# Final Report

*Animal experimental study for the usage of differently processed collagen type I gels for the treatment of cartilage defects on knee joints using Göttinger Minipig model*

In this animal experimental study the effectiveness of differently processed collagen type I gels for the treatment of full-thickness cartilage defects should be examined. The aim of this investigation was the purification of the question which influence different processing of primarily cell-free collagen gel matrixes has on the ability to repair cartilage tissue. As an animal model the Göttinger Minipig was chosen, because this model has proved itself in numerous preliminary investigations concerning the attitude of cartilage reparation very well.

**Material and Methods**

Overall, 18 adult Göttinger Minipigs (uncastrated boar) were operated. The age of the animals was an average of 3.5 years (2 to 4 years). At the beginning of the study the weight of the animals was averaged 48.3 kg (+/-4.9 kg). With 2 animals it came in the postoperative period to a patella dislocation. These both animals could not be taken up in the evaluation. Therefore the following distribution of the evaluateable laboratory animals surrendered taking into account the distinguished re-examination intervals:

| | |
|---|---|
| 6 weeks: | n = 6 animals |
| 12 weeks: | n = 4 animals |
| 12 months: | n = 6 animals |

**OP-Technique**

All animals were treated according to standardized OP protocols:
The animals were anesthetized using general anesthesia. After sterile washing and draping of the surgical area in the right knee joint, the representation of the joint was carried out by an approximately 5 cm long, anteriorly located midline incision. The arthrotomy was a media-ligamentous access along the patellar ligament, which was extended proximally to parapatellar. After inspection of the articular surfaces the defects were set to the trochlea using a 6mm drill. 2 defects were placed in the medial and the lateral parts of the trochlea by a standardized technique (fig. 1). The defects were all of full-thickness and pointed at the center due to the placement of the drill, a central pin, a perforation of the bone-cartilage plate.

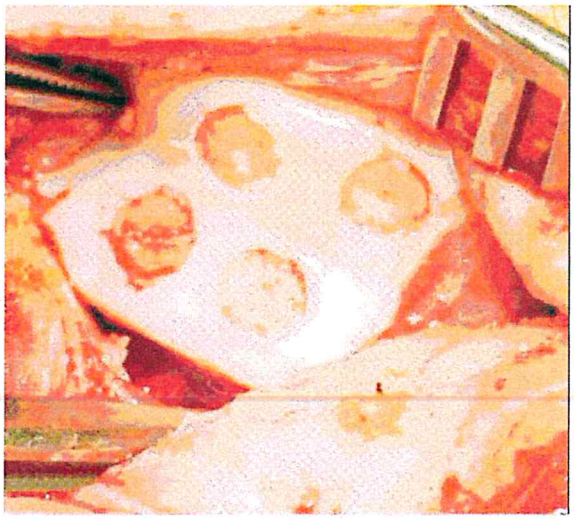

Fig.1: Causation of 4 full-thickness cartilage defects in the area of the trochlea.

**Treatment Groups**

The defects were treated according to the following scheme:

1. Proximal lateral defect: collagen liquid (Chondrofill liquid)
2. Distal lateral defect: collagen (Chondrofill 20-fold)
3. Proximal medial defect: control untreated
4. Distal medial defect: collagen (Chondrofill 4-fold)

All implants for this study were made available by the company Amedrix GmbH. The two form solid implants (Chondrofill 4 and 20x) were fixed with a thin layer of fibrin glue (Baxter Tissucol) in the defect and modelled planar with a large stamp equivalent to the level of the surrounding cartilage. The liquid collagen (Chondrofill liquid) was preparated according to the specifications of the company Amedrix GmbH during the surgery and filled directly into the defect. An untreated blank defect served as a control.

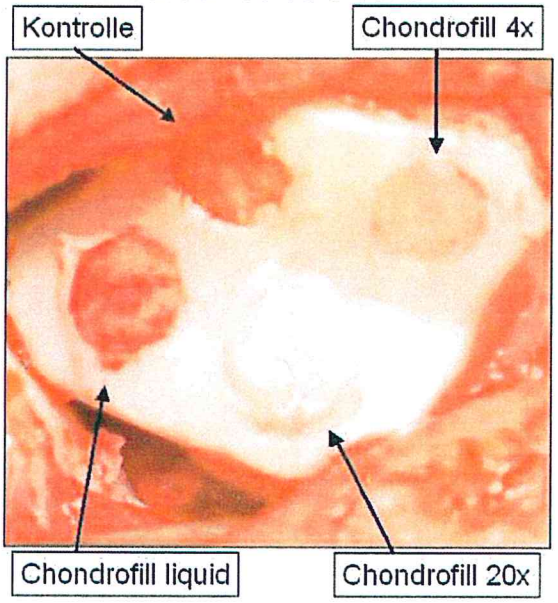


Fig. 2: Intra operational situs after treatment of the cartilage defects according to the abovementioned pattern

**Research Method:**
All preparations adducted for the analysis (n=16) were processed and analyzed with the following methods:

- Macroscopically evaluation of cartilage healing and of the periarticular tissue
- Histological Reprocessing (HE, Safranin-O, collagen I and II staining)
- Mechanical testing of the cartilage regenerate (sample testing with intender, e-module-estimation

## *Results*

### Macroscopic Appearance

The macroscopic appearance showed after 6 weeks for all 6 documented preparations a nearly complete filling of the defects with a glazed reparation tissue (see fig. 3) Difference between the treatment groups were macroscopically not visible. The treated joints show no irritant reactions or joint effusion.

