Surface hydrolysis of poly(glycolic acid) meshes increases the seeding density of vascular smooth muscle cells

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Abstract: A procedure for surface hydrolysis of poly(glycolic acid) (PGA) meshes was developed to increase cell seeding density and improve attachment of vascular smooth muscle cells. Hydrolysis of PGA in 1*N* NaOH transformed ester groups on the surface of PGA fibers to carboxylic acid and hydroxyl groups. After hydrolysis, the polymer scaffold retained its original gross appearance and dimensions while the fiber diameter decreased. A plot of fiber diameter versus the hydrolysis time showed a linear relationship, with a rate of decrease in fiber diameter of 0.65 μm/min. The molecular weight and thermal properties of the polymer did not change significantly following surface hydrolysis. In cell seeding experiments, surface-hydrolyzed mesh was seeded with more than twice as many cells as unmodified PGA mesh. Vascular smooth muscle cells attached to the surface-hydrolyzed PGA mesh both as individual cells and as cell aggregates while only cell aggregates were observed on the unmodified mesh. Control experiments indicated that adsorption of serum proteins onto the surface-hydrolyzed PGA fibers was correlated with the increase in cell seeding density. These results demonstrate that optimization of biomaterial–cell interactions provides a strategy for increasing the initial cell seeding density for the engineering of tissues of high cell density. © 1998 John Wiley & Sons, Inc. J Biomed Mater Res, 42, 417–424, 1998.

Key words: surface modification; poly(glycolic acid); cell seeding density; vascular smooth muscle cells; tissue engineering; biomaterials

INTRODUCTION

There are 1.4 million surgical procedures performed annually in the US that require arterial prostheses; the majority of these operations are on small to medium size arteries (less than 6 mm).[1] Autologous vein grafts routinely are used to bypass small arteries in the coronary or peripheral circulations. Vein grafts are fragile and sometimes are damaged when transplanted into the arterial systems, and, furthermore, adequate venous conduits are not available in all patients.[2,3] One approach to address these issues may be the development of tissue-engineered arteries that could provide a readily available and possibly superior alternative to conventionally used vein grafts. In this strategy, a biopsy from the patient would provide autologous vascular cells that would be cultured *in vitro* to form functional vascular conduits.[1,4,5] Previous attempts to culture vascular grafts *in vitro* have met with limited success in part because of their failure to mimic adequately the smooth muscle cell densities and organization of the vessel wall.[6–8]

Vascular smooth muscle cells in the wall of small blood vessels are organized into a cell-dense lamella structure that confers the bulk of the mechanical strength to the vessel.[9] The density of the smooth muscle cells is approximately 10^8 cells/cm^3.[8] In order to successfully culture a tissue-engineered vascular prosthesis *in vitro*, it is necessary to produce a tissue that simulates the observed *in vivo* smooth muscle cell density. A biodegradable polymer scaffold that is optimized for cell attachment will facilitate uniform and dense initial cell seeding and should result in improvement of the cell densities after tissue culture *in vitro*. In addition, gains in cellular attachment and proliferation that are realized for vascular cells may be translated to other cell types as well, thus improving the characteristics of other engineered tissues.

Poly(glycolic acid) (PGA, or Dexon) has been used widely as a biocompatible, biodegradable polymer for tissue engineering.[10–15] Surgical sutures made from PGA have been commercially available since 1970 and

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are approved for clinical use by the Food and Drug Administration.[16] This polymer has been processed into a variety of three-dimensional scaffolds that are useful for tissue engineering purposes, and neither the polymer nor its degradation product (glycolic acid) are toxic when implanted *in vivo*.[11] We describe herein a procedure for the modification of the surface of PGA scaffolds under strongly alkaline conditions. Hydrolysis of ester bonds on the surface of the PGA fibers resulted in higher seeding density and more spreading of smooth muscle cells as compared to unmodified PGA scaffolds.

MATERIALS AND METHODS

Materials

PGA mesh (2 mm in thickness) was purchased from Albany International Research Co. (Mansfield, Massachusetts). The mesh is composed of fibers that are 13 μm in diameter and has a porosity of 97%.[11] PGA polymer (available as nuggets), which was used for the preparation of PGA films, was purchased from Polysciences, Inc. (Warrington, Pennsylvania). Sodium trifluoroacetate and hexafluoroisopropanol were purchased from Fluka (Ronkonkoma, New York). Hoechst 33258 and calf thymus (type I) for DNA assays were obtained from Sigma (St. Louis, Missouri). Dulbecco's modified Eagle medium (DMEM), fetal bovine serum (FBS), penicillin G (sodium salt), streptomycin, phosphate-buffered saline (PBS), and 0.05% trypsin-5.3 m*M* EDTA solution were obtained from Life Technologies, Inc. (Grand Island, New York). The plastic chambers for cell seeding experiments were obtained from Advanced Tissue Science (La Jolla, California).

