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Evaluation of Water Uptake and Mechanical Properties of Biomedical Polymers

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ABSTRACT: Poly(vinyl alcohol) grafted with poly(D,L-lactide) or poly(D,L-lactide-*co*-glycolide) oligomers, were synthesized in our laboratory and investigated with respect to their potential for tissue engineering applications. In order to understand their structure–properties relationships the effect of length and composition as well as number of polyester grafts on PVA backbone chain on water uptake capability and hydrophilicity/hydrophobicity balance and on mechanical properties of hydrogels was evaluated. The *E* moduli of hydrogels display values between 0.01 and 100 MPa. The results indicate the route for the development of polymers with a very broad range of properties similar to those of natural cartilage tissue. © 2013 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 130: 3682–3688, 2013

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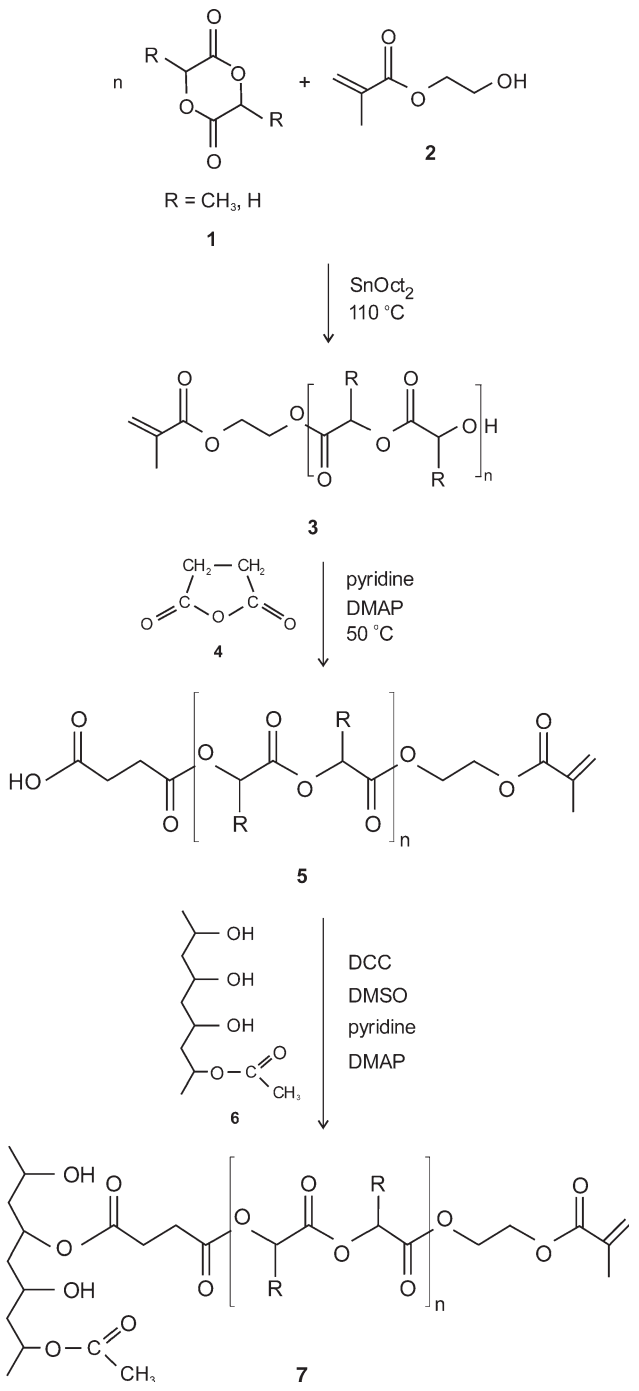
INTRODUCTION

The families of poly(hydroxyortho esters) such as polyglycolide (PGA), polylactide (PLA), and their copolymers (PLGAs), all biodegradable, are among the most intensively studied synthetic polymers that have found significant use in medical and pharmaceutical fields. Since their first application as suture materials, almost 50 years ago, these materials are used for various reconstruction procedures as scaffolding for three-dimensional cell cultures and transplantation of articular, auricular, nasal, cartilage,[1] and intervertebral disk,[2] in bone surgery such as fixation devices, for bone regeneration,[3–5] tissue engineering,[6–8] carriers for delivery of bioactive molecules as well as for drug delivery devices.[9–11] As opposed to the initial simple processing procedures of these materials, current studies are extended to knitted stents,[12] biodegradable polymer / bioceramic composites scaffold,[13–15] nanocomposites,[16] nanofibres,[17] etc. Largely, this is because of the availability, biocompatibility, biodegradability, and processability of these materials. Initial detailed studies of artifacts made out of poly-α-hydroxyacids, PGA, and PLA, or other biodegradable materials such as polydioxanone, poly(trimethylene carbonate), poly(ethylene glycol), and poly (ε-caprolactone), homopolymers and copolymers, have witnessed their biodegradability[18–26] and the absence of significant side effects such as inflammatory reactions, apart from a local pH reduction that results from cleavage of ester bonds and could cause a mild inflammatory influence on tissue.[26–32] This implies that the realm of materials' properties can be widened by modification via copolymerization, which provides a number of advantages because the architecture and composition of the biomaterials can be tailored to control their properties.[24,33,34] The functions of the scaffold in tissue engineering include providing mechanical integrity to the construct to impart dimensional stability, resist deformation because of body forces, and impart impulse to cells growing on the material. Copolymers of glycolide (GA) and lactide [poly(lactide-*co*-glycolide), PLGA] are amorphous where altering the ratio of PLA to PGA results in significant differences in the compression moduli and degradation times of the scaffolds. One of the limitations of scaffolds fabricated from PGA fibers is the small range of mechanical properties of these meshes. The compression modulus of scaffolds increases linearly with the incorporation of LA units, ranging from less than 1 kPa for PGA to approximately 20 kPa for scaffolds with 68% LA content. Hence, increasing the content of LA increases the compression modulus of the scaffolds by more than 20 folds.[14] Furthermore, altering the ratio of LA to GA results in significant changes in adhesion, shape, and proliferation rates of cells seeded onto these scaffolds, since PLA and PGA differ significantly in hydrophilicity/hydrophobicity.[35–38] Since one possible use of these materials is as a substrate for growing a layer of living cells, the material can be customized



