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Fabrication and *in vitro* evaluation of Sulphonated Polyether Ether Ketone/nano Hydroxyapatite composites as bone graft materials

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HIGHLIGHTS

- SPEEK/nHA composites were fabricated by phase inversion and electrospinning methods.
- Bead formation in the nanofibre mats increased with increase in the nHA concentration.
- The generation of triboelectricity was influenced by the concentration of nHA.
- The coefficient of friction and the wear rates decreased with increase in nHA content.
- *In vitro* studies revealed the cytocompatibility of the composites to MG-63 cell lines.

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ABSTRACT

Sulphonated Polyether Ether Ketone/nano Hydroxyapatite (SPEEK/nHA) composites were fabricated using two different techniques – phase inversion and electrospinning. The surface morphology of the composites examined using SEM revealed that the SPEEK/nHA membranes (obtained through phase inversion technique) was highly porous and the SPEEK/nHA nanofibre mats (obtained through electrospinning) showed increased tendency for bead formation with increase in the nHA concentration. The drug release studies performed using 5-Fluorouracil (5-FU) showed that the nanofibre mats had a better drug loading and release profile than the membranes. In addition, it was also noted that the nHA filler concentration influenced the drug release property. The *in vitro* bioactivity studies revealed that the propensity for mineralization of hydroxycarbonate apatite increased with increasing concentration of nHA in all the composites. The generation of triboelectricity was also observed to be influenced by the concentration of nHA which also translated into better *in vitro* bioactivity results. The wear properties analysed using pin-on-disc tribometer showed a decrease in μ as well as wear rates with increase in nHA content which was attributed to the preferential wearing out of the SPEEK matrix as much as due to the resistance to wear offered by the nHA particles. The *in vitro* cytotoxicity studies performed using MG-63 cells by MTT method revealed that all the composites possessed excellent cytocompatibilities which only increased marginally with increase in the nHA content. The studies showed that SPEEK/nHA composites could be considered as potential synthetic non-degradable bone graft materials which could find applications in both stress – bearing (membranes) and areas subject to minimal wear (nanofibre mats).

1. Introduction

Orthopedicians commonly encounter bone defects in their practise. The causes of these defects include injury, disease or bone surgeries such as removal of impacted teeth, tumorous growths and cysts. Other indications for bone grafts include gaps at fracture sites, comminuted fractures delayed unions and non-unions, corrective osteotomies and spinal fusion. Besides, limb lengthening procedures, enhancement of joint replacement prostheses and filling of the empty screws holes left after bone plate removal also necessitate the use of bone grafts [1]. These bone replacements also called as bone grafts, can be either natural bone (harvested either from humans or animals – autografts/xenografts) or other biomaterials (ceramics, polymers or their combination in various proportions – synthetic bone grafts).

To avoid the problems caused by xenografts and autografts such as donor site morbidity, risk of disease transmission, and immunogenicity, synthetic bone substitutes have been developed [2].

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Among the different synthetic polymers, biodegradable polymers remain the most investigated and are aimed at temporarily filling the bone defects which will eventually be replaced by bone [2,3]. However these are useful only in cases of small bony defects. The problems associated with these biodegradable implants include incomplete degradation and mismatch between the rate of polymer degradation and rate of new bone formation. Another problem relates to their poor mechanical properties [4]. Hence, in the case of larger defects, non-degradable graft materials are essential. Synthetic bioceramics such as bioactive glasses and calcium orthophosphates are the most suited for such applications. However, their application is limited on account of their poor mechanical and elastic properties [5].

Synthetic non degradable polymers such as polymethyl methacrylate (PMMA) and polyether ether ketone (PEEK) are being used for orthopaedic applications for a long time. PEEK is a rigid and brittle material and has favourable applications for load bearing applications [6]. However PEEK, due to its insolubility in solvents other than sulphuric acid, cannot be fabricated as a membrane which is suitable for application as bone grafts for bone defects. On other hand, sulphonated PEEK (SPEEK) is soluble in many solvents and can be fabricated as membranes or subject to electrospinning so as to fabricate SPEEK nanofibre mats. These SPEEK membranes can further be loaded with a drug such as an antibiotic or bone growth factors which would be essential to prevent infections at the site of surgical site and also enhance bone growth [3]. Our previous studies with SPEEK beads proved that SPEEK/nano hydroxyapatite composite was a suitable material for drug delivery and had good cytocompatibility [7].

