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Cycloolefin-Copolymer/Polyethylene (COC/PE) Blend Assists with the Creation of New Articular Cartilage

M. Petrtyl,[1] Z. Bastl,[2] Z. Krulis,[3] H. Hulejova,[4] M. Polanska,[4] J. Lisal,[1] J. Danesova,[1] P. Cerny[5]*

Summary: It is shown that the initial biomechanical stability of the applied COC/PE blend in the treated tissue locality, the initial integrity of biomaterials substituting the subchondral bone by polymer implant, the initial bearing capacity and the vertical position of these biomaterials have a mayor influence on the regeneration of new articular cartilage and subchondral bone. These aspects are essential for a prosperous treatment of osteochondral defects. Results reveal that the initial biomechanical stiffness of materials (substituting the subchondral bone) has a fundamental influence on both the quality and the quantity of new articular cartilage and subchondral bone. Research is also aimed at the application of suitable biologically tolerated polymer material, its surface modifications, verifications of the vertical position of implants in relation to the articular cartilage surface and ensuring the initial biomechanical stability of the polymer implant.

Keywords: articular cartilage; biomaterials; cycloolefin-copolymer blend; implant; subchondral bone

Introduction

Osteochondral defects are predominantly the result of system disorders of articular tissues. Defects arise as a consequence of slowed down metabolic processes due to ageing, impaired protective role of aggrecans and increased role of enzymes breaking down type II collagen,[1] reduced anabolic activity of chondrocytes,[2] as a consequence of changes in biomechanical properties of tissues, after traumatic disorders etc. Recently, good results have been achieved in the treatment of cartilage defects; it is still a rare phenomenon that good-quality articular cartilage is produced in the cured locality. Contemporary methods of research into the treatment of minor osteochondral defects are, among others, aimed at applications of osteochondral autografts (Kleemann R. et al.[4]), transplantations of resorbable co-polymers (Cohen S.B. et al.[5]), transplantations of chondrocytes (Ruano-Ravina A., Díaz M. J. [6,7,10]), transplantations of biologically active tissue matrices and numerous biological agents[8,9] etc. The creation of new articular cartilage and, in particular, the velocity of its new production are, from the biomechanical perspective, highly affected by the initial *stability and bearing capacity* of the implanted biomaterial, mainly in subchondral bone. The initial stability and capability of continuously distributing physiological loads may be guaranteed by using suitable polymer-based materials, which should be *biotolerant, bioconductive and biocompatible.*

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[1] Czech Technical University, Faculty of Civil Engineering. Laboratory of Biomechanics and Biomaterial Engineering, Thakurova 7, 166 29 Prague 6, Czech Republic
E-mail: petrtyl@fsv.cvut.cz
[2] J. Heyrovsky Institute of Physical Chemistry, Academy of Sciences of the Czech Republic, Dolejskova 2155/3, 182 23 Prague 8, Czech Republic
[3] Institute of Macromolecular Chemistry, Academy of Sciences of the Czech Republic, Heyrovskeho nam. 2, 162 06 Prague 6, Czech Republic
[4] Institute of Rheumatology, Na Slupi 4, 128 50 Prague 2, Czech Republic
[5] Ortotika s.r.o., U Pekarky 1, 180 00 Prague 8, Czech Republic

Blends of cycloolefin copolymer (COC) with linear low density polyethylene (LLDPE) have been proven as perspective materials for construction of selected skeletal replacements.[3] Biocompatibility testing of these copolymers has shown that the material is well tolerated by the living tissue. However, for application in various parts of artificial prostheses a high toughness is often desired. Therefore an attempt has been made to enhance the toughness by incorporation of a further component. Blending of COC with selected grade of LLDPE together with crosslinking of the dispersed phase appears to be the proper way for toughness improvement.

Research Objective

The work presented in this paper is focused on the validation of the production of new articular cartilage and subchondral bone *through the application of polymer implants* in localities of discrete osteochondral defects. Research is also aimed at: a) the application of a suitable biologically tolerated polymer material, b) its surface modifications, c) verifications of the vertical position of implants in relation to the articular cartilage surface and d) ensuring the initial biomechanical stability of the polymer implant.

Methodology

The following materials were used in this study: Ethylene-norbornene copolymer (COC) Topas 8007 (Ticona GmbH) containing 27 mol.% of norbornene, MFI (ISO 1133, 260 °C, 2.16 kg) 14. g/10 min; linear low density polyethylene (LLDPE) Exact 0210 (Exxon Mobil Chemical), MFI (ISO 1133, 190 °C, 2.16 kg) 11.5 g/10 min; Tert.-Butyl peroxide (tBP): 98%, product of Arkema Inc.

All the samples were prepared by blending of components in the W50EHT mixer of Brabender Plasti-Corder. Dynamical crosslinking of the blends was carried out by incorporation of 0.1% of tBP. Crosslinking of dispersed phase of the blends was made by irradiation with accelerated electrons. Determination of selected mechanical properties and rheological properties were determined according with standardized methods.

