Carbohydrate Polymers 90 (2012) 717–724

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Evaluation of biocomposite films containing alginate and sago starch impregnated with silver nano particles

P. Marie Arockianathan[a,1], S. Sekar[a,1], S. Sankar[a,1], B. Kumaran[b,2], T.P. Sastry[a,*]

[a] Bio-products Laboratory, Central Leather Research Institute, Adyar, Chennai 600 020, India
[b] K.M Centre for P.G Studies, Lawspet, Puducherry 605008, India

ARTICLE INFO

Article history:
Received 8 April 2012
Received in revised form 9 May 2012
Accepted 2 June 2012
Available online 9 June 2012

Keywords:
Sodium alginate
Nanocomposite
Sago starch
Silver nano particle
Wound dressing

ABSTRACT

In recent years, the metal nanoparticles/polymer composites have created lot of attraction due to their wide range of applications. In the present study, the composite films of alginate (AL) and sago starch (SG) impregnated with silver nano particles (AgNP) with and without antibiotic gentamicin (G) were prepared by solvent casting method. The films prepared were characterized for thermo gravimetric analysis, SEM, TEM and mechanical properties and the results have shown the composite nature of the films. AL–SG–AgNP and AL–SG–AgNP–G composites were used as wound dressing materials in experimental wounds of rats. The healing pattern of the wounds was evaluated by planimetric studies, macroscopic observations, biochemical studies and histopathological observations. The results have shown faster healing pattern in the wounds treated with AL–SG–AgNP and AL–SG–AgNP–G composites compared to untreated control. This study revealed that AL–SG–AgNP film might be a potential and economical wound dressing material.

1. Introduction

Wound healing is the primary response to any tissue injury (Sumitra, Manikandan, & Suguna, 2005). It is a complex dynamic process that involves many cascades of events like hemostasis, inflammation, proliferation and remodeling of tissues in order to fill the damage area and re-establish the skin barrier (Deng, He, Zhao, & Yang, 2007; Enoch & Leaper, 2005). A good wound dressing should maintain a moist environment upon absorption of the wound exudates, protect the wound from secondary infection, provide adequate gaseous exchange, regulate and/or mediate the release of certain growth factors and cytokines, and also be elastic, biocompatible, non-toxic and non-antigenic (Boateng, Matthews, Stevens, & Eccleston, 2008; Kokabi, Sirousazar, & Hassan, 2007; Lin, Chen, & Run-Chu, 2001; Singh & Pal, 2008). Recently many researchers are entailed to produce new and improved wound dressing materials by synthesizing and modifying biomaterials that are eco-friendly and sustainable. Alginate, a linear polysaccharide (copolymer of (1-4)-β linked D-mannuronic acid and (1, 3) α-L-guluronic acid (G) monomers), is a natural polysaccharide derived primarily from brown seaweed. Due to its hydrogel properties and biocompatibility, alginate has also been widely used in biomedical applications, including wound management. The major function of alginate in tissue engineering applications is to provide mechanical integrity (Drury, Dennis, & Mooney, 2004). Alginate can form a gel on absorption of wound exudates, prevent the wound surface from drying out, minimize discomfort during removal (Thomas, 2000) and enhance the rate of healing of skin wounds (Jarvis, Galvin, Blair, & McCollum, 1987).

Alginates are non-toxic, hydrophilic, biocompatible, biodegradable and low cost polymers (Li, Ramay, Hauch, Xiao, & Zhang, 2005; Sennerby, Rostlund, Albrektsson, & Albrektsson, 1987) which makes them suitable for many biomedical applications. Due to their good tissue compatibility, they have been widely used in the field of tissue engineering including regeneration of skin (Kong, Yu, Ji, & Xia, 2009), cartilage (Li & Zhang, 2005), bone (Alsberg et al., 2003; Divyarani et al., 2011) and liver (Ginzberg, Bonshtein, Agbaria, & Cohen, 2003; Yang et al., 2001) and in the treatment of exuding wounds and in enhancing the healing process (Paul & Sharma, 2004). There is a considerable interest in biodegradable films made from renewable and natural polymers, such as starch (Lawton, 1996). Starch, a natural biopolymer, can be used to blend with other polymers because it is relatively inexpensive, abundant and biodegradable (Mali & Grossmann, 2003).

As starch film shows poor mechanical properties and poor water resistance capacity, it is blended with other materials to improve its mechanical properties (Chatakanonda, Varavinit, & Chinachoti, 2000). In the present study, we have blended sodium alginate with

* Corresponding author. Tel.: +91 44 24911589; fax: +91 44 24911386.
E-mail addresses: pmanathan26@gmail.com (P. Marie Arockianathan), sekarjuly30@gmail.com (S. Sekar), sankarclri@gmail.com (S. Sankar), itsbkumaran@rediffmail.com (B. Kumaran), sastrytp@hotmail.com (T.P. Sastry).
[1] Tel.: +91 44 24911589; fax: +91 44 24911386.
[2] Tel.: +91 413 2251687; fax: +91 413 2251613.

0144-8617/$ – see front matter
http://dx.doi.org/10.1016/j.carbpol.2012.06.003

sago starch to enhance/modify the properties of starch thereby it suits to the desired application. As metal nano particles/polymer composites have created lot of attraction, silver nano particles are incorporated in the above composite film, since silver nano particles exhibit antimicrobial properties (Thomas & McCubbin, 2003).

The main objective of the present study is to prepare a novel biocomposite film containing alginate (AL) and sago starch (SG) with improved mechanical properties. This composite was also impregnated with silver nano particles with the aim of getting antimicrobial property to the end products. These AL–SG–AgNP composite films were used as wound dressing material in experimental rats. The progress of the wound healing in both experimental and control groups was evaluated by planimetric studies, macroscopic observations, histopathological and biochemical studies.

