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Synthesis, characterization and biocompatibility studies on chitosan-*graft*-poly(EGDMA)

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ABSTRACT

Poly(ethylene glycol dimethacrylate), poly(EGDMA), was grafted onto chitosan by using a redox initiation system. Chitosan-*graft*-poly(EGDMA) products were characterized by DSC, TGA, FTIR and XRD techniques. Chitosan-*graft*-poly(EGDMA) was found to be enzymatically degradable in aqueous solutions of lysozyme, lipase and a mixture of α-amylase and protease. The biocompatibility of chitosan-*graft*-poly (EGDMA) with 871% grafting yield was investigated by studying its cytotoxicity, sensitization, irritation, acute systemic toxicity and hemolytic activity. The results of biocompatibility experiments showed that the product can potentially be used for external intervention devices on bone and other tissue.

1. Introduction

Chitosan is a copolymer of *N*-acetylglucosamine and glucosamine units and is represented as a copolymer. Chitosan occurs in nature in the cell walls of some fungi, exoskeletons of insects and marine animals such as crabs and prawns. Chitosan and its derivatives possess a wide range of useful properties. They are biodegradable, and biocompatible with antibacterial and antioxidant activities. They are useful in drug delivery formulations and tissue engineering (Sashiwa & Aiba, 2004; Yilmaz, 2004).

Among various methods, graft copolymerization is a versatile tool to insert functional groups to a polymer (Bhattacharya & Misra, 2004). Grafting on chitosan can be carried out by means of initiation such as redox initiation system, radiation and enzymatic grafting (Jayakumar, Prabaharan, Reis, & Mano, 2005). Grafted chitosans have a wide range of applications from tissue engineering to metal ion adsorption. Graft copolymerization of dimethacrylates onto chitosan has proved to improve physical properties of chitosan such as its water solubility and swelling capacity (He, Yaszemski, Yasko, Engel, & Mikos, 2000; Yilmaz, Adali, Yilmaz, & Bengisu, 2007).

In this study, ethylene glycol dimethacrylate (EGDMA) has been selected as a graft co-monomer/crosslinker in the preparation of modified chitosan samples since EGDMA is known to be a biocompatible difunctional monomer (He et al., 2000). Grafting conditions of poly(EGDMA) onto chitosan was investigated in 1% acetic acid solution, in the presence of cerium(IV) ammonium nitrate (CAN) as the initiator under nitrogen atmosphere. The effects of monomer and initiator concentrations, time and temperature on the grafting yield were studied. The graft products were characterized by Fourier transform infra-red (FTIR) spectrophotometer, differential scanning calorimeter (DSC), thermal gravimetric analysis (TGA) and X-ray diffraction (XRD) analysis in addition to gravimetric evidence to grafting. The dissolution and swelling properties of the grafted products were tested in acid, neutral, basic and organic media. Biodegradability was tested by treating the products with lysozyme, lipase and a mixture of α-amylase and protease enzymes. The biocompatibility of the grafted product was investigated by studying cytotoxicity, sensitization, irritation, acute systemic toxicity and hemolytic activity.

2. Experimental

2.1. Materials

Medium molecular weight chitosan (Fluka) with a degree of deacetylation of 85% viscosity of 200 mPa s in 1% acetic acid at 20 °C, and a molecular weight of 400,000, as reported by the producer, was used as supplied. Ethylene glycol dimethacrylate (EGDMA) (Sigma–Aldrich), acetic acid (Aldrich), acetone (Pharma Mondial), lipase (Aldrich), lysozyme (Aldrich), Flaviastase which is mixture of α-amylase and protease to mimic pancreatic enzymes (Ibrahim Ethem), sodium chloride (Aldrich), cerium(IV)

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ammonium nitrate (Aldrich), sodium hydroxide (Aldrich), dipotassium hydrogen phosphate (Aldrich), sesame oil (Aldrich), sodium carbonate (Aldrich), polyethylene (Aldrich), phosphate buffered saline (Aldrich), nitrocellulose membrane (Aldrich), ammonium molybdate (Aldrich), sodium chloride (Aldrich) and hydrochloric acid (ACS reagent, Aldrich) were used without further purification.