Among the key requirements imposed on biologically active replacements of articular cartilage and subchondral bone there are: the initiation and support of biological activity of newly formed connective tissues, the bonding of fibrous tissues (collagen encapsulation) with the surface of the distal part ((COC/PE)-blend) of a non-live implant (ensuring the condition of biocompatibility and fibroconductivity), the verification of the tissue tolerance to a non-live object (ensuring the condition of biotolerance) and the ensuring of permanent biomechanical stability of artificial implants in a live environment. Special emphasis was put on creating conditions for the production of new articular cartilage, which bridges the implanted replacement.

The articular cartilage fulfils two fundamental functions: a supporting function and a protective one. Loaded articular cartilage must very fast distribute force effects into the subchondral and spongious bone and accumulate and release potential deformation energy in physiologically predetermined regimes.

Because of its molecular structure (firm bonding of water molecules with molecules of proteoglycans), articular cartilage turns into an incompressible material under large physiological loads. It is solely under these conditions of *hydraulic strengthening* that chondrocytes and extracellular matrices may be protected from their mechanical damage. By using non-load-bearing substitute materials in treated localities, the continuous transfer of force effects from cartilage and subchondral bone into spongious bone is impaired. Large deformations in reconstructed zones of tissues subsequently degrade the extracellular matrix synthesis and slow down the healing processes. Due to the fact that, e.g., the initial bearing capacity and biomechanical stability of autologenic bone mash is not sufficiently ensured during transplantations, a bi-component implant (Figure 1) has been designed by our team. The upper gel component, consisting of poly(2-hydroxyethylmetacrylate), has a modulus of elasticity of 5 to 8 MPa, and the (COC/PE) blend has a modulus of elasticity of 2.0 GPa. The cylindrical total replacements applied are 8 mm in diameter, the upper hydrogel component being 2 mm thick in the vertical direction, while the height of the lower COC-blend component is 10 mm. Both the upper and lower components are mechanically interconnected.

The lower distal polymer component is composed of basic copolymer: ethylene-norbornene with the content of 27% mol. norbornene. This polymeric material is very well tolerated by living tissues possessing good mechanical characteristics applicable in tissue engineering. Due to the fact that implanted materials of non-biological origin should posses material properties very close to the properties of the respective life tissue that they replace, our main attention was focused on modification procedures based on the incorporation of an additional polymer elastomer component and reinforcing agents, which will favourably affect the moduli of elasticity of the applied biomaterial. In order to achieve favourable moduli of elasticity, our focus was on affecting supramolecular structures which allow rheology of the dispersed phase by the application of ionizing radiation (γ-rays, accelerated electrons). Representative samples were verified in terms of their mechanical characteristics, thermal and oxidation stability and rheological characteristics of the melt. Attention was also paid to prove the absence of free radicals in order to prevent degradation processes.

In order to ensure the initial mechanical stability of an implant in the subchondral bone, the subchondral bone implant had to be stabilized in the vertical and horizontal direction by type I collagen. The molecules of type I collagen were immobilized on the (COC/PE) blend surface modified by microwave low-pressure nitrogen plasma. Collagen adsorption is known to promote cell adhesion and proliferation. The chemical composition of the (COC/PE) blend surface after plasmatic modification was characterized using the X-ray photoelectron spectroscopy method (XPS) and measuring the water static contact angle.

In-depth distribution of nitrogen- and oxygen-containing functional groups in (COC/PE) blend polymers after their plasmatic treatment was determined using angle-resolved X-ray photoelectron spectrometry (ARXPS). The concentration profiles tested included rectangular (“boxcar”), trapezoidal and exponential.

Prior to surface plasmatic modification, the lower components of implants were washed in an ultrasonic bath in pure isopropyl alcohol. This procedure leads to

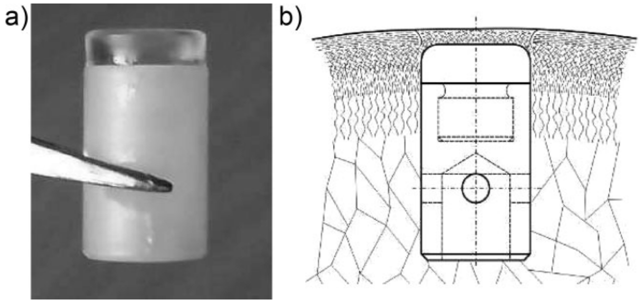

Figure 1.

A bi-component artificial replacement of cartilage and subchondral bone. a) Implant seen as a whole. The upper component of the implant is composed of hydrogel, the lower component of (COC/PE) blend. b) Cross section of the implant and an option of its vertical position in relation to articular surface.

the removal of traces of organosilicon compounds, frequently present on the surface of polymers and affecting the resulting chemical surface composition after modification. The samples were modified by the nitrogen MW plasma using MW reactor equipped with SLAN I OV 425 magnetron (Plasma Consult) with power of 300 W, pressure of nitrogen 80 Pa, nitrogen flow 15 scm^3/min and exposure 15 min. The samples with modified surfaces were further immersed in de-ionized water for 12 hours. The samples prepared in this way were sufficiently stable after several days to allow achieving reproducible results on them. The EPR spectra of the polymer (COC/PE) blend (hereinafter Blend) prepared in such a way did not show any presence of radicals.

