IMPORTANCE  Cell seeding throughout the thickness of a nanofiber construct allows for patient-specific implant alternatives with long-lasting effects, earlier integration, and reduced inflammation when compared with traditional implants. Cell seeding may improve implant integration with host tissue; however, the effect of cell seeding on thick nanofiber constructs has not been studied.

OBJECTIVE  To use a novel cell-preseeded nanofiber tissue engineering technique to create a 3-dimensional biocompatible implant alternative to decellularized extracellular matrix.

DESIGN  Animal study with mammalian cell culture to study tissue engineered scaffolds.

SETTING  Academic research laboratory.

PARTICIPANTS  Thirty-six Sprague-Dawley rats.

INTERVENTIONS  The rats each received 4 implant types. The grafts included rat primary (enhanced green fluorescent protein-positive [eGFP+]) fibroblast-seeded polycaprolactone (PCL)/collagen nanofiber scaffold, PCL/collagen cell-free nanofiber scaffold, acellular human cadaveric dermis (AlloDerm), and acellular porcine dermis (ENDURAGen). Rats were monitored postoperatively and received enrofloxacin in the drinking water for 4 days prophylactically and buprenorphine (0.2-0.5 mg/kg administered subcutaneously twice a day postoperatively for pain for 48 hours).

MAIN OUTCOMES AND MEASURES  The viability of NIH/3T3 fibroblasts cultured on PCL electrospun nanofibers was evaluated using fluorescence microscopy. Soft-tissue remodeling was examined histologically and with novel ex vivo volume determinations of implants using micro-computed tomography of cell-seeded implants relative to nanofibers without cells and commonly used dermal grafts of porcine and human origin (ENDURAGen and AlloDerm, respectively). The fate and distribution of eGFP+ seeded donor fibroblasts were assessed using immunohistochemistry.

RESULTS  Fibroblasts migrated across nanofiber layers within 12 hours and remained viable on a single layer for up to 14 days. Scanning electron microscopy confirmed a nanoscale structure with a mean (SD) diameter of 158 (72) nm. Low extrusion rates demonstrated the excellent biocompatibility in vivo. Histological examination of the scaffolds demonstrated minimal inflammation. Cell seeding encouraged rapid vascularization of the nanofiber implants. Cells of donor origin (eGFP+) declined with the duration of implantation. Implant volume was not significantly affected for up to 8 weeks by the preseeding of cells ($P > .05$).

CONCLUSIONS AND RELEVANCE  Polymer nanofiber–based scaffolds mimic natural extracellular matrix. Preseeding the nanofiber construct with cells improved vascularization without notable effects on volume. An effect of cell preseeding on scaffold vascularization was evident beyond the presence of preseeded cells. This 3-dimensional, multilayer method of cell seeding throughout a 1-mm-thick construct is simple and feasible for clinical application. Further development of this technique may affect the clinical practice of facial plastic and reconstructive surgeons.
Craniofacial soft-tissue deficits often require the use of implantable materials for reconstruction. Current solutions to this problem include autografts and acellular dermis from allogenic or xenogenic sources. Tissue engineered constructs provide hope of an alternative to the costs of acellular dermis and the morbidity of autograft tissue sources. Because it is made under consistent conditions, the tissue substitute performs more predictably, and tissue engineered constructs can be shelf ready. Additionally, a tissue engineered scaffold can be tailored to replace the missing tissue. Construct design choices include material, material processing, addition of signaling factors to be released locally, addition of natural extracellular matrix (ECM) polymers, cell seeding, and conjugation of peptides or proteins for cell attachment. Patient-specific autologous cells can be seeded onto a biocompatible polymeric scaffold, reducing the risk of immune rejection of the construct.

Our study focused on the addition of cells throughout a nanofiber scaffold composed of the synthetic polymer polycaprolactone (PCL) and the natural polymer collagen. Other studies have demonstrated that the addition of collagen to polymer nanofibers increases the viability and adhesion of cells. Seil and Webster conducted a pertinent study in which cells were sprayed between electrospun layers of nanofibers, resulting in a 3-dimensional construct with viable cells throughout. Our objective was to make a nanofiber scaffold that would not need the intensive process of cell spraying between layers. To reach the desired implant thickness, the method of Seil and Webster would require electrospinning and cell seeding longer than is feasible for cell viability. Therefore, a novel method of cell preseeding on each layer of a multilayer construct was investigated. This method of cell distribution in the fibers may be easily integrated into the product’s industrial design and would require little preparation in the clinical setting.