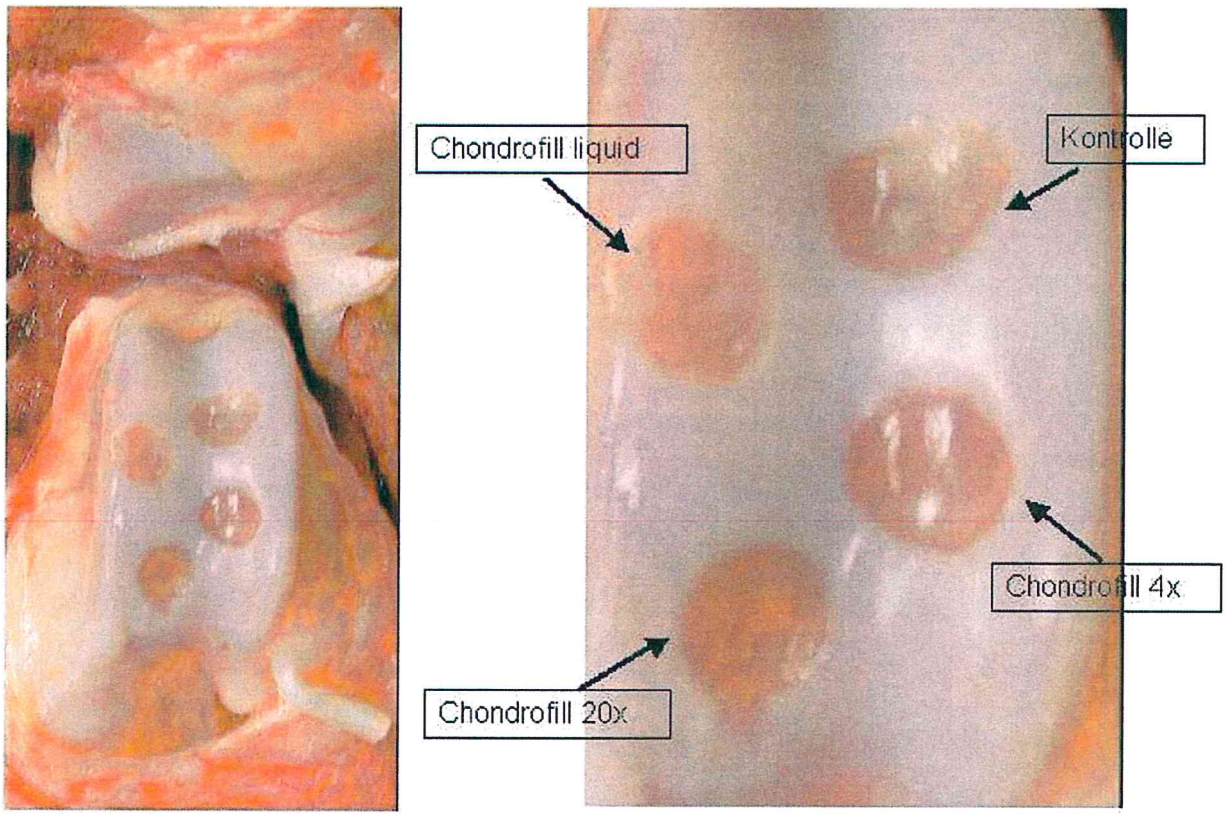


Fig.3: Typical macroscopic appearance after 6 week standing with filling of the defect zones by glazy reparation tissue with smooth surface structure

After 3 months the defects of the 4 evaluated animals were nearly complete filled with the reparation tissue. Nevertheless, the quality of the regenerates were partial very different. In direction the defects which have been treated with the liquid collagen showed the best results. The evaluated 4 joints showed no irritant reactions or joint effusions (see fig. 4).

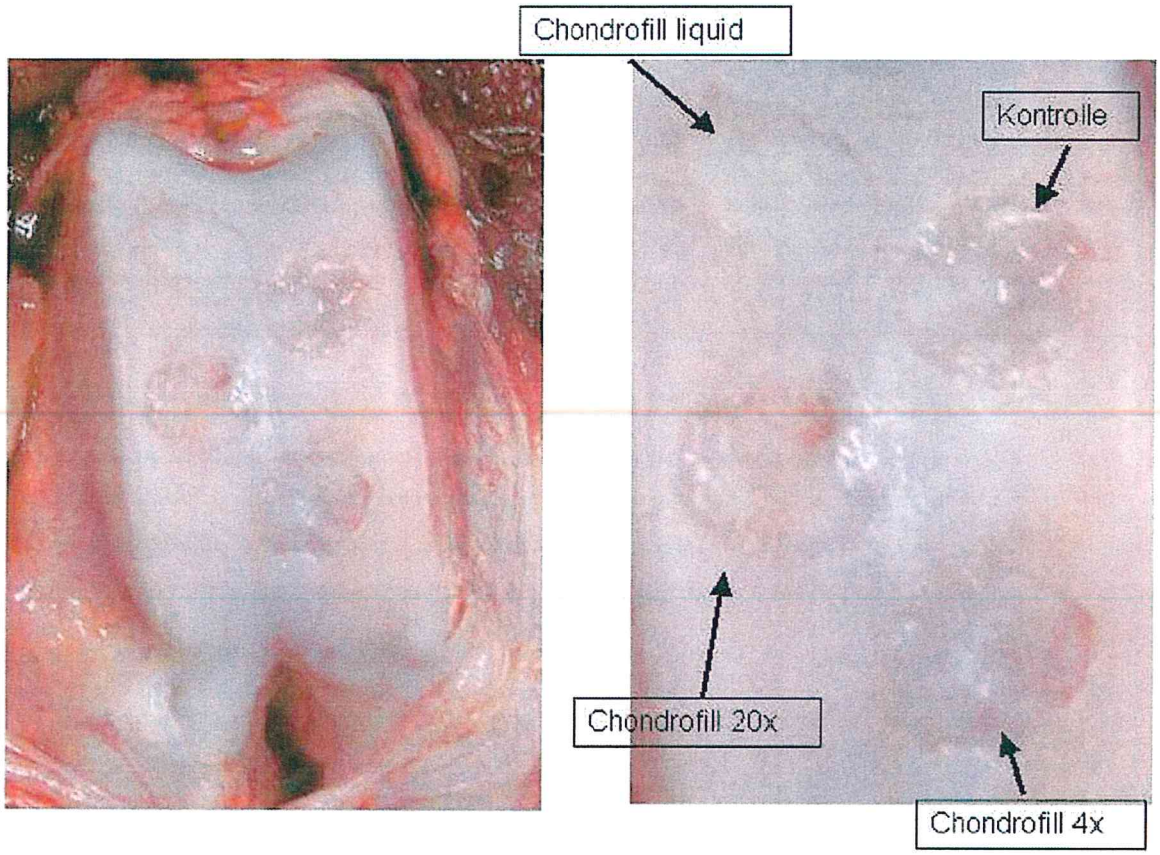


Fig.4: Typical macroscopic appearance after 12 weeks standing with filling of the defect zones by partly glazy partly cartilaginous structured reparation tissue with irregular surface structure.

The macroscopically appearance of the regenerate zones after one year showed generally in all treatment groups a complete filling of the defects with a tissue, which has macroscopic a cartilage-like appearance. The defects treated with Chondrofill 20x and the untreated control defects were still well specifiable from the surrounding cartilage. The defects treated with Chondrofill liquid and Chondrofill 4x gels showed generally a smooth surface structure and were hardly specifiable from the surrounding cartilage. Also after one year no irritant reactions or other inflammation reactions in and beyond the treated joints were provable (see fig. 5).

