

Engineering the Induction of Bone Formation with Transforming Growth Factor- β_3 and Responding Myoblastic Stem Cells

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INTRODUCTION: In a number of systematic studies in the different microenvironments of heterotopic and orthotopic craniofacial sites including the *rectus abdominis* muscle, the calvarium and the mandible respectively, our Unit has shown that primate tissues and microenvironments respond remarkably differently when compared to rodents, lagomorphs and canine tissues at identical doses of osteogenic proteins of the TGF- β superfamily [1]. In the non-human primate *Papio ursinus* and possibly thus by extension to the primate *Homo sapiens*, the mammalian TGF- β_1 and - β_2 isoforms do induce endochondral bone formation in heterotopic sites of the *rectus abdominis* muscle [1]. The three critical requirements for successful tissue engineering are suitable biomimetic substrata, soluble molecular signals and responding stem cells [2]. This communication describes the induction of bone formation by the mammalian transforming growth factor- β_3 (TGF- β_3) isoform and its enhancement by autogenous *rectus abdominis* myoblastic stem cells when implanted in orthotopic calvarial defects of the non-human primate *Papio ursinus*.

METHODS: Calvarial defects 25 mm in diameter were implanted with 25, 125 and 250 μ g hTGF- β_3 combined with insoluble collagenous bone matrix or macroporous sintered hydroxyapatites. 5, 25, 125 and 250 μ g hTGF- β_3 were implanted in the *rectus abdominis* muscle. Harvested specimens were subjected to molecular and morphometrical analyses including reverse transcription polymerase chain reaction (RT-PCR).

RESULTS: Heterotopic implantation of doses of hTGF- β_3 in the *rectus abdominis* muscle resulted in substantial induction of endochondral bone formation. In the same animals, the delivery of identical or higher doses of hTGF- β_3 resulted in minimal repair of calvarial defects with limited and pericranial bone regeneration only on day 90. RT-PCR of orthotopic calvarial specimens showed over-expression of *Smad-6* and *Smad-7* mRNAs

Restoration of the bone induction cascade was obtained by combining hTGF- β_3 with minced fragments of autogenous *rectus abdominis* muscle thus adding responding myoblastic stem cells for further bone induction by hTGF- β_3 .

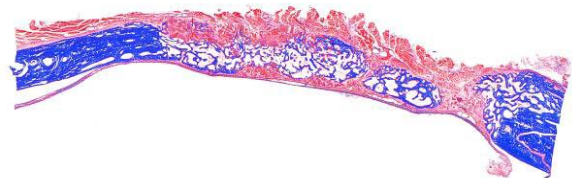


Fig. 1: Myoblastic stem cells within morcellized fragments of *rectus abdominis* muscle restore the induction of bone formation by the hTGF- β_3 osteogenic devices.

DISCUSSION & CONCLUSIONS: The limited induction of bone in calvarial defects is due to overexpression of *Smad-6* and -*7* downstream antagonists of the TGF- β signalling pathway. We have shown that an important source and/or *niche* of stem cells reside in the *rectus abdominis* muscle that can be transplanted in orthotopic calvarial and periodontal furcation defects [3]. Morcellized fragments of *rectus abdominis* include myoblastic and myoendothelial cells [4] to further enhance the osteogenic activity of the hTGF- β_3 isoform. The temporal and spatial expression of TGF- β_1 mRNA has indicated a specific temporal window during which expression of TGF- β_1 is mandatory for successful and optimal osteogenesis.

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