Non-woven PGA/PVA Fibrous Mesh as an Appropriate Scaffold for Chondrocyte Proliferation

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Short title: Non-woven PGA/PVA scaffold for chondrocyte proliferation

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Summary

Non-woven textile mesh from polyglycolic acid (PGA) was found as a proper material for chondrocyte adhesion but worse for their proliferation. Neither hyaluronic acid nor chitosan nor polyvinyl alcohol (PVA) increased chondrocyte adhesion. However, chondrocyte proliferation suffered from acidic byproducts of PGA degradation. Addition of PVA and/or chitosan into a wet-laid non-woven textile mesh from PGA, however, improved chondrocyte proliferation seeded *in vitro* on the PGA-based composite scaffold namely due to a diminished acidification of their microenvironment. This PVA/PGA composite mesh used in combination with a proper hydrogel minimized the negative effect of PGA degradation without dropping positive parameters of the PGA wet-laid non-woven textile mesh. In fact, presence of PVA and/or chitosan in the PGA-based wet-laid non-woven textile mesh even advanced the PGA-based wet-laid non-woven textile mesh for chondrocyte seeding and artificial cartilage production due to a positive effect of PVA in such a scaffold on chondrocyte proliferation.

Key words: PGA; PVA; non-woven textile; chondrocyte; tissue engineering

Introduction

Cells seeded in a biodegradable scaffold that slowly dissipates and is gradually replaced by new functional tissue, this is a promising approach in regenerative medicine. Implantation of such an artificial tissue, namely artificial cartilage based on autologous chondrocytes, is already currently applied in clinical practice for osteoarthritis treatment. Three-dimensional highly porous and preferentially fibrous constructs are inevitable in modern tissue engineering. These scaffolds grant the cells not only with suitable environment for their proliferation and differentiation, but also with a proper mechanical and morphological support. Several hydrogels from biopolymers have been reported to create suitable environment for chondrocytes and to provide three-dimensional immobilization of the cells (Benya and Shaffer 1982, Brittberg *et al.* 1997, Radice *et al.* 2000, Drury and Mooney 2003, Ng *et al.* 2005). Despite many positive features of hydrogels, several serious limitations constrain them for broader application. Optimal biomechanical properties belong among the most significant shortcomings. Consequently, development of more favorable scaffolds fully complying with both biocompatibility and biomechanical requirements is a key task of modern tissue engineering.

Application of composite scaffolds that are formed, besides of hydrogels, also from other compounds like biocompatible polymers seems to be a promising approach. A number of biodegradable polymers were reported. Polyglycolic acid (PGA), known as a resorbable suture in surgery and as ligament fixation in orthopedics, is frequently reported (Freed *et al.* 1994, Rokkanen *et al.* 2000, Schaefer *et al.* 2000, Lee and Shin 2007). Different structures and forms of PGA were already published ranging from foams to nanofibers. Non-woven PGA meshes were also used for chondrocyte proliferation *in vitro* and for extracellular matrix (ECM) production (Freed *et al.* 1994, Schaefer *et al.* 2000). However, inflammatory and nonbacterial

foreign-body reactions of PGA *in vivo* were described in several studies (Bostman and Pihlajamaki 2000). These inflammatory reactions were probably caused by acid byproducts of PGA degradation (Athanasiou *et al.* 1995). Thus, combination of PGA with other material seems to be inevitable namely for a broader *in vivo* application.

A higher amount of poly(lactic acid) (PLA) reportedly diminishes PGA degradation both *in vitro* and *in vivo* (Lu *et al.* 1999, Lu *et al.* 2000). Buffers or different basic compounds, such as calcium carbonate, sodium bicarbonate, calcium hydroxyapatite, wollastine or bioglass 45S5, were also examined as an alternative approach for pH control of PGA-based implants (Agrawal and Athanasiou 1997, Ara *et al.* 2002, Day *et al.* 2004, Li and Chang 2005).

In the present study, biocompatibility of different novel PGA-based non-woven composite scaffolds was tested for chondrocyte adhesion and proliferation. Particular attention was devoted to the positive effect of a nontoxic, water-soluble, biocompatible and biodegradable synthetic polymer – polyvinyl alcohol (PVA), which is widely used in the biomedical field (Jia *et al.* 2007). In addition, hyaluronic acid (HA), a polysaccharide commonly present in synovial fluid and extracellular cartilage matrix, and chitosan, another natural biodegradable biopolymer, were applied in our non-woven composite scaffolds to compensate the negative inflammatory properties of PGA scaffolds and to prepare the optimum environment for chondrocyte seeding and for development of artificial cartilage.

Materials and methods

Materials

The scaffolds were produced from poly(glycolic acid) (PGA) fibers (surgical thread from Chirmax, Ltd., Czech Republic, fiber average diameter 15 μm), polyvinyl alcohol (PVA) fibers (Kuralon K-II, Kuraray Europe GmbH, Germany, fiber average diameter 15 μm), and chitosan fibers (Weifang Young Deok Chitosan CO., LTD, China). Primary antibody against type II collagen (clone II-II6B3, DSHB, USA) and secondary antibody Cy 3-conjugated Donkey Anti-Mouse IgG (H+L) (Jackson ImmunoResearch) were used.

Preparation of the scaffolds

The fiber scaffolds were prepared by the so-called wet-laid process of non-woven production. The fibers were swollen and thoroughly dispersed in low voluminous concentration in various liquid media. The suspension was subsequently transported by a liquid flux on a screen, where a web was formed as a result of filtration-like process. The samples were finally dried and bonded.

