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Marrow-derived cells populate scaffolds composed of xenogeneic extracellular matrix

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Introduction. **The source of cells that participate in wound repair directly affects outcome. The extracellular matrix (ECM) and other acellular biomaterials have been used as therapeutic scaffolds for cell attachment and proliferation and as templates for tissue repair. The ECM consists of structural and functional proteins that influence cell attachment, gene expression patterns, and the differentiation of cells.**

Objective. **The objective of this study was to determine if the composition of acellular matrix scaffolds affects the recruitment of bone marrow–derived cellular elements that populate the scaffolds in vivo.**

Methods. **Scaffolds composed of porcine tissue ECM, purified Type I collagen, poly(L)lactic coglycolic acid (PLGA), or a mixture of porcine ECM and PLGA were implanted into subcutaneous pouches on the dorsum of mice. The origin of cells that populated the matrices was determined by first performing bone marrow transplantation to convert the marrow of glucose phosphate isomerase 1b (Gpi-1^b) mice to cells expressing glucose phosphate isomerase 1a (Gpi-1^a).**

Results. **A significant increase in Gpi-1^a expressing cells was present in sites implanted with the porcine ECM compared to sites implanted with either Type I collagen or PLGA. Use of recipient mice transplanted with marrow cells that expressed β-galactosidase confirmed that the majority of cells that populated and remodeled the naturally occurring porcine ECM were marrow derived. Addition of porcine ECM to the PLGA scaffold caused a significant increase in the number of marrow-derived cells that became part of the remodeled implant site.**

Conclusion. **The composition of bioscaffolds affects the cellular recruitment pattern during tissue repair. ECM scaffolds facilitate the recruitment of marrow-derived cells into sites of remodeling.** **© 2001 International Society for Experimental Hematology. Published by Elsevier Science Inc.**

The molecular and cellular events that regulate migration and differentiation of pluripotential hematopoietic stem cells during ontogeny remain unknown. Similarly, events that regulate the migration, proliferation, and differentiation of progenitor cells in and around a scaffold during tissue repair remain unknown. It is logical that the composition and/or structure of a tissue repair scaffold will play an important role in determining the cell types that participate in the repair process.

A significant body of work has been conducted during the past decade showing that tissue repair scaffolds derived from native extracellular matrix (ECM) can induce constructive remodeling of missing or severely damaged tissues [1–11]. Use of these scaffold materials is associated with tissue healing that includes differentiated cell and tissue types such as functional arteries and veins, innervated smooth muscle and skeletal muscle, cartilage, and specialized epithelial structures [3–11]. These differentiated cells are highly organized and the remodeled tissue resembles native tissue by the end of the repair process. The source of cells that contribute to this remodeling process has been the subject of investigation.

Studies have suggested that endothelial progenitor cells exist within most tissues, including the bone marrow and

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circulating blood [12–19], but the biologic signals that determine the route and rate of migration of these cells through tissues are largely unknown. The extent to which these cells can be manipulated will play a significant role in future studies of tissue and organ repair, replacement, and regeneration. One mechanism for manipulating the migration and differentiation of these cells may be the choice of biomaterials used to induce constructive remodeling.

Objective

The purpose of the present study was to examine the contribution of marrow-derived progenitor cells to fully healed and remodeled tissue in adult mice when a variety of naturally-occurring and synthetic scaffolds are used as templates for tissue repair.

Methods

Overview of experimental design

Seventy-seven female adult mice with labeled bone marrow–derived cells were divided into six separate groups, each of which consisted of 12–14 mice. Five of these groups were implanted with a different scaffold material in the dorsal subcutaneous tissue. The sixth group served as a sham-operated control. Each group was then further divided into four subgroups of 3–4 mice, each based upon the length of survival following implantation of the scaffold material. At the time of sacrifice, the implantation site was harvested and the tissue examined for both quantitative determination of the marrow-derived cells and localization of the marrow-derived cells that participated in the scaffold remodeling process.

Mouse model for quantitative measure of marrow-derived cells in scaffolds

Donor mice homozygous for the glucose phosphate isomerase–1 isoenzyme (Gpi-1^a) were a kind gift of Dr. David Harrison (Jackson Laboratories, Bar Harbor, ME, USA). Bone marrow cells were transplanted into lethally irradiated recipient mice that were homozygous for the Gpi-1^b isoenzyme (C57BL/6J strain). Following reconstitution, the composition of any tissue could be assayed for the presence of marrow-derived cells by sampling for recipient (Gpi-1^b) and donor (Gpi-1^a) isoenzymes.

C57BL/6J mice raised in our colony were lethally irradiated with 11 Gy of γ irradiation administered in two doses divided by 4 hours using a ^{137}Cs irradiator (Nordion, Kanata, Canada). Low-density mononuclear cells were isolated as previously described [20] and 5×10^6 cells were transplanted via tail vein injection. Recipient animals were tested at monthly intervals to assay for the level of donor cell chimerism via sampling of peripheral red and white blood cells. Stable chimerism posttransplant was generally achieved in 4 months. When the blood of the host Gpi-1^b animals was 100% donor (Gpi-1^a) in origin, animals were considered ready for use in the scaffold implantation studies described below [20,21].

