Data related to the problems of biodegradable materials in the treatment of articular cartilage defects

Animal experiments filling up the donor areas of autologous osteochondral mosaicplasty

Human experiences with osteoinductive biodegradable substance

Theses of Ph.D.

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Introduction

The biomechanically solid hyalin cartilage is unable to regenerate in adults. The mature chondrocytes embed into the matrix are able to proliferate only incompletely for a short period of 10 – 14 days.

The treatment of full thick defects of the joint surfaces is one of the most significant challenges of the orthopedic practice. Regeneration process following injuries is performed by the pluripotent mesenchymal cells of spongiosa exposed by the fissures of the subchondral cortical bone. This repair is the spontaneous experiment of the organism, via the following phases: vessel invasion → blood clot creation → connective tissue ingrowth → fibrous metaplasia → development of reparative fibrous cartilage. It is an inappropriate process, since consistent sliding surface of satisfactory thickness never develops, the biomechanical value of the reparative fibrous cartilage is poor, it's quality does not match with the loaded surface demands.

Beside the traditional surface formation procedures a number of new techniques was published in the last decade to construct hyalin or hyalin-like surface at the damaged areas. Data of theoretical research, experimental results and preclinical evaluations have promoted the wide range clinical popularity of

"microfracture" technique and autologous osteochondral mosaicplasty. Apart from these widespread procedures autologous chondrocyte transplantation seems to be suitable method to treat hyalin defects.

In the last decades especially intensive research is ongoing to find the methods to fill the full thick defects of the joint surfaces with hyalin quality substance to prevent OA. In this paper the actual surface composition procedures, the recent results of surface creation are presented along with my own animal and clinical experiments.

Objective

Advantageous experience in the clinical practice is only proven regarding autologous chondrocyte and osteochondral transplantation.
I set the objective to develop these cartilage surface reconstruction procedures to find a connective chain link serving the advancement of both methodology.

In animal experiments I investigated a biodegradable substance used in filling up the donor areas, to prevent a rare potential complication, the postoperative joint effusion. My endeavor was to include such materials to fill up the donor areas in the animal experiments, which have no license yet for human procedures.

Having been encouraged by the success in animal experiments I started to research the clinical use of bioresorbable implants. The suitable substance – as expected also in animal investigations – must support and enhance the invasion of mesenchymal stem cells from the cancellous bone. The invasion of mesenchymal pluripotent cells perform reparative tissue formation on the cartilage surfaces. The transplanted material ideally is degradable, resorbable in filling up the donor channels as well as the damaged joint surface. Since the attention of the researchers recently has turned into the direction of materials, which do not require cartilage cell culturing, available "off the shelf", the expected characteristics of the two substances used in my animal investigations and in my clinical practice reached a matching point here. My work was facilitated by the fact, that well reputed research centers agreed the cylindrical shaped surface replacement methodology. Therefore I carried on my work adhering the cylindrical implantation introduced by Hangody. My objective was to find a substance inhibiting the bleeding of the donor site and to form an implant having similar biological, physicochemical characteristics which enhances the renovation of the surfaces.

Methods

Animal tests

In my animal experiments 6 biodegradable substances were tested. These materials are used for implants in the dentistry, except the compressed collagen, which has no license for human use. Polyglyconate, two

kinds of polylactate, carbon rods, hydroxyapatit, and collagen based implants were used. These were implanted into the 100 knee joints of 50 German shepherd dogs to examine their feasibility to use to fill the donor sites of the mosaicplasty. The hydroxyapatit, polyglyconate-B, carbon rods and the compressed collagen were implanted to 20-20 knee joints of 10-10 dogs, in two sessions. Two types of polycaprolacton were implanted to 10-10 knee joints, using the same method. In each case the first operations were done in the left knees, 4 weeks later in the right knees.

