Recellularization of Acellular Human Small Intestine Using Bone Marrow Stem Cells

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ABSTRACT

We aimed to produce an acellular human tissue scaffold with a view to test the possibility of recellularization with bone marrow stem cells to produce a tissue-engineered small intestine (TESI). Human small-bowel specimens (n = 5) were obtained from cadaveric organ donors and treated sequentially with 6% dimethyl sulfoxide in hypotonic buffer, 1% Triton X-100, and DNase. Each small intestine (SI) piece (6 cm) was recellularized with EPCAM + and CD133 + allogeneic bone marrow stem cells. Histological and molecular analysis demonstrated that after decellularization, all cellular components and nuclear material were removed. Our analysis also showed that the decellularized human SI tissue retained its histoarchitecture with intact villi and major structural proteins. Protein films of common extracellular matrix constituents (collagen I, laminin, and fibronectin) were found in abundance. Furthermore, several residual angiogenic factors were found in the decellularized SI. Following recellularization, we found viable mesenchymal stem cells, CD133 + epithelial cells in villi adjacent to a muscularis mucosa and CD31 + smooth muscle cells, and a high repopulation of blood vessels with CD31 + endothelial cells. Our results show that in the future, such a TESI would be ideal for clinical purposes, because it can be derived from the recipient’s own immunocompatible bone marrow cells, thus avoiding the use of immunosuppression. STEM CELLS TRANSLATIONAL MEDICINE 2013;2:307–315

INTRODUCTION

The regenerative capacity of intestinal mucosa is greatly compromised during pathological conditions when mucosal damage has occurred to the intestine. Extensive intestinal resections are mostly followed by extreme efforts to correct many complications, including inflammatory bowel disease, trauma, mesenteric vascular disease, volvulus, congenital atresia, and neonatal necrotizing enterocolitis.

Resection of 70%–80% of the small bowel results in short-bowel syndrome (SBS), a condition that is associated with high morbidity and mortality [1]. It is reported that the long-term survival of patients who have less than 50 cm of residual small bowel is presently only 45% [2]. Different operative alternatives may improve the outcome of patients with SBS. Intestinal lengthening techniques, tapering procedures to improve peristalsis, and construction of intestinal valves to slow transit have all been shown to have important roles [3]. However, the long-term outcome of these procedures appears to be modest.

Current therapeutic options available for patients with short-bowel syndrome are aimed at ensuring an adequate supply of nutrients, water, electrolytes, trace elements, and vitamins. This can be achieved by parenteral nutrition (PN) via intravenous infusion. Small-bowel transplantation is another viable therapeutic option [4]. However, these approaches are still plagued by serious complications such as sepsis and liver failure associated with PN and limited availability of the donor organs and high graft-rejection rates associated with transplantation and the consequences of long-term immunosuppression.

Intestinal tissue engineering is an attractive alternative therapy to intestinal transplantation. Attempts to engineer small intestine since the late 1980s have achieved varying degrees of success in animal models with evolving refinements in biotechnology. The most encouraging results so far have been the generation of intestinal neonemcosa in the form of cysts when intestinal epithelial organoid units isolated from neonatal rats were seeded onto biodegradable polymers before implantation in syngenetic adult rat’s omentum [4].

There is an interest in developing tissue-engineered small intestine (TESI) with the ultimate goal of implanting structurally and functionally
compotent small intestine for the treatment of human SBS. Here, we have attempted to forward this field by bioengineering intestinal tissue with human stem cells. It has been reported that bone marrow (BM)-derived cells contribute to the regeneration of damaged intestinal epithelium as epithelial cells [5]. This suggests that BM-derived cells could be a potential source for intestinal epithelial tissue regeneration. We therefore aimed to produce an acellular human tissue scaffold with a view to test the possibility of recellularization with bone marrow cells to produce a tissue-engineered small intestine (SI). Such a TESI would be ideal for clinical purposes, since it can be derived from the recipient’s own (immunocompatible) BM cells, thus avoiding the use of immunosuppression.

**Materials and Methods**

All protocols used in the present study were approved by the local ethics committee. Small intestine tissue measuring 20–30 cm was taken from different deceased healthy organ donors (n = 5) after informed consent from the relatives. A biopsy piece of 2 cm² from each tissue sample was snap frozen in liquid nitrogen, stored at −80°C, and used at a later time point for immunohistochemical analysis.

**Retrieval of Small Intestine From Cadaver Donor**

A 30–50-cm segment of terminal ileum was retrieved from donors (details are given in the supplemental online Materials and Methods).

**Decellularization of Small Intestine Specimen**

In our initial experiments, we used three different decellularization protocols (details are given in the supplemental online Materials and Methods). Tissues were treated with each protocol (4% sodium deoxycholate followed by DNase) [6], protocol 2 (0.5% sodium dodecyl sulfate followed by DNase), and protocol 3 (6% dimethyl sulfoxide followed by 1% Triton-X-100 and lastly by DNase). Based on our preliminary results (not results), we decided to use protocol 3 for the present study.

