Effect of Surface Functionalization on the Physicomechanical Properties of a Novel Biofunctional Copolymer

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ABSTRACT: The physicomechanical properties of functionally active poly(hydroxyethyl methacrylate-*co*-methyl methacrylate) [poly(HEMA-*co*-MMA)] are evaluated. It has been reported that the surface phosphorylated poly(HEMA-*co*-MMA) is capable of eliciting direct bone bonding when implanted *in vivo*. Hence, it is important to examine the physicomechanical property of the copolymer as a function of surface modification. The properties assessed are differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), equilibrium swelling, compressive strength, and dynamic mechanical analysis. According to the DSC data, the glass transition temperature, T_g of poly(HEMA -*co*-MMA) is not significantly altered by surface phosphorylation. The TGA results demonstrated that unmodified and surface phosphorylated copolymers have similar degradation profile. The differential thermal analysis further supports the data. The equilibrium swelling of functionalized poly(HEMA-*co*-MMA) in phosphate buffer saline ascertained that surface phosphorylation significantly increased the hydrophilicity of the copolymer. The study further illustrated that the percentage of equilibrium swelling appreciably increases with increase in HEMA content in the copolymer and reached a plateau after 100 h. Both compressive strength and compressive modulus of poly (HEMA-*co*-MMA) decreased due to surface phosphorylation while dynamic storage modulus value was not altered. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 121: 3509–3515, 2011

Key words: biomimetic; copolymer; hydroxyethyl methacrylate; physicomechanical; surface phosphorylation

INTRODUCTION

Surface modification has been well adopted as a potential technique to design functional biomaterials with intelligent properties.[1,2] Among the various prospects of surface functionalization, one of the best explored applications is to invoke biomimetic mineralization on various polymeric substrates.[3,4] Several investigations have presented exciting candidates capable of eliciting bioinspired mineralization of hydroxyapatite on diverse substrates.[5–8] Biomaterials with the potential of eliciting bioinspired mineralization could be proposed as suitable candidates for bone tissue engineering applications.[9]

Congenital or acquired bone repair is one of the major concerns in human health care.[10] Reviewing the contemporarily used bone graft materials, bioactive ceramics and glasses are popular for osteointegration and bone bonding through formation of smart interfaces, but is associated with basic limitations such as brittleness and restricted processability.[11] Polymers are generally known for their easiness in processing, but not associated with inherent osteointegrative property. However, biofunctionality could be easily imparted to polymers through suitable biomimetic techniques.[12] Even though surface modification remains as a popular method to address biocompatibility issue, it is inevitable to eliminate the possibility of any superfluous outcomes that can influence the property of the modified biomaterial. Moreover, for the clinical success of a bone implant a matching of the mechanical properties between the implant and the host tissue would be needed to establish a stable interface with the host tissue.[9] Hence, it becomes very important to assess the physicomechanical properties of a biomaterial after its biomimetic functionalization to ensure its absolute biofunctionality.

Poly(methyl methacrylate), PMMA based bone cement is widely used as a two component self-setting cement for fixing joint prostheses.[13] However, in the *in vivo* environment, PMMA is isolated from the surrounding tissues by a layer of fibrous tissue formed around it.[14] The formation of fibrous tissue occurs because PMMA lacks osteoconductivity, which obviously eliminates bonding with host bone and eventually promotes stress-shielding and implant loosening.[14] Hence, it becomes a highly significant

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TABLE I
Poly(HEMA-co-MMA) Compositions with Different HEMA: MMA Molar Ratio

Composition	PH_1	PH_2	PH_3	PH_4	PH_5
HEMA (moles)	0.07	0.19	0.38	0.57	0.69
MMA (moles)	0.90	0.75	0.50	0.25	0.10
Benzoyl peroxide (initiator), wt %	0.5	0.5	0.5	0.5	0.5
EGDMA (cross-linker), wt %	1.0	1.0	1.0	1.0	1.0

need to eliminate the fibrous tissue encapsulation of PMMA to improve its efficacy toward bone bonding. There are several research studies performed to functionalize particularly with a focus toward a composite material composed of PMMA and a bioactive material.[15,16]

