Table 1. Overview of preclincal and clinical testing.

Study	Preclinical	Clinical	Clinical
Bone Defect	Tempolaral bone	Spinal disease	Revised THA
Material	HA	β-TCP	β-ΤСΡ
Treated	7	4	7
Control	7	20	20

*Discussion and conclusions:* Based on preclinical and clinical data, we suggest that MSCs in combination with artificial bone scaffold are suitable for the treatment of bone defects. However, more preclinical and clinical data are required to optimize the amount of implanted cells. *Acknowledgments:* This work was supported by the Grant Agency of the Czech Republic (GACR 304/10/0320) and the Grant Agency of the Ministry of Health of the Czech Republic (NT13477).

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## PP350 Silk bilayer scaffolds can induce fast integration with subchondral bone and support cartilage repair

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*Introduction:* Osteochondral defect (OCD) regeneration presents major challenges in orthopedics. Since healing of cartilage and bone should be simultaneously considered, ideal scaffolds should be those that can mimic both tissues properties. In this study, bilayered silk and silk-nano calcium phosphate (Silk/Silk-NanoCaP) scaffolds with tailored mechanical properties were developed for OCD tissue engineering application.

*Materials and methods:* Aqueous silk solution (16%) was prepared.<sup>1</sup> Nano calcium phosphate particles (16%) were synthesized in the silk solution (Silk-NanoCaP).<sup>2</sup> The bony layer was prepared by addition of NaCl particles (500–1000  $\mu$ m) into the Silk-NanoCaP suspension. After drying for 2 days and salt-leaching overnight, silk solution was added on top of the bony layer using the same procedure to produce the chondral layer. The final scaffolds were evaluated through *in vitro* culture of



Figure 1. H&E staining of the explants. Scale bar: 1 mm. Black and white arrows indicated cartilage and subchondral bone formation, respectively.

rabbit bone marrow stromal cells (RBMSCs) for 2 weeks, and *in vivo* implantation in a rabbit knee OCD for 4 weeks.

*Results:* The RBMSCs cultured in the scaffolds presented increasing viability from day 1 to day 7 by MTS assay. Good adhesion and migration of the RBMSCs in the scaffolds were achieved, as observed under the scanning electron microscope. Cell proliferation was observed from day 7 to day 14 as determined by DNA quantification. The bony layer induced higher alkaline phosphatase level as compared to the chondral layer, in osteogenic condition. Histological analysis (H&E) showed that the bilayered scaffolds integrated well with the host tissue, after 4 weeks of implantation in a critical size OC defect (Fig. 1). Abundant new bone formation was detected in the Silk-NanoCaP layer. Cartilage regeneration occurred in the silk layer.

*Discussion and conclusions:* The bilayered scaffolds favored the attachment, proliferation, and differentiation of RBMSCs. The bony layer of the bilayered scaffolds possessed osteoconductive properties. The bilayered scaffolds were biocompatible *in vitro* and *in vivo*. These scaffolds also induced both subchondral bone regeneration and supported cartilage regeneration, thus showing great promise in OCD regeneration.

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*Disclosure:* The authors declare that there is no conflict of interest. *References* 

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## PP351

## First results of the bone tissue morphological evaluation after implantation of new polymer and tricalcium phosphate scaffolds coated with resorbable nano hydroxyapatite

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*Introduction:* Due to bioactivity and biocompatibility hydroxyapatite (HAP) and tricalcium phosphate (TCP) are frequently used as the bone graft substitutes. Ceramics are brittle, and at present time their resorbtion rate is difficult to control. On the other hand polymers – polycaprolactone (PCL) offer good fracture toughness, but are less biocompatible than the ceramics. Covering a porous bioresorbable polymer scaffold with nano-HAP particles would intensify the bioactivity of the scaffold. Osteoblasts proliferate better on a hydrophilic surface made of nano-ceramics than on a polymer one or conventional ceramic material.

*Materials and methods:* Two kinds of porous scaffolds were produced one using state of the art polymer bio-plotting technology, and one made of TCP produced using a ammonium hydrogen carbonate provided viscous slurry foaming [1]. Resorbable nanoparticles of HAP 8 to 20 nm in diameter were produced using solvothermal synthesis. The nanoparticle coating on the external and internal surfaces of the scaffolds were performed using ultrasonic energy. The ultrasonic coating technology ensures strong adhesion of the nanoparticles to the scaffold. 12 rabbits were used to perform *in vivo* tests. TCP ceramic and PCL non-coated, PCL coated with nano-HAP, TCP coated with nano-HAP scaffolds were implanted in *tibia* of New Zealand rabbits. Surgically created defects were filled with scaffolds (dimensions: 5 mm diameter, 3 mm height). After 3 months explanted samples were used to evalu-