There were prepared five different wet-laid non-wovens used as scaffolds with area density 283 g/m^2:

PGA fibers were wet-laid in distilled water into a non-woven scaffold (**PGA**);

A blend of PGA fibers (50 %) and PVA fibers (50 %) was wet-laid in n-butanol and mechanically bonded by needle-punching (**PGA/PVA**);

A blend of PGA fibers (50 %) and PVA fibers (50 %) was wet-laid in n-butanol and consequently impregnated by distilled water. This solvent moderately dissolves PVA fibers that are unstable in water and thus PVA fibers served as a binder for the PGA ones (**PVA/PGA/PVA**);

PGA fibers were wet-laid in a water solution of 0.1 % sodium hyaluronate (w/w) ($M_r = 1.5 \times 10^6$ Da) (**PGA/HA**);

A blend of PGA fibers (1/3), PVA fibers (1/3) and chitosan fibers (1/3) was wet-laid in n-butanol and impregnated with distilled water. Dissolved PVA fibers served as a binder for PGA and chitosan fibers (**PGA/PVA/CH**).

Scaffolds were cut to circular pieces with a diameter of 6 mm and sterilized using hydrogen peroxide gas plasma. Sterilization by plasma was used because of PGA sensitivity to heat and moister. In addition, hydrogen peroxide residuals are nontoxic and noncarcinogenic.

Cells and cell culture conditions

Chondrocytes were isolated from the condyle of femur of a three-month-old New Zealand white rabbit under general anesthesia (60 mg/kg ketamine and 4 mg/kg xylazine). The cartilage was cut to small pieces (1 × 1 mm), put into a collagenase solution (0.9 mg/ml, collagenase crude 816 PSZ) and incubated in a humidified incubator (37 °C, 5 % CO_2) for 14 h. Then the cells were centrifuged at 300 *g* for 5 min and seeded into culture flasks. The chondrocytes were cultivated in Iscove's Modified Dulbecco's Medium supplemented with 10 % foetal bovine serum (FBS), penicillin/streptomycin (100 I.U./ml and 100 µg/ml, respectively), 400 mM L-glutamine, 100 nM dexamethasone, 40 µg/ml ascorbic acid-2-phosphate and ITS – X (10 µg/ml insulin, 5.5 mg/l transferrin, 6,7 µg/l sodium selenite, 2mg/l ethanolamine. Chondrocytes were seeded on scaffolds at a density of 80×10^3 /cm^2; the medium was changed every 3 days.

Cell proliferation analysis by MTT test

The MTT test is widely used for *in vitro* measurement of the metabolic activity and proliferation of cells. MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide] is reduced to purple formazan by mitochondrial dehydrogenase in cells indicating normal metabolism. 50 µl of MTT (1 mg/ml in a phosphate-buffered saline (PBS) pH 7.4) was added to the medium (150 µl) and samples were further incubated at 37 °C for 4 h. Formazan crystals were solubilized with 100 µl of 50 % *N,N*-dimethylformamide/20 % sodium dodecyl sulfate (SDS) /H_2O, pH 4.7. A volume of 200 µl of suspension was removed to the new well and the optical density of formazan was measured (λ_{sample} 570 nm, $\lambda_{reference}$ 690 nm). Absorbance of samples incubated without cells was deducted from cell-seeded samples. Cells seeded on tissue culture plastic (TCP) were used as control.

Fluorescence and confocal microscopy

Cells were stained using propidium iodide and visualized using fluorescence and confocal microscopy. Cells were fixed by frozen ethanol (–20 °C), rinsed with PBS and then propidium iodide (5 µg/ml in PBS) was added for 10 min, rinsed with PBS again and visualized using a confocal microscope (Leica SP2 AOBS (λ_{exc} = 514 nm and λ_{em} = 610–645 nm) and a fluorescence microscope (Olympus IX51, U-RFL-T).

Indirect immunofluorescence staining was used to detect type II collagen. Samples were fixed with 10 % formaldehyde/PBS for 10 min, washed in PBS and then incubated in 3 % FBS in PBS/0.1 % Triton at room temperature. Primary antibody (mouse anti type II collagen) was diluted 1:20 and added to samples for 1 hour at room temperature. Then samples were washed with PBS/0.05 % Tween for 3, 10 and 15 min. Secondary antibody (Cy 3-conjugated Donkey Anti-Mouse IgG (H+L), was diluted 1:300 and added for 45 min at room temperature. After

washing, an antifading solution was added (PBS/90 % glycerol/2.5 % 1,4-diazabicyclo(2,2,2)octane (DABCO). Type II collagen staining was visualized using confocal microscope Leica SP2 AOBS (λ_{exc} = 488 nm, λ_{em} = 555–620 nm).

In vitro degradation of the scaffolds

The weight in dry and wet conditions, and water absorption of every group of scaffolds was measured. Three specimens of each group were dried to constant weight and weighed. Then each specimen was put into 70 % ethanol for 10 min and then into PBS for 1 h. Surface water was wiped off with filter paper and the wet weight of samples was measured. Water absorption (WA) was calculated from the Eq. (1),

$$WA = 100 \times (M_w - M_d)/M_d\,, \qquad (1),$$

where M_w is the weight in wet condition, M_d in dry condition.

Five specimens of each group were used for pH measurement. Each sample was put into 20 ml of PBS in a separate bottle without stirring and refreshing. Samples were placed in an incubator (37 °C) and cultivated for 42 days. The pH was measured every two days (pH-meter HANNA HI 92240).