Gpi-I isoenzyme analysis

Peripheral blood cells (or tissue samples from the implantation studies) were deposited in microcentrifuge tubes, pelleted, and the cells lysed by freeze-thawing in distilled water. The cell lysates were applied to Titan III Zip Zone cellulose acetate plates (Helena Laboratories, Beaumont, TX, USA). Loaded gels were run for 30 minutes in an electrophoresis chamber at 300V. The Gpi-1 isoenzymes were detected as detailed by Harrison et al. [22]. Stained gels were scanned with a Rep clinical densitometer (Helena Laboratories, Beaumont, TX, USA) for quantitation.

β-galactosidase mouse model of selectively labeled bone marrow–derived pluripotential stem cells

The second method of selectively identifying cells of bone marrow origin that contributed to sites of scaffold remodeling in the present study utilized the β-galactosidase$^+$ bone marrow cell mouse model. This model has been used by several investigators to determine contributions of donor cells to tissue remodeling in chimeric hosts [23–25]. This model provided a very clear and definitive method of identifying marrow-derived cells in the host by standard histochemical methods. Using the X-GAL reagent and the strong staining properties of cells with β-galactosidase gene, all donor (i.e., bone marrow–derived) cells could be identified definitively in harvested tissue specimens.

Six- to eight-week-old male donor animals, C57bl/6J.TgR (ROSA26)26SOR mice, were obtained from Jackson Labs (Bar Harbor, ME, USA). Bone marrow cells were isolated as previously described [26]. A separate group of 6- to 8-week-old C57bl/6J male mice were lethally irradiated with 11 Gy of γ irradiation (96 cGy/minute) in two doses divided by 4 hours using a 137Cs irradiator (Nordion, Kanata, Canada). The C57bl/6J.TgR(ROSA26) 26SOR donor marrow cells were transplanted into the lethally irradiated C57bl/6J mice (5×10^6 donor marrow cells per recipient). The transplanted mice were housed in micro-isolator cages with acidified water posttransplant. At monthly intervals, peripheral blood cells were drawn via incision of the tail vein, and peripheral blood cells were stained using X-GAL for evidence of bacterial β-galactosidase as has been previously reported [25]. When the host animals were determined to be stably reconstituted with the donor cells (usually 4 months), they were ready for the scaffold transplantation studies described below.

Histology, immunohistology, and β-galactosidase staining

Following sacrifice of the animals, the tissue specimens were divided into two equal halves. One half was placed in neutral buffered formalin and prepared by standard methods for staining with hematoxylin and eosin. The second half of the collected specimen was snap frozen in liquid nitrogen and prepared for immunohistochemistry. The frozen specimens were stained with antibodies specific for PECAM-1 and von Willebrand factor. LacZ gene expression was detected in frozen sections by the enzymatic activity of the gene product β-galactosidase. This stability and specificity of staining allowed for identification of cells expressing the gene product. For the present study, bone marrow origin cells alone expressed this gene product. Those marrow-derived cells that participated in remodeling of the test scaffolds were easily identifiable by this method.

In brief, frozen sections were exposed to substrate solution for 12 hours at 25°C. Positive-staining cells exhibited a bright blue stain that was easily distinguishable from surrounding tissues. Counter-staining with eosin was done to accentuate the difference, which allowed easy localization and identification of cell type (e.g., endothelial cells, mesenchymal connective tissue cells, epithelial cell structures).

Scaffold materials

Five separate scaffold materials were examined in the present study. The first scaffold material was poly(L)lactic polyglycolic acid (PLGA), a resorbable, synthetic material commonly used in tissue engineering applications. The second material was purified Type I collagen, a resorbable, naturally-occurring matrix that is commonly used as a tissue repair scaffold (e.g., Contigen, Regen meniscus). The third and fourth scaffold materials were the extracellular matrix derived from the small intestinal submucosa (SIS) and urinary bladder submucosa (UBS), respectively. The fifth scaffold material was a composite of PLGA and UBS. The details regarding acquisition or preparation of these biomaterials is provided below.

Preparation of extracellular matrix from porcine small intestine and urinary bladder

The preparation of the SIS-ECM has been previously described [2,27,28]. Sections of small intestine harvested from freshly slaughtered market-weight pigs were delaminated of the superficial layers of the tunica mucosa and the entirety of the tunica muscularis externa. The remaining tunica submucosa with basilar layers of the tunica mucosa consisted almost entirely of extracellular matrix with a small number of intact cells. Subsequent treatment with dilute (0.1%) peracetic acid and rinsing in phosphate-buffered saline (pH = 7.4) and deionized water rendered the material acellular. The remaining ECM consisted of a complex mixture of structural proteins, including collagen type I, III, IV, V, VI, and proteoglycans, glycoproteins, glycosaminoglycans, and growth factors left in their native three-dimensional micro-architecture.