The authors have developed a novel bioactive polymer by copolymerizing methyl methacrylate, MMA with a comonomer, hydroxyl ethyl methacrylate, HEMA and subsequently surface phosphorylating the copolymer matrix.[17] Hydroxy ethyl methacrylate and its copolymers are extensively studied for various biomedical applications.[18–22] The surface phosphorylated poly(2-hydroxy ethyl methacrylate-*co*-methyl methacrylate) [poly(HEMA-*co*-MMA)] possessed a functionally active surface that could induce biomimetic mineralization of calcium phosphate phase.[15,16] In a recent study, the authors found that the surface phosphorylated poly(HEMA-*co*-MMA) promotes direct bone bonding when implanted *in vivo*.[23] This study evaluates the effect of surface phosphorylation on the physicomechanical properties of poly(HEMA-*co*-MMA).

MATERIALS AND METHODS

Materials

2-hydroxy ethyl methacrylate (HEMA) (Assay: 98%), methyl methacrylate (MMA) (Assay: 99%) and phosphorous pentoxide (Assay: 98+, ACS reagent) were procured from Aldrich chemical Co. Ethylene glycol dimethacrylate (EGDMA) (Assay: 98%) was obtained from Fluka. Benzoyl peroxide (Assay: 98%) was purchased from S.D. fine India, Mumbai, India. All other chemicals were procured from Ranbaxy India, Mumbai, India.

Methyl methacrylate was made free of inhibitor by treating with 4% sodium hydroxide solution for three times, followed by washing with distilled water and dried by placing over anhydrous magnesium sulfate. HEMA was made free of inhibitor by passing through an inhibitor remover column (Aldrich chemical Co.).

Synthesis of poly(HEMA-*co*-MMA)

Poly(HEMA-*co*-MMA) was synthesized as per the procedure reported elsewhere.[24] Briefly, by free radical initiated bulk polymerization of HEMA and MMA using 0.5 wt % benzoyl peroxide as initiator and 1 wt % EGDMA as *in situ* cross-linker. The experiment was conducted in a three-necked RB flask fitted with a condenser under nitrogen atmosphere. The temperature of the reaction bath was set at 80°C and the stirring rate was 300 rpm. After 15 min, the contents were carefully transferred to clean poly(propylene) molds and the polymerization was allowed to complete in a clean preheated air oven (set at 70°C) for 24 h. The compositions and their designations of copolymers with different HEMA: MMA molar ratio is given in Table I.

Surface phosphorylation of poly(HEMA-*co*-MMA)

The surface phosphorylation of poly(2-HEMA-*co*-MMA) was performed by using 76% phosphorous pentoxide (P_2O_5) at 80°C in a RB flask for 60 min.[23] The surface phosphorylated poly(HEMA-*co*-MMA) films and discs were cooled to room temperature and washed with distilled water. The samples were further immersed in distilled water for 48 h to make free of excess reagents, and then dried in air oven at 70°C.

Characterization

Poly(HEMA) is a biocompatible but highly hydrophilic polymer. Increased amounts of HEMA in poly(HEMA-*co*-MMA) may severely affect the swelling and mechanical properties of the copolymer. Hence, while choosing the best composition of poly(HEMA-*co*-MMA) for bone implantation study, the preferred composition was the copolymer containing low HEMA content. To understand the degree of swelling for various poly(HEMA-*co*-MMA) compositions, the equilibrium swelling degree analysis was performed as one of the primary objectives of the study. For an implant to be considered as a potential bone graft material, ability to trigger calcium phosphate nucleation under *in vitro* condition is a prerequisite. It was found that the composition with lowest HEMA content, represented as PH1 with HEMA: MMA ratio 0.07 : 0.90, showed minimum swelling and it was also capable of nucleating calcium phosphate under simulated physiological condition.[17] Moreover, it has been found that surface phosphorylated PH_1 is capable of direct bonding with