To identify the ''in-depth'' distribution of oxygen or nitrogen after plasmatic treatment, angle-resolved photoelectron spectra of C 1s, O 1s and N 1s electrons were measured on the (COC/PE) blend material.

The method applied is based on the fact that the intensity of the photoemission line i of the element A is proportional to the convolution of the in-depth concentration profile c(z) and the probability that the photoelectron will leave the sample from the depth z without a loss in kinetic energy. To fit our purposes, we used an algorithm for the rectangular (''boxcar''), trapezoidal and exponential profile. The software ARXPSolver developed by R. W. Paynter was used for this purpose. The acceptable agreement between the measured and calculated values was achieved for the boxcar profile. The used values of the effective attenuation length, including the effect of non-elastic and elastic electron dispersion, have been calculated using the NIST software ''Electron Effective-Attenuation-Length Database''.

The experimental ''in-vivo'' verifications on pigs were performed in two series. The first series of operations was performed in 2007 on three 3 pigs. The duration of the experiment was 8 months. The second series of operations was made 2008 on two pigs. The implant was composed of two parts, see Figure 1: the upper – hydrogel (poly-(2-hydroxyethylmetacrylate), replacing cartilage and the lower one prepared on the basis of cycloolefin blend ((COC/PE) blend), mounted into the subchondral/spongious bone.

In all, 12 + 8 implants were set in, always applying two implants into the knee joint into the lateral or medial locality. The surfaces of the lower implant components were alternatively modified by nitrogen microwave plasma and alternatively used without plasmatic modification. 0.3% solution of type I collagen was applied. The upper hydrogel part was alternatively coated with aggrecan (1 mg in 0.5 ml d H_2O), chitosan (0.3% solution with 5.5 pH) and the growth factor TGF-β (1.2 mg/1ml PBS), Figure 2.

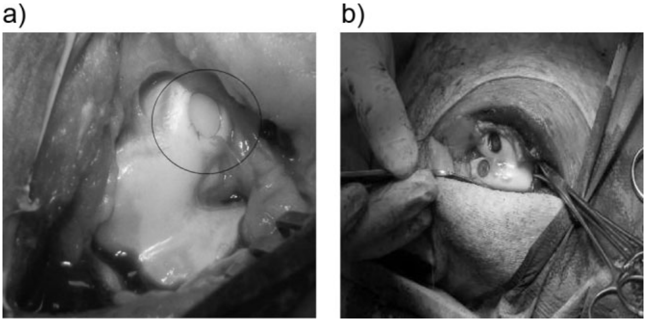

Figure 2.
Mounted implant; a) option with the implant's top surface mounted 2.2 mm under articular surface; b) alternative of implant's top surface mounted 1.5 mm under articular surface, the reference defect is seen on the right.

Histological verifications were focused on the viability of the subchondral bone on its interface with the (COC/PE) blend implant and on the formation, degradation or absence of articular cartilage, fibrocartilage, or fibrous tissue at different depths of lodgement the implant's top under the articular surface of cartilage.

Results and Discussion

It was proved that both procedures of modification phase, i.e. dynamical crosslinking using addition of organic peroxide (tBP) during melt mixing of the blends and crosslinking by accelerated electrons irradiation of the blends in solid state (with consequent melt processing), lead to toughness improvement (Figure 3). Crosslinking of dispersed phase leads to significant increase of the melt viscosity of COC/PE blends (Figures 4). This effect is specially marked at higher irradiation dose (50 kGy). Nevertheless, tested polymer materials may be processed using standard process techniques.

By applying LLDPE in the COC matrix, the strength of the resulting material grew by 100%. By netting the strength grew

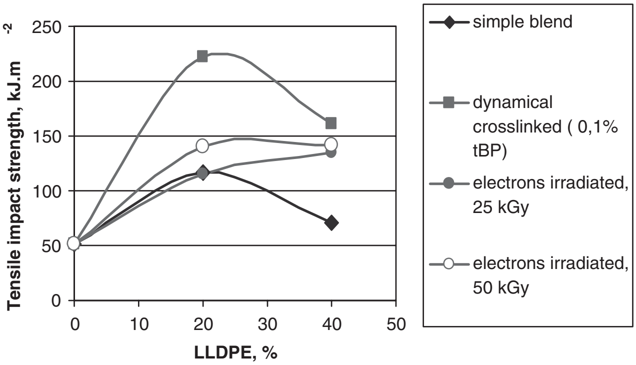

Figure 3.
Effect of composition and crosslinking of dispersed phase on tensile impact strength of COC/PE blends.

