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Grafting of glycidylmethacrylate onto demineralized xenogeneic bone in aqueous medium

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Summary

Demineralized xenogeneic bone (DXB) was prepared from bovine cortical tibia and graft copolymerized with glycidylmethacrylate (GMA) using a combination of potassium persulfate ($K_2S_2O_8$) and sodium metabisulfite ($Na_2S_2O_5$)as redox initiating system in aqueous medium. To optimize the reaction condition, the concentrations of backbone, monomer, initiator, temperature and time were varied. The percent grafting was found to increase initially and thereafter decrease in most of the cases. The optimum temperature and time were found to be 40°C and 180 minutes, respectively. The grafting results have been discussed and a reaction mechanism is proposed. Functional groups and structural changes of the graft copolymer were determined by Fourier transform infrared (FT-IR) spectroscopic method for proof of grafting and the results are discussed.

Introduction

Due to limited volume of autogeneic bone and risk of transmission of infections as well as immune response of allogeneic bone, materials of biological origin, xenogeneic bone, are proposed as an alternate to restore bone defects. Among many, Dr. M.R. Urist was the first to propose that protein factors from cortical bone appears to modulate bone healing [1]. With this, the stage was set for further studies that almost universally support the demineralized xenogeneic bone as an aid to bone healing [2,3]. The use of DXB as natural macromolecules has been increased in bone reconstruction due to its unique osteoinductive property [5]. In most of the clinical cases, surface properties of DXB play a significant role as its surface is in direct contact with body fluid

upon implantation. Therefore, modification of surface properties of DXB for the suitable application is of great importance.

Chemical modification will open ways to various utilization of these osteoinductive natural macromolecules. Recently, significant progress has been made in the chemical modification of natural macromolecules to improve their physico-chemical properties and to impart new functional groups for the use of coupling of therapeutic agents. Of all the possible modifications, graft copolymerization is anticipated to be quite promising for developing sophisticated functions under milder condition with lesser side reaction. With a view that grafted DXB may find better applications particularly in bone drug delivery systems as compared to ungrafted DXB, the present study of graft copolymerization of GMA onto DXB has been under taken using a combination of potassium persulfate and sodium metabisulfite as redox initiating system in aqueous medium.

Experimental

Materials

Demineralized xenogeneic bone having an average particle size of 120μm was prepared from bovine cortical tibia as reported earlier [5]. Glycidylmethacrylate (Polysciences, USA) was purified by distillation under vacuum. All other chemicals used were of analytical grade of S.D. Fine Chem., India.

Preparation of graft copolymer

The typical grafting reaction was as follows: a known amount of DXB (3-6 g) was suspended in 100ml of low conducting water at required temperature (water bath). After 30 minutes, freshly prepared 25ml of initiators solution containing equal ratio of potassium persulfate/sodium metabisulfite was added followed by glycidylmethacrylate (6.0×10^{-1} mol/L). The grafting reactions were carried out at varying time intervals (1-5 h). After completion of reaction, the contents were poured in methanol and the precipitated products were filtered and dried. The dried products were Soxhlet extracted for removal of unbound homopolymer using acetone and dried in vacuum to constant weight. The percent grafting and grafting efficiency were calculated as follows:

Percent grafting (PG) = $(W_g - W_o) / W_o \times 100$ (**1**)

%Grafting efficiency (% GE) = $W_g / (W_g + W_h) \times 100$ (**2**)

Homopolymer (%) = $W_h / W_m \times 100$ (**3**)

where, W_g - grafted weight; W_o – ungrafted weight; W_h - homopolymer weight; W_m – monomer weight.

FT-IR study

The FT-IR spectrum of polymer grafted DXB was recorded on a Nicolet 20DxB spectrophotometer (USA) using a KBr pellet for the proof of grafting reaction. The spectrum was recorded in the 4000-400 cm^{-1} regions with 2 cm^{-1} resolution averaging 100 scans.