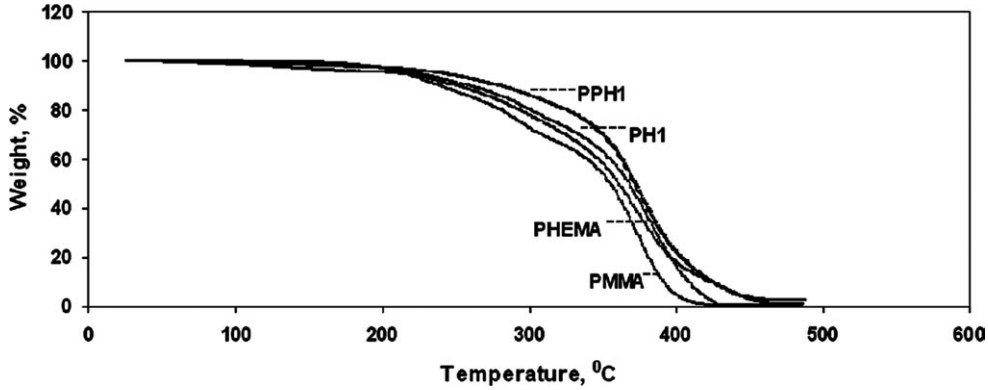

Figure 1 Thermograms of PMMA, PHEMA, PH1, and PPH1.

bone.[23] Hence in this study, PH_1 has been evaluated for its physicomechanical properties as a function of surface phosphorylation.

Differential scanning calorimetry (DSC)

The glass transition temperature (T_g) of poly(methyl methacrylate), designated as PMMA, poly(hydroxyl ethyl methacrylate) designated as PHEMA and poly(HEMA-*co*-MMA), PH1 before and after surface phosphorylation were determined using differential scanning calorimeter (DSC-2920) TA instruments, USA according to ASTM E 1356-03, "*Determination of glass transition temperature of polymers by differential scanning calorimetry*." The samples were pretreated by a first heat cycle carried out from room temperature to 200°C to get rid of any internal stress retained as an effect of the processing. For the determination of T_g, the heating rate was 5°C/min and the purge gas was nitrogen.

Thermogravimetric analysis

The thermogravimetric analysis (simultaneous TGA-DTA) of PHEMA, PMMA and poly(HEMA-co-MMA) before and after surface phosphorylation was performed as per ASTM E-1131-03 using simultaneous DTA-TGA (model SDT 2960) TA Instruments. A heating rate of 10°C/min from room temperature to a maximum temperature of 600°C was used. Nitrogen was used as the purge gas.

Equilibrium swelling in phosphate buffer saline (PBS)

The equilibrium swelling of poly(HEMA-co-MMA) copolymers, PH_1, PH_2, PH_3, PH_4, andPH_5 were measured in phosphate buffer saline and compared with that of PHEMA. The swelling study was carried out up to 336 h. Circular specimens with dimensions 10 mm diameter $\times$ 1 mm thickness were prepared for the swelling studies using Teflon® molds. Four specimens from each composition were used for the study. Each of these specimens was immersed in 15 mL of PBS with pH 7.4 at 37°C in separate glass containers and kept in an incubator at 37°C. The weight of the samples and the pH were recorded at 12 h intervals for the first 48 h, then at 96 h and at 336 h. The equilibrium swelling degree of the surface phosphorylated poly(HEMA-*co*-MMA) copolymers, designated as PPH_1, PPH_2, PPH_3, PPH_4, andPPH_5, were also measured in PBS and compared with that of phosphorylated PHEMA.

The degree of swelling was calculated as,

$$\text{Swelling degree } (\%) = (W_w - W_d)/W_d$$

where, W_d and W_w are the weight of dry and swollen samples, respectively.