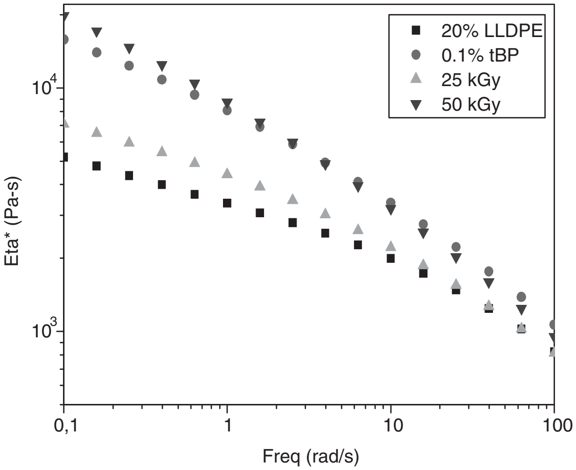

Figure 4.
Frequency-dependent complex viscosity of melt of COC/LLDPE mixes (80/20) for different modification techniques.

three times, by applying accelerated electrons it grew nearly five times, due to COC.

It is possible to conclude that COC/PE (80/20) shows good process ability and optimum combination of mechanical characteristics for use in osteochondral polymer replacements.

Photoelectron spectra of a plasmatically modified sample of a COC/PE blend polymer measured in the region of binding energies from 0 to 1000 eV and for three different detection angles are presented in Figure 5; Figure 6 displays C 1s spectra of photoelectrons measured in the high-resolution mode.

The results of the analysis of fitted spectra are presented in Tables 1 and 2. Experimental relationships of the ratio of intensities of spectral lines N 1s/C 1s and O 1s/C 1s applied for the calculation of the surface concentration gradient are shown in Figures 8 and 9. Figures 10 and 11 present results for nitrogen, while Figures 12 and 13 for oxygen. These results, among others, imply that the concentration profile of oxygen is twice deeper than the profile of nitrogen.

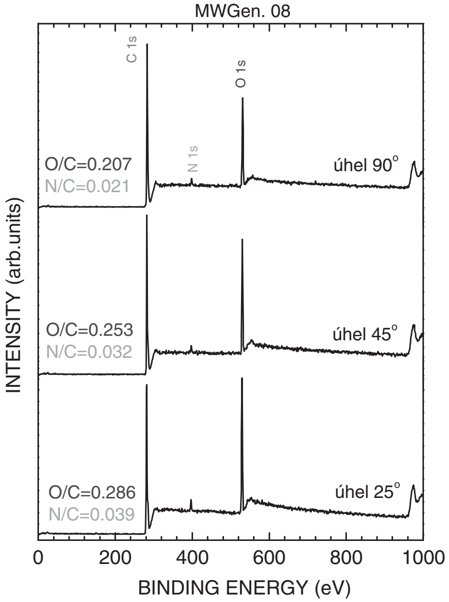

Figure 5.
Photoelectron spectra measured in the region of binding energies of 0–1000 eV for three different detection angles, a sample of a mw. Nitrogen plasma-treated COC-blend polymer. The spectra-related data include values of ratios of O/C and N/C atomic concentrations calculated from integrated intensities of C 1s, O 1s and N 1s photoelectron spectra measured in the high-resolution mode.

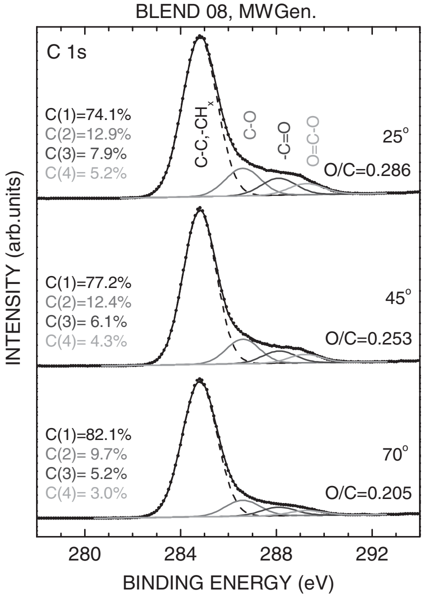

Figure 6.
Fitted C 1s spectra of photoelectrons of a plasmatically modified sample of a COC-blend polymer measured for three detection angles. The spectra-related data include values of ratios of surface atomic O/C concentrations and populations of —C—O, —C=O and O=C—O functional groups. The spectra are normalized to the same height.

Results of ARXPS analysis for nitrogen in plasmatically modified COC-blend polymer, Boxcar profile; Boxcar parameters: Z1 = 0; Z2 = 20.48290; Fit Factor = 0.12319615.

Results of ARXPS analysis for oxygen on plasmatically modified BLEND polymer, Boxcar profile; Boxcar parameters: Z1 = 0; Z2 = 42.085; Fit Factor = 0.58057505.

Oxygen-containing functional groups most likely arise on the surface of a polymer modified in the nitrogen plasma through the reaction with water molecules present in the residual atmosphere or desorbed from the reactor surface due to the plasma

Table 1.
Populations of surface functional groups in the (COC/PE) blend sample modified in the mw. nitrogen plasma on the detection angle.

