

# The initial bearing capacities of subchondral bone replacements considerably contributing to chondrogenesis

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The degeneration of articular cartilage results from osteoarthritis and many other forms of severe arthritis. Current treatments for cartilage repair are less than satisfactory, and rarely restore a full function or return the tissue to its natural state. The leading strategies in the treatment are aimed at the transplantation of cells and/or the use of various biological grafts, bioactive agents, or biologically compatible implant matrices. The insertion of a crushed autologous bone graft has been reported as a possible therapy. However, the regenerative quality of the tissue was less than 70% of healthy cartilage for fragments and controls. The implantation of cycloolefin-blend 3D-cylinders with hydrogel scaffolds on their proximal parts and with the applications of type I collagen films is one of several surgical therapies. The replacement and continuous biomechanical properties of the subchondral bone play an important role in the morphology and the quality of chondrogenesis. The initial biomechanical stability of COC-blend polymer replacements in the subchondral bone contributes to the formation of a new cartilage tissue. The initial bearing capacities of the implanted tissue/replacements and vertical positions of the replacements have a principal influence upon both the quality and the quantity of new articular cartilage.

*Key words: biomechanics, osteochondral defects, polymer replacement, hydrogel scaffold, cycloolefin-blend, bioactivity, articular cartilage*

## 1. Introduction

Hyaline cartilage shows only a limited response to self-repair (HUNTER [1]). Many people are stricken with degenerative osteochondral defects. Modern therapies of osteochondral defects are focused (for example) on the transplantation of osteochondral autografts, crushed spongiosa with collagen, chondrocytes and many others. The insertion of a crushed autologous bone graft has been reported as a possible therapy. However, the regenerative biomechanical (material) quality was less than 70% of healthy cartilage for fragments and controls (KLEEMANN et al. [2]). The transplantation of

autologous osteochondral 3D-cylinders is one of several surgical therapies (HORAS [3]). During operations osteochondral defects are filled with material of a natural histological structure. However, the subchondral bone plates are interrupted and the biomechanical stability between the original tissue and the transplanted tissue is different. Provision of a long-term functional stability of inanimate implants in live surroundings is a complex and quite uneasy task. The development of replacements for a human subchondral bone and articular cartilage follows the path of a proposal and investigation of such materials whose mechanical properties are very similar to the biomechanical properties of a bone/cartilage tissue and

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whose biophysical and biochemical interactions with the surrounding living tissue neither cause necroses, nor lead to any initiation of other pathological processes. The biophysical and biochemical fixation of replacements and/or scaffolds to the tissue depends dominantly: (a) on the biomechanical properties and biochemical environments of the implants and the tissue; (b) on the stress–strain distributions in the tissue and the replacement, (c) on the organization and stability of collagen molecules adsorbed to modified surfaces of COC-blend replacements and (d) on the chondrogenesis on the hydrogel scaffold.

*Our activities were aimed at forming new articular cartilage and subchondral bone using biocompatible and bioconductive polymer replacements.*

## 2. Methods

Finding the optimal biomechanical, biophysical and biochemical conditions for chondrogenesis is a very complicated and difficult task. The aim of our research is to assess the principal conditions improving the treatment of osteochondral defects. We have been focused preferentially on the application of biomaterials with material properties close to the natural properties of the relevant tissue. Special attention has been focused on the surface modification of the COC-blend by the action of nitrogen and/or oxygen microwave plasma, the application of type I collagen, the application of chitosan and the influence of the vertical position of replacements in the localities of osteochondral defects.



Fig. 1. Bi-component replacement of the subchondral bone (in the distal part, nontransparent material) and the articular cartilage scaffold (partly, in the proximal part, transparent hydrogel)

The presumed concept applies a substitute consisting of two supporting polymer components (see

figure 1 [4]). One of them (the lower element) is composed of a polycycloolefinic (blend) material (with the modulus of elasticity  $E = 0.5\text{--}3$  GPa, the diameter of 8 mm, and the length of 10–12 mm [5]), while the upper hydrogel scaffold element of poly (2-hydroxyethylmethacrylate) has the relative modulus of deformation  $E_{r,\text{def}} = 1.5$  MPa, the diameter of 8 mm and the thickness of the upper plate of 1.1–1.3 mm, figure 2. The COC-blend substance was made with spherical/ellipsoidal pores (with a diameter of 0.6–1.5  $\mu\text{m}$ ), figure 3.