Scheme 1. Schematic representation of the synthesis of poly(vinyl alcohol)-graft-[poly(D,L-lactic)/poly(D,L-lactic-*co*-glycolic)] grafted copolymers.

as a hydrogel, that will allow the proper nourishment and proliferation of the layer of living cells, after their attachment and spreading on the surface of the film, owing to the right hydrophobicity.[39] Therefore, it is reasonable to hypothesize that combining of hydrophilic poly(vinyl alcohol) (PVA) with PLA (PLGA) that add dimensional stability and rigidity to scaffolds, may facilitate cell adhesion and additionally alter the process of hydrolysis to these materials.

According to everything previously noted, the objective of this study is to examine PLA/PLGA—PVA hydrogels, regarding formulation, hydrophilic/hydrophobic ratio, and consistency against mechanical load, whereas the cell adhesion pliability is tested as well.

EXPERIMENTAL

Materials

PVA (M_w = 6000, degree of hydrolysis of 80.0%) (Polysciences) was dried in an oven at 80°C until constant weight before use. D,L-Lactide (LA) (Sigma Aldrich) and GA (Boehringer Ingelheim) were recrystallized from dry ethyl acetate (refluxed over calcium hydroxide). Hydroquinone (Merck), 2-hydroxyethyl methacrylate (HEMA) (Fluka), tin(II) bis(2-ethylhexanoate) (stannous octoate, $SnOct_2$, 95.0%) (Sigma Chemicals), 2,2-azobis(2-methylpropionitrile) (AIBN) (Aldrich), 4-(*N*,*N*-dimethylamino)pyridine (DMAP) (Aldrich), pyridine (Aldrich), dicyclohexyl carbodiimide (DCC) (Fluka), solvents: dimethyl sulfoxide (DMSO) (Fluka) and diethyl ether (DEE) (Fluka) were used as received.

Methods

Preparation of Hydrogels. The PVA-graft-[poly(D,L-lactide)/poly(D,L-lactide-*co*-glycolide)] (PLA/PGA-PVA) hydrogels were prepared by synthesis in three steps as previously described in detail.[40] Briefly, the first step was the synthesis of macromonomers by means of ring-opening polymerization (ROP) of LA or GA (1) in the presence of HEMA (2) as the initiator and stannous octoate as the catalyst. In the next synthesis step succinic anhydride (SA) (4), pyridine and DMAP were added to the mixture and left to react in order to transfer the hydroxyl into carboxylic acid end groups. Subsequently, the obtained HEMA-PLA-SA or HEMA-PLGA -SA, (5), were grafted onto the PVA backbone (6) using DCC for the activation of the carboxylic acid groups. The graft copolymers (7) were crosslinked via the methacrylate groups using a free radical AIBN initiator. The synthesis procedure of PLA/PGA-PVA hydrogels is presented in Schemes 1 and 2. The efficacy of each synthesis step was determined by the ^{1}H-NMR spectroscopy method,[40] using a Varian spectrometer (300 and 400 MHz,) in $CDCl_3$ or DMSO-d_6 at room temperature. Tetramethylsilane was used as an internal standard. The materials investigated here are listed in Table I.

The Captive Bubble Contact Angle technique was used in order to study the hydrophilicity of the surface of the highly hydrated polymers. For measuring purpose the swollen hydrogels were cut into pieces of ca. 2–3 cm^2 and fixed on microscope slides. The smaller the contact angle is, the greater is the hydrophilicity of the polymer surface.

The Tensile Tests were performed with the low-load horizontal tensile test machine Minimat 2000 (Rheometric Scientific). The strain rate was 10 mm min^{-1} and ambient temperature (23°C). The tensile bars were taken from water, mopped with filter paper and the dimensions of swollen samples were measured: width: 9 ± 0.5 mm, length: 35 ± 5 mm, thickness: 3 ± 0.2 mm. Average elastic moduli (E) were calculated from the resulting stress/strain curves as the average of five samples measurement.