Statistics

Quantitative data were presented as mean ± SD (Standard Deviation). Results were evaluated statistically using One-Way Analysis of Variance (ANOVA), and the Student-Newman-Keuls Method. The level of significance was set at 0.05.

Results

Chondrocytes adhere well to wet-laid non-woven scaffolds from PGA

Chondrocyte adhesion on wet-laid non-woven scaffolds from different PGA mixtures was measured by the MTT test 24 hours after seeding. In general, chondrocytes adhered well to all PGA-based scaffolds. The best adhesion (absorbance in MTT test $A = 0.41 \pm 0.06$), was observed on both wet-laid non-woven scaffolds from PGA without any additive (see Fig. 1) and the control TCP sample ($A = 0.47 \pm 0.04$). The addition of PVA to the mixture significantly diminished chondrocyte adhesion, except PVA/PGA/PVA. Absorption of composite scaffolds from PVA/PGA/PVA decreased to 0.37 ± 0.02 and for PGA/PVA even to 0.32 ± 0.02.

Curiously, the presence of hyaluronic acid in the PGA mixture did not improved cell adhesion. Absorption remained on the same statistical level with the PGA/PVA mixture (0.29 ± 0.05). This rather surprising finding support and confirm also results from fluorescence confocal microscopy where propidium iodide staining was used to visualize chondrocytes adhered on scaffold fibers (Fig. 2).

Presence of chitosan in the scaffold mixture resulted in the lowest chondrocyte adhesion of all the tested scaffolds. Absorption of the chitosan-containing scaffold (0.12 ± 0.07) was significantly lower not only than the control sample but also than the PVA-containing scaffolds as well than the mesh from PGA/HA mixture.

PVA but not hyaluronic acid improves chondrocyte proliferation on wet-laid non-woven scaffolds from PGA

Despite lower adhesion, the presence of PVA in wet-laid non-woven PGA scaffolds significantly improved chondrocyte proliferation. MTT tests performed on the 7th and 14th day after seeding that the originally a lower cell number adhering to fibers on the first day was

significantly amplified by very good proliferation. Already 7 days after seeding, the highest cell number was observed on PVA/PGA (0.93 ± 0.06). A somewhat lower value was observed after 7 days of cultivation for PVA/PGA/PVA and also on the control TCP (0.78 ± 0.23 and 0.64 ± 0.09, respectively). A lower absorbance 7 days after seeding was found for the PGA-containing scaffold. Absorbance (0.33 ± 0.16) was significantly lower both in the control TCP sample and in the PVA/PGA scaffold. Interestingly, the presence of hyaluronic acid did not improve chondrocyte proliferation at this early stage and, thus, a similar pattern and a significantly lower cell number compared to TCP and PVA containing scaffolds was observed also for the PGA /HA mixture (0.3 ± 0.03).

In spite of the worst adhesion, the presence of chitosan in the wet-laid non-woven scaffold markedly improved proliferation. While seven days after seeding our MTT test suggested a good proliferation (absorption 0.45 ± 0.01) but still did not prove a significant increase compared with the PGA or PGA/HA scaffolds, the PGA/PVA/CH showed a different outline after 14 days of cultivation. The amount of cells increased significantly (1.05 ± 0.14 on day 14; see Fig. 3) and reached the same statistical level as the control TCP sample (1.12 ± 0.12).

This clearly suggested a positive effect of PVA but also chitosan presence in wet-laid non-woven scaffolds on chondrocyte proliferation.

Confocal fluorescence microscopy of wet-laid non-woven scaffolds from PGA mixtures

The positive effect of PVA presence in wet-laid non-woven scaffolds from PGA mixtures on chondrocyte proliferation was proved by the MTT test. However, to confirm this conclusion and to verify suitable proliferation of chondrocytes along the fibers of wet-laid PVA-containing non-woven scaffolds, visualization by confocal microscopy was employed. Cells started to

spread along the fibers already 7 days after seeding. On day 14 and 21, large proliferation of cells was observed, mainly on PGA/PVA/CH and PVA/PGA/PVA (Fig. 4).

A key point for successful preparation of artificial cartilage seems to be preparation of chondrocytes in their optimum condition for transplantation. Only under these circumstances chondrocytes can produce the protein of extracellular matrix. To follow these condition, production of the type II collagen as typical extracellular marker for hyaline cartilage, was observed. Extracellular matrix started to be produced by proliferating chondrocytes on our wet-laid non-woven PGA/PVA scaffolds already during the first week after seeding. Using indirect immunofluorescence of anti-type II collagen, we visualized a developing extracellular matrix by confocal microscopy on days 7 and 14 (Fig. 5). Increasing presence of type II collagen, as a marker of the hyaline cartilage extracellular matrix, is clearly demonstrated.

Scaffolds containing PVA lead to pH stabilization

The time-dependent pH course in the incubation solution over a 42-day period was followed for all tested wet-laid non-woven scaffolds (Fig. 6). A significant pH drop for all PGA-containing scaffolds was found. The largest pH decrease was observed immediately after putting in solution. Despite the comparable initial pH decrease for all tested samples, medium acidification did not continue in the same manner. Acidification of the medium with scaffolds containing PVA continued only very moderately, especially for the PGA/PVA/CH. Conversely, samples containing only PGA or PGA in combination with HA prolonged considerable acidification over the whole 42-day period. This suggested a higher presence of degradation products in the incubation solution.

