

Evaluation of Mechanical Properties of Polyethylene Glycol/ Dextrane Methacrylate / Maleic Acid Copolymer as a Biological Scaffold

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Abstract

Introduction: The use of membranes and transplant materials as scaffolds in periodontal regenerative surgeries is gaining an increasing application for restoring the structure and function of lost tissues. The present study aimed to evaluate the mechanical properties of PEG/MA (Polyethylene glycol/Maleic Acid) copolymer as a bioscaffold. **Methods:** In this experimental laboratory study, a hydrogel scaffold was first manufactured of PEG/MA copolymer. Different hydrogel concentrations were prepared using visible light through changing concentrations of C2/1, c1/1 and C1/2 (C 1/2, C 1/1 and C2/1) of PEG/MA copolymer to those of dex-METH (dextran-methacrylate). Then, 57 samples were selected, 27 and 30 of which were related to degradation rate and solidification tests, respectively. The samples were examined in three triplet groups within three time periods to test the degradation rate. Five quintuplet groups were also classified to measure the tensile and compressive strengths using a microtensile device. Data were analyzed by one-way/two-way ANOVA and Tukey test. **Results:** Both the concentration of PEG/MA copolymer ($P < 0.007$) and sample incubation duration ($P < 0.001$) affected sample degradation rates, with C 2/1 group yielding the lowest degradation rate. Furthermore, mean values of tensile strength ($P < 0.04$) and compressive strength ($P < 0.001$) were significantly different among the three groups. **Conclusion:** A change in the proportion of the copolymer ingredients can affect the mechanical properties and destruction rate of the substance. Due to slow degradation rate and high tensile

strength, a concentration of 1/2 is more appropriate to be used as a membrane for covering bone loss.

Keywords: Maleic acid, Polyethylene glycol, Dextran methacrylate, Mechanical properties, Biodegradability.

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Introduction

Periodontal regenerative surgeries are gaining an increasing application nowadays with the aim of restoring the structure and function of lost tissues using membranes and transplant materials. In fact, the main purpose of transplant usage in the treatment of periodontal lesions is to provide a space for the growth and migration of periodontium cells. Such commonly used membranes and transplants come with structural, mechanical and functional limitations. It, therefore, seems that achievement of an ideal membrane for regenerative treatment applications still requires improvements in terms of physical properties (1).

While there are various strategies for the development of polymer materials in periodontal regenerative surgery, hydrogels can adopt the lesion shape owing to their adaptive design and synthesis, be planted in the living milieu with the least damage, and minimize surgeries (2). Therefore, they can conform to different needs of periodontal treatments.

Researchers have examined several methods for controlling and improving the mechanical properties of hydrogels. For example, a combination of different polymeric materials can be used to adjust and control the degradation rate as well as the mechanical and biological properties of materials. Accordingly, a combination of different cross-linking joints leads to the creation of a new material with controllable mechanical strength and flexibility, which is an appropriate standard for regenerative materials of periodontal lesions. However, it is not convenient to control the mechanical properties of a material during its degradation since the degradation rate of the material affects the mechanical properties of hydrogel. Nevertheless, repair process of the host tissue surrounding the transplant can compensate for such a lack of material strength (3, 4).

There are various methods for the synthesis of copolymer hydrogels, one of which is the use of optical cross-linkers. Optical polymerization of hydrogels results in a longer durability of the scaffold surface layer allowing the surgeon to use it as a protective membrane, which in turn removes the need for surgical repair of protective membranes.

The PEG/MA scaffold is a biocompatible hydrogel that can be directly injected into bone loss, which is associated with a decrease in surgical time in addition to lesion formation. As the scaffold is cured by visible light, this resolves the dangers of using direct UV light source in the work and clinic environments. The desired succession of lighting time to the hydrogel allows the surgeon to apply the intended shape to different regenerative treatments. Also, the hydrogel flexibility allows the preparation of prefabricated scaffolds out of

the surgical setting, and then its conformation to the surgical site.

Nguyen and West (5) studied optical hydrogels in tissue engineering applications and found that it was possible to adjust the hydrolytic and enzymatic degradation of optical hydrogels, and also to use such hydrogels as membranes, drug release systems, and bioscaffolds. Pivsa-Art et al. (6) investigated the effect of PEG as a plasticizer in a combination of poly-lactic acid and poly-butylene succinate and concluded that rising PEG levels resulted in decreased copolymer fragility with increased formability of the copolymer.