2.2. Preparation of grafted chitosan

A 0.8% (w/v) chitosan solution in 1% (v/v) aqueous acetic acid was prepared by stirring for 24 h at room temperature. The reaction vessel was purged with nitrogen and cerium(IV) ammonium nitrate (CAN) was added to the solution at constant temperature. The monomer, EGDMA, was then added to the reaction medium under vigorous magnetic stirring. The reaction was carried out for a given period of time. The product was precipitated with acetone, washed, filtered and dried at 60 °C. The effect of initiator concentration (0.025–0.125 M), time (1–4 h) and temperature (45–75 °C) on the grafting yield was studied in the monomer concentration range 4.7×10^{-3}–0.42 M.

2.3. Instrumental analysis

2.3.1. Fourier transform infra-red spectrophotometry (FTIR)

FTIR analysis was carried out using a Mattson 5000 Satellite FTIR Spectrometer. KBr pellets of the products were used for FTIR analysis.

2.3.2. Differential scanning calorimetry (DSC)

DSC thermograms were taken under nitrogen atmosphere by using a Perkin Elmer/Pyris-1 calorimeter. The heating rate was 10 °C/min within the range of 10–450 °C. DSC thermograms were taken in TUBITAK-MAM Laboratories in Izmit.

2.3.3. X-ray diffraction (XRD) analysis

Powder X-ray diffractometry analysis was carried out, using a Shimadzu XRD-6000 model diffractometer with Cu X-ray tube (λ = 1.5405 Å) in TUBITAK-MAM Laboratories. The crystallinity index was calculated by a technique based on the intensity of the signals proposed by Jaworska, Sakurai, Gaudon, & Guibal (2003), as follows:

$$CrI_{\text{peak}} = \frac{(I_{110} - I_{\text{am}})}{I_{110}}. \quad (1)$$

where CrI_{peak} is the crystallinity index based on the signal intensity, I_{110} is the maximum intensity at $2\Theta \approx 20^{\circ}$ of the (110) lattice diffraction and I_{am} is that of the amorphous diffraction at $2\Theta \approx 16^{\circ}$.

2.4. Gravimetric analysis

Percent yield of grafting was calculated by the following equation:

$$Yield \quad (\%) = \frac{w_p - w_{Chi}}{w_{Chi}} \times 100\% \quad (2)$$

where w_p is the weight of the product and w_{Chi} is the weight of chitosan.

2.5. Solubility and equilibrium swelling studies

Solubility of the products in water, 1% HAc, 1 M NaOH solution (pH 12), acid buffer (pH 1.2) and phosphate buffer (pH 7.4) were measured by mixing a weighed sample (2 mg in 100 mL solvent) and stirring for 24 h.

The water sorption capacities of insoluble chitosan-*graft*-poly (EGDMA) samples were determined by swelling them in acid solution (pH 1.2) and phosphate buffer solution (pH 7.4) at 37 °C. A known mass of dry copolymer was placed in buffer solutions. The swollen mass of the sample was determined by first blotting it with filter paper to remove surface adsorbed water and then weighing immediately on an electronic balance. The ratio of the mass of the swollen sample (W_s) to the mass of the dry sample (W_o) was calculated to give the swelling ratio (Q):

$$Q = \frac{W_s}{W_o} \quad (3)$$

2.6. Enzymatic degradation

A 5.0 mg sample was placed in 7.0 mL enzyme solution and kept at 37 °C. Buffer solutions at pH 7.4 (0.2 M KCl/0.2 M HCl) and pH 1.2 (0.1 M K_2HPO_4/0.1 M NaOH) were used to prepare 2 μg/mL solutions of lipase, lysozyme and Flaviastase. After 24 h particles were removed from solution by filtration. They were washed with double distilled water, dried at 70 °C overnight and weighted. Percent weight loss was then calculated using the initial and final weights of the sample. Each experiment was carried out in duplicate and average values were taken. Values agreed within 5%.

2.7. Biocompatibility of the products

Biocompatibility tests were carried out in Pharmacology Department of Hacettepe University, Ankara. This reaction deals with the evaluation of biocompatibility of grafted products by methods that measure acute systemic toxicity, sensitivity, irritation, hemolytical effect and cytotoxicity. In this study, ISO10993-1, ISO10993-10, ISO10993-11, ASTM 750:1987 (reapproved 1996) and ASTM F 756-00 procedures were followed (ASTM, 2000; ISO, 1999).

Test animals were 20 albino mice (male, 17–23 g) in acute systemic toxicity test, one healthy adult albino rabbit (male, 2300 g) in irritation test and three albino rabbits in hemolytical effect test.

