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Cartilage replacement by use of hybrid systems of autologous cells and polyethylene: an experimental study

Ilona Schoen · Torsten Rahne · Annekatrin Markwart · Kerstin Neumann · Alexander Berghaus · Ernst Roepke

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Abstract This study used porous polyethylene (PE) as a scaffold in an animal model system. The surface of the scaffolds was either modified with collagen II coating or first functionalized by oxygen plasma treatment and then coated with collagen II. The specimens were inoculated with autologous chondrocytes and transplanted into the concha of guinea pigs. Bare scaffolds were used as controls. Periods of 1, 6, and 12 months after implantation, samples of cells containing specimens and control samples were evaluated microscopically. As a result, the pre-seeded specimens were better integrated into the surrounding tissue than cell-free PE-specimens. Also a weaker immune reaction and an improved cartilage generation could be detected in the pre-seeded specimen. Compared to the other surface modifications, no further improvement of cartilage development was observed in the long term in vivo animal experimental study.

1 Introduction

The regeneration capacity of injured cartilage is known to be low [1]. To support cartilage regeneration, the tissue may be replaced by biomaterials, which might help to improve the clinical success in head and neck surgery.

So far, different implant materials have been used. However, close to chronic infection or poorly vascularized tissues, the recovery is a crucial point. Porous Polyethylene (PE) is a well-established implant material used in head and neck surgery and is commercially available as Medpor® (UHMW-PE). The successful use of Medpor® has been well documented in the literature. Its porous structure allows sufficient fibrovascularization [2]. On the other hand, complications have been described [7–10]. In rhinoplasty, Romo et al. [3, 4] reported a complication rate of 3–4%. Two more recent retrospective studies [5, 6] gave an account of UHMW-PE application as orbital implants and found complication rates of 5.6% and 8.6%, respectively. At facial reconstruction surgeries, Carboni et al. [7] reported a failure rate 1 of 5.7% (out of 105) used PE-implants.

In comparison to other biomaterials, Romo et al. [3] described PE as an excellent material for implants. By contrast, in his summary of the last five years' results of PE-implantations for nose reconstructions in multiple surgical revisions Berghaus [8] reported a relatively high complication rate of approximately 20%, caused by patients with pre-existing injured tissue. Further complications were reported by Blaydon et al. [9] who used PE as a spherical orbital implant. In this study half of the PE-implants were wrapped with autologous tissue like temporalis fascia or fascia lata to decrease the risk of conjunctival erosion. No significant differences between wrapped and unwrapped implants were found.

Tissue engineering enables the generation of human tissue under in vitro conditions [10]. Autologous tissue is generally assumed not to be rejected. In general one major problem of the engineered tissue is its stability and

I. Schoen (✉) · T. Rahne · A. Markwart · K. Neumann · E. Roepke
Department of Otorhinolaryngology, Head and Neck Surgery, University Hospital MLU Halle-Wittenberg, Magdeburger Str. 12, 06112 Halle, Germany
e-mail: ilona.schoen@medizin.uni-halle.de

A. Berghaus
Department of Otorhinolaryngology, Head and Neck Surgery, University of Munich (LMU), Marchioninistr. 15, 81377 Munich, Germany

functionality. This is particularly true for engineered cartilage. Therefore, in order to improve the stability and to produce a rapid increase in rigidity for functionality, resorbable or non-resorbable polymers were used as scaffolds for autologous chondrocytes.

In the past, chondrocytes of different origin [11] and resorbable scaffolds like fibrin, agarose or alginates have been used. Perka et al. [12] described a mixture of alginate and fibrin. Cao and Vacanti [13] suspended chondrocytes in a mixture of polyglycolic acid (PGLA) and polylactic acid (PLLA) to yield an auricular implant. Recently, Naumann et al. [14] described the auricle reconstruction with Hyaff 11, a derivate of hyaluronic acid. Collagen as gel or fleece structures [15–17] as well as copolymers from PLA and PEG has also been utilized [18]. While the reported morphology of the artificially generated cartilage in vitro or in vivo is quite similar to native tissue, the biomaterials' mechanical stability is weaker than the native tissue [19, 20].

The predominantly positive results of PE as an implant for cartilage reconstruction could be combined with the benefit of autologous chondrocytes transplantation. In a recent study [21], different surface modifications of PE were examined in order to obtain a better adherence of isolated primary chondrocytes onto the modified PE-surfaces and to enhance the cartilage formation of isolated primary chondrocytes in vitro. So far, two different strategies were used to improve the cell-surface interactions. Firstly—the PE-surface was covered with collagen before chondrocytes were placed on the surface. Collagen as an extra cellular matrix molecule enhances the development of a cartilage structure in vitro [21]. Secondly—the surface was covered by collagen after functionalization by different plasma treatments.

The effect of the different modifications has been studied in cell culture experiments [21, 22]. For their clinical application, further experiments are needed to examine the impact of the material on the whole body [17, 23, 24]. One main problem in the development of biomaterials is the evaluation of the material in terms of its function, i.e., a good integration of the PE-material into the native surrounding tissue, in order to fulfill its desired function.

The present animal experimental study aimed to improve the integration of PE-implants into the surrounding tissue and to reduce the complication rate by masking the surface with autologous cells. We studied (1) whether PE-materials covered by autologous chondrocytes were better integrated than unmodified PE-materials; (2) the nature of the integration process; and (3) to what extent the PE-cell interaction was improved by the functionalization of the PE-surface with collagen II.

2 Materials and methods

2.1 Materials

PE sheets with a porous structure (pore width 35–250 μm) were used, kindly provided by POREX SURGICAL INC (Georgia, USA, as Medpor®).

The in vivo experiments were performed with PE-specimens with a diameter of 6 mm. Collagen II (Sigma-Aldrich, Taufkirchen, Germany) was dissolved in 0.5 N acetic acid at a concentration of 2.5 mg/ml and used at a final concentration of 20 μg/ml.

2.2 Sample preparation

Collagen coating was done as previously described [21]. Here, PE-specimens were agitated for 3 h at room temperature in a collagen II solution (20 μg/ml Sigma) supplemented with cyclohexyl carbodiimid (0.14 mg/ml, Sigma) as coupling reagent.