The method for production of UBS-ECM was fundamentally the same as that described for the SIS-ECM (above). The muscularis externa (smooth muscle cell layers and serosa) were mechanically delaminated from the more superficial submucosa. The transitional epithelium, basement membrane, and lumina propria were similarly removed by either mechanical or ionic methods, leaving behind the extracellular matrix of the submucosa. This matrix was rendered acellular by methods identical to that described above for the SIS-ECM.

Preparation of porous PLGA

The synthetic polymer used for making porous scaffolds was 5% (w/v) and consisted of poly(L)lactic-co-glycolic acid, 50% of each. This porous scaffold with a three-dimensional structure and pore sizes of approximately 900 μM was prepared by incorporation of a sucrose polymer into the scaffold prior to molding followed by dissolution of the sucrose after polymerization. Pores may allow and/or promote three-dimensional tissue growth, nutrient diffusion, and vascularization [29]. Porous scaffolds were prepared as three-dimensional blocks that measured 1.0 cm × 1.0 cm × 0.5 cm.

Collagen scaffold

Porous bovine Type I collagen scaffolds measuring 1.0 cm × 1.0 cm × 0.5 cm were made using the Porosigen technique and freeze-drying method. Purified collagen was dissolved in acidic aqueous solution at a concentration of approximately 10%. The collagen suspension was blended into a slurry and freeze-dried.

PLGA/UBS copolymer

PLGA was purified as described above. Prior to polymerization, a particulate form of the lyophilized UBS-ECM was uniformly mixed with the PLGA solution to create a composite of approximately 10% UBS (w/w). The particulate size range of the UBS-ECM was 0.1–0.5 mm. The resulting construct had a 900 μM pore size as described for the PLGA-alone scaffold.

Surgical procedure and postoperative care

Each animal was anesthetized with isofluorane in inhalation chambers and maintained at surgical plane anesthesia. The surgical site on the back of each mouse was shaved and prepared for surgery with a Dial surgical scrub (Dial Corp., Phoenix, AZ, USA). Using sterile technique, a 1.5 cm^2 pocket was created in the subcutaneous tissue on the back of each animal. The test article of choice (measuring 1.0 × 1.0 × 0.5 cm) was carefully placed in the subcutaneous pocket and secured at the four corners with 3-O prolene suture material to prevent migration of the scaffold during the postoperative period. The animals were recovered from anesthesia and maintained on daily feed of Purina rodent chow in housing quarters with cycled light (12 hours on/off), regulated temperature, and water ad libitum.

At the appropriate time, the mice were euthanized by overdose of isofluorane. The graft sites, excluding the surrounding normal tissue and site of suture placement, were immediately excised and placed in saline. The graft tissue was frozen for storage prior to Gpi isoenzyme analysis. Separate sections of the harvested graft site were collected for histopathologic examination.

Since the endpoints of the studies were based upon the cell type that is present in the remodeled scaffold material, care was taken to eliminate the sites of suture placement from the harvested tissue. The inflammatory cells that tend to accumulate around sites of suture placement may confound interpretation of results since these cells would be of bone marrow origin. Only the central portions of the graft were used for isoenzyme analysis and immunohistochemistry.

Statistical methods

The percentage of total isoenzyme concentration expressing Gpi-1^a (GPI) was analyzed using two-way analysis of variance (ANOVA) model [30]. The factors are type of scaffold with six levels (PLGA, PLGA-UBS, SIS, Sham, Type1-C, and UBS) and time postimplantation with four levels (weeks 1, 2, 4, and 8). At each time point there were 3–4 mice per scaffold type. Results for the analysis of variance are presented with F-test statistics, the degrees of freedom and the associated *p* values. Multiple comparisons were performed using the Tukey method. All analyses were performed using SAS statistical software [30].

Results

Female adult mice in which all marrow-derived cells could be identified by either glucose phosphate isomerase isoenzyme concentration or β-galactosidase expression were used in the present study. Mice were implanted with one of five different scaffold materials or subjected to a sham surgical procedure. All mice survived the procedure without complication and resumed normal eating, drinking, and activity habits within 24 hours.

GPI isoenzyme findings

The recipient (i.e., marrow-transplanted) C57BL/6J mice showed a 100% reconstitution of the peripheral blood with GPI^a (donor) cells (Fig. 1). Figure 2 shows a graph of the

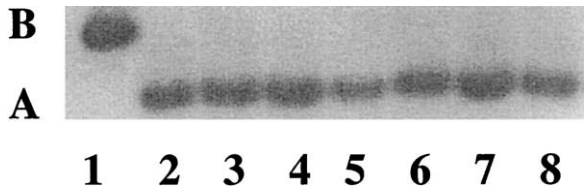

Figure 1. Gpi-1[a] analysis of peripheral blood cells from normal and transplanted animals four months posttransplantation. Lane 1: Gpi-1[b] activity of the recipient strain of mice (BoyJ) prior to transplantation. Lane 2: Gpi-1[a] activity of the donor strain of mice (ROSA β-gal). Lanes 3–8: Gpi-1[a] activity indicative of complete reconstitution of the recipient marrow with donor-type cells.

mean values for the GPI percent activity at the implant site for each group of mice prior to and following scaffold implantation. All recipient mice showed a baseline value of approximately 8% GPI[a] (marrow-derived cells) in the dorsal subcutaneous tissue prior to implantation of any scaffold material. This 8% represents the GPI[a] activity present in circulating peripheral blood cells present within the normal tissue vasculature and for extravascular marrow-derived cells residing in the subcutaneous compartment.

