INTRODUCTION:
Bone morphogenetic proteins (BMPs) are potent soluble factors that play key roles in bone morphogenesis and in the early stages of fracture repair. They are known to act through recruitment of pluripotent cells and to influence their localization and induce their differentiation into osteogenic cells [1]. However, short in vivo half-life, rapid dispersal from site of administration and perceptible immunogenicity of non-autologous sourced BMPs remain major drawbacks to their further clinical applications [2]. Sustained release of BMPs from cells protected against host immune system in polymer microcapsules has been postulated as an alternative strategy to induce new bone formation. The objective of our study was to evaluate the local osteoinductive effect of encapsulated cells releasing multiple BMPs over a prolonged period of time. In this study the semi-permeable alginate-Poly-L-lysine (PLL)-alginate microcapsule, which has been overwhelmingly explored as an effective method of immunoprotecting non-autologous cells, was selected as the encapsulating material. We encapsulated the Saos-2 human osteoblast cells, known to secrete an array of BMPs [3], and injected them into the skeletal muscles of C57 mice to evaluate the ectopic bone formation effect of the encapsulated cells.

METHODS:
A total of 30 female C57BL/6 mice (C57) aged 9 weeks and above were randomly assigned to two equal groups of treatment (encapsulated Saos-2) and control (direct Saos-2) intramuscular injection. Four (4) animals in each group received double sequential fluorochrome labeling. Five (5) animals per group were sacrificed at 3, 6 and 15 weeks post injection. Histology, radiology, polyfluorochrome sequential labeling and immunohistochemistry of collagen 1 and BMP-2/-4 were done to evaluate osteogenesis.

RESULTS:
Routine histology (H&E, toluidine blue, safranin O and von Kossa) reveals membranous condensation of mesenchymal tissue around the microcapsules as early as 3 weeks (Fig. 1). Mineralization of the newly formed skeletal structures was detectable by day 21 from calcein green and tetracycline incorporation and immunohistochemical staining of collagen 1. X-ray observation of increasing radio-opacities was evident by week 9. Distinct primitive bone trabeculae (primary spongiosa) with osteocytes in newly forming lacunae and basophilic osteoid matrix containing ossification centers were found at week 15 (Fig. 2). von Kossa staining demonstrated calcified rings of cortical bone in the surrounding of the injected microcapsules. Immunoreactivity of BMP-2/-4 antibodies was continuously detected at the vicinity of microcapsule in all treatment animal samples even at 15 weeks (Fig. 3). In contrast there was no evidence of bone formation in control animals with direct Saos-2 cells injection (figure not shown here).

DISCUSSION:
In this study, intramembranous style ossification (Fig.1) was observed, consistent with high dose BMP administration [4]. Typical de novo new bone features such as bone spicules that matured into a web of branching primitive bone trabecules were also observed (Fig.2). These features, which are typical of neonatal intramembranous bone formation, are abnormal in matured animals except in malignancy or as in this case, induced osteogenesis with high dose BMPs [4]. This study is the first to provide experimental evidence of new bone formation by encapsulated living Saos-2, and to establish the feasibility of local, multiple growth factor delivery from encapsulated non-autologous cells without the need for immunosuppression. Persistent multiple BMP expression (fig. 3) could open up a potential to mimic the route and timing of growth factors release as has been advocated by many scientist to enhance the use of these agents in a wider clinical setting. This promising result prove that osteogenic cell microencapsulation potentially have important applications in bone tissue engineering for clinical purpose including fracture nonunion, segmental bone defect, spinal fusion, osteoporosis fractures and other orthopedic disorders.

REFERENCES:
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POLYMER ENCAPSULATED HUMAN OSTEOBLASTIC CELLS FORM ECTOPIC BONE IN MICE

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Figure 1 H&E week 3: Mesenchymal condensations into osteoid matrix (*) with newly forming ossification centers (yellow circle).

Figure 2 H&E week 15: Primitive bone trabeculae (B) formed between microcapsules (M) and basophilic osteoid matrix (*). Note an ossification center (yellow circle).

Figure 3 BMP-2/-4 immunostaining week 15: Expression of BMPs in the bone trabeculae (hematoxylin, counterstain).