The reaction was performed in 50 ml tubes. After gentle shaking on a roller mixer, the samples were rinsed several times with PBS, dried over night and plasma-sterilized before being used in further experiments.

The surface modifications by oxygen plasma treatment were performed according to Dayss et al. [25]. Briefly summarized, oxygen plasma was generated with micro waves 300 W, 30 s with oxygen flow rates of 50 cm^3/min, vacuum at about 6×10^{-3} mbar, followed by an oxygen flush for 60 s. Oxygen plasma treated specimens were incubated with collagen II solution as already described.

2.3 Surface characterization

The amount of collagen bound at the surface was estimated by a semi-quantitative ELISA algorithm [26]. The discs were placed in a 48 well plate, blocked by incubation with a 1% BSA/PBS solution for 1 h and treated for 48-h with a collagen specific antibody (Rockland-Incorporation, Hamburg, Germany) at a dilution of 1:8000. After three washings with PBS, the discs were incubated with anti rabbit antibody conjugated with alkaline phosphatase (Sigma) at a dilution of 1:5000. After further 3 h of incubation followed by three washings, the samples were transferred in a new 48 well plate, equilibrated with substrate buffer for 5 min and then incubated with the alkaline phosphatase substrate for 30 min. The reaction was stopped by adding 3 N NaOH. The optical density of an aliquot of 100 μl was measured in a micro titer plate reader as absorbance units. Each measurement was done four times.

2.4 Animal model

In vivo examination was performed using young adult female guinea pigs with a body weight of approximately 600 g, according to the Helsinki guidelines. Polyethylene with different surface modifications was used: (1) PE, (2) PE covered with collagen II, and (3) PE oxygen plasma treated and covered with collagen II.

The animal experiments were realized in four steps.

1. Isolation of autologous chondrocytes from guinea pigs.
2. Seeding the PE-samples with autologous cells.
3. Implantation of the seeded samples and the cell free control samples in opposite ears.
4. Removal of the cartilage implant with the surrounding tissue for histological examination.

2.5 Isolation and propagation of chondrocytes

The autologous chondrocytes were isolated from the concha of the guinea pigs. Animals were anesthetized using a mixture of xylazine hydrochloride 5 mg/kg (Rompun Bayervital, Leverkusen, Germany) and ketamine hydrochloride 100 mg/kg (Ketanest 50, Parke-Davis, Freiburg, Germany). After shaving, the surgical area was disinfected and the skin at the ear concha was incised with a scalpel to generate a small piece of auricular cartilage. The wound was closed with a 6-0 vicryl rapid absorbable stitch (Ethicon, Norderstedt, Germany). Finally, the animal was marked by a tattoo on the opposite ear.

The piece of cartilage was homogenized to single cells by assistance of mechanical breakup and enzymatic digestion as described elsewhere [27]. Originally grown in six well plates, the cells were later cultivated in T75 culture dishes in DMEM supplemented with growth factors and antibiotics for up to three passages. For the in vivo studies about 4–5 million cells per sample were used, depending on the individual recovery rate in the isolation step. All specimens were cultivated in vitro for 2 weeks before implantation was performed.

For implantation, the animals were anesthetized as described above and a PE-specimen containing autologous cells was implanted into a subcutaneous pocket at the ear concha. The modified cell-free PE-specimens were engrafted into the opposite side as a control.

After a post-implantation time period of 1, 6, or 12 months, the guinea pigs were killed by an overdose of anesthetic and the cartilage implants together with surrounding tissue were removed. Nine experimental groups ($n = 8$ at the beginning of the experimental study) with different surface modifications were investigated corresponding to the time periods.

2.6 Histological assessment

Samples were fixed in buffered paraformaldehyde (2%) and embedded in Technovit 7200 or K-Plast resin according to the manufacturer's recommendations, cut into sections of 4 μm and collected on super frost slides (Menzel, Braunschweig, Germany) for histological analysis.

As histological standard procedures, hematoxylin & eosin staining and Giemsa or Goldner staining were performed [28]. Stained specimens were evaluated under a microscope (Axio-phot, Zeiss, Germany) equipped with different objectives and a digital camera (PixeLINK 1394). The area of interest was digitalized in up to 20 smaller areas, as illustrated in Fig. 1. Each single image was analyzed for the newly generated cartilage islands or foreign body giant cells (FBGC). The areas or numbers were quantified by software analysis 3.0 (Soft imagine systems).

2.7 Evaluation

For statistical analysis, the histological specimens were evaluated in two different modes.

First, the histological specimens were morphometrically analyzed for cartilage islands and foreign body giant cells (FBGC). The number of FBGC per implant area was determined and given as number of FBGC/mm^2 PE. The total area of the section was measured as illustrated in Fig. 1. Each group consisted of 8 animals for each time point and of 24 animals for each type of surface modification.

Four independent observers evaluated and semi quantified the immune reaction using an immune reaction score according to Saris et al. [29]. The resulting scores were grouped from 0 to 3 (0 = absent immune reaction, 1 = slight, 2 = moderate, and 3 = severe foreign body reactions). The same sections were used to evaluate the cartilage development (0 = no cartilage development, 1 = few new cartilage areas, 2 = moderate new areas, and 3 = good cartilage development). Cartilage islands were measured and the mean values of the estimated areas were compared to the surface modification. The evaluation by scoring was done for the cartilage development in the same manner as described above for the immune reaction.

Immune reaction and cartilage development were analyzed statistically by multivariate and univariate tests using SPSS 15.0.