Methods

Electrospinning

Nanofibers are electrospun (Figure 1A) from polymer solutions of PCL (number average molar mass, 80,000 Da) (Sigma-Aldrich Co) with rat tail collagen (Becton-Dickinson), which is solvated in 1,1,1,3,3,3-hexafluoro-2-propanol (Sigma-Aldrich Co). The polymer solution has an overall PCL to collagen weight ratio of 2000:1 and is ejected at a 1-mL/h flow rate by a syringe pump (World Precision Instruments) using an 18G blunted needle. We used a tip-to-collector distance of 10 cm with an applied voltage of 12 kV.

Cell Culture

The NIH/3T3 fibroblasts were purchased from ATCC. All cell culture was performed with high-glucose Dulbecco modified Eagle medium supplemented with 10% fetal bovine serum, 1% penicillin and streptomycin, 1% pyruvate, and 1% nonessential amino acids.

Primary Dermal Fibroblast Isolation

A section of skin between the scapulae was shaved and depilated (Nair; Church & Dwight Co, Inc) on the previously euthanized rat. Triplicate washes of povidone-iodine followed by ethanol were used to remove loose hair and to sterilize the skin. A 2 × 2-cm section of skin was then removed and cut into pieces that were distributed among the media-filled wells of a 6-well plate followed by abrading with a scalpel. The tissue was placed in an incubator until the first media change. This placement occurred 2 to 4 days after tissue harvest depending on the condition of the media (indicator color change) or cell outgrowth. Seven to 10 days after the initial seeding, the plates were washed once to remove the bulk of the floating cellular material. Cells were then trypsinized and passed through a 70μM filter to remove any large particles. Cell proliferation occurred in T75 tissue-culture–treated flasks (Figure 1C). Cells up to passage 5 were used for studies. Cell passaging was performed when the monolayer reached 70% to 80% confluency.

Multilayer Cell-Seeded Construct Creation

Constructs consisted of 10 layers of nanofibers with cells seeded on each layer. Sections of nanofibers, 10 × 10 cm, were lifted from the aluminum foil onto which they were collected by soaking in 70% ethanol for approximately 3 minutes. The nanofibers were spread evenly in a Petri dish. They were dried by removing as much ethanol as possible by using a pipette at the edges and then were allowed to dry inside a cell culture hood for 4 hours (Figure 1B). The scaffolds were placed in a desiccator overnight or until the day of seeding, when the scaffold was sterilized in the cell culture hood using a UV lamp for 1 to 2 hours.

Trypsinized cells were washed and resuspended to a concentration of approximately 3 × 10^5 to 5 × 10^5 cells/mL. Ten microliters of this cell suspension was added as a droplet to each 1-cm² quadrant, resulting in approximately 3 × 10^3 to 5 × 10^4 cells/scaffold. Cells were allowed to settle in each droplet of media for 1 to 2 hours in the cell incubator. The plate was then flooded with prewarmed media followed by culture overnight as a single layer.

The next day, the single layer of nanofibers (10 × 10 cm) was folded to build the 10 layers, resulting in a group of 10 scaffolds measuring 10 cm × 1 cm × 1 mm. A scalpel was used to cut the group into 10 scaffolds ready for implant (1 cm × 1 cm × 1 mm each) (Figure 1D). The thickness of a single nanofiber layer was approximately 0.1556 mm on dry fibers, which would result in a scaffold of approximately 1.0 to 1.5 mm thick. Nonseeded scaffolds were prepared in the same way, omitting the cell seeding step.

Preparation of Decellularized Dermis Implants

The decellularized dermis implants AlloDerm (LifeCell Corporation) and ENDURAGen (Stryker) were prepared according to the product inserts. Both were cut into 1 × 1-cm scaffolds, 1 for each animal.