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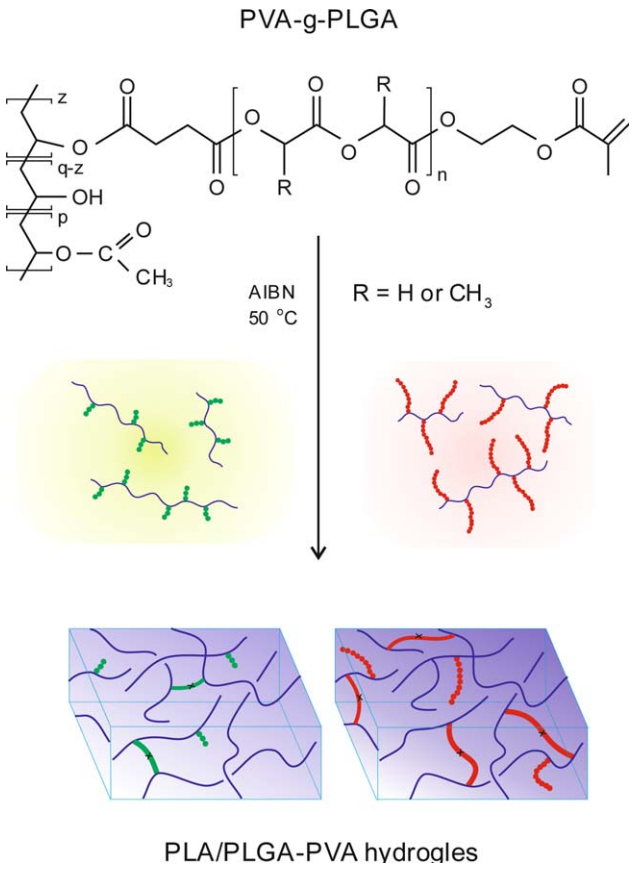

Scheme 2. Schematic representation of crosslinking of grafted copolymers into PLA/PGA-PVA hydrogels. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

The Water Uptake measurements were undertaken based on the experiments that are conducted with series of three samples of each hydrogel having circular shape with defined diameter using a punch cutter. The water uptake of samples was calculated according to eq. (1) after weighting the water swollen sample (m_s) and the same sample in the dry state (m_d):

$$\mathrm{WU}\,(\%) = \frac{m_s - m_d}{m_d} \times 100 \qquad (1)$$

Cell Adhesion Test. *In vitro* cell culture experiments were carried out in order to evaluate the cellular interaction of the hydrogel surface with primary human dermal fibroblasts (hF). The isolation procedure was initiated within 3 h following surgery according to methods described by van den Bogaerdt.[41] Briefly, the polymer disc specimens were sterilized by immersing them into 70 vol% ethanol for 2 h and then rinsed with distilled and sterile water. Tissue-culture grade polystyrene (TCPS), from Greiner Bio-One GmbH, served as control substrate. HFs (4×10^4 cells mL^{-1}) of the fourth passage were seeded onto each material and cultured at 37°C. On day 4, the morphology was investigated by inspection under a light microscope. The samples were treated with 4% formaldehyde in PBS (pH 7.4) and subsequently stained with Mayers' haemalaun at room temperature to study the cells' morphology.

RESULTS AND DISCUSSION

The objective of this study was to compare hydrogel materials from our laboratory that originate from the same starting monomers [PVA grafted with poly(D,L-lactide) or poly(D,L-lactide-*co*-glycolide)] but vary in structure and composition (Table I), in order to determine their potentials, in particular their mechanical, water uptake and surface properties as tissue replacement materials. The studies were focused upon the "wet-state" properties of scaffolds, because the potential implants are used in an aqueous environment.

Water Uptake of Hydrogels

The difference in capability of water uptake is an indicator of differences between materials and can be taken as a preliminary judgment of hydrolytical degradation. Materials examined in this work display the initial water uptake capability between 16% and 69%, depending on their structure and composition. The length of the polyester grafts has a significant influence on the water uptake capacity of these materials as it is shown by pairs of hydrogels A and B or O and P. With decreasing length of the polyester grafts the water uptake increases from 24% in hydrogel A to 40% in hydrogel B and from 25% in hydrogels O to 34% in hydrogel P (Table I). Similarly, a series of samples F, I, and L of higher degree of grafting ($DG_{theor.}$ 20%) of the PVA chains compared to the former ($DG_{theor.}$ 15%), shows a decrease of water uptake because of the increase in polyester graft content. The water uptake amounts from 39% in hydrogel L, 29% hydrogel I, and only 16% in hydrogel F with the longest side chains. If hydrogels A, B, F, I, O, and P are compared further

Table I. Composition of Hydrogels, Theoretical Degree of Grafting on Poly(Vinyl Alcohol) (PVA) Backbone and Water Uptake Capability of the Hydrogels