As such a scaffold is fabricated for the first time, its physical properties are unknown. This study, therefore, investigated the mechanical properties of PEG/MA copolymer as a bioscaffold in a series of laboratory experiments.

Materials and methods

This experimental-laboratory study was conducted at the Faculty of Dentistry, Islamic Azad University of Isfahan (Khorasgan), Bioscience and Stem Cell Research Center, during the academic year of 2015-16. A total of 57 samples was selected, 27 and 30 of which were related to degradation rate tests and strength assessment, respectively. Different hydrogel concentrations were prepared using visible light through changing tests PEG/MA tests concentrations of 1/2, 1/1 and 2/1 (C 1/2, C 1/1 and C 2/1) in relation to those of dex.METH. The samples were examined in three triplet groups within three time periods to test their degradation rates. Five samples for each concentration to measure tensile strength and five other of each concentration for evaluation of compressive strength were prepared (30 samples in total).

Preparation of Dextran-Methacrylate: Dextran was dissolved in the LiCl/DMF (lithium chloride in dimethylformamide) (10 wt %) solvent system at 90°C under nitrogen gas purge. After dissolution, it was cooled down to 70°C and triethylamine, as a nucleophilic catalyst, was added slowly. The amount of triethylamine added was 10 mol % of methacrylic anhydride. The dextran solution was stirred vigorously for 10 min and methacrylic anhydride was then slowly injected to the system using a syringe. The amount of methacrylic anhydride added was 0.3 molar ratio based on the hydroxyl groups in dextran glucose unit. The reaction was conducted for 5 hours at 70°C

To remove any residual unreacted methacrylic anhydride in dextran-methacrylate, the dextran-methacrylate was dissolved in DMF and precipitated in isopropyl alcohol. The same procedure was repeated for

3 times to achieve a completely purified dextran-methacrylate.

Dextran (MW=64,000-76,000), (-)-riboflavin, and L-arginine were obtained from Sigma Chemical Co. (St. Louis, Missouri, USA).

In vitro biodegradation test: The biodegradation test has been conducted according to ASTM-F1635-11. Different hydrogel concentrations were prepared using visible light through changing concentrations of C2/1, C1/1 and C1/2 (C 1/2, C 1/1 and C2/1) of PEG/MA copolymer to those of dex-METH (10mm length×10 mm width×1mm thickness) Samples were weighed before being placed in PBS to measure the initial mass of the films and immersed in 5 ml PBS (PH=7) in glass vials and incubated at 37°C for 12 weeks. During incubation time, weight changes were measured every four weeks. The weight differences of samples were calculated by the following formula:

$$\frac{w_0 - w_1}{w_0} \times 100$$

Mechanical properties of photocross-linked hydrogel :Tensile and compression properties of photocross-linked hydrogel PEG/Dex/MA were performed by microtensile machine (MTD-500 Plus, SD Mechatronik, Feldkirchen-Westerham,Germany) at room temperature. For tensile testing, dumb-bell shaped samples were elongated with constant cross-head speed of 2 mm/min. Compressive strength was measured on cylindrical samples with 5 mm diameter and 10 mm height. A constant cross-head speed of 2 mm/min was known as failure for compression testing

In order to investigate the hydrolytic degradation of PEG/MA copolymer, several blocks (10 × 10 × 10 mm) were prepared and tested for each concentration of the three samples. The prepared samples were incubated in vials containing 5 cc of saline phosphate buffer (pH 7.4) at 37 °C. The samples were then taken out from the buffer at 4-week intervals for 12 weeks and washed with distilled water to desalt them of absorbed saline buffer. Afterwards, the samples were dried in an oven at a room temperature until no weight change of hydrogel was detected and weighed with a digital scale to calculate the percentage of weight difference.

Five cylindrical samples (5 mm in diameter and 10 mm in height) per three concentrations were prepared to examine the compressive strength of Polyethylene Glycol/ Dextrane Methacrylate / Maleic Acid copolymer. The specimens were then transferred to a microtensile tester and placed in order between the jaws of the device adjusted at a speed of 2 mm/min. The applied force was calculated as long as a crack or a crevice were formed on the sample. Finally, the stress applied to the material was calculated with the formula F/A in SI system.

