Biodegradable Polymeric Scaffolds for Tissue Engineering, BioTechnology, 12, 689-693

ARTICLE *in* BIO/TECHNOLGY · AUGUST 1994

DOI: 10.1038/nbt0794-689 · Source: PubMed

Biodegradable Polymer Scaffolds for Tissue Engineering

Lisa E. Freed*, Gordana Vunjak-Novakovic, Robert J. Biron, Dana B. Eagles[1], Daniel C. Lesnoy[1], Sandra K. Barlow[1] and Robert Langer

Division of Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge, MA 02139. [1]Albany International Research Co., Mansfield, MA 02048. *Corresponding author.

Synthetic polymer scaffolds designed for cell transplantation were reproducibly made on a large scale and studied with respect to biocompatibility, structure and biodegradation rate. Polyglycolic acid (PGA) was extruded and oriented to form 13 μm diameter fibers with desired tenacity. Textile processing techniques were used to produce fibrous scaffolds with a porosity of 97% and sufficient structural integrity to maintain their dimensions when seeded with isolated cartilage cells (chondrocytes) and cultured *in vitro* at 37°C for 8 weeks. Cartilaginous tissue consisting of glycosaminoglycan and collagen was regenerated in the shape of the original PGA scaffold. The resulting cell-polymer constructs were the largest grown *in vitro* to date (1 cm diameter × 0.35 cm thick). Construct mass was accurately predicted by accounting for accumulation of tissue components and scaffold degradation. The scaffold induced chondrocyte differentiation with respect to morphology and phenotype and represents a model cell culture substrate that may be useful for a variety of tissue engineering applications.

Received 14 March; accepted 12 April 1994.

Synthetic polymers can stimulate isolated cells to regenerate tissues with defined sizes and shapes[1] and are currently being studied as scaffolds for cell transplantation both *in vitro* and *in vivo*. Examples include vascular grafts made from endothelial cells and expanded polytetrafluoroethylene (ePTFE, Dacron)[2,3], cartilage made from chondrocytes and polyglycolic acid (PGA, Dexon™), polylactic acid (PLA), and polylactic-co-glycolic acid (PLGA, Vicryl™)[4-7], liver equivalents from hepatocytes and PGA or polyvinylalcohol (PVA, Ivalon™)[8,9], and intestinal tubes made from enterocytes and PLGA[10].

Ideally, cell scaffolds for tissue engineering should meet several design criteria: (1) The surface should permit cell adhesion and growth, (2) neither the polymer nor its degradation products should provoke inflammation or toxicity when implanted *in vivo*, (3) the material should be reproducibly processable into three dimensional structures, (4) the porosity should be at least 90% in order to provide a high surface area for cell-polymer interractions, sufficient space for extracellular matrix regeneration, and minimal diffusional constraints during *in vitro* culture[4], (5) the scaffold should resorb once it has served its purpose of providing a template for the regenerating tissue, since foreign materials carry a permanent risk of inflammation[11], and (6) the scaffold degradation rate should be adjustable to match the rate of tissue regeneration by the cell type of interest.

PGA is a synthetic polymer that meets all of the above criteria. Surgical sutures made of PGA have been commercially available since 1970 and are approved for clinical use by the Food and Drug Administration (FDA)[12]. Nonwoven meshes made of PGA are currently under investigation for various tissue engineering applications including the *in vitro* cultivation and *in vivo* transplantation of cartilage cells (chondrocytes)[4-8,13-14]. PGA scaffolds have been seeded with chondrocytes, cultured *in vitro*, and characterized with respect to cell proliferation and cartilaginous matrix regeneration[5,13,14]. Chondrocyte-PGA constructs have been implanted *in vivo* as allografts to repair full-thickness articular cartilage defects in rabbits[6], and as xenografts to form subcutaneous "neocartilage" from human chondrocytes in nude mice[4].

The present studies address a number of areas that are critical for the successful engineering of cartilage based on cell-polymer constructs: (1) detailed characterization and optimization of the biodegradable PGA scaffold, (2) application of large-scale polymer extrusion and textile processing techniques to make scaffolds in a reproducible way in sufficient quantities for eventual clinical use, and (3) regeneration of cartilaginous tissues with biochemical and histological compositions comparable to normal cartilage, and clinically useful dimensions.

Results

Polymer scaffolds. The unprocessed PGA had a weight average molecular weight (M_w) of 68.9 kD and a number average molecular weight (M_n) of 25.1 kD ($M_w/M_n = 2.75$), as measured by gel permeation chromatography (GPC) (see Experimental Protocol). The melting point (T_m) was 220°C and the glass transition temperature (T_g) was 42°C, as measured by differential scanning calorimetry (DSC).

