**In vitro** Cellular Responses of TCP-collagen Composites Cross Linked with EDC
Otilia Zarnescu,1 Oana Craciunescu,2 Elena Iulia Oprita,2 Lucia Moldovan,2
Corresponding Author: otilia@bio.unibuc.ro
1University of Bucharest, Faculty of Biology, Bucharest, Romania and 2National Institute R&D for Biological Sciences, Bucharest, Romania

**Introduction**
Collagen-beta-tricalcium phosphate scaffolds were used in regenerative medicine due to their similarity to the inorganic component of bone (1,2). Composite porous matrices based on collagen and various amounts of nano-β-tricalcium phosphate (TCP) crosslinked with 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide hydrochloride (EDC) were developed as scaffolds for bone regeneration. The effect of TCP on cells in accordance with two TCP concentrations in the collagenous matrices was investigated using osteoblast cultures.

**Materials and Methods**
In order to fabricate the composite scaffolds, a solution of type I collagen was mixed with various amounts of TCP. The mixtures were frozen at -40°C and freeze-dried for 24 h. Collagen sponges of approximately 50 mg were submerged in the EDC solution and incubated with shaking for 18 h. Cellular responses to EDC-cross linked TCP-collagen composites were tested in two ways: (1) small pieces of composites (5x5x5 mm) were placed in culture of osteoblasts; (2) each composite was injected with osteoblasts at a density of 2 x 10^6 cells/cm². In both variant composites were cultured in standard culture conditions for 3 and 6 days, respectively. The composites were tested in osteoblast cultures to evaluate proliferation and immunohistochemical expression of osteocalcin and active caspase-3 (as marker of apoptosis).

**Results**
Morphological evaluation showed that osteoblasts spread, and proliferated well on the culture dishes and inside the porous scaffolds and retain their normal morphology even after 3 and 6 days in culture. Moreover, good biocompatibility was suggested by the small number of cells positive for active caspase-3. The production of osteocalcin was confirmed by immunohistochemical staining.

At both concentrations of TCP was observed that at six days of cultivation osteocalcin expression is more pronounced than at three days (Fig.1).

**Discussion and Conclusions**
The ability of these composite scaffolds to enhance osteocalcin expression recommends them as scaffolds for bone tissue engineering. Further *in vitro* and *in vivo* analysis needs to be done to evaluate the therapeutic potential of these EDC-cross-linked collagen-beta-TCP sponges in regenerative medicine.

**References**

**Acknowledgments**
This work was supported by Project BIOSTEM, No. 61012/2007.

**Disclosures**
The authors indicate no potential conflicts of interest.