

Tissue engineering is a rapidly developing field, based on using scaffolds made from natural or synthetic materials in combination with cells and stimulating factors for the replacement of damaged or lost tissue. It is very important to evaluate qualities of these scaffolds, which are necessary for cell proliferation and their mechanical support.

The aim of this study was to develop a suitable scaffold for chondrocyte proliferation, scaffold functionalization and detection of cells and proteins of extracellular matrix (ECM) using methods of fluorescence and confocal microscopy. Another aim was to test an appropriate scaffold on a big animal model *in vivo*.

Several scaffolds from natural and synthetic materials, in the form of microfibers, non-woven textiles, gels and foams were prepared for this study. Scaffolds were seeded with chondrocytes and cell adhesion, proliferation and synthesis of ECM proteins were detected. Methods of fluorescence microscopy, confocal microscopy and second harmonic generation (SHG) were used for visualization of cells and proteins. A hydrogel based on fibrin and hyaluronan was used as a scaffold for osteochondral defect regeneration in minipigs study.

Prepared scaffolds showed high biocompatibility, good chondrocyte adhesion and ECM proteins synthesis. Moreover, microfibrillar scaffold from PHEMA was enriched by liposomes containing fetal bovine serum (FBS). This system of drug delivery was found as an appropriate system for cell proliferation and differentiation support. Fluorescent confocal microscopy was shown as a suitable method for visualization of cells and ECM. Moreover, the usage of SHG enabled detection of ECM specific proteins without any need of a fluorescent probe. Combination of these two methods enabled detection of cells and ECM distribution. Composite hydrogel based on fibrin and hyaluronan was successfully tested on the minipig model *in vivo*. Hyaline like cartilage was created 6 months after implantation.