Sample	LA : GA mol% : mol%	N[a] Ester units in side chain	DG_{theor}[b] on PVA backbone	Water uptake (WU), %	SD_{WU}[c]
A	100 : 0	16	15	24	0.071
B	100 : 0	8	15	40	0.401
C	100 : 0	4	20	44	0.744
E	100 : 0	4	10	61	0.065
F	75 : 25	16	20	16	0.181
I	75 : 25	8	20	29	0.927
J	75 : 25	8	15	38	1.514
K	75 : 25	8	10	47	1.875
L	75 : 25	4	20	39	2.298
M	75 : 25	4	15	45	3.684
N	75 : 25	4	10	60	0.552
O	50 : 50	18	15	25	3.102
P	50 : 50	9	15	34	2.149
Q	50 : 50	4	20	37	1.499
S	50 : 50	4	10	69	0.309

[a] number of ester groups per graft.
[b] theoretical degree of grafting, i.e., number of grafts per PVA chain.
[c] standard deviation of Water Uptake measurements.

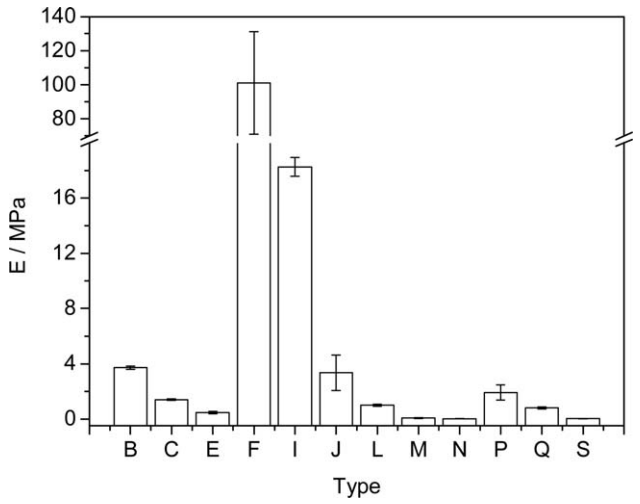

Figure 1. *E* moduli of hydrogels swollen in PBD (pH 7.4) measured with the low-load horizontal tensile test machine at room temperature.

regarding the differences in water uptake because of variation of their composition and *DG*, the influence of composition is seen to be smaller than that of *DG*. Samples F and I display significantly lower water uptake capability because of the higher *DG* despite the medium lactide content. Thus, hydrogels A, with pure lactide polyester grafts and O, with 50 mol% of lactide in the grafts, with $DG_{theor.}$ of 15%, both show a water uptake of around 25%, whereas hydrogel F with 75 mol% of lactide shows a water uptake of 16%, which is the lowest among all hydrogel types because of the long polyester grafts and particularly to the $DG_{theor.}$ of 20%. Also, in the series of hydrogels I, J, and K with decreasing $DG_{theor.}$ (20%, 15%, and 10%) water uptake inecreases from 29% in hydrogel I, to 38% in hydrogel J and up to 47% in hydrogel K. Similarly, in pairs of hydrogels L–N and Q–S increase of $DG_{theor.}$ by a factor of 2 causes approximately a decrease of water uptake to one half. Generally, the water uptake capability of examined hydrogels differs largely depending on their composition and structure, whereas the highest values display hydrogels with shortest polyester grafts and lowest $DG_{theor.}$, i.e., hydrogel N of 60% and hydrogel S of 69%.

Mechanical Properties of Hydrogels

The water-swollen samples were subjected to mechanical testing and obtained *E* moduli are shown in Figure 1. The values of Young's modulus are obtained as an average of five measurements for each sample with standard deviation. The *E* modulus of the hydrogels show significant differences having values between 0.01 and 100 MPa, depending on the composition and number of polyester repeating units and especially on the degree of grafting of PVA. Higher values of the *E* modulus are observed for the hydrogels F and I because of their long polyester grafts with 16 and 8 repeating units, respectively, combined with the high degree of grafting ($DG_{theor.}$ 20%, whereas experimentally determined values were $DG_{exp.}$ 15% and 14%), which results in the highest crosslinking density among all samples. Thus, the *E* modulus of the hydrogel F approaches the modulus of a thermoplastic polymer (>100 MPa).[42] Likewise, hydrogels B, J, and P with eight and nine repeating units in the polyester grafts, respectively, show a higher *E* modulus than the rest of the hydrogels, which have only short polyester grafts with four repeating units. Furthermore, if hydrogels I and J are compared, which have the same number of repeating units and lactide to GA ratio in the grafts, their *E* modulus differs by more than a factor of 4. This is likely to be because of the differences in degree of grafting, $DG_{theor.}$ is 20% for I and 15% for J, whereas in case of hydrogel I $DG_{exp.}$ was found to be 15%. Unfortunately, in hydrogel J it was not possible to determine experimentally the degree of grafting because of physical crosslinking.[43] To add to this, when samples P and J are compared they show close values of *E* modulus although they differ regarding composition but this is the least contributing factor. Bearing in mind that sample P has a fractionally longer PES chain, which is the most important contributing factor and knowing that the $DG_{exp.}$ of sample P is 13% it can be concluded that the crosslinking density of sample J may be the same or, more likely, lower.

