Multifunctionality and electrical stimulation induced cellular response of Hydroxyapatite-Barium Titanate piezobiocomposite

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Statement of Purpose: Owing to the electrically active nature of living bone to regulate it's biochemical activities, the present study developed a new class of piezobiocomposite which potentially mimics the integrated dielectric constant, AC conductivity, piezoelectric strain coefficient, compressive strength and modulus values of bone. Further, two different aspects of the influence of electric field application towards stimulating the growth/proliferation of bone/connective tissue cells *in vitro*: (a) intermittent delivery of extremely low strength pulsed electrical stimulation (0.5-4 V/cm, 400 µs DC pulse) and (b) surface charge generated by electrical poling (10 kV/cm) of hydroxyapatite -$BaTiO_3$ piezobiocomposite have been demonstrated. In addition, the concern of potential toxicity of $BaTiO_3$ has also been addressed *in vivo* using mouse model.

Methods: The HA-xBT (x = 0-100 wt.%) composites were optimally processed using multistage spark plasma sintering (SPS) route and, the suitability of the proper composite in terms of mimicking the electrical (dielectric constant, AC conductivity, piezoelectric and pyroelectric coefficients) and mechanical properties (fracture toughness and compressive/flexural strengths) of bone was evaluated from the range of developed composites.

The external E-field mediated cellular response on optimal composites was performed using human osteogenic as well as mouse fibroblast L929 cells and the strength of E-field was optimized to get the maximum cell density on the composite samples. Further, the effect of optimal electric field on the cell viability and calcium (Ca) deposition by the cultured cells has been studied. The influence of surface charge on cellular behavior has also been studied. The charged state of the samples was assessed by measuring pyroelectric current. Further, to address the toxicity concerns by the HA-BT particulates, twenty BALB/c mice were intra-articularly injected at their right knee joints with different concentrations of HA-BT composite of upto 25 mg/ml.

Results: The Ultrafine grains (≤ 0.50 µm) of HA and BT phases were predominantly retained in the SPSed samples, which is rather difficult during conventional processing. The functional properties, such as AC conductivity, piezoelectric strain coefficient as well as compressive strength and modulus, measured with the optimal HA-40BT composite are 1.3×10^{-9} $(ohm\text{-}cm)^{-1}$, 0.9 pC/N, (138.3±17.0) MPa and (10.7±1.0) GPa, respectively and these values are close to that of the natural bone. The value of pyroelectric coefficient and the maximum polarization for HA-40BT composite are 2.40 $\mu C/m^2K$ and ~ 1 mC/m^2, respectively. Further, the *in vitro* results establish that the cell growth can be enhanced using the new culture protocol of the intermittent delivery of electrical pulses within a narrow range of stimulation parameters. The optimal E-field strength for enhanced cellular response for mouse fibroblast L929 and osteogenic cells is in the range of 0.5-1 V/cm. The MTT as well as alizarin red assays confirmed the significant increase in viability Ca-deposition on the E-field treated samples, implicating the positive impact of applied electrical pulses. The negatively charged surfaces of developed piezocomposite stimulated the cell growth in a statistically noticeable manner as compared to the uncharged or positively charged surfaces of similar composition. The histopathological examination confirmed the absence of any trace of injected particles or any sign of inflammatory reaction in the vital organs, such as heart, spleen, kidney and liver of tested mice at 7 days post-exposure period.Rather, the injected nanoparticulates were found to be agglomerated in the vicinity of the knee joint, surrounded by macrophages (Fig. 1). The serum biochemical analyses also complimented to the non-immunogenic response to injected particulates.

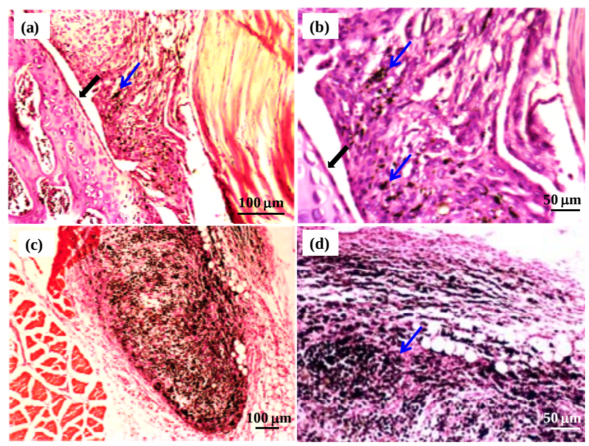

Fig.1: Histopathological features of mice knee joint sections, at 7 days post-injection with HA-40 wt.% $BaTiO_3$ particle eluates: (a) and (b) show regions of particle accumulation between the cartilage and skeletal muscle [(0.25 mg/ml)], (c) and (d) depict higher amount of particle accumulation in the fibroadipose tissue region in the vicinity of knee joint [(25 mg/ml)].

Conclusions: The above mentioned exceptional combination of functional properties potentially establishes hydroxyapatite-40 wt. % barium titanate piezocomposite as a new generation composite, enables this material to act as an electroactive biomaterial platform. This is the first proof that barium titanate, even in naoparticulate form does not cause any systemic toxicity in mouse model. Altogether, the absence of any inflammatory/adverse reaction during *in vivo* study opens up myriad of opportunities for barium titanate based piezoelectric implantable devices in biomedical applications.