Detection angle	O/C	C				O		N
		$-CH_x$	$-C-O-$	$-C{=}O$	$O-C{=}O$	$-C{=}O$	$-C-O$	$-C-NH_x$
		$-C-C-$		$N-C{=}O$		$N-C{=}O$	$O{=}C-O^*$	$N-C{=}O$
25	0.286	0.741	0.129	0.079	0.052	0.207	0.072	0.039
45	0.253	0.772	0.124	0.061	0.043	0.180	0.069	0.032
70	0.205	0.821	0.097	0.052	0.030	0.151	0.057	0.026
90	0.207	0.834	0.091	0.046	0.029	0.153	0.055	0.021

Table 2.
Binding energies E_B of core-level electrons and widths of photoemission lines in half of their height (in brackets). Data are in eV.

Detection angle	C 1s				O 1s		N 1s
	$-CH_x$	$-C-O-$	$-C{=}O$	$O-C{=}O$	$-C{=}O$	$-C-O$	$-C-NH_x$
	$-C-C-$		$N-C{=}O$		$N-C{=}O$	$O{=}C-O^*$	$N-C{=}O$
45	284.8	286.6	288.0	289.2	532.4	533.5	400.3
			(1.6)			(2.1)	(2.3)

COC-BLEND, MW nitrogen plasma.

action and by successive chemical reactions after the sample has been exposed to air.

The COC/PE blend polymer, like the majority of other polymers, following plasmatic modification undergoes aging manifested by a slight growth in the static water contact angle and a decrease in the value of the ratio of surface O/C atomic concentrations resulting from the reorientation of surface polar groups. The cause of this reorientation is a tendency towards reducing surface energy. After ca 100 hours of air exposure, the velocity of these relaxation processes has diminished. Provided modified polymers are placed in a hydrophilic environment (e.g. water or buffer solution with collagen) shortly after modification, the tendency towards reorientation is suppressed, most likely due to the interaction of molecules of the

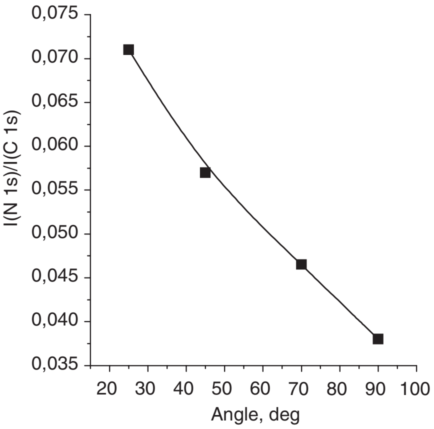

Figure 7.
Dependence of the ratio of integrated intensities of N 1s and C 1s lines on the detection angle.

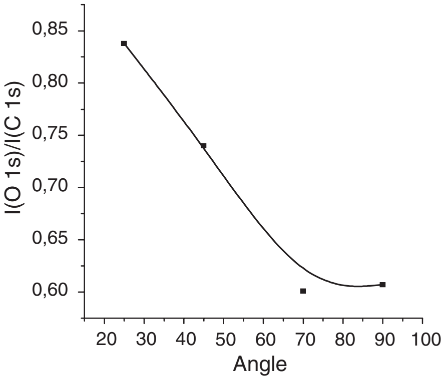

Figure 8.
Dependence of the ratio of integrated intensities of O 1s and C 1s lines on the detection angle.

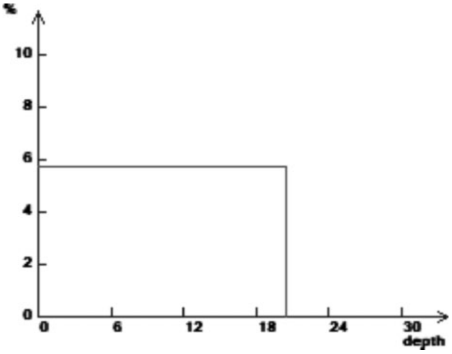

Figure 9.
Calculated boxcar profile for nitrogen.

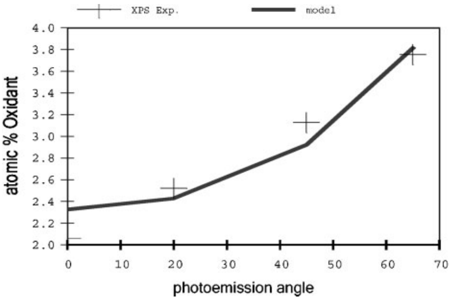

Figure 10.
Comparison of results calculated for a model with results of the ARXPS experiment.

medium with polar functional groups on the polymer surface.

Another objective of our study was to determine a potential influence of the used diagnostic method where a sample is exposed to soft X-ray radiation on the surface concentration of oxygen and the change in the populations of oxygen-containing surface groups. Figures 13–16 and Table 3 sum up the results of this study for a sample exposed to air for 360 hours after plasmatic modification, i.e., a sample that already appears stable. The results manifest that during radiation exposure the value of the ratio of surface O/C atomic concentrations declines, in particular as a result of a drop in the surface concentrations of COO and C−O functional groups, while the concentration of C=O groups remains practically unchanged.

