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Cross-linker and non-gelling Na^+ effects on multi-functional alginate dressings

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ABSTRACT

This study investigated the effects of Ca^{2+}, Cu^{2+} and Zn^{2+} as well as added Na^+ during cross-linking on the mechanical properties of cross-linked alginates and also their potential to release cations in infected wounds. All these variables were found to affect the functional properties of the alginate films to varying extent. The added Na^+ increased the tensile strength of the films cross-linked by Ca^{2+} but it exerted the opposite effect on those cross-linked by Cu^{2+} or Zn^{2+}. Employing a combination of cross-linking cations resulted in greater extent of cross-linking but variable effect on tensile strength. The added Na^+ also influenced the release of cations from alginate matrices to different extents in pH 4.0 and 8.0. The percentage of cations released from the films was lowest for Cu^{2+} but relatively high for Ca^{2+} and Zn^{2+}. Hence, careful selection of cations is important to ensure that the functions of the alginate dressings are not compromised.

1. Introduction

Wound dressings play important roles in the management of wounds. In particular, calcium alginate dressings are widely used for exuding wounds (Agren, 1996). These biodegradable dressings are made of natural polymers derived from brown seaweeds. They exhibit good fluid handling properties that encourage healing of wounds in a moist environment (Fraser & Gilchrist, 1983). However, calcium alginate dressings are not known to possess anti-microbial properties. Poorly managed exuding wounds are avenues for opportunistic infection and are often associated with delayed healing. This poses a great challenge in the management of exuding wounds.

In recent years, there is a growing interest to incorporate anti-microbial metals into alginate dressings or use them in combination with other anti-microbial agents to enhance the overall anti-microbial activity (Burrell, Yin, Djokic, & Langford, 2001; Ishimori & Ohara, 1994; Karandikar, Gibbins, & Cornell, 2006; Qin & Groocock, 2002). In a previous study, it was found that divalent cations such as Cu^{2+} and Zn^{2+} exhibited higher anti-microbial activities than Ca^{2+} and were generally compatible with common topical anti-microbial agents (Goh, Heng, Huang, Li, & Chan, 2008). It would be interesting to further investigate how these cations influence the mechanical properties and other functions of the alginate dressing. Non-gelling cations such as Na^+ may also influence the properties of alginate matrices. For instance, the sodium alginate present is generally believed to improve the fluid absorbency of calcium alginate matrices (Qin, 2005). This seems to suggest that the inclusion of Na^+ during cross-linking can modulate functional properties of alginate dressing. To confer effective anti-microbial activity, anti-microbial metals in the alginate dressings should be readily released during use. However, the pH of an infected wound is often altered by the residing pathogens (Chai, 1992). Changes in pH can affect the ion exchange process of alginates at the wound site and its efficacy as an anti-microbial wound dressing.

In this study, the effects of Ca^{2+}, Cu^{2+}, Zn^{2+} and added Na^+ on the mechanical properties of the resultant cross-linked alginates as well as the potential of these matrices to release cations in infected wounds were investigated. By understanding the underlying mechanisms, the findings will provide valuable perspectives on the functional impact these cations have in the development of novel alginate dressings with anti-microbial properties.

2. Materials and methods

2.1. Preparation of sodium alginate films

Sodium alginate films were produced by a solvent evaporation technique as described previously (Chan, Lee, & Heng, 2006). Briefly, a 3% (w/v) aqueous solution of sodium alginate (Keltone® LVCR, ISP-Alginates Industries, USA) was first prepared and left to stand overnight to remove air bubbles. 30 g of the alginate solution was transferred into a glass Petri dish of diameter 10 cm and oven-dried to constant weight on a levelled surface at 40 °C. The dried films were removed and stored in a desiccator for at least 48 h before use. Three batches of sodium alginate films were prepared.

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Table 1
Types of alginate films prepared.

Formulation	Molar ratio of cations used				Code
	Ca^{2+}	Cu^{2+}	Zn^{2+}	Na^{+}	
Control (Sodium alginate)	–	–	–	–	SA
Cross-linking in the absence of added Na^{+}					
Cross-linking with Ca^{2+}	1	–	–	–	Ca-F
Cross-linking with Cu^{2+}	–	1	–	–	Cu-F
Cross-linking with Zn^{2+}	–	–	1	–	Zn-F
Cross-linking with a combination of Ca^{2+} and Cu^{2+}	1	1	–	–	CaCu-F
Cross-linking with a combination of Ca^{2+} and Zn^{2+}	1	–	1	–	CaZn-F
Cross-linking in the presence of added Na^{+}					
Cross-linking with Ca^{2+}	1	–	–	2	Ca-FN
Cross-linking with Cu^{2+}	–	1	–	2	Cu-FN
Cross-linking with Zn^{2+}	–	–	1	2	Zn-FN

2.2. Preparation of cross-linked alginate films

Cross-linked alginate films were produced by an external gelation method. The salts used for cross-linking were calcium chloride, copper sulphate, and zinc chloride (Merck, Germany), copper nitrate (Fluka, France) as well as sodium chloride (Lab-Scan Asia, Thailand). Each sodium alginate film was immersed in 100 ml of cross-linking solution containing a total concentration of 0.15 M of the specified cations for 1 h. The cross-linked films were washed with deionised water thrice to remove any surface unbound cations before drying to constant weight at 25 °C. The films harvested were then stored in a desiccator for at least 48 h before use. Three batches of cross-linked films were prepared. The types of alginate films prepared are shown in Table 1.