In the cytotoxicity test, Vero (African Monkey Kidney Cell line) was used. Ten healthy adult albino guinea pigs of either gender from strain, weighing 300–500 g were used for test material and five animals for control group.

In the test methods of acute systemic toxicity test, extracts of samples in polar and nonpolar solvents prepared at 37 °C for 72 h (sample extracts) were used. For control groups, solvents alone were incubated at 37 °C for 72 h (blank extracts). The test material and control extracts prepared in physiological saline were injected (50 mL/kg) to mice via tail vein (ASTM F 750:1987; Method A). The test material and control extracts prepared in sesame oil were given intraperitoneally (50 mL/kg) to mice (ISO1093-5, 2000).

In the irritation test, once the extract had been obtained, it was used within 24 h. On the day before the test, the fur on the back of the animal was shaved. A sufficient distance was kept on both sides of the spine for the application and observation of all test sites. Extract samples of the test material and negative control were applied directly to the skin on each side of the rabbit. Four-ply gauze patch was used as negative control sample. The application sites were covered with 25 mm × 25 mm gauze patch and were wrapped with semi-occlusive bandage for 4 h. At the end of the contact time the dressing were removed and the position of the sites were marked.

Hemoglobin calibration curve consistent with the specifications of the International Committee for Standardization in Hematology (ICSH) was prepared. The absorbance was measured at 540 nm using a spectrophotometer for hemolytical effect test.

Blood from three rabbits were pooled and diluted with PBS (phosphate buffered saline) to obtain 10 mg/ml hemoglobin content and treated with sample extracts.

These solutions were incubated for 3 h at 37 °C and were centrifuged at 700–800*g* for 15 min. The supernatants were treated with cyanmethemoglobin for 3–5 min and the absorbances of the

solutions were read at 540 nm with a spectrophotometer. Hemolytical indexes of the test samples were calculated according to obtain absorbances (ASTM F 750-00:2000).

In the sensitization test, 25 mm × 25 mm four-ply gauze patch immersed in extract of the test material and a sample of negative control were applied directly to the clipped area under an occlusive dressing for 6 h and removed. 0.9% (m/v) NaCl was used as negative control sample for control animals. This procedure was repeated on three consecutive days a week for 3 weeks. Fourteen days after the last induction application all test and control animals were challenged with the patch by using the same procedure.

Indirect contact test (Agar diffusion test) method was used in cytotoxicity studies. Sub confluent monolayer cells were contracted indirectly with 1 ml of extract saturated disc for 48 h.

3. Results and discussion

3.1. Optimization of grafting conditions

Poly(EGDMA) was grafted onto chitosan under nitrogen atmosphere, in aqueous medium using cerium(IV) ammonium nitrate, CAN, as the redox initiator. The synthesis conditions of chitosan-*graft*-poly(EGDMA) were optimized with respect to monomer and initiator concentrations, reaction time and reaction temperature. The weight increase of chitosan was taken as an evidence for successful grafting reaction.

The effect of monomer concentration on the grafting yield is shown in Fig. 1(a). The grafting yield steadily increases up to 871% (at 0.42 M EGDMA) with increasing initial EGDMA concentration in the medium. It is a general observation that an upper limit in monomer concentration is reached at which grafting is not favoured any more (Bhattacharya & Misra, 2004; Yilmaz et al., 2007) usually due to increased homopolymer formation. The effect of reaction time is given Fig. 1(b). The grafting percent increases up to 3 h reaction time and decreases at longer reaction times. A number of factors play role in the decrease of the grafting yield with time. This could be attributed to (i) a decreased grafting rate because of decreased monomer and initiator concentrations in time, (ii) reduced availability of the grafting sites in time due to the steric hindrance of the growing side chains and (iii) oxidative chain degradation leading to lower molecular weight products that could have been washed away during product purification. In the temperature range studied (45–75 °C), the grafting yield increases with temperature and reaches 365% for poly(EGDMA) grafting at