Mechanical properties

The compressive strength of poly(HEMA-co-MMA) before and after surface phosphorylation was measured using Instron-3345, (Instron, UK) at a crosshead speed of 1 mm/min (temperature 25 $\pm$ 2°C, RH 50%). The samples were cylindrical in shape with 2 mm diameter and 4 mm height. In addition, the storage modulus of the copolymer before and after surface phosphorylation was also assessed using a dynamic mechanical analyzer, Tritec 2000 DMA machine in the tensile mode. Polymer samples in the form of films were used for the study.

RESULTS

Differential scanning calorimetry

The glass transition temperature (T_g) of PH1 was 104.57°C while that of PPH1 was 103.17°C. The marginal shift in T_g to the rubbery side could be attributed to surface phosphorylation of PH1. The T_g of PMMA and PHEMA were observed as 99.77°C and 98.67°C, respectively.

TABLE II
Comparative Evaluation of Thermal Behavior of PMMA, PHEMA, PH1, and PPH1

Sample	Onset of decomposition temperature (°C)	Temperature of 50% decomposition (°C)	Weight loss after first stage decomposition (%)
PH1	189	368.01	24.14
PPH1	206.3	362.6	23.12
PMMA	172.62	355.4	30.08
PHEMA	200.85	361.24	28.06

TGA

The TGA scans of PMMA, PHEMA, PH1, and PPH1 are given in Figure 1. The decomposition of PH1 begins at 189°C, whereas for PPH1 the decomposition begins only at 206.3°C. All the samples showed similar decomposition profile. For PMMA, the decomposition begins at 172.62°C and the weight loss rapidly increased with increase in temperature. The weight loss after the first stage decomposition was 30.08% and it reached 99.55% after the second stage decomposition. The weight remained at 592°C was 0.20%. The temperature at 50% decomposition was 355.4°C. Similar to PMMA, PHEMA also had a rapid burn out profile. However, the decomposition of PHEMA was initiated at a higher temperature, 200.85°C. The weight loss after the first stage decomposition for PHEMA was 28.06% and it increased to 84.16% after second stage decomposition. The decomposition further continued with increase in temperature with a mass of 0.85% remained at 524°C. The temperature at 50% decomposition was 361.24°C.

The decomposition of PH1 has begun at 189.28°C, which is a temperature in between degradation commencement temperature of PMMA (172.62°C) and that of PHEMA (200.85°C). However, the degradation profile for PH1 showed the same trend as that of PMMA and PHEMA. The weight loss after the first stage decomposition was 24.14% and it declined further to 0% at 600°C. For PH1, the 50% of decomposition occurred at a temperature 368.01°C, which is slightly higher compared to that of PHEMA (361.24°C) and PMMA (355.4°C) but slightly lower than that of PPH1 (362.6°C). The weight loss after the first stage decomposition for PPH1 was 23.12% and it declined further to 0% at 600°C. Table II shows a comparative evaluation of the thermal behavior of PMMA, PHEMA, PH1, and PPH1.

The differential thermograms (Fig. 2) showed that there is no significant difference in the degradation profile of PMMA, PHEMA, and PH1. However, PHEMA showed the highest thermal stability with an onset of degradation at 200.85°C and for PMMA, the lowest (172.62°C). As expected, the DSC profile of PH1 was in between that of PMMA and PHEMA with a thermal stability up to 189.28°C. It is apparent from the thermograms that there is a slight increase in the onset of decomposition for PPH1 compared to PH1. The decomposition of PH1 begins at 189°C while for PPH1 the decomposition begins only at 206.3°C.

Equilibrium swelling

Figure 3(a) imparts information about the nature as well as extent of swelling of PH1, PH2, PH3, PH4, and PHEMA. It is apparent that the degree of swelling increases with increase in HEMA content in poly(HEMA-*co*-MMA), leading to an equilibrium swelling represented by a plateau after 100 h. Hence

