Synergistic effects of statins as a potential strategy for local osteoporotic bone repair

Christopher R. Simpson[1], Silvia Paiva[1,2], Helena M. Kelly[1,3], Ciara M. Murphy[1,2]

[1]Tissue Engineering Research Group, RCSI, Dublin, Ireland, [2]Advanced Materials and Bioengineering Research (AMBER) Centre, Trinity College Dublin, Dublin, Ireland, [3]School of Pharmacy & Biomolecular Sciences, Royal College of Surgeons

Background: Bone remodelling relies on balanced osteoblast-driven bone formation and osteoclast-driven bone resorption. Osteoporosis is characterised by depleted bone mass caused by bone resorption outweighing formation, causing fractures worldwide at a rate of 1 every 3 seconds. Currently, licensed osteoporosis therapies such as anabolics (Teriparatide) or anti-catabolics (bisphosphonates) only target one side of impaired bone remodeling to reduce fracture risk but, do not repair or restore bone structure or function. Statins have recently been shown to target the same osteoclast-inhibitory pathway as bisphosphonates but exhibit an anabolic effect on osteoblasts. This study aims to compare two licensed statins (simvastatin-SVT and atorvastatin-ATV) to determine their effect on bone cell activity, with the view to functionalize our previously developed thermoresponsive hydrogels, as a statin releasing therapeutic platform for osteoporotic bone repair.

Methods: **Osteogenic culture:** Briefly, rMSCs were treated with osteogenic media consisting of 10mM β-glycerophosphate, 50μm ascorbic acid, 10nM dexamethasone with or without ATV and SVT (10-1000nM). At days 7, 14, 21 and 28, cultured monolayers assessed for calcium by Alizarin staining or quantified using a calcium colorimetric assay (StanBio). Early relative osteogenic gene expression measured by PCR.

Osteoclastogenic Inhibition: Pre-osteoclast cells (RAW264.7) were stimulated with RANK-L and treated with ATV 1000 & 100nM or SVT 500 & 100nM. At days 1 and 7, tartrate resistant acid phosphatase (TRAP) activity was quantified by colorimetric measurement of phosphatase enzyme conversion of pNPP in the presence of tartaric acid. Fixed cells were stained with DAPI and Phalloidin, imaged and multinucleated cells counted. Relative osteoclastogenic gene expression measured by PCR.

Controlled Release: SVT was loaded into a thermoresponsive hydrogel (methylcellulsoe 2.5% w/w, 0.1% w/w collagen, 0.1 or 0.2% w/w hydroxyapatite) yielding a final concentration 1mg/ml. Controlled release was assessed by UV quantification of PBS supernatant when hydrogel was agitated at 37°C over 28 days.

Results: Osteogenic differentiation of rMSC increased in a dose dependent manner when treated with SVT and ATV. Both treatments, at concentrations of 1000nM, yielded a significant increase in calcium production after 21 and 28 days. Moreover early markers of osteogenic differentiation ALP and RunX2 were observed to be further upregulated in statin treatment groups at day 14. With respect to anti-osteoclastogenic activity, RAW 267.4 cell treatments of up to ATV 1000nM and SVT 500nM showed significant decreases in quantified and stained tartrate resistant acid phosphatase (TRAP). Further, observation of multinucleated and fused RAW cells was reduced when treated with statins. Controlled release from a thermoresponsive methylcellulose hydrogel showw SVT undergoes an initial burst of release followed by a slower, sustained rate of release over 28 days.

Conclusions: These results indicate that statins possess a unique capacity to synergistically enhance bone formation and inhibit osteoclast activity. Moreover, sustained release of SVT was demonstrated. Collectively, there is potential for an innovative single therapeutic strategy to target impaired bone remodeling.