Fig. 1. Polycaprolacton, compressed collagen, polyglyconate and carbon rod samples

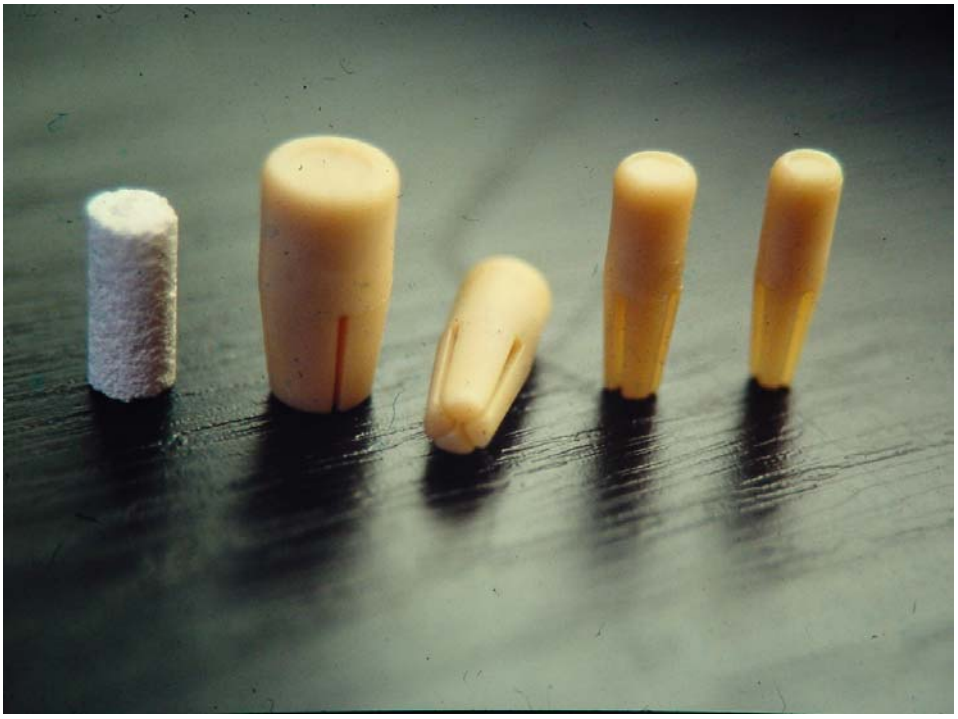

Fig. 2. Hydroxylapatit and polyglyconate plugs

All surgery was done in general anesthesia. Hydroxylapatit and polyglyconate-B, the carbon and compressed collagen were implanted in the forms of special plugs (Fig. 3.) or as rods with 15 mm length. Both forms of the polycaprolacton was heated and injected as fluid to the donor area.
Control group of 6 dogs (12 knees) were used to test the extent of postoperative bleeding. In this group all donor channels were left open. Veterinary doctor diagnosed the bleeding of the donor sites, and each case was noted, where following physical investigation 5 ml or more blood was evacuated.