2.3. Determination of mechanical properties of alginate films

The thickness of each film, 1 cm × 7 cm, was measured at five random points using a micrometer (Mitutoyo, 293-721-30 CE, Japan) and the result was expressed as mean value ± SD. Only film samples with thickness deviation of less than 10% from the mean were used. The tensile strength of each sample was determined using a tensile tester (Shimadzu, EZ tester, Japan) with an initial grip separation of 50.0 mm and a test speed of 10 mm/min. From the stress–strain curve of each sample, the tensile strength (N/mm^2), percent elongation at break (%) and elastic modulus (N/mm^2) were obtained using the Shimadzu WinAGSLite software. 10 samples of each type of film were used to determine the mechanical properties and the results were expressed as mean ± SD.

$$\text{Tensile strength} = \frac{\text{Maximum load}}{\text{Cross-sectional area}} \quad (1)$$

$$\%\ \text{elongation at break} = \frac{\text{Increase in length of film at the point of rupture}}{\text{Original length of film}} \times 100 \quad (2)$$

$$\text{Elastic modulus} = \text{Gradient of the linear portion of the stress–strain curve} \quad (3)$$

2.4. Determination of specific cation content and release of cations from alginate films

An accurately weighed amount of film was digested with hydrogen peroxide (30%, w/w, Merck, Germany) and nitric acid (70%, w/w, Ajax, Australia), in a microwave oven (Ethos 900, Milestone, Italy). Upon cooling, the content was made up to 100 ml in a volumetric flask using deionised water. Appropriate dilutions were carried out and the specific cation content of the film was determined using atomic absorption spectrometry (Shimadzu, AA6800, Japan) at 422.7 nm for Ca^{2+}, 324.8 nm for Cu^{2+}, 589.0 nm for Na^{+} and 213.9 nm for Zn^{2+}.

The release profiles of the cations from the films were also determined by introducing films of pre-determined weights into covered tubes with 25 ml of test medium. To simulate the wound fluids, the test media contained 142 mM Na^{+} and 2.5 mM Ca^{2+} and were adjusted to pH 4.0 and 8.0, using 0.1 N hydrochloric acid (J. T. Baker, USA) or undiluted triethanolamine, respectively. Sample solutions obtained were appropriately diluted with deionised water before atomic absorption spectrometric assay. The amount of specific cations that were released from the film at the end of 60 min was expressed as a percentage of the specific cation content of the film. For each type of film, the determination was carried out in triplicates and the results obtained were expressed as mean value ± SD.

2.5. Statistical analysis

Statistical analysis of the data was carried out using 2 sample *t*-test or one-way analysis of variance (ANOVA) with the aid of the statistical programme SPSS, version 13. The significance level was set at $p < 0.05$.

3. Results

3.1. Specific cation content of cross-linked films

Cross-linked alginate films were prepared as model wound dressings. Based on our preliminary study using Ca^{2+} and literature findings (Al-Musa, Abu Fara, & Badwan, 1999), the sodium alginate films were immersed in a 0.15 M cross-linking solution for 1 h to ensure complete cross-linking to produce sufficiently strong films for handling and evaluation. The amount of cross-linking cations present in each gram of cross-linked film was determined and the results presented in Fig. 1.

Alginate films produced in the absence of added Na^{+} (such as Ca-F) contained significantly larger amounts of cross-linking cations than Ca-FN, which were cross-linked with added Na^{+} ($p < 0.05$). This was similarly observed for the cation contents in Zn-F when compared against Zn-FN ($p < 0.05$), or Cu-F when compared against Cu-FN ($p < 0.05$). Comparing the three types of cross-linking cations, the respective cations in Cu-F or Zn-F was significantly lower than the Ca^{2+} present in Ca-F ($p < 0.05$), with $Ca^{2+} > Cu^{2+} > Zn^{2+}$. A similar trend in cation contents was observed when these cations were compared in films cross-linked in the presence of added Na^{+} (Ca-FN, Cu-FN, Zn-FN). Interestingly, the total content of cross-linking cations for films cross-linked with a combination of Ca^{2+} and Cu^{2+} or Ca^{2+} and Zn^{2+} was significantly higher than that for films