Animal Implant Procedure

Thirty-six Sprague-Dawley rats each received the 4 implant types and then were monitored postoperatively according to...
Electrospun nanofibers made of polycaprolactone (PCL)/collagen (A) were imaged under a scanning electron microscope. The constructs were lifted from the collector and sterilized. An example 10 × 10-cm prepared construct is pictured next to a ruler measuring about 16 cm long (B). Primary fibroblasts were harvested (C) and seeded on the scaffold in a monolayer. After cells were allowed to attach and culture on the fibers, the scaffold was folded and cut (D). A cross section of a scaffold shown below. The cell-preseeded scaffolds were compared with unseeded scaffolds and decellularized dermis as controls in a single rat (E). Rats had implants for 4, 8, or 12 weeks. Each animal received 1 of each of the 4 implant types in distinct subcutaneous pockets on the dorsum. Implants were scanned (1), implanted (2), explanted, scanned a final time (3), and processed for histologic analysis (4). Postexplant scanning (3) was completed after fixation in paraformaldehyde; therefore, the samples were soaked in 70% ethanol at the second scanning, while implants for the first scan were soaked in aqueous media. Fibroblast antibody staining (F and G). Fibroblasts were identified using P4HB antibody. Histologic specimens are shown with antibody (F) and without antibody (G). Scale bar indicates 200 μm. C indicates cells (in part A, C indicates concentration), d, distance between needle and collector; ECM, extracellular matrix; eGFP, enhanced green fluorescent protein; f, flow rate; H, human; N (in part E) or NF, nanofiber; P, porcine; V+, positive applied voltage, and WD, working distance.

Figure 1. Experimental Methods

Harvest eGFP-expressing fibroblasts

Lift, dry, and sterilize

Seed

Culture

Stain

4, 8, or 12 wk
n = 36 (12 × 3)

Explant

Implant

Implant

Decellularized dermis ECM

4

3

2

1

Human

Porcine

Collector

WD 39.0 mm
10 µm
x1000
18.0 kV

Electrospun nanofibers made of polycaprolactone (PCL)/collagen (A) were imaged under a scanning electron microscope. The constructs were lifted from the collector and sterilized. An example 10 × 10-cm prepared construct is pictured next to a ruler measuring about 16 cm long (B). Primary fibroblasts were harvested (C) and seeded on the scaffold in a monolayer. After cells were allowed to attach and culture on the fibers, the scaffold was folded and cut (D). A cross section of a scaffold shown below. The cell-preseeded scaffolds were compared with unseeded scaffolds and decellularized dermis as controls in a single rat (E). Rats had implants for 4, 8, or 12 weeks. Each animal received 1 of each of the 4 implant types in distinct subcutaneous pockets on the dorsum. Implants were scanned (1), implanted (2), explanted, scanned a final time (3), and processed for histologic analysis (4). Postexplant scanning (3) was completed after fixation in paraformaldehyde; therefore, the samples were soaked in 70% ethanol at the second scanning, while implants for the first scan were soaked in aqueous media. Fibroblast antibody staining (F and G). Fibroblasts were identified using P4HB antibody. Histologic specimens are shown with antibody (F) and without antibody (G). Scale bar indicates 200 μm. C indicates cells (in part A, C indicates concentration), d, distance between needle and collector; ECM, extracellular matrix; eGFP, enhanced green fluorescent protein; f, flow rate; H, human; N (in part E) or NF, nanofiber; P, porcine; V+, positive applied voltage, and WD, working distance.

Histological Processing and Staining

Explanted tissue was fixed in 4% paraformaldehyde for 48 hours at room temperature and subsequently kept in 70% ethanol at 4°C before embedding in paraffin. Tissue specimens were embedded at the University of Virginia Research Histology Core (Figure 1E). Sections of the paraffin blocks were cut to 5 μm thick. Hematoxylin-eosin and Masson trichrome stains were completed at the Research Histology Core.