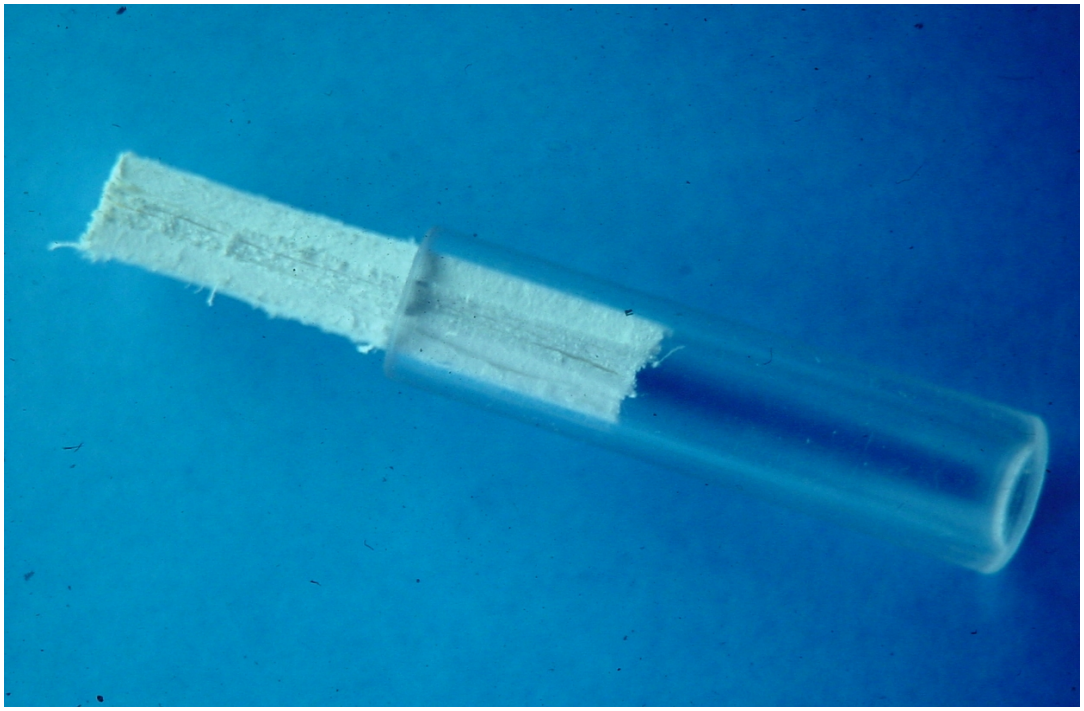

Fig. 3. Compressed collagen plug for the animal test

Clinical tests

Out of the substances tested in my animal experiments, compressed collagen was considered as potential donor filling material. This material however had no license for human use. Therefore a synthetic material, Poly Ethylen Oxyd Terephtalate / Poly Butylene Terephtalate, in short PolyActive-B was elected for our clinical tests. In 2005 we pioneered in the World filling up the donor site in human mosaicplasty procedures with PolyActive-B! This material has been approved by the FDA in the USA, this fact was also important in our choice. Based on advantageous animal tests and on routine oral surgical practice the assumption was made, that this material will not purely replace bone, but it creates

fibrous cartilage on the surface, fulfilling our expectations. The study was approved by an independent ethical committee and registered at ICH GCP EN 540 according to the Helsinki agreement in 2000.

Results

In our animal tests all substances inhibited effectively a substantial postoperative bleeding (4%) compared to the unfilled control (41,6%; P=0.00006). The evaluation of rigidness on the surfaces of the polyglyconate and compressed collagen plugs and also the histology proved cartilage – like parameters over thee filled donor areas. In cases of hydroxyapatit, polycaprolacton and carbon rods no acceptable fibrous cartilage creation was found in the sliding surfaces. In surfaces of hydroxyapatit, polycaprolacton a weak layer of connective tissue was found on the surfaces with poor cell content. The carbon rods induced acceptable granulation tissue formation 6 months after implantation, but the tissue was only scar. Only in cases of polyglyconate and compressed collagen plugs was consistent fibrous cartilage formation observed after 12 weeks, this layer was of excellent quality on the samples taken at 26^{th} and 30^{th} week. The donor areas hold usually relatively less load in the knee, but occasionally they are sustained to substantial contact pressure, therefore fibrous cartilage of good quality is only acceptable. At the control (second-look) arthroscopies good sliding surfaces were found on the top of the implanted plugs. Based on the histological results the compressed collagen seems to be the optimal implant to fill the donor channels.

The PolyActive-B plugs implanted in human donor areas also prevented bleeding and enhanced the healing of the donor sites.

