Introduction:
Growth and differentiation factor-5 (GDF-5) as a member of the TGF-beta superfamily of growth factors is essential for normal skeletal growth and collagen maturation [2, 4, 5]. GDF-5 has been reported to have a proliferative effect and causes improved biomechanical properties on healing tendons and ligaments [1, 6, 7]. Therefore, the aim of our study was to investigate the influence of a GDF-5 coated suture on the healing Achilles tendon in a rat model.

Material & Methods:
The right Achilles tendon in 80 male Sprague-Dawley rats, weighing 290-320g, was sharply transected and sutured with an absorbable suture material (Vicryl™ 5-0, Ethicon Inc.). The plantaris tendon was left unsutured to prevent internal splinting of the healing tendon. No operation was performed on the left, uninjured hindlimb. No cast or dressings were applied, and the animals were unrestricted during the healing phase.

All animals were randomized according to uncoated (control, n=40) or GDF-5 coated group (n=40) as well as to different time periods after surgery. Approval of our institutional animal care review board was obtained prior to start the study.

At 1, 2, 4 and 8 weeks after surgery, the healing tendons were evaluated macroscopically, histologically (n=3 in each group at each time point) and biomechanically (n=7 in each group at each time point). Histology was assessed on HE- and Picrosirius [3] stained sections. By elution of the bound Sirius red dye and its photometric quantification (OD 550nm) the relative collagen content of the sections was determined [µg collagen/mm²].

Biomechanical parameters as maximum failure load, tendon stiffness and lengthening until failure load were determined.

Results:
Gross examination revealed that the repair tissue in the GDF-5 group differed from controls in volume and color and had a firmer consistency. These findings were confirmed by measuring tendon thickness and morphometric analysis of the scar area in histological sections (e.g. 1 week, uncoated: m=42±21mm², coated: m=77±21mm², p=0,001).

Biomechanical testing showed a significantly higher maximum tensile strength at 2 weeks (uncoated m=48,6N, GDF-5-coated 66,1N; p=0,04, Fig. 1) in combination with significantly increased values for tendon stiffness at 1, 2 and 4 weeks for the GDF-5 group (Fig. 2). This effect diminished stepwise until 8 weeks after surgery. However, the increase in tendon stiffness was accompanied by a significant decrease in lengthening until failure load at 2, 4 and 8 weeks (2 weeks: uncoated 4,5mm, GDF-5-coated 2,8mm, p=0,002; 4 weeks: uncoated 4,2mm, GDF-5-coated 2,2mm, p=0,001; 8 weeks: uncoated 3mm, GDF-5-coated 2,2mm, p=0,01).

Histology in the uncoated group showed normal healing of collagenous tissue with parallel oriented collagen fibers after 4 weeks and an almost reorganized pattern after 8 weeks. The HE histology of the GDF-5 coated group was initially dominated by a very high cellularity after 1 and 2 weeks and by large, rounded and clear cells after 4 and 8 weeks. These cartilage-like cell formations were seen over the whole section not strictly associated to the vicinity of suture material (see Fig 3).

The suture material itself was seen until 8 weeks after tendon repair and did not induce any inflammatory or toxicological effect in both experimental groups.

Using the Picrosirius-polarization method with its characteristic birefringence for collagen type I (orange/yellow) and type III fibrils (green) the GDF-5 group still showed a high proportion of collagen type III after 4 weeks, whereas the control specimen were already dominated by collagen type I. The whole collagen content at this time point was significantly lower in the GDF-5-coated group (4 weeks: uncoated 1,15 +/- 03µg/mm², GDF-5-coated 0,6 +/- 0,1 µg/mm², p=0,001).

Discussion:
To our knowledge this is the first experimental study to investigate the influence of a GDF-5 coated suture onto tendon healing in a defect model. Our biomechanical data showed improved tensile properties of the healing Achilles tendon in the GDF-5 group, by means that the tendon repair process was significantly increased but less compliant after 2 weeks. On the other hand histological data strongly suggest that GDF-5 induced cartilage-like cell formations in the healing Achilles tendon in rats after 4 weeks, which needs to be confirmed by analysis of matrix composition and cell phenotype. In our opinion the improved biomechanical properties, observed here and by others, are caused by GDF-5 induced hypertrophic scar formation and delayed differentiation rather than by improved repair tissue composition.

Overall, the appearance of cartilage like structures in the healing tendon has to be considered as an inappropriate side effect, which may be overcome by modifications of the GDF-5 dose and the suture material.

Acknowledgements: We have to thank Helga Lorenz and Haili Wang for technical assistance. Financial support was obtained from the Research fund of the Orthopaedic University Hospital of Heidelberg.