Vascular smooth muscle cells were isolated from the medial layer of bovine thoratic aortas that had been obtained from a local abattoir. Briefly, the adventitia layer of the aortas was stripped away and the intimal layer was removed using forceps. The remaining medial layer was cut into 1 cm^2 sections and explanted into tissue culture dishes containing a small amount of growth medium. After 1 day, excess medium was added to the explants. After 10 days, explants were removed from the dishes and monolayers of smooth muscle cells were trypsinized and passaged.[17] Cell identity was confirmed by ''hill and valley'' appearance on microscopy and by positive staining for α-actin in vascular smooth muscle cells.[18] In this study, only cells below passage 4 were used. Cells were cultured at 37°C in a humidified 10% CO_2 environment in DMEM containing 20% FBS, penicillin G (100 unit/mL), and streptomycin (100 μg/mL).

Instrumentation

Thermal analysis of the polymer was carried out on a Perkin–Elmer DSC-7 differential scanning calorimeter employing a heating rate of 20°C/min. The molecular weight of the PGA polymer was measured using a microbatch method (0.1 w/v% sodium trifluoroacetate in hexafluoroisopropanol) by a miniDAWN light scattering detector from Wyatt Technology Corporation.[19] Contact angles were measured by Video Contact Angle System-2000 (Advanced Surface Technology, Inc.). X-ray photoelectron spectroscopy (XPS) was carried out using a Surface Science Laboratories X-100 spectrometer employing monochromatized Al Kα (1486.7 eV) X rays. A critical point dryer (Balzers Union) and a sputter coater (Technics, Inc.) were used. Scanning electron microscopy (SEM) analysis was carried out on a JOEL 6320 FEGSEM system. DNA assays were carried out on a fluorometer from Photon Technology International.

General procedure for surface hydrolysis of PGA meshes

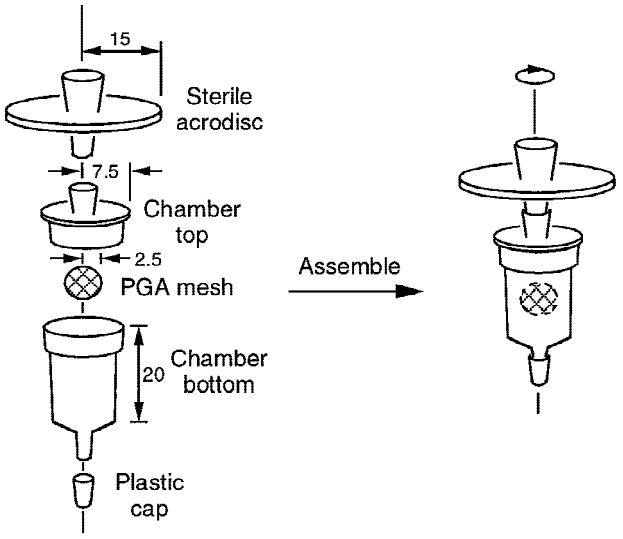
Samples of PGA mesh (discs of 5 mm in diameter and 2 mm in thickness, ~5 mg) first were washed with hexane to remove possible surface contaminants. After the hexane wash, the samples were filtered, wetted in EtOH, and then placed into distilled water (10 mL) and shaken for 5 min to allow solvent exchange. One sample of PGA mesh was removed from the water and placed in 1*N* NaOH (10 mL), and the suspension was shaken for certain time periods (e.g., 1, 3, 5, 7, or 10 min in this study) at room temperature. After base treatment, the mesh was taken out and rinsed extensively with water until the pH of the rinsing water became neutral. The PGA scaffold then was washed with EtOH, filtered, and lyophilized. The dried samples were coated with Au (~200 Å in thickness) by a sputter coater (4V, 4 min) and examined by SEM (accelerating voltage, 15 kV).

Preparation of PGA films and characterization of surface properties

The PGA polymer (~50 mg nugget) was placed between two pieces of aluminum foil that had been heated at 245°C for 1 min in a heating platen. The polymer sample then was compressed between two platens (5000 lbs. load) for 1 min, removed, and allowed to cool to room temperature. The aluminum foils were peeled off, and the resulting PGA film was approximately 25 μm in thickness. The surface-hydrolyzed PGA film was produced by soaking the PGA film in 1*N* NaOH solution for 1 min, then extensively washing it with distilled water and drying it *in vacuo*. During the measurement of contact angles, one drop of water (~5 μL) was introduced by a microsyringe onto the polymer film and its image was captured by a video camera. The contact angle was measured by the VCA-2000 Dynamic/Windows Software as the angle between the interface of PGA film–water–air. During XPS analysis, photoelectrons were analyzed by a hemispherical multichannel detector, and an electron gun (energy 5 eV) was used to compensate for charging. XPS analysis determined the effect of surface hydrolysis on elemental compositions of carbon and oxygen at the surface of the PGA film.