Fig. 2. Poly (2-hydroxyethylmethacrylate) scaffolds – upper parts of replacements



Fig. 3. Surface of COC-blend with designed pores with diameters ranging from 0.6 to 1.5  $\mu\text{m}$

In order to improve the bonding between the polymer blend and collagen, the surface of the polymer matrix was modified by the action of a nitrogen and/or oxygen microwave plasma. The plasmatic modification resulted in a significant increase of surface hydrophilicity demonstrated by a decrease of water contact angle.

The plasma modification [6] was carried out in a MW reactor equipped with the SLAN I OV 425 (Plasma Consult) magnetron operand at 300 W, 80 Pa, a gas flow of 15 scm/min and with exposure times of 15 min. For water contact angle measurements, the SEE System was used (Milli-Q water droplet volume 2  $\mu$ L).

After optimizing conditions for the surface modification with regard to achieving the highest hydrophilicity of the surface, the samples were examined by the XPS method with the aim of identifying their chemistry and the population of individual chemical groups present on the surface. After plasmatic treatment, the components with higher values of binding energy occurred in the C 1s spectra of electrons (see figure 4).

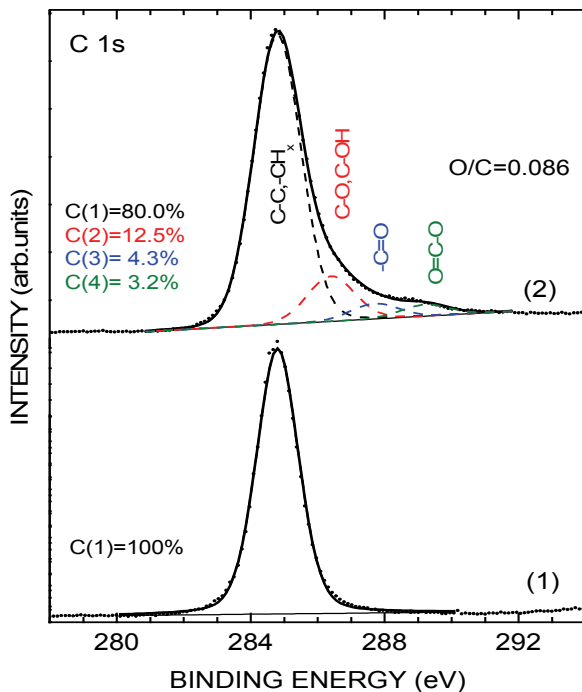


Fig. 4. Spectra of C 1s electrons of (1) unmodified and (2) plasma-modified surface of polymer blend

The aging of modified surfaces was also studied by XPS and by water contact angle measurements. The adsorption of collagen I on untreated and treated polymers was studied by XPS and AFM methods. XPS measurements were carried out using the ESCA 310 (Scienta) spectrometer. Electrons were excited by Al K $\alpha$  monochromatized radiation. For the visualization of surface topography, the AFM Nanoscope IIIa (Digital Instruments) in the tapping mode was used.

The upper components of the replacement were made of poly-hydroxyethylmethacrylate with chitosan without any additional plasma surface treatment. Osteochondral defects (depth: 12 mm, diameter: 8 mm)

were created in each lateral and medial tibial condyle of the right and left knees in 6 adult pigs. Histological analyses of the cartilage matrix were accomplished after 6 and 4 months.

### 3. Results

The replacements developed and plasmatically modified/unmodified under in vivo conditions have been proven as bioactive, bioconductive and biotolerant materials. It is well known that collagen adsorption promotes cell adhesion and proliferation.

The measured spectra of C 1s electrons showed the presence of components belonging to C–O, C=O and O–C=O functionalities (see figure 4). For examining the collagen adsorption, stable samples whose surface composition did not change with time were used. The presence of adsorbed collagen was indicated by the presence of the spectrum of N 1s electrons in the spectrum and its morphology was visualized by the AFM method. It was observed that on untreated, hydrophobic smooth polymer surfaces comparable or larger amounts of collagen are adsorbed than on hydrophilic surfaces, but immobilized collagen tends to form aggregates on hydrophobic surfaces. On hydrophilic, plasma-modified surfaces, more homogeneous coverage by collagen is observed.