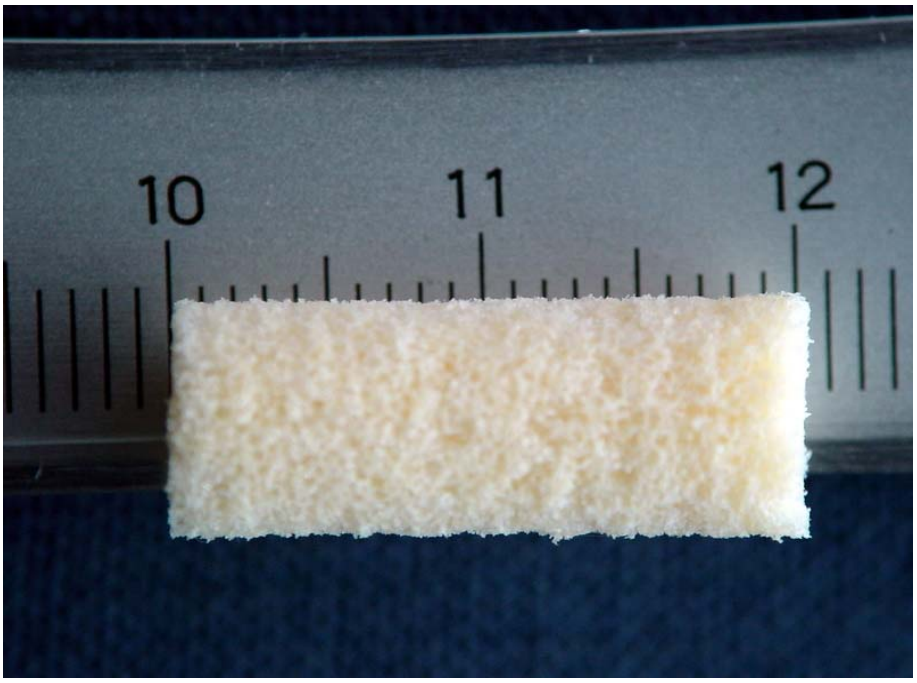

Fig.4. PolyActive-B implant

The material of implants easily penetrated by blood, permitting invasion of mesenchymal cells. They provide good base the create fibrous cartilage on the surface and does not limit vascular and tissue integration in the deeper layers. PolyActive-B is a safe biocompatible substance, no intraarticular inflammation or foreign body reaction was detected.

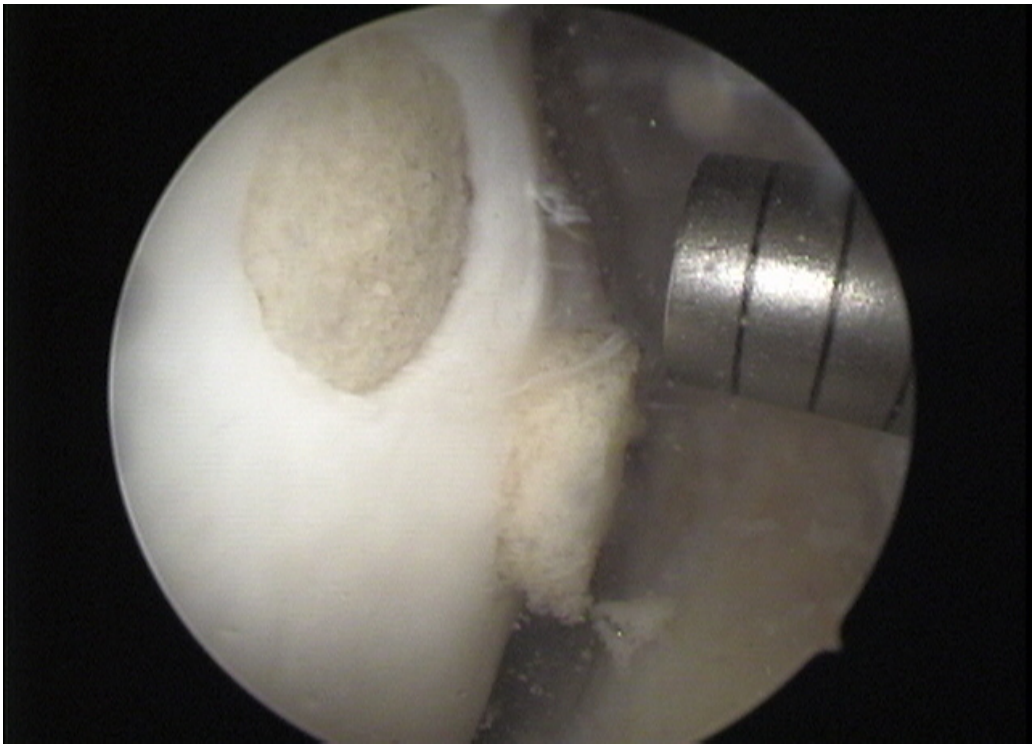

Fig.5. Arthroscopic filling with PolyActive-B.