Seeding of vascular smooth muscle cells on the PGA mesh in the presence of serum

Smooth muscle cells were grown to subconfluence in tissue culture flasks (T-75, surface area 75 cm^2). The cell layer was first rinsed with PBS buffer (10 mL) before being harvested with 0.05% trypsin-5.3 m*M* EDTA solution (5 mL). Cells were collected by centrifugation and resuspended in DMEM with 20% FBS to a concentration of 2.5×10^6 cells/mL. Each sample of PGA mesh (five samples for both unmodified and surface-hydrolyzed mesh) was placed into a small plastic chamber (volume = 1.5 mL, Scheme 1) after its weight was measured accurately for the calculation of cell seeding density (see below). All the surface-hydrolyzed meshes in cell seeding experiments were obtained from surface hydrolysis in 1*N* NaOH for 1 min. The chamber was equipped with a 0.2 μm acrodisc filter to allow for adequate gas exchange (Scheme 1). The smooth muscle cell suspension (1 mL) was added to the chamber and rotated at 0.66 rpm for 2 h at 37°C in a humidified atmosphere in 10% CO_2 to allow cells to attach to the mesh. After cell seeding, the PGA mesh was removed from the chamber and rinsed with PBS to remove unattached cells. One sample was saved for SEM analysis (see procedure below). The other four samples were placed in 1 mL of trypsin–EDTA solution and incubated for 1 h at room temperature and sonicated for 30 s to remove attached smooth muscle cells from the PGA mesh. Complete removal of the attached cells from the mesh was confirmed by light microscopic examination of the scaffold. The DNA content was determined by measuring the fluorescence intensity of a dye (Hoechst 33258) upon binding to DNA (λ_{ex} = 365 nm, λ_{em} = 458 nm).[20,21] Calf thymus (10 μg/mL) was used as a DNA standard. The number of cells was calculated based on a constant of 8.5 pg DNA/smooth muscle cell.[22] The cell seeding density was determined as the number of cells per unit weight of the dry PGA mesh. The yield of cell seeding was determined as the ratio of the number of cells seeded on the PGA mesh over the total number of cells during cell seeding.



Scheme 1. Illustration of the plastic chamber used for cell seeding experiments. During cell seeding, the assembly is rotated along its axis at a speed of 0.66 rpm. The unit of the numbers in the scheme is mm.

Seeding of vascular smooth muscle cells on the PGA mesh under serum-free conditions

Smooth muscle cells were grown and harvested following the procedure described previously. Cells were collected by centrifugation and resuspended in serum-free DMEM to a seeding concentration of 2.5×10^6 cells/mL. Two types of surface-hydrolyzed PGA mesh were used. In one case, the surface-hydrolyzed PGA mesh was immersed in 20% FBS in DMEM for 2 h to allow serum proteins to adsorb to the fiber surface, and the mesh was rinsed twice in serum-free DMEM to remove unattached proteins. One piece of the PGA mesh was washed further with distilled water, lyophilized, and saved for XPS analysis. The other set of surface-hydrolyzed PGA meshes was not pretreated with serum-containing medium. The cell seeding experiments were carried out in the same plastic chamber under the same seeding conditions described above. The cell seeding density was determined by the DNA assay.

SEM analysis

Cells on the PGA mesh were fixed in 2.5% glutaraldehyde solution in 25 m*M* of sodium cacodylate containing 2% sucrose and 1.2 m*M* of $CaCl_2$ at pH 7.2 (osmolarity 300 mOsm) for 2 h. After fixation, the samples were rinsed in a solution of 100 m*M* of sodium cacodylate containing 3.37% sucrose and 1.2 m*M* of $CaCl_2$ at pH 7.2 (300 mOsm). The osmolarity and the pH of the fixation solution were controlled to be comparable to that of the tissue culture medium to preserve the cell morphology on the polymer surface during fixation.[23,24] The samples were dehydrated with 10 min exchanges in each of 50, 70, 80, and 90% aqueous ethanol solution and three times in absolute ethanol. The ethanol was further exchanged to liquid CO_2 in a pressure chamber, and the CO_2 was vaporized at a temperature (40°C) above its critical point (36°C). The dried samples were coated with Au (~200 Å in thickness) by a sputter coater (4V, 4 min) and examined by SEM (accelerating voltage, 5 kV).

RESULTS AND DISCUSSION

Surface hydrolysis of PGA meshes

We achieved the surface hydrolysis of the PGA fibers by soaking the PGA mesh in an aqueous solution of 1*N* NaOH. Hydroxide anion hydrolyzes the ester groups on the surface of the PGA fiber, resulting in the breaking of the polymer chain and the formation of carboxylic acid and hydroxyl groups on the termini of the two new chains.[25] In principle, hydrolysis can occur at any ester bond along the polymer chain that is

accessible to the hydroxide anions in solution. Hydrolysis at multiple points on the same polymer chain reduces the polymer to smaller fragments—oligomeric or monomeric glycolic acid—which become soluble in solution, resulting in the observed decrease of the diameter of the PGA fibers.

We used SEM to examine the change in appearance and surface morphology of the PGA fibers as a result of hydrolysis. Figure 1 (A,B,C) shows three sets of PGA meshes after 1, 5, and 10 min hydrolysis in 1*N* NaOH, respectively (scales are identical in all three figures). Figure 1 demonstrates that: (1) after hydrolysis, the PGA mesh kept its overall fibrous mesh structure; (2) the fiber diameter decreased as a function of increased hydrolysis time, and the decrease in fiber diameter was approximately the same for most of the fibers; and (3) the surface of individual fibers after hydrolysis appeared to be smooth (Fig. 1 inserts). These observations are consistent with the hypothesis that hydrolysis occurs only at the surface of PGA fibers. This hypothesis is further supported by the relatively unchanged bulk properties of hydrolyzed samples (see below).