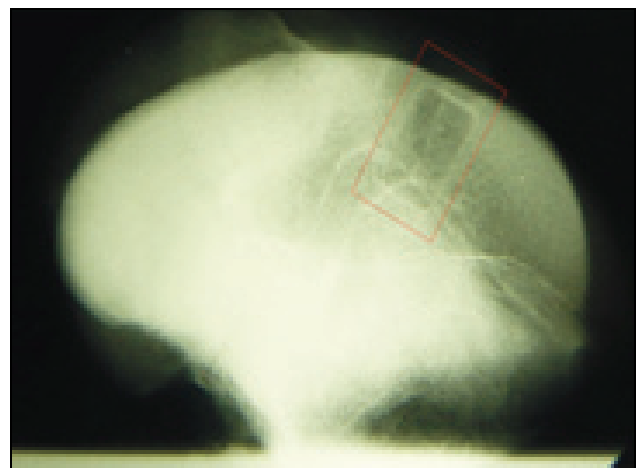


Fig. 5. X-ray stability analyses of replacement + scaffold with the new articular cartilage. Excellent stability of replacement in the subchondral bone without necrosis

The developed subchondral bone around the COC-blend had the same quality as a natural healthy one [7]. The new subchondral bone mineralized perfectly. The mediator C+TGF films (made from type I colla-



gen and from growth hormones TGF- $\beta$ ) applied on the COC-blend surface contribute to the creation of stable encapsulation (figure 5). Verifying the C+TGF films on the COC surface (in vitro) showed very good cell proliferation and cell differentiation. The modified surface exhibits enhanced adsorption of collagen and improvement of its adhesion. Stronger bonding explains a higher quantity, better organization and better stability of collagen molecules adsorbed on oxidized surfaces.

The polymer replacements installed into artificially executed osteochondral defects of porcine tibial condyles, including both modified and non-modified implants, demonstrated a perfect tolerability and appeared to heal into the existing subchondral bone without any displacement or evidence for necrosis. Histological findings and morphological changes of osteochondral samples did not demonstrate any pathological features. The top surfaces of the bi-component replacements were overgrown with viable new articular cartilage (figure 6) or with articular cartilage and partly with fibrocartilage (figure 7).

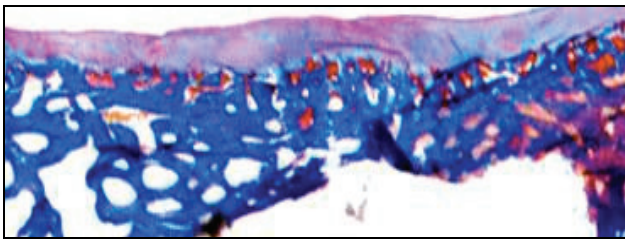


Fig. 6. Tissue bridge from articular cartilage and subchondral/spongy bone across the HEMA scaffold

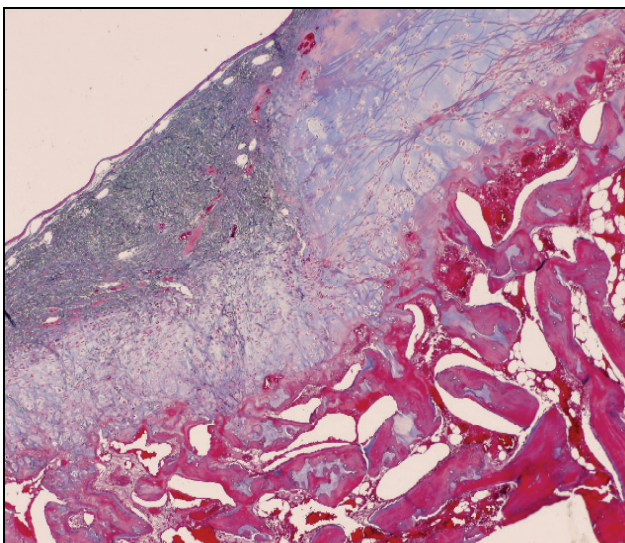


Fig. 7. Top surfaces of the hydrogel scaffold were overgrown with subchondral bone (the lower layer), with viable new articular cartilage (the medial layer) and partly by fibrocartilage (the upper small part)

The biomechanical in vivo environments have particularly potent regulatory effects on chondrogenesis, both in terms of proliferation and the new matrix synthesis. The matrix synthesis is regulated by mechanical stimuli and depends on the *initial high stability of subchondral bone COC-blend replacements* (see figure 6).