Mice subjected to the sham surgical procedure showed a fourfold increase in the mean donor type GPI[a] activity at the surgical site at 7 days that returned to baseline levels of activity at 28 and 56 days. Similarly, mice that received a bovine collagen Type I implant were noted to have a sixfold increase in donor type GPI[a] activity at the implant site at 7 days that returned to baseline levels of activity at 28 and 56 days. Mice that received a PLGA implant were observed to have a mean 10-fold increase in donor type GPI[a] activity at the implant site at 7 days and persistence in donor type GPI activity threefold above baseline at 28 days and 56 days. In contrast, mice that received a SIS implant showed a sixfold increase in donor type GPI[a] activity at 7 days that further increased to eightfold above baseline (and the sham control value) at 28 days and 56 days. Likewise, mice that received a UBS implant demonstrated implant sites with a 10-fold increase above baseline GPI[a] activity at 7 days with a maintenance of a ninefold increase at 28 days and an eightfold increase at 56 days. Finally, mice that received a composite PLGA/UBS implant were noted to have a ninefold increase in donor-type GPI[a] activity at the implant site at 7 days with persistence of donor-type GPI activity at eightfold above baseline and sham control levels at 28 and 56 days.

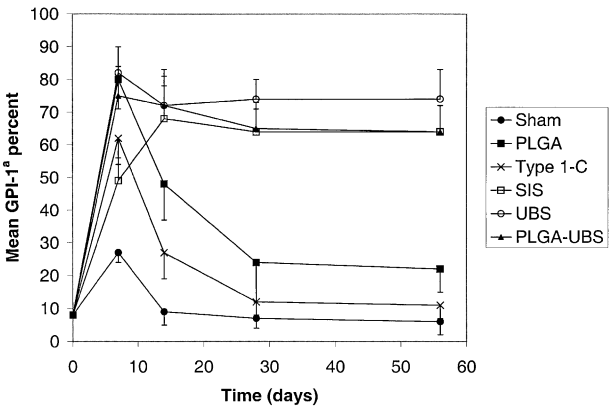

Figure 2. Graph of mean Gpi-1[a] percent activity (± SD) at scaffold implant site over time. The scaffolds that contain ECM showed persistently high percent Gpi-1[a] activity compared to sham operated controls, purified Type I collagen, and PLGA.

Microscopic findings and β-galactosidase findings

The GPI isoenzyme data indicated that a significant number of marrow-derived cells populated the scaffolds in the early postoperative period and that these cells persisted at the implant site in the presence of the porcine acellular ECM scaffolds. However, neither the type nor the location of these cells within the tissue was known. The morphology and specific location of these marrow-derived cells was determined by the use of congenic donor mice that differed from the recipient mice not only in GPI isoenzyme activity but also in constitutive expression of bacterial β-galactosidase activity (ROSA β-gal) (Fig. 3). Detailed histological analysis was performed on the scaffold implant sites at 7, 28, and 56 days to identify the cells populating the scaffold implant site and for evidence