3 Results

3.1 Collagen binding

To obtain a better adherence of the chondrocytes, the PE matrix was covered with the ECM molecule collagen. To

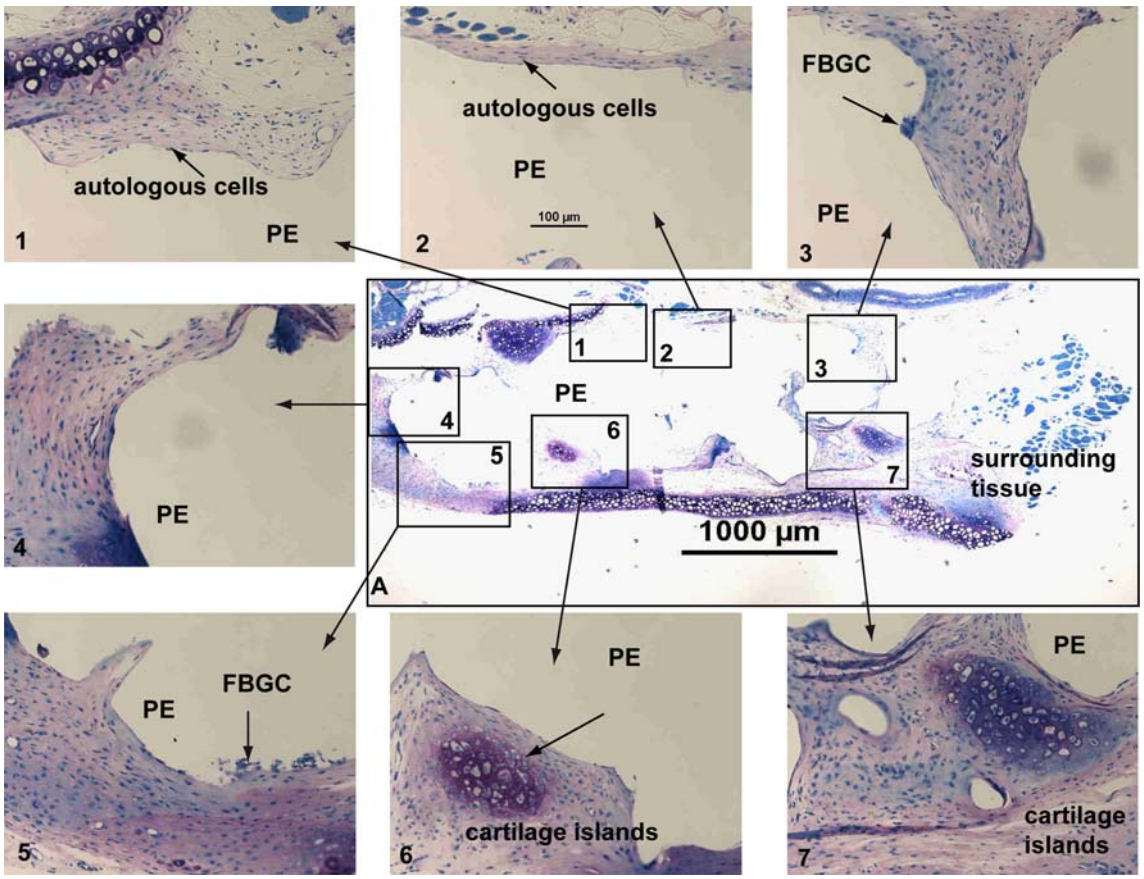

Fig. 1 Illustration of the histological evaluation. The histological slice (A) was split into smaller regions to analyze the tissue implant interface and the tissue, surrounding the implant (small pictures in greater magnification, (1–7, PE—polyethylene, FBGC—Foreign Body Giant Cells)

bind the collagen covalently and to attach greater amounts of collagen to the PE surface, the PE material had been modified by oxygen plasma treatment prior to the incubation with collagen.

Figure 2 shows the amounts of bound collagen at the surface without (left column) and with (right column) surface functionalization (e.g., carboxyl groups). The given values are the measured optical densities in the supernatant, resulting from the enzymatic reaction of the alkaline phosphatase coupled to the second antibody. As expected, a slightly better but not significant collagen binding capacity was found for the oxygen plasma treated surface.

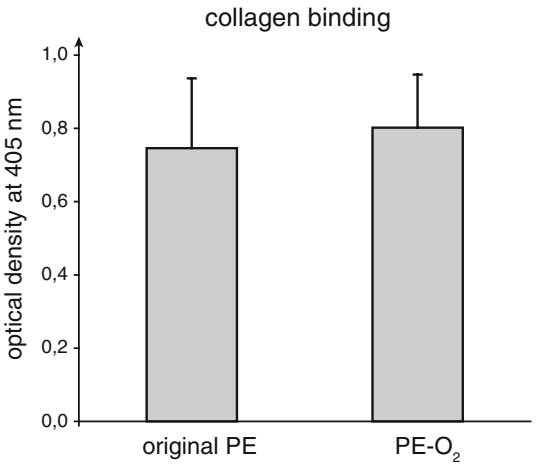

Fig. 2 Collagen II bound to the PE-surface without (original PE) and after oxygen plasma treatment (PE-O_2). The functionalized surfaces bound slightly more collagen II

3.2 Animal evaluation

Half of the PE-specimens with and without the described surface functionalization were seeded with autologous cells from guinea pig concha. The specimens were inserted into the donor pig's ear after being in vitro for two weeks. The unseeded control-specimens were implanted in the opposite ear.

A total 152 PE-specimens were implanted into 76 guinea pigs and evaluated. Fifty percent of these were found to be covered with autologous cells. In 21 animals, we found a rejection of the implant. Because of the missing control, these animals were excluded from the evaluation. Table 1 shows the distribution of the PE-samples according to their

Table 1 Surface modification and implant rejection

Time of explantation	1 month			6 months			12 months		
Surface modification	PE	PE-collagen	PE-O_2-collagen	PE	PE-collagen	PE-O_2-collagen	PE	PE-collagen	PE-O_2-collagen
Number of animals	10	8	10	9	7	9	7	7	8
Evaluated animals	5	7	6	6	6	7	6	4	7
Rejection without cells	5	1	4	3	1	1	1	3	1
Rejection with cells						1			

surface modification and explantation time. Only one specimen of the cell seeded group was rejected whereas a rejection of 20 specimens was detected in the unseeded group. Thus, 13.8% of the implanted specimens had been rejected, 13.14% without autologous cells versus 0.66% of cell seeded PE-specimen.

Most rejections were found in the unmodified group followed by the oxygen plasma treated and collagen covered PE group. Only in the former group was one cell seeded PE-specimen rejected. In most cases rejection occurred in the first month after implantation.

The rejections were detected at the end of each in vivo incubation time because of the missing macroscopic inflammation.

