INVESTIGATION OF DIALDEHYDE CORN STARCH AS CROSSLINKING AGENT IN COLLAGEN-BASED WOUND DRESSING MATERIALS

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Collagen is the most commonly used tissue-derived natural polymer. Collagen gels are widely used in wound dressing materials due to their good degradability, poor antigenicity, excellent biocompatibility and unique fibril forming properties. But, poor mechanical properties of physically formed collagen gels limit their usage in biomedical fields. However, collagen gels can be stabilized by using cross-linkers. In recent years, use of crosslinking agents obtained from biodegradable, low toxic, abundant, inexpensive and renewable sources in wound dressing materials for stabilization of collagen gels has become important. In the present study use of starch as a crosslinking agent in stabilization of collagen gels was investigated. For this purpose native starch was modified via oxidation of corn starch by NaIO4 in order to obtain dialdehyde starch. Then, the degree of substitution and swelling index of oxidized starches were determined and the starch samples were characterized by Fourier transforms infrared (FT-IR) spectroscopy and Scanning Electron Microscopy (SEM). Collagen gels were crosslinked with dialdehyde starch samples for preparing wound dressing materials. The collagen gel composites were lyophilized and sponge forms were obtained. They were characterized by water uptake capacity and enzymatic degradation. The results revealed that, dialdehyde corn starch can be used successively in collagen-based wound dressing materials as crosslinking agent.

Keywords: dialdehyde starch, collagen, wound-dressing material.

INTRODUCTION

Collagen protein is one of the major components of connective and bone tissues (Cuneo *et al.*, 2010) being one of the mostly used biomaterials due to its excellent biocompatibility, biodegradability and weak antigenicity, well established structure, biologic characteristics and to the way it interacts with the body, being known by the body as one of its constituent and not as an unknown material (Albu *et al.*, 2011; Ficai *et al.*, 2013).

Collagen dressings encourage the deposition and organization of newly formed collagen, creating an environment that fosters healing because of the dressings' chemotactic properties on wound fibroblasts. Collagen-based biomaterials stimulate and recruit specific cells, such as macrophages and fibroblasts, along the healing cascade in order to enhance and influence wound healing (Fleck and Simman, 2010). However, physically formed collagen gel exhibits poor mechanical properties, and low thermal stability, which limit its applications as wound dressings (Mu *et al*., 2011; Li *et al*., 2016). Therefore, extensive research has focused on modifying collagen with some physical, chemical, and biological cross-linkers to strengthen the intramolecular or intermolecular structures (Tan *et al*., 2015). They are often cross-linked by UV light irradiation, multivalent metal ions, formaldehyde, or glutaraldehyde (Gayatri *et al*., 2001; Sheu *et al*., 2001; Mu *et al*., 2011). The use of chemical cross-linkers may lead to the presence of unreacted molecules in the collagen matrix, which can result in cytotoxic reaction. Currently, natural or bio-based cross-linking agents such as genipin, proanthocyanidin, citric acid, malic acid, ferulic acid, tannic acid are being explored and

investigated (Cheirmadurai *et al*., 2016). Note that oxidized polysaccharides have received an increasing attention as ideal crosslinking reagents of protein in recent years (Li *et al*., 2016). Polysaccharides are abundant, inexpensive, biodegradable, environmentally friendly and renewable biopolymers. Two aldehyde groups can be introduced in structure of polysaccharides such as starch and cellulose by periodate oxidation. These highly reactive dialdehyde groups can be used as crosslinking agents.

In the present study, native corn starch was modified by periodate oxidation in different molar ratios and dialdehyde starch (DAS) samples were obtained. Then, they were used as a crosslinking agent for obtaining collagen wound dressing materials.

MATERIALS AND METHODS

Materials

Native corn starch was supplied from Hasal Starch Company in Izmir/Turkey. Sodium metaperiodate ($NaIO_4$) were purchased from Sigma Co. Ltd. Type I collagen having a concentration of 2.4% (w/v) was obtain by own technology in ICPI - Collagen Department and type I collagenase from *Clostridium histolyticum* was purchased from Sigma-Aldrich. All other chemicals used in analysis were analytical grade.

