Electrical modulation of bone cell activity through biodegradable heparin-doped conductive substrate

Shiyun Meng[1,2], Mahmoud Rouabhia[2], Ze Zhang[1]
[1] Faculté de médecine, Université Laval; Centre de recherche, Hôpital St-François d'Assise, CHUQ, Québec, Canada
[2] Faculté de médecine dentaire, Université Laval, Québec, Canada

Introduction: Biophysical stimuli are useful to improve bone formation through promoting cell growth and differentiation. For example, the electrical stimulation delivered by capacitive coupling showed that insulin-like growth factor and transforming growth factor-β1 mRNA increased [1]. Up to now, a group of proteins named as voltage-sensing proteins have been found to play fundamental roles in many cell functions through involving in synaptic transmission and regulating homeostasis in most cells [2]. In bone tissue engineering, the study of the sensitivity of bone cells to electrical potential stimulation (EPS) is fundamentally important to formulate effective EPS regimes to promote bone healing. In this study, biodegradable conductive membranes were used as substrates to mediate EPS. The objective is to demonstrate the sensitivity and modulation of bone cells to electrical stimulation using a biodegradable scaffolding material.

Methods: The conductive membranes were made of a polypyrrole/heparin/poly(L,L-lactide) composite material (PPy/HE/PLLA), in which 5% conducting PPy doped with heparin was blended with 95% PLLA matrix [3]. Home-made multi-well electrical stimulating cell culture plates were designed for cell culture under EPS. The new plates were designed in such a way that during EPS the electrodes were totally excluded from culture medium. Osteoblast-like cells (Saos-2) were cultured on the PPy/HE/PLLA membranes in a 3:1 mixture of Dulbecco-Vogt's Modified Eagle (DME) medium and Ham's F12 (H) supplemented with 24.3 µg/ml adenine, 10 µg/ml human epidermal growth factor, fungi 0.5µg/ml, 0.4 µg/ml hydrocortisone, 5 µg/ml bovine insulin, 2×10^{-9} M 3,3´,5´ and triiodo-L-thyronine, 100 U/ml penicillin, 25 µg/ml streptomycin and 10% fetal calf serum. The cultures were incubated in 5% CO_2 atmosphere at 37°C for 48h before being subjected to various EPS ranging from about 100-400 mV/mm. For each EPS condition, the electrical stimulation period lasted from 2 to 8 h. After electrical stimulation, the cells were permitted to recover for another 48h before harvesting. Sulforhodamine B (SRB) measurement was employed to study cell proliferation. The morphology of the SRB-stained cells was observed and photographed with an epifluorescence microscope. In control groups, cells followed the same protocol but without EPS. The concentrations of secreted alkaline phosphatase (ALP) and osteocalcin (OC) are analyzed by ELISA and RT-PCRs

Results/Discussion: An optimal EPS condition was identified, under which cell proliferation was 1.2 to 1.4 fold of its control group (Fig. 1). At EPS stronger than this optimal condition, the cell proliferation value decreased to 60% of its corresponding control group. At other two weaker EPS conditions, there were no significant difference between the EPS and control groups. The SRB-stained cells showed that all the four ESP conditions supported the cells growth, but more cells could be observed under the optimal condition after 6 and 8h EPS. The ALP value of the EPS groups at the favorable conditions was higher than that of the control groups. The OC expression was also modulated by EPS. Through this study we showed that bone cells responded to EPS, of which positive modulation only happened under specific experimental conditions (intensity and stimulation period). Beyond those conditions, there was either no modulation to cell activities, or negative modulation occurred.

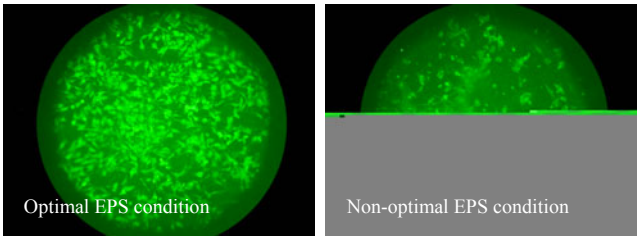

Fig.1 Fluorescent photomicrographs of Saos-2 cells following different EPS treatment.

Conclusion: This study demonstrated that bone cells like Saos-2 are responsive to EPS. Positive modulation of cell growth and secretion of certain important proteins happened only under specific experimental conditions, beyond which inhibition may occur. These findings suggest that the heparin activated PPy/PLLA composite material used in this study may be useful for bone tissue engineering as scaffolding materials. (This study was financially supported by CHIR and NSERC.)

References:

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