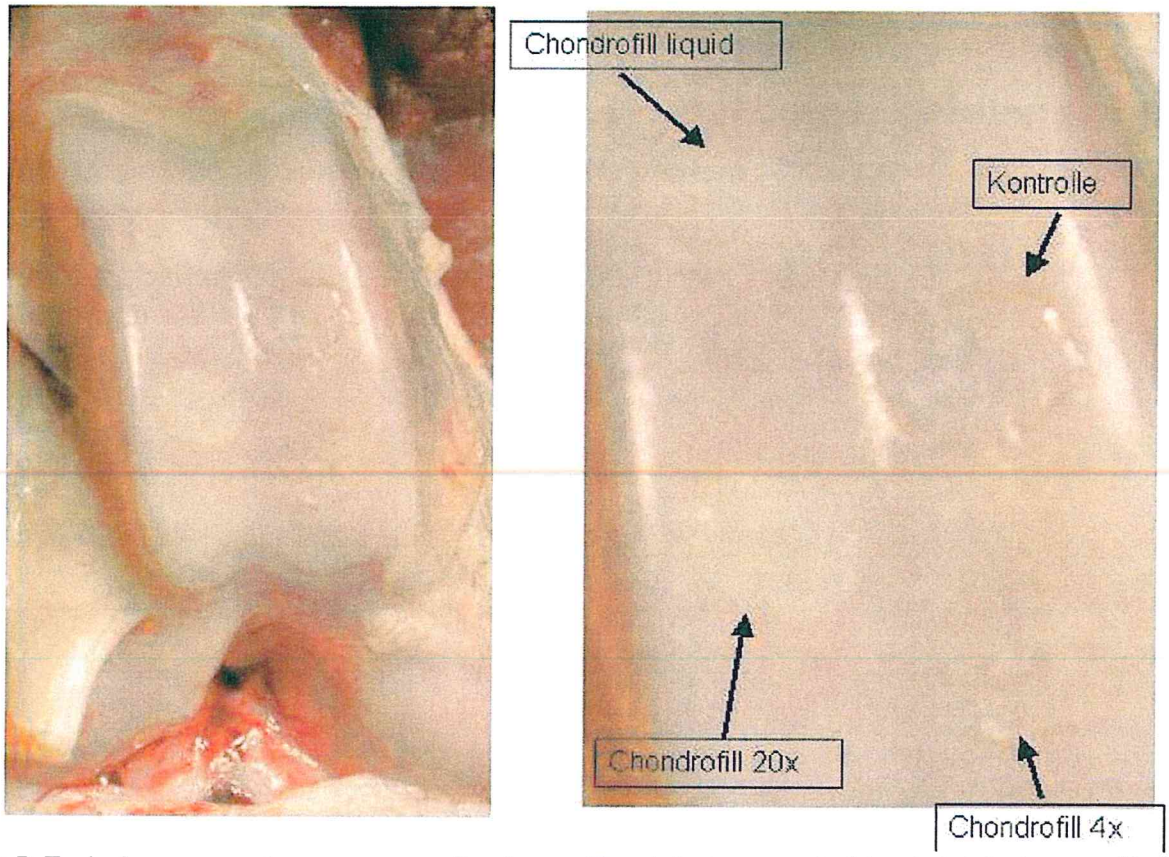


Fig.5: Typical macroscopic appearance after 1 year. The defect zones of all treated defects were filled well. The defects treated with Chondrofill liquid and Chondrofill 4x were hardly specifiable from the surrounding cartilage

*Qualitative histological Examination*

After 6 weeks a multitude of immigrated cells could be found in the primarily cell-free implants. These cells showed a predominantly fibroblastic morphology. In all collagen implants it came by and by to a differentiation of the immigrant cells to a predominantly chondroblastic phenotype. This could be shown not only in the HE-staining, but also in the more cartilage specific collagen type II staining. The implantation of the differently processed collagen gels led in most cases to a complete reconstruction of the removed cartilage. In the treatment groups treated with the liquid squired collagen the reparation tissue was qualitatively the best. The groups treated with the 20x compressed collagen and the untreated control showed the worst histological results.

The anchorage of the regenerate to the subchondrale bone plate was completely given in all groups and in the present re-examination periods without recognizable fracture. The integration in the surrounding cartilage tissue was well in the individual treatment groups. In the defects treated with the 20x compressed collagen respectively the untreated controls partly considerable structure breakings in the border area of the regeneration zone appeared. Homogeneous reparation procedures were found predominantly in the groups treated with the 4-1 concentrated collagen and the primary liquid collagen.