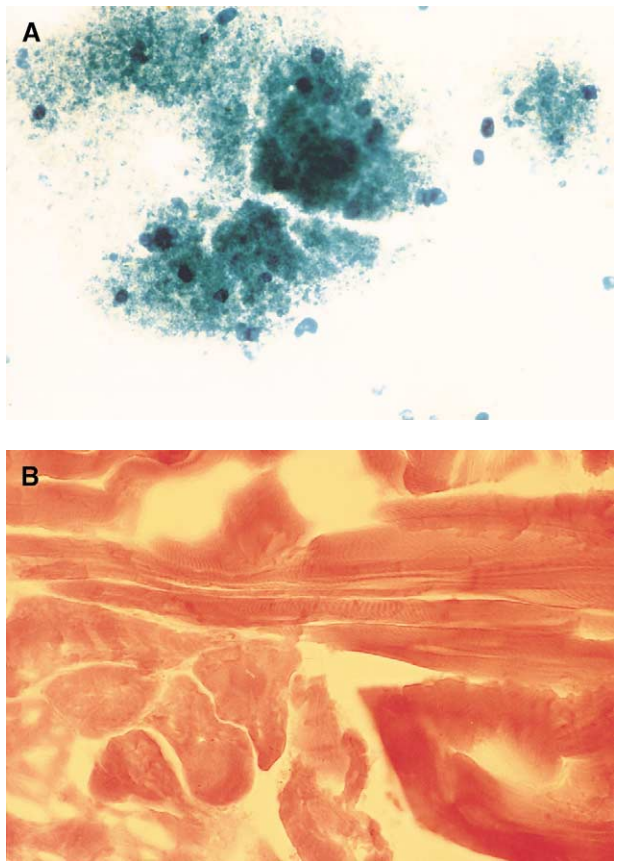

Figure 3. (A) β-galactosidase^{+} (i.e., blue) staining cells in an aspirate of bone marrow cells of recipient mice. **(B)** Cardiac muscle tissue, just as all tissues without bone marrow–derived cells, from recipient mice stain negative for β-galactosidase expression.

of β-gal expression. All recipient mice were 100% engrafted with donor-type GPI-expressing cells from the ROSA β-gal mice prior to any implantation studies (Fig. 3).

Mice subjected to the sham surgical procedure showed only scattered β-gal$^+$ cells at the surgical site after the initial tissue inflammatory response subsided. By day 56 there was complete restoration of normal tissue histology.

Mice that received a PLGA implant showed an inflammatory response in the surrounding tissue that changed from a mixed-type inflammatory cell infiltrate at day 7 to a low-grade mononuclear cell infiltrate by day 56. There was a moderately dense fibrous connective tissue response that invaded the outer portion of the PLGA implant by day 56 that contained only scattered, round-shaped β-gal$^+$ cells at day 56 (Fig. 4A and B).

Mice that received a Type I collagen implant showed a small number of β-gal positive cells at the site of implant remodeling by day 56. These cells were generally spindle shaped and confined to the outer regions of the collagen implant. The implant itself was still distinctly visible and there was little remodeling of the collagen in the central portions of the implant, even at day 56 (Fig. 5A and B).

Mice that received either an SIS or UBS implant showed a large number of β-gal$^+$cells at all time points. At day 7, the cells expressing β-galactosidase were usually round in shape and consistent with mononuclear inflammatory cells. By day 56, the SIS and UBS had been resorbed and replaced by normal connective tissues including fibrous tissue, adipose tissue, and smooth muscle cells (Fig. 6A). When the remodeled site was stained for β-gal expression, many of the spindle-shaped cells stained positively and were consistent with the morphology of fibroblasts or endothelial cells (Fig. 6B). The implant site showed a rich vascular response at both the 28- and 56-day time points and some of the spindle cells lining primitive capillary spaces showed β-gal$^+$ staining (Fig. 6C). The spindle cells identified as endothe-

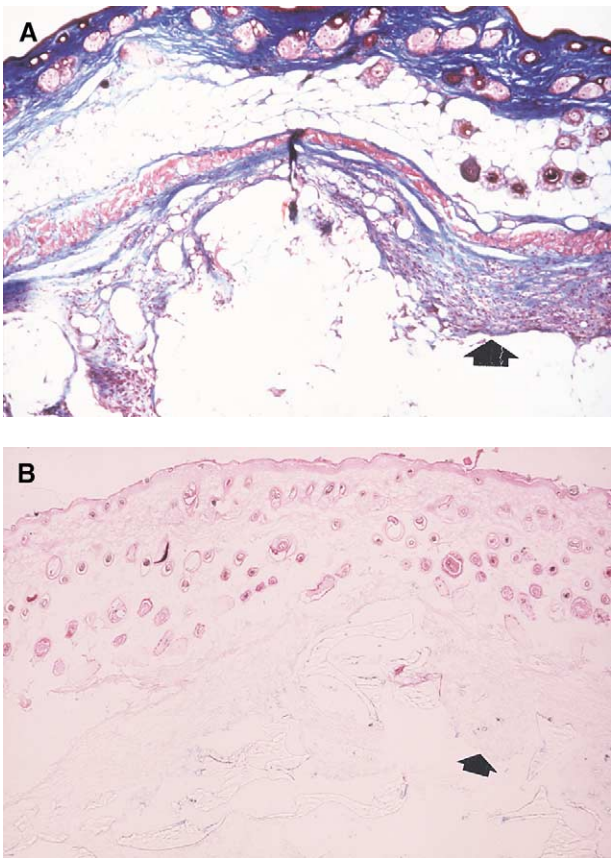

Figure 4. (A) Histologic section of PLGA implant site at day 56. The PLGA material is unstained (bottom of picture) and the arrow delineates the boundary of a surrounding chronic inflammatory cell reaction. (Masson's Trichrome Stain, ×40). **(B)** Histologic section of PLGA implant site stained for β-galactosidase expression and counterstained with eosin. Arrow points to boundary between subcutaneous tissue and PLGA implant. β-galactosidase expression was seen only faintly in round-shaped cells at this implant site (×40).