3.3 FBGC and modified PE-implants

To get information on the immunological potential of the PE-implants, the number of FBGC was analyzed. Figure 3 shows the results of the morphometrically evaluated measurements. The amount of FBGC, surrounding the implant, was estimated and related to the area of the PE-implant. The immune reaction of the cell seeded samples and the unseeded controls were compared. Figure 3 focuses only the immune reaction in relation to the surface modification without respect to the explantation time.

A two-ways analysis of variances (ANOVA) with factors of cell seeding (unseeded and cell seeded), surface modification (original, co II, and O_2 co II) showed a trend of cell seeding main effect ($F(1,100) = 2.31$, $P < 0.1$). Post hoc tests (Tukey-HSD) revealed a decreased number of FBGC in the cell seeded PE-specimens. Thus, the cell seeded PE-specimens showed a weaker immune reaction than the unseeded controls. The best result was found with the PE-specimens covered by collagen (Fig. 3). In the oxygen-plasma treated group, no benefit of cell seeding appeared when compared to the different experimental groups regardless of the evaluation time.

No clear pattern could be obtained when evaluation was done using FBGC areas (Fig. 4). The data demonstrate that the benefit of cell seeding was amplified in the collagen group with autologous cells. The increase in FBGC areas after one year in vivo was astonishing. The FBGC area was

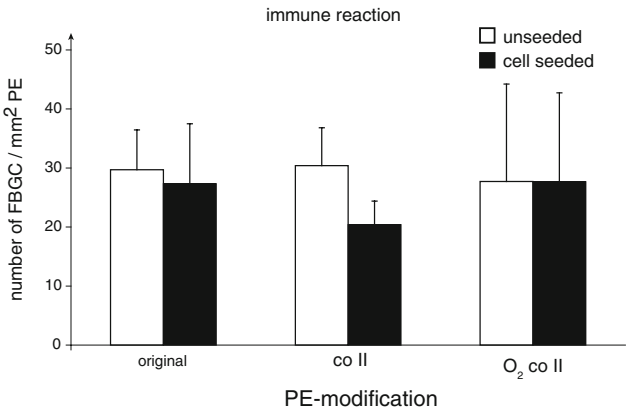

Fig. 3 Foreign body reaction of the surrounding tissue. PE-implant without cells (*white*) and with cells (*black*), summarized over the observation period. The number of foreign body giant cells was normalized to the PE-area. (Means with standard deviation, $n = 17$, 17 and 20 animals, see Table 1)

compared with a three-ways ANOVA with factors of cell seeding (unseeded and cell seeded), surface modification (original, co II, and O_2 co II), and evaluation time (1, 6, and 12 months). The main effect of the evaluation time ($F(2,88) = 2.92$, $P < 0.1$) barely missed significance and had to be discussed.

However, a FBGC reaction scoring based evaluation revealed a more homogeneous tendency (Fig. 5). The seeding of the specimens with autologous cells before implantation proved advantageous (three-ways ANOVA ($F(1,92) = 5.57$, $P < 0.05$)). Whereas the surface modifications produced only a slight benefit which was not significant.

3.4 Cartilage development

As a second parameter, the newly built cartilage areas were estimated. The results obtained by morphometric measurements showed more cartilage islands in specimens seeded with cells. In contrast to the immune reaction, the best regeneration of cartilage was found with unmodified PE-surfaces (Fig. 6). The control group revealed the best cartilage development in the plasma treated, collagen coated group. A three-ways ANOVA of the cartilage area

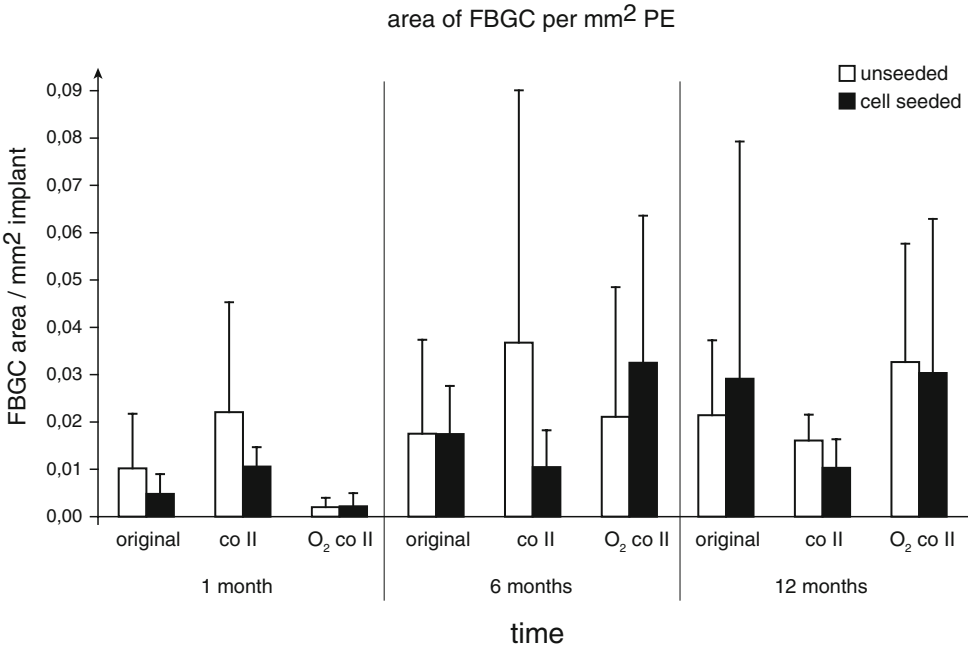

Fig. 4 Foreign body reaction of the surrounding tissue for unseeded (*white*) and cell seeded (*black*) PE-implants and explantation time. The area of foreign body giant cells was related to the surrounded PE-area (means with standard deviation, *n* about 6; see Table 1 for detailed description)