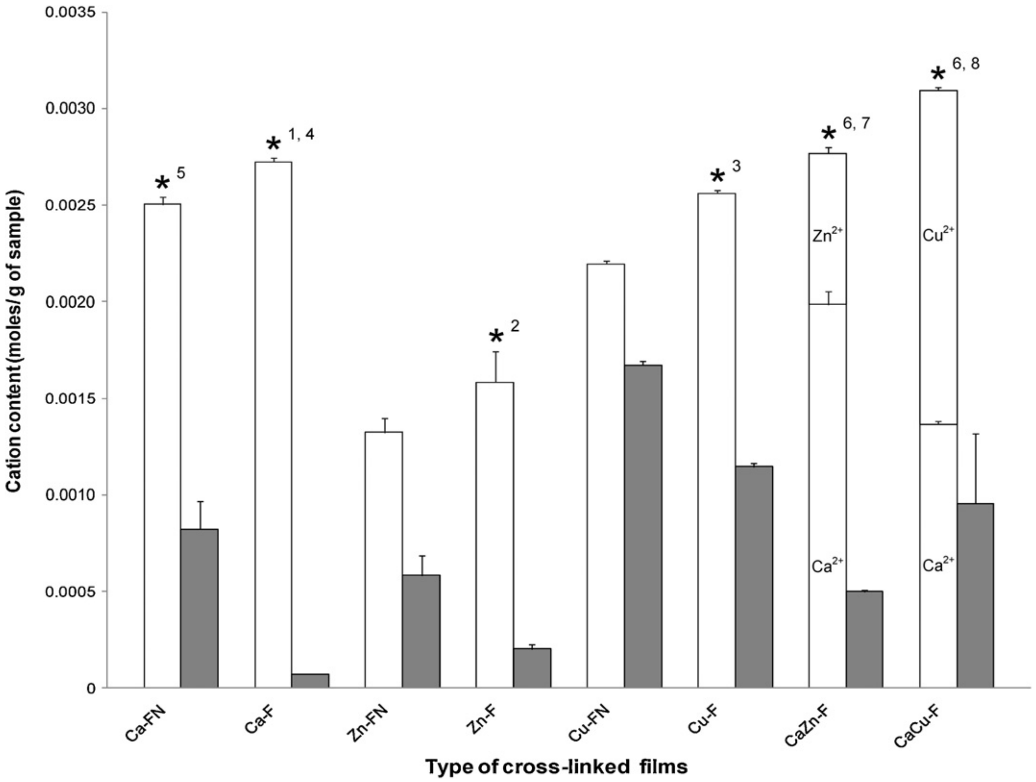

Fig. 1. Cation content (mean ± SD) of cross-linked alginate films ($n = 3$). Cross-linking cations represented by blank bars and Na^+ by shaded bars. ⋆: statistically significant ($p < 0.05$), 1: Ca-F compared with Ca-FN, 2: Zn-F compared with Zn-FN, 3: Cu-F compared with Cu-FN, 4: Zn-F or Cu-F compared with Ca-F, 5: Zn-FN or Cu-FN compared with Ca-FN, 6: CaZn-F or CaCu-F compared with the rest of the films (Ca-FN, Ca-F, Zn-FN, Zn-F, Cu-FN, Cu-F), 7: CaZn-F (Ca^{2+}) compared with CaZn-F (Zn^{2+}), and 8: CaCu-F (Ca^{2+}) compared with CaCu-F (Cu^{2+}).

cross-linked with a single type of cross-linking cation ($p < 0.05$), even as the total amount of cross-linking cations employed was kept constant (Fig. 1 and Table 1). In addition, the total contents of cross-linking cation and Na^+ in the latter films were also different. A closer examination of the cation content in CaZn-F showed a higher proportion of Ca^{2+} than Zn^{2+} ($p < 0.05$). The Cu^{2+} content in CaCu-F was however higher than that of Ca^{2+} ($p < 0.05$).

The contents of cross-linking cations were further compared with that of Na^+ in each cross-linked film (Table 2). For cross-linking with a single type of cation in the absence of added Na^+, it was found that Ca-F had the highest cross-linking cation to Na^+ ratio (39.2:1), followed by Zn-F (7.8:1) and Cu-F (2.2:1). A similar trend was observed for the films cross-linked in the presence of added Na^+. For films cross-linked with a combination of cross-linking cations, the Ca^{2+} to Na^+ ratio was higher than the Zn^{2+} to Na^+ ratio in CaZn-F, while the Cu^{2+} to Na^+ ratio was higher than the Ca^{2+} to Na^+ ratio in CaCu-F.

Table 2
Proportion of cross-linking cations to Na^+ in cross-linked alginate films.

Code	Content ratio (cross-linking cation: Na^+)[a]
Ca-FN	3.1:1
Ca-F	39.2:1
Zn-FN	2.3:1
Zn-F	7.8:1
Cu-FN	1.3:1
Cu-F	2.2:1
CaZn-F	4 (Ca^{2+}):1 and 1.6 (Zn^{2+}):1
CaCu-F	1.4 (Ca^{2+}):1 and 1.8 (Cu^{2+}):1

[a] Content ratio refers to the amount of cross-linking cation with respect to Na^+ present in 1 g of cross-linked film.