2. Materials and methods

2.1. Materials

The sago rice was purchased from nearby local retail market and sodium alginate and other chemicals used in this study were purchased from Sigma–Aldrich, India and were of analytical grade.

2.2. Preparation of silver nano particles using alginate (AL–AgNP) solution

Silver nano particles were prepared using alginate solution by modifying the procedure described earlier (Liji, Sundarseelan, Sekar, & Sastry, 2009). Briefly, to 1 mL of alginate solution (2%), 1 mL of aqueous Ag_2SO_4 solution (1×10^{-4} M) was added followed by stirring for 1 h. To this solution, 0.2 mL of 1 M $NaBH_4$ (dissolved in 0.3 M NaOH) was added drop wise followed by stirring till the solution attained golden brown color. At this stage addition of $NaBH_4$ was stopped, the stirring was continued for another fifteen minutes; formation of silver nano particles was observed. This solution containing silver particles was stored at 10 °C till further use.

2.3. Preparation of sago (SG) solution

Sago was powdered using a domestic mixer and sieved to a particle size of 50–200 μm. 10 g of sago powder was boiled in 100 mL water till it becomes a homogenous solution. The solid content of the solution was found to be 13.68% and this solution was used for further experiments.

2.4. Optimization of sago film

As the film formed by drying SG solution was very brittle, ethylene glycol (EG) was used to get the flexibility to the film. Keeping 40 mL of SG solution constant, the amount of EG added was varied from 0.5 to 4 mL and the resultant solutions were poured into polythene trays having measurements 10 cm × 10 cm, and dried at 30–35 °C. The dried films were stored in polythene covers for further use.

2.5. Optimization of AL–SG–AgNP composite film

The stoichiometric ratio of 40 mL sago and 2 mL EG, with higher tensile strength, was used for the preparation of AL–SG–AgNP composite. Keeping the amounts of SG solution and EG constant, the amount of alginate–AgNP solution was varied in the ratio 4:0.5–4:3.5 and the resultant solutions were poured into polythene trays having measurements 10 cm × 10 cm, and dried at 30–35 °C. The dried films were stored in polythene covers and sterilized for 4 h at 45 °C with 100% ethylene oxide (EtO) and stored for further use.

2.6. Preparation of AL–SG–AgNP–G film

The drug gentamicin (G) 180 μg/mL was added to SG and AL–AgNP in the stoichiometric ratio (4:3) and stirred well. The solution was poured into polythene trays having measurements 10 cm × 10 cm, and dried at 30–35 °C. The dried films were stored in polythene covers and sterilized for 4 h at 45 °C with 100% ethylene oxide (EtO) and stored for further use.

2.7. Characterization

The mechanical properties of the composite films were measured using universal testing machine (Instron model 4501). Thermogravimetric analysis was carried out with a Perkin Elmer TGA over a temperature range of 30–700 °C at a heating rate of 20 °C/min under nitrogen atmosphere. The morphological studies were carried out on a Leica stereo scan-440 Scanning electron microscope equipped with phoenix EDX attachment. Transmission electron microscopy (TEM) images were recorded on a FEG-TEM (Philips CM 200 field emission gun).

2.8. Animal experiments

All experiments were performed according to the Institutional Animal Ethical Committee approval and guidelines [845/ac/04/CPCSEA]. Male Albino Wister rats, weighing 150–200 g, were divided into three groups: control, AL–SG–AgNP and AL–SG–AgNP–G dressings. Throughout the experiment, rats were maintained in an air-conditioned room at 25 ± 1 °C with a lighting schedule of 12 h light and 12 h dark and were fed with commercial balanced diet and water *ad libitum*.

2.9. Surgical procedure and treatment

Each animal was given a dose of sodium pentobarbital 40 mg/kg body weight intraperitonally and the dorsal surface of the rat below the cervical region was shaved on its back under aseptic conditions. An open excision wound of 2 cm × 2 cm was created on the shaved dorsal side of rats using sterile surgical blade. For the control group, sterile cotton gauze dipped with gentamicin was applied on the wound. The group 1 was applied with the wound dressing film of AL–SG–AgNP and for the group 2; the AL–SG–AgNP–G film was applied. The dressings were periodically changed at an interval of 4 days with the respective materials. Three rats were sacrificed periodically on 4th, 8th, 12th, 16th and 18th day of post wound creation and the granulation tissues formed were removed and stored at −70 °C until analysis. The progress of wound healing in the three groups was evaluated visually, planimetrically, histologically and biomechanically by periodical monitoring of wound surface.

2.10. Biochemical parameters

In the present study, DNA, protein, collagen, hexosamine and uronic acid levels were estimated in the granulation tissue of control and experimental wounds on days 4, 8, 12 and 16. The granulation tissue was collected after sacrificing the animals on the respective days. Estimation of protein was determined by Lowry, Rosebrough, Farr, and Randall's method (1951) and DNA was estimated by the method of Burton (1956). Collagen and hexosamine were determined in defatted dried granulation tissue by the methods of Woessner (1961) and Elson and Morgan (1933), respectively. Extraction of uronic acid from the tissue was carried out according