We randomly selected 5 fibers from each SEM image of PGA mesh under different hydrolysis times and measured their diameters. Figure 2 plots the average values of fiber diameter as a function of the hydrolysis time in solution and demonstrates a linear correlation with a slope of 0.65 μm/min. The diameter of the fibers after 10 min of hydrolysis decreased to half of the value of the untreated fibers (from 13 μm to 6.5 μm), corresponding to a mass loss of 75%. We also observed that PGA fibers were completely degraded after 20 min of hydrolysis in 1*N* NaOH. The calculated time for half mass loss (the diameter changes from 13 μm to 9.2 μm) was 6.2 min under these conditions, as compared to 5 weeks in phosphate-buffered saline (pH 7.1) at 37°C.[11]

Surface hydrolysis significantly changed the wettability of the PGA fibers. To quantify this change, we measured the contact angles of water on the surface-hydrolyzed PGA film and unmodified film. The values of contact angles were 10 ± 5° and 53 ± 2° on the surface-hydrolyzed PGA film and the unmodified film, respectively. The smaller value of the contact angle of water on the surface-hydrolyzed PGA film demonstrates a more hydrophilic and polar surface as compared to the unmodified film. We also used XPS to analyze the change in surface elemental compositions as a result of surface hydrolysis. For the unmodified PGA film, the surface is composed of 51% carbon and 49% oxygen. For the surface-hydrolyzed PGA film, the surface elemental composition changes to 49% carbon and 51% oxygen. The higher percentage of oxygen on the surface-hydrolyzed PGA film is consistent with the addition of a hydroxyl group during the hydrolysis of an ester group (R_1COOR_2) to carboxylic acid (R_1COOH) and alkanol (R_2OH). The presence of carboxylic acid and alkanol groups was further supported by chemical modification of these functional groups at the surface.[25]

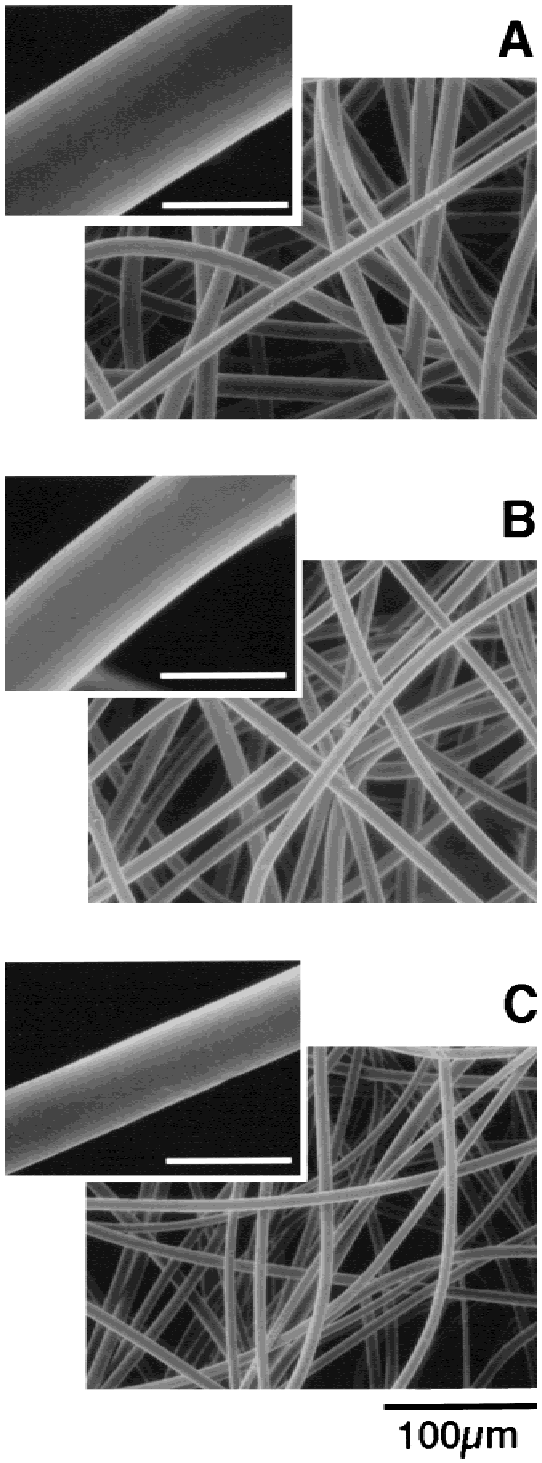


Figure 1. SEM analyses of the PGA mesh as a function of hydrolysis time. A, B, and C correspond to hydrolysis times of 1, 5, and 10 min in 1*N* NaOH, respectively. The insert in each figure shows the detailed surface morphology of an individual fiber. The scale bars in the inserts are 10 μm. The scale bar (100 μm) at the bottom of C applies to A and B as well.