Textile processing techniques were used to prepare fibrous PGA scaffolds with desired porosity (97%) and thickness (0.5 cm) (Fig. 1 and Experimental Protocol). An oriented, multifilament yarn with a tenacity of 4.5–5.3 grams per denier (gpd) was formed by polymer extrusion, stretching and relaxation at elevated temperatures. The yarn was crimped, cut, carded into a lofty web, and needled to form nonwoven mesh using barbed needles to entangle the fibers and lock them together. Heat-setting further increased the dimensional stability of the mesh and smoothed the top and bottom surfaces. A multihole die was used to punch the mesh into 1 cm diameter discs; 3,000 discs were produced in a single run. Scaffold porosity was 97% as calculated from PGA density (1.50–1.64 g/cm^3)[12], dimensions, and sample weight (26.4 ± 2.4 mg, n = 8). Scaffolds maintained their three dimensional structure over 1 month of immersion in 37°C tissue culture medium. PGA fiber diameters were 13 ± 1 μm dry and 15 ± 2 μm after wetting (Fig. 2).

Cell culture studies. Chondrocytes attached to the fibers in multiple layers and retained their spherical morphology (Fig. 2B). The PGA scaffold appeared to induce chondrocyte differentiation as follows. The cells near the fibers were round and found in lacunae surrounded by a glycosaminoglycan (GAG)-rich matrix, while those further away from the fibers were elongated and did not secrete GAG (Fig. 2C).

The biochemical compositions of cell-PGA constructs are shown in Table 1. All tissue components (cells, GAG, collagen) increased over the 8 weeks of *in vitro* culture. The dry mass of freshly isolated chondrocytes was 0.10 ± 0.011 ng/cell; the dry

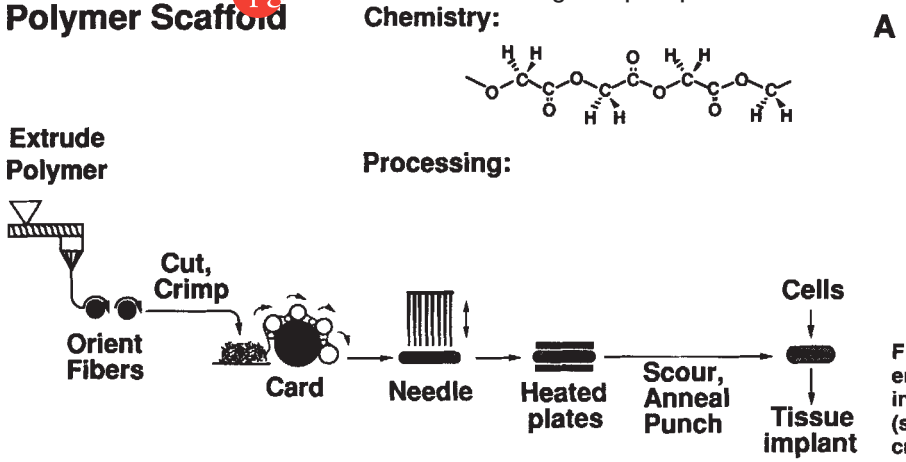


FIGURE 1. PGA scaffolds for tissue engineering. (A) Large-scale processing technology. (B) Scaffold structure (scanning electron micrograph of a full cross section).

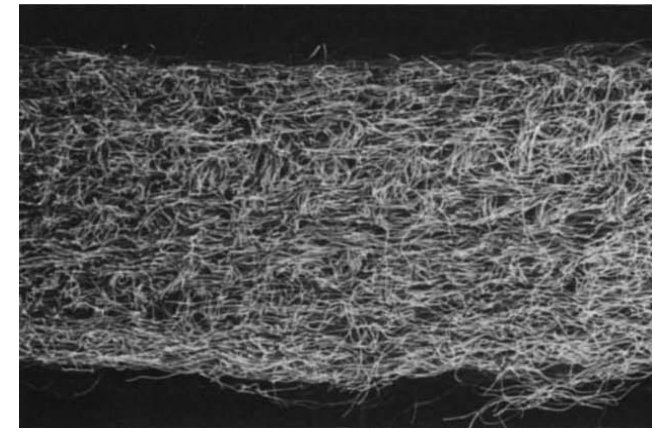


TABLE 1. Composition of cell-polymer implants.

Parameter	*In Vitro* Cultivation Time 2 days	3 weeks	8 weeks
Dry Weight (dw, mg)[1]	32.6±3.5	26.0±2.9	25.8±3.1
Cells (% of dw)[1]	1.2±0.3	2.6±0.4	4.0±1.0
Glycosaminoglycan (GAG, % of dw)[1]	0.3±0.1	4.6±0.9	15.4±2.6
Collagen (% of dw)[1]	0.2±0.1	14.4±1.8	30.8±3.2
PGA (% of dw)[2]	100.0±0.1	81.7±0.8	37.3±1.6

[1]Measured values from the tissue cultivation study (average±SD, n=6).
[2]Calculated values from the PGA degradation study (average±SD, n=3).

to wet weight (dw/ww) ratio was 2% (n=8). Cell mass was calculated from the product of the cell dry mass and the construct cell number, which increased from 3.5×10^6 at 2 days to $9.1\pm1.0\times10^6$ at 8 weeks. Regenerated tissue accounted for 50% of the dry weight (%dw) of 8 week constructs as follows: cell mass, 4%dw; GAG, 15%dw; collagen, 31% dw. The dw/ww ratio of 8 week constructs was 9.2±1.3%, which was about half of that previously measured for parent calf cartilage (17.7±0.3%)[5]. The GAG and collagen contents of 8 week constructs were 14.0±2.8 mg GAG/g ww and 28.0±3.3 mg collagen/g ww, which represented 33% and 19% of those previously measured for calf cartilage (42±5.4 mg/g ww and 145±7.5 mg/g ww, respectively)[5].