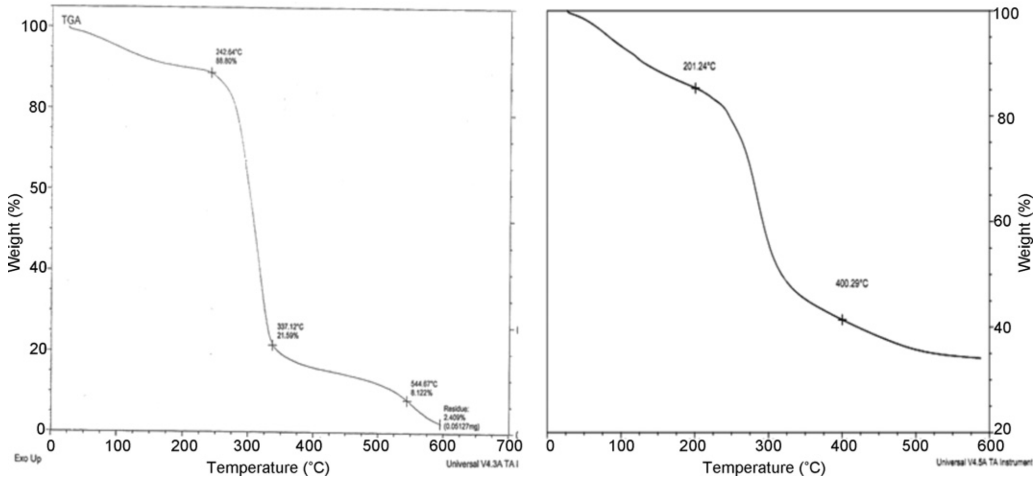

Fig. 1. Thermogravimetric analysis of (A) sago (SG) film and (B) alginate–sago (AL–SG) film.

to the method of Schiller, Slover, and Dorfman (1961) and estimated by the method of Bitter and Muir (1962).

2.11. Histological studies

The animals were sacrificed periodically on 4th, 8th, 12th 16th and 18th days post wound creation and the tissue from the wound site of the individual animal was removed. These samples were then separately fixed in 10% formalin, dehydrated through graded alcohol series, cleared in xylene, and embedded in paraffin wax (m.p. 56 °C). Serial sections of 5 μm thickness were cut and stained with hematoxylin and eosin. The sections were examined under a microscope and photomicrographs were taken.

2.12. Tensile strength of the healed wound

On the 30th day after creating the wound, the animals were anesthetized. Healed tissue along with normal skin at the two ends was excised for tensile strength (MPa) and percentage of elongation at break (%) measurement using a universal testing machine (Instron model 4501). The break load was measured and the tensile strength was calculated by the following equation

$$\text{Tensile strength} = \frac{\text{Break load}}{\text{strip cross} - \text{sectional area}}$$

2.13. Planimetric studies

Hair was clipped around the scar for proper visualization and the individual contour of the wounds of both control and experimental animals was measured periodically, using a transparent graph sheet and the rate of healing was calculated and expressed as percentage contraction (Morgan et al., 1994).

2.14. Statistical analysis

Data are expressed as mean ± SD. Values are compared using Duncan's multiple comparison using SPSS 13 software. *p* values less than 0.05 were considered statistically significant.

3. Results and discussion

Recently many researchers have developed several wound materials by synthesizing and modifying biomaterials. An ideal wound dressing should have several key attributes (Horch, Kopp, Kneser, Beier, & Bach, 2005) and only those dressing material with good mechanical properties can be applied on to wound properly.

In this study, the composite films containing sodium alginate and sago starch impregnated with silver nano particles (AL–SG–AgNP) were prepared and EG were used to improve the flexibility of the films. Among the various compositions studied, 40 mL sago and 2 mL EG gave higher tensile strength and good elongation properties (Supplementary Table S1). Increase in the amount of EG above 2 mL shown decrease in the tensile strength of the samples, this may be due to the excess of ethylene glycol molecules impregnated between the starch molecules. This composition of SG and EG was used to prepare AL–SG–AgNP composites. Among various AL–SG–AgNP composites prepared, the stoichiometric ratio of SG and AL–AgNP (4:3) gave better mechanical properties (Supplementary Table S2) and the same was used for further characterization studies such as mechanical properties, SEM, TEM, Thermal gravimetric analysis and *in vivo* studies.

3.1. Thermo gravimetric analysis (TGA)

Normally, when a biomaterial is heated to higher temperature it decomposes into CO_2, CO, NO_x and H_2O. In the present investigation, the biomaterials prepared were heated in a nitrogen atmosphere from 30 to 700 °C. The weight loss of SG (Fig. 1A) was found to be 79% of its weight at 331 °C whereas AL–SG (Fig. 1B) samples lost 55% of the weight at the same temperature. The increase in the thermal stability of AL–SG samples may be due to the presence of AL in the composite. The stability of the composites at higher temperature is an added advantage to biomaterials. At 600 °C, the thermograms of SG and AL–SG have shown 2.41%, and 34% residual matter respectively.

3.2. Scanning electron microscopy (SEM) and energy dispersive X-ray analysis (EDX)

Scanning electron microscope pictures exhibit the surface morphology of biomaterials. In the present study, SEM pictures were taken for SG, AL–SG and AL–SG–AgNP (Fig. 2A–C). The SG film has shown porous and rough surface, similar surface characteristics were observed for AL–SG and AL–SG–AgNP film. The porous nature of the AL–SG–AgNP film helps in absorbing the wound exudates and also helps in oxygen exchange to the wound surface. The EDX spectrum of AL–SG–AgNP film has clearly shown the presence of silver with a corresponding signal (Fig. 2D).

