Synthetic Matrix Vesicles Modulate Mineralization Response of Human Osteoblast-like Cells

Philip J Chuang, Tolulope O. Akinade, Lance C. Kam, Helen H. Lu
Department of Biomedical Engineering, Columbia University, New York, NY

Statement of Purpose: Bone is the most commonly replaced organ with nearly 1 million grafting procedures performed annually in the U.S.[1,2]. Biomineralization is often facilitated by matrix vesicles, a lipid-based nanocarrier within which Ca^{2+} and PO_4^{3-} are combined to form calcium phosphate in tissues such as bone[3]. Consequently, ion containing liposomes have been used to model the calcification process[4-9]. The **objective** of this study is to test the potential of synthetic matrix vesicles to facilitate cell-mediated mineralization. Specifically, effects of PO_4^{3-} (Pi) containing liposomes on the response of human osteoblast-like cells will be evaluated over time as a function of liposome dose. It is **hypothesized** that the addition of Pi-encapsulated liposomes will enhance mineralization and that a higher liposome dosage will further increase mineral deposition. It is anticipated that this bottom-up approach to mineralization has the potential to guide cell-mediated bone formation.

Methods: *Liposome Synthesis:* Liposomes were formed by combining phospholipids 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) at a ratio of 90:10 (w/w) in 0.2M NaH_2PO_4[10]. Vesicles were extruded through a 200nm membrane and liposome size and morphology were assessed by cryo-electron microscopy (n=2). Pi concentration was quantified (n=5) using a colorimetric assay (BioVision). *Cells/Cell Culture:* Study groups include human osteoblast-like cells (Saos-2, ATCC) cultured with Pi liposomes at low (Pi) and high (Pi-H) doses, with monolayer cultures without liposomes (M) or ion-free liposomes (Lip) as controls. Cells were seeded at a density of 50,000 cells/cm^2, with liposomes added at day 1. All cells were maintained in fully supplemented media, and in half of the cultures, with β-glycerophosphate (β-GP, 3mM) added on and after day 7. *End-point Analyses:* Cell viability (n=2) was evaluated using Live/Dead assay (Invitrogen) and total DNA (n=5) was detected by the Picogreen dsDNA assay (Molecular Probes). Alkaline phosphatase (ALP) activity (n=5) was detected by a colorimetric assay, while von Kossa and picrosirius red were used to stain for mineral and collagen distribution respectively (n=2). *Statistical Analysis:* Multi-way ANOVA and Tukey-HSD post-hoc test were performed for all pair-wise comparisons ($p<0.05$).

Results: *Liposome Characterization:* Pi liposomes have an average diameter of 215±6nm with Pi detected within the liposomes (Fig. 1). *Cell Growth:* Pi-H groups without β-GP saw an increase in cell number by day 14, whereas those treated with β-GP showed no significant increase in cell number over 21 days (*$p<0.05$; Fig. 2-3). Cell number was significantly higher for Pi over all groups starting at day 7 (^$p<0.05$). Cells remained viable in all groups through day 21 (Fig. 4). *Matrix/Mineralization:* ALP activity was found to increase by day 14 in all control groups and by day 21, for Pi without β-GP (*$p<0.05$; Fig. 2-3). On day 21, all liposome groups except for Lip without β-GP had lower ALP activity than the monolayer controls (^$p<0.05$). In addition the Lip groups showed higher ALP activity over all Pi groups, with Pi-H exhibited the lowest activity (^$p<0.05$). A collagen-rich matrix was found to be deposited by cells in all groups (Fig. 4). Cell cultures stained positive for bulk mineral on day 21 for all groups supplemented with β-GP with a higher apparent stain in Pi-H over Pi (Fig. 4).

Discussion: In this study, it was found that the Pi liposomes enhanced cell proliferation and modulated ALP activity on day 21 both in the presence and absence of β-GP. However, the proliferative effect is lost at higher dose. This decrease in ALP activity was previously observed and is attributed to the Pi liposomes providing a biomimetic source of ions for mineralization reducing the need for ALP production by the cells[11]. The further reduction of ALP activity from the higher dose of Pi liposomes is explained by the additional Pi ions released. Higher qualitative mineral production was observed for the higher dose Pi liposomes in the presence of β-GP, and is also attributed to the increase in available Pi for mineralization. These results demonstrate that liposome-based ion carriers represent a promising system for modulating mineralization response. Future studies will focus on optimizing this system for cell-mediated biomineralization.

References: [1]Giannoudis *et al.*, 2005. [2]Langer *et al.*, 1993. [3]Mann *et al.*, 2001. [4]Boskey *et al.*, 1997. [5]Blandford *et al.*, 2002. [6]Camolezi *et al.*, 2002. [7]Eanes *et al.*, 1992. [8]Ierardi *et al.*, 2002. [9]Michel *et al.*, 2004. [10]Murphy *et al.*, 2000. [11]Chuang *et al.*, 2012.

ACKNOWLEDGEMENT: NIH-NIAMS(AR0550280, AR056469); NSF GK-12 LEEFS (PJC), NYSBC

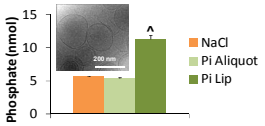

Figure 1: *Cryo-EM(left) and Pi concentration(right; ^$p<0.05$)*

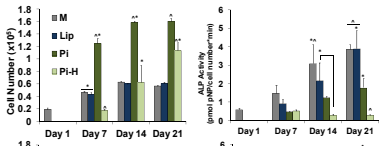

Figure 2: *Cell proliferation(left) and ALP activity(right; *,^$p<0.05$) for monolayers without β-GP*

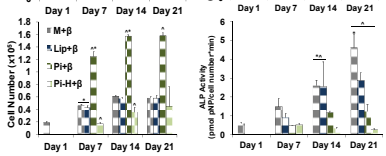

Figure 3: *Cell proliferation(left) and ALP activity(right; *,^$p<0.05$) for monolayers with β-GP added on day 7*

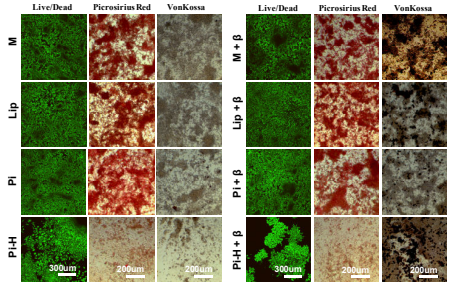

Figure 4: *Day 21 Live/Dead, Picrosirius Red and VonKossa stain of monolayers*