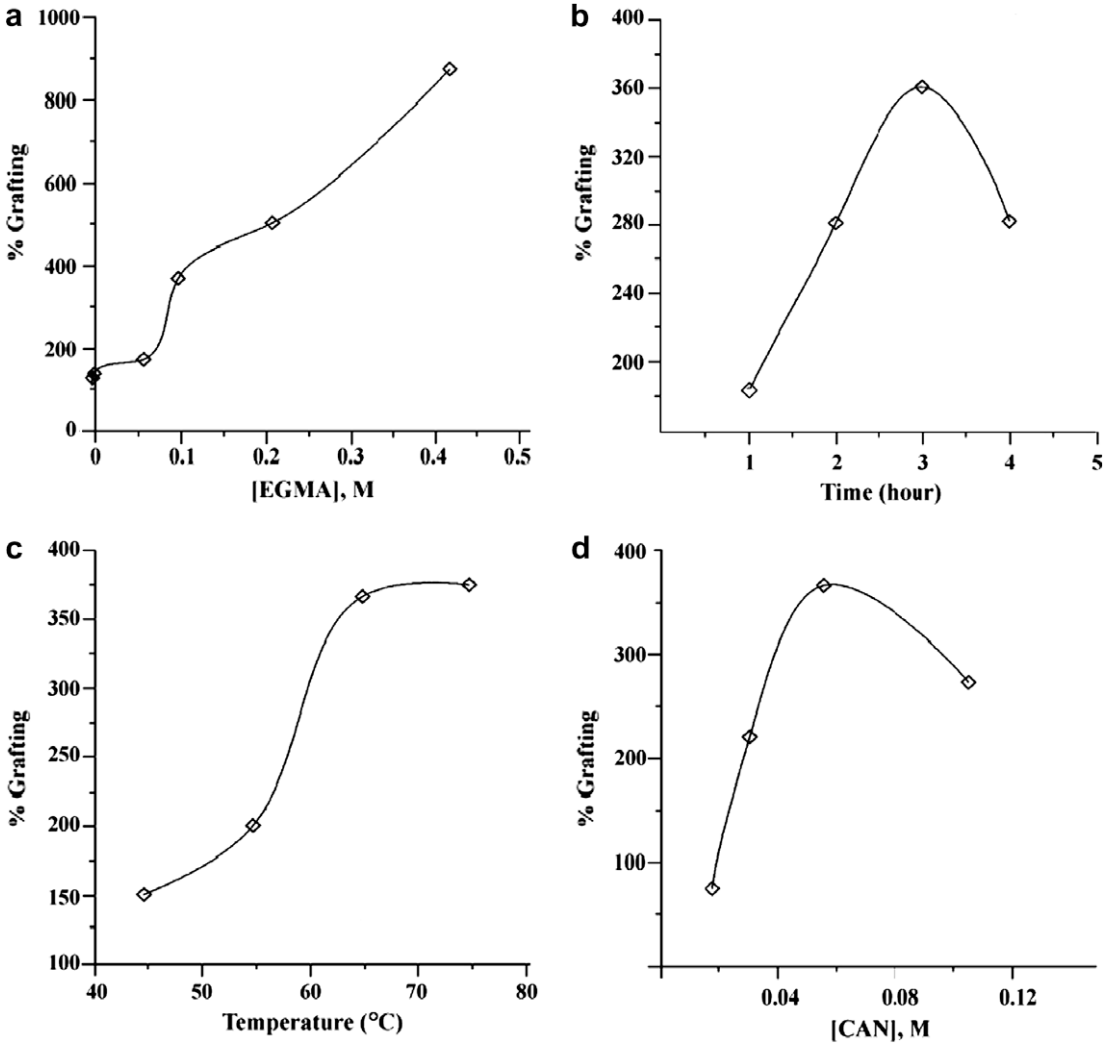

Fig. 1. (a) Effect of monomer concentration on the grafting yield (reaction conditions: 0.8% (w/v) chitosan in 1% (v/v) HAc solution, 0.05 M CAN, 3 h, 65 °C). (b) Effect of reaction time on the grafting yield (reaction conditions: 0.8% (w/v) chitosan in 1% (v/v) HAc solution, 0.05 M CAN, 0.10 M EGDMA, 65 °C). (c) Effect of reaction temperature on the grafting yield (reaction conditions: 0.8% (w/v) chitosan in 1% (v/v) HAc solution, 0.05 M CAN, 0.10 M EGDMA, 3 h). (d) Effect of initiator concentration on the grafting yield (reaction condition: 0.8% (w/v) chitosan in 1% (v/v) HAc solution, 0.10 M EGDMA, 3 h, 65 °C).