Results and Discussion

The main objective of this work is the grafting of methacrylic monomer onto demineralized xenogeneic bone to impart new functional groups, which are capable to deliver the drugs or growth factors for hard tissue applications. The grafting of PGMA was proved by FT-IR spectroscopic technique. The FT-IR spectrum of DXB shows the absorption peaks at 1669 and 1541 cm^{-1} due to the presence of amide group (Figure 1a). The IR spectrum of grafted DXB (Figure 1b) shows new peaks in addition to characteristic peaks of DXB. The absorption peaks pertaining to epoxide group at 906 and 853 cm^{-1}, carbonyl group at 1731 cm^{-1} and C-O stretching band at 1260 cm^{-1} are evidence for the occurrence of polyGMA (PGMA) in the grafted DXB sample. Based on the above findings, it is confirmed that the grafting of PGMA has taken place onto DXB backbone.

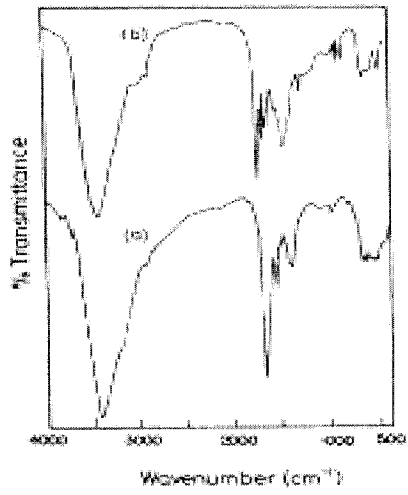

Figure 1. FT-IR spectra of (a) ungrafted DXB and (b) grafted DXB

The effect of DXB concentration was investigated by changing the DXB concentration from 3–6 g, keeping the other parameters constant in a total volume of 100 ml . It is evident that with increasing DXB concentration, both percent grafting and grafting efficiency increased and reached a maximum value at a concentration of 5 g and thereafter decreased with further increase in the amount of DXB added (Figure 2). The initial increase may be due to the fact that the reactive sites increased with increase in the amount of DXB. The decrease in percent grafting and grafting efficiency with higher amount of DXB may be due to the destruction of radical activity of DXB backbone soon after it is formed due to termination of grafting chains between the backbones.

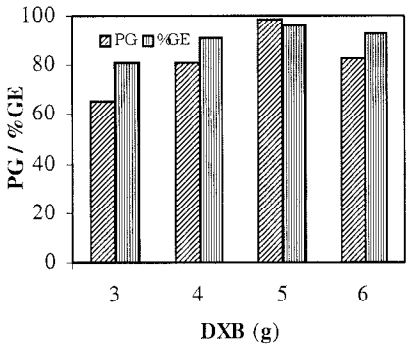

Figure 2. Effect of backbone on grafting reaction: GMA, 6.0 X 10^{-1} mol/L; $K_2S_2O_8/Na_2S_2O_5$, 1.5 X 10^{-3} mol/L each; temperature, 40°C; time, 180 minutes; total volume, 100ml.

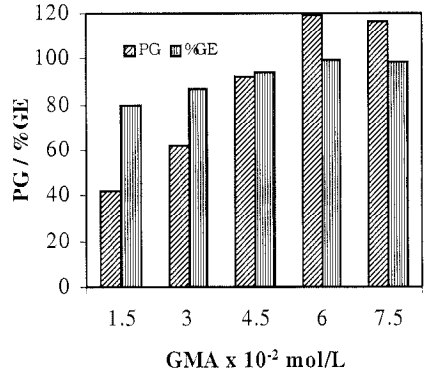

Figure 3. Effect of monomer on grafting reaction: DXB, 5.0 g; $K_2S_2O_8/Na_2S_2O_5$, 1.5 X 10^{-3} mol/L each; temperature, 40°C; time, 180 minutes; total volume, 100ml.