Immunostaining was completed at the University of Virginia Biorepository and Tissue Research Facility. Anti-GFP antibody (AB3080; Millipore Corp [Chemicon]) was used to trace the identity of cells in and around the construct to those seeded on the implant. Prolyl 4-hydroxylase subunit beta (P4HB;
Nanofiber Constructs for Tissue Reconstruction

Acris Antibodies Inc) was used to identify fibroblasts (Figure 1F and G). Antigen retrieval and deparaffinization were performed in a pretreatment process (PT Link; Dako) using a low-PH solution (EnVision FLEX Target Retrieval Solution; Dako) for 20 minutes at 97°C. Immunohistochemistry was performed on a robotic platform (Autostainer; Dako). Endogenous peroxidases were blocked with peroxidase and alkaline phosphatase–blocking reagent (Dako) before incubating the sections with antibodies for GFP at 1:200 for 60 minutes at room temperature. Antigen-antibody complex was detected using an immunohistochemistry visualization kit (EnVision Rabbit Link; Dako) followed by incubation with 3,3′-diaminobenzidine tetrahydrochloride (DAB+; Dako) chromogen. All the slides were counterstained subsequently with hematoxylin; they were then dehydrated, cleared, and mounted for assessment and imaging.

Staining for CD8 (clone OX-8 [catalog No. CBL1507]; Chemicon) was performed with a few modifications to the protocol above. Antigen retrieval was conducted at high pH. Incubation was performed at 1:100 for 30 minutes. Antigen-antibody complex was detected using biotinylated link and streptavidin–horseradish peroxidase (LSAB-HP system, K0609; Dako).

Measurement of the Fibrotic Capsule Thickness
A sample of slides was stained with Masson trichrome. The fibrotic capsule was defined as the blue-stained collagen layer around the implant. Slides of nanofibers and nanofibers with cells were examined under a 40× objective. Measurements were made on the electronic images in ImageJ software (http://rsbweb.nih.gov/ij/).

GFP+ Cell and CD8 T-Cell Quantification
The images created with the 40× objective were quantified. The GFP+ cells were quantified by region in the scaffold. The CD8+ cells in the types of regions used for the GFP+ cell quantification were counted as well.

Hematoxylin-Eosin Section Examination
Hematoxylin-eosin–stained slides were randomly assigned a numerical code before the scoring examination. Individual slides were scored on the following scale: the depths of blood vessels were scored as 0 for sparse; 1, on the periphery; 2, scattered; 3, well distributed; and 4, very dense. Ratings were blinded, and mean ratings were calculated by implant and time point to produce the presented analysis.

Vascularization was also quantified by measuring the area of blood vessels divided by the area of tissue quantified in each high-power field of the implant periphery on electronic images in ImageJ software. Tissue thickness was measured in a similar manner through the depth of the constructs. Foreign body giant cell (FBGC) presence was quantified by measuring the area of FBGCs (defined as cells with ≥3 nuclei) and counting the number of FBGCs per tissue area.

Micro–Computed Tomography of Scaffolds
The scaffolds were scanned (Figure 1E) to obtain the percent change in volume of each implant, calculated as the volume before implant minus the volume after explant divided by the volume before implant. Fewer than 12 postexplant specimens were available for scanning because a few explants extruded and were lost because the implants from 2 rats were reserved at each time point for histological analysis of the tissue immediately surrounding the implant. Keeping the surrounding tissue was not feasible for all implants owing to insufficient radiographic density differential at the postexplant time points.

After cell seeding, the implants were cultured in the folded form for no longer than 3 days prior to being implanted. The scan procedure required that an implant be outside the cell incubator for no more than 140 minutes. All implants were carefully curved to fit into a sterile 0.9-mL conical centrifuge tube that was then placed into a custom holder mounted in the specimen scanner apparatus for micro–computed tomography (micro-CT) (vivaCT 40; Scanco Medical). Evaluations were performed after scanning with a low threshold of ~35 mg of hydroxyapatite, selected to exclude the plastic tube holding the sample. All implants were sterilely hydrated in media (nanofiber constructs) or normal saline (decellularized dermis implants) before being scanned.

In Vitro Cell Studies
For interlayer migration studies, NIH/3T3 cells were seeded only on certain quadrants of the 10 × 10-cm grid, and the constructs were folded as described for in vivo studies. For viability studies, cells were seeded onto single-layer scaffolds. In either case, a single layer (having been unfolded in the case of a folded scaffold) was stained using propidium iodide (Sigma-Aldrich Co) or a cell-permeant nuclear counterstain (Hoechst; Invitrogen) and a cell-permeant dye (Calcein AM; Invitrogen).