3.2. Mechanical properties of cross-linked films

The mechanical properties of the cross-linked alginate films are presented in Figs. 2 and 3. Tensile strength indicates the strength of the film to resist damage. Percent elongation at break describes the flexibility or extensibility while elastic modulus indicates the stiffness or rigidity of the film (Wu & McGinity, 2000). Generally, cross-linked films showed significantly higher tensile strength than the uncross-linked SA ($p < 0.05$). The only exception was Zn-FN, which had tensile strength comparable to SA ($p > 0.05$). Alginate films produced in the absence of added Na^+ (such as Cu-F) had higher tensile strength than that of Cu-FN, which was cross-linked with added Na^+ ($p > 0.05$). The tensile strength of Zn-F was significantly higher than that of Zn-FN ($p < 0.05$), but the reverse was observed for the tensile strength of Ca-F when compared with

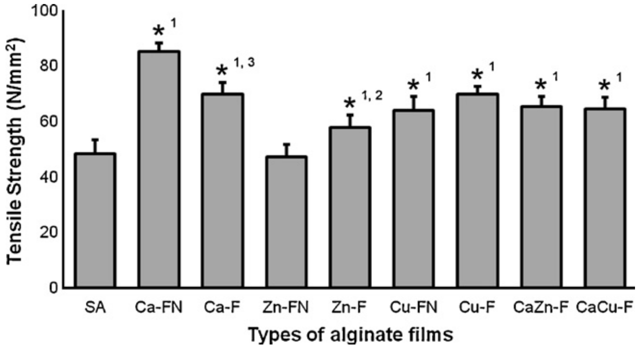

Fig. 2. Tensile strength (mean ± SD) of cross-linked alginate films ($n = 10$). ⋆: statistically significant ($p < 0.05$), 1: each of the film compared with SA, 2: Zn-F compared with Zn-FN, and 3: Ca-F compared with Ca-FN.

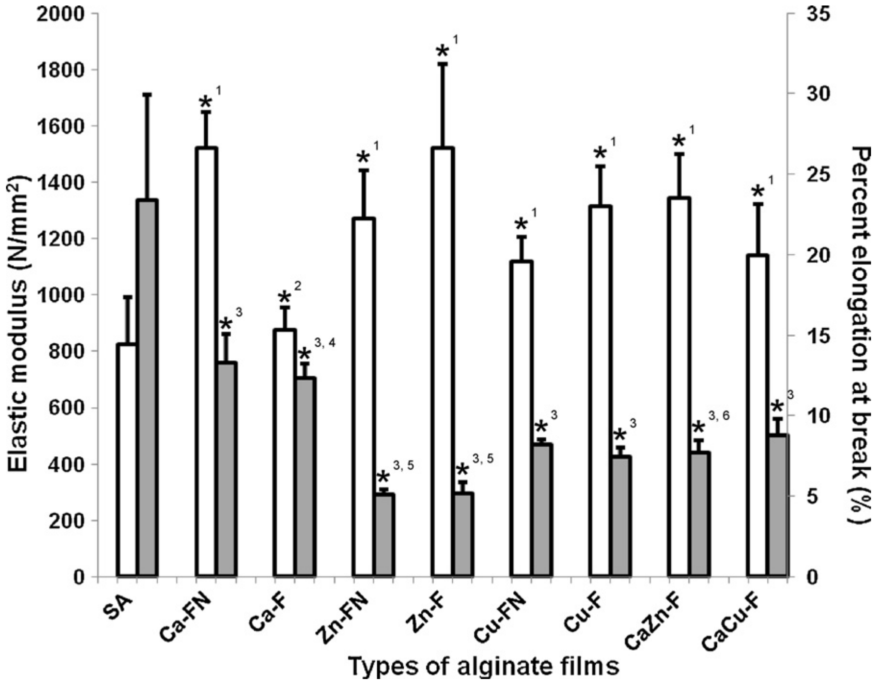

Fig. 3. Elastic modulus (blank bar; mean ± SD) and percent elongation at break (shaded bar; mean ± SD) of cross-linked alginate films ($n = 10$). ⋆: statistically significant ($p < 0.05$), 1: each of the film compared with SA, 2: Ca-F compared with Ca-FN, 3: each of the film compared with SA, 4: Ca-F compared with Cu-F or Zn-F, 5: Zn-FN or Zn-F compared with the rest of the films (Ca-FN, Ca-F, Cu-FN, Cu-F, CaZn-F, CaCu-F), and 6: CaZn-F compared with Zn-F.

Ca-FN ($p < 0.05$). When alginates were cross-linked with a combination of Ca^{2+} and Zn^{2+} (CaZn-F), tensile strength measured was intermediate to that of Ca-F and Zn-F ($p > 0.05$). This trend was not observed for CaCu-F, which had slightly lower strength than Ca-F and Cu-F ($p > 0.05$). Higher tensile strengths were generally observed for films cross-linked with Ca^{2+}.