All results indicate the strong influence of graft length, which is followed by the influence of the crosslinking density. The hydrogels with the shortest grafts (C, E, L, M, N, Q, and S) are soft (though their $DG_{theor.}$ varies between 10%, 15%, and 20%). As the samples with the shortest polyester grafts and with a similar degree of grafting but different composition regarding the LA/GA ratio (e.g. C, L, and Q or E, N, and S) show a similar *E* modulus, one may conclude that the composition of these copolymers has negligible influence on the mechanical properties. This is opposite to the behavior of poly(hydroxyortho esters) where it was shown that the presence of PLA increases significantly the *E* modulus.[14] Obviously, the presence of PVA, strongly influences the material's properties. It is important to point out that the mechanical properties of aliphatic polyesters depend greatly on molecular weight. The bending strength of a PLA sample with M_w = 160,000 is 50–60 MPa and the modulus of elasticity is ca. 3 GPa. PLA with M_w = 250,000 has a modulus of elasticity of 7 GPa.[44] Thus, high molecular weight of semi-crystalline PLAs results in excellent mechanical properties but as well in long degradation periods and might induce long-term complications *in vivo*. Poly(L-lactide) is a chiral crystalline polymer that is preferred in the production of surgical implants for internal fixation because of its high initial strength and good strength retention.[21] On the other hand, low molecular weight amorphous poly(D,L-lactide) fully degrades at a much greater rate but suffers from poor mechanical properties.[45] Poly(L-lactide) (M_w = 137,000) displays an elastic modulus of ≈20 ± 3 MPa, whereas its 40/60 w/w blend with poly(D-lactide) displays an elastic modulus of 22 ± 3.4 MPa and poly(D,L-lactide) displays an elastic modulus of 2.8 ± 0.4 MPa.[46] Young's modulus, and elongation-at-break of blended poly(L-lactide) and poly(D-lactide) films were reported to be higher than those of nonblended films.[47] PVA hydrogels were proposed as promising biomaterials to replace diseased or damaged articular cartilage. A critical barrier to their use as load-bearing tissue replacement is a lack of mechanical properties. When measured over a strain range of 10–60%, the compressive modulus of PVA hydrogels increases from approximately 1 to 18 MPa, which is within the range of the modulus of articular cartilage.

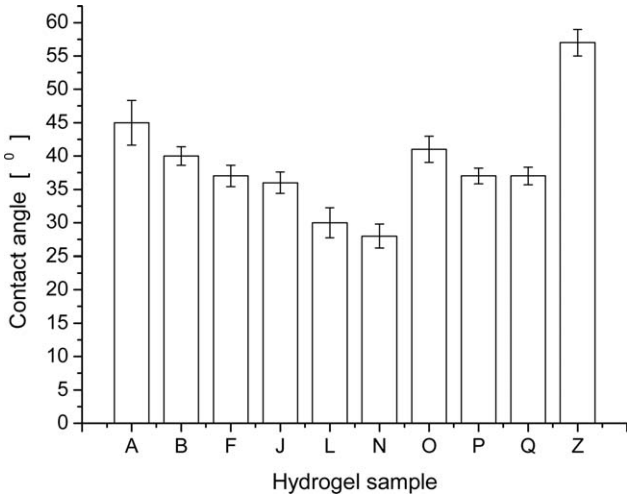

Figure 2. Contact angle of different hydrogels measured in water using the *captive-bubble* method.

The shear tangent modulus (0.1–0.4 MPa) was also found to be strain dependent and within the range of normal human articular cartilage.[46] Kobayashi et al.[48] performed mechanical tests on PVA hydrogels of different water contents. They showed that by adjusting the water content in the gel production process PVA provides viscoelastic characteristics similar to those of human soft tissues. The stress–strain curves in their work showed an *E* modulus of 5 MPa of the PVA hydrogel containing 20% of water, an *E* modulus of 0.45 MPa of the PVA hydrogel with 45% water content, an *E* modulus of 0.30 MPa of the PVA hydrogel with 60% water content, and an *E* modulus of 0.27 MPa of the PVA hydrogel with 90% water content, which presents a value very close to the value of the human meniscus ($E \approx 0.20$ MPa). Sudhamani et al.[49] found that the tensile strength of PVA films is ca. 2 MPa, which is in the same range as obtained by Kobayashi et al.[48] When mechanical properties of blended films of chitosan and PVA were measured[50] the *E* modulus was found to decrease with increasing amount of PVA in the blend. The *E* modulus of the dry PVA films was found to be 120 ± 56 MPa. This value is much higher than the *E* moduli of hydrogels swollen to a different extent (0.27–5 MPa).[48] The mechanical test proved the possibility of obtaining materials with different mechanical properties for diverse applications through variation of the composition and structure of hydrogels. Additionally, some hydrogels of similar *E* modulus have similar water uptake capability, whereas for some other it is not the case. Thus, e.g. hydrogels B and J, which show water uptake of ca. 40% display similar *E* modulus of ca. 4 MPa. Also, if hydrogel I, J, and P are compared, hydrogel J shows an *E* modulus twice as high as that of hydrogel P despite slightly higher water uptake of 4%. At the same time, hydrogel I shows a much higher *E* modulus, together with smaller water uptake of 5%. Hence, by controlling the primary characteristics of copolymers it is possible to achieve high ability of water uptake as well as desired mechanical properties of materials.

