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Investigation on sulphonated PEEK beads for drug delivery, bioactivity and tissue engineering applications

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Abstract Poly ether ether ketone (PEEK) has wide applications in the field of medicine as a prosthetic material and can be sulphonated using sulphuric acid. This study focuses on the fabrication of water soluble SPEEK beads using infusion pump and the obtained beads were characterised using Fourier Transform Infra Red (FTIR) spectroscopy to confirm the sulphonation, while their surface morphology was studied using scanning electron microscope (SEM). In order to study the bioactivity of the prepared beads, hydroxyapatite was dispersed within them followed by their immersion in SBF. In order to study the drug release kinetics of the beads, they were loaded with amoxicillin in different concentrations. Cytotoxicity studies were performed by MTT method and ALP activity was measured using mouse osteoblast cells (MC3T3-E1). The SEM and FTIR of the prepared beads confirmed the morphology and the sulphonation of the prepared beads. Also, the apatite formation on the SPEEK beads, subsequent to their immersion in SBF, proved that the beads possessed excellent bioactivity. Moreover, the beads developed exhibited low cytotoxicity, implying good biocompatibility and safety. Thus, the results of the study indicated that the novel SPEEK beads could potentially be used as a drug carrying vehicle with low toxicity.

Introduction

Drug delivery is always an advent of interest for researchers and the progress in the field of drug targeting is slow. The application of novel drug delivery systems started in the mid-seventies of the last century with the advent of the monoclonal antibody technology as well as the development of liposomal and polymeric nanoparticle carriers. Polymers, monoclonal antibodies, liposomes, proteins and many other entities created an opportunity by acting as a carrier molecule of the drug. However, there also exist the problem in the synthesis of these carriers, their toxicity and also the release kinetics of the drug.

The main focus of this article is towards the development of a polymer as the carrier of the drug particles. Amongst the various types of polymers that were tried in the research only a few polymer based drug targeting strategies have successfully reached the clinic, and eventually reached the international drug market. Moreover, polymeric nanoparticles and liposomes are pointed out as the most promising drug carrier devices, by targeting to various body parts, mainly using the active drug targeting strategy via antibodies [1].

In the last few decades, controlled and targeted drug delivery has been the main focus in the field of research. Rapid advances achieved in polymer science created new innovations in drug delivery which made a big impact in the field of biomedical applications such as strategies to protect the drug carriers as well as the drug against chemical and physiological triggers by providing a matrix [2]. They could be designed into different ways to deliver drug, some of which include membrane, nanofiber, microspheres, beads, hydrogels, etc. [3]. There are various systems that have been investigated for the possible applications over a range of therapeutics, such as providing a wider range in the selectivity of anticancer drugs, sustained release and preferably, should also act as a vehicle for protection and delivery of drugs.

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Many varieties of polymeric materials, preparation methods and characterization techniques have been applied so far in this field. Polymeric materials that are water soluble can be employed as a carrier of drugs that are taken through different modes into the body, particularly into the bloodstream.

The precise significance of the polymer is to provide a slow release of the drug that is loaded in it, through the cleavage of the bonds which attaches the polymer to the drug. Release of the drug could be controlled by various environmental conditions including temperature, pH, drug concentration or the presence of enzymes [4]. Apart from all the above mentioned conditions, the designed polymer materials should be a blood and cell compatible one. The objective of this study was to investigate the use of sulphonated poly ether ether ketone (SPEEK) in drug delivery, which according to the best knowledge of authors, has not been reported elsewhere.

In general, Poly ether ether ketone (PEEK), a thermoplastic material, is being used increasingly in the field of chemical processing, aerospace, automotive and medical industries. Its enhanced chemical stability, mechanical properties along with high thermal stability and ease of processing either alone or as a composite makes PEEK an attractive customer for high-performance applications [5]. In this study, we investigated sulphonated poly ether ether ketone (SPEEK) for the controlled delivery of amoxicillin. PEEK becomes water soluble when it is sulphonated for a long period of time, but the cytotoxicity of the SPEEK has not been studied so far. This property plays a vital role as their use in implants is critical when it comes in contact with the tissues, since direct bone-implants are more advantageous than a biomaterial which would form a fibrous tissue post-surgery [6]. The sulphonation of PEEK and its material properties were studied and reported in our previous studies [7, 8]. The schematic picture of the sulphonation process is described in Fig. 1. The main advantage of the present system is the possibility of high drug loading efficiency and the preservation of bioactivity.