Figure 3 shows changes in the percent grafting and grafting efficiency with varying monomer concentration from 1.5 to 7.5 X 10^{-1} mol/L. Both the percent grafting and grafting efficiency are observed to increase with increasing monomer concentration up to 6.0 X 10^{-1} mol/L. With a further increase of monomer, the percent grafting remained steady. The increase in the rate of grafting could be ascribed to the greater availability of the monomer to the grafting sites. The increasing trend in percent grafting upon increasing the monomer may also be due to the gel effect that arises when polymerization medium becomes highly viscous. The increase in viscosity may reduce termination rate of the grafting chains due to their slower diffusion [6], which in turn leads to higher rates of percent grafting and grafting efficiency.

The change in percent grafting at varying concentration of $K_2S_2O_8/Na_2S_2O_5$ (0.5 - 2.0 X 10^{-3} mol/L, each) has been studied. The percent grafting and grafting efficiency increase up to 1.5 X 10^{-3} mol/L and then decreased, although not very significantly, above this concentration (Figure 4). Increase in the initiator concentration increases the free radicals in the reaction medium, which in turn increases the number of grafting sites on the backbone. The free radicals participate in many grafting reaction [7], which can directly interact with the DXB backbone to form active sites and may also initiate homopolymerization of GMA. It is possible that some of the active homopolymers may undergo chain-transfer reaction with the DXB and create additional active sites upon it, due to which the percent grafting will be higher. However, increase in the concentration of initiators results in the enumeration of the number of primary radicals, DXB radicals, and grafting macroradicals of side chains, which may interact with each other causing termination and hence reducing the grafting yield.

The graft copolymerization of GMA onto DXB has been studied at five different temperatures i.e. 30, 35, 40, 45, and 50°C (Figure 5). It is observed from the result that optimum grafting temperature is 40°C. The percent grafting increases with temperature from 30 to 40°C and then decreases with further increase of temperature to 50°C, though no significant change in the percent grafting occurs at the temperatures between 35 and 40°C. At temperature above 40°C, there is a possibility of denaturation of DXB, which may lead to decrease of percent

grafting. Beside, there may be a production of homopolymerization of GMA, which leading to decreasing percent grafting.

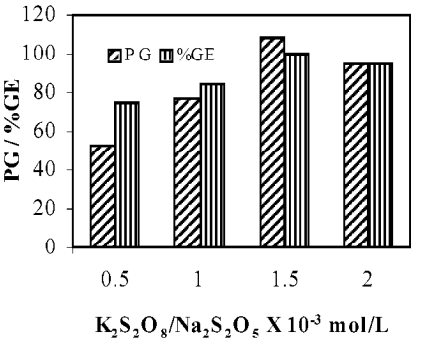

Figure 4. Effect of initiator on grafting reaction: DXB, 5.0 g; GMA, 6.0 X 10^{-1} mol/L; temperature, 40°C; time, 180 minutes; total volume, 100ml.

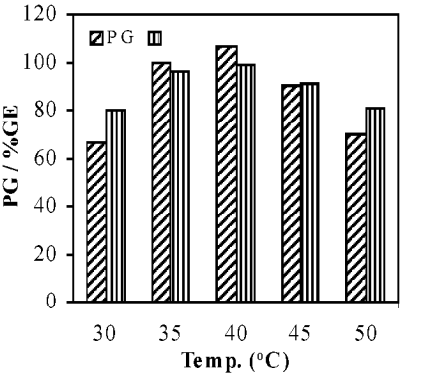

Figure 5. Effect of temperature on grafting reaction: DXB, 5.0 g; GMA, 6.0 X 10^{-1} mol/L; $K_2S_2O_8/Na_2S_2O_5$, 1.5 X 10^{-3} mol/L each; time, 180 minutes; total volume, 100ml.

With increase in the grafting time, percent grafting increased up to 180 minutes after which it leveled off with further increase in the grafting time (Figure 6). The increase in the grafting yield with respect to time is accounted by increase in the number of grafting sites on the DXB backbone in the initial stage of the reaction. Longer reaction periods have little effect on the percent grafting since, the number of active sites remains almost constant with increasing grafting time and hence there is no further change in the grafting yield.