Surface Properties of Hydrogels

The surface properties of a biomaterial applied in tissue engineering are a key element in controlling the interaction with attaching cells and the surrounding tissue. Hydrogels within the scope of this work consist of both hydrophilic and hydrophobic domains that enable tuning of the final hydrophilic/hydrophobic balance. Aliphatic polyesters PLA, PGA, and their copolymers PLGA have been used to construct temporary scaffolds for tissue engineering on the basis of their biocompatibility, hydrolytical degradation capability, high mechanical strength, and excellent processing properties. However, because of poor hydrophilicity of these materials they are free of cell recognition sites on their surfaces, which leads to poor mass transport in scaffolds and poor cell affinity of the materials.[36] In order to improve the cell affinity of aliphatic polyesters, many efforts have been made to modify their surface properties and adjust the hydrophilicity/hydrophobicity balance[37,38] simply by introducing hydrophilic segments. Here, the approach to introduce the hydrophilic PVA backbone in order to improve cell affinity is used. It is documented in literature that PVA is biocompatible apart from many other good properties such as similarity to natural cartilage tissue in terms of water content in the hydrogel.[46,51] It has been observed already that cells adhere, spread, and grow more on surfaces with moderate hydrophilicity, regardless of the cell types used, than on more hydrophobic or even more hydrophilic surfaces.[46]

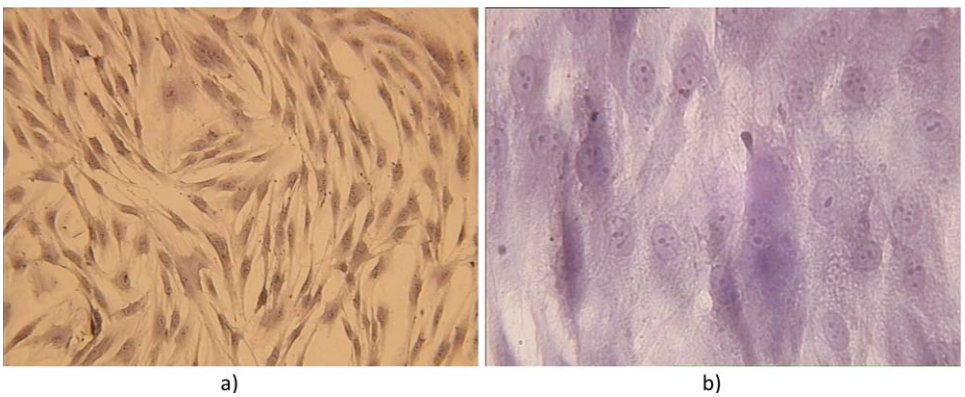

Figure 3. Representative images depicting the shape of primary human dermal fibroblasts (hF) attached to the surface of the hydrogel type P after 4 days of incubation: (a) at MAG × 134 and (b) at MAG × 400. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

The hydrophobicity/hydrophilicity of the uppermost surface layer of a solid is expressed usually in terms of wettability with water that is quantified by contact angle measurements. In Figure 2 values of the contact angle of swollen hydrogels, using the *captive-bubble* method are given. The values of the contact angle lie between 27° and 45°. Only poly(*rac*-lactide) (Z), used here as a kind of standard, has a value of 57°, which shows its lower hydrophilicity. One can conclude from Figure 2 that couples of samples of the same composition but different structure because of the difference in the length of polyester grafts have larger contact angles (hydrogels A relative to B, F relative to L, O relative to P). This is because of the apparent higher hydrophobicity of ester comonomers relative to PVA. Hydrogels with pure lactide grafts have higher contact angles compared to those having some GA in the polyester grafts because of higher hydrophobicity of lactide relative to GA. Thus hydrogel A has a higher contact angle than hydrogel O, as well as B relative to P. Hydrogels L and N with the shortest polyester grafts exhibit the lowest contact angles among all samples. Here again, by varying the polyester / PVA ratio a significant change of surface properties of hydrogels can be achieved.

Biocompatibility

As biocompatibility is a very important property of a material in case of its intended medical use, in order to evaluate the biocompatibility of the materials obtained here, hydrogel type P was examined concerning the cells adhesion, viability, and proliferation. Hydrogel P was selected for its composition: lactide to GA ratio 50 : 50 mol% in the polyester graft with nine repeating ester units and an experimental degree of grafting of 13%, as well as a medium value of contact angle.

The test of the biocompatibility was performed in cell culture media following a procedure given in the literature.[41] Figure 3 presents the sample surface after 4 days of incubation, showing vital, densely arranged cells on the sample surface [at a magnification of 134 (a) and higher magnification of 400 (b)]. Obviously, cells adhere evenly to the sample surface. This demonstrates that the hydrophilic/hydrophobic balance of the material suitable for cell adhesion can be achieved even without additional surface treatment.