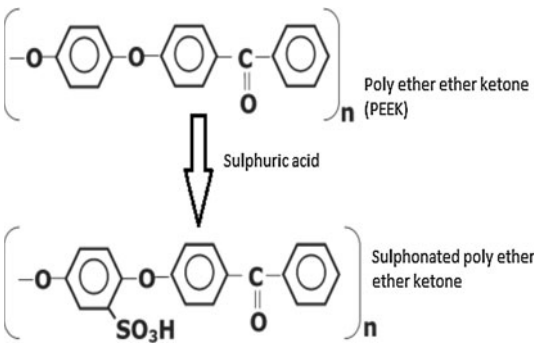

Fig. 1 Sulphonation of PEEK

Amoxicillin is an antibiotic used to treat bacterial infections by inhibiting the synthesis of bacterial cell wall. It is one of the most widely used antibiotics against many infections and hence we opted for its use in this study. The main objectives of the study includes the following

(i) Sulphonating the PEEK thus making it water soluble.
(ii) Characterization of the sulphonation using FTIR, and preparation of the beads.
(iii) Analysing the drug loading and release kinetics.
(iv) In vitro bioactivity and biocompatibility were also investigated.

Materials and method

Materials

PEEK powder was purchased from Victrex while sulphuric acid and acetone were procured from SISCO Laboratory, India. Hydroxyapatite (HA) was purchased from Sigma Aldrich.

Method

Preparation of sulphonated PEEK

Water soluble SPEEK was prepared by using the following procedure. A total amount of 5 g of PEEK (Sigma Aldrich) was dissolved in 100% sulphuric acid under constant stirring for 5 days in order to achieve high degree of sulphonation and make it a water soluble one. After this reaction time, the sulphonated polymers were precipitated in cold water, washed, and then completely dried in an oven.

Preparation of SPEEK beads

Water soluble SPEEK of 3 g weight was dissolved in the 10-mL distilled water and stirred overnight. The SPEEK solution was dropped into a non-solvent (in this case acetone) using infusion pump at a feed rate of 0.5 mL/h. Beads obtained through the above method were collected, centrifuged at 6000 rpm for 30 min, and dried. The surface morphology of the beads prepared were analysed using the SEM (HITACHI S-3400model).

Fourier transform-infrared spectroscopy analysis

FTIR studies of SPEEK were carried out using KBr pellets in a Perkin Elmer FTIR spectrophotometer. The spectra obtained were recorded in transmission mode. The

difference between the PEEK, SPEEK and drug loaded SPEEK was analysed using the FTIR.

Bioactivity studies

For the bioactivity studies, 2wt% of HA was incorporated into the SPEEK beads using the procedure similar to the one described in "Preparation of SPEEK beads" section. The additional step involves the ultra-sonication of the SPEEK solution containing HA in order to facilitate a uniform dispersion of HA in the solution prior to bead formation. The obtained beads were immersed in 5 mL of simulated body fluid (SBF) at 37 °C. The SBF solution, possessing an ionic composition similar to that of the human body and first proposed by Kokubo et al., was used here to test the bioactivity of the beads in vitro. The samples were subject to various periods of incubation (0, 15 and 30 days) in SBF then removed from it, washed with water and finally dried at the room temperature. The calcium–phosphate due to the apatite formation on the bead surface was analysed using SEM [9, 10].

Drug loading and release kinetics

Determination of drug loaded Ten milligrams of beads was suspended in 10-mL buffer solution of pH 9.0 (sodium hydroxide solution). The mixture was maintained at 60 °C for a few hours and the amount of amoxicillin released by hydrolysis was determined by T90$^+$ 3000 series UV spectrophotometer (PG Instruments) at $\lambda_{max} = 217$ nm for an interval of 30 min until the value reached a constant.

Release kinetics The amount of amoxicillin released was monitored by UV spectrophotometer at $\lambda_{max} = 217$ nm as a function of time. The procedure used was as following: 10 mg of beads was placed in 10-mL phosphate buffer (PBS) of pH 7.4 with constant stirring at 37 °C [11, 12]. Absorbance of the samples was observed for the release of the drug from the beads using the UV–Visible spectrophotometer at an interval of 30 min.

Cytotoxicity studies

Mouse osteoblast cells (MC3T3-E1) were cultured using Dulbecco's modified Eagle's medium supplemented with 5% fetal bovine serum and 1% penicillin–streptomycin and then seeded into the T75 tissue culture flasks. The medium was changed every day in order to supply fresh nutrients for the cells thereby increasing the growth of the cells. Once the cells became adherent they were removed using trypsinization and used for further experiments. The cytotoxic nature of the samples towards the cells were analysed based on Table 1.