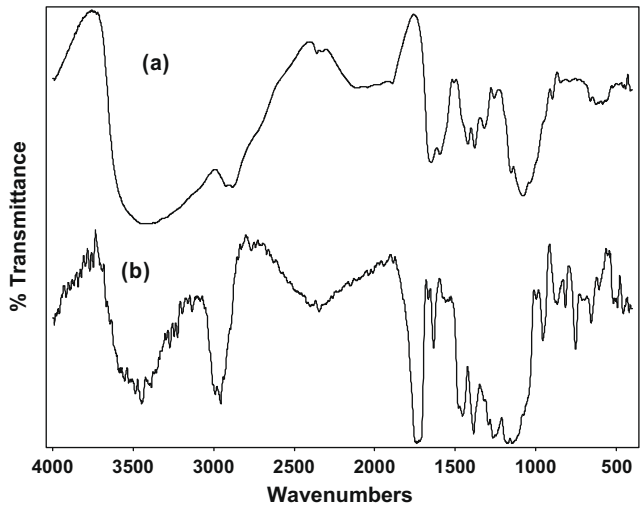

Fig. 2. FTIR spectrum of (a) chitosan and (b) chitosan-*graft*-poly(EGDMA) (%*G* = 493).

75 °C as shown in Fig. 1(c). A maximum grafting yield of 365% is obtained at 0.05 M CAN concentration when other factors are kept constant as can be followed from Fig. 1(d). Further increase in the CAN concentration decreases the grafting yield owing to a higher probability of radical termination reactions before monomer addition.

The highest grafting yield (871%) was obtained at 65 °C, in 3 h using 0.05 M CAN at an initial EGDMA concentration of 0.42 M in 0.8% (w/v) chitosan solution in acetic acid.

3.2. Characterization of the products

3.2.1. Chemical structure

The chemical identity of the products was confirmed by FTIR analysis. The FTIR spectra of chitosan and chitosan-*graft*-poly(EGDMA) are shown in Fig. 2(a) and (b), respectively. The carbonyl peak of the acrylate of EGDMA appears at 1730 cm^{-1} and, the lactone carbonyl at 1764 cm^{-1}. The amide-I band of chitosan is at 1637 cm^{-1}. The N—H stretching of chitosan at 1527 cm^{-1} decrease upon with grafting of EGDMA onto chitosan indicating that the grafting reaction mainly occurs at the amine site.

3.2.2. Thermal behaviour

Thermal behaviour of chitosan and poly(EGDMA) grafted chitosan were compared to each other by DSC analysis. The DSC thermograms of chitosan and chitosan-*graft*-poly(EGDMA) are given in Fig. 3(a) and (b), respectively. The sharp exothermic peak observed at 300 °C in the thermogram of chitosan is not available in that of the grafted product. Instead, chitosan-*graft*-poly(EGDMA) product (131% grafting) exhibits two decomposition peaks at 238 and 259 °C. The decrease in the thermal stability indicates that the amine groups which are the hydrogen bonding sites have been lost during the grafting reaction as evidenced by a decrease in crystallinity explained below.

3.2.3. Crystallinity

XRD patterns of chitosan and poly(EGDMA) grafted chitosans with 134% (GE10) and 871% grafting yields (GE0125) are shown in Fig. 4(a–c), respectively. Chitosan gives two characteristic crystallinity peaks at $2\theta = 8°$ and 20°. As the mole fraction of chitosan in the initial grafting mixture is decreased, % crystallinity of the grafted product decreased. The amorphous shoulder appears at $2\theta = 16°$. In the grafted samples, the main crystalline peak is lost.

