 and Yan et al. 2000). On the basis of their favorable physical properties, this study explored the potential of the gelatin-alginate PEC thin film for wound-dressing applications. Gelatin is a well-characterized protein fragment obtained by partial degradation of water insoluble collagen fiber (Sezer and Akbuga, 1999) and has been widely used in the biomedical field, because of its merits, including its biological origin, biodegradability, hydrogel properties, and commercial availability at a relatively low cost (Takeuchi et al., 2000). Gelatin is an intriguing candidate for drug delivery and is widely being used as tissue engineering scaffold. Cross-linked gelatin sponges have also been investigated for their application as a component of artificial skin or tissue transplants to promote epithelialization and granulation tissue formation in wound (Kniep and Simon, 2007). Gelatin has also been used in medicine as an artificial blood, plasma expander, wound dressing, adhesive, and absorbent pads for surgical use (Choi and Regenstein, 2000).

Alginate hydrogels have shown excellent potential in a variety of biomedical applications, including scaffolds for tissue engineering or carriers for drug delivery systems (Ma, 2005). Commonly derived from seaweed, alginate is a linear polysaccharide consisting of β-D-mannuronic acid (M) and α-L-guluronic acid (G) monomers that are arranged in blocks of G, M, and random combinations of M and G monomers (G-, M-, and MG-blocks) (Ma, 2005 and Draget et al., 1991). By providing a relatively inert aqueous environment within its matrix and high gel porosity that follows for the diffusion of macromolecules, sodium alginate blends itself favorably to biomedical applications. In addition, it is water soluble and degradable under normal physiological conditions and may be applied to encapsulated materials under mild conditions that do not involve noxious organic solvents. When treated with calcium chloride, cooperative crosslinking occurs between calcium ions and G-blocks to result in an ‘egg-box’ structure that imparts gelling ability and mechanical strength (Rees, 1981). The ionic crosslinking reaction is mild and provides a favorable environment for cell immobilization or drug encapsulation (Ma, 2005). These properties make sodium alginate an attractive carrier for the systemic delivery of proteins and DNA drugs, and for the encapsulation of cells and enzymes. In this present study, dilute $CaCl_2$ was mixed in gelatin/sodium alginate blend to improve the stability of the film against water. The ultimate

goal of this research work was to develop a bioadhesive wound dressing material, which would be flexible, bioactive, biocompatible and biodegradable with rapid healing properties.

2 EXPERIMENTAL

2.1 Materials

Sodium alginate used in this work was purchased from UNI-CHEM, China. A type-B Gelatin was supplied by OSL, Bangladesh. Calcium chloride was purchased from MERCK, India.

2.2 Methods

2.1.1 Gelatin/Sodium Alginate blend film with 2% $CaCl_2$

Granules of gelatin (15 g) were dissolved in 100 ml of de-ionized water and heated for 2 h at 70°C under normal pressure. Gelatin/sodium alginate blend films of four different compositions (w/w) (95/5, 90/10, 80/20, and 70/30) with 2% $CaCl_2$ were prepared by solution casting. The gelatin solution was poured on to silicon based release film and kept at room temperature for 24 hours for film formation. The dried films (thickness about 0.50 mm) were peeled off and cut into small pieces as required for mechanical testing and then kept in the desiccators.

2.3 Characterization

2.3.1 Mechanical testing

Tensile strength (TS) and percent elongation at break (Eb) of different blends films were measured with Universal Testing Machine (Hounsfield Series S, UK) using DIN EN 10002-1 method of testing polymer film. The load capacity was 500 N, efficiency was within maximum ±1% with crosshead speed is 10 mm/min and gauze length is 20 mm. The mechanical tests of blended films were performed at 65% relative humidity and at room temperature to enable identical moisture content.

2.3.2 Water and Buffer Uptake

Both water and 0.1M Phosphate Buffered Saline (PBS) was used to monitor the drainage ability of gelatin/alginate films. An approx 20×10 mm weighed film sample was placed on the top surface of the buffer-soaked sponge. Only bottom side of the film was allowed to come into contact with the wet sponge surface. The sample was removed periodically (5, 10, 15, 20, and 30 min), blotted dry with blotting paper, and weighed until constant weight was obtained. Similarly, water uptake was measured similarly using water instead of PBS. Both water and buffer uptake of the gelatin/alginate films evaluated was expressed as:

$$Wg = \frac{Wt - Wo}{Wo} \times 100\ \%$$

Where, Wg = Weight gain, Wt = Weight after uptake, W_0 = Initial dry weight of the gelatin/sodium alginate films.

