

## Bone Marrow Regeneration following Tibial Marrow Ablation in Rats is Age Dependent

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**Statement of Purpose:** Injuries to the marrow cavity result in rapid endosteal bone formation followed by bone remodeling and regeneration of the marrow (1). It is not known whether this process is affected by age, although the quality of marrow is markedly different in young and old animals (2). Whereas young animals have red marrow and comparatively high levels of mesenchymal stem cells, old animals have yellow marrow characterized by increased levels of fat cells and they have fewer mesenchymal stem cells. To test if marrow restoration differs as a function of age, we used the rat tibial bone marrow ablation model (1), which has been used to examine calcification during osteogenesis (3), effects of metal implants on osteointegration (5) and remodeling of bone graft substitutes during marrow cavity restoration (6). These previous studies were conducted in 3-month old immunocompetent rats but analysis of many biomaterials requires the use of immune deficient animals; however, it is not known whether this will affect the healing process. Accordingly, we assessed bone marrow healing in nude rats aged 3-months and 10-months using micro-CT and histomorphometry, and compared the results to our previous work using Sabra strain rats. Thus, we determined if restoration of bone marrow is age dependent; if differences in healing can be detected by micro-CT; if the quality of marrow differs in young and old rats; and if the time course of healing in 3-month immunocompromised animals is comparable to that seen in normal rats of the same age.

**Methods:** Marrow was ablated in the left tibia of seven rats (rNu/rNu) per time point. At 0, 7, 14, 21, 28, 35 and 42 days post-surgery, the treated tibia and the contralateral tibia were harvested, fixed in 70% ethanol for 24 h and post-fixed in buffered formalin. Both tibias were scanned using microCT and trabecular BV/TV calculated. Mid-sagittal sections of decalcified paraffin embedded bones were stained with haematoxylin and eosin. BV/TV was calculated using ImagePro. Left tibias from untreated animals were used as controls for histology.

**Results:** Micro-CT analysis showed an increase in bone formation on days 7 and 14 in 3-month animals and by day 21, remodeling had reduced the area of trabecular bone by 50%. 10-month animals had less trabecular bone at days 7 and 14, levels were sustained through 21 days. Histomorphometry indicated that peak bone

formation was at day 7 in 3-month rats with remodeling underway by day 14, as noted previously for Sabra strain rats. However, in 10 month rats, peak bone formation was at day 14, with remodeling at day 21.

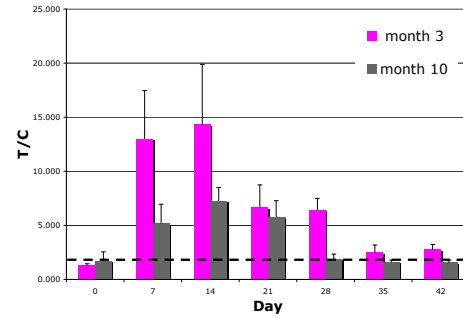


Figure 1: **MicroCT**; effect of age on trabecular BV/TV of the rat tibial marrow canal. Data are means  $\pm$  SEM, N=7, and are normalized against the BV/TV of the contralateral tibia.

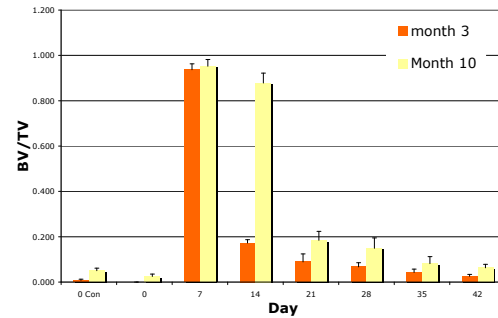


Figure 2: **Histomorphometry**; effect of age on trabecular BV/TV following bone marrow ablation. Data are means  $\pm$  SEM, N=7.

**Conclusion:** Endosteal bone formation and remodeling in 3-month nude rats is comparable to 3-month immunocompetent rats. Aged animals produced less primary bone that younger animals and remodeling was initiated later. Differences in micro-CT and histomorphometric analyses may reflect a reduction in calcification of the osteoid in the 10-month old animals.

### References:

1. Foldes et al. J Bone Miner Res. 1989; 4:643-6
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