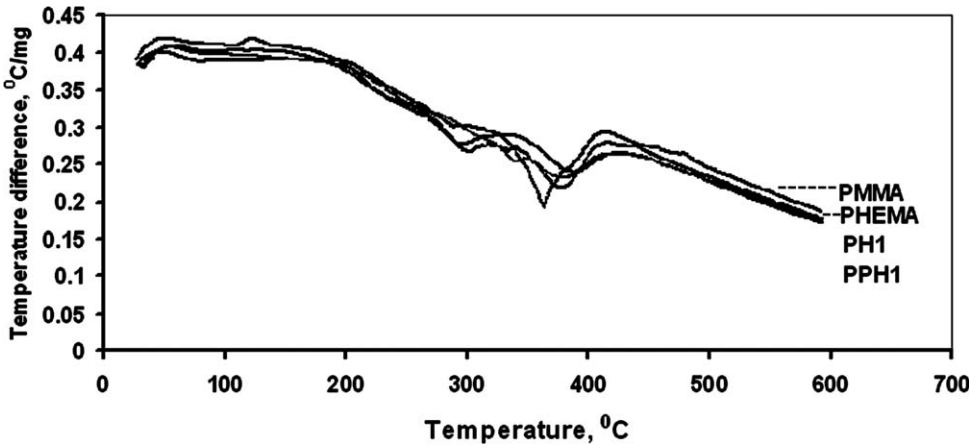

Figure 2 Differential thermograms of PMMA, PHEMA, and PH1.

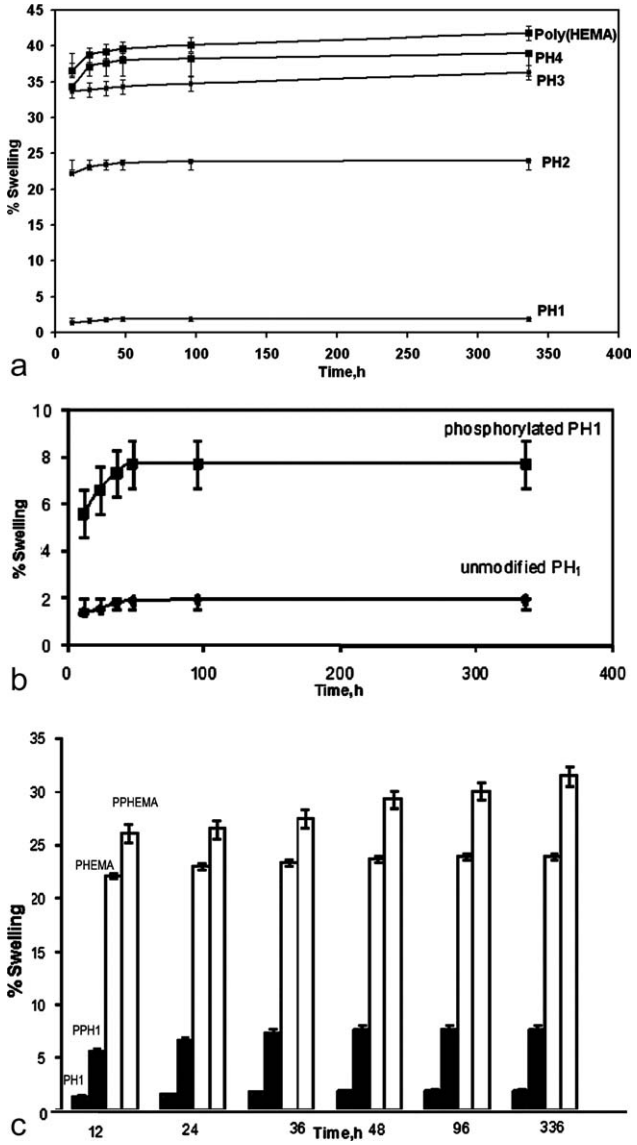

Figure 3 (a) Equilibrium swelling of poly(HEMA-*co*-MMA) compositions (PH1, PH2, PH3, PH4) and poly (HEMA). (b): Equilibrium swelling of poly(HEMA-*co*-MMA), PH1, and surface phosphorylated poly(HEMA-*co*-MMA), PPH1. (c): Effect of phosphorylation on degree of swelling: comparative assessment between PH1, PPH1 and poly(HEMA).