The degradation of PGA was measured using scaffolds without cultured cells in phosphate buffered saline (PBS) with 10% fetal bovine serum (FBS) at 37°C (see Experimental Protocol). The dw of PGA scaffolds passed through a flat maximum over the first week due to hydration and nonspecific protein adsorption. The 3 day dw was thus used to normalize PGA degradation data (dw/dw3d). After 8 weeks *in vitro*, the PGA had degraded to 30% of its initial mass and represented 37% of the construct dw (Table 1).

PGA mass decreased according to first order kinetics in two stages as follows. The first 50% of the mass degraded between 1–4 weeks with a rate constant of 0.099 wk^{-1}; thereafter, the mass degraded with a rate constant of 0.065 wk^{-1}. Initially, scaffolds had a heat of fusion (H_f) of 80 J/g, as measured by DSC, equalling that of commercially available Dexon sutures. Initial scaffold crystallinity was thus assumed to be comparable to that of Dexon sutures (46–52%)[15]. The change in polymer crystallinity during scaffold degradation was calculated from the measured H_f of serial samples, as previously described[16]. Between 1–4 weeks, there was an increase in H_f that corresponded to an increase in polymer crystallinity to 68–77%, after which the H_f and corresponding crystallinity decreased.

After 8 weeks, cell-PGA constructs consisted of cells, cartilaginous matrix, and remnant polymer, and had dimensions comparable to those of the original scaffold (1 cm diameter x 0.35 cm thick) (Fig. 3A). The regenerated tissue consisted of GAG that stained with safranin-O, collagen types I and II, which were seen immunohistochemically[4], and a collagenous outer capsule, 229±55 μm thick.

The kinetics of tissue regeneration is shown in Figure 3B. The amounts of tissue components (cells, GAG, collagen) in cell-polymer constructs increased over time (squares), while the amount of PGA with nonspecifically adsorbed proteins decreased, as calculated from the degradation study (triangles). The total mass predicted by the material balance (uppermost line) accounted for 100% of the measured construct dw (circles).

Discussion

The goals of the present work were to develop an optimal polymer scaffold for *in vitro* chondrocyte culture, to apply large-scale polymer processing techniques to make scaffolds in a reproducible way in sufficient quantities for eventual clinical use, and to regenerate cell-polymer constructs with biochem-