Statistical Analysis
A spreadsheet program (Excel; Microsoft Corp) was used to calculate the unpaired 2-tailed t-test statistic, reported as a P value. Statistical software (Minitab, Inc) was used to calculate the standard deviation. Where appropriate, the Mann-Whitney test was used to determine statistical significance in Minitab.

Results
PCL/Collagen Nanofibers and Fibroblast Viability and Migration
To characterize the interaction of fibroblasts with the PCL/collagen nanofibers, cells were seeded on a nanofiber sheet, and, after folding, cells were on the outer 2 layers (top and bottom) of a 10-layer construct. Within 12 hours of culture in vitro, the cells were present in the middle layers. Because no cells were seeded in the middle layers, this finding suggests migration between layers. After 7 days of culture, all 10 layers contained cells (data not shown), suggesting that once folded the construct may function as a single scaffold rather than one made of 10 distinct layers. The viability of the cells was verified to remain high compared with tissue culture polystyrene for up to 2 weeks in vitro (Figure 2A) on a single layer of nano-
fibers. Furthermore, the appearance of the cells was more spindlelike on the nanofiber scaffolds (Figure 2B).

**Cell Seeding and Implant Volume Changes**

Computed tomography is capable of hard-tissue and soft-tissue imaging. Based on the assumption that to a migrating cell the 10-layer construct is comparable to a single-layer construct, the folding method needed to be validated in vivo. The 10 layers of the construct had cells seeded to each layer prior to folding (Figure 1D). One cell-seeded scaffold and 1 of each of the 3 control implants were placed subcutaneously in each rat (Figure 1E). Micro-CT scan evaluations revealed that all scaffolds except decellularized porcine dermis lost volume at all time points (Figure 2C). Regardless of cell seeding, large volume decreases were evident at 4, 8, and 12 weeks in the nanofiber groups. The cell-seeded and nanofiber-only implants were statistically significantly different from each other only at 12 weeks after implantation ($P = .03$). In contrast, human decellularized dermis lost the greatest volume between weeks 4 and 8 but was stable thereafter. Decellularized porcine dermis essentially lost no volume until 12 weeks after implantation, and its volume was the most similar to its preimplant measurement of all the implant types. Renderings of the micro-CT scans demonstrate a decrease in radiographic density (Figure 2D).

**Host Incorporation of Cell-Seeded Implants In Vivo**

After 4, 8, and 12 weeks of implantation, cell-seeded nanofibers were compared against nanofiber-alone, as well as against the human and porcine decellularized ECMs. Tissue deposition between nanofiber layers was observed in both cell-seeded and nanofiber-only scaffolds, indicating that the nanofibers provided a good substrate for de novo extracellular matrix secretion at 4 weeks after implant (Figure 3A and C-H), which was not necessarily dependent on cell preseeding. The seeding of cells did not have a significant effect until 12 weeks after implantation. At 4 weeks, 10 samples were available for all implants; at 8 weeks, 8, 9, 9, and 8 samples were available for nanofibers, nanofibers with cells, human ECM, and porcine ECM, respectively; and at 12 weeks, 10 samples were available for all implants. Limit lines indicate standard error. D. Renderings of thresholded reconstruction of implants from micro-computed tomographic scans before (left) and after (right) weeks in a subcutaneous pocket. Radiographic density became more punctuate following implant.

$^aP < .05$ vs porcine ECM.

$^bP < .05$ with a connecting line shows the indicated comparison.
increased slightly through 12 weeks. The nanofiber-only scaffold group saw increased vascularity between weeks 4 and 8. Human and porcine ECMs were generally at a low level of vascularization throughout the study.

Persistence of Preseeded Fibroblasts in the Construct

Hematoxylin-eosin staining established that the implants were highly cellular. Staining with P4HB antibody, which is specific for fibroblasts, suggested that cells in the implants were fibroblasts (Figure 1F and G). Because all dermal fibroblasts seeded onto the fibers expressed eGFP, the origin of the cells could be determined using immunohistochemistry. Stained cells with a fibroblastic or rounded macrophage appearance were quantified (Figure 4A). The counted cells were also classified according to the region from which the 40× image was taken: outside the implant, at the implant interface, or inside the implant (Figure 4B). Fibroblastic cells were found almost exclusively at the interface, and the greatest number of cells per field were on the cell-seeded implants at 4 weeks (Figure 4C).