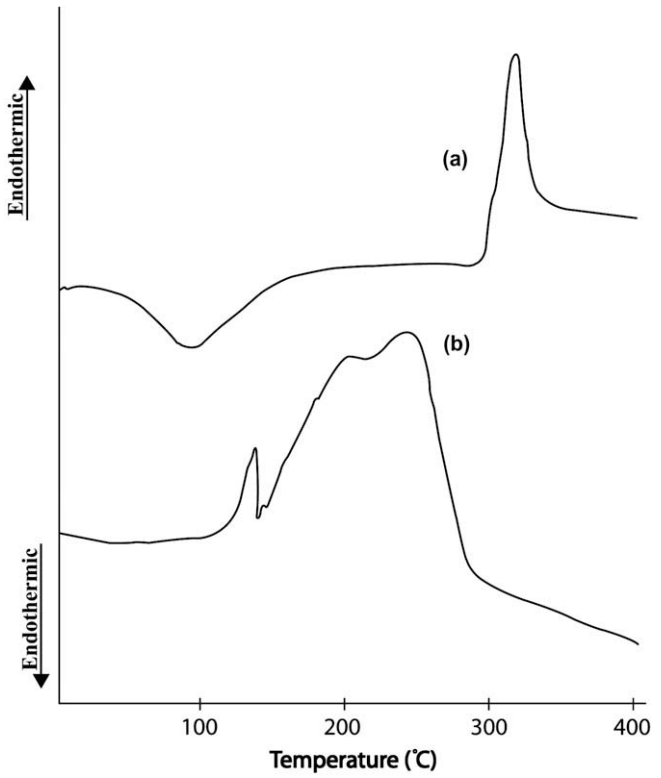

Fig. 3. DSC thermogram of (a) chitosan (b) chitosan-*graft*-poly(EGDMA) (%*G* = 131).

A new weaker crystalline peak appears at 28° and, the amorphous peak at $2\theta = 18°$ becomes predominant. So, both the crystalline structure and the degree of crystallinity of chitosan are affected as a result of the grafting reaction. Sample GE10 has a percent crystallinity of 54 according to Eq. (2). The amount of poly(EGDMA) grafted onto chitosan is much higher (871%) in GE0125 and the sample is almost completely amorphous.

3.3. Dissolution and swelling properties of chitosan-graft-poly(EGDMA)

The results of dissolution and swelling behaviour of poly(EGDMA) grafted chitosans in aqueous acid, neutral and basic media are summarized in Table 1. All products are insoluble in NaOH solution at pH 12. The chitosan-*graft*-poly(EGDMA) products with 123% and 134% grafting values are completely soluble in 1% HAc, water and acid buffer at pH 1.2. These products are partially soluble in phosphate buffer at pH 7.4. Products with higher grafting yields (169–365%) are partially soluble in 1% HAc, distilled water, and in acid buffer solutions. They swell in phosphate buffer solution. Chitosan itself is insoluble in neutral water or in pH 7.4 phosphate buffer solution. Incorporation of poly(EGDMA) side chains on the polymer backbone furnishes chitosan with water solubility and swelling capability in slightly basic media. This is not only due to the chemical nature of poly(EGDMA) but also because of decreased crystallinity of the products as a result of grafting reaction, as explained above. Further increase in the grafting yield (493–871%) results in complete insolubility in all dissolution media tested. The decreasing tendency for solubility with increasing grafting yield is attributed to increased crosslinking in the products owing to the bifunctional nature of EGDMA.

3.4. Enzymatic degradation

The enzymatic degradation results are summarized in Table 2. GE1, GE05, GE025 and GE0125 have grafting yield values of