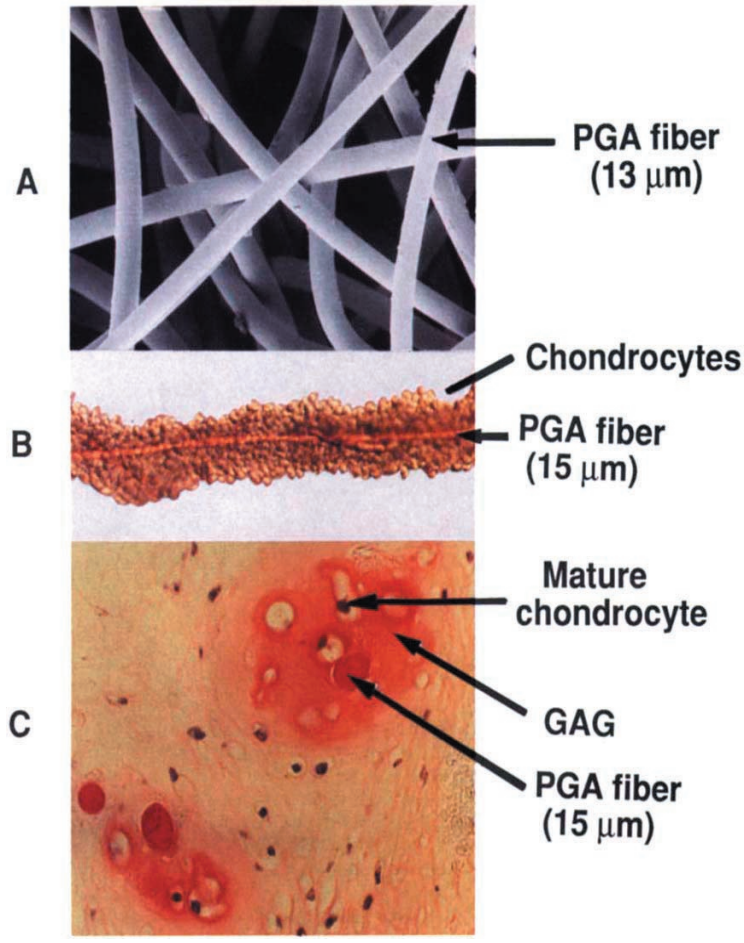


FIGURE 2. PGA fibers. (A) Initial scaffold with a fiber diameter of 13 ± 1 μm (scanning electron micrograph). (B) Multiple layers of rounded cells attached to a fiber in a 3 day chondrocyte-PGA construct (Hoffman modulation contrast, original magnification x 200). (C) Accumulation of cells and glycosaminoglycan (GAG) around PGA fibers in an 8 week chondrocyte-PGA construct (safranin-O stain, original magnification x 400).

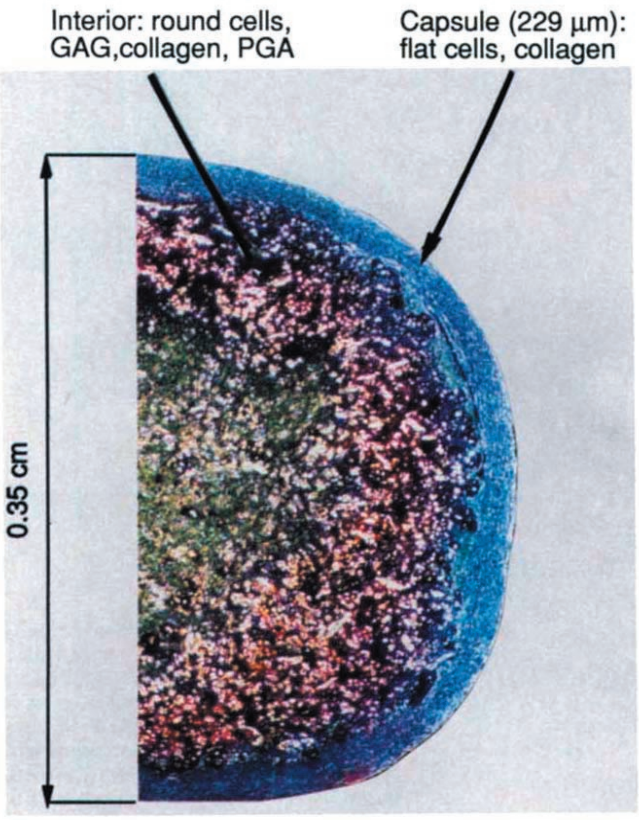
ical and histological compositions comparable to normal cartilage, and clinically useful dimensions. As a model system, chondrocyte-PGA cartilage constructs 1 cm in diameter and 0.35 cm thick consisting of calf chondrocytes, GAG and collagen were studied. Similarly sized implants made with human cells could potentially be used in reconstructive orthopedic surgery, e.g. for the repair of an osteoarthritic trapeziometacarpal joint at the base of the human thumb[17].

The chemical properties of PGA allowed it to be extruded and oriented to form fibers comparable to those used in Dexon surgical sutures[12,15,18,19]. PGA fibers 13 μm in a diameter with a tenacity of 5 gpd provided a good substrate for chondrocyte culture (Fig. 2). Large-scale textile processing techniques were adapted to make 3,000 scaffolds in a single run with desired porosity (97%) and dimensions (1 cm diameter x 0.5 cm thick) (Fig. 1A). Three-dimensional scaffold structure could be stabilized by needling combined with heated plates (Figs. 1B); needling introduced vertical channels while heated plates smoothed the top and bottom surfaces. The final scaffold had sufficient mechanical integrity to maintain its dimensions during *in vitro* cell culture and thus to determine the size and shape of the regenerated tissue.

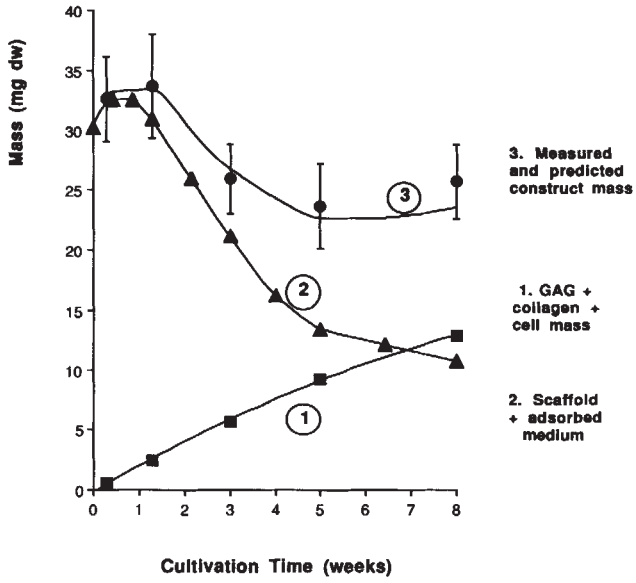
The chondrocyte-PGA constructs shown in Figure 3A are, to the best of our knowledge, the thickest cartilaginous tissues grown *in vitro* to date. Cell-polymer constructs consisted of chondrocytes, GAG and collagen, and had an outer collagenous capsule. After 8 weeks of *in vitro* culture in mixed petri dishes, regenerated tissue accounted for 50% of the construct dry weight (4% cell mass, 15% GAG, and 31% collagen) (Table 1). The measured chondrocyte wet weight of 4 ng/cell was comparable to that of other mammalian cells[20]. The total GAG and collagen content of 8 week constructs was 22% that of the parent calf cartilage; previous studies have demonstrated further chondrogenesis following *in vivo* implantation[4].