Cells with a macrophage appearance were found primarily at the interface and outside the implant (Figure 4D). These cells likely engulfed seeded cells and therefore were still harboring eGFP or they arose by differentiation from the seeded cells. The cells with a macrophage appearance were highly migratory, showing up on the decellularized dermis implants at 4 weeks. Only the human decellularized dermis showed significant numbers of cells at 12 weeks. Although the cause of this is not known, the human decellularized dermis was located closest to the cell-seeded scaffolds on the rat dorsum. Thus, the seeded fibroblasts did not persist during this experiment, but eGFP+ cells actively participated in the response to the subcutaneous implants.
Immune Reaction to the Implants
The presence of cells promoted a thicker capsule (Figure 5A–C) and greater numbers of, as well as larger, FBGCs (Figure 5F and G). However, CD8+ cytotoxic lymphocytes (CD8 T cells), although increased early, were significantly decreased at 12 weeks at the interface and also outside the implants with the seeding of cells (Figure 5D and E). This suggests that the preseeded cells affected the type of inflammatory response, which was characterized by FBGCs and reduced specific immunity.

Discussion
Our experiments aimed to develop a novel cell-seeding method that allows a spatially controlled number of cells to be distributed through the thickness of a nanofiber scaffold. Collagen in the nanofibers provides cell adhesion ligands, while the PCL provides cost-effective mechanical stability. Both qualities are critical for any scaffold constructed. Cell seeding has been shown to decrease inflammation15 and to increase acceptance despite the seeded cells being replaced by the host cells. The benefits of cell seeding would be difficult to bring to clinical practice with currently published methods. The method presented here allows for easier construction and may aid in bringing this technology to practitioners.

Cells seeded onto the PCL/collagen matrix promoted incorporation of the implanted scaffold even though they were eventually replaced by host cells. The seeding density of cells within the implant was relatively low compared with published results (Table). This finding may support a hypothesis that cell presence is more important for integration with host tissue than is the cultivation of cells on the constructs long enough for proliferation and the laying down of ECM proteins.22 The multilayer constructs can be folded immediately before implantation. This allows the clinician to tailor the implant at the time of surgery.

In the cell migration experiment, cell presence on all 10 layers indicates migration of individual cells across at least 4 layers of nanofibers from each side. The cell migration observed within the scaffolds is greater than the depth of penetration in a modified gelatin-based scaffold, where a maximum penetration of 250 μm was noted.23 Gee et al24 reported the use of a natural cross-linking agent, which was used to demonstrate that a greater proportion of collagen inhibited migration of mesenchymal stem cells. The collagen-containing electrospun scaffolds used here were not cross-linked and supported fibroblast migration. The migration experiment sug-
gests that, although the implant begins as 10 distinct layers, once folded it acts as a single implant.

The volume of the implants was slightly reduced by the addition of cells. Change in implant volume, especially a reduction, may be seen as a negative result in terms of maintenance of appearance in a reconstructive surgical setting. The study design did not evaluate the effect of these volume changes on outward appearance at the implant site. However, as long as the implant volume does not decrease faster than the new tissue is formed, implant volume decrease does not indicate that there would be a visible surface deficit.

The thickness of the capsule was visualized using Masson trichrome–stained sections on nanofibers (NFS) (A) and NFs with cells (B) (original magnification ×40). The capsule thickness was measured (C). Immunostaining for CD8 was performed (D). Scale bar indicates 100 μm. The CD8+ cells were quantified (E). Significant reductions in the NFS with cells group was apparent at 12 weeks. Limit lines indicate standard error (n = 20) for each bar. The number of foreign body giant cells (FBGCs) per slide area was greatest at the NF implants with few exceptions (F). At 12 weeks, there were fewer FBGCs on the NFS than there were on the NFS with cells. The area of the individual FBGCs followed almost the same trend, with the NF groups having larger FBGCs than the decellularized extracellular matrix groups at 4 and 8 weeks (G). By 12 weeks, however, the cell-seeded NF implants were surrounded by larger FBGCs than were the NF-only implants. Limit lines indicate standard error (n = 30) for each bar. *P < .05 (≤25 measurements were made for each group).