The cross-linked films had generally higher elastic modulus and thus more rigid than the uncross-linked SA ($p < 0.05$). When compared further with SA, all the cross-linked films also exhibited a marked decrease in percent elongation at break and thus less flexible ($p < 0.05$). Cross-linking in the absence of added Na^+ produced Cu-F and Zn-F which were more rigid than Cu-FN and Zn-FN, respectively ($p > 0.05$). However, Ca-F was significantly less rigid than Ca-FN ($p < 0.05$). Overall, Ca-F was found to be more flexible than Cu-F and Zn-F ($p < 0.05$), with Zn-FN and Zn-F exhibiting the lowest flexibility among the formulations investigated ($p < 0.05$). The presence of added Na^+ during cross-linking did not seem to significantly influence the flexibility of cross-linked matrices. When alginates were cross-linked with a combination of Ca^{2+} and Zn^{2+} (CaZn-F), flexibility of the matrices obtained was significantly higher than that of Zn-F ($p < 0.05$). It was further observed that CaZn-F was less rigid than Zn-F ($p > 0.05$). The less rigid CaCu-F had a lower elastic modulus but showed slightly higher flexibility than that of Cu-F ($p > 0.05$).

3.3. Release of cations from alginate films

The amount of cross-linking cations released into the test medium at the end of 60 min was expressed as a percentage of the total content of the cross-linking cations in the same weight of cross-linked film (Fig. 4). The pH of 4.0 and 8.0 were used to depict wound conditions due to different bacterial colonisation. The percentage of each type of cation released ranged from about 10% to 50%. When comparing the effect of pH on the release profile of each formulation, films particularly Ca-FN and Cu-FN showed significantly greater release at pH 8.0 than at pH 4.0 ($p < 0.05$). At pH 8.0, the percentage of cations released was highest for Ca^{2+}, followed by Zn^{2+} and Cu^{2+}. The trend was less clear at pH 4.0, where the percentage of cross-linked cations released from Ca-FN was lower than Ca-F, Zn-FN and Zn-F. Irrespective of the pH, the percentage of Cu^{2+} released was always the lowest ($p < 0.05$).

Comparing between films cross-linked with and without added Na^+, Cu-F showed markedly higher percentage of cations released than Cu-FN at pH 4.0 or 8.0 ($p < 0.05$). On the other hand, the release of cations for Zn-F and Zn-FN were comparable in both pH. The trend between Ca-F and Ca-FN was however less clear, as there was greater release of cations from Ca-FN than Ca-F at pH 8.0 but lower release from Ca-FN at pH 4.0. For matrices cross-linked with a combination of cations, the total percentage of cations released from CaZn-F was generally lower than that from CaCu-F at both pH 4.0 and 8.0. The total percentage of cations released from CaZn-F at pH 4.0 was one of the highest among the films. It was also higher than that released at pH 8.0 but the reverse was observed for CaCu-F. A closer examination showed that a higher proportion of Ca^{2+} was released in comparison with Zn^{2+} from CaZn-F ($p < 0.05$) and similarly, more Ca^{2+} was released than Cu^{2+} from CaCu-F ($p < 0.05$).

4. Discussion

4.1. Effect of cations on cross-linking

The suitability of the cation to cross-link alginates is crucial in the development of anti-microbial alginate dressings. Cations were postulated to influence cross-linking, and unpublished data in our earlier study have indicated that these effects gave rise to differences in thickness of cross-linked films and their fluid handling properties in simulated wound fluids. Hence, the specific cation contents of cross-linked films were further studied to corroborate the mechanisms underlying cross-linking and their functional implications in alginate dressings. Calcium alginate films were used to represent the commercial alginate dressings.

The cation content of cross-linked films was dependent on the extent of cross-linking and relative binding affinity of the cations for