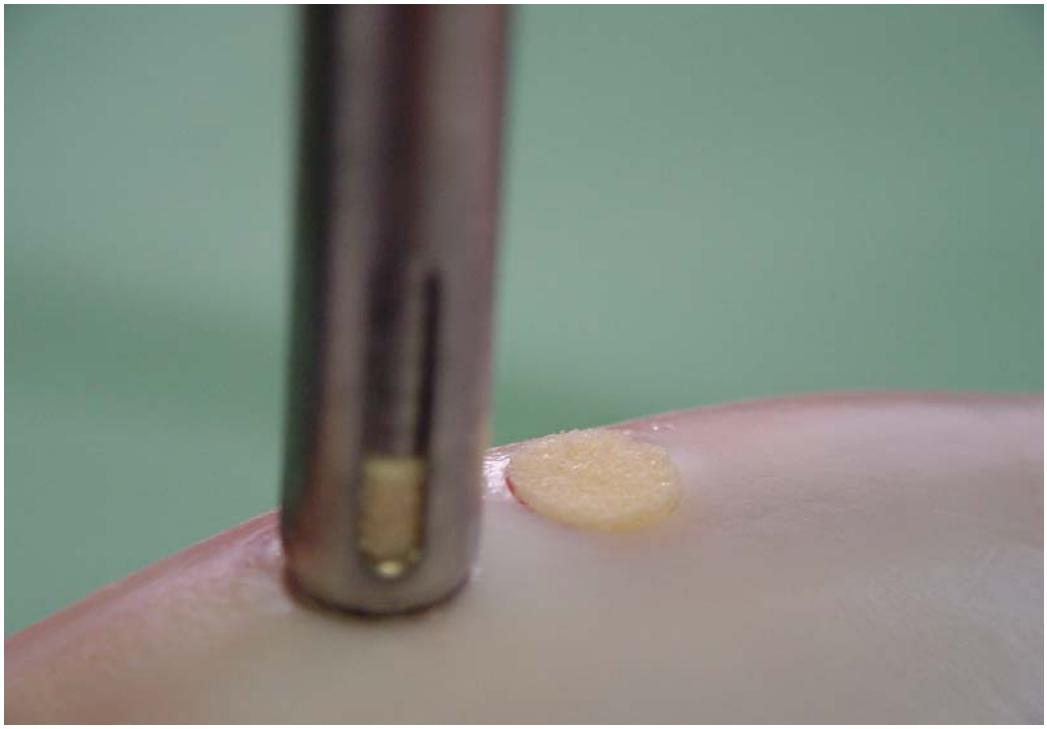

Fig.6. Filling with PolyActive-B from arthrotomy

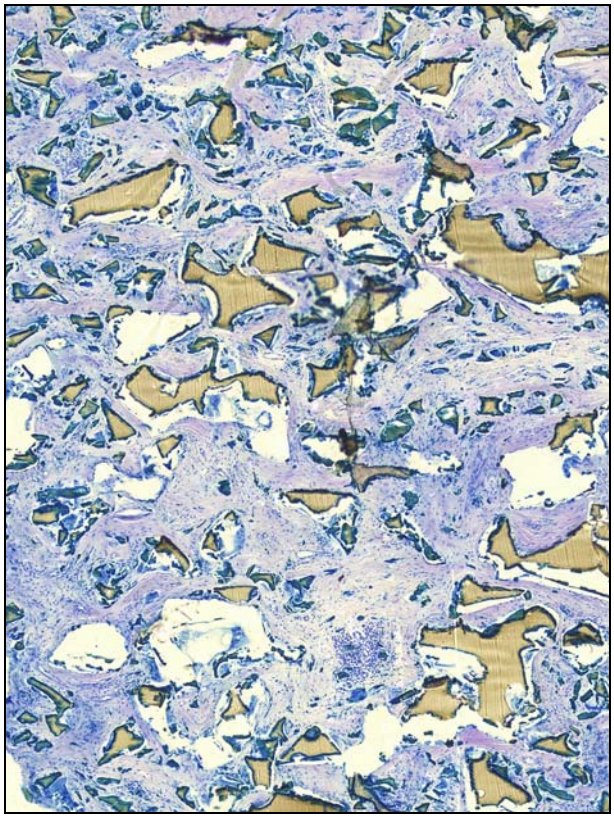

Fig.7. Fragments of disintegrated PolyActive-B are visible in this specimen stained with dimethylmethylen-blue (DMMK 150x)

Good integration of the implants to the surrounding bone was confirmed with MRI apart from histological analysis.

Conclusions

The substances used in animal tests all blocked postoperative bleeding. Only the compressed collagen however fulfilled the other important criteria and promoted impregnation of blood, allowing invasion of stem cells and cellular and vascular invasion. A further criterion was to allow creation of acceptable fibrous cartilage on the surface. According to the histological results only the compressed bovine collagen correspond to this conditions.