Alkaline phosphatase activity (ALP)

A calorimetric assay was used to find the ALP activity, where the conversion of p-nitrophenol phosphate to a yellow coloured product in the presence of ALP takes place. Using the Sigma diagnostic kit no. 104, the ALP activity of the cells was tested both in the presence and absence of HA.

Results and discussion

FTIR

The FTIR spectra of PEEK and SPEEK are shown in Fig. 2. The spectrum of PEEK, Fig. 2a, shows trace amount of water in its matrix as revealed by its O–H stretching vibration close to 3500 cm^{-1}. The aromatic ring C–H stretching vibration is shown by its peak just above 3000 cm^{-1}. The C=O stretching vibration gives its peak close to 1700 cm^{-1}. The peaks at 1600, 1500 and 1440 cm^{-1} are due to aromatic ring skeletal vibrations. The intense broad peak between 1000 and 1400 cm^{-1} includes the peaks due to phenyl C–O vibration. The peaks below 1000 cm^{-1} are due to aromatic C–H bending modes. The FTIR spectrum of SPEEK, Fig. 2b, shows an intense broad envelope close to 3450 cm^{-1} which is attributed to the O–H stretching vibration of SO_3H which established sulphonation of PEEK. The water content appears to be less as its bending vibrations are not intense enough as seen in the peak close to 1620 cm^{-1}. All the other features are nearly same as that of PEEK.

SEM analysis

The surface morphologies of the dried beads were examined under SEM. Figure 3a shows the SEM analysis for the typical bead formation in the range of 1–2 μm and the beads were found to possess a spherical morphology without any agglomeration which makes it a potential candidate for use in drug delivery applications.

On higher magnification, surface of the single beads were analysed (Fig. 3b) and the beads showed a spherical nature. Also, it was clear that the beads were uniform in their size and exhibited a smooth surface, thereby showing promise to be a suitable candidate for the controlled release of the drug.

Bioactivity study

Figure 4a–c present SEM micrographs of SPEEK polymer beads before (Fig. 4a) and after immersion in SBF for bioactivity test corresponding to 15, 30 days (Fig. 4b, c)

Table 1 Qualitative and quantitative score used in cytotoxicity studies [13]

Confluency (%)	Floating cells (%)	Change in cellular morphology and response	Relative growth rate (%)	Score/response
100	0	Nil	≥100	Pass (0–1)
90–100	0–5	Few cells affected with slight change	75–100	Pass (1–3)
60–90	5–10	Some round or spindle shaped cells with mild changes	50–75	Retest (3–5)
30–60	10–20	Round or spindle shaped cells with moderate changes	25–60	Fail (5–7)
0–30	>20	Morphology change in all cells severely	0–25	Fail (7–8)

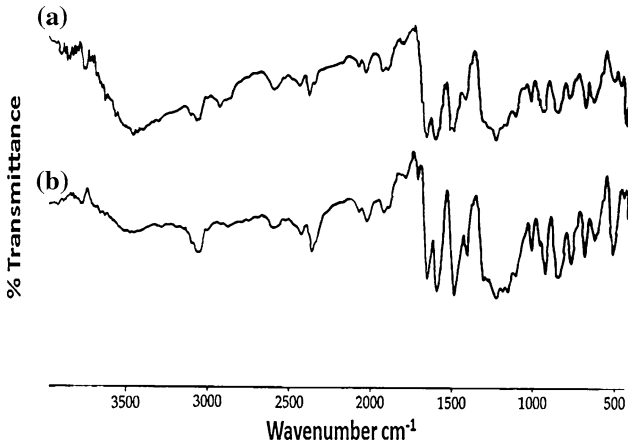

Fig. 2 FTIR Spectra of (*a*) PEEK and (*b*) SPEEK

respectively. In Fig. 4a, the typical oriented morphology of the beads was observed which was maintained in the first days of immersion in SBF. However, after 15 days of immersion, a number of nuclei started to form on the surface of the beads which corresponded to the formation of calcium–phosphate (Ca–P).

These nuclei have grown with increasing immersion times and after 30 days, a compact and well-defined Ca–P layer could be observed, as presented in Fig. 4 c. This layer resembled the typical cauliflower-like morphology of Ca–P films. This clearly indicates the bioactivity of the developed beads, confirming that SPEEK could possibly be used in the field of tissue engineering. Ca and P elements in the formed layers can be clearly seen in SEM analysis.

