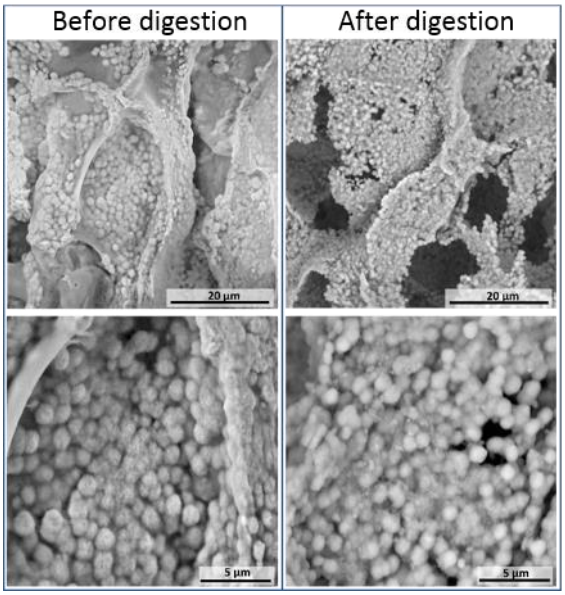
Studies on calcium deposits and growth factors in longitudinal septa of cartilage growth plate for better understanding of physiological ossification and development of clinically useful biomaterials stimulating bone formation

The project constitutes continuation of our previous study in which geometric features of cartilage growth plate were characterized. What is cartilage growth plate? Long bones contain shaft (diaphysis) and usually two articular ends (epiphyses). Shaft is separated from each epiphysis by the cartilage growth plate, which is responsible for the continued growth of bone for 18 to 20 years. The area of transition between the growth plate and the diaphysis is called metaphysis. Cells (chondrocytes) continuously proliferate in the growth plate, to enlarge (hypertrophy) in the area approaching metaphysis in which bone formation begins. Rows of proliferating chondrocytes are separated from each other by extracellular longitudinal septa composed of matrix produced by chondrocytes. Longitudinal septa, close to metaphysis, calcify, with deposition of crystallizing calcium phosphates. The calcified zone is invaded from the metaphysis by capillaries accompanied by cells, which, in contact with calcium deposits, differentiate into bone forming cells (osteoblasts) and begin bone deposition. Osteoblasts and chondrocytes produce several types of bone morphogenetic proteins (BMPs), serving as growth factors stimulating formation of cartilage and bone. Such factors are produced and present within growth plate, but which of them and at what concentration are present within calcified zone remains unknown.



The goal of the present study is to elucidate this particular point, in the hope that it may help to produce new generation of bioimplants, able to stimulate bone formation in a similar manner as it occurs at the growth plate/metaphysis interface. Calcified cartilage will be obtained from young calves. Calcium deposits are dispersed within cartilage matrix of longitudinal septa, thus we have to distinguish between growth factors which are present within non-calcified matrix and those which may hidden within calcium deposits. This point is illustrated by scanning electron microscopy images. They shows calcium deposits in intact cartilage and after digestion of cartilage with proteolytic enzymes. Subsequent dissolution of calcium salt with HCl discloses a thin plate of matrix hidden within calcium deposits and not accessible to enzymes.
In the first part of study growth factors will be extracted from the non-calcified matrix, purified affinity chromatography and identified by the enzyme-linked immunosorbent assay (ELISA).

Calcium deposits will be dissolved, presumably present growth factors released, and detected as above. The whole partially purified material from non-calcified cartilage and calcium deposits will be also sequenced by the Mass Spectrometry Laboratory to find out other proteins. We hypothesise that during formation of phosphate crystals within calcified cartilage, growth factors produced by the adjacent chondrocytes are adsorbed and incorporated into calcium deposits to serve as a depot of bone stimulating factors. Presence of proteins (presumably growth factors) in the calcium deposits will be studied by sophisticated physical methods, such as Fourier Transform Infrared (FTIR) spectroscopy, Raman spectroscopy, and high-resolution scanning electron microscopy. Our hypothesis may be true or not, but the unquestionable profit from the study would be determination of quality and quantity of growth factors present in the critical area of physiological bone formation. On the basis of these observations we will prepare bio-implants with detected combination of growth factors and test their ability to stimulate bone formation in animal model.