Wettability of the scaffold influenced scaffold degradation

Slower scaffold degradation of composite PVA-containing wet-laid non-woven scaffolds was accompanied also by another important characteristic: higher water retention inside the scaffolds. Presence of PVA in the mixture increased several times the water absorption in all wet-laid non-woven scaffolds (Table 1). The highest water retention was shown by the PVA/PGA scaffold, three times higher than the PGA scaffold. On the other hand, the addition of hyaluronic acid twice lowered the water content in the scaffold compared to the PGA scaffold. Notably, samples characterized by higher water retention showed also less pronounced pH dependence. In addition, higher water retention in our samples correlates well not only with the pH dependence but also with improved chondrocyte proliferation while the decreased water content in the PGA/HA sample correlated with their worse proliferation.

Discussion

Chondrocytes adhere well but poorly proliferate on PGA non-woven textile mesh

Polyglycolic acid is commonly known as a resorbable substance frequently used in tissue engineering (Freed *et al.* 1994, Rokkanen *et al.* 2000, Schaefer *et al.* 2000, Lee and Shin 2007). Many different forms and structures of PGA were reported for application in regenerative medicine and tissue engineering but only a few reports are related to non-woven PGA meshes and chondrocyte proliferation (Freed *et al.* 1994, Schaefer *et al.* 2000). In accordance with previously reported data, we found PGA non-woven textile mesh as a proper material for chondrocyte adhesion. Interestingly, presence of PGA in composite scaffolds seemed to be dominant for chondrocyte adhesion to non-woven textile mesh. Neither hyaluronic acid nor chitosan nor PVA increased chondrocyte adhesion in any composite scaffold tested in the present

study. Thus, our study clearly showed and confirmed the previously reported results that the mesh from polyglycolic acid (no matter whether in a single-component form or as a composite scaffold) is suitable material for chondrocyte adhesion. Simultaneously, however, chondrocyte proliferation on wet-laid non-woven PGA fibers significantly suffers from acidic degradation products. Naturally, hydrolysis of PGA causes its bulk degradation to glycolic acid which is followed by further transformation in the tricarboxylic acid cycle. Final degradation products are excreted as carbon dioxide and water (Athanasiou *et al.* 1995, Agrawal and Athanasiou 1997).

Elevated concentration of acidic by-products could be solved *in vitro* by pH control and/or incubation solution exchange. This approach, however, can be applied neither *in vivo* nor *in vitro* for composite scaffolds where mesh from polyglycolic acid is immobilized in gels or other similar materials. These composite scaffolds, nevertheless, attract growing attention particularly due to the possibility of tuning up their properties to make them more appropriate for cell seeding and proliferation. Consequently, better understanding of PGA degradation processes and especially improved knowledge of their modulation is highly important.

Addition of PVA improves chondrocyte proliferation on PGA non-woven textile mesh

Although our composite scaffolds based on PGA non-woven textile mesh did not show any improved chondrocyte adhesion, their application in tissue engineering seems to be very promising. A proper additive can modify accordingly the biomechanical properties of the scaffold. Biomechanical properties are modified by presence of type II collagen as was detected in our study by immunofluorescence staining and subsequent visualization by confocal microscopy. Despite of less sensitivity of this method compared to PCR, type II collagen was detected in the vicinity of chondrocytes on day 7 and 14. Production of both type II collagen and

glycosaminoglycans (GAG) are typical indicators of chondrogenic character of cells that appear in conjunction. These components are synthesized by chondrocytes and diffused to the extracellular matrix (ECM) of hyaline cartilage. Joint synthesis of both GAG and type II collagen were commonly detected (Benya and Shaffer 1982, Vunjak-Novakovic *et al.* 1999, Deng *et al.* 2003). Consequently, diffusion of type II collagen from chondrocytes as well as collagen crosslinking decreased by the inhibition of GAG incorporation in the newly formed ECM (Bastiaansen-Jenniskens *et al.* 2009).

Appropriate biomechanical properties of artificial scaffolds are currently a considerable obstacle in the construction of artificial cartilages. In addition, composite scaffolds can adjust the lifetime of biodegradation. Last but not at least, apposite compounds can adapt scaffold biocompatibility. We clearly proved in our study a positive effect of PVA addition in wet-laid non-woven scaffolds from PGA on chondrocyte proliferation. A slightly lower chondrocyte adhesion in PVA-containing textile mesh was overcome already during the first week of incubation. Improved proliferation was accompanied also by a higher pH stability of incubation media. This suggested a moderate appearance of acidic biodegradation products in scaffolds resulting in a more favorable environment for chondrocyte proliferation. Notably, the presence of chitosan in wet-laid non-woven scaffolds from PGA/PVA did not significantly modify the positive effect of PVA on chondrocyte proliferation. Undoubtedly, however, such additives can modify the biomechanics and biodegradability of composite scaffolds. The influence of additives on these parameters, however, could be only hardly predicted, as follows from the effect of hyaluronic acid which is one of the key substances of the hyaline extracellular matrix. Consequently, proper scaffold composition and also the way of preparation remain to be further experimentally checked and tested.