Polymer scaffold degradation is required for regeneration of a "natural" tissue implant. The four stages of PGA degradation are: (1) hydration, (2) loss of tensile strength, (i.e., cleavage of the ester bonds in the polymer backbone), (3) loss of mass (i.e., decrease in molecular weight below that required for polymer coherence), and (4) solubilization[21]. PGA degradation depends on: temperature, pH (but not in the range of 5.2–7.4), molecular weight and its distribution, and the degree of crystallinity[22,23]. Bulk-phase hydrolysis of PGA fibers occurs independently of the surface to volume ratio[18]. The glycolic acid monomer is enzymatically converted to glycine via oxidation and transamination[24]. PGA degradation can be accelerated by certain enzymes (e.g. carboxypeptidases)[25] or slowed by protein adsorption[26]. The observed increases in PGA dw and fiber diameter after immersion in solutions containing FBS were attributed to hydration and nonspecific protein adsorption.

PGA degradation in cell-polymer constructs was approximated by that measured for PGA alone in 37°C buffer. This approach was used in order to avoid the need to separate partially degraded PGA from regenerated tissue; other investigators have also modeled *in vivo* PGA degradation *in vitro*, using buffer[18,22]. PGA scaffolds degraded according to first order kinetics in two stages (Fig. 3B). Between weeks 1–4, the PGA degraded relatively quickly and the crystallinity of the remaining polymer increased; both the mass and crystallinity decreased more slowly thereafter. These findings are consistent with previous



A



B

FIGURE 3. Cell-polymer constructs. (A) An 8 week construct consisting of chondrocytes, GAG, collagen and PGA remnants surrounded by a collagenous capsule (dark field microscopy of full cross-section, original magnification x 40). (B) Tissue regeneration kinetics: (1) accumulation of GAG, collagen, and dry cell mass (average ± SD, n = 6), (2) scaffold degradation calculated from that measured for PGA in buffered PBS with 10% FBS (average ± SD, n = 3), and (3) measured dry weights of cell-PGA constructs (data points, average ± SD, n = 6) compared to those accounted for by the sum of (1) and (2) above (line).

reports that the amorphous polymer regions degrade more rapidly than the crystalline regions[19,22,23].

The amounts of cells, GAG and collagen measured in the cell-polymer constructs and mass of the remaining PGA calculated from the degradation study accounted for the measured construct dw at all time points (Fig. 3B). This finding justifies the calculation of polymer degradation in cell-PGA constructs from the data obtained for PGA scaffolds in buffer. The mass of the regenerated tissue increased more slowly than the mass of the PGA decreased such that the total construct dw decreased slightly over 8 weeks of *in vitro* cultivation (Fig. 3B). The rate of extracellular matrix secretion depended on tissue culture conditions and was increased when bioreactors were used to cultivate cell-polymer constructs[27].

Tissue regeneration kinetics in the cell-PGA system (Fig. 3B) were consistent with a three-dimensional tension-dependent model that accounts for the effects of the mechanical force balance on cell shape and function[28]. In this "tensegrity" model, the mechanical properties of the substrate determine whether cultured cells proliferate or differentiate: rigid substrates that can resist cell-generated tensions induce proliferation, while malleable substrates induce differentiation[28]. Previous studies showed that: (1) Chondrocytes cultured on tissue culture polystyrene flattened and switched from type II to type I collagen synthesis[29], (2) Chondrocytes cultured in agarose gels remained spherical and maintained their differentiated phenotype[30], (3) Chondrocytes cultured on polyhydroxyethylmethacrylate (poly-HEMA) coated dishes to which they could attach but not spread remained spherical and did not dedifferentiate[31], and (4) extracellular forces (e.g., shear stress) affected the morphogenesis of cells[27,32] and tissues[33].