2.3.3 Measurement of pH

The values of pH for the different gelatin/sodium alginate blend solutions were determined using a digital pH meter (PHILIPS, PW-9409, UK) with an efficiency level of ±0.3.

2.3.4 Fourier Transformed Infrared (FTIR) Spectroscopy

The FTIR spectroscopy of gelatin/sodium alginate blend films were performed by FTIR Spectrophotometer (Paragon 500 Model, PerkinElmer, Beaconsfield, Buckinghamshire, UK) in the wave number range 400–4000 cm^{-1} with resolution of 4 cm^{-1}. The FTIR spectrum was taken in transmittance mode.

2.3.5 Cytotoxicity Test

In vitro cytotoxicity test was performed using Brine Shrimp Lethality Bioassay method (Khan et al. 2012 and McLaughlin and Rogers, 1999). It is a primary toxicity screening procedure used as an initial screening of bioactive compounds. Brine shrimps (*Artemia salina*) were hatched using brine shrimp eggs in a conical shaped vessel (1 L), filled with sterile artificial seawater and pH was adjusted at 8.5 using 1 N NaOH under constant aeration for 48 h. After hatching, active nauplii free from egg shells were collected from brighter portion of the hatching chamber and used for the assay. 0.125, 0.25, 0.50, 0.75, and 1.0 mg/ml of gelatin/alginate film (70% of gelatin and 30% of alginate with 2% $CaCl_2$) was dissolved in simulated seawater and taken in petri plates where the active nauplii were inoculated. After

overnight incubation, the nauplii were counted. 0.5 mg/ml of vincristine sulfate was considered as LC 100.

3 RESULTS AND DISCUSSION

3.1 Mechanical Properties

The tensile strength and elongation at break of the $CaCl_2$ (2%) incorporated gelatin/sodium alginate blend films are shown in (**Figure 1**). The tensile strength of the pure gelatin film is 27±1.1 MPa, which supported the earlier literature (Sarkar et al., 2006). The ultimate tensile strength of the film showed tendency to decrease with increasing percentage of sodium alginate up to 20%. After the initial decrease, TS began to increase with the increasing ratio of sodium alginate and had a peak of 25±0.9 MPa at about 30% sodium alginate content. The calcium ion from calcium chloride form complex with carboxylate ion of sodium alginate. The access of calcium chloride affects hydrogen bonding between gelatin and alginate, which are responsible for the lowering of tensile strength and elongation at break for the film containing 10% and 20% sodium alginate. In the recent study, it was found that with the increase of sodium alginate content from 30% results in decrease in TS and EB. The elongation at break (Eb) of the various samples as explicit in (**Figure 1**) shows a sharp decrease at 10% sodium alginate content (Eb = 4±0.46%), whereas the Eb value of pure gelatin film is 11±0.37%. Then it began to increase with the increase of sodium alginate. The incorporation of sodium alginate molecules into the continuous matrix of gelatin disrupts the structural chain regularities of gelatin, which breaks down the molecular packing and provides a greater path length (path around the periphery of the dispersed particles) for dissipation of energy before its ultimate rupture. However, it may induce some fibrillar characteristics into the system which can raise its elongation before rupture. Amino group of the gelatin polypeptide chains acts as an electron donor and the hydrogen of alginate acts as an electron acceptor (Klose et al., 1952). This induces dipole–dipole attraction between the two phases, which is supposed to enhance molecular interaction. But this interaction has a larger intermolecular gap which leads to decrease TS after certain concentration (Rahman et al., 2010). The TS of the film exhibit a steady fall with the increase of alginate after the peak point. Because molecular chain structure of gelatin is expected to be somewhat degraded with the incorporation of alginate. The hydrogen of alginate is suggested to form a hydrogen bond with the amino group of gelatin and hence forms the key factor in achieving miscibility (Brandrup et al., 1999 and Salaneck, 1996).