PH1 shows lowest and PHEMA shows the highest percentage of swelling. At equilibrium, PH1 (with HEMA:MMA = 0.07 : 0.90) shows a swelling of 2%, while PH4 (HEMA:MMA = 0.57 : 0.25) shows 39% swelling. It is worth to mention that PH2, the poly (HEMA-*co*-MMA) composition with HEMA:MMA = 0.19 : 0.75, also shows a significant percentage of swelling of 24%. It is obvious from Figure 3(a) that PHEMA has an exceptionally high degree of swelling (42%).

The trend of equilibrium swelling profile exhibited by surface phosphorylated PH1 is comparable to that of PH1 [Fig. 3(b)]. It is imperative to state that surface phosphorylation significantly increases the degree of swelling. For PH1, the swelling degree was 2% while it has considerably increased to 8% for the surface phosphorylated PH1.

To understand the relation between the degree of phosphorylation as a function of HEMA content in poly(HEMA-*co*-MMA), poly(HEMA) was phosphorylated and its degree of swelling has been plotted against unmodified poly(HEMA), PH1, and PPH1 [Fig. 3(c)] which shows that the degree of swelling is extremely less for PH1.

Mechanical properties

Compressive modulus

The compressive strength (stress at maximum load) of PH_1 was found as 153 ± 3 MPa and modulus 4.6 ± 2 GPa. The compressive strength (stress at maximum load) for PPH_1 was found as 39.35 ± 2 MPa and modulus 1.6 ± 2 GPa.

Dynamic mechanical analysis

The storage modulus values of PH1, PPH1, PMMA and poly(HEMA), measured using DMA is given in Figure 4. The storage modulus of PMMA was observed as 2.44 GPa and that for PHEMA was 1.43 GPa. It is evident from the figure that PMMA has a higher modulus compared to PHEMA. The storage modulus value of PH1 remained same after surface phosphorylation, 1.75GPa at room temperature (25 ± 2) °C. It could also be manifested that the storage modulus values of PH1 is in between that of PMMA and PHEMA.

DISCUSSION

The results demonstrated that even though the decomposition profiles of PMMA, PHEMA, and poly(HEMA-co-MMA) are quite similar, a slightly increased thermal stability was observed for surface phosphorylated poly(HEMA-*co*-MMA). This could be attributed to surface phosphorylation. Phosphate

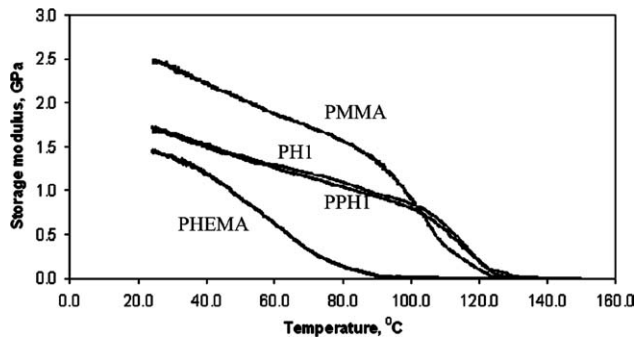

Figure 4 Storage modulus of PMMA, PHEMA, PH1, and PPH1.

group being more stable compared to hydroxylic group, the initiation of decomposition is slightly delayed. The DTA curves further corroborate that there is no significant difference in the onset of decomposition between PMMA and poly(HEMA-*co*-MMA). The thermograms ensure good thermal stability and a safe processing temperature range to poly(HEMA-*co*-MMA).

It is well recognized that swelling plays a vital role in determining the mechanical properties of the PHEMA and its copolymers.[25] A four times increase in the extent of swelling for surface phosphorylated poly(HEMA-*co*-MMA) is obviously an indication of the increased hydrophilicity and is merely attributed to the surface phosphorylation. However, when compared with PHEMA, the degree of swelling is extremely less for PPH1 [Fig. 3(c)]. Hence, it could be viewed that even though surface phosphorylation promotes the hydrophilicity of the copolymer, the extent of swelling could be controlled conveniently by suitably designing the copolymer composition before subjecting to phosphorylation.