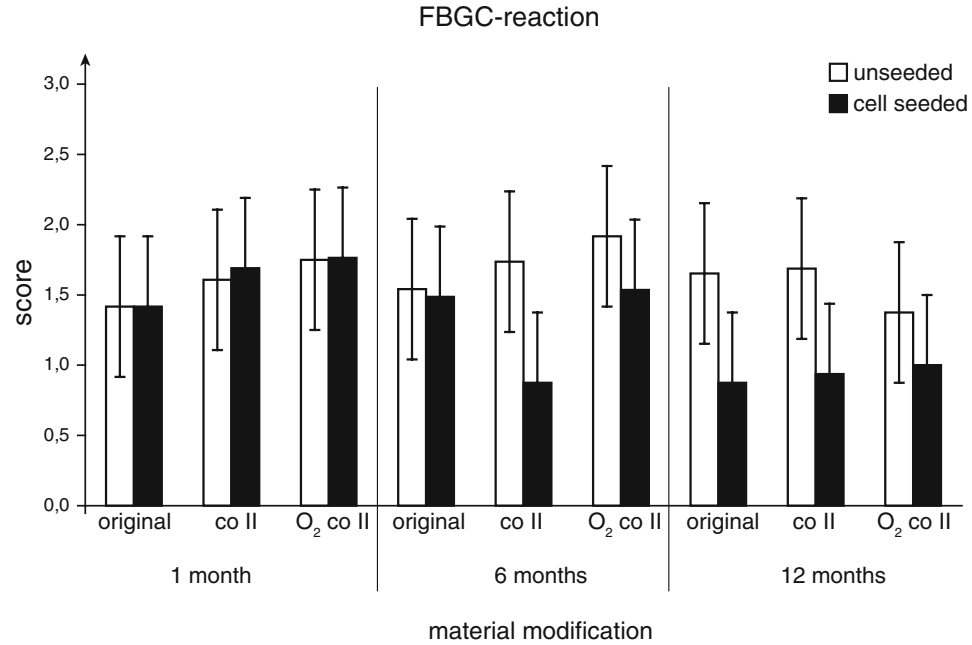

Fig. 5 Score of foreign body reaction at the three different evaluation points (no reaction = 0, slightly reaction = 1, moderate reaction = 2, severe reaction = 3) for unseeded (*white*) and seeded (*black*) PE surfaces. Score was done by four different observers and averaged (Means with standard deviation, *n* about 6, see Table 1 for detailed description)

with factors of cell seeding (unseeded and cell seeded), surface modification (original, co II, and O_2 co II), and evaluation time (1, 6, and 12 months) demonstrated a main effect of cell seeding ($F(1,88) = 6.90$, $P < 0.01$) and a main effect of the evaluation time ($F(2,88) = 3.46$, $P < 0.05$). No other main effect or interaction was significant. Post hoc comparisons (Tukey HSD) showed an increased cartilage area of the cell seeded PE-specimen compared to the original PE-specimen Investigation 12 months after implantation revealed a larger cartilage area than investigated 1 month after implantation.

To verify these results again, a second evaluation was done by scoring. The differences between the individual surface modifications were smaller but revealed the same trend. Again, the unmodified cell seeded PE-specimens showed the most newly developed cartilage (Fig. 7). Based on the score level, a moderate cartilage development for the cell seeded original PE could be observed. A three-ways ANOVA of the cartilage score with factors of cell seeding (unseeded and cell seeded), surface modification (original, co II, and O_2 co II), and evaluation time (1, 6, and 12 months) indicated a main effect of cell seeding

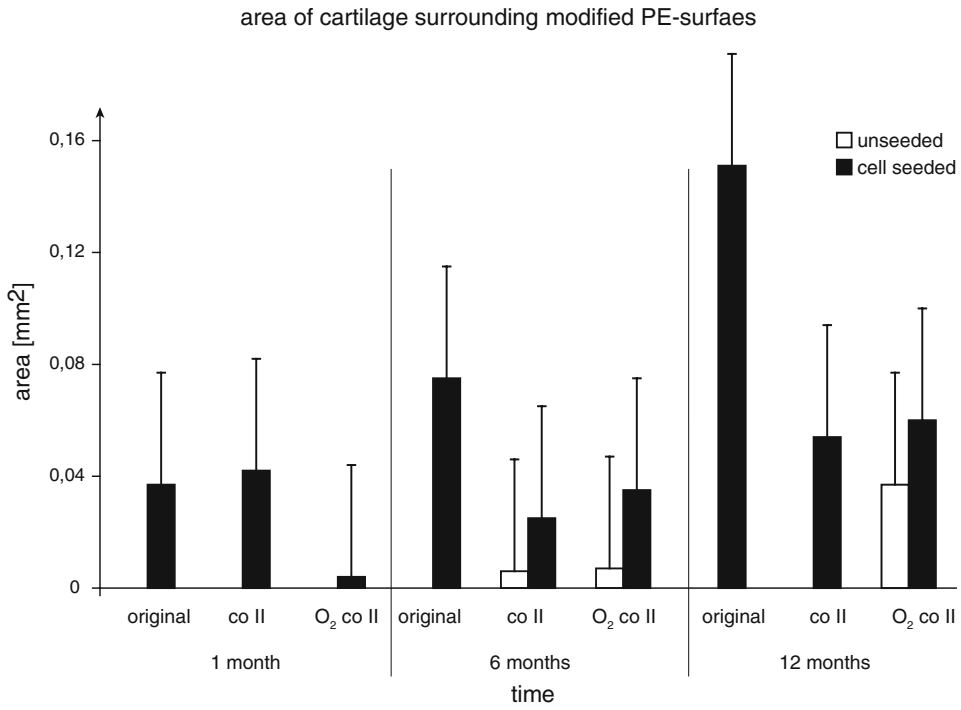

Fig. 6 Cartilage area development of the modified cell seeded (*black*) and unseeded (*white*) PE-specimens (*black*) at the three different explantation times (*n* about 6, see Table 1 for detailed description)