Successful cell adhesion on hydrogel P, a water uptake of 34% and an *E* modulus of ca. 2 MPa, indicates a promising material with properties similar to those of natural cartilage tissue.

CONCLUSIONS

The compositions of the copolymers were determined previously by means of ^{1}H-NMR spectroscopy, whereas the influence of segment length, composition, and content of polyester grafts on the properties of poly(D,L-lactide) or poly(D,L-lactide-*co*-glycolide) grafted PVA [PLA/PLGA-PVA] hydrogels were investigated in this work. It was found that the hydrogels have adjustable mechanical properties depending on the length and number of polyester grafts. The *E* moduli of hydrogels have values between 0.01 and 100 MPa, the range that encompasses from very weak materials up to tough materials with *E* modulus close to that of thermoplastic polymers. Water uptake of these materials amounts to between 16% and 69%. It is interesting that some hydrogels of similar water uptake capability have similar *E* modulus, whereas in the case of some other materials the properties mismatch. Furthermore, water uptake combined with the capability of materials hydrophobicity tuning has been proved to allow good biocompatibility resulting in vital, densely arranged cells on the sample surface. Therefore, a combination of properties important for the intended application of these materials as hydrogels can be tuned by varying the structure and the polyester graft to PVA backbone ratio. The findings reported here open a new field for the development of polymers with a very broad range of mechanical properties, together with water uptake ability as well as an optimum hydrophilicity/hydrophobicity balance regarding the cell seeding capability.

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REFERENCES

1. Athanasiou, K. A.; Schmitz, J. P.; Agrawal, C. M. *Tissue Eng.* **1998**, *4*, 53.
2. López, A.; Persson, C.; Hilborn, J.; Engqvist, H. *J. Biomed. Mater. Res. Appl. Biomater.* **2010**, *95*, 75.
3. Mäkelä, E.; Antero Mäkelä, E.; Partio, E. K.; Juutilainen, T.; Lähteenkorva, K.; Törmälä, P.; Rokkanen, P. *J. Mater. Sci. Mater. Med.* **2008**, *19*, 1061.
4. Leiggener, C. S.; Curtis, R.; Müller, A. A.; Pfluger, D.; Gogolewski, S.; Rahn, B. A. *Biomaterials* **2006**, *27*, 202.
5. Hammerle, C. H.; Lang, N. P. *Clin. Oral. Implants Res.* **2001**, *12*, 9.
6. El-Amin, S. F.; Lu, H. H.; Kahn, Y.; Burems, J.; Mithchell, J.; Tuan, R. S. *Biomaterials* **2003**, *24*, 1213.
7. Kohane, D. S.; Langer, R. *Pediatr. Res.* **2008**, *63*, 487.
8. Papkov-Sokolsky, M.; Agashi, K.; Laya, A.; Shakesheff, K.; Domb, A. J. *Adv. Drug Deliv. Rev.* **2007**, *59*, 187.
9. Chun, K. W.;. Cho, K. C.; Kim, S. H.; Jeong, J. H.; Park, T. G. *J. Biomater. Sci. Polym. Ed.* **2004**, *15*, 1341.
10. Yoon, J. J.; Kim, J. H.; Park, T. G. *Biomaterials* **2003**, *24*, 2323.
11. Gelperina, S.; Maksimenko, O.; Khalansky, A.; Vanchugova, L.; Shipulo, K.;Abbasova, E.; Berdiev, R.; Wohlfart, S.; Chepurnova, N.; Kreuter, J. *Eur. J. Pharm. Biopharm.* **2010**, *74*, 157.
12. Nuutinen, J.-P.; Välimaa, T.; Clerc, C.; Törmälä, P. *J. Biomater. Sci. Polym. Ed.* **2002**, *13*, 1313.
13. Hong, Z.; Zhang, P.; Liu, A.; Chen, L.; Chen, X.; Jing, X. J. *Biomed. Mater. Res. A.* **2007**, *81*, 515.
14. Moran, J. M.; Pazzano, D.; Bonassar, L. J. *Tissue Eng.* **2003**, *9*, 63.
15. Huang, Y. X.; Ren, J.; Chen, C.; Ren, T. B.; Zhou, X. Y. *J. Biomater. Appl.* **2008**, *22*, 409.
16. Zhou, Q.; Xanthos, M. *Polym. Degrad. Stab.* **2008**, *93*, 1450.
17. Young, Y.; Min, B.-M.; Lee, S. J.; Lee, T. S.; Park, W. H. *J. Appl. Polym. Sci.* **2005**, *95*, 193.
18. Kreiser-Saunders, I.; Kricheldorf, H. R. *Macromol. Chem. Phys.* **1988**, *199*, 1081.