Sodium Metaperiodate Oxidation of Corn Starch

The oxidation of the corn starch by $NaIO_4$ was carried out based on the procedure of Zhang et al. (2011) with slight modifications. The molar ratios of $NaIO_4$ to starch were 1:0.2, 1:0.4, 1:0.6, 1:0.8 and 1:1.0. The pH was adjusted to 3.0 with 2% (w/v) hydrochloric acid and the mixture was rigorously stirred with magnetic stirrer at 35 °C for 4 h in dark ambient. After oxidation process, the mixture was filtered and washed 10 times (10x100 mL) with distilled water and then the product was washed with 50 mL acetone. After washing process, it was dried in a hot-air oven at 50°C for 48 h until constant weight. The dried products were milled and sieved.

Determination of Aldehyde Contents of Oxidized Starches by $NaIO_4$

The aldehyde contents of the oxidized samples were determined using the rapid quantitative alkali consumption method described by Hofreiter *et al.* (1955).

$$Da\% = \frac{C_1 \cdot V_1 - 2 \cdot C_2 \cdot V_2}{W/161 \times 1000} \times 100\% \quad (1)$$

where C_1=NaOH normality (mol/L), C_2=H_2SO_4 normality (mol/L), V_1=total volume of NaOH (mL), V_2=total volume of H_2SO_4 (mL), W=dry weight of the dialdehyde sample, 161=average molecular weight of the repeat unit in DAS.

Determination of the Swelling Index of Native and Oxidized Starches

The swelling index of native corn starch and oxidized starches was determined according to method of Willpiszewska and Spychaj (2007).

Fourier Transform Infrared (FT-IR) Spectroscopy

The infrared spectra of native and dialdehyde starches were done by JASCO FT/IR-4200 spectrophotometer in the range of 4000-600 cm^{-1}.

Scanning Electron Microscopy (SEM)

The morphological features of native and dialdehyde starches were observed by scanning electron microscope (SEM- Quanta 200-FEI/Holland). The dried samples were mounted on a metal stub and the images were taken at an accelerating voltage of 10 kV. Micrographs of dialdehyde starches were recorded at 4000x magnifications.

Crosslinking of Collagen with Dialdehyde Starch

Type I collagen gel was crosslinked with different ratio of oxidized starch as Table 1 presents and their pH were adjusted at 7.2-7.4 with 1 M NaOH, to mimic the human body pH.

Table 1. The formulations of prepared samples

Sample Name	Collagen gel (g)	Oxidized starch (g)
C – O	41.68	-
C – 1:0.2	41.68	1.0
C – 1:0.4	41.68	1.0
C – 1:0.6	41.68	1.0
C – 1:0.8	41.68	1.0
C – 1:1.0	41.68	1.0

All the gels were lyophilized using Martin Christ freeze-dryer according to the previously described method (Albu *et al.*, 2011) and collagen-starch composites were obtained.

Determination of Water Uptake Capacity of Collagen-Starch Composites

Three pieces were cut from each lyophilized sample and were weighted (W_d) sensitively. Then 2.5 mL distilled water was added for each weighed sample. At the established time intervals they were reweighed (W_w) to determine their water uptake capacity. The water uptake capacity of samples was calculated according to the following equation:

$$Water\ up\text{-}take\ =\ (W_w - W_d)/W_d\ g/g \tag{2}$$

where W_w represents the weight of wet matrices at immersion time, W_d represents the weight of dried one.

Enzymatic Degradation of Collagen-Starch Composites

Enzymatic degradation of prepared collagen-starch composites was investigated by monitoring the weight loss depending on exposure time to collagenase solution. 0.5 mL collagenase solution was added to previously weighed composites. At regular time intervals, the swollen composites were removed from degradation solution and then weighed. The percentage of matrices degradation was determined by the following equation:

$$\%\ weight\ loss\ =\ [(W_i - W_t)/W_i] \cdot 100 \tag{3}$$

where W_i represents the initial weight, W_t represents the last weight.

RESULTS AND DISCUSSION

Aldehyde group contents of the oxidized starches by $NaIO_4$ are showed in Table 2.