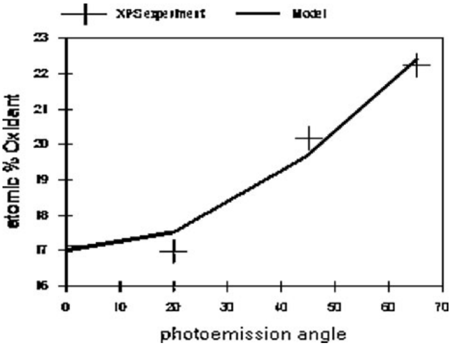

Figure 11.
Comparison of results calculated for a model with results of the ARXPS experiment.

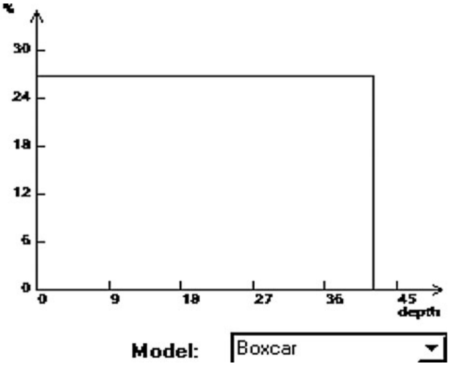

Figure 12.
Calculated boxcar profile for oxygen.

The concentration profile of oxygen is approximately twice deeper than the concentration profile of nitrogen. Following plasmatic modification, a gradual reorientation of surface polar groups occurs which may be suppressed by their interaction with a hydrophilic medium or by binding other molecules. Surface plasmatic COC/PE blend modification has contributed to viability on the interface between the COC/PE blend surface and the sound subchondral bone.

Bioactivity, biocompatibility and bioconductivity (of type I collagens) in the lower (distal) COC/PE blend component (localized in the subchondral bone) were improved by plasmatic modification of these polymers.

The formation of polar groups on the modified (COC/PE) blend surface contrib-

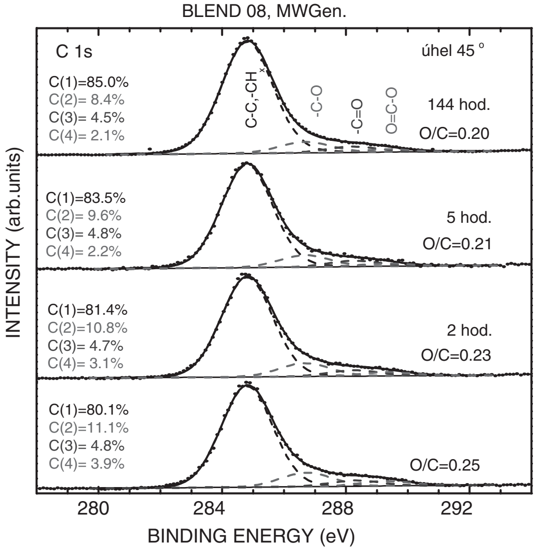

Figure 13.

Spectra of C 1s photoelectrons of a COC/PE blend sample modified in the nitrogen plasma measured at a detection angle of 45°. Spectra-related data include populations of C—O, C=O and O=C—O functional groups, the value of the overall ratio of O/C atomic concentration and the time of exposure to soft X-ray (hυ = 1486.6 eV) radiation (the sample was exposed to radiation for a total of 5 hours, the 144-hour spectrum was measured 139 hours after X-ray irradiation was finished). The spectra are normalized to the same height.

uted to the immobilization of type I collagen. The application of type I collagen also contributed to accelerating the initial stability of the lower COC/PE blend component of the implant in the cavity remaining after the removal of the pathological subchondral bone. The newly formed bone tissue in the surroundings of artificial replacements did not show any signs of pathological remodelling changes or insufficient mineralization. Bone trabeculas were fully mineralized, as could be verified on preparations produced by the technique using non-decalcified cuts. The implant's healing occurred without any negative effects. The bone tissue around the lower component was reproduced in its full volume and in original quality.

Adequate stresses on the interface (contact of collagen fibrils with a modified polymer) on the macro-level of tissue

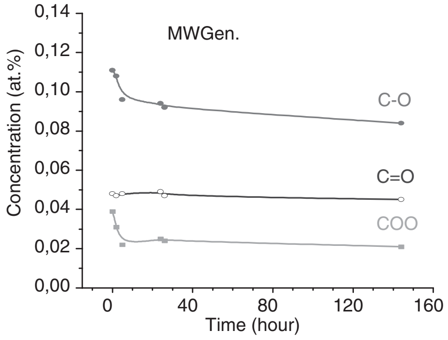

Figure 14.

Time dependence of populations of individual surface oxygen-containing functional groups.

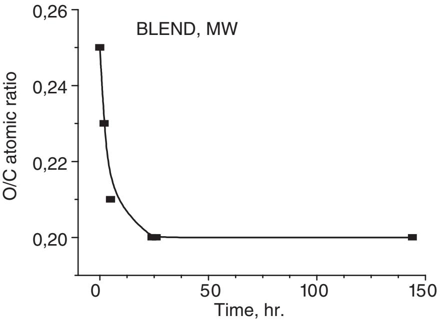

Figure 15.