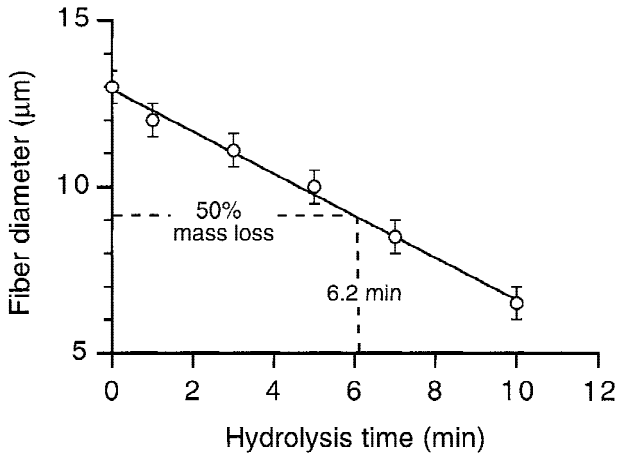


Figure 2. Correlation of fiber diameter with the hydrolysis time of PGA mesh in 1*N* NaOH. The fiber diameters were measured from SEM images of the PGA mesh (e.g., Fig. 1). The hydrolysis rate of PGA fibers in 1*N* NaOH is 0.65 μm/min. The time for 50% mass loss is 6.2 min.

Bulk properties of the surface-hydrolyzed PGA fibers

To examine the influence of surface hydrolysis on the bulk properties of the PGA polymer, we measured the molecular weight and thermal properties of the surface-hydrolyzed PGA fibers as a function of the hydrolysis time (Table I). The molecular weight of the polymers changed less than 10% after 10 min of hydrolysis (as a comparison, the weight reduced to a quarter of its original value). The glass transition temperature (T_g) of the polymer decreased slightly from 46.5°C to 45.7°C (experimental uncertainty ± 0.3°C) after 10 min of hydrolysis. We did not observe consistent changes in the values of melting temperature (T_m) and enthalpy of melting (ΔH_m). Since the value of ΔH_m directly reflects the crystallinity of the polymer,[26] the relatively constant values of ΔH_m for the surface-hydrolyzed PGA fibers suggest that the fibers retained their crystallinities following surface hydrolysis. We conclude that the bulk properties of the PGA polymer did not change significantly as a result of hydrolysis in 1*N* NaOH.

TABLE I
Dependence of Fiber Diameter, Molecular Weights (M_w, weight average), T_g, T_m, and ΔH_m on the Hydrolysis Time of the PGA Mesh

Hydrolysis Time (min)	Fiber Diameter (μm)*	M_w (kD)†	T_g (°C)‡	T_m (°C)‡	ΔH_m (j/g)§
0	13.0	51	46.5	219.8	82
1	12.1	52	46.6	219.8	81
3	11.2	53	46.2	219.1	82
5	10.0	52	46.0	219.6	80
7	8.4	48	45.5	219.9	82
10	6.5	48	45.7	219.6	80

*The fiber diameter was estimated as an average of five randomly picked PGA fibers in SEM images. The variation in fiber diameter is less than ± 0.5 μm for all fibers.
†Experimental uncertainity is ± 2kD.
‡Experimental uncertainty is ± 0.3°C.
§Experimental uncertainty is ±1 j/g.

Surface hydrolysis versus bulk hydrolysis

In PBS at 37°C (pH = 7.1), the PGA fibers have been reported to undergo bulk-hydrolysis, that is, hydrolysis that occurs both inside and on the surface of the PGA fibers.[11] This mode of hydrolysis is in contrast to the current study, where hydrolysis has been observed essentially only at the surface of the fiber. The mode of hydrolysis depends mainly on the relative rates of two processes: hydrolysis of the polymer by the reactant and diffusion of the reactant from solution into the polymer matrix. When the diffusion rate is higher than the reaction rate, bulk hydrolysis occurs; when the reaction rate is higher than the diffusion rate, surface hydrolysis dominates. There are two strategies for switching bulk hydrolysis to surface hydrolysis: increasing the hydrolysis rate of the polymer or decreasing the diffusion rate of the reactant. In the current study, we used hydroxide anions to increase the hydrolysis rate of the PGA fibers. As a result, the time for half mass loss for PGA fibers in 1*N* NaOH is 6.2 min while that in neutral PBS buffer is 5 weeks, corresponding to an increase in rate of more than three orders of magnitude. The increased hydrolysis rate is due to the much higher nucleophilicity of the hydroxide anions than that of water. The significant increase in hydrolysis rate resulted in the surface hydrolysis of PGA fibers.

The diffusion rate of a reactant into a solid polymer matrix depends on various factors, including the size and polarity of the reactant, and the crystallinity, density, and hydrophobicity/hydrophilicity of the polymer matrix. The diffusion rates of small molecules (e.g., H_2O or OH^-) into PGA fibers have not been documented in the literature previously. We speculate that the diffusion rate for OH^- is smaller than that of water since surface hydrolysis produces negative charges on the PGA fibers that repel OH^- anions. Furthermore, OH^- would be less stable in a neutral PGA environment than would water.