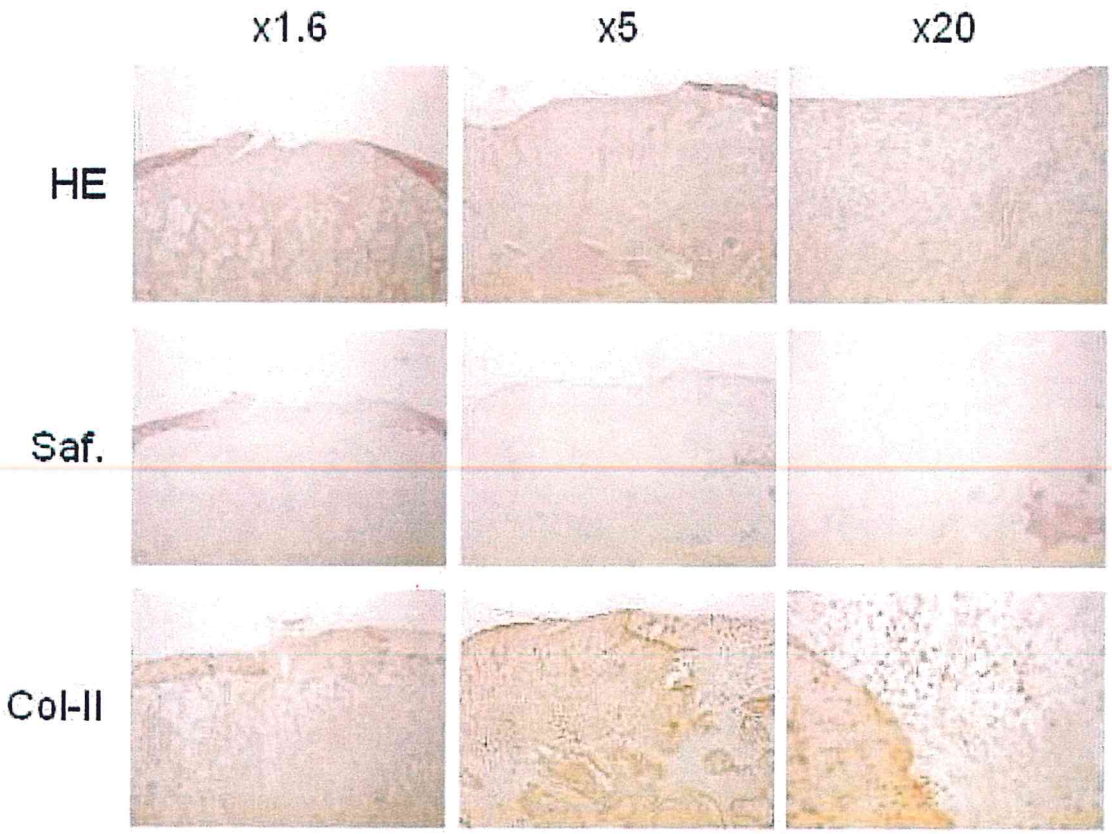


Fig. 6a: Histological preparations of the untreated defect after 6 weeks

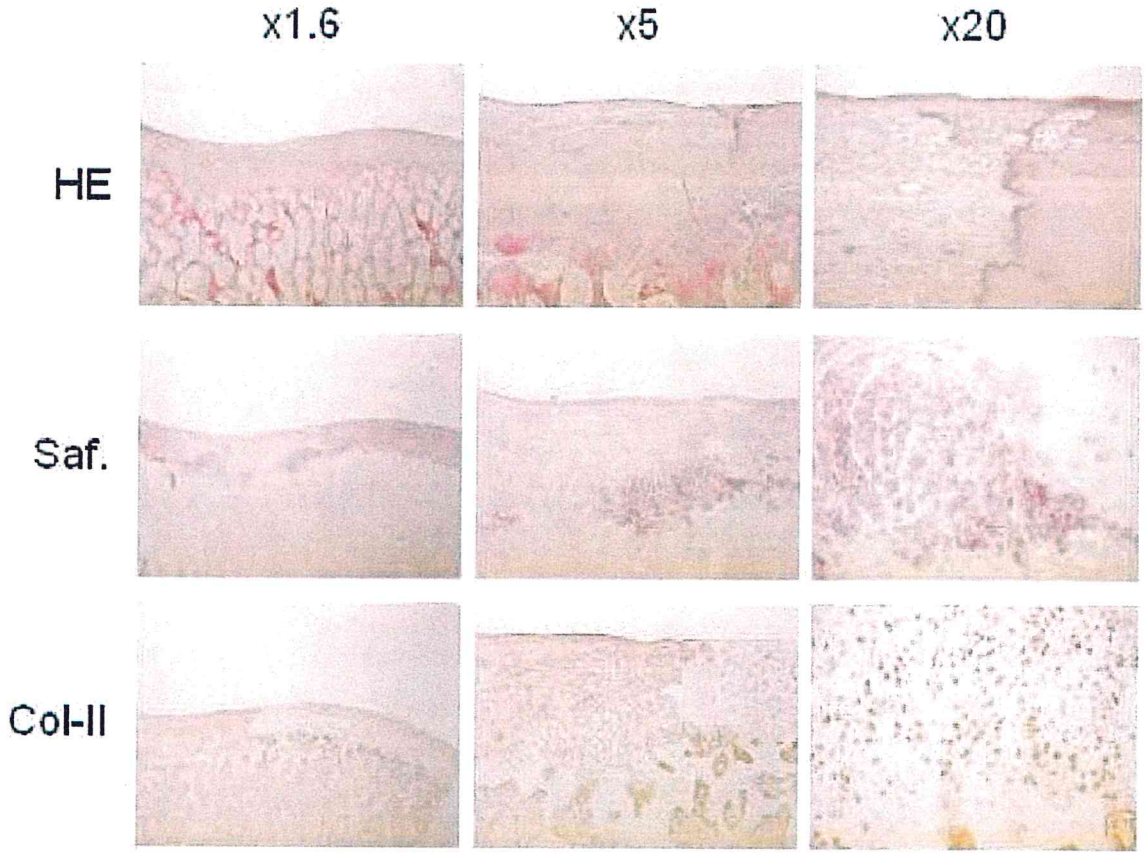


Fig. 6b: Histological preparations of defects treated with Chondrofill liquid after 6 weeks

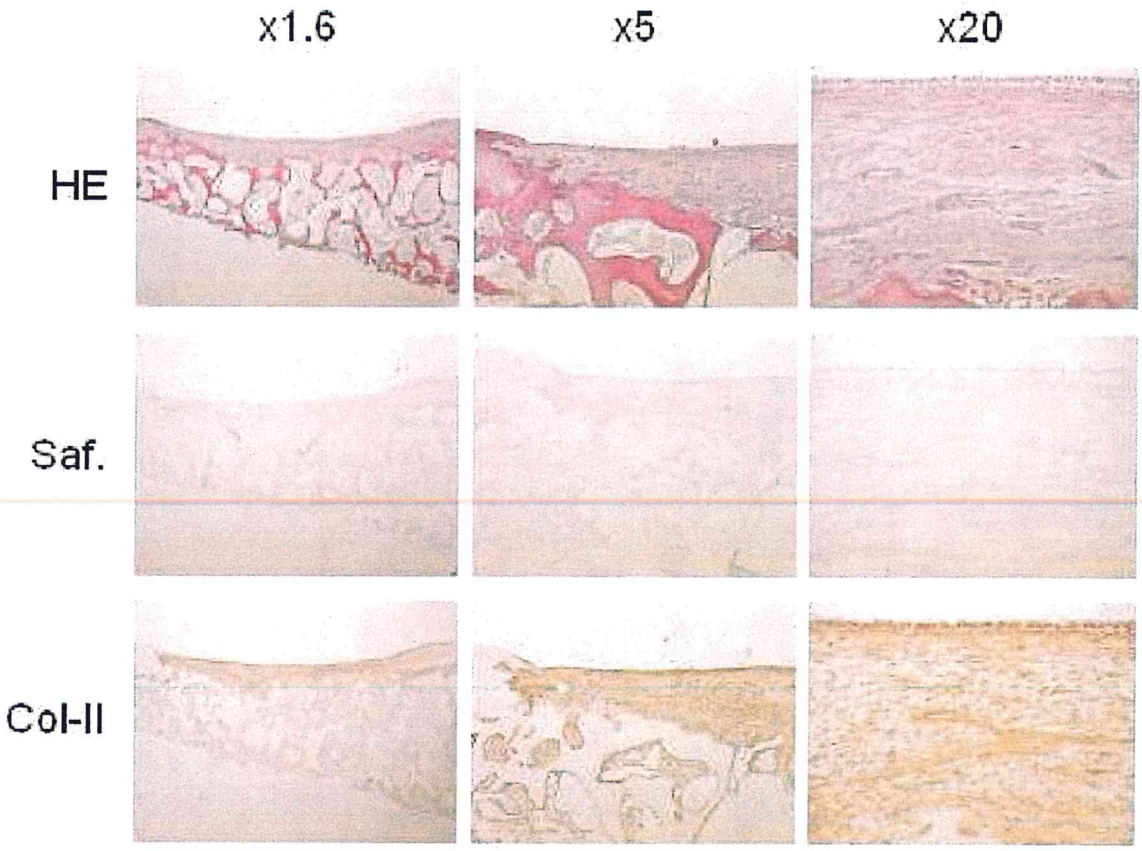


Fig 6c: Histological preparations of defects treated with Chondrofill 4x-gel after 6 weeks