In this study, SPEEK/nHA composites were fabricated by two methods – phase inversion and electrospinning. These composites were evaluated through different *in vitro* studies for their suitability as a synthetic bone graft material. Further the effect of triboelectricity on the *in vitro* bioactivity of these composites was also evaluated.

1.1. Triboelectricity and bone remodelling

It has been known for long that bone is piezoelectric which means that bone is capable of transforming mechanical forces into electrical signals. It has also been recognized that the development of piezoelectricity plays a vital role in bone remodelling. Bone is a dynamic tissue capable of self-renewal to facilitate growth, repair, reinforcement and resorption [8,9].

The piezoelectric behaviour of bone is not clearly understood. The deformation and subsequent separation of charge symmetry is thought to enhance the piezoelectric effect of the crystalline structure of bone. The factors affecting the piezoelectric sensitivity coefficient includes frequency, direction of load, and relative humidity [10]. In the bone, pressure gradients created due to mechanical stress are known to affect the flow of bone fluid [11]. The movement of the bone fluid, which is comprised of ions, results in the production of charges along the direction of flow, thus giving rise to an electrical current that is associated with a potential difference at two different bone sites [12]. This potential difference is known as stress-generated potential, SGP which functions as cell signals and influences the response of bone to mechanical loading [13,14]. Studies by Bur [15] showed that the piezoelectric coefficient of bone was 0.7 pC N^{-1}. In addition, it was also realized that the electric current generated by piezoelectric materials promote osteogenesis and regulates bone metabolism. Generally, an electrical potential is generated between the compressive and tensile sides of a dry bone when it is subjected to a shearing force as observed during certain orthopaedic procedures. The side of compression develops a negative charge and is associated with bone resorption and production. Further, it was also noted that the magnitude of the charge developed was determined by the angle of application of the load [16]. These experiments and observations strongly suggested that electrical charges experienced by bone influenced bone growth, repair and remodelling.

2. Materials and methods

Polyether ether ketone (PEEK) (M. Wt. 100 KDa) was procured from Victrex, India while nano hydroxyapatite (nHA) was purchased from Sigma–Aldrich, India. The solvents Dimethyl formamide (DMF) and *N*-Methyl Pyrollidone (NMP) were purchased from Sisco Research Laboratories PVT LTD, Mumbai, India. The nano hydroxyapatite powder used as received and had a particle size of less than 200 nm. The TEM image of the commercial nHA used in the present study was reported in our earlier paper [17].

2.1. Sulphonation of PEEK

The initial step towards the fabrication of SPEEK based composites was the sulphonation of the commercially procured PEEK using sulphuric acid according to the procedure described elsewhere [18]. In brief, about 10 g of PEEK powder was taken in a three-necked round bottom flask to which a calculated quantity of sulphuric acid was added. Sulphonation of PEEK was achieved by stirring the contents of the flask at room temperature under nitrogen atmosphere for a prescribed duration of time. Subsequently, the contents of the flask were poured slowly onto a large excess of crushed ice in a glass container. The SPEEK present in the sulphuric acid solution immediately precipitated as soft fibres and were recovered by filtration. The thus obtained SPEEK precipitates were washed several times with deionised water until the pH of the wash water reached neutrality. To ensure the complete removal of any residual sulphuric acid, the SPEEK precipitates were further kept in boiling water for one hour. They were then filtered and dried in an oven at 90 °C for about 10 h. The schematic representation of conversion of PEEK to SPEEK is shown in Fig. 1 below.

2.2. Fabrication of SPEEK/nHA composites by phase inversion technique

To fabricate SPEEK/nHA membranes by phase inversion technique, first a measured quantity of SPEEK was dissolved in DMF. Subsequently, calculated quantities of nHA (0, 2.5, 5, 7.5 and 10 wt %) were added to form the corresponding groups as listed in Table 1. The suspension was then ultrasonicated for 15 min to achieve uniform dispersion of the filler. The SPEEK/nHA solution was then

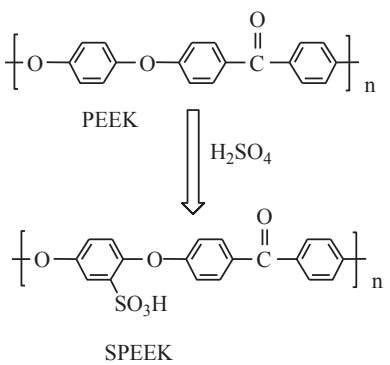

Fig. 1. Schematic representation showing the sulphonation of PEEK using sulphuric acid.

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