Exploring the Potential of Collagen Scaffolds in Calvarial Bone Regeneration

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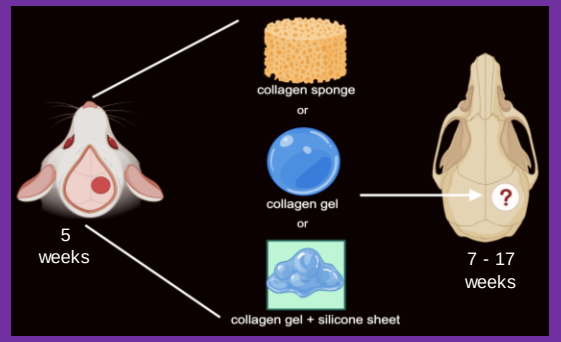
Background

Autologous bone grafts are widely used to repair bone defects such as craniofacial abnormalities. However, procedures involving bone grafts are invasive and there is risk for donor site infection and morbidity. Bone tissue engineering, which incorporates stem cells, scaffolds, and biological factors, is a promising alternative to current repair methods for cleft palate and other skeletal defects. In our study, we assess the impact of several scaffolds and their osteoconductivity in a calvarial defect mouse model. The scaffolds include implants of collagen sponge, collagen gel and collagen gel with an overlying silicone membrane. Understanding progenitor cell involvement in bone regeneration in scaffolds can help optimize bone tissue engineering approaches for clinical cases, such as cleft lip and palate disorders.

Methods



- Created a 3 mm calvarial defect at 5 weeks of age
- Interventions: collagen sponge pre-loaded with 20 microliters of PBS, collagen gel with a silicone sheet, collagen gel only
- Control group received no intervention
- Harvested mice calvaria for analysis at 2 to 12 weeks post-intervention
- Utilized Amira software for 3D-reconstruction volumetric analysis
- Immunostaining used to characterize components of the osteoconductive environment

Results

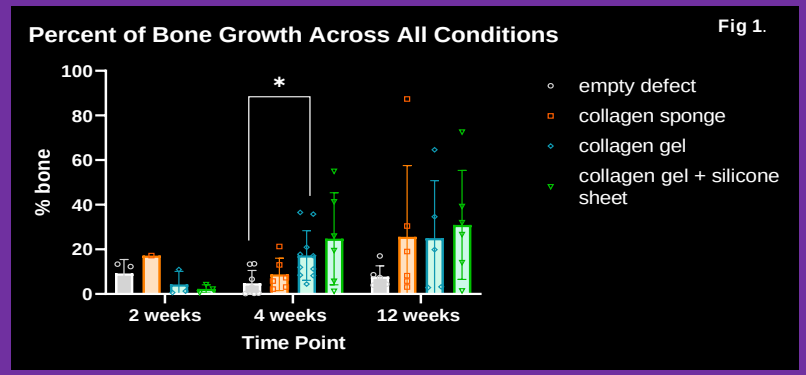


Fig 1. To evaluate new bone growth in the calvarial defect, we calculated the percent of bone growth based on the ratio between the volume of bone in the defect to the volume of bone on the intact side. At 4 weeks, the percent of bone in the collagen gel condition was significantly higher than percent of bone in the control condition. At 4 and 12 weeks, collagen gel with overlying silicone sheet had the highest percent of bone growth in the defect, 24.7% and 30.9% respectively.

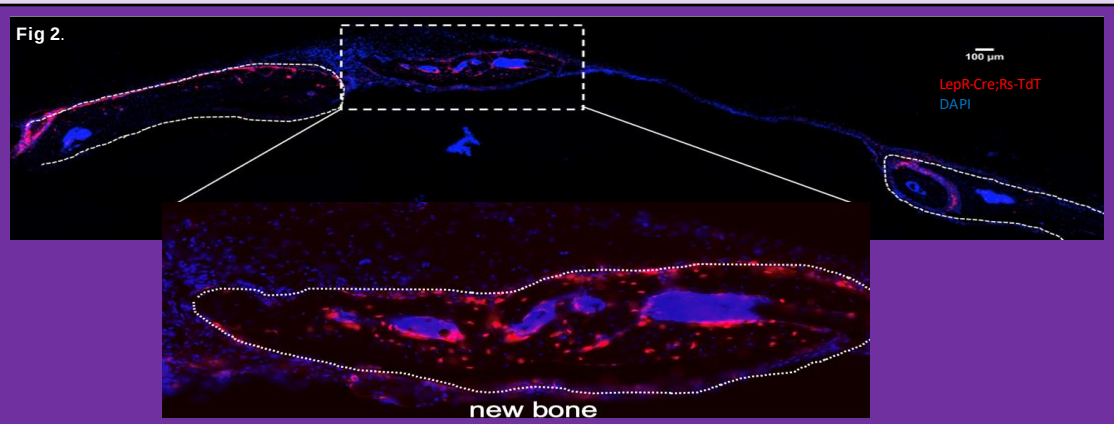


Fig 2. Given the role of LepR+ cells in contributing to bone growth and acting upon injury, we characterized these cells within the calvarial defect model. In newly generated bone, we observed red LepR-Cre+ stem cells in the marrow, bone matrix, and periosteal surface. This suggests that these cells are contributing to new bone formation.

Fig 3. LepR-Cre+ cells in newly generated bone were positive for Runx2, which is involved in early osteogenic differentiation.

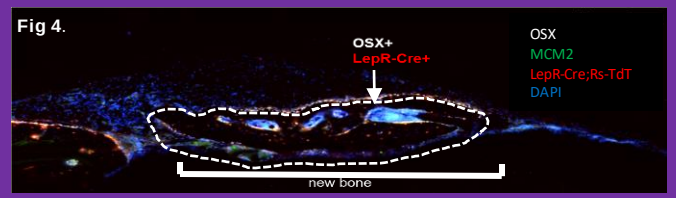


Fig 4. LepR-Cre+ cells also express Osx2, which is further down the differentiation pathway for bone.

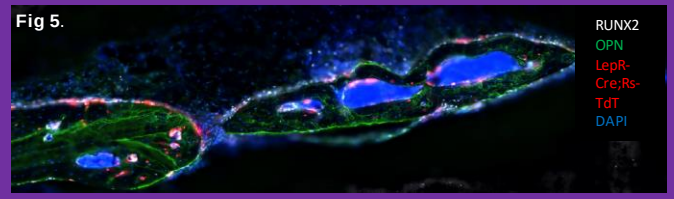


Fig 5. Newly generated bone was also positive for osteopontin, which is a bone matrix protein upon which calcified bone can be built. Osteopontin is present in both the new bone and the intact bone.

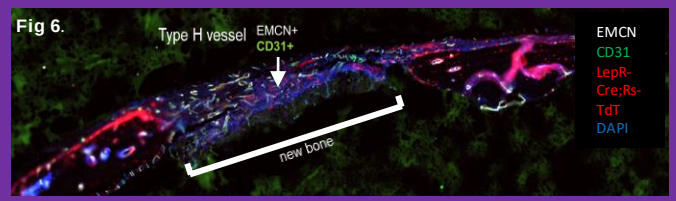


Fig 6. Our IF staining confirmed vascularization of the defect with a double positive EMCN/CD31 stain for type H vessels which lie in close proximity to LepR-Cre+ cells.

Conclusions

- Collagen gel with silicone sheet had superior volume of bone growth in comparison to collagen sponge and collagen gel
- Prominent osteoblast presence and vasculature staining was found within the defect
- LepR-Cre+ cells drive bone regeneration through osteogenic lineage commitment
- Work is underway combining the scaffold with biological factors

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