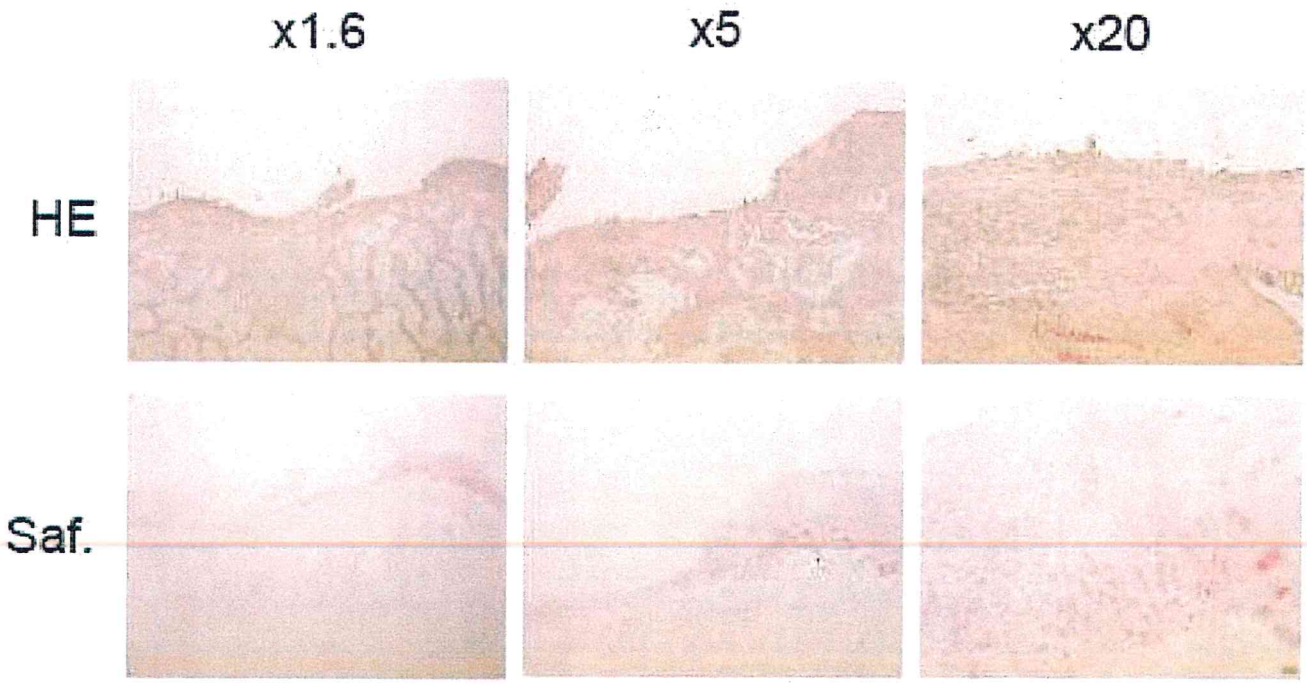


Fig.7a: Histological preparations of untreated defects 3 months after treatment

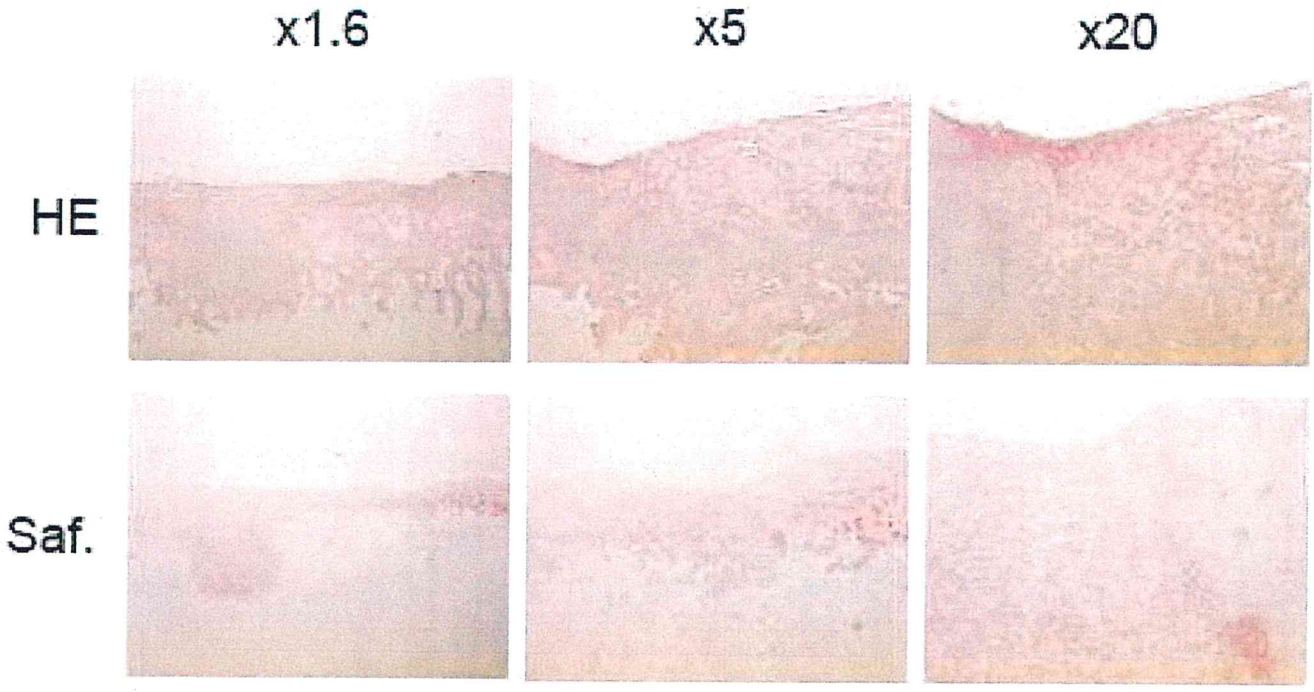


Fig.7b: Histological preparations of defects treated with Chondrofill liquid 3 months after treatment