Seeding of vascular smooth muscle cells onto PGA meshes

To evaluate the effect of surface hydrolysis of PGA fibers on the cell seeding density, we seeded vascular

smooth muscle cells on the surface-hydrolyzed mesh and the unmodified mesh in the presence of serum. Smooth muscle cells were attached to the surface-hydrolyzed PGA meshes at a seeding density of $(3.1 \pm 0.1) \times 10^5$ cells/mg of PGA (column C in Fig. 3), more than twice that on the unmodified PGA mesh [$(1.4 \pm 0.1) \times 10^5$ cells/mg of PGA, column A, $P < 0.0005$, $n = 4$).[27] The yield of cell seeding was 49% and 25% for the surface-hydrolyzed meshes and unmodified meshes, respectively. We hypothesized that the increase in cell seeding density and yield was a result of adsorption of serum proteins on the hydrolyzed PGA surface, which promoted cell adhesion. To test this hypothesis, we prepared eight samples of surface-hydrolyzed PGA mesh. Half of these samples were pretreated by immersing in serum-containing medium for 2 h prior to cell seeding. All samples then were seeded with cells suspended in DMEM *without* serum, as described in Materials and Methods. As shown in Figure 3, substantially more cells [$(3.7 \pm 0.1) \times 10^5$ cells/mg PGA, yield 59%, column D] were attached to surface-hydrolyzed PGA mesh that was pretreated with serum than were attached to the untreated surface-hydrolyzed mesh [$(1.3 \pm 0.1) \times 10^5$ cells/mg PGA, yield 22%, column B, $P < 0.0005$, $n = 4$). In addition, there was no observable difference in cell seeding density between control PGA mesh (column A) and surface-hydrolyzed mesh that was not exposed to serum (column B). In summary, surface hydrolyzed PGA mesh exposed to serum proteins either before (column D) or during (column C) cell seeding resulted in substantially increased cell densities as compared to both control PGA mesh (column A) and to surface-hydrolyzed mesh that was not exposed to serum (column B). These results suggest that adsorption of serum proteins onto surface-hydrolyzed meshes occurs before cell attachment, and the presence of serum proteins results in the increase in the cell seeding density. This observation is consistent with literature reports that surface sulfonic groups (which have negative charges similar to the carboxylic acid groups on the surface-hydrolyzed PGA mesh) and hydroxyl groups enhance the attachment of cells to the biomaterial surface as mediated by serum proteins.[28,29] To further test this hypothesis, we used XPS to examine the surface-hydrolyzed PGA mesh pretreated with serum. XPS analysis showed the appearance of photoelectrons from nitrogen in addition to those from carbon and oxygen. Since PGA polymer is composed of only carbon, hydrogen, and oxygen elements, the nitrogen signal is consistent with the presence of adsorbed proteins (or amino sugars) on the surface of PGA fibers.

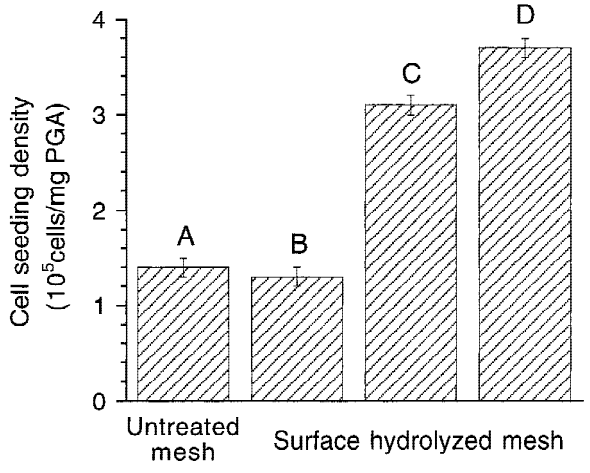


Figure 3. Dependence of cell seeding density on the seeding conditions. The seeding conditions for different columns are: cells in columns A and C were seeded in the presence of serum, and cells in columns B and D were seeded in the DMEM without serum. The PGA mesh in column D was immersed in serum-containing medium for 2 h before cell seeding. The error bars represent the standard errors from four measurements.

We used SEM to examine the cell morphology on the surface of PGA fibers. Figure 4(A) shows the appearance of smooth muscle cells on unmodified PGA fibers after cell seeding in the presence of FBS. Even though smooth muscle cells were prepared as single cell suspensions, they appeared mainly as aggregates on the surface of unmodified PGA fibers. The shapes of the majority of cells were round [Fig. 4(A) insert]. In comparison, smooth muscle cells appeared both as individual cells and as cell aggregates on the surface-hydrolyzed fibers [Fig. 4(B)]. The individual cells appeared to be well attached and spread on the fiber surface [Fig. 4(B) insert]. These results indicate that surface-hydrolyzed PGA fibers provide an improved surface for smooth muscle cells as compared to the unmodified PGA fibers. These differences may translate into improved cellular viability and replications in subsequent culture periods.[30]

CONCLUSIONS

This paper describes a method for surface hydrolysis of PGA scaffold to increase cell seeding density and improve biomaterial–cell interactions. Hydrolysis of PGA fibers in 1*N* NaOH changes the surface properties and the diameters of the fibers while leaving the bulk properties (e.g., molecular weight, thermal properties, crystallinity) mostly intact. Surface hydrolyzed PGA fibers showed substantially increased cell seeding for vascular smooth muscle cells in the presence of serum: the cell seeding density on surface-hydrolyzed PGA mesh is $(3.1 \pm 0.1) \times 10^5$ cells/mg PGA, more than twice that on the unmodified PGA mesh [$(1.4 \pm 0.1) \times 10^5$ cells/mg PGA, $P < 0.0005$, $n = 4$]. SEM analysis shows that smooth muscle cells are well attached and spread on the surface-hydrolyzed PGA fibers. The