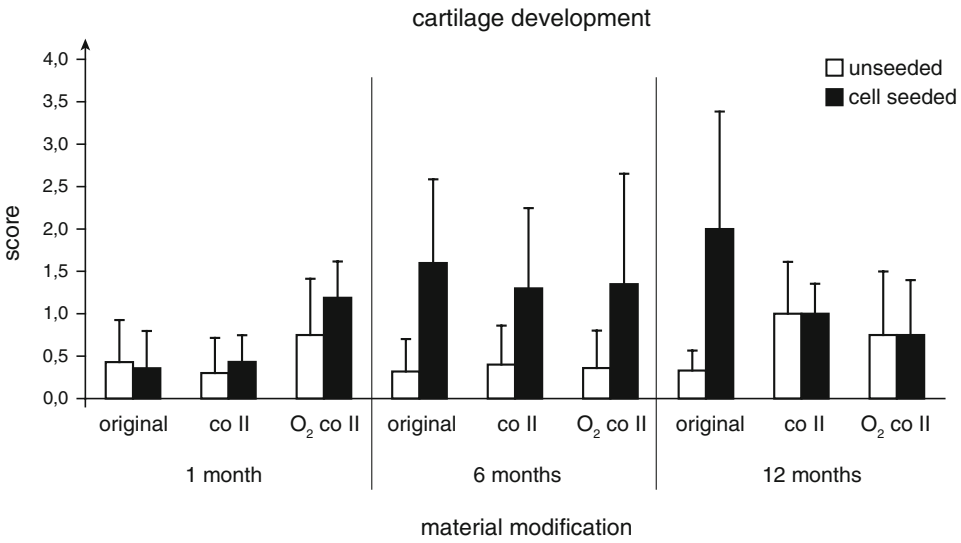

Fig. 7 Cartilage score at the three different evaluation points (no cartilage = 0, slightly cartilage areas = 1, moderate cartilage areas = 2, good cartilage development = 3) for unseeded (*white*) and seeded (*black*) PE surfaces. Score was done by four different observers and averaged (Means with standard deviation, *n* about 6, see Table 1 for detailed description)

($F(1,92) = 16.36$, $P < 0.001$) and an interaction of cell seeding and the evaluation time ($F(2,88) = 3.25, P < 0.05$). No other main effect or interaction was significant.

4 Discussion

Artificial materials for tissue replacement should be biocompatible. To meet this requirement, several biomaterials were developed and tested in different experimental models. The aim of this study was to improve the characteristics of porous PE, in clinical use as MEDPOR®. In order to improve the connection between synthetic PE and the surrounding tissue, the ECM molecule collagen II was coated onto the PE's surface.

Starting with in vitro examinations [21] of the modified PE, there was the need to prove the received results. Biomaterials have to be analyzed in terms of their function [21, 22]. Most publications on regeneration and tissue engineering of cartilage deal with articular cartilage. In head and neck area, mainly hyaline and elastic cartilage was found. In this study we looked for tissue replacement of elastic auricular cartilage. As a well established animal model in head and neck surgery the guinea pig was chosen.

The animals fulfil the model function in regeneration of elastic cartilage especially well and are good to handle. Recently Petersen et al. [30] applied the minipig model to analyze implanted long term tissue engineered hyaline cartilage constructs. The authors used this model for hyaline cartilage replacement of osteochondral lesion in the knee joint. Their model differs from ours in terms of mechanical load. Landis et al. [31] showed the importance of the autologous cells' origin with respect to their function in the body. However, the mechanical stability of tissue engineered cartilage remains a problem. To overcome this, synthetic implants like porous PE (MEDPOR®) or rib cartilage are used for replacement of auricular lesions in clinical practice.

Although porous PE is an established implant material, repulsion reactions sometimes occur even after a long time as discussed above. Carinci et al. [32] analyzed the complication and risk factors of PE-implantations in a retrospective clinical study and found a failure rate of 14.8% for ear rebuilding.

The results presented here demonstrate the benefit of covering PE-implants with autologous cells for cartilage replacement. This is the first report concerned with in vivo integration of PE-hybrid systems composed of porous PE and autologous cells in terms of their function.

In our study the rejection rate of 0.66% of cell seeded PE-specimens demonstrates a clear reduction, caused by covering the PE-surface with autologous cells. In addition, the surrounding tissue interacts with the autologous cell layer after implantation. It is likely that the body recognizes these cells as its own and prevents immune reactions against these cells, as shown by the reduced presence of foreign body giant cells (see Figs. 3, 4 5).

Collagen binding may also prevent an immune reaction. The coating with collagen alone improved the biological properties of the surface with respect to the immune reaction. The oxygen plasma treatment opened the possibility for covalent collagen binding. Little or no effect could be detected in the oxygen plasma treated group (Fig. 4). These findings were consistent with our results found in vitro [21]. Furthermore, they demonstrated the limitations of in vitro screening of biomaterials where the immune reaction is not relevant. As immune reactions increase the chance of retraction of the artificial cartilage also in patients, the use of immune competent animals is therefore important.

On the whole the quantification of the observed effects was difficult, due to unavoidable technical problems. Different stabilities of the PE-implant and the surrounding tissue caused a crumpling of the surrounding tissue and the histological slice became uneven. In that way, the examination of several layers was induced, possibly leading to incorrect results. The histological slices of the PE-specimens had different lengths because of their round form. To equalize the differences caused by the material, the results were normalized per mm^2 PE, analyzing the whole area of the implant with the surrounding cells.

In order to control the morphometric system, the histological specimens were evaluated by additional scoring for foreign body reactions. In contrast to the morphometric measurement, the score values showed a more homogeneous figure, starting with equally distributed FBGC over all groups (see Fig. 5). The results reflected the same trend: Seeding PE-implants with autologous cells before implantation led to a reduction of the immune reaction. A further improvement was reached by coating the PE-surface with collagen.

A similar tendency was visible with autologous cells when cartilage development was examined. We could show that seeding specimens with autologous cells was advantageous (see Fig. 6).

However, the effect of collagen coating is unclear. On the one hand, by using PE without autologous cells before implantation, the coating showed a positive effect on the cartilage development. The best results in cartilage regeneration were obtained by the combination of functionalization and covalent collagen binding (see Fig. 6, 12 month, right column), but the regeneration level was very low compared to the original PE-surface. As expected, the amount of collagen on the surface was higher in the oxygen plasma treated group (Fig. 1).

On the other hand, in the group of cell-seeded specimens, this surface modification did not produce an improvement, e.g., better integration, more new cartilage development. Long term observation showed: the best cartilage development was found in the case of cell seeded unmodified PE (Figs. 6, 7). The difference between cell-containing and cell-free specimens was statistically significant but this could not be found for the other surface modifications. Again, these results document the limitations of our in vitro findings, where about 10% more chondrocytes grew on collagen covered smooth PE surfaces [21]. The porosity of the material plays an important role for the cell adhesion and ingrowths. It is difficult to calculate the effects of different modifications like surface structure, coating or covalent binding of collagen. This difficulty demonstrates again that in vitro results are preliminary and have to be proven in vivo. The observed main effect could be monitored in vivo only.