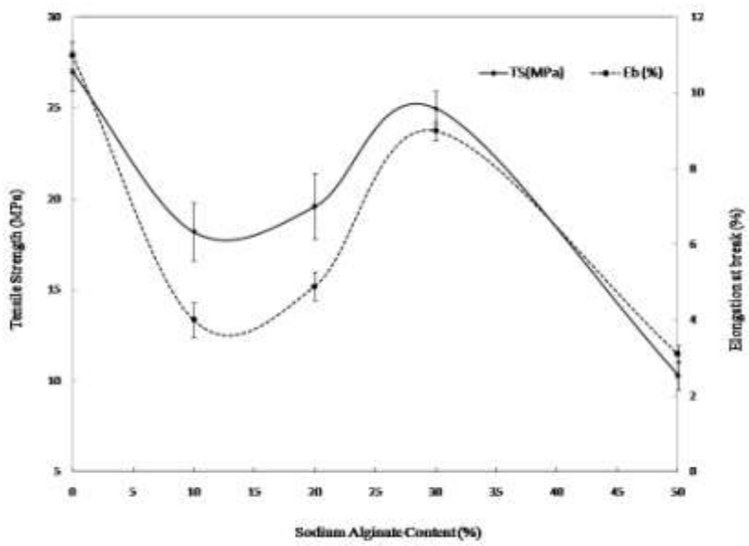

Fig. 1. Effect of sodium alginate on tensile strength and elongation at break of the gelatin/sodium alginate blend films.

For an ideal wound dressing material modified gelatin film must has a high Eb having good TS (Paul, 1986). It was found that 70:30 gelatin/alginate AT film showed high TS (25±0.9MPa) with a moderate Eb (9±0.25%) (**Figure 1**). Thus, optimization of ratio of the alginate was ensured.

3.2 Water and Buffer uptake Properties

The experiment was designed to simulate an open exudative wound dressed with gelatin/sodium alginate based AT film. Water uptake of the AT film was increased with time up to a certain level. But it began to decrease after the saturation point and the decreasing rate was not same for the different composition (**Figure 2**).

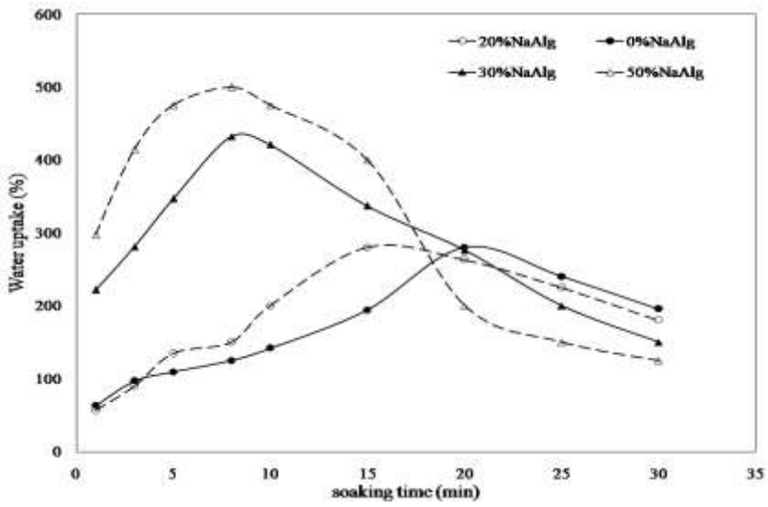

Fig. 2. Comparison of water uptake of $CaCl_2$ incorporated gelatin/sodium alginate blend film.