Drug loading and release kinetics

The amount of amoxicillin drug loaded in the SPEEK beads were calculated using a standard curve obtained from UV Spectrophotometer at 217 nm. The amount of the amoxicillin loaded in the beads at different concentrations was found to be as in Table 2. It was observed that when the concentration of the drug was increased above 200 mg/g of the polymer the bead formation was disturbed.

The drug release experiment was carried out by incubating the drug loaded beads in 10-mL phosphate buffer (PBS) of pH 7.4 at 37 °C with constant stirring and by monitoring the absorbance of amoxicillin at 217 nm as a function of time. It was observed from Fig. 5 that the initial release or "burst" phase was due to the rapid dissolution of the drug domains at the surface of the polymer beads. Further release due to the degradation of the polymer contributed to the second phase of the release. Chemically controlled drug deliveries are of two types, based on which the release of the drug occurs—namely the pendant chain systems and erodible drug delivery systems. The former is because of the covalent attachment of the drug to the polymer which is labile and may be broken due to the hydrolysis or enzymatic degradation of the polymer. The erodible mode occurs mainly due to the degradation or dissolution of the polymer system. The release rate of the drug is controlled by the diffusion or the degradation mechanism of the polymer. If the diffusion of the drug is low then the rate controlling step is the polymer degradation [11, 12]. Here there was an initial fast release which accounted for around 10–40% of the drug attributed to the rapid dissolution of the drug from the surface of the polymeric beads. The second phase around 40–99% drug release was due to the degradation of the polymer beads itself. As the release at this pH was more, this could be potentially used for diseases associated with colon like diverticulitis as a double potential drug delivery [14].

The factors such as the drug present on the surface, size and the solubility of the drug domains at the surface contributed to the magnitude of the fast release or initial burst phase. The drug release observed in this study appeared to be different from the one observed by Makarov et al. [15] where the percentage of release was dependent on the concentration of the drug, whereas in the present results, only the initial burst phase was affected by the concentration of the drug. Moreover, it was also observed that the initial burst phase was influenced by the amount of drug loaded. With increase in the concentration of the drug loaded in the beads, there was an increase in the initial release. Also this type of release may be due to the adsorption of the drug particles on the surface of the beads [16]. The second phase of the release may be attributed to the erodible mode due to the degradation or dissolution of the polymer system. Hence, it could be clearly stated that the release kinetics is a chemically controlled release

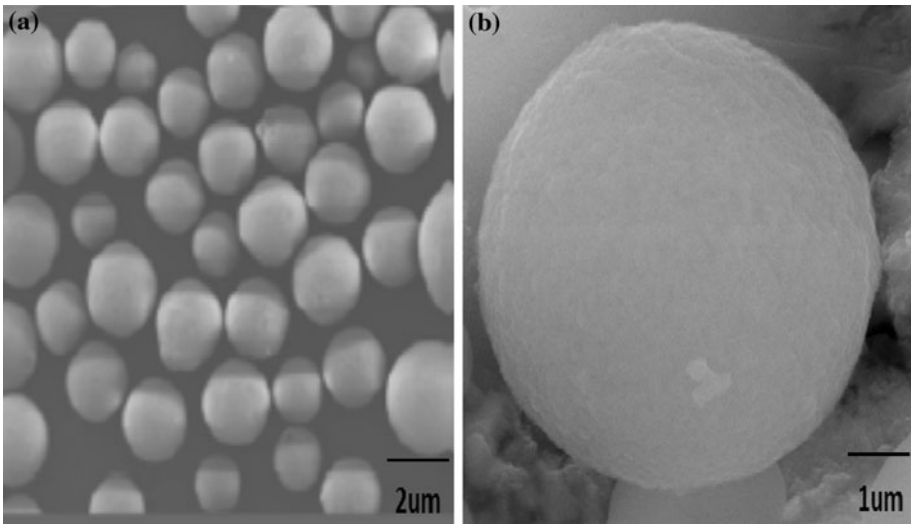

Fig. 3 SEM images of **a** SPEEK beads that were formed with an average size of 1.5–2 μm and **b** higher magnification showing a single SPEEK bead