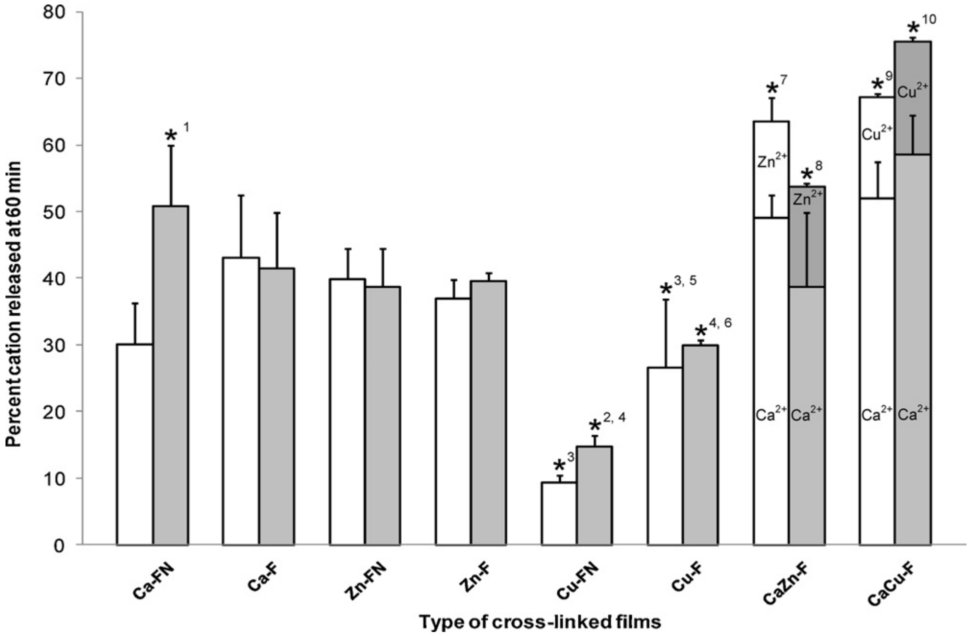

Fig. 4. Percentage of cross-linking cations released from cross-linked alginate films ($n = 3$) at 60 min in simulated wound fluids, pH 4.0 (blank bars; mean ± SD) and pH 8.0 (shaded bars; mean ± SD). ★: statistically significant ($p < 0.05$), 1: Ca-FN (pH 8) compared with Ca-FN (pH 4), 2: Cu-FN (pH 8) compared with Cu-FN (pH 4), 3: Cu-FN or Cu-F compared with the rest of the films (Ca-FN, Ca-F, Zn-FN, Zn-F, CaZn-F, CaCu-F, in pH 4), 4: Cu-FN or Cu-F compared with the rest of the films (Ca-FN, Ca-F, Zn-FN, Zn-F, CaZn-F, CaCu-F, in pH 8), 5: Cu-F (pH 4) compared with Cu-FN (pH 4), 6: Cu-F (pH 8) compared with Cu-FN (pH 8), 7: Ca^{2+} compared with Zn^{2+} in CaZn-F (pH 4), 8: Ca^{2+} compared with Zn^{2+} in CaZn-F (pH 8), 9: Ca^{2+} compared with Cu^{2+} in CaCu-F (pH 4), and 10: Ca^{2+} compared with Cu^{2+} in CaCu-F (pH 8).

alginates. A higher content of cross-linking cations in moles indicates more cations bound to the binding sites, hence giving rise to more extensive cross-linking in the alginate matrix. In this study, the type of cations used for cross-linking clearly had an effect on the cation content of the resultant cross-linked matrices. It is known that Ca^{2+} binds preferentially to the guluronate (G) residues in the alginates (Grant, Morris, Rees, Smith, & Thom, 1973). Cu^{2+} and Zn^{2+} were reported to be less selective in their binding to alginates (Aslani & Kennedy, 1996; Chan, Jin, & Heng, 2002; Zheng, 1997). During cross-linking, the smaller ionic radii of Cu^{2+} (87×10^{-12} m) and Zn^{2+} (74×10^{-12} m) may translate to smaller impedance in their diffusion to the inner core of the alginate matrix as compared to Ca^{2+} (99×10^{-12} m). Hence, Cu^{2+} and Zn^{2+} were expected to form matrices with higher cation contents. However, this trend was not observed when equimolar concentrations of cross-linking cations were employed. One possible explanation could be related to the method of cross-linking employed.

Cross-linked films were prepared by an external gelation method, where cross-linking occurred first at the film surface. Formation of thicker alginate films has been reported when increasing concentrations of calcium salt were used (Chan et al., 2006). This phenomenon was attributed to the greater extent of cross-linking at the film surface, which in turn affected the permeability of the film to the cross-linking cation. The results obtained in the present study further substantiated the non-selective binding properties of Cu^{2+} and Zn^{2+}, which caused extensive cross-linking at the surface of the matrix. When this occurred, the highly cross-linked surface acted as a barrier which impeded the diffusion of Cu^{2+} and Zn^{2+} to the matrix interior for cross-linking. The build-up of cross-linking cations on the matrix surface might also result in considerable electrostatic repulsion between cations, thereby reducing the availability of the cations for cross-linking (Chan et al., 2006).

The extent of cross-linking in the alginate matrix was also affected by the relative binding affinity of the cations for the alginates. Cations with less selective binding property are expected to result in more extensive cross-linking (Chan et al., 2006). However, the binding affinity of Zn^{2+} for alginates was lower than that of Ca^{2+} (Haug & Smidsroed, 1965; Smidsroed & Skjaak-Braek, 1990). It was apparent from the present study that the weaker binding affinity of Zn^{2+} for alginates was the predominant factor affecting the extent of cross-linking. The less strongly bound Zn^{2+} were more easily displaced from the binding sites in the production of the film and this gave rise to lower cation content in Zn-F as compared to that in Ca-F or Cu-F.

The presence of other cations (Na^{+} or even a cross-linking cation, e.g. Ca^{2+}) also influenced the extent of cross-linking as well as the uniformity of cross-linking throughout the entire alginate matrix. Na^{+}, being monovalent, does not participate in the cross-linking of alginates. However, it can interact with the binding sites of alginates. The lower content of cross-linked cations in alginate films cross-linked in the presence of added Na^{+} clearly showed that the added Na^{+} competed with the cross-linking cations for the binding sites.

