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In Vitro Stimulation Of Endochondral Ossification Of The Growth Plate Using An Ex Vivo 3 Dimensional Organotypic Culture Model

General Topics / Basic Sciences

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Background
The growth plate is placed between the metaphysis and epiphysis at each end of a long bone, thereby being responsible for bone lengthening in children through endochondral ossification. This strictly regulated process involves chondrocyte proliferation and maturation as well as changes in the extracellular matrix. Growth plate injuries may occur either as a result of trauma, burns or osteomyelitis, which further results in disturbed mobility. Although there is a lot of research concerning fracture healing worldwide it still remains unclear which cells interact with each other and how this contributes to bone fracture repair.
Objectives
Since in vivo studies have already demonstrated that an inflammatory phase in the early stage of fracture healing occurs and reduction of animal experiments represents an important goal of research in general, we aimed to contribute to this progress by using an ex vivo 3 dimensional organotypic culture model isolated from rat femur to provide a better insight into biological and molecular mechanisms associated with fracture healing. Moreover, upon successful stimulation of fracture lesion we wanted to evaluate cellular responses involved in the gap closure of the mechanically-induced lesion.
Study Design & Methods
Femoral organotypic bones were derived from 4 days old postnatal rats and 300-400 µm slices were prepared using an advanced vibratome. Organotypic slices were cultured under osteogenic conditions for 15 days and subjected to live confocal imaging, histological stainings, FTIR spectroscopy, and morphometric analyses. Runx2, osteocalcin and collagen II markers were used to immunohistochemically assess the bone and cartilage turnover.
Results
Here we demonstrated that organotypic slices were viable up to 22 days with negligible amount of dead cells. Moreover, ongoing endochondral ossification corresponding to bone turnover was histologically observed and confirmed by increased expression of Runx2 and osteocalcin ($p<0,01$) at day 15. Additionally, FTIR spectroscopy data revealed a remarkable increase in hydroxyapatite and bone minerals at day 15 compared to the control samples.
Conclusions
These results introduce the first ex vivo tissue engineering approach, which closely mimics

the in vivo tissue microenvironment of mineralizing bone. The strict hierarchical architecture and function of the different zones of the growth plate could be investigated in detail. Therefore, we assume that the model can be adapted for studies concerning fracture healing, growth plate arrest or adverse effects and toxicological impact of treatments and materials on the growth plate.