Our cell culture scaffold consisted of synthetic biodegradable polymer fibers with moderate tenacity (made by extrusion and orientation) formed as a highly porous, mechanically stabilized mesh (made by carding, needling, and annealing). Chondrocytes attached to the fibers as multiple layers of spherical cells (Fig. 2B) and regenerated a cartilaginous matrix in the shape of the original polymer scaffold (Fig. 3B). GAG secretion was highest for cells located near the PGA fibers (Fig. 2C), which is consistent with the general hypothesis that a cell can be induced to express its differentiated phenotype *in vitro* when cultured on a substrate which maintains its *in vivo* configuration[34,35]. Cell-PGA constructs had sufficient rigidity to support cell proliferation, extracellular matrix secretion and the formation of an outer, collagenous capsule (Fig. 3A). These findings demonstrate the importance of scaffold design parameters and the *in vitro* culture environment on chondrocyte phenotype and cartilage tissue regeneration. The scaffold described in this paper represents a model cell culture substrate that may be useful for a variety of tissue engineering applications.

Experimental Protocol

Polymer scaffolds. PGA was obtained from Boehringer (Ingelheim, FRG). PGA molecular weight was measured relative to polymethylmethacrylate (PMMA) standards by gel permeation chromatography (GPC) using a mixed bed column (Jordi, Bellingham, MA) and a differential refractive index detector (Waters, Milford, MA). The PGA was heated for 2 min. at 245°C, quenched using dry ice, and dissolved for 2 hours to

prepare a 0.2% (w/v) solution of PGA in hexafluoroisopropanol with 0.01M sodium trifluoroacetate. PGA processing into cell culture scaffolds is schematized in Fig. 1. PGA was extruded and oriented to form 2 denier per filament yarn (one denier is the gram weight of 9,000 m of yarn) with a tenacity of 4.5–5.3 grams per denier (gpd), as measured using an Instron. Extrusion was done at temperatures 10–20°C above the polymer T_m using a 1 inch diameter extruder with a 20:1 length to diameter (l/d) ratio, adapted with a 30-hole spinneret with a 3:1 l/d ratio and 250 μm diameter capillaries. Glyceryl monostearate (Stepan, Maywood, NJ) prepared at 0.15% in isopropyl alcohol was used as a spin finish. The yarn was elongated by 4.7-fold and relaxed by 5% at a temperature of 110°C. The yarn was crimped, cut into 4 cm segments, and carded to form a lofty web (microdenier card, American IWER, Greenville, SC). The web was needled to form an 0.5 cm thick nonwoven mesh using a Fiber Locker (North Adams, MA) fitted with barbed 40 g "star blade" needles (Foster, Manitowoc, WI). The mesh was compressed between heated plates for 10 min. at 195°C, scoured for 5 min. in 1,1,1, trichloroethane (Doe & Ingalls, Medford, MA), and annealed under vacuum at 110°C for 16 hours in order to remove residual monomer. Final mesh thickness was measured using a Randall Stickney gauge (Waltham, MA), and cut into 1 cm diameter discs using a multi-punch die (Brockton/Boston Cutting, Avon, MA). The scaffolds were packaged in 3 × 7 inch plastipeel pouches (Baxter, McGaw Park, IL), sterilized with ethylene oxide (Anderson, Chapel Hill, NC), aerated in a dessicator for 12 hours, and transferred into a Dri-Lab glove box (Vacuum/Atmospheres Co., Hawthorne, CA). The pouches were then placed in 4 × 8 inch trilaminate aluminum foil pouches (K-Pak, Minneapolis, MN) containing a piece of dry filter paper (Whatman, Clifton, NJ), and heat-sealed with an iron (Clamco, Cleveland, OH). The degradation rate of the PGA scaffolds was measured in buffer at 37°C as follows. Sterile test-tubes were prepared containing: 0.150±0.005 gm of polymer and 40 cm^3 of PBS (9.3 mM) with 10% FBS, 100 U/cm^3 penicillin, and 100 μg/cm^3 streptomycin (GIBCO, Grand Island, NY). This ratio of PGA mass to medium volume was equal to that used in cell culture studies. The test tubes were mixed at 75 rpm using an orbital shaker (Bellco, Vineland, NJ) at 37°C for up to 8 weeks. Each week starting at week 2, samples were centrifuged (3,000 rpm for 20 min.) and 75% of the PBS was replaced in order to maintain a pH of 5.0–7.2. At timed intervals, triplicate samples were collected by vacuum filtration using preweighed cellulose acetate membranes with 0.20 μm pores (Costar No. 8301). Samples were dried for 3 days at 70°C, and reweighed in order to quantitate the mass of remaining polymer.