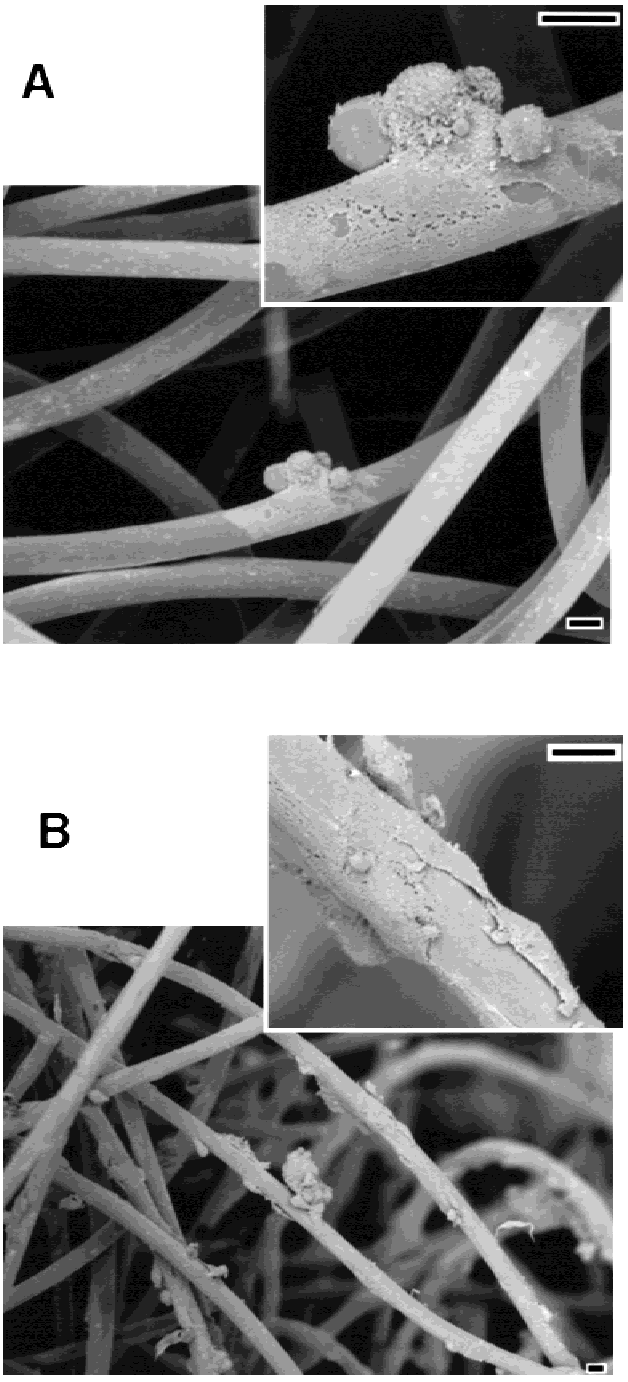


Figure 4. SEM images of smooth muscle cells seeded on PGA meshes. A and B correspond to unmodified and surface-hydrolyzed PGA meshes, respectively. The inserts in both figures show enlarged images of cell morphology on the surface of PGA fibers. The scale bars in all the figures and inserts are equal to 10 μm.

strategy of optimizing cell–biomaterial interactions to increase initial cell seeding density may be useful in engineering tissues of high cell density.