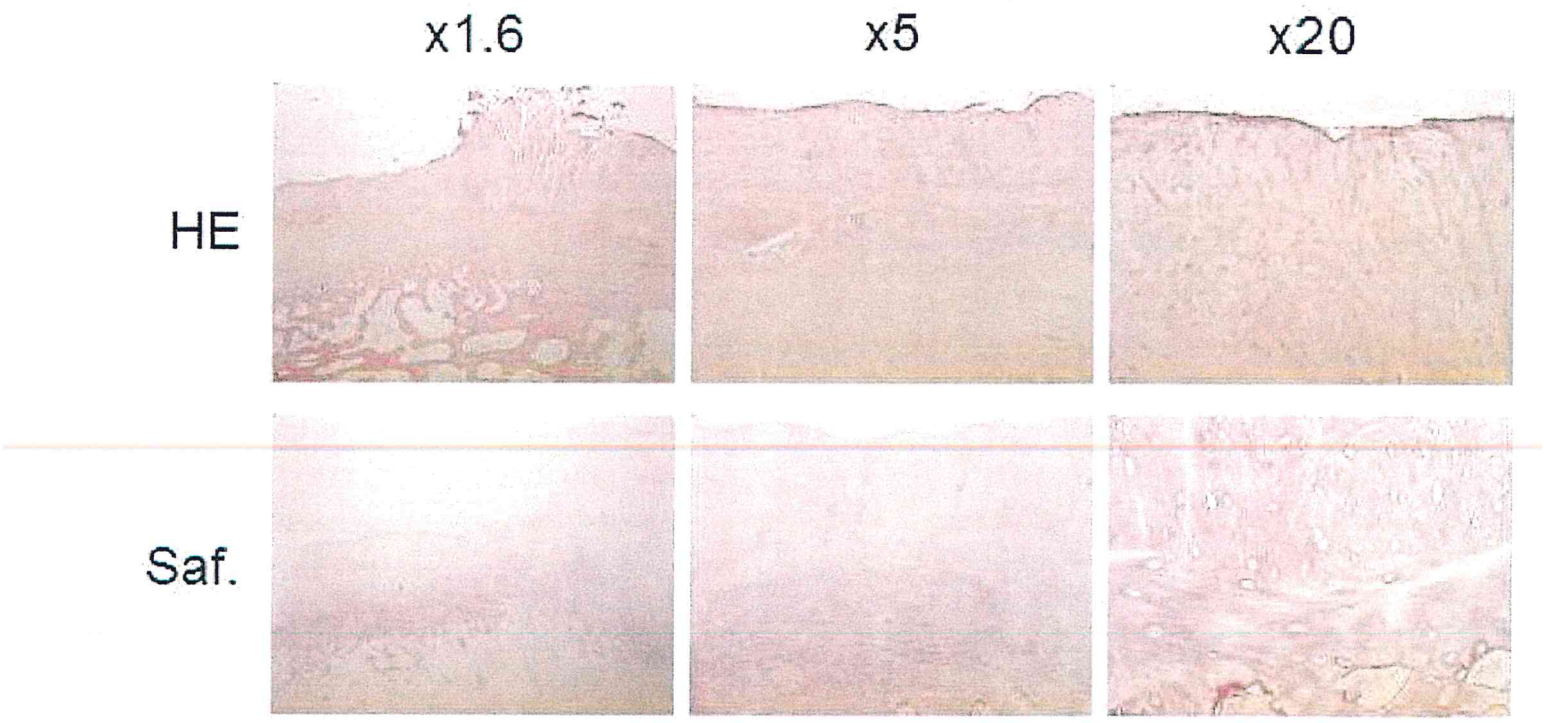


Fig.7c: Histological preparations of defects treated with 20fold compressed collagen implants 3 months after treatment

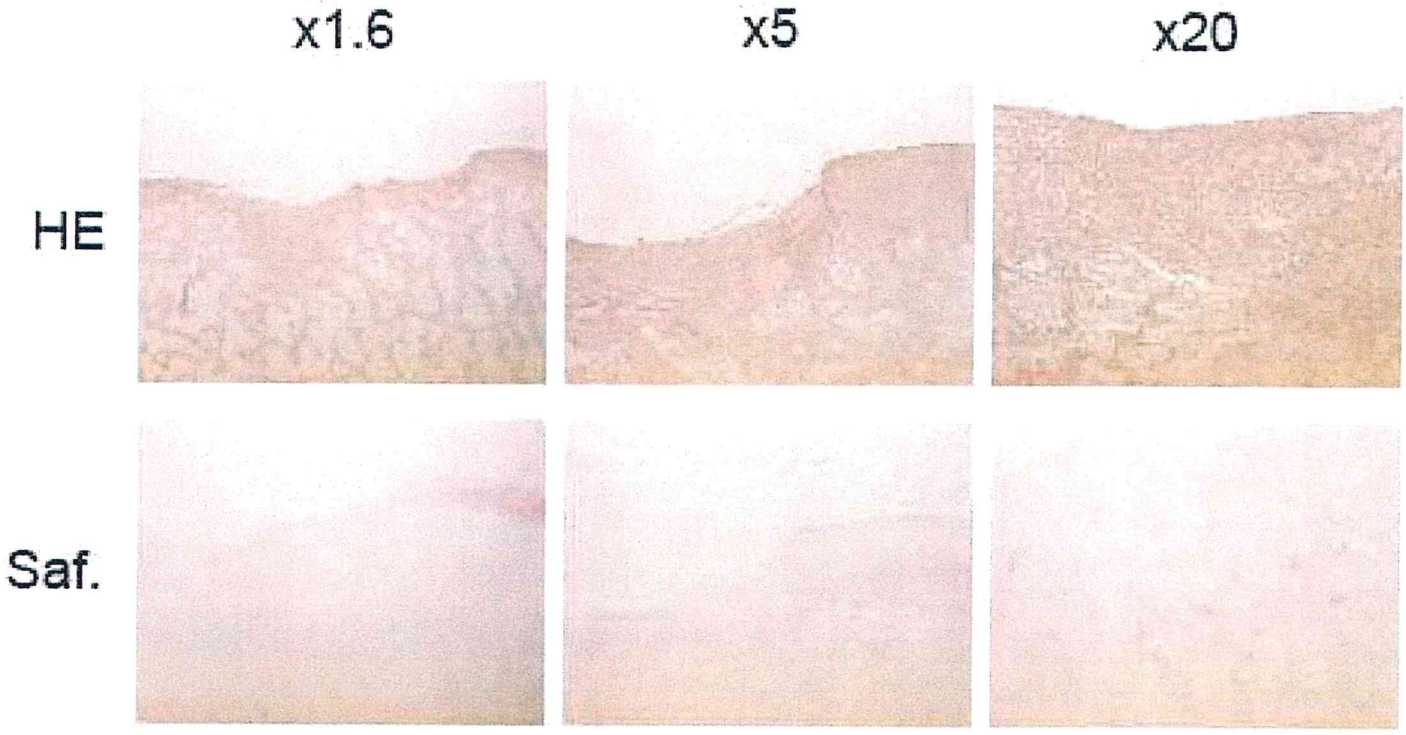


Fig.7d: Histological preparations of defects treated with the 4-1 concentrated collagen implants 3 months after treatment