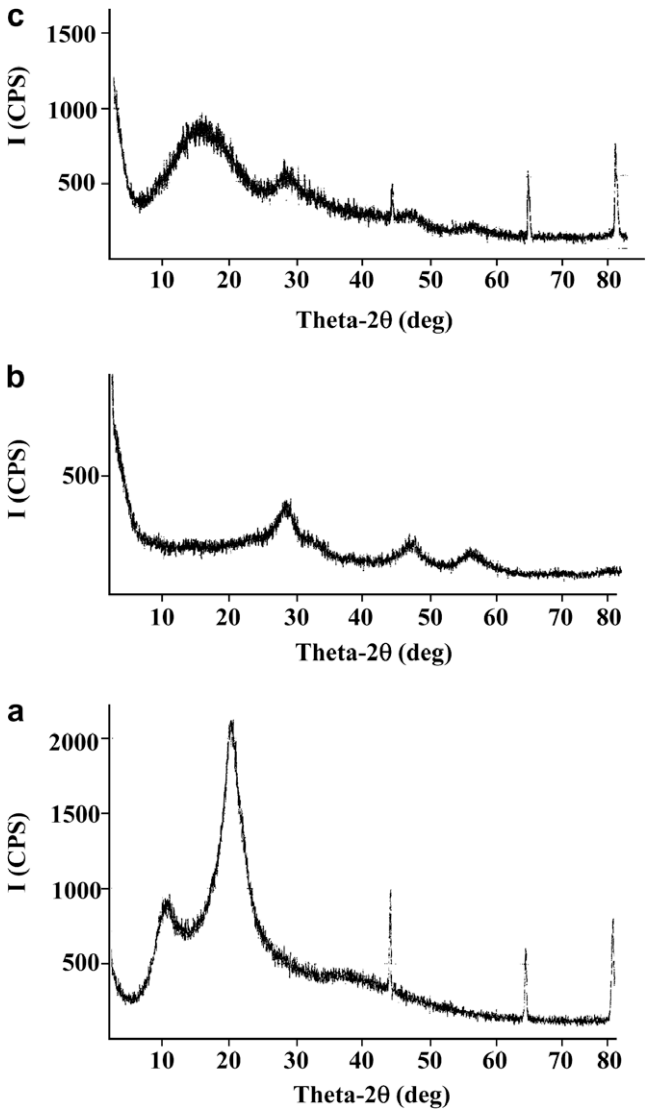

Fig. 4. XRD patterns of (a) chitosan (b) chitosan-*graft*-poly(EGDMA) (%*G* = 134) (c) chitosan-*graft*-Poly(EGDMA) (%*G* = 871).

169%, 365%, 493% and 871%, respectively. Parent chitosan flakes used in the grafting experiments were tested as control samples. GE1 is partially soluble GE05 and GE025 swell and GE0125 is insoluble in acid buffer solution. They all swell in phosphate buffer solution except for GE0125 which is insoluble. Under the action of all enzymes studied, a slight decrease in the % weight loss has been observed with the increasing grafting yield. % Weight loss is slightly higher in acid buffer solution than in phosphate buffer in the presence of all enzymes. In the acid medium, free amino groups on chitosan are protonated leading to a higher degree of swelling and hence better diffusion of the enzyme molecules into the polymer. Therefore, it can be concluded that solubility and swelling properties can be used to predict the tendency to degrade under the action of enzymes. Among all, Flaviastase is an enzyme whose activity is more pronounced in the acid medium as reflected by the results given in Table 2. The degradative effect of these enzymes on chitosan films cast from 1% acetic acid solution revealed similar results showing that the effect of Flaviastase is considerably higher under acidic conditions. Chitosan films gave a 10% weight loss under the action of Flaviastase in phosphate buffer solution of pH 7.4, whereas weight loss increased to 36% in acid buffer of pH 1.2 (Bengisu, Yilmaz, Oylum, & Baglama, 2005). Crosslinking and hence reduced dissolution or swelling decreased the effect of an enzyme on the chitosan sample. Chitosan films crosslinked with 25 ppm glutaraldehyde solution gave a weight loss of only 16% in acidic Flaviastase solution.

Table 2
Enzymatic degradation behaviour of chitosan, and chitosan-*graft*-poly(EGDMA) graft copolymers.

Enzyme solution	% Weight loss				
	Chitosan	GE1	GE05	GE025	GE0125
Lysozome pH 7.4	45	43	38	33	31
Lysozome pH 1.2	47	42	40	36	33
Lipase pH 7.4	40	36	33	31	30
Lipase pH 1.2	45	44	35	36	31
Flaviastase pH 7.4	42	36	27	27	27
Flaviastase pH 1.2	53	48	44	41	35

The effect of the enzymes on the chemical structure of the samples was followed by FTIR spectroscopy. Degradation of the chain can be followed from the decrease in the intensities of the glycosidic bond at 1039 cm^{-1}, and the amide band at 1635 cm^{-1}. It can be proposed that enzymatic degradation of chitosan in the enzyme solutions studied involves chain scission mainly at the *N*-acetylglucosamine bearing units.

3.5. Biocompatibility

In this study, the biocompatibility of chitosan-*graft*-poly(EGDMA) with the highest grafting yield (871%) was examined. The biocompatibility studies were undertaken with respect to acute systemic toxicity, irritation, sensitization, hemolytical effect and cytotoxicity. Acute systemic toxicity was performed by qualitative evaluation of toxicology symptoms. The animals were observed for toxicological symptoms or death at 4, 24, 48 and 72 h following injection. Mice treated with test material extracts were compared with mice treated with their matching blank extracts. Response to systemic injection was evaluated according to (ASTM F 750:1987). During the 72 h observation period, mice treated with blank extracts exhibited no adverse physical symptoms such as hyperkinesias, dyspnea, abdominal irritation, diarrhea and tremor (ASTM

Table 1
Solubility characteristics of grafted chitosan in acid, neutral, basic and aqueous media.