Time dependence of the O/C atomic surface concentration ratio.

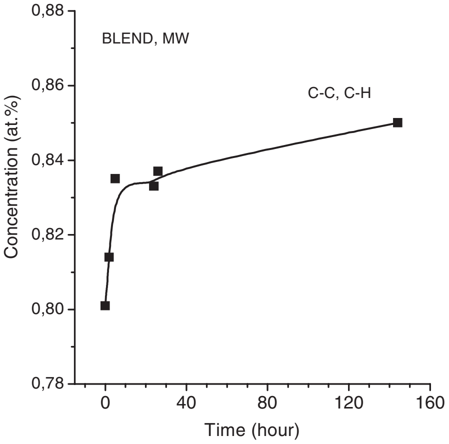

Figure 16.
Time dependence of carbon population in the surface C—C, C—H bonds.

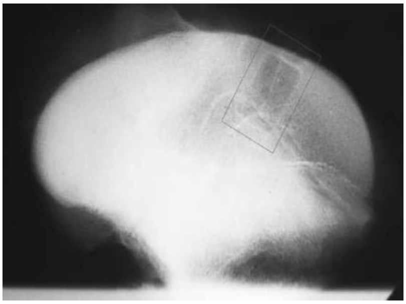

Figure 17.
An X-ray image of implanted bi-component replacement. The lower component is coated by high-quality collagen encapsulue. High-quality collagen encapsule on the lower component's surface were verified producing no single case of necroses.

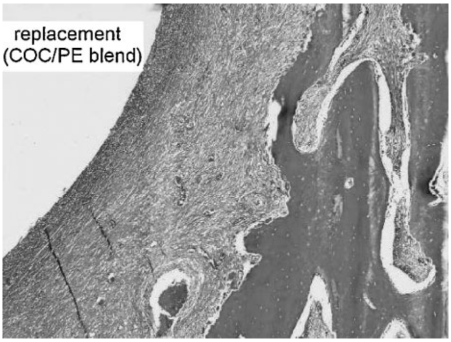

Figure 18.
A histological image of a fibrous capsule (on the implant's side) surrounding the implant laterally on the interface between the implant (on the left) and the bone (on the right). No inflammatory reaction or potential toxic changes are evident anywhere.

exceeded values of 40–60 MPa. By creating a strong binging stress field, physical connectivity (biocompatibility) of biological material with non-live material (COC/PE blend) and contact of two utterly different materials without toxic reactions (bioconductivity) were indirectly manifested. Preoperative application of type I collagen on the (COC/PE) blend surface creates a bearing scaffold on the interface with the subchondral bone (Figure 11) thus contributing to time-accelerated cellular adhesion, to successive proliferation and differentiation of new populations of cells. Plasmatic modification by nitrogen and oxygen atoms using energies determined by our team during ion implantation, significantly contributes to biological activ-

Table 3.
Populations of C and O chemical states in a COC-blend sample modified in the nitrogen microwave plasma.

	Detection angle	O/C	C				O	
			$-CH_x$	−C−O	−C=O	−C=O	O−C=O	−C−O
			−C−C−	−C−OH				O=C−O*
1st measurement	45	0.25	0.801	0.111	0.048	0.039	0.215	0.039
2nd + 2 h.	45	0.23	0.814	0.108	0.047	0.031	0.194	0.033
3rd + 3 h, in total 5 h	45	0.20	0.835	0.096	0.048	0.022	0.179	0.032
4th +19 h, in total 24 h	45	0.20	0.833	0.094	0.049	0.025	0.164	0.031
5th +2 h, in total 26 h	45	0.20	0.837	0.092	0.047	0.024	0.161	0.039
	5	0.26	0.792	0.112	0.057	0.039	0.215	0.041
6th + 118 h, in total 144 h	45	0.20	0.850	0.084	0.045	0.021	0.158	0.037
	5	0.26	0.784	0.122	0.055	0.039	0.225	0.040

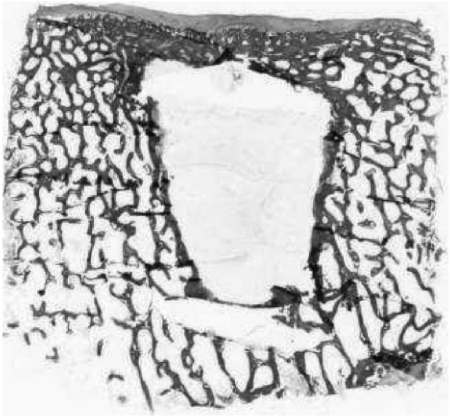

Figure 19.
New articular cartilage and subchondral bone have bridged the proximal part of the polymer implant; the top of polymer implant is at a depth of ca 2.5 mm under articular cartilage.