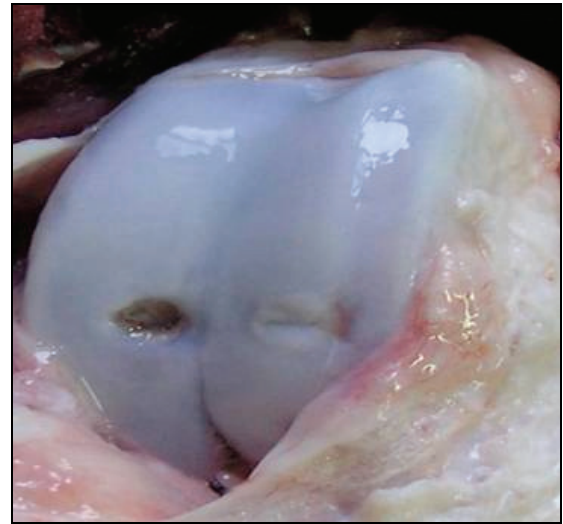


Fig. 8. New articular cartilage after 12-week chondrogenesis (the right tibial condyle) and the control defect (the left tibial condyle)

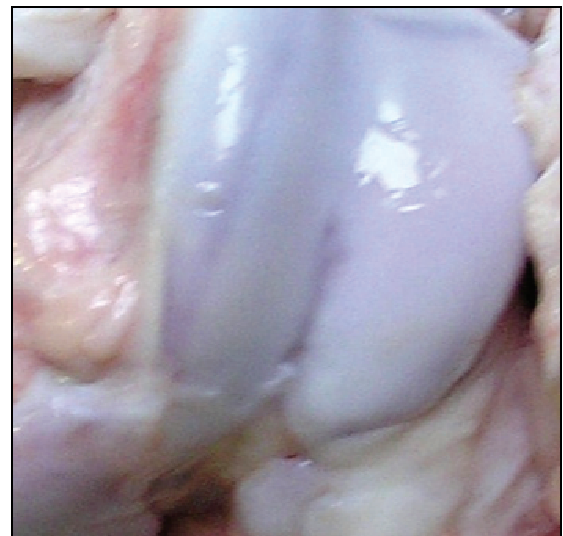


Fig. 9. Chondrogenesis after 20 weeks in both the left and right tibial condyles. The application of chitosan (0.3% liquid, pH 5.5), TGF- $\beta$  (1.2 mg/1 ml PBS) type I collagen (0.3%), surface plasmatic modification of COC-blend

## 4. Discussion

The leading strategies in the treatment of osteochondral defects are to minimize the operative trauma

by minimally invasive procedures, to stimulate chondro/osteogenesis and/or to regenerate the tissues. Operative approaches are becoming ever smaller. Current and future concepts are based on a better understanding of biomechanical conditions and local mechanisms of healing, tissue regeneration and prophylaxis.

The local application of *growth factors* is investigated in clinical practice and has a great potential in treatment [8]. The reason for a *limited acceptance* in clinical use may be that the applied *proteins are expensive and with limited availability, and considerable quantities have to be implanted locally* [8].

Other relatively new innovative techniques include *the stem cell therapy*. The application of autologous stem cells taken out and re-transplanted can also be used for healing [8]. However, this manner of treatment depends on the appropriate biomechano-chemical conditions of the tissue. The healing of osteochondral defects is controlled both mechanically and biologically. The processes of osteo/chondrogenic differentiation are slightly promoted by mechanical effects [14]. The cells are very sensitive to small strains. The physiological balance between the microstrain magnitude and biochemical stimulation can be easily disrupted when the subchondral/spongy bone is pathologically a soft one. In the case of an unstable subchondral bone and spongy bone, the articular surface of cartilage is affected by small sags [7].

