

## Ion channel agonist release scaffolds for bone tissue engineering.

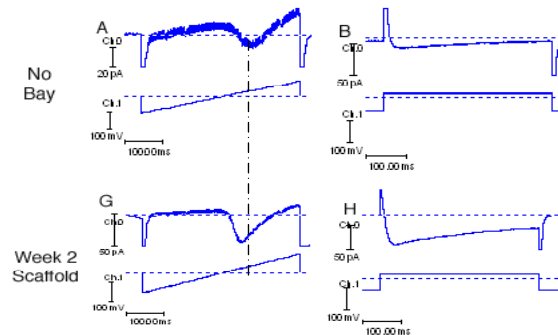
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**INTRODUCTION:** Calcium influx through L-type VOCC ion channels is a key event in the transduction of mechanical stimuli by bone cells (1). The calcium channel agonist Bay K8644 has been shown to increase levels of mechanically induced bone matrix production (2), and thus manipulation of VOCC channels represents a potentially potent tool for tissue engineering. We have previously developed a Bay-encapsulated scaffold, which results in the up-regulation of bone matrix proteins in bone cell seeded constructs (3). In this study we use whole-cell electrophysiology to characterise the effects of 'scaffold released Bay' on L-type VOCC currents recorded from osteoblasts. Furthermore we also report the development of scaffolds incorporating a different class of L-type VOCC agonist, namely FPL.

**METHODS:** Collagen coated Bay-encapsulated PLLA scaffolds were produced as described previously (3). Scaffolds (n=4) were cultured in 1ml ddH<sub>2</sub>O under cell culture conditions for 28 days. ddH<sub>2</sub>O was collected and replaced weekly and the Bay concentration of individual samples determined via UV spectroscopy. Whole cell patch clamp electrophysiology of L-type VOCC currents recorded from ROS 17/2.8 cells was used as a means to assess the functionality of the released agonist. For isolation of calcium currents, barium was used as the charge carrier. Cells were bathed in either BaCl saline (108 mM BaCl<sub>2</sub> and 10 mM HEPES, corrected to pH 7.6 with NaOH) or BaCl saline containing 500nM, 1µM or 10µM Bay. Alternatively, cells were bathed in saline containing Bay released from scaffolds. Similar methods were also used to develop and evaluate FPL-encapsulated scaffolds.

**RESULTS:** For all scaffold salines, Bay concentrations remained within the physiological range required for agonist activity (1-10 µM) during 28 days in culture. L-type currents recorded in scaffold release salines showed characteristic differences when compared to recordings performed in the absence of Bay. These shifts are characteristic of the effects of Bay on L-type currents and were indistinguishable from currents recorded in the presence of known concentrations of Bay.



*Fig 1 – L-type VOCC currents recorded in normal saline (top) and week 2 'Bay scaffold release saline' (bottom).*

Whole cell recordings were also used to demonstrate the effects of FPL on osteoblast L-type currents and confirm the stability of this compound under cell culture conditions and following release from PLLA scaffolds. The effect of FPL on osteoblast proliferation, LDH production and apoptosis demonstrates that this compound is well tolerated by osteoblasts and represents another potential tool for augmenting bone matrix production in tissue engineered constructs.

**DISCUSSION & CONCLUSIONS:** In summary this work demonstrates that calcium channel agonists can be successfully incorporated and subsequently released from 3-D scaffolds without loss of functional activity. These findings confirm conclusions of our previous work suggesting that the enhancement of bone matrix production following mechanical conditioning of cells in Bay-encapsulated PLLA scaffolds is due to augmentation of L-type channel activity (3).

**REFERENCES:** El Haj et al, *Med Biol Eng Comp*, 37, 403-409, 1999; 2) Walker et al, *J Cell Biochem*, 79(4), 648-661, 2000; 3) Wood et al, *J Control Release*, 112(1), 96-102 2005.

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