Composite scaffold preparation is certainly an important feature dramatically influencing composite scaffold application. We found that the presence of PVA significantly increases water retention. This is one of the key points in the hypothetical explanation of improved chondrocyte proliferation – higher water retention leads to better supply of chondrocytes with nutrients and also to a quicker disappearance of all kinds of degradation products. Such a scaffold structure needs a firmer connection between the fibers and has to be characterized by a more hydrophilic interior with larger pores for water penetration but also for cell seeding. Firmer and more solid scaffolds could be achieved either chemically or using physical means. Our attempts for a physical way of connection (mechanical puncturing) failed so far. However, we present here a successful chemical approach – an application of PVA as a suitable chemical fiber connection in wet-laid non-woven scaffolds from PGA.

Chitosan but not hyaluronic acid is another component improving chondrocyte proliferation on PGA scaffolds

Besides PVA, in our present study we tested also the effect of other compounds that could improve chondrocyte seeding and proliferation, viz. hyaluronic acid and chitosan. HA is a biocompatible and biodegradable polysaccharide which is a very common component of synovial fluid and extracellular matrix (ECM). It plays an important role in tissue hydration. HA is commonly used in medicine and tissue engineering for its physicochemical properties (Hahn *et al.* 2007) and was reported to improve *in vitro* substrate adhesion ability and proliferative activity of human chondrocytes (Patti *et al.* 2001). Interestingly, however, an attempt to apply HA in our PGA wet-laid non-woven scaffolds composite scaffold with the aim of connecting PGA fibers failed. The presence of hyaluronic acid in the PGA mesh did not result in any

improvement of chondrocyte proliferation compared to PVA scaffold. Notably, the presence of HA in our sample also did not improve water retention. This suggests that rather the size of pores and not the hydrophilicity of the surface component in wet-laid non-woven scaffolds can play a crucial role for proper cell proliferation.

The presence of chitosan, however, resulted in different properties of the PGA composite scaffold. Chitosan is at least partly a deacetylated form of chitin, the second most abundant form of organic resource found in nature next to cellulose. These biopolymers have a good biocompatibility, biodegradation and various biofunctionalities including antithrombogenic, homeostatic, immunity-enhancing, and wound-healing (Jia *et al.* 2007). We applied chitosan in our PGA composite scaffold. While presence of chitosan significantly deteriorated chondrocyte adhesion, chodrocyte proliferation was greatly enhanced after the first week, but especially during the second week of cultivation. Consequently, chitosan, beside PVA seems to be a promising compound for preparing composite scaffolds based on wet-laid non-woven PGA textile mesh intended for chondrocyte tissue engineering. It is necessary, however, to state clearly that the properties of chitosan can sharply depend on its source and purity.

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FIGURE LEGEND

Fig. 1.
Chondrocyte adhesion (MTT test) one day after scaffold seeding (numbers over the bars show the rate of statistically significant difference; *p* is less than 0.05).

Fig. 2.
Fluorescence and confocal microscopy – chondrocyte adhesion on (a) PGA/PVA and (b) PGA/HA scaffold one day after scaffold seeding.

Fig. 3.
Chondrocyte proliferation (MTT test) 7 and 14 days after scaffold seeding (numbers over the bars show the rate of statistically significant difference; *p* is less than 0.05).

Fig. 4.
Fluorescence confocal microscopy of proliferated chondrocytes. Chondrocytes are stained by propidium iodide, which is bound on cell DNA. Fibers of scaffold are also visible for their autofluorescence. PGA/PVA/CH scaffold – (a) 7, (b) 14, (c) 21 days after seeding, PVA/PGA/PVA scaffold – (d) 7, (e) 14, (f) 21 days after seeding.

Fig. 5.
Fluorescence confocal microscopy of proliferated chondrocytes – immunofluorescent detection of type II collagen. PVA/PGA/PVA scaffold (a) 7 and (b) 14 days after seeding.

Fig. 6.
Time-dependent acidification of scaffolds from PGA mixtures**.**

Table 1.

Scaffold Wettability

Scaffold	Dry weight [mg]	Wet weight [mg]	Water absorption [%]
PGA	4.2 ± 0.9	9.5 ± 1.6	141 ± 46
PVA/PGA	4.4 ± 0.2	22.5 ± 1.2	416 ± 26
PVA/PGA/PVA	4.5 ± 0.3	5.5 ± 0.6	23 ± 10
PGA/HA	3.9 ± 0.5	6.7 ± 0.8	73 ± 13
PGA/PVA/CH	5.3 ± 0.3	18.3 ± 2.3	242 ± 22

For calculation detail see Methods.