The regeneration of osteochondral defects treated with *crushed bone grafts* is in verified cases accompanied by the presence of soft regenerated tissue [2]. The regeneration of osteochondral defects treated with crushed bone grafts remains incomplete still after three months. However, the inserted bone graft can be completely absorbed. After six months, the connective tissue within the defect is transformed into a bone and fibrocartilage tissue through enchondral ossification [2]. *The surface of the regenerated joint area is rough and irregular*. The regenerate mechanical quality was 61%–70% of healthy cartilage for treatment and control, respectively. Although this method was reported as successful in the clinical treatment, it failed to enhance the quality of regenerated defects in the case of a sheep study [2]. It must be noted that the stability of biomaterials substituting pathological subchondral/spongy bone tissues is the fundamental condition for the regeneration of osteochondral defects.

Osteoarthritis is the result of pathological biological processes and high biomechanical effects that destabilise the natural tissue degradation and synthesis of articular cartilage [9]. The decrement of hyaluronic acid (HA, hyaluronate) concentrations and the descent of its molecular mass are the principal causes of

chondral defects. The *application of intraarticular injections of hyaluronan* upgrades the quality of articular cartilage through the synovial liquid [10]. This is a therapeutic experiment for effective temporary elimination of pains [11], [12]. Good clinical results have been obtained after the treatment of deep chondral defects in the knee with autologous chondrocytes implantation using *3D hyaluronan-based scaffolds* (Hyalograft C), [13]. Some scaffolds can effectively increase the initial bearing capacity of newly created tissue. It is also the fundamental condition for successful chondrogenesis.

From the previous small/non-invasive methods of treatments it is apparent that the quality of microstructures and continuous biomechanical properties of the subchondral bone play an important role in the morphology and the quality of chondrogenesis. Chondrogenesis depends very sensitively on the initial stability of biomaterials implanted into the subchondral bone. Vertical displacements and rotations of COC-blend replacements shortly after implantations must be eliminated. The initial integrity of biomaterials substituting the subchondral bone, the initial bearing capacity and the vertical position of these replacements have a major influence on chondrogenesis. The initial biomechanical stiffness of materials (substituting the subchondral bone) has a fundamental influence on the quality of new articular cartilage.

## 5. Conclusion

With regard to these initial requirements, acceleration of the stability of COC-blend replacements in the subchondral bone is a requisite of advisable conditions for the tissue genesis.

The *stability of COC-blend replacements* in the subchondral bone can be ensured by:

- (1) plasmatic modification of the COC-blend surface by the action of a nitrogen and/or oxygen microwave plasma;
- (2) surface spherical/ellipsoidal pores with the diameter of  $< 0.5, 1.5 > \mu\text{m}$  in the COC-blend;
- (3) application of both type I collagen (0.3%) and growth hormones TGF- $\beta$  (1.2 mg/1 ml PBS) on the COC-blend surface;
- (4) application of chitosan (0.3% liquid, pH 5.5) on the hydrogel surface.

The bearing capacities of subchondral bone COC-blend replacements considerably contribute to the genesis of a new extracellular cartilage matrix (figures 7 and 8). Histological analyses demonstrated the healing

process with partial (12 weeks) or complete (20 weeks) spongy bone + cartilage bridging (in vivo).

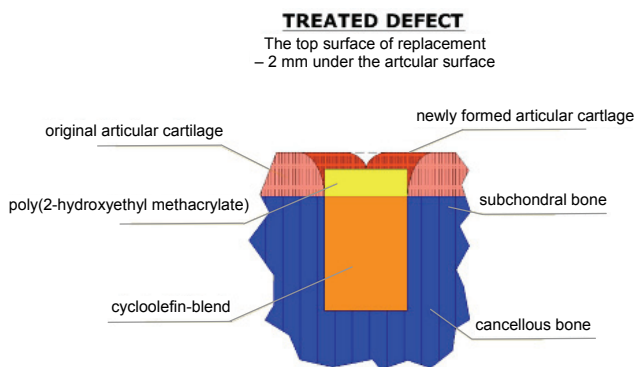


Fig. 10. New articular cartilage is formed over the hydrogel scaffold. Its surface is approximately 1–1.5 mm above the tidemark