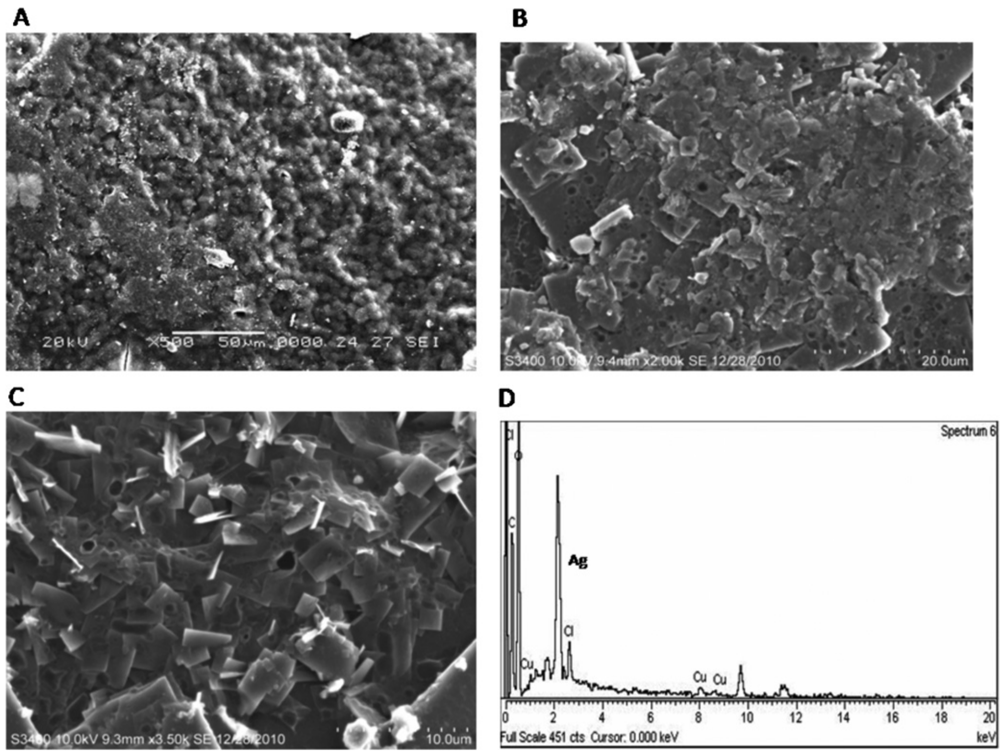

Fig. 2. SEM image showing the porous surface of (A) SG, (B) AL–SG and (C) AL–SG–AgNP, (D) EDX spectrum of AL–SG–AgNP shows the presence of silver.

3.3. *Transmission electron microscopy (TEM)*

The TEM picture shows size and shape of the AgNP. The size of AgNP was found to be between 15 and 20 nm and the shape of the NPs was found to be spherical (Fig. 3).

3.4. *Animal studies*

3.4.1. *Photographic evaluation*

Surface of the wounds were photographed periodically from a constant distance for both control (Fig. 4A and D) and experimental (Fig. 4B, C, E and F) groups. Faster rate of healing was observed in the experimental wounds compared to that of control. The results are in agreement with those of planimetric observations.

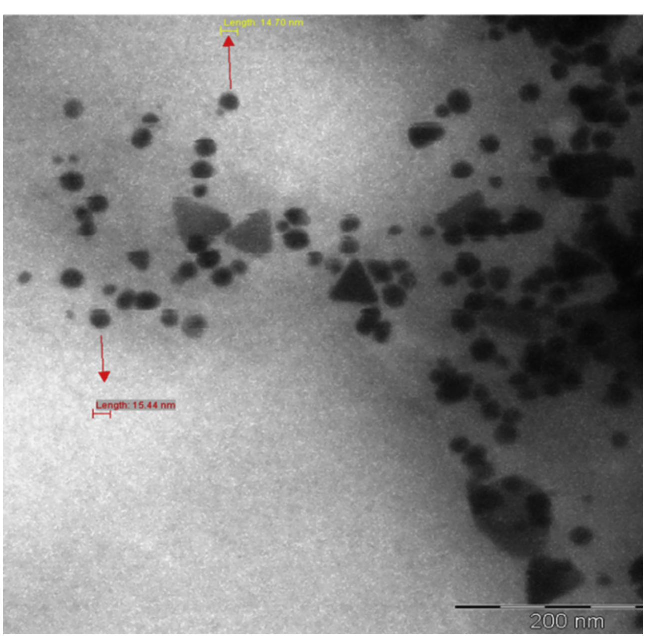

Fig. 3. TEM microphotograph of AL–AgNP showing silver nano particles with spherical shapes.

3.4.2. *Wound contraction*

On the 4th day, the wound contraction of control was 10.8% whereas 23.6% and 28.7% closure were observed in AL–SG–AgNP and AL–SG–AgNP–G treated wounds respectively. However, by the 16th day, 99% of the wound was closed in the experimental groups; 89% of the wound was closed in the control animals (Supplementary Fig. S1); these results show the efficacy of AL–SG–AgNP and AL–SG–AgNP–G as wound dressing materials. The increase in wound contraction in the treated rats might be due to the enhanced activity of fibroblasts.

3.4.3. *Period of healing*

The macroscopic analysis of the wound revealed that the complete healing of the wound in AL–SG–AgNP and AL–SG–AgNP–G dressing groups took only 18 and 16 days respectively, whereas it took 25 days for the control groups. There was no significant difference in the gross healing pattern between the dressing materials AL–SG–AgNP and AL–SG–AgNP–G.