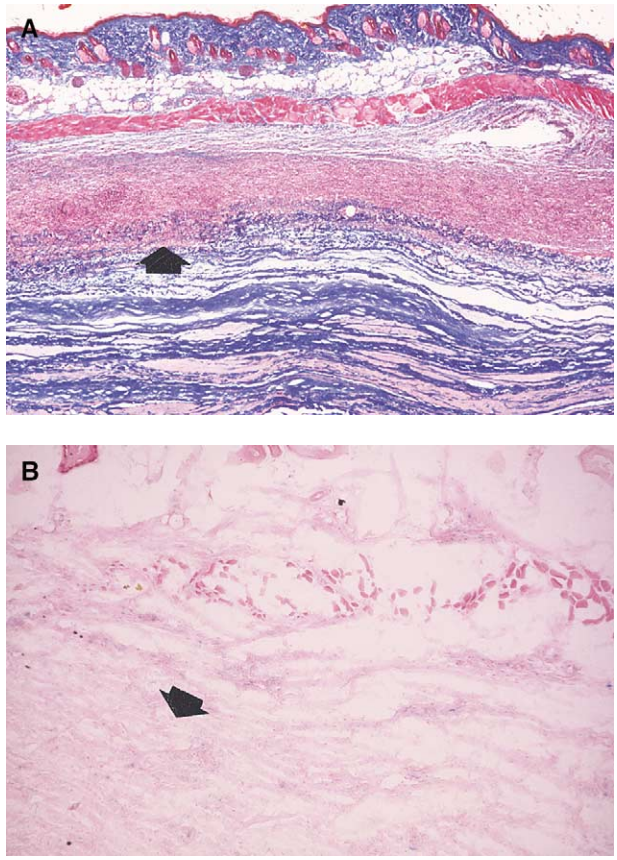

Figure 5. (A) Histologic section of purified Type I collagen implant site on day 56. Arrow points to interface between implant and surrounding subcutaneous tissue. There is an infiltration of cells at the edges of the implant but a lack of cellular infiltrate in the central portions of the implant. (Masson's Trichrome Stain, ×40). **(B)** Arrow points to interface between purified Type I collagen implant and subcutaneous tissue. There is a lack of β-galactosidase expression in the cells in this region. The section is counterstained with eosin (×125).

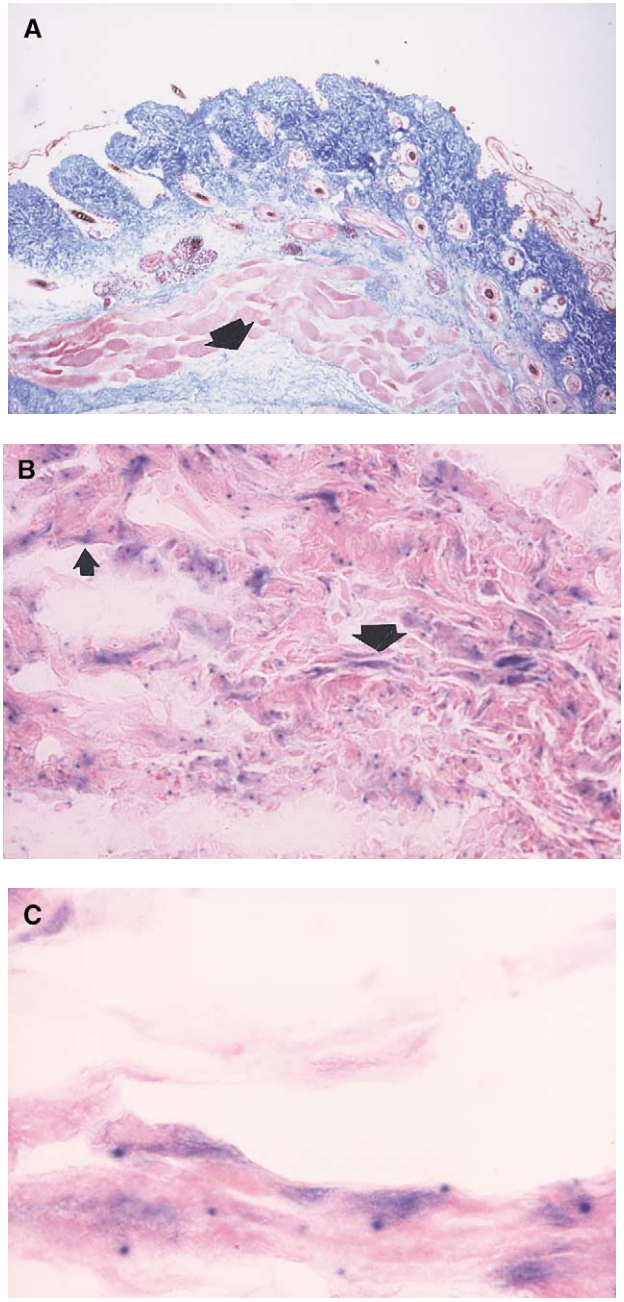

Figure 6. **(A)** Section of SIS implant site, day 56. Arrow points to interface between host muscle tissue and underlying connective tissue that represents the original SIS implant site. No SIS material is visible at this time. (Masson's Trichrome Stain, ×25). **(B)** β-galactosidase$^+$ (i.e., blue) staining cells in the remodeled SIS implant site. Arrows point to spindle-shaped cells that are occasionally aligned in longitudinal lumenal structures reminiscent of primitive capillaries. (Counterstained with eosin, ×125). **(C)** High magnification in photograph of β-galactosidase$^+$ staining spindle cells adjacent to lumenal space. This section is counterstained with eosin (×240).

lial cells on H&E stained sections and Masson's Trichrome stained sections also stained positively for PECAM-1 and von Willebrand Factor (Fig. 7A and B).

