Masquelet Technique: Based on the SMAD signaling pathway, the content of the relevant growth factors peaks at early time points in young rats.

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Introduction

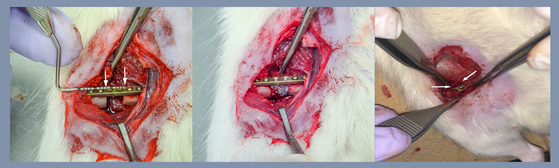
The induced membrane(IM)has been proved to have osteogenic activity, due to its ability to secrete growth factors, which mainly promote osteoblast differentiation via SMAD signaling pathways.

Although IM technique was originally created for adults, it has now been used in the treatment of bone defects in children. However, the current animal experimental models are limited to adult , since many processes will change due to aging, especially for bone regeneration.

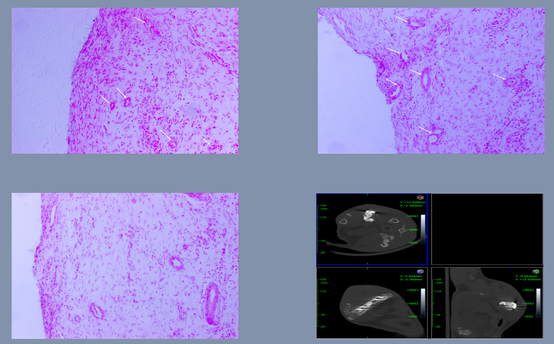
Therefore, our experiment is based on the SMAD signal pathway, by measuring the changes of related growth factors in the IM of young rats of different ages to track the peak performance of IM for bone formation, which provides a reference for future scientific experiments and clinical practice.

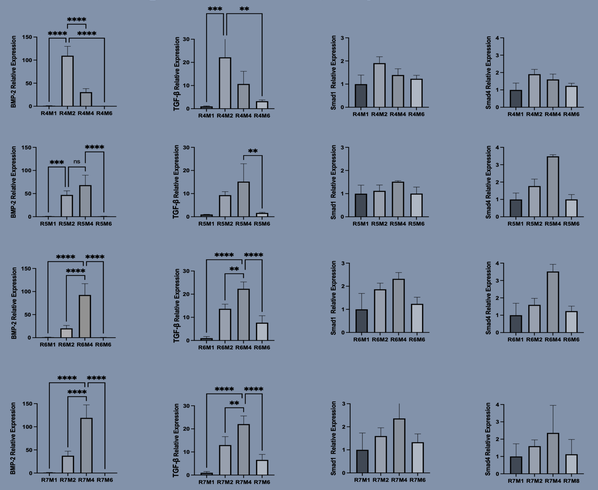
Method

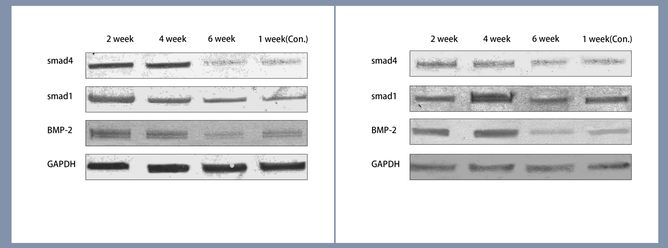
Method: Twenty-four SD rats aged 4, 5, 6, and 7 weeks respectively, weighing 200-350g. A critical size bone defect was established for the right femur of all rats, then filled in PMMA. After 1, 2, 4, and 6 weeks, 6 rats in the same age group were sacrificed, the IM were used for analysis.

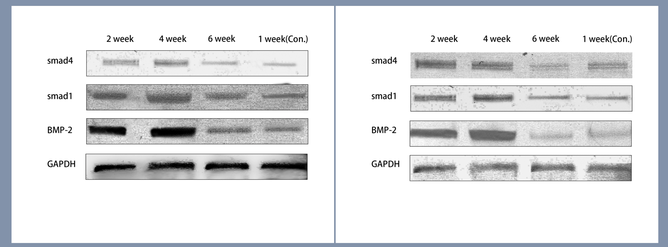


Result









HE staining: Within a few weeks after PMMA insertion, the inflammatory process causes tissue regeneration and the formation of granulation tissue containing macrophages, Synovial-like cells and fibroblasts arranged along the longitudinal axis of the IM, which can be observed in all groups. New capillaries can also be observed. In the 6-week-old rat group, vascularization was initially observed at 2 weeks, and then the degree of vascularization reached a peak at 4 weeks. However, in the 4-week-old rat group, the peak of vascularization appeared earlier time point.

RT-qPCR: For each group of data, a 1-week IM was used as a blank control. In 6 or 7-week-old rats, gene expression gradually increased as the membrane matured and reached a peak in four weeks, and then declined rapidly after 4 weeks. At 6 weeks, the expression of SMAD channel-related genes was almost the same as at 1 week (the induction membrane was just formed at this time). In the younger mouse group, especially the 4-week-old group, the peak of related gene expression seems to be advanced, reaching the peak at 2 weeks, and then starting to decline.

Western blot: The expression of SMAD pathway-related genes in the IM is basically consistent with the RT-qPCR results.

Conclusion

In the younger rat, the peak of IM was generated at an earlier time point.