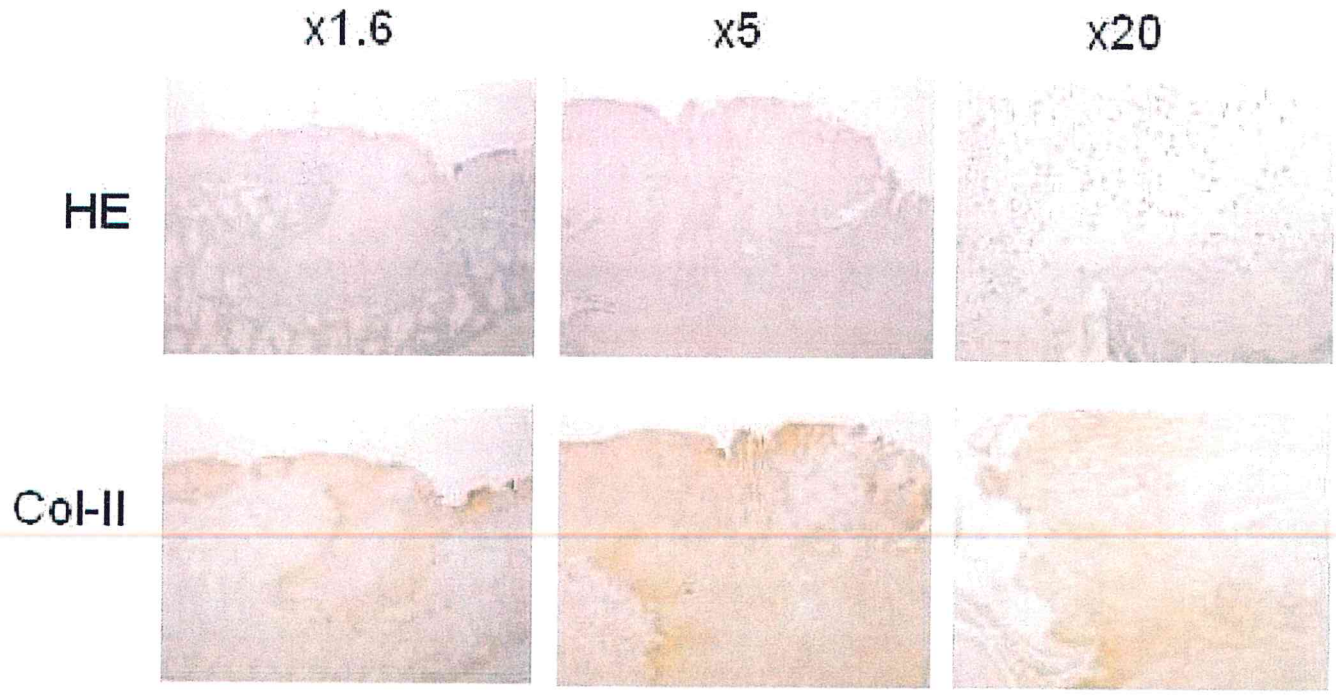


Fig.8a: Histological preparations of the untreated defect after one year

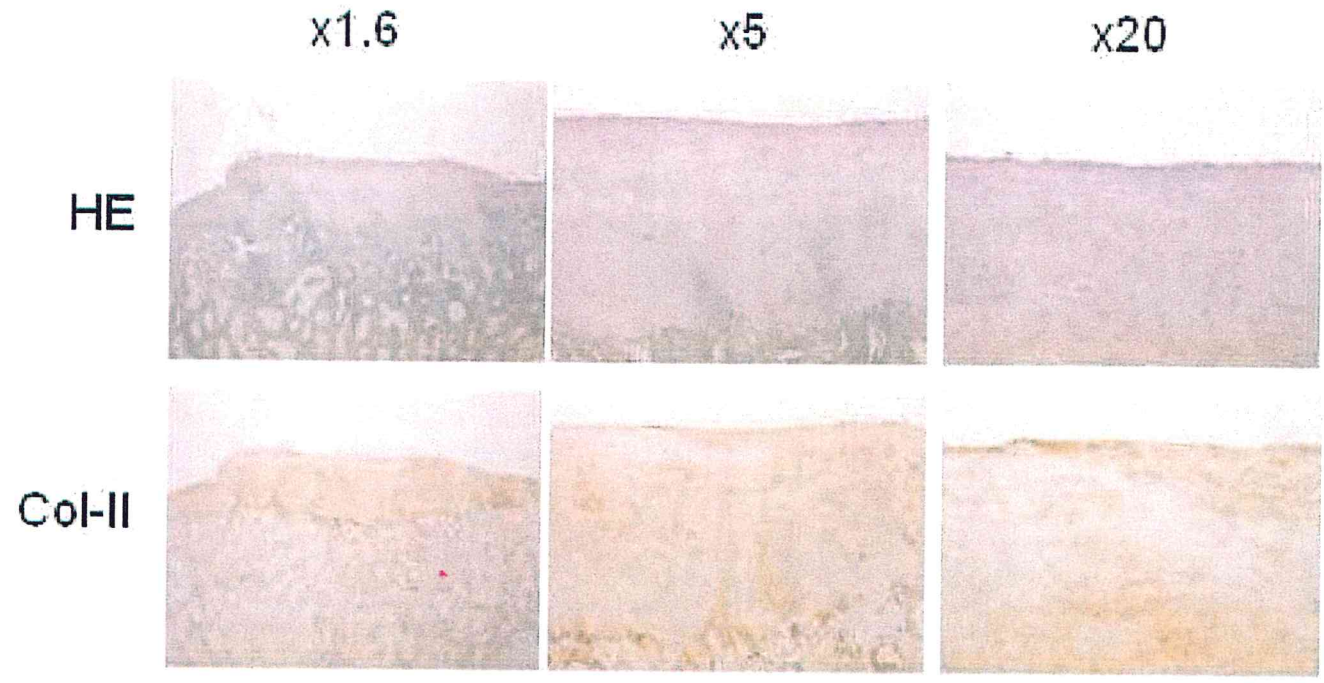


Fig.8b: Histological preparations of defects treated with Chondrofill liquid one year after treatment