The mice that received a composite PLGA/UBS implant showed a mixed inflammatory cell response at day 7 with an

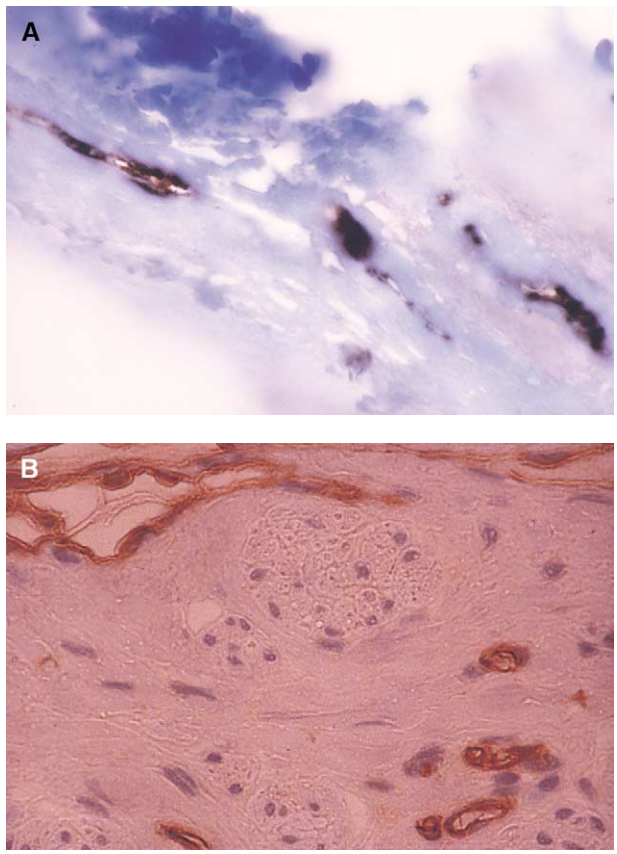

Figure 7. **(A)** PECAM-1$^+$ (i.e., black) staining cells in the SIS implant site 56 days after surgery. **(B)** von Willebrand factor$^+$ (i.e., brown) staining cells in the SIS implant site 56 days after surgery.

abundance of β-gal$^+$ cells present at the periphery of the implant. The inflammatory response showed a predominantly mononuclear round cell population by days 28 and 56 and the PLGA material remained visible at all time points (Fig. 8A). β-gal$^+$ spindle cells were present immediately adjacent to the remnants of the PLGA and these cells were found throughout all areas of the implant as opposed to only at the periphery (Fig. 8B). The UBS component of the composite scaffold could not be identified at either the 28- or 56-day time points.

Statistical analysis

The two-way analysis of GPI isoenzyme concentration showed that there were differences due to the bioscaffold materials ($F = 262.08$; $df = 5,53$; $p < 0.001$) and differences among the four time points of evaluation ($F = 64.96$; $df = 3,53$; $p < 0.001$) and the interaction between type and time ($F = 19.28$; $df = 15,53$; $p < 0.001$). There were statistically significant differences for the UBS and SIS containing scaffolds vs all other types of scaffolds at 28 and 56 days ($p < 0.05$).

Discussion

A variety of scaffold materials, both with and without cells, have been used to facilitate wound repair. The molecular