3.4.4. *Biochemical parameters*

The biochemical parameters *i.e.* protein, DNA, collagen, hexosamine and uronic acid in the granulation tissues of the control and experimental rats on different days after wound creation were analyzed. A significant increase in the collagen content was observed from day 4 to 8 in the experimental rats when compared to control (Fig. 5A). However, the collagen contents have shown higher values on all the days studied in experimental animals. The marked increase in collagen content of granulation tissue isolated from experimental groups may be due to increased synthesis of collagen and could be correlated with the enhanced migration of fibroblasts and epithelial cells to the wound site for effective healing (Bernabei et al., 1999; Pilcher, Sudbeck, Dumin, Welgus, & Parks,

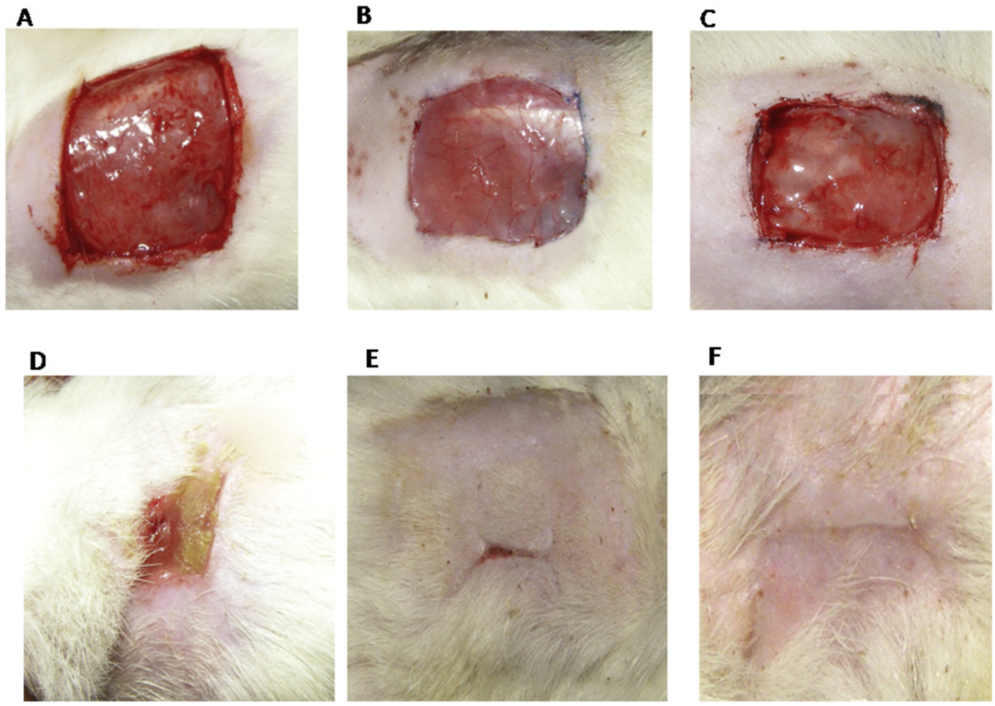

Fig. 4. Photographic representation of wound contraction rate on different days of healing: (A), (B) and (C) are control, AL–SG–AgNP and AL–SG–AgNP–G treated groups on 0th day, respectively. (D), (E) and (F) are control, AL–SG–AgNP and AL–SG–AgNP–G treated groups on 16th day, respectively.

1998). The decreased content of collagen in control group is due to the prolonged inflammatory phase (Senthil Kumar, Kirubanandan, Sripriya, & Sehgal, 2008). Likewise, protein and DNA contents of experimental groups have showed significantly higher values up to 12th day than in control followed by reduction in the later phase of wound healing (Fig. 5B and C). The increase in DNA content in the treated wounds indicates cellular hyperplasia and the increase of total protein content in the initial phase of healing is due to the active synthesis and deposition of matrix proteins in the granulation tissues, this resulted in the increased synthesis of collagen.

Hexosamine acts as ground substratum for the synthesis of extracellular matrix; significant decrease in the hexosamine content was associated with concomitant increase in the extracellular

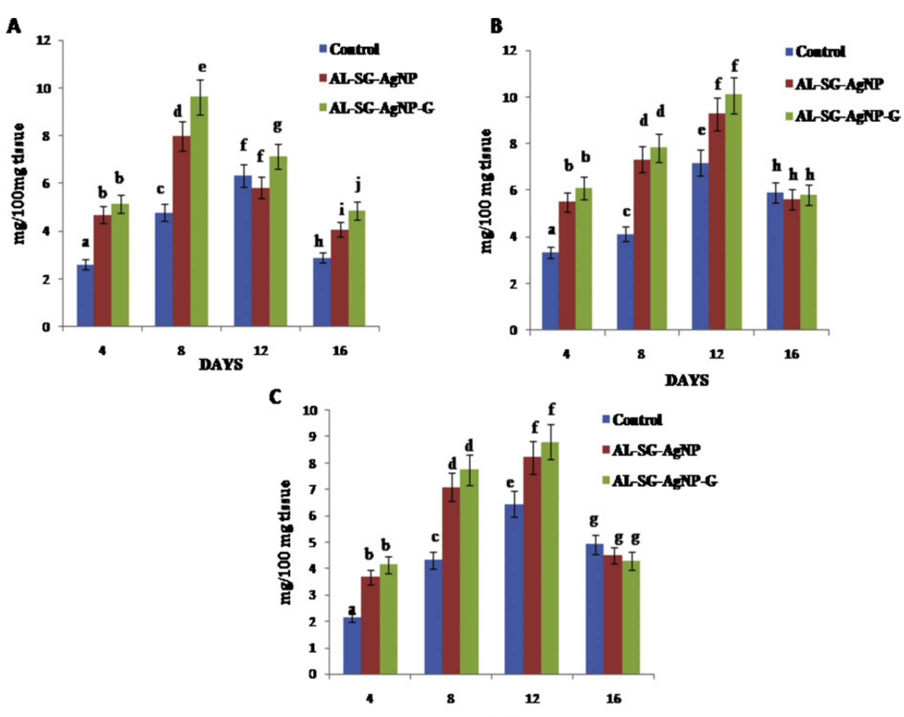

Fig. 5. (A) The collagen content, (B) the protein content and (C) the DNA content in granulation tissue of control, AL–SG–AgNP and AL–SG–AgNP–G treated groups on various days of healing ($n = 3$), respectively. Results are presented as mean ± SD; means bearing different superscripts (within 4th, 8th, 12th and 16th day) differ significantly at $p < 0.05$ using Duncan's test for multiple comparison.