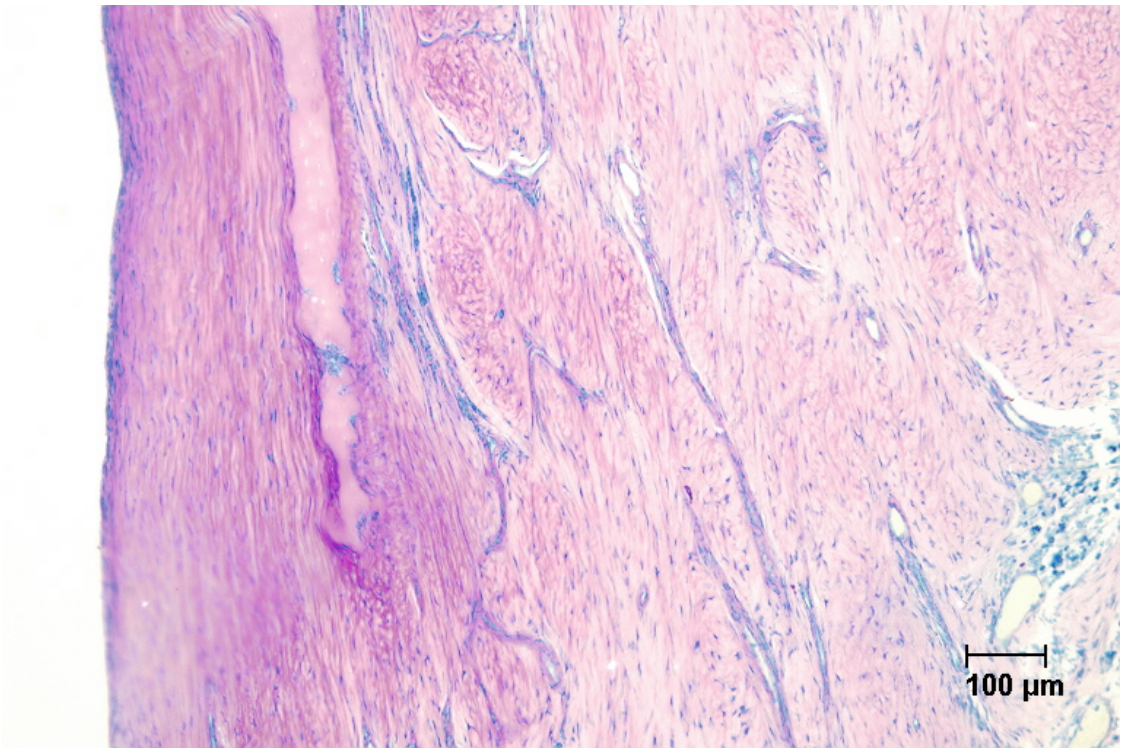

Fig. 8. Compressed bovine collagen plug after 10 weeks. The newly formed thin walled vessels are visible (DMMK 100 x)

The procedure using the available compressed cylinder form is easy, the plugs intended to fill the donor sites can be driven home without difficulties and attach there reliably due to their elastic characteristics. Macroscopic and histological test proved that the newly formed regenerative tissue is at level with its environment providing the necessary congruence.

Conclusions of clinical research

In the clinical tests the PolyActive-B plugs implanted in human donor areas prevented the postoperative bleeding of the joint, at the same time they did not hinder the healing of the donor channels. Conclusion based on our histological analysis prove that the PolyActive-B plugs got impregnated with blood, facilitating such a way the invasion of mesenchymal stem cells. They formed an appropriate scaffold structure to ensure a good quality base for the reparation of the osteochondral defect. Healing the defect resulted in good quality filling. One of the most significant result of our clinical test is that the quality of the reparative fibrous tissue which formed over the filled donor channels is not worse than that of the reparative tissue formed over the unfilled donor sites. As the MRI and the arthroscopies proved, the fillings were at level with the joint surface. The surfaces over the unfilled donor sites were fragmented and softer.
Inside the PolyActive-B plugs excellent vascularization and cellular integration was observed. Part of the implants degraded, fragments found in histology prove biodegradability. Proper subchondral bone formation was found. No inflammation, arthrofibrosis or foreign body reaction was detected, proving the biocompatibility of the PolyActive-B plugs.

Animal experiments and clinical tests reveal that selection of a proper filling substance is not easy. We concluded that to create good quality reparative tissue on the damaged surfaces is possible with implantation of proper biodegradable scaffold structures without using cartilage tissue. These observations prove, that if biodegradable structures are implanted into cancellous bone and mesenchymal stem cells invade via bleeding, this unconditionally is advantageous for the reparation of the subchondral area and the damaged surface. Mesenchymal stem cell invasion is the precondition for the fibrous cartilage healing. On the surface of the compressed bovine collagen the same reparative fibrous cartilage cover can develop, which is commonly observed above the unfilled donor areas.