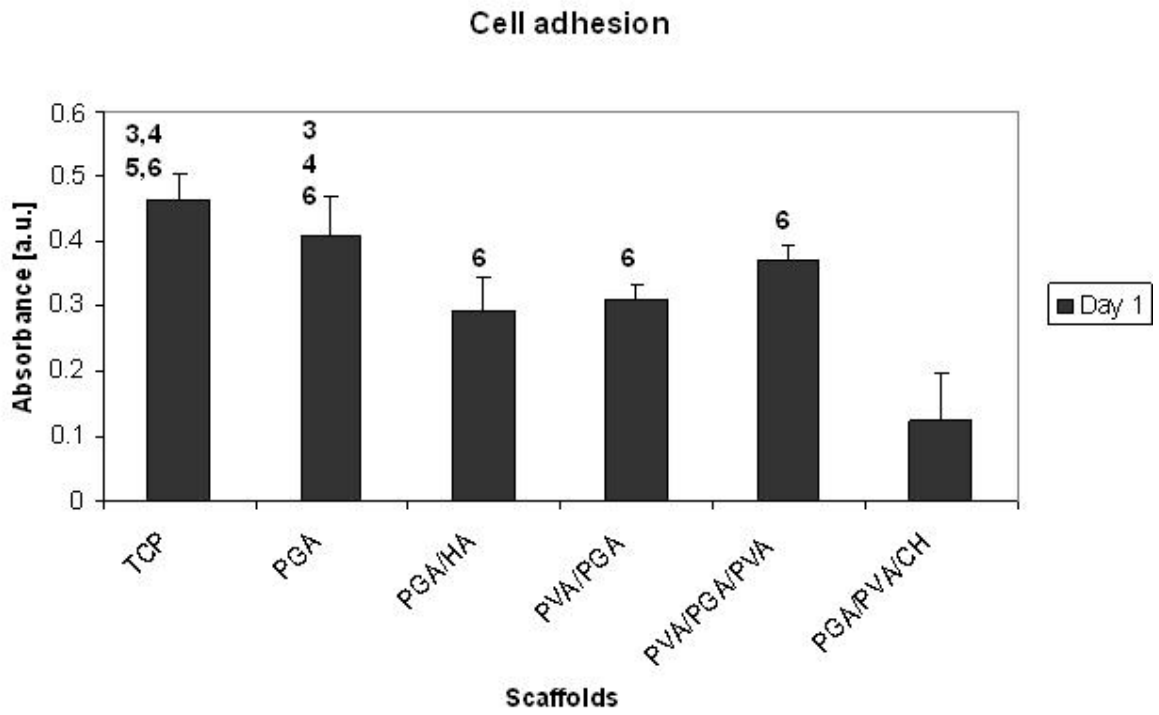

Fig. 1.
Chondrocyte adhesion (MTT test) one day after scaffold seeding (numbers over the bars show the rate of statistically significant difference; *p* is less than 0.05).

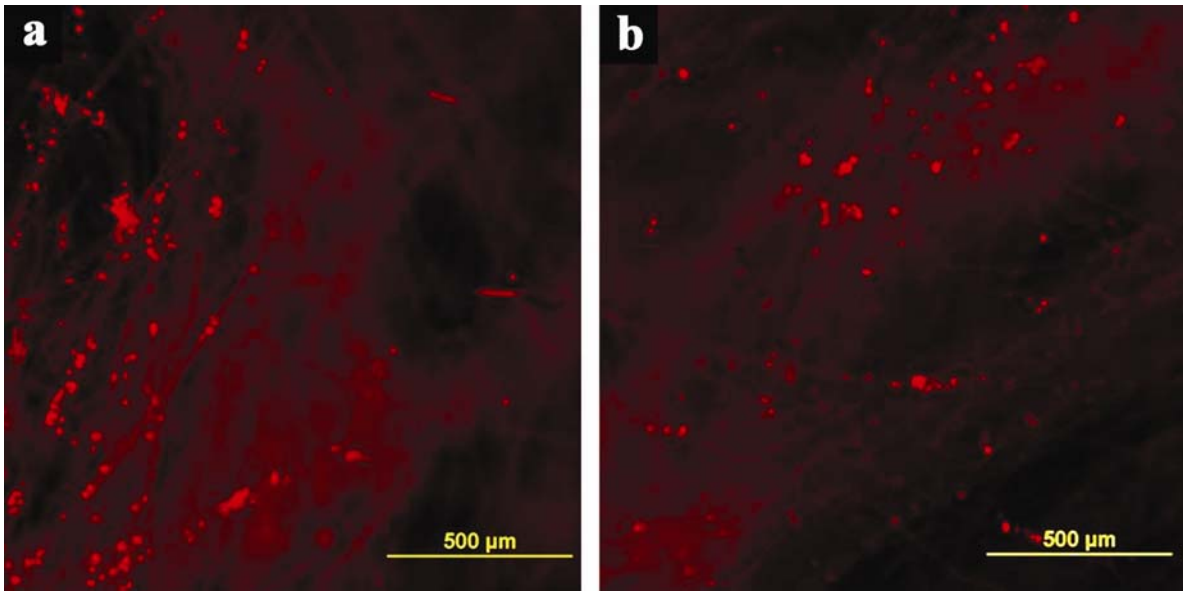

Fig. 2.
Fluorescence and confocal microscopy – chondrocyte adhesion on (a) PGA/PVA and (b) PGA/HA scaffold one day after scaffold seeding.