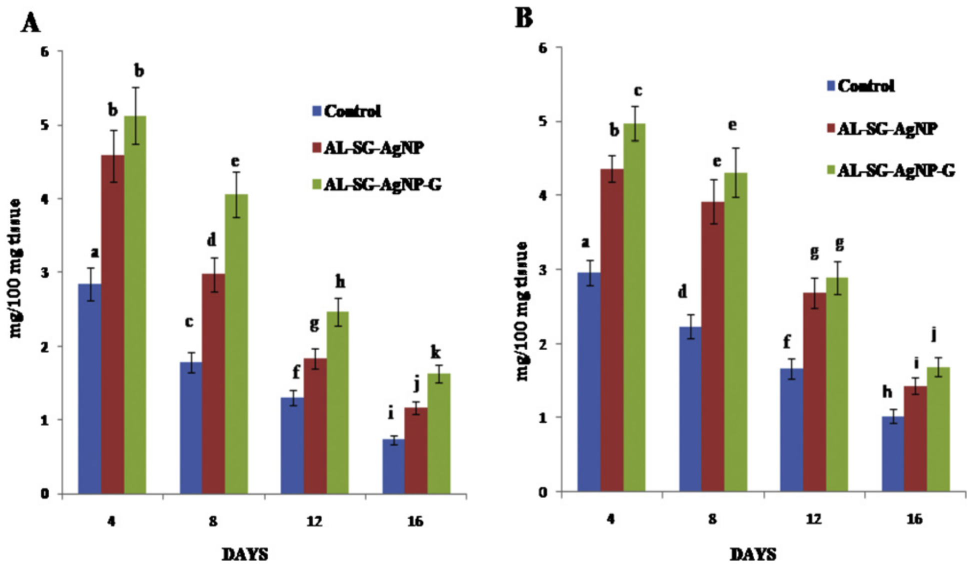

Fig. 6. (A) The hexosamine content and (B) the uronic acid content in granulation tissue of control, AL–SG–AgNP and AL–SG–AgNP–G treated groups on various days of healing ($n = 3$), respectively. Results are presented as mean ± SD; means bearing different superscripts (within 4th, 8th, 12th and 16th day) differ significantly at $p < 0.05$ using Duncan's test for multiple comparison.

matrix. The hexosamine values have shown decreasing trend in both control and experimental groups (Fig. 6A), however, there was a notable increase of these values in the experimental groups compared to control group. Similar trend was observed in the amounts of uronic acid (Fig. 6B). Increase in the levels of these biochemical parameters *i.e.* collagen, DNA, protein, uronic acid and hexosamine in the experimental groups gave an indication of the faster rate of wound healing compared to control. Similar results were observed in the earlier studies (Noorjahan & Sastry, 2004; Sumitra et al., 2005).