Cell culture. Knee joints from 2–3 week old bovine calves were obtained from a local abattoir within 4 hours of slaughter. The cartilage was digested with type II collagenase (Worthington, Freehold, NJ) to obtain isolated cells as previously described[4]. The cells were resuspended in culture medium (Dulbeccos Modified Eagle Medium, DMEM), containing 10% FBS, 10 mM N-2-hydroxyethylpiperazineN′-2-ethanesulfonic acid (HEPES), 0.1 mM nonessential amino acids, 0.4 mM proline, 50 mg/l ascorbic acid, 100 U/cm^3 penicillin, and 100 μg/cm^3 streptomycin (GIBCO, Grand Island, NY and Sigma, St. Louis, MO), and seeded on PGA scaffolds as previously described[5]. In brief, PGA was prewet in medium for 24 hours at 37°C, placed in 35mm dishes coated with a thin film of 1% agarose (high T_m, BioRad, Richmond, CA), and 6 cm^3 of medium and 8×10^6 cells were added. The dishes were placed on an orbital shaker set at 75 rpm and cultured for up to 8 weeks at 37°C/5% CO_2. Medium was replaced every 2–3 days. Samples for biochemical analysis were frozen, lyophilized for 3 days at 250 μm Hg, and digested with 2 cm^3 Type III papain (Sigma). The number of chondrocytes per sample was assessed by measuring the amount of DNA using Hoechst 33258 dye (Polysciences, Warrington, PA)[36]. Sulfated GAG content was determined after reaction with dimethylmethylene blue dye (Aldrich, Milwaukee, WI).[37]. Total collagen content was determined from the hydroxyproline content after acid hydrolysis and reaction with *p*-dimethylaminobenzaldehyde (Fischer, Paris, KY) and chloramine-T (Mallinckrodt, Fair Lawn, NJ)[38]. Cell mass was calculated by weighing lyophilized samples of 0.4 cm^3 of culture medium with and without 8×10^6 cells. Histological samples were fixed in neutral buffered formalin, embedded in paraffin, and sectioned (8 μm thick). Sections were stained with hematoxylin and eosin (for cells), safranin-O/fast green (for GAG), and polyclonal antibodies to type I and II collagen as previously described[4]. Sample dimensions were measured using an inverted microscope fitted with an eyepiece reticle (Nikon Diaphot, Tokyo, JA).

Acknowledgments

This work was funded by the National Aeronautics & Space Administration (NASA) (grant NAG9-655), and Advanced Tissue Sciences (La Jolla, CA). The authors thank J. Emmanual, J. Hrkach, L. Barish, and A. Dixon for their help.