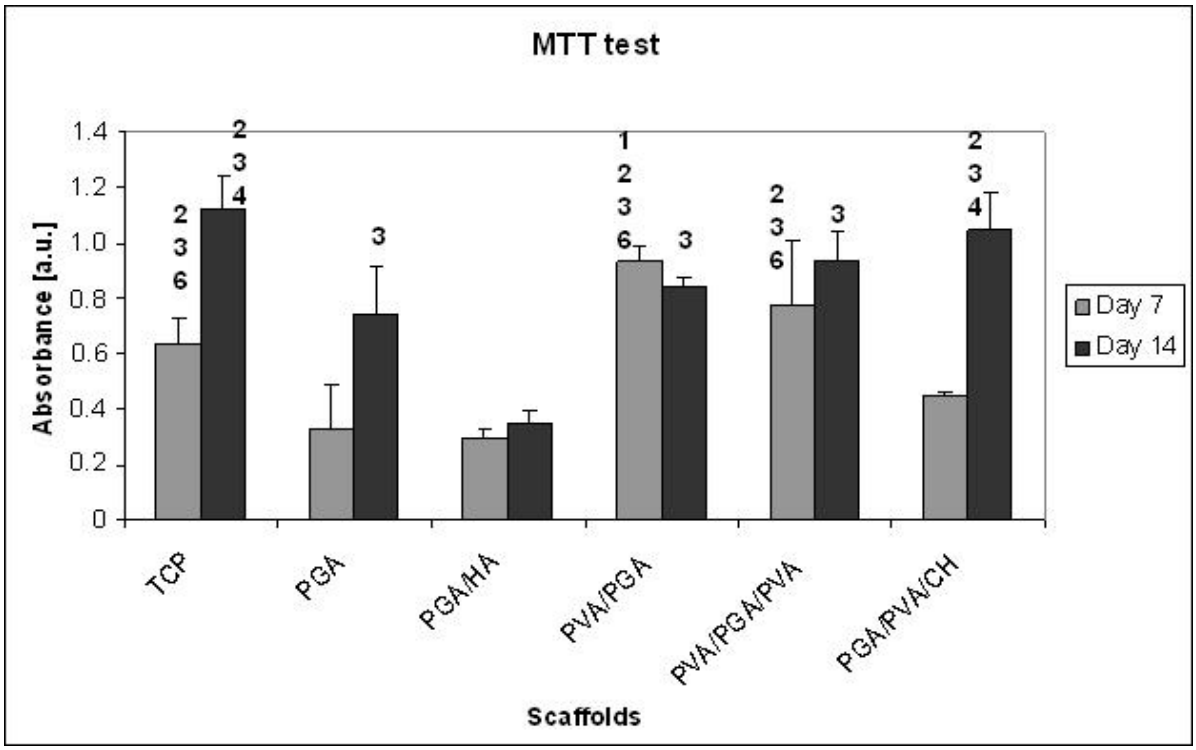

Fig. 3.
Chondrocyte proliferation (MTT test) 7 and 14 days after scaffold seeding (numbers over the bars show the rate of statistically significant difference; *p* is less than 0.05).

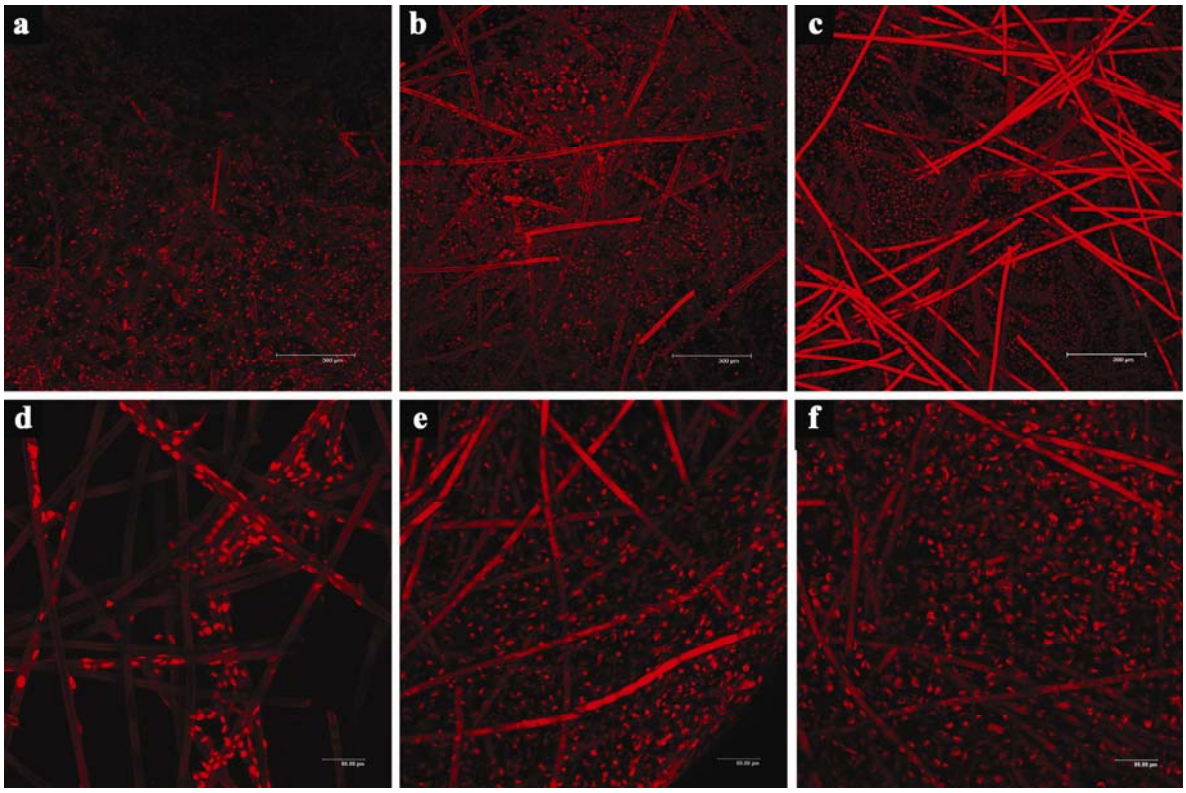

Fig. 4.
Fluorescence confocal microscopy of proliferated chondrocytes. Chondrocytes are stained by propidium iodide, which is bound on cell DNA. Fibers of scaffold are also visible for their autofluorescence. PGA/PVA/CH scaffold – (a) 7, (b) 14, (c) 21 days after seeding, PVA/PGA/PVA scaffold – (d) 7, (e) 14, (f) 21 days after seeding.