Table 2. The aldehyde contents of oxidized starches

Samples	Aldehyde Content %
1:0.2	16.7
1:0.4	28.9
1:0.6	45.9
1:0.8	67.7
1:1.0	80.2

From the results, it was seen that increasing periodate molar ratio caused significant increase in aldehyde content in starch molecule. It is proved that periodate is a selective oxidant and can cleave the C2, C3 bond of anhydroglucose units with the formation of dialdehyde groups.

Swelling index of native and dialdehyde starches are presented in Table 3.

Table 3. Swelling index of starches

Samples	Swelling Index (cm^3/g)
Native starch	0
1:0.2	1
1:0.4	1.6
1:0.6	0
1:0.8	0.8
1:1.0	2

The swelling indices in water of native and dialdehyde starches were compared and the sample with 1:0.6 presents same swelling index as native starch, 0. The highest swelling index was presented by sample 1:1.0, to be 2.

Characterization of Native and Dialdehyde Starches by FTIR and SEM

The band at 1723.92 cm^{-1} is the most characteristic band of C=O vibration in aldehyde group. The spectra of the obtained starches are presented in Figure 1.

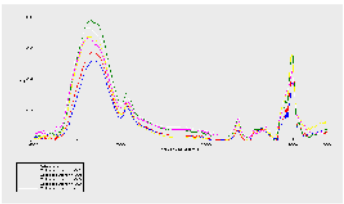

Figure 1. FTIR spectra of native and dialdehyde starches

It can be seen clearly, native corn starch could be oxidized successfully by periodate oxidation method. SEM images were recorded for native and dialdehyde starches at 4000x magnification as Figure 2 presented.

It can be seen that the molecules of starch cluster by increasing oxidation degree; the reason could be that introduction of increasing aldehyde groups into starch molecules provoked formation of intermolecular cross-links.

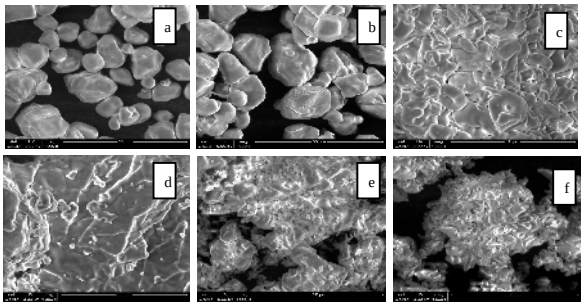

Figure 2. SEM images of native and dialdehyde starches:
a) native starch, b) 1:0.2, c) 1:0.4, d) 1:0.6, e) 1:0.8, f) 1:1.0

Determination of Water Uptake Capacity of Collagen-Starch Composites

The water up-take capacity of collagen-starch samples are presented in Figure 3 starting with first minute to 24 hours at different intervals of time. All the samples absorbed the most amount of water in 24 hours.

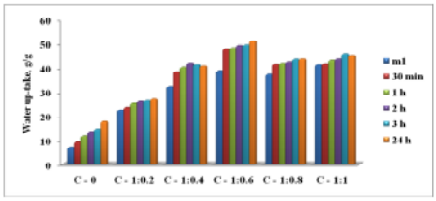

Figure 3. Water uptake capacity (g/g) of collagen-oxidized starch composites in time

As Figure 3 shows the collagen as such absorbed the smallest amount of water, the starch inducing the hydrophilicity to the samples and the most absorbent one is C-1:0.6.

Enzymatic Degradation of Collagen-Starch Composites

The degradation of collagen-starch composites showed their stability in a simulated environment which mimics the body. Weight loss values of matrices during degradation are presented in Figure 4.

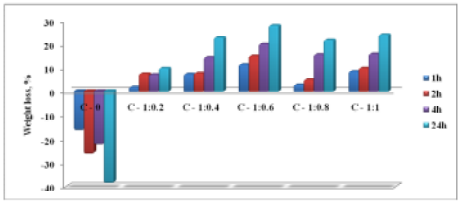

Figure 4. The weight loss (%) of collagen-oxidized starch composites in time

The most resistant sample against degradation was collagen reference during 24 hours. From the figure it was also seen that the degree of degradation is in correlation with hydrophilic degree: increase in absorbent capacity resulted with higher degree of

degradation (noticeable till 1:0.6 oxidation degree). While the sample C-1:0.2, which was treated with slightly oxidized starch, was degraded only 9.8%, the sample C-1:0.6, having the highest hydrophilic nature, degraded 28% in 24 hours.