Absorption pattern was similar in case of buffer uptake which was carried to evaluate body fluid drainage capability of the film from an open wound. As both of the gelatin and sodium alginate are hydrophilic, so water uptake is quite rapid to a certain level. But after 20 minutes of emersion, films were saturated and began to go in solution. Thus, the net weight of film was decreased. Sodium alginate is more hydrophilic than gelatin and interaction of gelatin-sodium alginate causes a higher hydrophilicity. But films containing 20% and 30% sodium alginate uptake more water than others and where 30% sodium alginate containing film showed the maximum stability in water. Similar results were found when samples were soaked in buffer (**Figure 3**). In the study, 30% sodium alginate and 20% sodium alginate containing film not only absorbed more percentage of buffer solution but also these films were stable for longer period in buffer solution. Both Gelatin and sodium alginate have excellent wound healing enhancing properties. So, these high water and buffer retaining properties and stability suggest that the AT film of composition of 70:30 gelatin/alginate with 2% $CaCl_2$ is very potential to be an ideal tissue scaffold for accelerated wound healing.

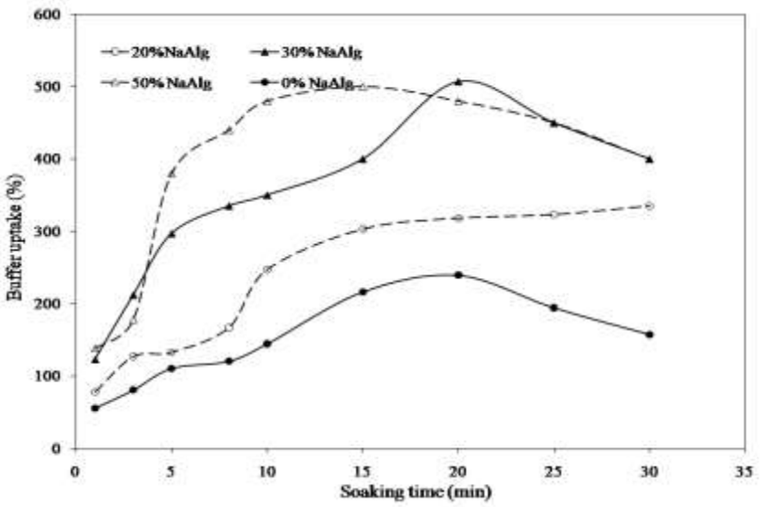

Fig. 3. Comparison of buffer uptake of $CaCl_2$ incorporated gelatin/sodium alginate blend film.

3.3 pH of the Solution

The pH of pure gelatin, alginate and Gelatin/sodium alginate/$CaCl_2$ blend solutions of the present work are presented at **Table 1**. According to the pH value (pH 4.7), gelatin is acidic. But addition of increasing concentration of sodium alginate solution increased the pH of the solution (pH of the pure sodium alginate solution is 8.42). Addition of 2% $CaCl_2$ had no effect on the pH of these solutions. Results suggesting that the pH of gelatin/NaAlg solution is more suitable for *in vivo* application than that of pure gelatin as the body pH is around 7.4.

Table 1. pH of pure gelatin, sodium alginate and Gelatin/sodium alginate/$CaCl_2$ blend.

Different Solution	pH
Pure gelatin	4.92
Pure NaAlg	8.42
NaAlg solution with $CaCl_2$	8.31
Gelatin/NaAlg (80-20) % blend with $CaCl_2$	7.42
Gelatin/NaAlg (70-30) % blend with $CaCl_2$	7.82
Gelatin/NaAlg (50-50) % blend with $CaCl_2$	8.21

3.4 FTIR spectra analysis

The FTIR spectrum of the pure gelatin showed that the region of 1100–1700 cm^{-1} contain the most potentially useful information bearing on the structure of gelatin. By observing the spectrum of the gelatin film during this research work, the peaks at C=O stretching at 1600 cm^{-1}, N-H bending at 1557 cm^{-1}, $C-H_2$ bending at 1451 cm^{-1} and C-O streching at 1241 cm^{-1} were found which coincided with standard FTIR value of pure gelatin. The existence of two or more frequencies for the same band might be interpreted as indicating different types of hydrogen bonding.

