Osseointegration of Novel Porous Sulfonated Poly (aryl ether ketone) – In vivo study

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Statement of Purpose: Tissue engineering is a boon, especially to orthopedics and craniofacial reconstructions. Non resorbable bone biomaterials play a pivotal role in rebuilding the shape and maintaining the bone contours. Titanium has been used in such cases but due to certain disadvantages, polymeric materials are being sought. Though Poly ether ketone groups of polymers have been proved to be chemically stable and biocompatible, they are inert towards bone. Hence, through some ways chemical modifications have to be made to overcome this difficulty. To the best of the literature survey, sulphonated poly(Aryl ether ketones) especially porous forms, have not been reported for tissue engineering and reconstruction. This study investigates the biocompatibility of porous sulfonated poly (aryl ether ketone) in vitro and in vivo.

Methods: The Poly(Aryl Ether Ketone) PK was purchased from Gharda Chemicals, India and used as received. All other chemicals were of analytical grade. McCoy's 5A Medium and Alamar Blue were purchased from HIMEdia Labs (India). Fetal Bovine serum was purchased from Sigma Aldrich(India). Alkaline Phosphatase assay kit was purchased from Agappe diagnostics (India). The polymer sulphonation was carried out by constant stirring of 2.5% w/v solution of PK in concentrated sulfuric acid (36.76 N) at 50^{o}C for 3 – 4 hrs. Subsequently, immersion precipitation was carried out on clean petridish placed on crushed ice using ice cold double distilled water. The temperature of the precipitating system was maintained in the range of 2 – 4 ^{o}C, by frequent addition of ice cold water. The porous sulphonated polymer instantly separated (SPK). The same was analysed by FTIR, Elemental analysis, NMR spectroscopy, Thermo-gravimetry (TGA) and differential scanning calorimetry (DSC) techniques. The porosity and density were evaluated according to Zhang et al (1). In vitro mineralization was evaluated using simulated body fluid for 21 days according to Duan et al. (2), mineralization analysed in FTIR, XRD and SEM. Cell culture studies were conducted SaOS2 (Human Osteosarcoma) cell lines for 14 days. Viability was analysed on 2nd and 4th days, alkaline phosphatase activity was analysed on 4th and 7th days. Alizarin red staining and quantitation was done on 14th day. In vivo analysis was conducted by implantation of cylindrical samples (1mm dia x 10mm length) in the experimental femoral defects of male wistar rats (weighing 200-220 gms) for 8 weeks. After necropsy, the histological analysis was conducted by regular H&E staining and Masson's trichrome staining. The undecalcified ground section was observed in SEM to evaluate bone implant contact.

Results: While FTIR analysis suggested sulfonation by presence of peaks of 1,2,4 trisubstituted phenyl ring (857 cm^{-1}), NMR analysis confirmed the sulfonation by exhibiting a downfield shift sulfonated aromatic ring (7.50ppm) and elemental analysis revealed 50% sulfonation. Thermal analyses showed the stability of SPK till 250 ^{o}C (Loss of sulfur). The SPK samples were 88.7 ± 2.5% porous and their density was about 0.13 x 10^{-3} g/cc. The in vitro mineralization analyzed in FTIR, XRD and SEM showed deposition of apatite on the SPK. In-vitro analysis conducted on SaOS2 osteosarcoma cell lines showed more than 90% viability on 2nd and 4th days; The cells showed ALP activity similar to that of control wells, testifying to the biocompatibility. Alizarin red staining and quantitation was better on samples compared to control wells. In the in vivo studies, H&E and Masson Trichrome staining revealed deposition of mature bone in close intimacy to implant. SEM of the bone implant interface showed close proximity of bone to implant.

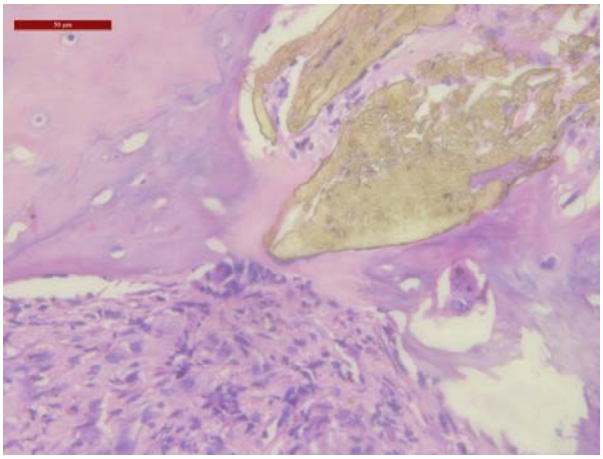

Figure 1. Bone implant interface - H&E Staining (Scale bar = 50 μm)

Conclusions: Novel porous SPK sponges have been successfully tested in vitro and in vivo. The analyses show that the material supports growth of cells on its surface. The in vivo studies revealed the close relation of bone to the implant after 8 weeks. Summarily, the results suggest that SPK is a promising candidate for reconstruction of bone in the non load bearing areas.

References:

1. Zhang Y. J Biomed Mater Res. 2001; 55:304–312.
2. Duan YR. J Mater Sci Mater Med. 2004;15:1205-1211.