References

1. Langer, R. and Vacanti, J. P. 1993. Tissue Engineering. Science **260:**920–926.
2. Lanzetta, M. and Ower, E. 1992. Achieving better patency rates and neoendothelialization in 1-millimeter polytetrafluoroethylene grafts by varying fibril length and wall thickness. Microsurg. **13:**76–83.
3. Golden, M. A., Hanson, S. R., Kirkman, T. R., Schneider, P. A. and Clowes, A. W. 1990. Healing of polytetrafluoroethylene arterial grafts is influenced by graft porosity. J Vasc. Surg. **11:**838–845.
4. Freed, L. E., Marquis, J. C., Nohria, A., Emmanual, J., Mikos, A. G. and Langer, R. 1993. Neocartilage formation *in vitro* and *in vivo* using cells cultured on synthetic biodegradable polymers. J. Biomed. Mater. Res. **27:**11–23.
5. Freed, L. E., Marquis, J. C., Vunjak-Novakovic, G., Emmanual, J. and Langer, R. 1994. Composition of cell-polymer cartilage implants. Biotech & Bioeng. **43:**605–614.
6. Freed, L. E., Grande, D. A., Emmanual, J., Marquis, J. C., Lingbin, Z. and Langer, R. 1994. Joint resurfacing using allograft chondrocytes and synthetic biodegradable polymer scaffolds. J. Biomed. Mater. Res. Vol. 28 (In press).
7. Vacanti, C., Langer, R., Schloo, B. and Vacanti, J. P. 1991. Synthetic biodegradable polymers seeded with chondrocytes provide a template for new cartilage formation *in vivo*. Plastic and Reconstr. Surg. **88:**753–759.
8. Cima, L. G., Vacanti, J. P., Vacanti, C., Ingber, D., Mooney, D. and Langer, R. 1991. Tissue engineering by cell transplantation using degradable polymer substrates. J. Biomechanical Engineering **113:**143–151.
9. Uyama, S., Takeda, T. and Vacanti, J. P. 1993. Delivery of a whole liver equivalent hepatic mass using polymer devices and hepatotrophic stimulation. Transplantation **55:**932–935.
10. Mooney, D. J., Organ, G., Vacanti, J. P. and Langer, R. 1994. Design and fabrication of biodegradable polymer devices to engineer tubular tissues. Cell Transplantation. **3:**203–210.
11. Gristina, A. G. 1987. Biomaterial-centered infection: microbial adhesion versus tissue integration. Science **237:**1588–1595.
12. Frazza, E. J. and Schmitt, E. E. 1971. A mew absorbable suture. J. Biomed. Mater. Res. Symp. **1:**43–58.
13. Freed, L. E., Vunjak-Novakovic, G. and Langer, R. 1993. Cultivation of cell-polymer cartilage implants in bioreactors. J. Cell. Biochem. **51:**257–264.
14. Freed, L. E., Vunjak-Novakovic, G., Marquis, J. C. and Langer, R. 1994. Kinetics of chondrocyte growth in cell-polymer implants. Biotech & Bioeng. **43:**597–604.
15. Gilding, D. K. and Reed, A. M. 1979. Biodegradable polymers for use in surgery polyglycolic/polylactic acid homo- and copolymers. Polymer **20:**1459–1464.
16. Cohn, D. Younes, H. and Marom, G. 1987. Amorphous and crystalline morphologies in glycolic acid and lactic acid polymers. Polymer **28:**2018–2022.
17. Pellegrini, V. D. 1991. Osteoarthritis of the thumb trapeziometacarpal joint: A study of the pathophysiology of articular cartilage degeneration. I. Anatomy and pathology of the aging joint, and II. Articular wear patterns in the osteoarthritic joint. J. Hand Surg. **16A:**967–982.
18. Craig, P. H., Williams, J. A., Davis, K. W., Magoun, A. D., Levy, A. J., Bogdansky, S. and Jones, J. P. 1975. A biologic comparison of polyglactin 910 and polyglycolic acid synthetic absorbable sutures. Surg. Gyn. & Obstet. **141:**1–10.
19. Benicewicz, B. C. and Hopper, P. K. 1990. Polymers for absorbable surgical sutures, J. Bioactive and Compatible Polymers **5:**453–472.
20. Freshney, R. I. 1987. Culture of Animal Cells, 2nd Ed., p. 232. Wiley-Liss, New York.
21. Kronenthal, R. L. 1975. Biodegradable polymers in medicine and surgery, p. 118–137. *In:* Polymers in Medicine and Surgery, Plenum Press, NY.
22. Chu, C. C. 1981. The *in vitro* degradation of polyglycolic acid sutures-effect of pH. J. Biomed. Mater. Res. **15:**795–804.
23. Zhang, X., Goosen, M. F. A., Wyss, U. P. and Pichora, D. 1993. Biodegradable polymers for orthopedic applications. J. Macromol. Sci. Rev. Macromol. Chem. Phys. **C33:**81–102.
24. Singhal, J. P., Singh, H. and Ray, A. R. 1988. Absorbable suture materials, preparation and properties J. Macromol. Sci. Rev. Macromol. Chem. Phys. **C28:**475–502.
25. Williams, D. F. and Mort, E. 1977. Enzyme accelerated hydrolysis of PGA. J. Bioengineering **1:**231–238.
26. Williams, D. F. 1980. Effect of bacteria on absorbable sutures. J. Biomed. Mater. Res. **14:**329–338.
27. Freed, L. E. and Vunjak-Novakovic, G. 1994. Hydrodynamic forces determine *in vitro* chondrogenesis in a three-dimensional cell-polymer model system. Biotech. & Bioeng. *Submitted.*
28. Ingber, D. E., Dike, L., Hansen, L., Karp, S., Liley, H., Maniotis, A., McNamee, H., Mooney, D. Plopper, G., Sims, H. and Wang, N. 1994. Cellular tensegrity: exploring how mechanical changes in the cytoskeleton regulate cell growth, migration, and tissue pattern during morphogenesis. Int. Rev. Cyt. **150:**173–224.
29. Daniels, K. and Solursh, M. 1991. Modulation of chondrogenesis by the cytoskeleton and extracellular matrix. J. Cell Sci. **100:**249–254.
30. Benya, P. D. and Brown, P. D. 1986. Modulation of chondrocyte phenotype *in vitro*, p. 219–233. *In:* Articular Cartilage Biochemistry. K.E. Kuettner, R. Schleyerbach, and V.C. Hascall (Eds.). Raven Press, New York.
31. Glowacki, J., Trepman and E., Folkman, J. 1983. Cell shape and phenotypic expression in chondrocytes. Proc. Soc. Exp. Biol. Med. **172:**93–98.
32. Wang, N, Butler, J. P. and Ingber, D. E., 1993. Mechanotransduction across the cell surface and through the cytoskeleton Science **260:**1124–1127.
33. Thompson, D. W. 1977. On Growth and Form, Ch. 8, p. 221–267. Cambridge University Press, New York.
34. Folkman, J. and Mascona, A. 1978. Role of cell shape in growth control. Nature **273:**345–349.
35. Watt, F. 1986. The extracellular matrix and cell shape. Trends in Biochem. Sci. **11:**482–485.
36. Kim, Y. J., Sah, R. L., Doong, J. Y. H. and Grodzinsky, A. J. 1988. Fluorometric assay of DNA in cartilage explants using Hoechst 33258. Anal. Biochem. **174:**168–176.
37. Farndale, R. W., Buttle, D. J. and Barrett, A. J. 1986. Improved quantitation and discrimination of sulphated glycosaminoglycans by the use of dimethylmethylene blue. Biochim. Biopyhs. Acta. **883:**173–177.
38. Woessner, J. F. 1961. The determination of hydroxyproline in tissue and protein samples containing small proportions of this imino acid. Archiv. Biochem, & Biophys. **93:**440–447.