The swelling degree of a hydrogel is favorably influenced by the osmotic potential, strong interactions with water, high free volume, high chain flexibility, and low cross-link density.[26] Poly (hydroxyl ethyl methacrylate) is a highly hydrophilic polymer and is known to form hydrogels.[26] Even though poly (HEMA-*co*-MMA) cannot be called as a hydrogel, the basic hydrophilicity of PHEMA is reflecting in its swelling behavior. The swelling data shows that phosphate coupling imparts greater hydrophilicity to the system. During surface phosphorylation, the —OH group undergoes esterification which leads to greater hydrophilicity to the macromolecular chains.

Hermitte et al.[27] investigated the relationships between formulation, bulk properties, and surface properties of copolymers prepared with HEMA, MMA, and ethylmethacrylate (EMA). They have reported that bulk water content and the swelling ratio of HEMA copolymers is proportional to the amount of HEMA and is linearly correlated to the contact angle hysteresis.[27,28] The swelling of PHEMA is greatly dependent on temperature and the penetrating solution. The balance between hydrophilic/hydrophobic groups, the length between links, and the size and distribution of the network also determines the extent of swelling.[29]

Polymers are viscoelastic materials and their mechanical properties have strong dependence on time and temperature. Temperature scans across the dynamic spectrum of mechanical absorptions are commonly required for the characterization of polymers.[30] Higher water content in PHEMA will cause steep decrease in the tensile strength and tear resistance.[29] The same behavior is expected for copolymers of PHEMA. The temperature indirectly influences the strength of poly (HEMA) copolymers, because it controls the extent of swelling. Most importantly, the surface properties of PHEMA are very pertinent as it has adjustable interfaces.[31] The compressive strength as well as modulus of poly (HEMA-*co*-MMA) decreased significantly by surface phosphorylation. The stress–strain curves of both poly (HEMA-*co*-MMA) and surface phosphorylated poly (HEMA-*co*-MMA) show that there was no breaking point during compression and hence no ultimate failure was observed. The fundamental reason for PHEMA to show a lower modulus compared to PMMA is specifically associated with the hydrophilic nature of PHEMA. The water molecules adsorbed by PHEMA from the environment have a plasticizing action and offer greater flexibility to the polymer.[32]

It has been observed by several investigators that functionalized polymers with surface bound phosphate group induces *in vitro* nucleation of calcium phosphate crystals on their surface under simulated physiological environment.[33–35] However, many of these surface functionalized polymers require pretreatments such as calcium hydroxide/calcium chloride pretreatment after functionalization for the *in vitro* mineralization of calcium phosphate. Moreover the popular surface phosphorylation techniques are done at higher temperature (>100°C), using organic solvents and catalysts, which may alter the basic polymer characteristics significantly. Here, the authors propose a method which does not require any pretreatment after surface functionalization. Moreover, the surface phosphorylation is carried out at significantly lower temperature. This work demonstrates that surface phosphorylation does not alter the inherent physicochemical properties of poly(HEMA-*co*-MMA). Moreover, a recent study of the authors has shown that surface phosphorylated poly(HEMA-*co*-MMA) could invoke direct bone bonding *in vivo*.[23] Hence, phosphorylated poly(HEMA-*co*-MMA) could be considered as a potential candidate for bone repair *in vivo*.

CONCLUSIONS

The glass transition temperature of poly(HEMA-*co*-MMA) is not affected due to surface phosphorylation. The TGA profiles of PMMA, PHEMA, and poly (HEMA-*co*-MMA) were similar but a slight higher onset decomposition temperature was observed for surface phosphorylated poly (HEMA-*co*-MMA). The hydrophilicity and equilibrium swelling of the copolymer increased significantly due to surface phosphorylation. The compressive strength and modulus of poly (HEMA-*co*-MMA) decreased considerably due to surface phosphorylation while the dynamic storage modulus remained unchanged.

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