There are several reasons for the lack of statistical significance. First, the PE material and the surrounding tissue differ in their stability. After positioning the slice onto the microscopic slide, further staining manipulations induced the loss of the implant in several cases. Second, it was impossible to get planar sections as illustrated in Fig. 8a and b. The results of the morphometric measurement of these specimens revealed a large variability.

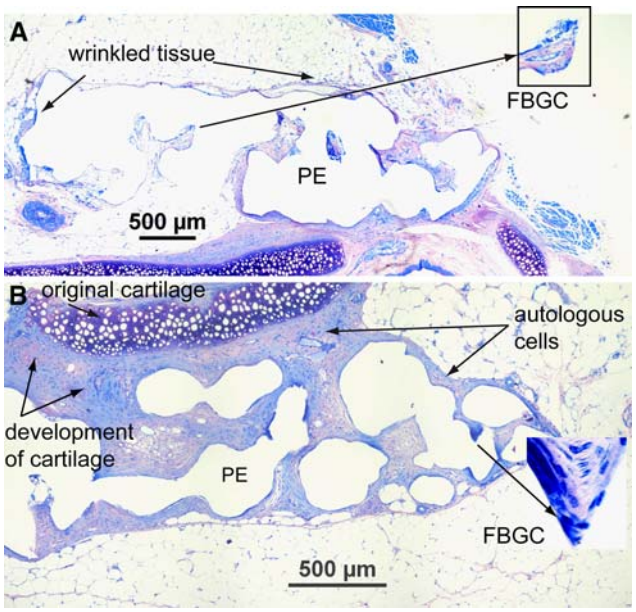

Fig. 8 Image of PE-specimen after one year in vivo integrated into the surrounding tissue without (**a**) and seeded with autologous cells (**b**) before implantation

A third reason for the lack of statistical significance was the inhomogeneous seeding of the PE samples, resulting in cell seeded specimens with areas missing the autologous cells. Hilborn and Bjursten [33] described necrotic processes by macrophages at the inert implant-tissue interface. Necroses of cells elicit a pro-inflammatory response and hence a more intense immune reaction. From the performed in vitro studies we know: there is a critical cell quantity for good cartilage development (unpublished results). Petersen et al. [30] described a two step seeding of the scaffold and a conditioning of the chondrocytes after monolayer expansion in alginate gels. This seems to be a good tool in order to get a homogeneous spreading of the chondrocytes on the PE-material and should be tested in further experiments.

5 Conclusions

In order to improve the implant properties MEDPOR®, the PE surface can be modified in two different ways: first, by chemical (collagen coating or covalent binding after oxygen plasma treatment) and/or, second, by biological conditioning (seeding autologous cells onto the surface).

The results presented here indicate that porous PE implants are better integrated if seeded with autologous cells. In order to further improve the biocompatibility of non-resorbable biomaterials, autologous cells seem to be a useful tool. The surface modification by collagen coating leads to a reduction of the immune reaction if no autologous cells are available. However, collagen isolated from animals may cause allergic reactions in humans.

The results of this animal experimental study confirmed our former in vitro findings, concerning the effect of collagen coating. Our results demonstrate that cartilage regeneration needs a long time.

In order to track the rejection rate in vivo, autologous cells should be labeled, seeded onto unmodified PE-surfaces and implanted into the guinea pig. The in vivo imaging of the implants should be done by MRI.

In further in vitro and in vivo studies, the non-resorbable PE-material should be replaced by a PGA-based scaffold tailored for cartilage and bone tissue replacement. The hybrid system should be analyzed by biochemical, histochemical, immune histochemical and gene expression examinations in a more detailed manner as described by Landis et al. [31] and Tigli [34]. But finally the integration and mechanical stability of tissue engineered cartilage has to be evaluated in vivo.

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References

1. Lin Z, Willers C, Xu J, Zheng MH. The chondrocyte: biology and clinical application. Tissue Eng. 2006;12:1971–4. doi:10.1089/ten.2006.12.1971.
2. Praveen J, Patel MA, Rees HC, Olver JM. Fibrovascularization of porous polyethylene orbital floor implants in humans. Arch Ophthalmol. 2003;121:400–3.
3. Romo TIII, Sclafani A, Sabini P. Use of porous high-density polyethylene in revision rhinoplasty and in the platyrrhine nose. Aesthetic Plast Surg. 1998;22:211–21. doi:10.1007/s002669900193.
4. Romo TIII, Sonne J, Choe KS, Sclafani A. Revision rhinoplasty. Facial Plast Surg. 2003;19:299–307. doi:10.1055/s-2004-815649.
5. Nam SB, Bae YC, Kang YS. Analysis of the postoperative outcome in 405 cases of orbital fracture using two synthetic implants. Ann Plast Surg. 2006;56:263–7. doi:10.1097/01.sap.0000199173.73610.bc.
6. Cui HG, Li HY, Chen YH. Analysis of high density porous polyethylene (Medpor) orbital implant in 266 cases. Zhonghua Zheng Xing Wai Ke Za Zhi. 2006;22:133–5.
7. Carboni A, Cerulli G, Perugini M, Renzi G. Long-term-follow-up of 105 porous polyethylene implants used to correct facial deformity. Eur J Plast Surg. 2002;25:310–4. doi:10.1007/s00238-002-0407-3.
8. Berghaus A. Implantate für die rekonstruktive Chirurgie der Nase und des Ohres. Laryngorhinootologie. 2007;86:S67–76. doi:10.1055/s-2007-966301.
9. Blaydon SM, Shepler TR, Neuhaus RW, White WL, Shore JW. The porous polyethylene (Medpor) sperical orbital implant: a retrospective study of 136 cases. Ophthal Plast Reconstr Surg. 2003;19:364–71. doi:10.1097/01.IOP.0000083643.36461.84.