To test the tensile strength of PEG/MA polymer, dumbbell shaped samples were prepared with width, length, thickness of 3 mm, 5 mm, and 1 mm, respectively. The specimens were then transferred to a microtensile device set at a speed of 2 mm/min, and the force was calculated until the sample was entirely halved equally. Finally, the pressure applied to the material was calculated with the formula F/A in SI system.

Statistical analysis

Kolmogorov–Smirnov test is used to test the normality of the data. Data were analyzed by both one-way and two-way analysis of variance (ANOVA) and Tukey Test (as a Post Hoc Test) with SPSS 22 software at a significance level of P <0.05.

Results

According to two-way ANOVA, in respect of interactive effect of both concentration and time variables there was no significant effect (P=0.185) but Both the concentration of PEG/MA copolymer - independent of incubation time (P = 0.007) (Table I) and the incubation time of the samples -independent of concentration- (P < 0.001) (Table II) affected the degradation rate of samples. The degradation decreased as the concentration increased, however, the degradation rate elevated with increasing sample incubation time.

According to post hoc test (Tukey) to evaluate the effect of time independent of concentration, following results were found; in 4 weeks there was significantly less degradation rate than 12 (P<0.000) and 8 weeks (P<0.015) but the difference was not significant between 8 and 12 weeks (P=0.264). In other hand the degradation rate of C 2/1 concentration was significantly less than C 1/2 (P=0.005) (independent of time) but there was no significant difference between C 1/2 and C1/1 (P=0.333) or C 1/1 and C 2/1 concentrations (P=0.103)

According to one-way ANOVA Mean tensile strength was significantly different among the three groups (P =0.04). Pairwise comparison of the groups revealed that mean tensile strength was significantly lower in C 1/1 group than those of the other two groups, which showed no significant difference (P = 0.58).

Mean value of compression strength was significantly different among the three groups (P < 0.001). Pairwise comparison of the groups showed that mean compressive strength of the samples in C 1/1 group was significantly higher than those of the other two groups (P < 0.001), with C 2/1 showing a greater strength than that of C 1/2 group (P = 0.02). (Table III)

Table I. Mean percentage of hydrolytic degradation based on PEG/MA copolymer concentration.
(C = Concentration)

	C 1/2	C 1/1	C 2/1	P-Value
N	9 [†]	9	9 [†]	0.007
Mean ± SD	11.83±1.4	10.46±2.13	8.46±1.86	

[†]: statistically different

Table II. Mean percentage of hydrolytic degradation based on incubation time.

	4 Weeks ^{†‡}	8 Weeks [†]	12 Weeks [‡]	P-value
N	9	9	9	<0.001
Mean ± SD	7.86±1.4	10.7±2.06	12.2±1.76	

[†], [‡]: statistically different

Table III. Mean (± SD) values of tensile and compressive strengths (kPa) based on sample concentration (C = Concentration)

Test	C ½	C 1/1	C 2/1	P-value
Tensile strength	63.9±5.9	51.9±8.6	61.3±7.3	0.04
Compressive strength	59.1±77.7	140.6±9.2	73.3±6.8	<0.001

Discussion

A polymer with three different concentrations was prepared herein using a combination of dextran-methacrylate, maleic acid, and polyethylene glycol by altering their proportions. Due to the novelty of the developed copolymer as a bioscaffold, examinations were carried out on the hydrolytic degradation process of the material and its mechanical properties.

The effect of increasing PEG/MA copolymer concentration on the hydrolytic degradation indicated that the degradation process can be controlled by increasing PEG/MA copolymer concentration compared to that of dextran. In fact, maleic acid can create more colloidal prepolymer chains by creating cross-linked joints because of raising maleate units in the middle of polymer chains, thereby, affecting the control of water absorption and degradation time. Moreover, water absorption and copolymer degradation process can be controlled through adjusting the concentration of this material in copolymers (7).

Sun et al. (8) studied the hydrolytic degradation process and polylactic coglaiecolic acid (PLGA) polymer and found that the percentage of PLGA hydrolytic degradation averaged 2.5 % after 6 weeks, whereas the present study measured a value of approx. 12% after 3 months. This difference in the material degradation

process arises from differences in the molecular structure of materials and the stability of carbon bonds

In a study on the properties of silica-hybridized collagen hydrogels, won Kim et al. (9) concluded that collagen enzymatic degradation rate decreased by increasing silica percentile weight. The compressive strength also improved by increasing silica units from 100 kPa in pure collagen to 400 kPa in hybrid collagen with 20% of silica by weight. Our data indicate an uppermost mean compressive strength of 140 kPa, which is higher than that of pure collagen in the above study. The difference between the two studies is due to differences in the molecular structure of the materials and the type of mechanical analyzer (DMA 25N 01dB-Metravib, France) used herein