The most significant result of my work is to discover, that both substances used in my animal and clinical tests blocked the unwanted postoperative joint bleeding.
New result is in my work to prove, that both the compressed collagen used in my animal tests and the PolyActive-B used in human experiments fills up with blood according to our expectations. Our tests proved that these materials can provide the planned and suspected mesenchymal cellular invasion. Since bovine collagen can not be used because of the risk of the Jacob Kreuzfeld disease, other derivate is to be researched instead, e.g. chicken collagen.

The second and equally important result of my work is to prove the creation of congruent surfaces using PolyActive-B in the human tests. Histology proved the degradation of this substance with local new bone formation. Surface of the PolyActive-B plugs enhance creation of fibrous cartilage. The reparative tissue on their surface is similar or better quality compared to the reparative tissue over the unfilled channels. Mesenchymal stem cell invasion from the bone marrow play a key role in the osteochondral reparative process.

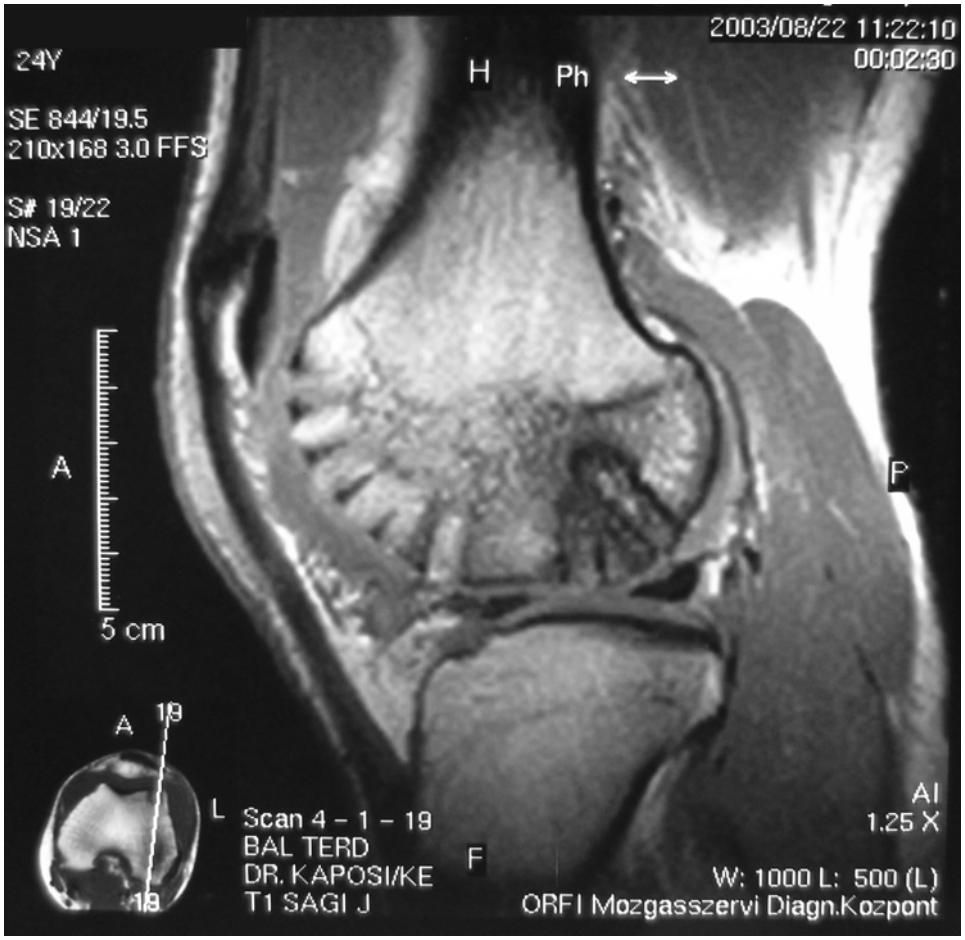

Fig. 9. MRI scan of the donor areas filled with PolyActive-B plugs.

The PolyActive-B plugs therefore provide proper environment for the settlement, differentiation, proliferation of these cells. and for the creation of proper fibrous tissue and remodellation of the bone.

Based on research and theoretical considerations the PolyActive-B could be the basis to find further cartilage surface creative techniques. Combination with other methods and biologically active materials could be a promising possibility of future research. Such a biologically active material is the growth hormone, which has been included into the experiments aiming the reconstruction of the cartilage surface.

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