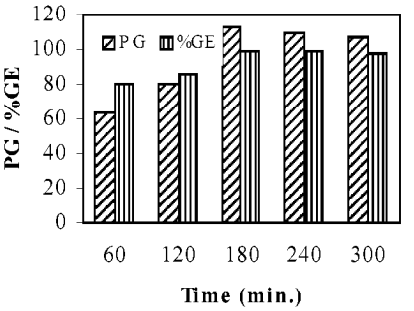

Figure 6. Effect of time on grafting reaction: DXB, 5.0 g; GMA, 6.0 X 10^{-1} mol/L; $K_2S_2O_8/Na_2S_2O_5$, 1.5 X 10^{-3} mol/L each; temperature, 40°C; total volume, 100ml.

Reaction mechanism

The mechanism of $K_2S_2O_8/Na_2S_2O_5$ initiated graft copolymerization of GMA onto DXB is the attachment of $^{\bullet}OH/SO_4^{\bullet-}$ radicals generated from the decomposition of initiators with the formation of active centers onto the DXB backbone. If active centers are formed, polymer chains start to grow on them, resulting in chain branches. The overall mechanism may be represented as follows:

Initiator decomposition (in the presence of $S_2O_5^{2-}$)

$$S_2O_8^{2-} \rightarrow 2SO_4^{\bullet-} \quad (4)$$

$$2SO_4^{\bullet-} + 2H_2O \rightarrow 2HSO_4^{-} + 2^{\bullet}OH \quad (5)$$

It is apparent from the above reactions that the radicals formed in the polymerization medium abstracts a hydrogen from the DXB backbone to produce DXB radicals

$$DXB\text{-}CH_2OH + {}^{\bullet}OH / SO_4^{\bullet-} (k_i) \rightarrow DXB\text{-}{}^{\bullet}CHOH + H_2O + HSO_4^{-} \quad (6)$$

(DXB radical)

In addition, the $^{\bullet}OH / SO_4^{\bullet-}$ radicals may initiate the homopolymerization of GMA by addition to the double bond of the monomer.

$$^{\bullet}OH / SO_4^{\bullet-} + GMA \rightarrow GMA^{\bullet} \quad (7)$$

$$GMA^{\bullet} + n\,GMA \rightarrow (GMA)^{\bullet}_{n+1} \rightarrow \text{homopolymer} \quad (8)$$

The graft polymerization is started by the addition of GMA monomer to the DXB-$^{\bullet}$CHOH active sites.

$$\text{DXB-}^{\bullet}\text{CHOH} + \text{GMA} \quad (k_i') \rightarrow \text{DXB-HOHC-GMA}^{\bullet} \quad (9)$$

(graft initiation)

Here, -$^{\bullet}$CHOH is corresponding radical on the DXB backbone, GMA is a monomer, k_i and k_i' are the respective rate constants. The propagation and termination of the reaction can be represented as

$$\text{DXB-HOHC-(GMA)}_{n-1}^{\bullet} + \text{GMA} \quad (k_p) \rightarrow \text{DXB-HOHC-(GMA)}_n^{\bullet} \quad (10)$$

(graft propagation)

$$\text{DXB-HOHC-(GMA)}_n^{\bullet} + \text{DXB-HOHC-(GMA)}_n^{\bullet} \ (k_t) \rightarrow \text{graft copolymer} \quad (11)$$

Where k_p and k_t are the respective rate constants of propagation and termination reactions.

Conclusions

The $K_2S_2O_8/Na_2S_2O_5$ redox system can effectively initiate the graft copolymerization of GMA onto DXB backbone in aqueous medium. The optimum reaction condition was standardized by varying the reaction parameters. FT-IR proved the grafting reaction of GMA onto DXB backbone, having epoxy groups. It is possible that the grafted DXB can subsequently be coupled to antibiotic through epoxy groups and can be used as an effective form of antibiotic therapy apart from being used as bone substitute.

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