Multiple Electrical stimulation through conductive PLLA/PPy/heprin scaffold promotes in vitro bone regeneration

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Developing effective bone regeneration therapies is one of the most clinically important long-term goals. Bone loss caused by trauma, neoplasia, reconstructive surgery, congenital defects, or periodontal disease is a major health problem worldwide. Indeed, close to 6 million fractures occur annually in the United States. Of these, 5 to 10% (0.3 to 0.6 million) fail to heal properly, due to non-union or delayed union[1]. Each human from berth to death may experience bone damage which, if not reversed, will result in significant health problems. Thus, there is a need for safe, effective methods to replace and promote bone regeneration. Autologous bone grafting is the safest treatment for bone loss. But it is limited by graft materials and generates additional morbidity of a second incision with the accompanying risks of postoperative pain, neurovascular injury, infection, and other complications. To overcome these limitations, bone allograft could be a solution, which however carries the risk of immune reactions. This immune response can have an adverse effect on the graft's incorporation and increase the incidence of rejection. Consequently, there has been an extensive amount of research into bone graft substitutes and biologic therapies to promote bone regeneration/replacement. Multiple strategies related to bone regeneration were indentified. These include osteogenic and angiogenic proteins such as growth factors (e.g. BMPs) that play important roles in fracture healing. Similar to biological stimuli (e.g., growth factors), electrical stimulation (ES) has the potential to be applied to tissue engineering and regenerative medicine[2]. As a physical cue, ES offers a rational and potentially highly efficient approach to regulate cellular functions. It has been shown that electrode-based ES are capable of modulating numerous important cellular activities[3]. Recently we showed that ES significantly upregulated the metabolic activity of human fibroblasts[4]. Thus the purpose of the present word was to investigate the osteogenic activity of the heparin (HE) activated electrically conductive polypyrrole/poly(D,L-lactide) (PPy/PDLLA) scaffold.

Methods: Biodegradable conductive scaffold was produced by blending 95 wt% non-conductive PLLA with 5 wt% conductive PPy doped by bioactor heparin. This was used to investigate in vitro bone regeneration. To do so, the scaffold was seeded with 7.5×10^5 osteoblasts and cultured for 24 h to allow cell adhesion and initial growth. At the end of this culture period cells were exposed or not to ES (200 mV/mm) for 6 h. Following exposure oateoblasts were cultured in bone mineralizing medium. Exposure to ES was repeated each 48h for three times. Following each ES exposure, culture medium was refreshed. After the last ES exposure, osteoblasts were cultured for an extra 1, 2 and 3 weeks with medium refreshing every day. At the end of each week, bone nodule formation was investigated by Hoechest and Alizarin Red S (ARS) staining. To confirm bone regeneration, SEM, EDX, XPS and wide angle X-ray diffraction analyses were performed. Finally bone biological markers including RUNX-2, ALP, OC, and BMP2 expressions were investigated using quantitative real-time RT-PCR.

Results: Our results demonstrated that multiple exposures to ES promoted osteoblasts growth basically at 3 and 4 weeks. The was ascertained by the high number of osteoblast aggregation (Fig. 1) and bone like-nodules with a nodule size reaching 0.03 mm. Bone formation was confirmed by ARS and calcium content analyses showing a mineral deposits formation starting at week 2 of culture, increasing over time and appearing much larger at week 4. The calcium in the mineral deposits was about 2.91 in the ES and 2.37 in the non-ES groups confirming the efficacy of ES on nodule formation. SEM analyses demonstrated nodules of more than 100 µm in size at 4 weeks in the ES group. Surface chemical element quantification showed high levels of calcium and phosphate on the surface of the mineral deposits following ES exposure as compared to non-ES stimulated cultures. Finally, gene expression analyses confirmed bone formation through an increase on bone markers (ALP, BMP2, Runx2, and OC) in ES stimulated osteoblast culture as compared to the controls.

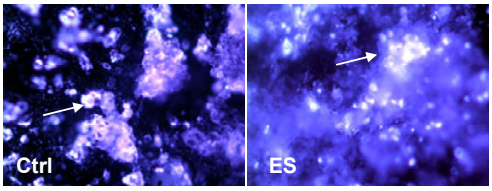

Fig.1: Nodule formation 4 weeks after ES. Hoechest staining showing greater cell aggregation (nodule formation, arrow) in the ES as compared to non-ES culture

Conclusions: Overall, these results demonstrated the ostegenic activity of ES. Therefore ES could be used together with other osteogenic mediators such as bone morphogenetic proteins to promote in vitro bone engineering for potential clinical applications.

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