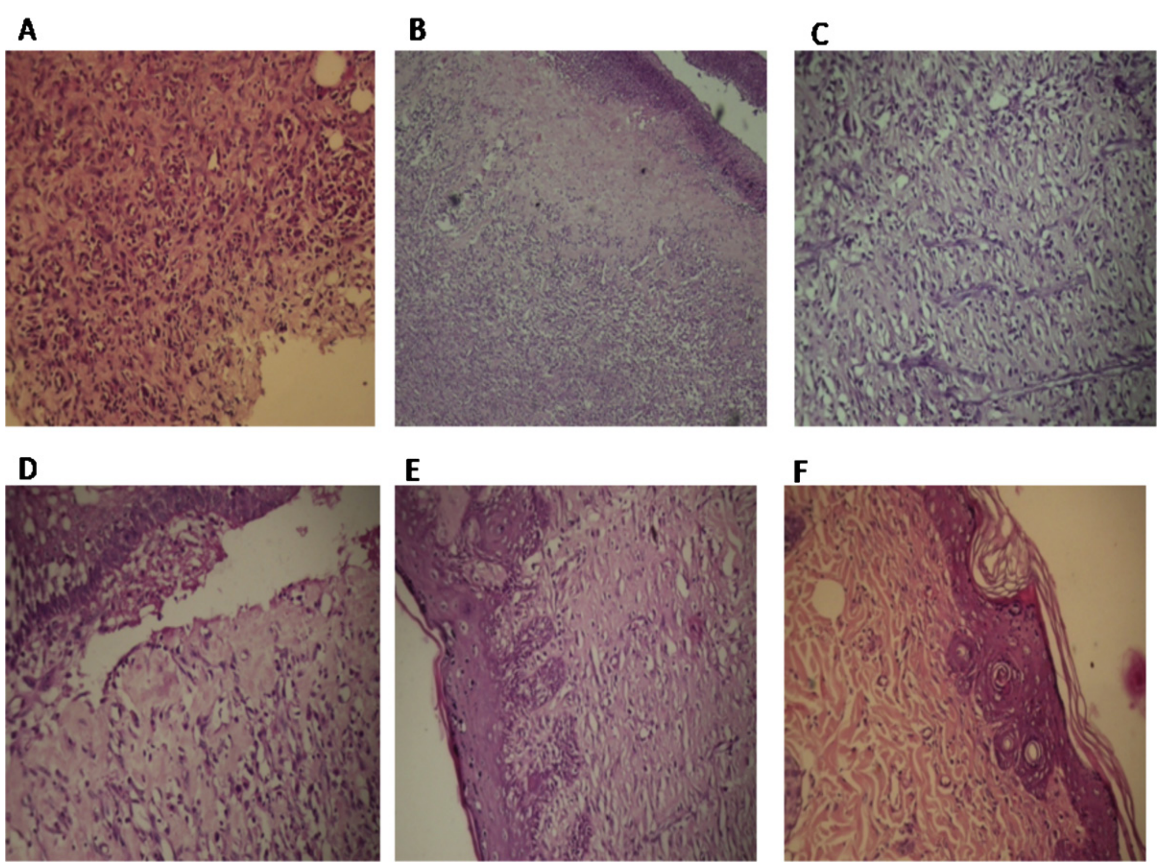

Fig. 7. Hematoxylin and eosin stained sections of the granulation tissue at different time intervals. (A) and (D) are control group on day 8 and 16 respectively, (B) and (E) are the AL–SG–AgNP treated group on day 8 and 16 respectively and (C) and (F) are the AL–SG–AgNP–G treated group on day 8 and 16 respectively. (A), (C), (D), (E) and (F) are at similar magnification (20×) and (B) is at 10×.

3.4.5. Histological studies

Histological changes recorded in the control and treated groups at different days are shown in Fig. 7. Re-epithelization of the wounds was noticed as early as 8th day in the treated groups whereas it occurred on the 12th day in the control group. In addition to re-epithelization, there was significant increase in the collagen content and early collagenization in the treated rats. This was attributed to the enhanced migration of fibroblasts to the wound site. The healing process depends to a large extent on the regulated biosynthesis and deposition of new collagen and their subsequent maturation (Dunphy & Udupa, 1956). The histopathological studies revealed that wound healing was complete by 18th day in the case of AL–SG–AgNP treated wounds and 16th day in AL–SG–AgNP–G treated wounds whereas normal histology was observed in the control wounds only on 25th day.

3.4.6. Tensile strength

The tensile strength values exhibited by healed excision wounds of experimental groups are greater than control group (Supplementary Table S3). Increased tensile strength indicates increase in collagen matrix. There is rapid biosynthetic activity in experimental groups during initial phase of granulation. In the remodeling phase, maturation of collagen takes place by the formation of inter and intramolecular cross links, hence, increased wound strength was observed (Noorjahan & Sastry, 2004). But in the case of control, the decreased tensile strength and prolonged wound healing further support the slow rate of wound healing due to dry wound-healing conditions offered by cotton gauze. Among the experimental groups, those treated with AL–SG–AgNP and AL–SG–AgNP–G show higher tensile strength values when compared with the control, and this is mainly attributed to its increased hydrophilic capacity thereby maintaining moist environment at the wound site, which is reflected in its faster wound-healing nature. Moreover, the tensile strength is directly related to the amount of collagen synthesized at the wound site. If we observe the results shown in Fig. 5A, the collagen content values in the AL–SG–AgNP and AL–SG–AgNP–G treated wounds were higher when compared to those of control group at all intervals.

4. Conclusion

Evaluation of the AL–SG–AgNP and AL–SG–AgNP–G composites as a temporary biological wound dressing materials on the experimental wounds of rats has revealed that the experimental wounds healed faster than the control wounds. The gross observations have revealed that the complete closing of wounds were observed on the 16th day for the animals treated with AL–SG–AgNP–G, whereas it took 18 days for AL–SG–AgNP and 25 days for the control wounds. There was no significant difference in the gross healing pattern between the dressing materials, AL–SG–AgNP and AL–SG–AgNP–G and the presence of antibiotic has only reduced the inflammatory cells. These observations gave an indication that AL–SG–AgNP and AL–SG–AgNP–G composites might be tried as a wound dressing materials in the clinical wounds of smaller and larger animals before apply on to humans.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2012.06.003.

References

Alsberg, E., Kong, H. J., Hirano, Y., Smith, M. K., Albeiruit, A., & Mooney, D. J. (2003). Regulating bone formation via controlled scaffold degradation. *Journal of Dental Research*, *82*, 903–908.

Bernabei, R., Landi, F., Bonini, S., Onder, G., Lambiase, A., & Pola, R. (1999). Effect of topical application of nerve-growth factor on pressure ulcers. *Lancet*, *354*, 307.

Bitter, T., & Muir, H. M. (1962). A modified uronic acid carbazole reaction. *Analytical Biochemistry*, *4*, 330–334.

Boateng, J. S., Matthews, K. H., Stevens, H. N., & Eccleston, G. M. (2008). Wound healing dressings and drug delivery systems: A review. *Journal of Pharmaceutical Sciences*, *97*, 2892–2923.

Burton, K. (1956). A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. *Biochemical Journal*, *62*, 315–323.

Chatakanonda, P., Varavinit, S., & Chinachoti, P. (2000). Effect of crosslinking on thermal and microscopic transitions of rice starch. *Food Science and Technology*, *33*, 276–284.

Deng, C. M., He, L. Z., Zhao, M., & Yang, D. (2007). Biological properties of the chitosan–gelatin sponge wound dressing. *Carbohydrate Polymers*, *69*, 583–589.

Divyarani, V. V., Ramachandran, R., Chennazhi, K. P., Tamura, H., Nair, S. V., & Jayakumar, R. (2011). Fabrication of alginate/nanoTiO_2 needle composite scaffolds for tissue engineering applications. *Carbohydrate Polymers*, *83*, 858–864.

Drury, J. L., Dennis, R. G., & Mooney, D. J. (2004). The tensile properties of alginate hydrogels. *Biomaterials*, *25*, 3187–3199.

Dunphy, J. E., & Udupa, K. N. (1956). Chemical and histochemical sequences in the normal healing of wounds. *New England Journal of Medicine*, *253*, 847–852.