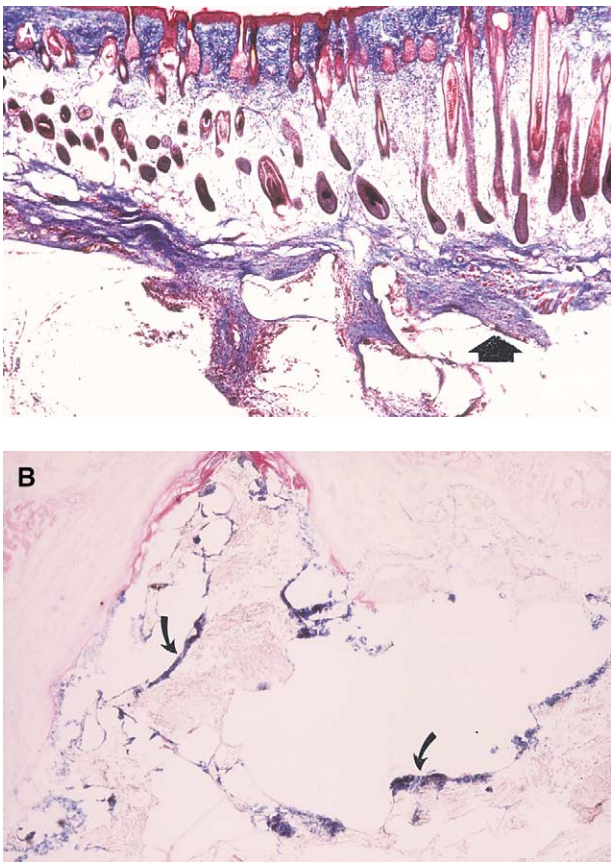

Figure 8. (A) Section of PLGA-UBS implant site at day 56. There is a moderately dense cellular response at the periphery of the implant. The PLGA remains unstained. No UBS material can be identified. The arrow points to the interface between the implant and the surrounding host tissue. (Masson's Trichrome Stain, ×40). **(B)** β-galactosidase^{+} staining cells at the interface (arrow) between the PLGA-UBS implant and the host tissue. (Counterstained with eosin, ×125).

composition of acellular scaffolds may directly influence the host response. It has been previously reported that acellular resorbable porcine ECM scaffolds derived from the small intestinal submucosa and urinary bladder submucosa facilitate the constructive, tissue-specific replacement of diverse tissue structure such as esophagus [3], tendon [27,31], urinary bladder and urethra [5,32,33], and dura mater [4,28] in experimental animals and in human patients. The origin of the cells that repopulated the ECM scaffolds in these subjects has not been reported. In the present study, it was determined that the composition of implanted scaffolds affects the source of cells that populate these scaffolds. The present study evaluated scaffold remodeling sites 56 days following surgery. This time point was chosen because it was well past the time of any acute inflammatory response. In fact, the implant sites containing SIS or UBS alone showed complete resorption of the scaffold with replacement by only differentiated host tissues. No scaffold could be seen, nor was any evidence for an inflammatory response present.

Scaffolds prepared solely with acellular resorbable porcine SIS or UBS or with the synthetic polymer PLGA mixed with UBS were populated by a significant number of marrow-derived cells at the 56-day time point. These marrow-derived cells were phenotypically consistent with endothelial cells and fibroblasts. Confirmation of the specific cell types that derive from the marrow will require additional study.

The capacity of some organ systems of the body to replace injured or senescent cells with new progeny derived from progenitor cells is becoming increasingly appreciated [12]. In response to tissue injury, it has been widely viewed that local recruitment of cells into the repair site was the primary mode of tissue reconstitution. The recent findings that some endothelial and fibroblast cells present in damaged or injured tissue are derived from circulating precursors has stimulated reconsideration of this phenomenon. Asahara et al. [34] reported that circulating cells gave rise to endothelial cells that could incorporate into sites of vascular injury. Shi et al. [35] reported that precursors of canine vascular endothelial cells that seed intravascular grafts are derived from the bone marrow compartment. Chesney et al. [36,37] reported that human and murine blood contains bone marrow mononuclear cells that migrate out of the bloodstream into areas of wound healing and function as fibroblast cells. These studies provide evidence that blood contains progenitor cells capable of producing tissue endothelial and fibroblast cells.

The potential for constructive tissue repair and regeneration in adult tissues, as opposed to scar tissue formation, has become evident with the recent isolation and characterization of stem and progenitor cells for skeletal muscle [13], cartilage [14], liver [15], pancreas [16,17], epithelial tissues [18], and brain [19]. Perhaps most exciting is the observation that stem cells residing in one tissue can differentiate and become functional cells in another tissue or organ. For example, bone marrow cells have been demonstrated to differentiate into neurons and glia [38,39], skeletal muscle [40], hepatocytes [41], and endothelial cells [34,42]. These particular studies raise the intriguing hypothesis that marrow-derived stem cells may circulate and participate in the cellular responses to tissue injury and repair.

Conclusion

The present study suggests that the participation of marrow-derived cells can be, at least in part, modulated by the type of scaffold material that is utilized for tissue repair. We observed marked increases in marrow-derived cells in the fully remodeled tissue sites that were implanted with scaffolds composed of SIS-ECM or UBS-ECM. At this time it is unclear whether stem cells in the marrow were recruited to the implanted scaffolds and differentiated into the endothelial and fibroblast cells, distinct progenitors for the endothelial and fibroblasts were mobilized from the marrow into the blood stream and emigrated into the scaffolds, or differentiated marrow endothelial and fibroblast cells migrated to the scaffolds. Further

studies will be required to address these questions and to determine the mechanism of SIS and UBS recruitment of the marrow-derived cells into the subcutaneous implant site. It previously has been noted that SIS-ECM is comprised of a heterogenous mixture of structural and functional proteins [43–45]. It is possible that one or more of these proteins mediates the marrow recruitment of progenitor cells.

Acknowledgments

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