Collagen/seresin membrane as a wound covering material and its hydrolytic degradation underwent an investigation in both cross-linked and uncross-linked states by Akturk et al (10). After 4 weeks, it was found that the percentage of uncross-linked collagen/seresin hydrolytic degradation averaged almost 78%, which rose with increasing seresin, while cross-linked collagen/seresin yielded a value of nearly 32%. Our research, on the other hand, obtained a maximum average degradation of 10.7% after 7 weeks, which dropped to 4.4% in C 2/1 group by increasing cross-linkers.

Therefore, this result demonstrates an impact of crosslinks on the hydrolytic degradation rate of materials.

Increasing concentration of PEG/MA copolymer had a positive influence on the tensile strength suggesting the role of two dextran-methacrylate and PEG ingredients in the tensile properties of the substance, hence, the presence of maleic acid affects the compressive strength of the material. Consequently, the prevailing dextran content in the copolymer in C 1/2 group could result in the highest amount of tension in this ratio of the hydrogel. Thus, there is no correlation between increased concentration of PEG/MA copolymer and resistance to the tensile strength, but the predominance and also a balance between dextran-methacrylate and PEG provides the highest tensile strength.

Roeder et al. (11) examined the tensile strength of type I collagen scaffolds and concluded that the tensile strength of collagen matrix improved from 1.5 to 24.3 kPa by an increase in collagen concentration. In the present study, however, the highest and lowest mean tensile strengths were estimated as 63.9 and 51.9, respectively. The discrepancy between the two studies comes from the differences in material type and measuring device (Miniature materials tester, Rheometric Scientific, Inc., Piscataway, NJ).

Regarding the role of elevated concentration of PEG/MA copolymer in compressive strength, the C 1/1 group presented a significantly greater mean compressive strength than the other two concentrations. In fact, a concentration of 1/1 could balance the concentrations of all three ingredients, thereby, enabling maleic acid to show its effect and produce a slight elasticity in this ratio of hydrogel. The predominance of dextran in C ½ could create a transparent and soft substance. With increasing copolymer ratio in C 1/2, PEG plus dextran could overcome maleic acid content of the copolymer. Even so, an increase in maleic acid had an effect on the material strength significantly raising C 2/1 compressive strength compared to that of C ½. Nonetheless, both ratios exhibited significantly lesser strength values than that of C 1/1 indicating a balanced and optimal ratio of the copolymer ingredients.

A research by De Sousa et al (12) on the tensile strength of PLGA as a tissue regeneration membrane revealed maximum tensile strength of 16.7 MPa in PLGA, which is higher than that of our finding. An obvious reason for this variation is the abundant carbon bonds present in polylactic acid with a significant effect on the material strength. In addition, other affecting factors include the type of applied device (universal testing machine, model DL 2000, EMIC) for the tests and also differences in sample dimensions (5 mm W and 25 mm L)

Because a material with a degradation rate proportional to the tissue structure is required in the

process of periodontal lesion restoration, collagen scaffolds are not ideal for periodontal treatments because of a high degradation rate and acid-based polymeric scaffolds with a slow degradation rate. Although the degradation process can be accelerated by the addition of various polymeric materials, an abrupt degradation of acid-based polymers can create an acidic surgical site rendering unfavorable conditions for cell growth. Since our studied material has a moderate hydrolytic degradation rate, it can be expected to decompose along with the tissue repair.

The results of solidification tests suggested a superior mechanical property of the present scaffold to that of collagen membranes, however, it was weaker than acid-based polymeric scaffolds. As both collagen membranes and acid-based polymers revealed acceptable results, the mechanical property of PEG/DM/MA scaffold is also in an acceptable range.

Conclusion

Changes in the ingredient proportions of a copolymer can affect the mechanical properties and degradation rate of materials. In fact, the therapeutic use of a substance has an influence on determination of an optimal ratio between components of a copolymer. A concentration of 2/1 is more suitable to be used as a membrane for covering bone loss owing to a slow degradation rate and a high tensile strength. A concentration of 1/1 is recommendable if it is used as a bone loss filler material because it possesses a higher compressive strength and moderate degradation rate.

Conflicts of interest

None declared.

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