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References

1. R. Langer and J. P. Vacanti, ''Tissue engineering,'' *Science,* **260,** 920–926 (1993).
2. M. G. Davies and P. O. Hagen, ''Structural and functional consequences of bypass grafting with autologous vein,'' *Cryobiology,* **31,** 63–70 (1994).
3. J. L. Cox, D. A. Chiasson, and A. I. Gotlieb, ''Stranger in a strange land: The pathogenesis of saphenous vein graft stenosis with emphasis on structural and functional differences between veins and arteries,'' *Prog. Cardiovasc. Dis.,* **34,** 45–68 (1991).
4. R. P. Lanza, R. Langer, and W. L. Chick, eds., *Principles of Tissue Engineering,* R. G. Landes Company, Austin, Texas, 1997.
5. R. M. Nerem and A. Sambanis, ''Tissue engineering: From biology to biological substitutes,'' *Tissue Eng.,* **1,** 3–12 (1995).
6. N. L'Heureux, L. Germain, L. Raymond, and F. A. Auger, ''*In vitro* construction of human blood vessel from cultured vascular cells: A morphologic study,'' *J. Vasc. Surg.,* **17,** 499–509 (1993).
7. B. van der Lei, P. Nieuwenhuis, I. Molenaar, and C. Wildevuur, ''Long-term biologic fate of neoarteries regenerated in microporous, compliant, biodegradable, small-caliber vascular grafts in rats,'' *Surgery,* **101,** 459–463 (1987).
8. C. B. Weinberg and E. Bell, ''A blood vessel model constructed from collagen and cultured vascular cells,'' *Science,* **231,** 397–400 (1986).
9. J. A. G. Rhodin, ''Architecture of the vessel wall,'' in *Handbook of Physiology,* D. F. Bohr, A. P. Somlyo, H. V. Sparks, Jr., and S. R. Geiger (eds.), American Physiological Society, Bethesda, Maryland, 1980, pp. 1–33.
10. D. J. Mooney, C. L. Mazzoni, C. Breuer, K. McNamara, D. Hern, J. P. Vacanti, and R. Langer, ''Stabilized polyglycolic acid fibre-based tubes for tissue engineering,'' *Biomaterials,* **17,** 115–124 (1996).
11. L. E. Freed, G. Vunjak-Novakovic, R. J. Biron, D. B. Eagles, D. C. Lesnoy, S. K. Barlow, and R. Langer, ''Biodegradable polymer scaffolds for tissue engineering,'' *Bio/Technology,* **12,** 689–693 (1994).
12. A. G. Mikos, Y. Bao, L. G. Cima, D. E. Ingber, J. P. Vacanti, and R. Langer, ''Preparation of poly(glycolic acid) bonded fiber structures for cell attachment and transplantation,'' *J. Biomed. Mater. Res.,* **27,** 183–189 (1993).
13. L. E. Freed, G. Vunjak–Novakovic, and R. Langer, ''Cultivation of cell–polymer cartilage implants in bioreactors,'' *J. Cell. Biochem.,* **51,** 257–264 (1993).
14. C. Vacanti, R. Langer, B. Schloo, and J. P. Vacanti, ''Synthetic biodegradable polymers seeded with chondrocytes provide a template for new cartilage formation *in vivo*,'' *Plast. Reconstr. Surg.,* **88,** 753–759 (1991).
15. L. G. Cima, J. P. Vacanti, C. Vacanti, D. Ingber, D. Mooney, and R. Langer, ''Tissue engineering by cell transplantation using degradable polymer substrates,'' *J. Biomech. Eng.,* **113,** 143–151 (1991).
16. E. J. Frazza and E. E. Schmitt, ''A new absorbable suture,'' *J. Biomed. Mater. Res. Symp.,* **1,** 43–58 (1971).
17. R. I. Freshney, ''Disaggregation of the tissue and primary culture,'' in *Culture of Animal Cells,* R. I. Freshney (ed.), Wiley, New York, 1994, pp. 127–147.
18. O. Skalli, P. Ropraz, A. Trzeciak, G. Benzonana, D. Gillessen, and G. Gabbiani, ''A monoclonal antibody against alpha-smooth muscle actin: A new probe for smooth muscle differentiation,'' *J. Cell Biol.,* **103,** 2787–2796 (1986).

19. P. J. Wyatt, ''Light scattering and the absolute characterization of macromolecules,'' *Anal. Chim. Acta,* **272,** 1–40 (1993).
20. C. Labarca and K. Paigen, ''A simple, rapid, and sensitive DNA assay procedure,'' *Anal. Biochem.,* **102,** 344–352 (1980).
21. Y. J. Kim, R. Sah, J. Doong, and A. J. Grodzinsky, ''Fluorometric assay of DNA in cartilage explants using Hoechst 33258,'' *Anal. Biochem.,* **174,** 168–176 (1988).
22. J. Gao and R. Langer, unpublished results.
23. R. Lee, R. Garfield, J. Forrest, and E. Daniel, ''Effects of glutaraldehyde fixative osmolarities on smooth muscle cell volume, and osmotic reactivity of the cells after fixation,'' *J. Microsc.,* **125,** 77–88 (1982).
24. R. Lee, R. Garfield, J. Forrest, and E. Daniel, ''The effect of fixation, dehydration and critical point drying on the size of cultured smooth muscle cells,'' *Scanning Electron Microsc.,* **3,** 439–448 (1979).
25. To confirm the formation of carboxylic acid and alkanol groups on the PGA fiber surface, we activated these functional groups by carbonyl diimidazole and covalently attached argininamide from its amino group onto the surface. The presence of argininamide on the PGA surface was confirmed by XPS based on detection of elemental nitrogen that is introduced from argininamide to the otherwise carbon, oxygen, and hydrogen environment of the PGA surface.
26. D. Cohn, H. Younes, and G. Marom, ''Amorphous and crystalline morphologies in glycolic acid and lactic acid polymers,'' *Polymer,* **28,** 2018–2022 (1987).
27. The *P* value is a probability number that measures the extent to which the two groups of sample data are the same. For calculation of *P* values, see: *Applied Statistics,* 3rd ed., J. Neter, W. Wasserman, G. A. Whitmore (eds.), Allyn and Bacon, Inc., Boston, 1988, pp. 329–334.
28. H. M. Kowalczynska and J. Kaminski, ''Adhesion of L1210 cells to modified styrene copolymer surfaces in the presence of serum,'' *J. Cell Biol.,* **99,** 587–593 (1991).
29. A. Curtis and J. Forrester, ''The competitive effects of serum proteins on cell adhesion'' *J. Cell Sci.,* **71,** 17–35 (1984).
30. After tissue culture for 8 weeks, surface-hydrolyzed PGA mesh seeded with smooth muscle cells yielded a tissue having a cell density comparable to the *in vivo* blood vessels. In comparison, unmodified PGA mesh seeded with smooth muscle cells gave a tissue having a substantially smaller cell density.