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Signal transduction pathways controlling the induction of bone formation by macroporous biomimetic matrices

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ABSTRACT

In spite of vigorous research efforts to date the induction of bone formation by macroporous coral-derived constructs when implanted heterotopically in the *rectus abdominis* muscle of the non-human primate Chacma baboon *Papio ursinus* has not yet been resolved and needs to be assigned. More importantly, the apparent redundancy of molecular signals singly initiating the induction of bone formation in primate species and the heterotopic induction of endochondral bone formation by the mammalian recombinant human transforming growth factor –β_3 (rhTGF-β_3) isoform have not yet been assigned and need to be mechanistically resolved. Using the r*ectus abdominis* muscle of *Papio ursinus* the study sought to molecularly determine how coral-derived macroporous constructs and doses of the hTGF-β_3 isoform initiate the induction of bone formation. To elucidate the function of osteoclastogenesis and Ca^{2+}, biomimetic coral-derived 7% hydroxyapatite/calcium carbonate (7% HA/CC) devices were supplemented either with 240 μg zoledronate bisphosphonate, an osteoclast binding antagonist, or 500 μg of the calcium channel blocker verapamil hydrochloride. Additionally but in separate coral-derived bioreactors, 125 μg rhTGF-β_3 and/or 125 μg hNoggin were added to answer the question of how TGF-β_3 induces bone formation. All devices were then subsequently implanted within heterotopic sites of the *rectus abdominis* muscle of 6 *Papio ursinus* and left *in vivo* for 15, 60 and 90 days. Harvested specimens were subjected to histomorphometrical and quantitative reverse transcription polymerase chain reaction (qRT-PCR) analysis. *Collagen Type IV* expression supported by extensive vascularisation was detected and observed respectively in all implants after 15 days *in vivo*. Importantly the zoledronate treated specimens possessed delayed tissue patterning and morphogenesis, an observation not as pronounced as within the verapamil hydrochloride treated specimens. However,

both treatment modalities showed a lack or minimal bone formation. The gene expression experiments revealed that *Noggin* was elevated whereas *bone morphogenetic protein-2 (BMP-2)* was greatly downregulated in the verapamil or zoledronate treated devices, indicating that bone formation by macroporous coral-derived devices is through the BMP-pathway. Therefore Ca^{2+} release may be the driving force behind the bone induction mechanism by both regulating stem cell differentiation and the BMP-pathway.

In 7% HA/CC constructs preloaded with 125ug hNoggin there was downregulation of *BMP-2* and lack of *TGF-β_3* activity. Minimal bone formation characterised the hNoggin treated bioreactor. The hTGF-β_3/hNoggin treated devices exhibited *BMP-2* upregulation on day 90 and significant *Noggin* downregulation on day 60 and 90. Downregulation of *Noggin* corresponded to limited bone formation at the periphery of the device at day 90. In the TGF-β_3 loaded 7% HA/CC constructs there was significant downregulation of *BMP-2* expression on day 15, followed by upregulation on day 60 and 90. *BMP-2* upregulation was accompanied by the concomitant upregulation of *RUNX-2* and *Osteocalcin*, along with prominent induction of bone formation. These results unequivocally show that in the *rectus abdominis* muscle of *Papio ursinus* TGF-β_3 induces bone formation by upregulating BMP expression *via* Noggin transcription, together with controlling the differentiation of a progenitor stem cell niche to enhance osteoblastic cell differentiation and proliferation.