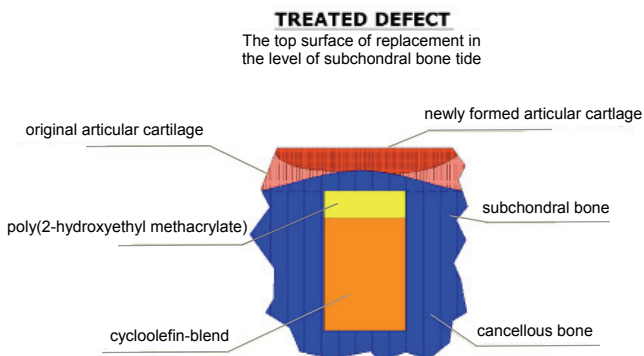


Fig. 11. New subchondral bone and new articular cartilage are formed over the hydrogel scaffold. The top plane of hydrogel surfaces is allocated approximately 0.5 mm under the indigenous level of the tidemark

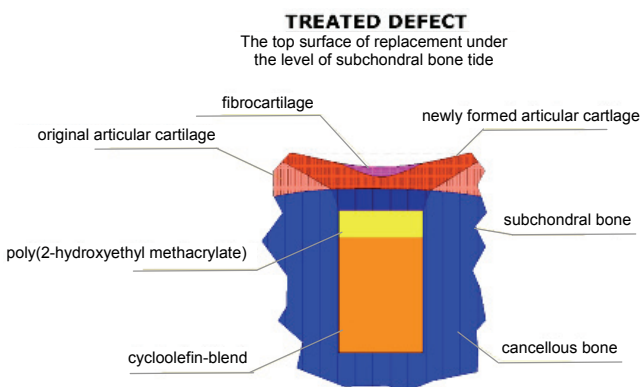


Fig. 12. New subchondral bone and new articular cartilage with peripheral fibrocartilage are formed over the hydrogel scaffold. The surface of hydrogel scaffold is allocated approximately 2 mm under the indigenous level of the tidemark. The articular surface has a sag profile

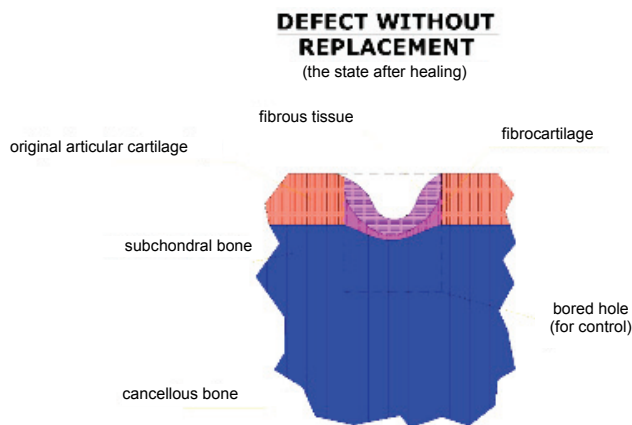


Fig. 13. Control defects are filled with both fibrous and fibrocartilage tissues

The cycloolefin blend copolymers [COC-blend] and hydrogel [poly (2-hydroxyethylmethacrylate)] scaffolds can be suggested as a reliable reconstructive alternative for local osteochondral defects and effective support for the creation of new hyaline cartilage having an articular surface without fibrillation.

With regard to the conditions mentioned previously, the new articular cartilage is formed over the hydrogel scaffold when its surface is approximately 1–1.5 mm above the tidemark (see figure 10). If the hydrogel surfaces are allocated approximately 0.5 mm under the indigenous level of the tidemark, then a new subchondral bone and new articular cartilage are formed over the hydrogel scaffold (figure 11). A new subchondral bone and new articular cartilage with peripheral fibrocartilage are also formed over the hydrogel scaffold when the top surface of the hydrogel scaffold is allocated approximately 2 mm under the indigenous level of the tidemark. The articular surface has a sag profile (figure 12). Control defects are filled with both fibrous and fibrocartilage tissues (figure 13).

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