19. Li, S.; Vert, M. *Macromolecules* **1994**, *27*, 3107.
20. Kricheldorf, H. R.; Mang, T.; Michael, J. *Macromolecules* **1984**, *17*, 2173.
21. Amecke, B.; Bendix, D.; Entenmann, G. In Encyclopedic Handbook of Biomaterials and Bioengineering; Wiese, D.L., Ed.; Marcel Dekker Inc: Boston, **1995**; p 977.
22. Höcker, H.; Keul, H. *Macromol. Symp.* **2001**, *174*, 231.
23. Pêgo, A. P.; Poot, A. A.; Grijpma, D. W.; Feijen, J. *J. Mater. Sci. Mater. Med.* **2003**, *14*, 767.
24. Måberg, S.; Plikk, P.; Finne-Wistrand, A.; Albertsson, A. -C. *Chem. Mater.* **2010**, *22*, 3009.
25. Tyson, T.; Finne-Wistrand, A.; Albertsson, A. -C. *Biomacromolecules* **2009**, *10*, 149.
26. Hutmacher, D. W. *Biomaterials* **2000**, *21*, 2529.
27. Wu, X. S. In Encyclopedic Handbook of Biomaterials and Bioengineering, Part A: Materials; Wise. D. L.; Trantolo, D. J.; Altobelli, D. E.; Yaszemski, M. J.; Gresser, J. D.; Schwartz, E. R., Eds.; Marcel Dekker: New York, **1995**; p 1015.
28. Tice, T. R.; Tabibi, E. S. In Treatise on Controlled Drug Delivery: Fundamentals Optimization, Applications; Kydonieus, A., Ed.; Marcel Dekker: New York, **1992**; p 315.
29. Bostman, O.; Hirvensalo, E.; Vainionpaa, S.; Vihtonen, K.; Tormala, P.; Rokkanen, P. *Int. Orthop.* **1990**, *14*, 18.
30. Rokkanen, P. U.; Bostman, O.; Hirvensalo, E.; Makela, E. A.; Partio, E. K.; Patiala, H.; Vainionpaa, S. I.; Vihtonen, K.; Tormala, P. *Biomaterials* **2000**, *21*, 2607.
31. Agrawal, C. M.; Athanasiou, K. A. *J. Biomed. Mater. Res. Appl. Biomater.* **1997**, *38*,105.
32. Bergsma, E. J.; Brujn, W.; Rozema, F. R.; Bos, R. M.; Boering, G. *Biomaterials* **1995**, *16*, 25.
33. Gunatillake, P. A.; Adhikari, R. *Eur. Cell. Mater.* **2003**, *5*, 1.
34. Burdick, J. A.; Philipott, L. M.; Anseth, K. S. *J. Polym. Sci. Part A Polym. Chem.* **2001**, *39*, 683.
35. Khang, G.; Choe, J.-H.; Rhee, J. M.; Lee, H. B. *J. Appl. Polym. Sci.* **2002**, *85*, 1253.
36. Cai, Q.; Wan, Y.; Bei, J.; Wang, S. *Biomaterials* **2003**, *24*, 3555.
37. Webb, K.; Hlady, V.; Trosco, P. A. *J. Biomed. Mater. Res.* **1998**, *41*, 422.
38. Yang, J.; Bei, J. Z.; Wang, S. G. *Polym. Adv. Technol.* **2002**, *13*, 220.
39. Vacanti, C.A.; Bonassar, L.J.; Vacanti, M.P.; Shufflebarger, J. *N. Engl. J. Med.* **2001**, *344*, 1511.
40. Vidović, E.; Klee, D.; Höcker, H. *J. Polym. Sci. Part A Polym. Chem.* **2007**, *4*, 4536.
41. Van Den Bogaerdt, A. J.; Van Zuijlen, P. P.; Van Galen, M.; Lamme, E. N.; Middelkoop, E. *Arch. Dermatol. Res.* **2002**, *294*,135.
42. Janović, Z. Polimerizacije i polimeri; HDKI-Kemija u industriji: Zagreb, **1997**; p 111.
43. Nuttelman, C. R.; Henry, S. M.; Anseth, K. S. *Biomaterials* **2002**, *23*, 3617.
44. Kasuga, T.; Ota, Y.; Nogami, M.; Abe, Y. *Biomaterials* **2001**, *22*, 19.
45. Joziasse, C. A. P.; Veenstra, H.; Topp, M. D. C.; Grijpma, D. W.; Pennings, A. J. *Polymer* **1998**, *39*, 467.
46. Stammen, J. A.; Williams, S.; Ku, D. N.; Guldberg, R. E. *Biomaterials* **2001**, *22*,799.
47. Tsuji, H.; Ikada, Y. *Polymer* **1999**, *40*, 6699.
48. Kobayashi, M.; Toguchida, J.; Oka, M. *Knee* **2003**, *10*, 47.
49. Sudhamani, S. R.; Prasad, M. S.; Sankar, K.U. *Food Hydrocoll.* **2003**, *17*, 245.
50. Srinivasa, P. C.; Ramesh, M. N.; Kumar, K. R.; Tharanathan, R. N. *Polymer* **2003**, *53*, 431.
51. Oh, S. H.; Kang, S. G.; Kim, E. S.; Cho, S. H.; Lee, J. H. *Biomaterials* **2003**, *24*, 4011.