The higher total contents of cross-linking cations in CaZn-F and CaCu-F strongly indicated that the alginates had different binding sites with varying affinity for the different cations. It was also apparent that the use of a combination of different cations enabled interaction with different and more binding sites. On the other hand, the use of a single type of cations probably involved limited types of binding sites and these binding sites would be saturated by a higher concentration of the cations. The higher proportion of Cu^{2+} to Ca^{2+} in CaCu-F further suggested that the alginates were more extensively cross-linked by Cu^{2+}. In fact, the proportion of Ca^{2+} in CaCu-F was markedly lower when compared to CaZn-F. The difference in binding affinity between Cu^{2+} and Na^{+} was reported to be much higher than that between Ca^{2+} and Na^{+} (Ouwerx, Velings, Mestdagh, & Axelos, 1999). Hence, it was possible that some binding sites were common to different cations and the higher binding affinity of Cu^{2+} competed more favourably than Ca^{2+} for the similar binding sites. Our results also reaffirmed the weak binding affinity of Zn^{2+} as the predominant factor over its binding selectivity with alginates during cross-linking. Nevertheless, both binding affinity and binding selectivity of the cations would significantly affect the properties of the cross-linked matrix.

4.2. Effect of cations on mechanical properties of films

From the overall results, it was clear that the tensile strength of cross-linked alginates was largely affected by the extent and uniformity of cross-linking. Alginates are composed of M–M, G–G and M–G blocks formed from the basic mannuronate (M) and guluronate (G) residues (Chapman & Chapman, 1980). Cross-linking generally resulted in a more rigid and less flexible structure. The intrinsic inflexibility of the alginate matrix was affected by the polymer chains that were cross-linked and the influence of the polymer blocks increased in the following order: M–G < M–M < G–G (Smidsroed, Glover, & Whittington, 1973). The G–G blocks are buckled and stiff while the M–M and M–G blocks are flexible and ribbon-like (Onsoyen, 2001). Since Ca^{2+} binds preferentially to the G–G blocks, the M–M and M–G blocks are free to rotate and impart flexibility to the matrix. On the other hand, Cu^{2+} and Zn^{2+} bind less selectively, resulting in less free rotating blocks and lower matrix flexibility. This aptly explained the greater flexibility of films cross-linked by Ca^{2+}. The relatively low flexibility and high rigidity of Zn-F was probably due to extensive surface cross-linking.

The addition of Na^{+} during cross-linking has been reported to promote the formation of a less heterogeneously cross-linked matrix (Draget, Oestgaard, & Smidsroed, 1990; Skjaak-Braek, Grasdalen, & Smidsroed, 1989). From our earlier results, it could be further inferred that a high concentration of competing Na^{+} encouraged displacement of cations from binding sites at the matrix surface. When Na^{+} was added in the cross-linking of alginates with Ca^{2+}, the more permeable matrix surface allowed diffusion of more Ca^{2+} to cross-link the alginates in the interior. This accounted for the formation of stronger and more rigid films with little change in flexibility. In contrast, the lower binding affinity of Zn^{2+} and Cu^{2+} for alginates was the predominant factor and encouraged a significant proportion of these cations to be displaced by the added Na^{+} during cross-linking. This led to the formation of a less cross-linked matrix with weaker and less rigid properties.

Films cross-linked by a combination of cations were weaker even though they were cross-linked to a greater extent. As mentioned previously, Ca^{2+} displayed significantly different binding selectivity and binding affinity from Zn^{2+} and Cu^{2+}. It was interesting to note that the differences in cross-linking properties of the cations seemed to have resulted in a less organized or more random cross-linked structure that compromised its tensile strength. CaZn-F and CaCu-F had lower content of Ca^{2+} but they were less flexible than Ca-F. This observation could be attributed to Zn^{2+} or Cu^{2+} through their interaction with the M–M and M–G blocks, thereby illustrating the strong influence of bound M–M/M–G blocks on flexibility of the cross-linked matrix. Clearly, the mechanical properties of the alginate matrices are strongly affected by the cross-linking cations and careful choice of the cross-linking cations to be used alone or in combination is very important.

Ideally, wound dressings should be physically strong yet soft while performing its protective function on the wound. The conformability of the dressing to the wound surface and body contours is also important for effective absorption of exudates and entrapment of bacteria. This is of particular relevance when dressings are applied to wounds on moving joints and areas with varying contours or in deep and lacerated wounds. Hence, for alginates to be an effective wound dressing, matrix properties such as durability and flexibility (extensibility) are particularly important. In general, a hard and tough polymer matrix has high tensile strength, high elastic modulus and high percentage elongation at break. Brittle matrices in particular, may require a large force to break or cause permanent deformation. Such materials are however limited by their inability to be stretched when a force is applied. As such, brittle alginate matrices are characterised by a high tensile strength and high elastic modulus but low percentage elongation at break. On the other hand, a soft but tough polymer matrix has a low elastic modulus, moderate tensile strength and high percentage elongation at break. The results showed that Ca^{2+} or Cu^{2+} are more useful than Zn^{2+} for the development of alginate dressings that are soft and tough.

4.3. Release of cations from alginate films

The release of cations from the cross-linked alginate films was investigated after 60 min in the respective simulated wound fluids of pH 4.0 and 8.0. A higher percentage of cations released means that more cations were displaced from the binding sites, hence giving rise to greater availability of free cations. Only the free cations would be available to exert any potential anti-microbial activities during use. In our earlier study, the cross-linked alginate films exhibited maximum fluid handling properties in simulated wound fluids at the end of 60 min (data not shown). As such, the release profiles of cations in this study provided important information on the potential of these cations to be released from the alginate dressings as anti-microbial metals in different wound conditions.