Table. Cell-Seeding Density Literature Values

<table>
<thead>
<tr>
<th>Density</th>
<th>Medium</th>
<th>Cell Type</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 × 10⁵ or 60 × 10⁵ Cells/mL</td>
<td>Injectable hydrogel</td>
<td>Chondrocytes</td>
<td>Maher et al, 2010</td>
</tr>
<tr>
<td>2 × 10⁶ Cells/mL</td>
<td>10 × 10 × 0.5-mm PLGA nanofibers</td>
<td>hMSCs, hMSC-derived chondrocytes, and osteoblasts</td>
<td>Xin et al, 2007</td>
</tr>
<tr>
<td>1 × 10⁵ to 5 × 10⁵ Cells/cm²</td>
<td>P(LLA-CL) (75:25) electrospun nanofibers</td>
<td>ECs and SMCs</td>
<td>Mo et al, 2004</td>
</tr>
<tr>
<td>As many of 4.0 × 10⁶ cells in a pellet as would stick over several days</td>
<td>1 cm × 1 cm × 1 mm-thick PCL nanofiber scaffold</td>
<td>Primary MSCs</td>
<td>Yoshimoto et al, 2003</td>
</tr>
<tr>
<td>0.2 × 10⁶ to 5.7 × 10⁶ Cells/mL scaffold</td>
<td>Freeze-dried alginate scaffold</td>
<td>Primary hepatocytes</td>
<td>Dvir-Ginzberg et al, 2003</td>
</tr>
<tr>
<td>15 × 10⁶ Cells/mL</td>
<td>Agerose gel</td>
<td>Primary chondrocytes</td>
<td>Kisiday et al, 2002</td>
</tr>
</tbody>
</table>

Abbreviations: ECs, endothelial cells; hMSCs, human mesenchymal stem cells; SMCs, smooth muscle cells; PCL, polycaprolactone; PLGA, poly(lactic-co-glycolic acid); P(LLA-CL), poly(l-lactide-co-e-caprolactone); SMCs, smooth muscle cells.
Observations from this study demonstrate that cell seeding can be accomplished on multilayer PCL/collagen nanofiber constructs and that this cell seeding improves vascularization and modifies the inflammatory response. Cell migration during a 7-day period in combination with the single-layer viability suggests that diffusion-limited nutrient delivery is not a limiting factor within these multilayer scaffolds. The PCL/collagen nanofibers were well tolerated by the recipients. When eGFP-expressing cells were used to differentiate between host and donor cells, many donor-derived cells were found near the nanofiber surface of individual layers. The eGFP-positive cells became less numerous throughout the study, being replaced by host cells and participating in the remodeling process.

Cell-seeded implants produced a significantly greater amount of early neovascularization. Long-term positive effects of cell seeding extended beyond their disappearance in the constructs. In conclusion, these cell-seeded constructs may prove valuable for dermal augmentation or replacement and may act as a model to study the effects of cell seeding on tissue engineered constructs.

ARTICLE INFORMATION
Submitted for Publication: February 22; final revision received May 4; accepted June 24.

Author Contributions: Dr Botchwey and Mr Bowers had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Dr Barker and Mr Bowers contributed equally to this study and are to be considered co-first authors.

Study concept and design: Barker, Bowers, Chance, Klembczcyk, Brayman, Park, Botchwey.

Acquisition of data: Barker, Bowers, Hughley, Klembczcyk, Brayman.

Analysis and interpretation of data: Barker, Bowers, Brayman, Park, Botchwey.

Drafting of the manuscript: Barker, Bowers, Klembczcyk, Brayman, Park.

Critical revision of the manuscript for important intellectual content: Barker, Bowers, Hughley, Chance, Brayman, Park, Botchwey.

Statistical analysis: Bowers.

Obtained funding: Botchwey.

Administrative, technical, or material support: Barker, Hughley, Klembczcyk, Brayman, Park, Botchwey.

Study supervision: Barker, Chance, Park, Botchwey.

Conflict of Interest Disclosures: None reported.

Funding/Support: This study was supported in part by a Leslie Bernstein Grant from the American Association of Facial Plastic and Reconstructive Surgery.

Additional Contributions: Carter P. Shields prepared the explants for histological processing. This work was completed in the Biomedical Engineering Department at the University of Virginia.

REFERENCES