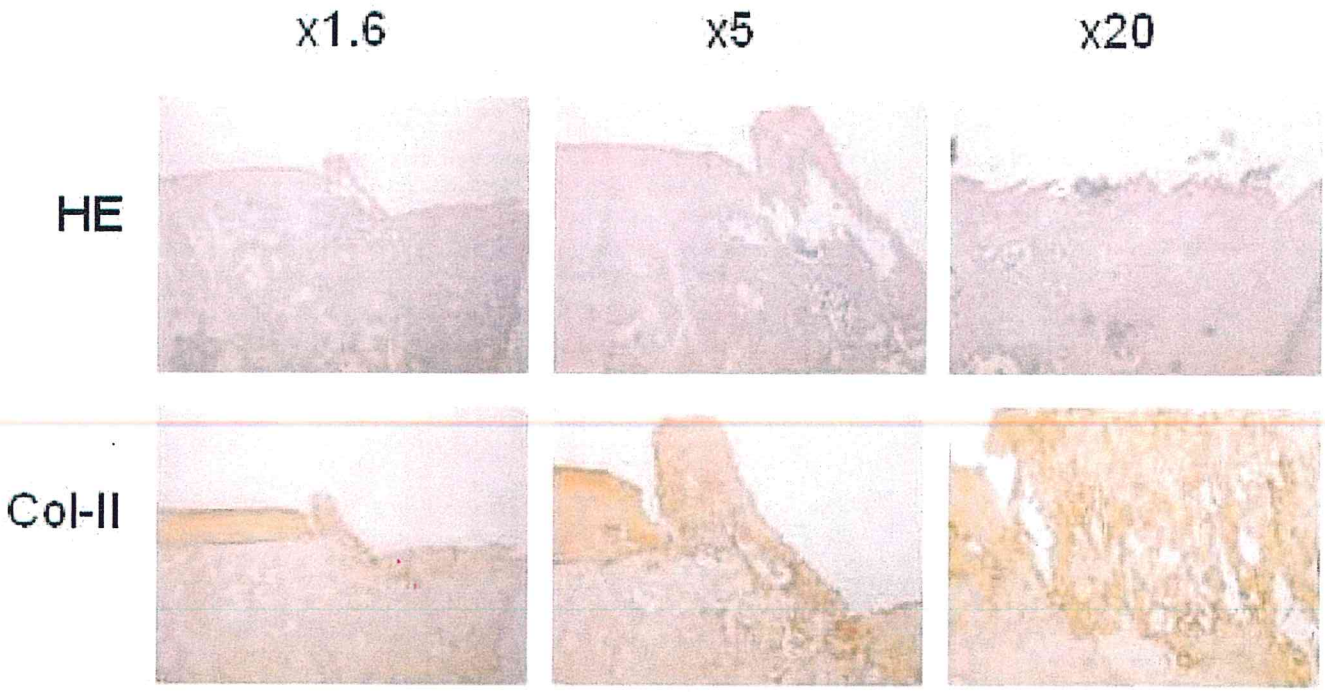


Fig.8c: Histological preparations of defects treated with 20fold compressed collagen implants one year after treatment

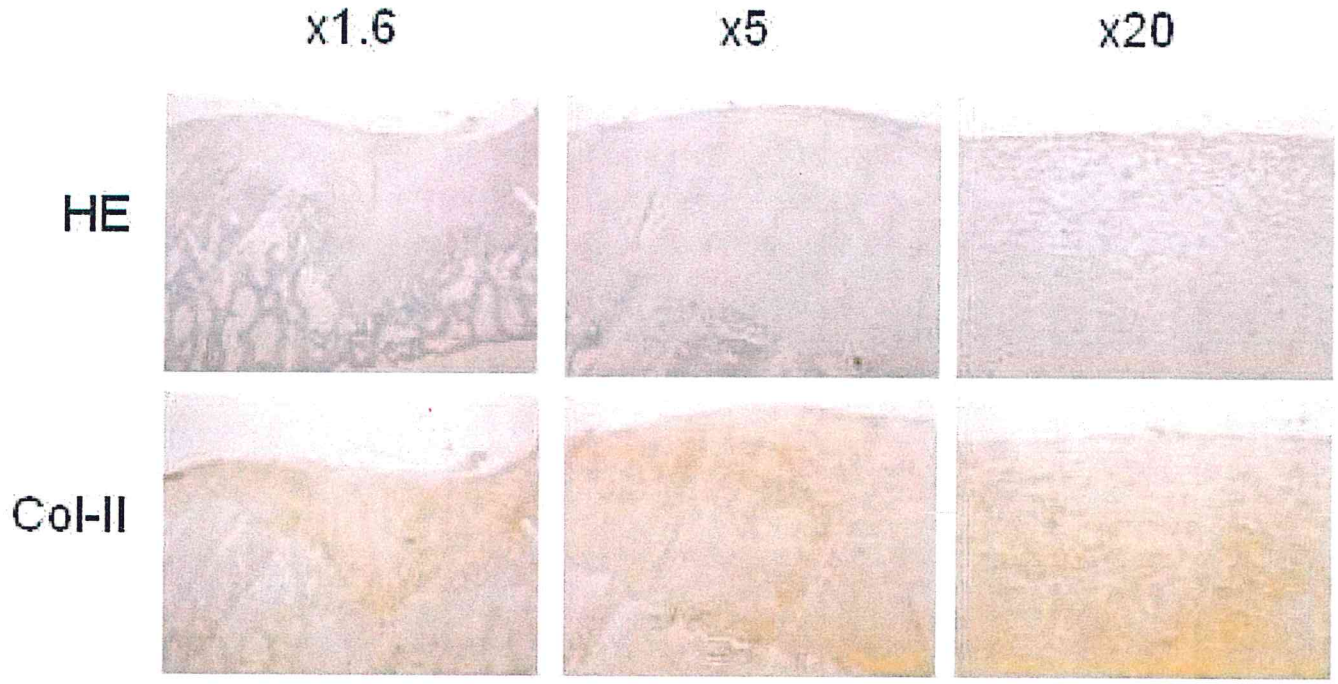


Fig.8d: Histological preparation of defects treated with 4-1 concentrated implants one year after treatment

The analysis of the mechanical properties in the particular reparation zones after estimation of the e-module at the time 3 months showed the following result:

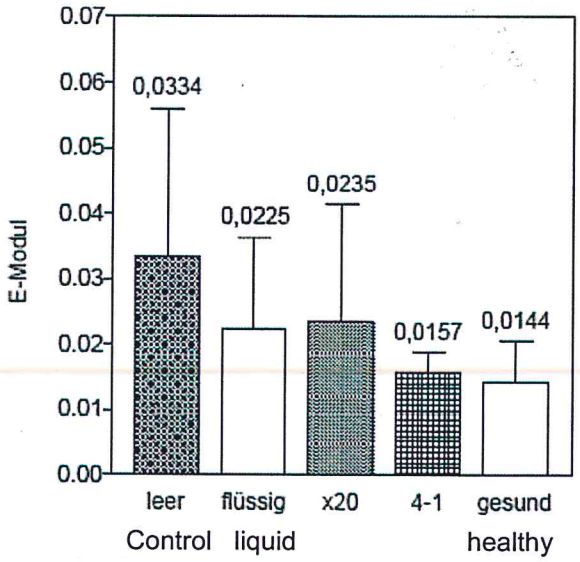


Fig.9: E-Modules in the particular defects after an examination interval of 3 months: The 3 defects treated with collagen showed compared to the healthy surrounding cartilage the best results. The untreated control defect has the worst e-module.

An examination of the mechanical properties of the preparations after 1 year could not be carried out on grounds of organizational problems.

## *Summary*

The results of this study show that the quality of the reparation tissue is dependent on the processing and kind of the application of the used collagen gel. The best results could be achieved by using Chondrofill liquid and the 4fold compressed Chondrofill gel. Already after 6 and 12 weeks the best morphological and also histological results could be achieved in these both groups of treatment. The mechanically more stable, highly compressed gel (Chondrofill 20x) seems to have biologically no advantage to the less concentrated available collagen mixtures. The initial results after 6 and 12 weeks could be confirmed by the longer-term results after one year. The findings of the biomechanical testing confirm the concept of a collagen gel implantation for the support of cartilage reparation. Already after 3 months mechanical properties comparable to healthy surrounding cartilage could be found for the defects treated with collagen.

In no case undesirable accompanying reactions were found like inflammations or further irritant phenomena which could be led back to the implants. The processing of the used implants was extremely user-friendly and taking into account the specifications of the manufacturer practicable without any problems.

This translation was provided by Diana Baumann (Amedrix GmbH) and is based on the German Version written by Prof. Dr. Ulrich Schneider, dated 17.03.2012.