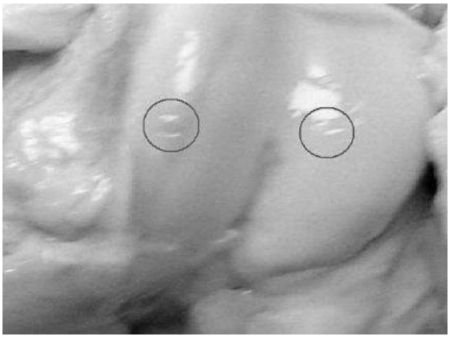

Figure 20.
Production of new articular cartilage at places of osteochondral defect removal. The upper hydrogel component of the implant was coated with chitosan, while the lower (COC/PE) blend component was plasmatically modified and coated with type I collagen. The implant's top was at ca 2.5 mm distance from the articular surface.

ity, in particular, to adhesion of fibroblasts onto the preoperatively applied collagen underlayer (type I collagen).

Based on completed "in-vitro" tests, we may say that none of the tested biomaterials have, in a principal way, affected the expression of pro-inflammatory cytokines or apoptotic molecules.

During "in-vivo" experiments, all polymer implants used (both modified and

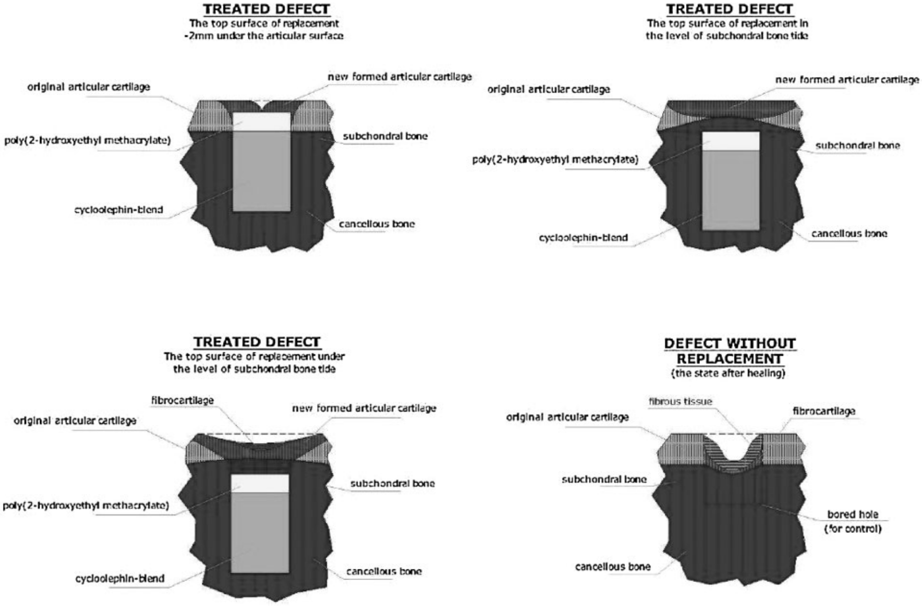

Figure 21.
The effect of the vertical position of a stabilized implant on the new articular cartilage and subchondral bone synthesis (cross section of healing of a control defect in the articular cartilage without inserting an implant is seen in the bottom right part).

unmodified) were perfectly healed into the bone tissue. These implants did not induce any unanimously pathological response in these structures. No toxic effects were revealed. Perfect mineralization was manifested in the reconstructed parts of tissues.

In all alternative cases where embedding of implants under the articular surface was also investigated (Figures 19–21), all implants had been healed under this surface and bridged with high-quality new articular cartilage and subchondral bone (Figure 19 and Figure 20).

Conclusion

(1) The initial biomechanical stability of applied COC/PE blend in treated tissue locality, the initial integrity of biomaterials substituting the subchondral bone by polymer implant, the initial bearing capacity and the vertical position of these biomaterials have a mayor influence on the regeneration of new articular cartilage and subchondral bone. These biomechanical aspects are essential for a prosperous treatment of osteochondral defects.

(2) The insertion of a bi-component polymer implant (with a plasmatically modified distal component coated with type I collagen) into the borehole, in the locality of the subchondral defect, contributes to healing of tissue around the polymer implant, without the appearance of inflammatory reactions or without the appearance of macrophages.

(3) While observing the permanent position of the top of a bi-component implant in the *tide line* plane, i.e. on the interface of the upper edge of the original subchondral bone and the lower edge of the original cartilage, or no deeper than 1 – 1.2 mm under this interface, high-quality bridging of the implant with articular cartilage and high-quality subchondral bone is produced (assuming initial stability conditions of the polymer replacement are fulfilled). The application of chitosan on the surface of the upper hydrogel component of the implant contributes to the bioactivity of newly produced tissues.

(4) The initial biomechanical stiffness of materials (substituting the subchondral bone) has the fundamental influence on both the quality and the quantity of new articular cartilage and subchondral bone.

(5) Verified polymer implants may be considered as bioactive, bioconductive and biotolerant materials and suitable candidates for replacements in osteochondral defects. With regards to the assumptions above, polymer replacements based on COC/PE blend + hydrogel might be applicable in clinical practice in slowing down or preventing faster progressions of early stages of osteoarthritis (complicated and frequently posttraumatically induced), but also in cases of the appearance of local pseudocysts and other discrete osteochondral defects.

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