10. Shin H, Jo S, Mikos AG. Biomimetic materials for tissue engineering. Biomaterials. 2003;24:4353–4. doi:10.1016/S0142-9612(03)00339-9.
11. Isogai N, Kusuhara H, Ikada Y, Ohtani H, Jacquet R, Hillyer J, et al. Comparison of different chondrocytes for use in tissue engineering of cartilage structures. Tissue Eng. 2006;12:691–3. doi:10.1089/ten.2006.12.691.
12. Perka C, Schultz O, Sittinger M, Zippel H. Chondrozytentransplantation in PGLA/Polydioxanon-Vliesen. Orthopade. 2000;29:112–9.
13. Cao Y, Vacanti MP, Paiqe KT, Upton J, Vacanti CA. Transplantation of chondrocytes utilizing a polymer-cell construct to produce tissue-engineered cartilage in the shape of a human ear. Plast Reconstr Surg. 1997;100:297–302. doi:10.1097/00006534-199708000-00001.
14. Aigner J, Tegeler J, Hutzler P, Campoccia D, Pavesio A, Hammer C, et al. Cartilage tissue engineering with novel nonwoven structured biomaterial based on hyaluronic acid benzyl ester. J Biomed Mater Res. 1998;42:172–81. doi:10.1002/(SICI)1097-4636(199811)42:2<172::AID-JBM2>3.0.CO;2-M.
15. Lee CR, Grodzinsky, Hsu H-P, Spector M. Effects of a cultured autologous chondrocytes-seeded type II collagen scaffold on the healing of a chondral defect in a canine model. J Orthop Res. 2003;21:272–81. doi:10.1016/S0736-0266(02)00153-5.
16. Nehrer S, Breinan HA, Ramappa A, Young G, Shortkroff S, Louie LK, et al. Matrix collagen type and pore size influence behavior of seeded canine Chondrozytes. Biomaterials. 1997;18:769–76. doi:10.1016/S0142-9612(97)00001-X.
17. Angele P, Abke J, Kujat R, Faltermeier H, Schumann D, Nerlich M, et al. Influence of different collagen species on physico chemical properties of crosslinked collagen matrices. Biomaterials. 2004;25:2831–41. doi:10.1016/j.biomaterials.2003.09.066.
18. Rotter N, Haisch A, Bücheler M. Cartilage and bone tissue engineering for reconstructive head and neck surgery. Eur Arch Otorhinolaryngol. 2005;262:539–45. doi:10.1007/s00405-004-0866-1.
19. Bryant SJ, Durand KL, Anseth KS. Manipulations in hydrogel chemistry control photoencapsulated chondrocytes behavior and their extracellular matrix production. J Biomed Mater Res. 2003;67A:1430–6. doi:10.1002/jbm.a.20003.
20. Baek CH, Ko YJ. Characteristics of tissue-engineered cartilage on macroporous biodegradedable PLGA scoffold. Laryngoscope. 2006;116:1829–34. doi:10.1097/01.mlg.0000233521.49393.0d.
21. Röpke E, Schön I, Vogel J, Jamali J, Bloching M, Berghaus A. Screening of modified polyethylene surfaces for tissue engineering of chondrocytes. Laryngo-Rhino-Otol. 2007;86:37–43. doi:10.1055/s-2006-945025.
22. Vats A, Tolley NS, Polak JM, Gough JE. Scaffold and Biomaterials for tissue engineering: a review of clincal applications. Clin Otolaryngol. 2003;28:165–72. doi:10.1046/j.1365-2273.2003.00686.x.
23. Cancedda R, Dozin B, Giannoni P, Quarto R. Tissue engineering and cell therapy of cartilage and bone. Matrix Biol. 2003;22:81–91. doi:10.1016/S0945-053X(03)00012-X.
24. Rickert D, Lendlein A, Peters I, Moses MA, Franke RP. Biocompatibility testing of novel multifunctional polymeric biomaterials for tissue engineering applications in head and neck surgery: an overview. Eur Arch Otorhinolaryngol. 2006;263:215–22. doi:10.1007/s00405-005-0950-1.
25. Dayss E, Leps G, Meinhardt J, Wutzler A. Biokompatible Polymerschichten. In: Leps G, Kausche H, editors. 40 Jahre Werkstofftechnik. Merseburg; 1999, p. 84.
26. Harlow E, Lane D. Antibodies: a laboratory manual. New York: Cold Spring Harbour laboratory; 1988.
27. Freshney RI. Culture of animal cells. A manual of basic technique. 2nd ed. Berlin, New York: de Gruyter; 1990.
28. Romeis B. Mikroskopische Technik. 17th ed. München: Urban und Schwarzenberg; 1989.
29. Custers RJ, Creemers LB, Verbout AJ, van Rijen MH, Dhert WJ, Saris DB. Reliabaility, reproducibility and variability of the traditional Histologic/Histochemical Grading System vs the new OARSI Osteoarthritis Cartilage Histopathology Assessment System. Osteoarthritis Cartilage. 2007;15:1241–8. doi:10.1016/j.joca.2007.04.017.
30. Petersen JP, Ueblackker P, Goepfert C, Adamietz P, Baumbach K, Stork A, et al. Long term results after implantation of tissue engineered cartilage for the treatment of osteochondral lesions in a minipig model. J Mater Sci: Mater Med. 2008;19:2029–38. doi:10.1007/s10856-007-3291-3.
31. Kusuhara H, Isogai N, Enjo M, Otani H, Ikada Y, Jacquet R, et al. Tissue engineering a model for the human ear: assessment of size, shape, morphology, and gene expression following seeding of different chondrocytes. Wound Rep Reg. 2009;17:136–46.
32. Cenzi R, Farina A, Zuccarino L, Carinci F. Clinical Note Clinical outcome of 285 Medpor grafts used for craniofacial reconstruction. J Craniofac Surg. 2005;16:526–33. doi:10.1097/01.scs.0000168761.46700.dc.
33. Hilborn J, Bjursten LM. A new and evolving paradigm for biocompatibility. J Tissue Eng Regen Med. 2007;1:110–9. doi:10.1002/term.4.
34. Tigli RS, Gümüsdereioglu M. Evaluation of alginate-chitosan semi IPNs as cartilage scaffolds. J Mater Sci: Mater Med. 2009;20:699–709. doi:10.1007/s10856-008-3624-x.