Sample	% Grafting	Solubility*				
		1% Hac	Water	NaOH solution pH 12	Acid buffer pH 1.2	Phosphate buffer pH 7.4
GE50	123	SL	SL	IS	SL	PS
GE10	134	SL	SL	IS	SL	PS
GE1	169	PS	PS	IS	PS	SW
GE05	365	PS	PS	IS	SW	SW
GE025	493	IS	IS	IS	SW	SW
GE0125	871	IS	IS	IS	IS	IS

* SL, soluble; PS, partially soluble; IS, insoluble; SW, swell.

Table 3
The results of the cytotoxicity test.

	Negative control	Positive control (1 mM)	Graft product
Confluency	Normal	Cannot be observed	Same with negative control
Granulation	Not existing	Cannot be observed	Not existing
Cell membrane Lysis	Not existing	Cannot be observed	Not existing
Rounded cell	Not existing	Cannot be observed	Not existing
Agregation	Not existing	Cannot be observed	Not existing
Vacuolization	Not existing	Cannot be observed	Not existing
Peracute toxicity	Not existing	Existing	Not existing
Picnotic cell	Not existing	Cannot be observed	Not existing

F 750:1987). None of the animals treated with the test material extract showed a different biological reaction than the animals treated with the blank extract. According to (ASTM F 750:1987), all mice subjected to intravenous or intraperitoneal injection were normal and showed no adverse physical symptoms after injection. There were no difference between the body weights of the control and test animals at 24, 48 and 72 h post-injection. As a result, the specimens did not cause any toxicological symptoms or death.

The qualitative evaluation of irritation examines lesion. For acute (single exposure) test, each application site was observed for erythema and oedema at 1, 24, 48 and 72 h followed the removal of the patches. No lesion was observed at 1, 24, 48 and 72 h recording for both test material samples and negative controls. Therefore, according to Table 1 entitled 'Scoring system for skin reaction in (EN ISO 10993-10), the score for irritation is 0 and according to Table 2 entitled 'Irritation response categories in rabbit' in (EN ISO 10993-10), response category is 'negligible' for both the test material and the negative control. Irritation reaction was not observed.

Determination of sensitization was performed by qualitative evaluation method according to (ISO 10993-10; 2002). No lesions were observed at 24 and 48 h for both test material samples and controls. Therefore, according to (ISO 10993-10; 2002), the grading was not observed.

The hemolytical effect test method is based on measurement of the hemoglobin released by hemolysis. The negative control type has 0.105% hemolytic index and it is non-hemolytic. The positive control type and test sample are hemolytic with 30.193% and 78.562% hemolytic indexes, respectively.

The chitosan-*graft*-poly(EGDMA) presents hemolytic character. This might be due to the crosslinking of reactive free amino groups from chitosan by grafting. As reported in the literature (ISO 10993-4; 1999), it is not possible to define a universal level of acceptable or unacceptable amounts of hemolysis. By definition, the blood compatible material should be non-hemolytic. Therefore, the chitosan-*graft*-poly(EGDMA) product has hemolytical effect and its not blood compatible.

In this study, the qualitative determination of cytotoxicity is based on the evaluation of cells microscopically to assess for changes in general morphology, vacuolization, detachment and cell, membrane lysis. The results of the cytotoxicity test were summarized in Table 3. These observations predict noncytotoxicity according to (ISO 10993-5).

4. Conclusions

1. Poly(EGDMA) was grafted onto chitosan in aqueous medium using cerium(IV) ammonium nitrate (CAN) as the redox system.
2. All grafted products with lower grafting yields were water and acid soluble. Increasing the monomer concentration in the reaction medium induced crosslinking reactions. Products with higher grafting yields were either partially or highly crosslinked and hence their water and acid solubility decreased with increasing monomer concentration. Further increase of monomer concentration produced insoluble graft products.
3. Chitosan-*graft*-poly(EGDMA) is susceptible to enzymatic degradation upon treatment with lysozyme, lipase or α-amylase protease as an indication of biodegradability.
4. The results of biocompatibility experiments carried on GE0125 which has 871% grafting value showed that this particular product can be utilized as biocompatible polymer in biomedical applications such as external intervention devices on tissue, bone and dentin applications.

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