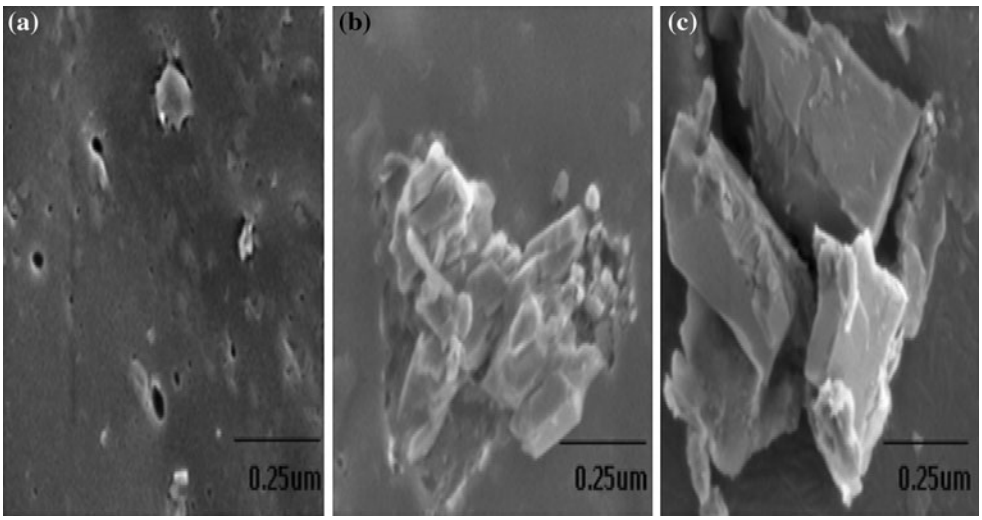

Fig. 4 SEM image on the surface of SPEEK beads **a** first day after the addition of HA, **b** after 15th day of incubation in SBF and **c** after 30 days of incubation in SBF, respectively

Table 2 Shows the loading efficiency of the drug loaded in SPEEK beads

Calculated drug concentration (mg amoxicillin/g of polymer)	Amount of drug loaded (mg amoxicillin/g of polymer)	Loading efficiency = [amount drug loaded/ calculated drug concentration] × 100 (%)
200	180.2	90.1
150	142.9	95.3
100	90.6	90.6
50	48.3	96.6
25	24.7	98.8
10	9.8	98.0

as the second release could be correlated to the breaking of the covalent attachment between the drug and the polymer.

Cytotoxicity study

The reduction of yellow tetrazolium salt that is MTT due to the metabolically active cell mitochondria in the living cell results in the formation of violet formazen dye from which the amount of viable cells in the sample was quantified spectrophotometrically using ELISA reader [13]. The results of the study showed that the beads passed the test with an index score of 1–3 (Table 3) thereby it could be stated this could potentially be used in the tissue culture

studies. This was further confirmed through the microscopic analysis wherein, the density of the osteoblasts was observed to be similar to the one observed in control (Fig. 6). It should be noted that the results were similar to most of the biodegradable polymers.

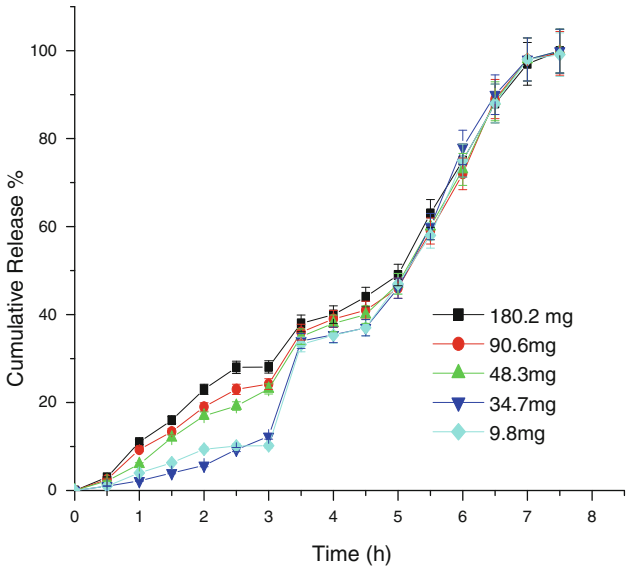

Fig. 5 In vitro release kinetics of amoxicillin from SPEEK beads over a period of 8 h

Table 3 Results for the cytotoxicity analysis for the SPEEK

	Morphological change	Reactivity	Total cytotoxic response	Score
Control	Nil	Nil	0–1	Pass
SPEEK	Negligible amount	Mild	1–3	Pass
SPEEK + nHA	Negligible amount	Mild	1–3	Pass

ALP activity

ALP activity of the sample without HA on the first week of incubation showed to be on the higher ratio compared to the second week of incubation which is related to the ageing leading to a decreased osteoblast activity. The ALP activity reaching the maximum coincides with the early osteoblastic differentiation stage whereas the decrease observed in the second week may be attributed due to various reasons. During all the stages of the assay, SPEEK samples showed a higher level of ALP activity than the control group that were exposed to the osteoblast cells. The increased expression of ALP during the first week of incubation with SPEEK beads speculates that they are conducive for the proliferation of osteoblast cells Fig. 7.