CONCLUSION

New crosslinking agent as dialdehyde starches were obtained with different degree of oxidation and characterized by aldehyde contents, swelling index, FT-IR and SEM images. Successively introduction of aldehyde groups in to the starch structure by $NaIO_4$ oxidation was proved by determining the most characteristic band of C=O vibration specific for aldehyde at 1723.92 cm^{-1} by FT-IR. Dialdehyde starch samples which were oxidized in different degrees were used as crosslinking agents to prepare new collagen-starch composites. Their absorption and degradation capacities showed that the sample (C-1:0.2), having the lowest water uptake capacity (27 g/g), is the most resistant sample to degradation while the sample (C-1:0.6), having the highest water uptake capacity, is the most easily degradable sample (loss 28% in weight in 24 hours).

Since it was seen that absorption and degradability properties of collagen-dialdehyde starch composites can be controlled by crosslinking degree which is in fact related by oxidation degree of the starch it was thought that collagen-diladehyde starch composites are promising for using as wound dressing.

REFERENCES

Albu, M.G., Titorencu, I. and Ghica, M.V. (2011), *Biomaterials Applications for Nanomedicine*, in: P. Rosario (Ed.), InTech: Rijeka, Croatia.

Cheirmadurai, K., Thanikaivelan, P. and Murali, R. (2016), "Highly bipcompatible collagen-Delonix regia seed polysaccharide hybrid scaffolds for antimicrobial wound dressing", *Carbohydrate Polymers*, 137, 584-593.

Cuneo, F., Costa-Paiva, L., Pinto-Neto, A.M., Morais, S.S. and Amaya-Farfan, J. (2010), "Effect of dietary supplementation with collagen hydrolysates on bone metabolism of postmenopausal women with low mineral density", *Maturitas*, 65(3), 253–257.

Ficai, A., Albu, M.G., Birsan, M., Sonmez, M., Ficai, D., Trandafir, V. and Andronescu, E. (2013), "Collagen hydrolysate based collagen/hydroxyapatite composite materials", *Journal of Molecular Structure*, 1037, 154-159.

Fleck, C.A. and Simman, R. (2010), "Modern Collagen Wound Dressings: Function and Purpose", *Journal of the American College of Certified Wound Specialists*, 2, 50–54.

Gayatri, R., Sharma, A.K., Rajaram, R. and Ramasami, T. (2001), "Chromium(III)-induced structural changes and self-assembly of collagen", *Biochemical and Biophysical Research Communications*, 283, 229.

Hofreiter, B.T., Alexander, B.H. and Wolff, I.A. (1955), "Rapid Estimation of Dialdehyde Content of Periodate Oxystarch through Quantitative Alkali Consumption", *Journal of Analytical Chemistry*, 27, 1930-1931.

Li, D., Ye, Y., Li, D., Li, X. and Mu, C. (2016), "Biological properties of dialdehyde carboxymethyl cellulose crosslinked gelatin–PEG composite hydrogel fibers for wound dressings", *Carbohydrate Polymers*, 137, 508–514.

Mu, C., Liu, F., Cheng, Q., Li, H., Wu, B., Zhang, G. and Lin, W. (2010), "Collagen Cryogel Cross-Linked by Dialdehyde Starch", *Macromolecular Materials and Engineering*, 295, 100–107.

Sheu, M.T., Huang, J.C., Yeh, G.C. and Ho, H.O. (2001), "Characterization of collagen gel solutions and collagen matrices for cell culture", *Biomaterials*, 22, 1713.

Tan, H., Wu, B., Li, C., Mu, C., Li, H. and Lin, W. (2015), "Collagen cryogel cross-linked by naturally derived dialdehydecarboxymethyl cellulose", *Carbohydrate Polymers*, 129, 17–24.

Wilpiszewska, K. and Spychaj, T. (2007), "Chemical modification of starch with hexamethylene diisocyanate derivatives", *Carbohydrate Polymers*, 70, 334-340.

Zhang, L., Liu, P., Wang, Y. and Gao, W. (2011), "Study on physico-chemical properties of dialdehyde yam starch with different aldehyde group contents", *Thermochimica Acta*, 512, 196-201.