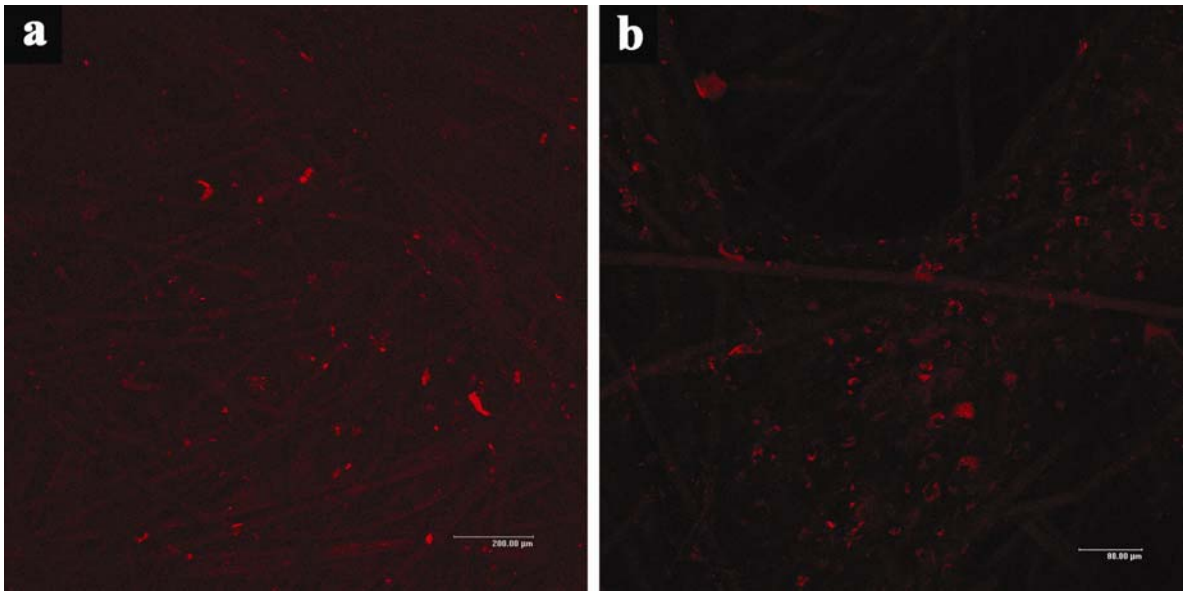

Fig. 5.
Fluorescence confocal microscopy of proliferated chondrocytes – immunofluorescent detection of type II collagen. PVA/PGA/PVA scaffold (a) 7 and (b) 14 days after seeding.

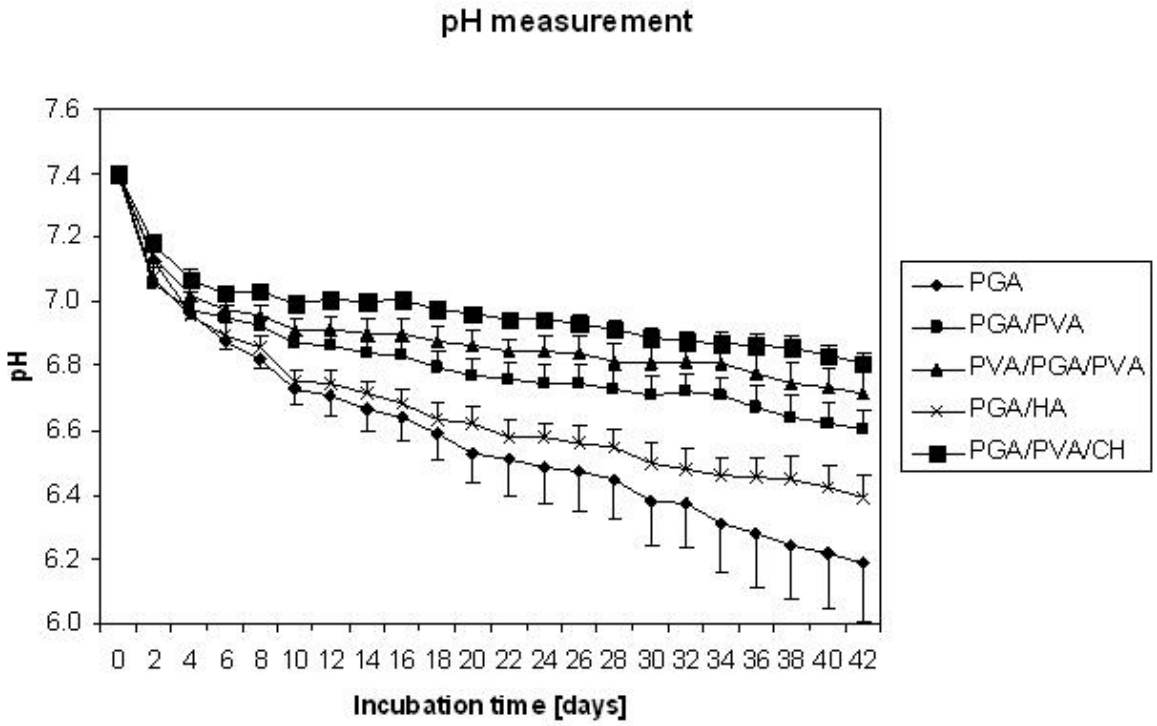

Fig. 6.
Time-dependent acidification of scaffolds from PGA mixtures**.**