The age of mouse from which osteoblasts were isolated plays a role in the ALP activity. The results obtained suggested that the SPEEK beads used was biocompatible thereby allowing the proliferation of the cells followed by osteogenic differentiation. The addition of the HA to the polymer also might enhance the mineralized bone matrix formation on the surface of HA which was proved from the results obtained [17, 18]. The increased activity of ALP associated with SPEEK provides significant evidence that it serves as an optimum environment for application as scaffolds in tissue engineering.

Conclusion

In this study, attention has been focussed on the preparation of SPEEK beads for the drug delivery and tissue engineering applications. The beads were spherical in shape

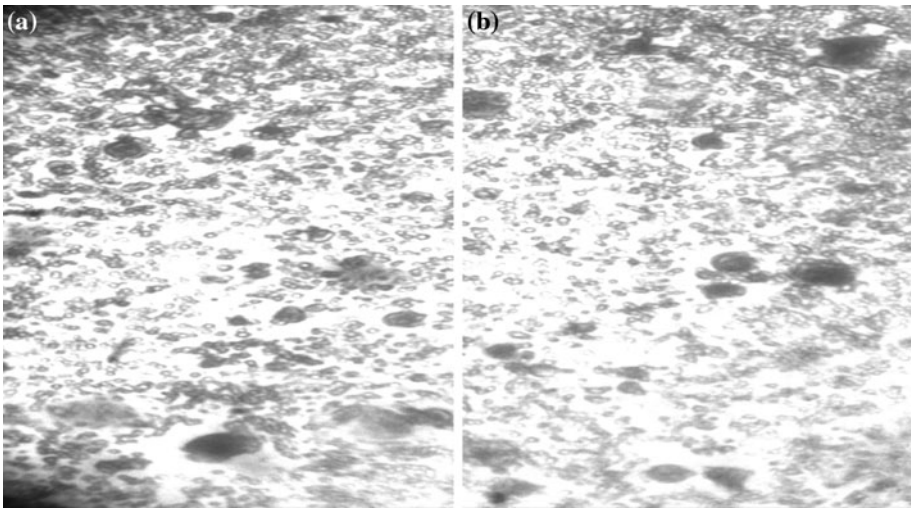

Fig. 6 Microscopic images of MC3T3-E1 osteoblast cells in the **a** control, **b** SPEEK

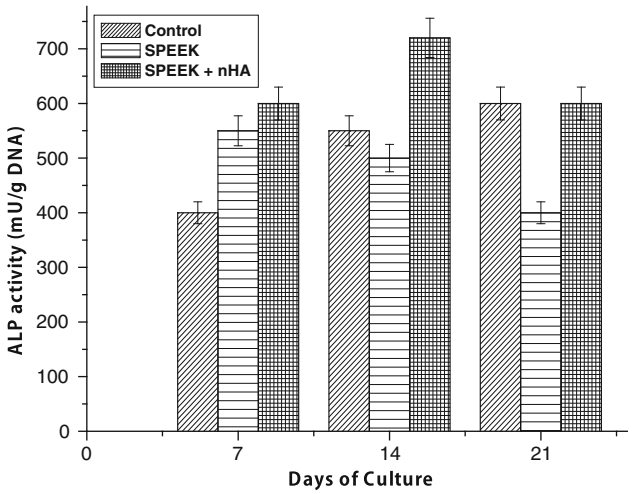

Fig. 7 ALP activity of MC3T3-E1 osteoblast cells after 21 days of culture on Control, SPEEK and SPEEK + nHA

and the prepared SPEEK beads showed very good bioactive behaviour thus proving that the biomaterial prepared could serve as a good tissue engineering model. The SPEEK beads were prepared successfully and loaded with the amoxicillin at various concentrations, thereby an effective loading of drug was observed. The release kinetics showed that the release was in a controlled fashion and the beads exhibited both the surface and diffusion based release for a period of 8 h. Moreover, the beads developed exhibited low toxicity in contact with the mouse osteoblast cells, implying good biocompatibility and safety. Thus, the controlled release profile, lower toxicity makes it a potential candidate towards the drug delivery and tissue engineering.

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