FTIR spectrum of pure sodium alginate showed that 1608 cm^{-1} for C=O stretching, 1419 cm^{-1} $C-H_2$ bending, 1124 cm^{-1} for C-N stretching. Infrared spectra for the crosslinked films of gelatin/sodium alginate (70%-30%) blend film showed 3 new absorption band at 1411 cm^{-1}, 1655 cm^{-1} and 1031cm^{-1} which are attributed to the $C-H_2$ bending, C=O cm^{-1} stretching, and C-O stretching respectively. There was some extra intercalation formed in presence of calcium ion. Calcium chloride, a known cross-linking agent of alginate, is assumed to act by complexing carboxylate anions of alginate with bivalent calcium ions, thus forming a three dimensional network, when it was blended with gelatin (Roy et al., 2009). Among those reason (70-30) % gelatin/sodium alginate with 2% $CaCl_2$ blend film showed drastic change in their physico-chemical character.

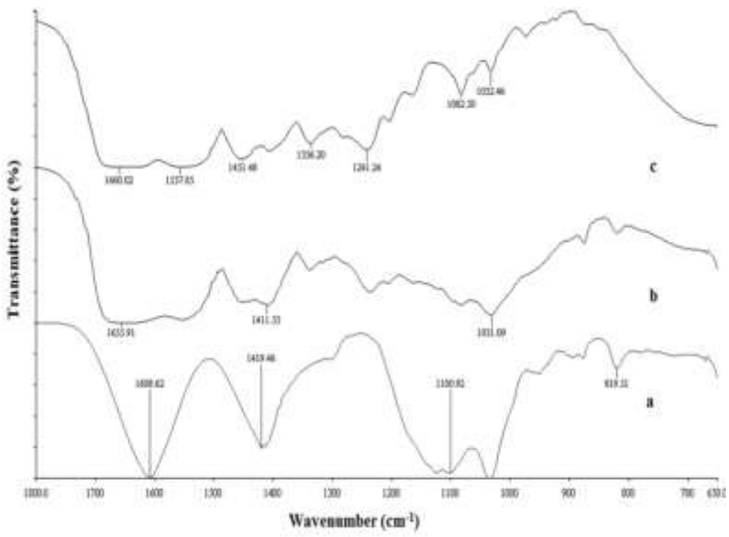

Fig. 4. FTIR of a) sodium alginate, b) gelatin (70%)/sodium alginate (30%)/$CaCl_2$(2%), C) pure gelatin

3.5 In vitro Cytotoxicity Test

Brine Shrimp Lethality Bioassay method was used to test if the composite scaffold had any cytotoxic effect. Gelatin/alginate/$CaCl_2$ AT film was dissolved in artificial sea water in which nauplii were inoculated. The number of death increased at higher concentration of the composite scaffold (**Table 2**). It may be due to three reasons: a) the AT film may have cytotoxic effect, b) dissolved oxygen concentration of the saline water may have decreased and c) a viscous layer may have formed on the gills of nauplii.

The results suggested that the possible reason of nauplii death is not toxicity as the number of death was nil for lower concentrations. Moreover, gelatin and sodium alginate were used as parent materials of the AT film which are both biocompatible. Thus, the most possible reason for the death of nauplii is the formation of a viscous layer on their gills. Lack

of oxygen availability was also a fetal factor here because this viscous layer limits oxygen permeability through the gills.

Table 2. Mortality rate of Brine shrimp (Artemia salina) for different concentrated gelatin/alginate film solution.

Dose (mg/ml)	No. of nauplii present after incubation	Mortality (%)
Vincristine (0.5)	0	100
Negative control	10	0
Gelatin/alginate film (0.125)	10	0
Gelatin/alginate film (0.25)	9	10
Gelatin/alginate film (0.5)	9	10
Gelatin/alginate film (0.75)	8	20
Gelatin/alginate film (1.00)	7	30

4 CONCLUSIONS

In the present study, a bioadhesive wound dressing material based on gelatin/sodium alginate was prepared, characterized and evaluated for biomedical application. The FTIR spectra of the gelatin/sodium alginate films suggested that molecular interaction occurred between gelatin and sodium alginate in solution. AT film composed of gelatin/sodium alginate in a ratio of 70:30 showed high fluids and wound exudate drainage capability. pH of this AT film was 7.82 which is compatible with body liquid. This film exhibited higher tensile strength (25 MPa) and elongation at break (9%) which complements the tissue scaffolding. Based on the above results it can reasonably concluded that gelatin/alginate AT film protects and acts to mimic the natural moist environment of wound surface which will eventually lead to accelerated wound healing.

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Biography

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