Effect of Age on Bone Graft Integration and Resorption in a Rabbit Tibial Defect Model

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Introduction: Bone graft substitutes are used widely in orthopaedic surgery to facilitate bony defect healing. Rabbits are commonly used for preclinical studies on bone graft substitutes. Twelve week old rabbits are commonly used given the greater expense and often logistical aspects of using older animals. Age however is an important factor in bone healing. Our aim was thus to compare the rate of defect healing and bone graft resorption of a well studied bone graft substitute, β-tricalcium phosphate (β-TCP), in younger versus older rabbits in an established tibial defect model (1,2).

We hypothesized that the rate of healing and bone graft resorption would be slower in older compared to younger rabbits making the difference between implant and control groups more significant. This may provide a more critical comparison of bone graft substitutes as well as potentially enabling studies to be carried out on smaller number of animals.

Materials and Methods: Thirty female New Zealand White rabbits were used following ethical approval. Three cohorts were studied: 3 month-old (n = 10), 12 month-old (n = 10) and 24 month-old (n = 10) rabbits. A bilateral tibial defect (15x5mm) was created in the anteromedial cortex(1,2). The defect on one side was then filled with β-TCP (Oseferion, Olympus Biomaterials), and the contralateral side was left empty as control. The periosteum and the skin were closed in layers.

Animals were killed at 4 and 12 weeks following surgery. The endpoints included X-rays and histology (1,2). The tibias were Faxitroned in the AP and lateral planes and graded semi-quantitatively for healing and implant resorption. The tibias were fixed in buffered formalin, decalcified in 10% formic acid/formalin and embedded in paraffin. Five micron sections were cut and stained with H&E and trichrome. Sections were analyzed under light microscopy and graded for percentages of new bone formation, remaining implant materials, cortex remodeling and soft tissue voids at the defect site.

Results: Faxitrons revealed the rate of new bone formation and bone graft resorption were reduced with increasing animal age. Closure of the anteromedial cortex was not achieved in empty defects and was retarded with increasing age (IMAGE 1). Defects filled will the β-TCP performed well in the younger (12 week) compared to the older rabbits (12 and 24 months) in terms of new bone formation and cortical bridging. A complete cortex was however not achieved in the older animals at 12 weeks following surgery.

Histologically, the empty defects had fibrous and fatty tissue at 4 weeks following surgery. Healing progressed with time, especially in the younger animals but had yet to reconstitute a remodeled cortex by 12 weeks. Overall, healing was slower in the older animals at 12 weeks compared to the younger animals (IMAGE 2).

The β-TCP had a positive influence of new bone formation within the defect sites for all animals. The percentage of new bone was significantly higher in the younger rabbits. Both bone formation and bone graft resorption appeared decreased in older animals at 4 weeks. Cortical healing was improved by 12 weeks in all animals. The older animals however lagged behind the younger in all aspects of healing with the β-TCP. Residual β-TCP was present in the 24 month animals at 12 weeks but appeared to be completely resorbed in the 12 week and 12 month animals (IMAGE 3).

Discussion: Preclinical animal models can provide great insight into the in vivo performance and biology of bone graft substitutes. These models however cannot completely replicate the human scenario and have limitations. The current study explored the influence of age on the healing of a cortical window defect placed into the anteromedial aspect of rabbit tibias in 3, 12 and 24 month old animals. Empty anteromedial tibial defects did not heal by 12 weeks in rabbits 12 months of age or older. The β-TCP used in the current study displayed strong osteoconductive properties even in animals 24 months old. Older rabbits demonstrated a significant reduction of new bone formation as well as bone graft resorption and may provide a more robust animal model for the future to differentiate bone graft materials in preclinical studies.

References: