INTRODUCTION:
It is estimated that five to ten percent of all fractures occurring annually in the US result in delayed or impaired healing. For the enhancement of bone repair and remodeling growth-factor based approaches are an alternative avenue to autogenic and allo genic bone grafting which are often associated with the risk of immunogenicity or increased morbidity (1). Insulin-like growth factor I (IGF I) is among the most abundant growth factors in bone and promotes the proliferation and differentiation of osteoprogenitor cells. Its clinical relevance in fracture healing is still controversial (2). Previously, we reported on the development of a polymeric controlled delivery system for IGF I and demonstrated bridging of experimental bone defects after 8 weeks (3, 4). Even though IGF I release from this system is limited in vivo to approximately 4 weeks only, long-term studies are essential to evaluate the efficiency and safety of this therapeutic approach.

Therefore, we examined the impact of local and temporary IGF I treatment on bone repair and remodeling during 18 months in a tibial fracture model in sheep. We focused on the analysis of bone microstructure and 3D architecture of newly formed bone by using novel micro-computer tomography (micro-CT).

METHODS:
IGF I was encapsulated in poly(lactide-co-glycolide) 50:50 (PLGA 50:50) microspheres (MS) using solvent evaporation. Protein loading and encapsulation efficiency were determined by HPLC. Particle size was measured by laser light diffractionometry and surface morphology of the MS was analyzed by scanning electron microscopy. IGF I in vitro release kinetics were monitored during 62 days and analyzed by ELISA. Bioactivity of encapsulated IGF I was verified using a mitogenic bioassay using an osteosarcoma cell line (MG63). The in vivo study was performed in 6 adult sheep (Swiss alpine) according to Swiss federal guidelines and approved by the institutional animal welfare committee. Under anesthesia unilateral, standardized 10 mm mid-diaphyseal tibial defects were established in either right or left hind legs. Each tibial defect received 100 mg PLGA MS loaded with 100 µg IGF I. Afterwards, defects were stabilized with a dynamic compression plate. Due to ethical considerations an untreated control defect was only authorized for an observation period of 8 weeks (negative control). Therefore, the corresponding intact tibias served as a reference. Radiographs were taken at regular time intervals. Sheep were killed after 18 months. Inner organs as well as lymph nodes were removed to examine pathological irregularities. Micro-CT images (µCT40, Scanco Medical, Bassersdorf, Switzerland) were made with a resolution of 30 µm. For bone analysis a length of 8.5 mm around the defect center was evaluated. The bones were then cut, and slices prepared for histology (grinding and thin sections stained with toluidine blue and von Kossa).

RESULTS:
Endochondrial ossification started as early as 3 weeks post operation. New bone formation was pronounced in IGF I treated defects compared to the negative control where no new bone was observed after 8 weeks.

DISCUSSION:
Controlled and local IGF I delivery is able to enhance new cortical bone formation in sheep. The application seems to be safe and effective, and bone repair and remodeling seem to be normal. Newly formed bone is well integrated into the remaining bone network. Due to an accelerated healing process callus and fibrous tissue formation is reduced compared to natural healing of defects of that size. Observed microruptures in and around the defect areas probably resulted from motions of the plate.

In conclusion, good stability was obtained. The callus size indicates that there is still remodeling going on. For the treatment of larger bone defects a combination with an osteoconductive scaffold seems to be favorable.

REFERENCES:
1. G. A. Rodan, T. J. Martin, Science 289, 1508-14 (Sep 1, 2000).