Elson, L. A., & Morgan, W. T. J. (1933). A colorimetric method for the determination of glucosamine and chondrosamine. *Biochemical Journal*, *27*, 1824–1828.

Enoch, S., & Leaper, D. J. (2005). Basic science of wound healing. *Surgery (Oxford)*, *26*, 31–37.

Ginzberg, M. D., Bonshtein, I. G., Agbaria, R., & Cohen, S. (2003). Liver tissue engineering within alginate scaffolds: Effects of cell-seeding density on hepatocyte viability, morphology and function. *Tissue Engineering*, *9*, 757–766.

Horch, R. E., Kopp, J., Kneser, U., Beier, J., & Bach, J. (2005). Tissue engineering of cultured skin substitutes. *Journal of Cellular and Molecular Medicine*, *9*(3), 592–608.

Jarvis, P. M., Galvin, D. A. J., Blair, S. D., & McCollum, C. N. (1987). How does calcium alginate achieve haemostasis in surgery? *Proceedings of the 11th Congress on Thrombosis and Haemostasis*, *58*(1), 50.

Kokabi, M., Sirousazar, M., & Hassan, Z. M. (2007). PVA–clay nanocomposite hydrogels for wound dressing. *European Polymer Journal*, *43*, 773–781.

Kong, Q., Yu, Z., Ji, Q., & Xia, Y. (2009). *Material Science Forum*, *610*, 1188–1191.

Lawton, J. W. (1996). Effect of starch type on the properties of starch containing films. *Carbohydrate Polymers*, *29*, 203–208.

Li, Z., Ramay, H. R., Hauch, K. D., Xiao, D., & Zhang, M. (2005). Chitosan/alginate hybrid scaffolds for bone tissue engineering. *Biomaterials*, *26*, 3919–3928.

Li, Z., & Zhang, M. (2005). Chitosan–alginate as scaffolding material for cartilage tissue engineering. *Journal of Biomedical Materials Research Part A*, *75A*, 485–493.

Liji Sobhana, S. S., Sundarseelan, J., Sekar, S., & Sastry, T. P. (2009). Gelatin–chitosan composite capped gold nanoparticle: A matrix for the growth of hydroxyapatite. *Journal of Nanoparticle Research*, *11*, 333–340.

Lin, S. Y., Chen, K. O., & Run-Chu, L. (2001). Design and evaluation of drug-loaded wound dressing having thermoresponsive, adhesive, absorptive and easy peeling properties. *Biomaterials*, *22*, 2999–3004.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. T. (1951). Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry*, *193*, 265–276.

Mali, S., & Grossmann, M. V. E. (2003). Effects of yam starch films on storability and quality of fresh strawberries. *Journal of Agricultural and Food Chemistry*, *51*, 7005–7011.

Morgan, P. W., Binnington, A. G., Miller, C. W., Smith, D. A., Valliant, A., & Prescott, J. F. (1994). The effect of occlusive and semi occlusive dressings on the healing of acute full-thickness skin wounds on the forelimbs of dogs. *Veterinary Surgery*, *23*, 494–502.

Noorjahan, S. E., & Sastry, T. P. (2004). An in vivo study of hydrogels based on physiologically clotted fibrin–gelatin composites as wound dressing materials. *Journal of Biomedical Materials Research Part B: Applied Biomaterials*, *71B*, 305–312.

Paul, W., & Sharma, C. P. (2004). Chitosan and alginate wound dressings: A short review. *Trends in Biomaterials & Artificial Organs*, *18*, 18–23.

Pilcher, B. K., Sudbeck, B. D., Dumin, J. A., Welgus, H. G., & Parks, W. C. (1998). Collagenase-I and collagen in epidermal repair. *Archives of Dermatological Research*, *290*, 37–46.

Schiller, S., Slover, A., & Dorfman, A. (1961). A method for the separation of acid mucopolysaccharides: Its application to the isolation of heparin from the skin of rats. *Journal of Biological Chemistry*, *236*, 983–987.

Sennerby, L., Rostlund, T., Albrektsson, B., & Albrektsson, T. (1987). Acute tissue reactions to potassium alginate with and without colour/flavour additives. *Biomaterials*, *8*, 49–52.

Senthil Kumar, M., Kirubanandan, S., Sripriya, R., & Sehgal, P. K. (2008). Triphala incorporated collagen sponge – A smart biomaterial for infected dermal wound healing. *Journal of Surgical Research*, *144*, 94–101.

Singh, B., & Pal, L. (2008). Development of sterculia gum-based wound dressings for use in drug delivery. *European Polymer Journal*, *44*, 3222–3230.

Sumitra, M., Manikandan, P., & Suguna, L. (2005). Efficacy of *Butea monosperma* on dermal wound healing in rats. *The International Journal of Biochemistry & Cell Biology*, *37*, 566–573.
Thomas, S. (2000). Alginate dressings in surgery and wound management. Part 1. *Journal of Wound Care*, *9*(2), 56–60.
Thomas, S., & McCubbin, P. (2003). A comparison of the antimicrobial effects of four silver-containing dressings on three organisms. *Journal of Wound Care*, *12*(3), 101–107.
Woessner, J. R. (1961). Catabolism of collagen and non-collagen protein in the rat uterus during post partum involution. *Archives of Biochemistry and Biophysics*, *93*, 440–447.
Yang, J., Chung, T. W., Nagaoka, M., Goto, M., Cho, C. S., & Akaike, T. (2001). Hepatocyte-specific porous polymer-scaffolds of alginate/galactosylated chitosan sponge for liver-tissue engineering. *Biotechnology Letters*, *23*, 1385–1389.