REVITALIZATION OF CRITICAL-SIZED CALVARIAL DEFECTS IN RATS USING HEPARIN-CONJUGATED FIBRIN HYDROGEL WITH BMP-2 AND ADIPOSE-DERIVED PERICYTES

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Massive bone defects present challenges in orthopedic surgery, requiring innovative solutions. Hydrogels offer promise due to their resemblance to the extracellular matrix. Adipose tissue-derived pericytes (ADPs) and osteoinductive proteins like BMP-2 show potential for bone tissue engineering. Combining them via heparin-conjugated fibrin hydrogel aims to enhance healing in critical-sized calvarial defects.

The study investigated the use of a heparin-conjugated fibrin (HCF) hydrogel containing BMP-2 and adipose-derived pericytes for repairing critical-sized calvarial defects in rats. ADPs were cultured from rat adipose tissue, and the HCF hydrogel was prepared accordingly. *In vitro* experiments assessed BMP-2 release kinetics and bioactivity, while mineralization assays were conducted on cultured ADPs. *In vivo* experiments involved surgically creating calvarial defects in rats and implanting HCF gel alone or with BMP-2, pericytes, or both. Micro-CT imaging and histological analysis were performed post-implantation to evaluate bone formation.

In our study, we prepared the HCF hydrogel by combining heparin-conjugated fibrinogen, human fibrinogen, human thrombin, aprotinin, and calcium chloride. Gelation occurred within 3 minutes at room temperature. SEM analysis revealed an open interconnected pore morphology and macroporous structure, facilitating cell attachment and proliferation. ELISA showed sustained release of BMP-2 from the HCF hydrogel over 28 days. Bioactivity assays demonstrated the ability of released BMP-2 to enhance ALP activity in rat neonatal calvarial osteoblasts. Additionally, BMP-2 induced osteogenic differentiation of rat ADPs, as evidenced by increased ALP activity, osteocalcin expression, and calcium deposition. Implantation of HCF hydrogels containing BMP-2 and/or ADPs into rat calvarial defects significantly promoted bone tissue regeneration compared to controls, with the greatest effect observed in the group receiving both BMP-2 and ADPs. Micro-CT and histological analysis confirmed substantial bone formation and defect closure after three months.

In conclusion, our *in vitro* findings demonstrated that the HCF hydrogel provided sustained release of BMP-2, retaining its bioactivity and promoting osteogenesis in rat calvarial osteoblasts and ADPs. *In vivo* results showed that combining allogeneic ADPs with BMP-2 enhanced bone regeneration in critical-sized calvarial defects, indicating the potential of this approach for large bone defect restoration.