Fluorescent visualization of the process of mineralization in transparent organs

Proper development of human skeleton which serves as a site of muscle attachment and protection for internal organs is pivotal for functioning of our organism. One of the most known and well studied diseases of the skeleton is osteoporosis. In osteoporosis, severe loss of bone occurs, which greatly decreases mechanical strength of bones, therefore increases risk of life-threatening bone fractures. Unfortunately, not only loss of bone tissue is dangerous but also it's excess. Situation of "excess bone tissue development" is called pathological mineralization, process in which soft tissues (such as lung or kidney) may calcify. Who might be affected? Literally everyone. It has to be noticed, that calcification occurs always at sites of tissue necrosis, for example in walls of blood vessels during extremely common state of arthrosclerosis.

So far, to study the process of pathological mineralization, scientists were equipped with 2 main techniques: histology and radiological imaging. Unfortunately, none of these is optimal. Histology allows tissue examination with high resolution, but it is slow and labor-intensive approach, impossible to apply for studies made on whole organs or even whole bodies (which is necessary to describe unpredictable pattern of calcification). On the contrary to histology stand such techniques as RTG or magnetic resonance imaging. This approach enables to observe sites of pathological mineralization rapidly within the entire body, but due to its poor resolution, calcification is visible only at the advanced stage.

Recently developed methods of optical tissue clearing (OTC) might overcome limits of the aforementioned techniques. OTC combines what is best in each approach – high resolution scanning of histology with great speed of 3-dimensional imaging of magnetic resonance. In view of this, our primary goal is to optimize the OTP approach, so that it is feasible to study the pathological mineralization in the entire organs, as never before. It has to be noted, that preparation of such transparent specimens is just a small step further on the way to decipher and get better understanding of pathological mineralization in soft tissues. In order to distinguish unharmed tissue from calcific deposition, it is also pivotal to prepare a protocol which will enable selective staining of bone tissue. Once cleared and stained, samples have to be imaged. Unfortunately, there is no commercially available microscopy setup to image such gross specimens like whole organs and whole bodies of rodents. Therefore, we aim to develop customized imaging setup based on light-sheet microscopy (imaging technique, in which transparent organs are illuminated by light in the form of thin plane). Next, we will use the acquired images to construct 3-dimensional volumetric reconstruction of organs and maps of sites of pathological mineralization. These maps will reveal localization and dynamics of formation of calcific deposition and show its influence on architecture of organs. We hope that achieving of the aforementioned objectives will improve our understanding of pathological mineralization and rapidly contribute to development of therapies acting on early stage of disease before time when organ failure is unavoidable.