The percentage of cations released from films was the lowest for Cu^{2+} but relatively high for Ca^{2+} and Zn^{2+}. This could be attributed to the higher binding affinity of Cu^{2+} than Ca^{2+} and Zn^{2+}. As Zn^{2+} showed weaker binding affinity but less selective in binding to alginates than Ca^{2+}, the percentage release of Ca^{2+} and Zn^{2+} from the films was comparable. From these results, it was clear that the types of cations could affect the anti-microbial properties of alginates in infected wounds by the different extent of release of cross-linked cations during use.

The effect of added Na^{+} during cross-linking was also manifested as different extents of cations released from the cross-linked alginates. In the production of cross-linked film, the added Na^{+} enabled diffusion of cations into the matrix interior for cross-linking. As a result, it became harder for the cross-linked cations that were bound in the interior to be released. This was especially so if the binding affinity of the cross-linking cation was high, as seen by the greater release of cations from Cu-F than Cu-FN. It was earlier suggested that there were varying binding affinity of cations at the different binding sites of alginates. This was reaffirmed by a higher percentage of cations released from films cross-linked with a combination of Ca^{2+} and Cu^{2+} than that with Ca^{2+} and Zn^{2+}. In particular, a relatively higher percentage of Ca^{2+} was released from CaZn-F and CaCu-F than Cu^{2+} or Zn^{2+}. It was apparent from the results that factors other than the influences of binding affinity and binding selectivity of cross-linking cations would affect the release of cations from matrices cross-linked by different cations.

The pH employed in this study depicted wound conditions due to different bacterial colonisation. In the simulated wound fluid of pH 4.0, partial conversion of sodium alginate to alginic acid could occur as H^{+} displaced the bound cations from the carboxylate groups of the polymer chains. A reduction in gel strength was reported when Ca-alginate gels were converted to alginic acid (Draget, Braek, & Smidsrod, 1994), which encouraged the release of cations from the matrix. This was however not always observed in the present study. It should be recalled that Na^{+} and Ca^{2+} were incorporated into the test media to simulate the physiological fluids. Hence, this Ca^{2+} present could potentially induce cross-linking and retard dissolution of the cross-linked alginates.

Anti-microbial metals have been used in different approaches to impart anti-microbial property to alginates dressings. The incorporation of silver compounds into alginate or alginate-sodium carboxymethyl cellulose (CMC) fibres as well as splutter coating of alginate dressings with nanocrystalised silver powder have previously been reported (Burrell et al., 2001; Ishimori & Ohara, 1994; Qin & Groocock, 2002). Karandikar et al. (2006) reported that medical devices like catheters or alginate/CMC dressings, when soaked in solutions consisting of salts or complexes of silver, copper or zinc, imparted anti-microbial properties against *Staphylococcus aureus*. More recent studies by Quaranta et al. (2011) further suggested that dry copper surfaces could exhibit anti-microbial activities against yeasts via a contact-mediated killing mechanism. However, little was understood about the release properties of these cations from alginate dressings in different wound conditions. The results in this study indicated that Zn^{2+} can potentially be released from alginate dressings and this property could be particularly useful when sustaining an effective anti-microbial concentration in exuding wounds. In this regard, the lower release property of Cu^{2+} will be preferred when treating wounds with less exudates. However, more studies on the clinical effects of these cations on different exuding wounds as well as toxicity of these dressings are necessary.

5. Conclusions

The functional properties of alginate matrices are affected by a number of factors that include the extent and uniformity of cross-linking, the relative binding affinity as well as the binding selectivity of the cations for alginates. These factors are influenced to different extents by the types of cations, the employment of added Na^+ during cross-linking and even cross-linking effects by Ca^{2+} found in the physiological fluid during use. Ca^{2+} or Cu^{2+} could be more useful than Zn^{2+} for making alginate dressings that are soft and tough. Moreover, the different binding affinity of Zn^{2+} and Cu^{2+} for alginates could affect the anti-microbial properties of alginates in infected wounds by the different extent of release of cross-linked cations during use. Generally, alginates cross-linked by Ca^{2+} and Zn^{2+} are not adversely affected by the effects of pH in different wounds. A lower release property of Cu^{2+} was observed, further suggesting that its potential application as anti-microbial dressings would be limited to wounds with less exudates. The inclusion of added Na^+ during cross-linking is not always preferred as it decreased the tensile strength, as well as decreased the release of anti-microbial cations from Cu^{2+} cross-linked alginates. In order to develop alginate dressings that are physically strong yet soft and also perform an effective anti-microbial function, approaches which can modulate the release of Cu^{2+} may be explored. This includes cross-linking alginates with a combination of Cu^{2+} and Zn^{2+}. By studying the mechanistic properties of cations, suitable strategies of employing cations can be further identified to develop anti-microbial alginate dressings.

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