Each small intestine specimen was sliced into 6–8-cm-long segments. The tissue was immediately and thoroughly rinsed in phosphate-buffered saline (PBS) containing 0.5% penicillin, 0.5% streptomycin, and 0.5% amphotericin B and frozen at −80°C in PBS overnight. The next day the samples were thawed at room temperature. The segments were washed once with distilled water. One end of each specimen was kept open while the other was clamped, and the lumen was filled with 10 ml of 6% dimethyl sulfoxide (DMSO; Sigma-Aldrich, Gothenburg, Sweden, http://www.sigmaaldrich.com). The other end was then clamped, and each specimen was then immersed in a wide-bottom plastic bottle containing 6% DMSO and kept on an agitator at 37°C overnight. The next day, the lumen was filled with 10 ml of 0.4 mg/ml deoxyribonuclease I (Sigma-Aldrich) in 1 M NaCl, and the tissue was clamped, immersed in a plastic bottle containing 1 M NaCl, and incubated for 4 hours on the agitator at 37°C. Lastly, the lumen of the specimens was washed with 20 ml of distilled water (D/W) and placed in a plastic bottle with D/W on the agitator for 6 hours to remove cell debris. Two cycles of the decellularization protocol were run. At the end of the decellularization process, the SI segments were washed continuously for 24 hours with 20 ml of PBS (changed every 6 hours). All solutions used for decellularization contained the above-mentioned antibiotics. At the end of each cycle, a small piece of tissue was screened for the presence of nuclei and verified histologically using standard procedure.

**Characterization of Recellularized SI Matrix**

The decellularized SI segments were characterized by staining with hematoxylin and eosin (H&E) and Mason's trichrome as well as by Luminex technology for various proteins. Collagens, glycosaminoglycans (GAGs), and proteoglycans and elastin were characterized using Sicrol soluble collagen, Blysacan sulfo-glycolylated glycosaminoglycan, and Fastin elastin assays (all from Biocolor, Newtownabbey, U.K., http://www.biocolor.co.uk) respectively. Prior to sectioning and staining, all tissue samples were turned inside-out to permit a better examination of the luminal side of the TESI (details are given in the supplemental online Materials and Methods).

**Determination of Tensile Strength of the Decellularized SI**

Biocolor and decellularized tubular SI samples, 10 mm wide, were tensile-tested as ring samples according to ISO [7] with an Instron 5566 (Instron, Norwood, MA, www.instron.com). The specimen holder had a diameter of 10 mm. The preload was 1.5 N, and the test speed was used was 50 mm/minute. The maximum force and the vertical elongation at the maximum force were registered. The force along the circumference of the tube was half of this measured force. The elongation along the circumference of the tube was 2 times the measured elongation. The accuracy of the tensile tester was 1% in force and 0.5% in elongation.

**Analysis of Residual Angiogenic Growth Factors After Decellularization**

Total protein was extracted from the DSI (n = 3) using a commercially available kit (Millipore AB, Stockholm, Sweden), and the protein concentration for all samples was standardized to 1 mg/ml. The angiogenic growth factors produced by the DSI were tested by Luminex technology using a commercially available kit (Millipore AB, Darmstadt, Germany, http://www.millipore.com). The following 17 growth factors were tested: angiopoietin-2, bone morphogenetic protein-9, epidermal growth factor (EGF), endoglin, endothelin-1, fibroblast growth factor (FGF-1; acidic FGF), FGF-2 (basic FGF), follistatin, granulocyte colony-stimulating factor, heparin-binding EGF, hepatocyte growth factor (HGF), interleukin-8, leptin, placental growth factor, vascular endothelial growth factor (VEGF)-A, VEGF-C, and VEGF-D.

**Isolation of Bone Marrow Stem Cells for Recellularization of SI Specimens**

The cells were prepared from 20 ml of BM obtained from a donor. The bone marrow was separated on lymphoprep and...
washed three times with Dulbecco’s modified Eagle’s medium (DMEM). We isolated CD133+ stem cells, since these cells have been reported to differentiate into several cell types [8–10]. The cell fraction was divided into two; one fraction was used to isolate CD133+ stem cells and the other EPCAM+ cells using CD133– and EPCAM-coated Mini MACS beads (Miltenyi Biotec, Bergisch Gladbach, Germany, http://www.miltenyibiotec.com), respectively, as described by us earlier [11]. The number of CD133+ and EPCAM+ cells obtained was counted, and viability was tested using trypan blue. Both cell types were cultured in 0.2% gelatin-coated culture wells at 37°C in a humidified atmosphere of 95% air and 5% CO2. EPCAM+ cells were grown in complete epithelial cell medium. The basal medium used was a mixture of DMEM and Ham’s F-12 medium in a 1:1 proportion. For preparation of complete medium, 5% heat-inactivated FBS, 1% l-glutamine, and 1% penicillin-streptomycin; amphotericin (Gibco, Grand Island, NY, http://www.invitrogen.com) was added to the basal media mixture. The complete medium was supplemented with hepatocyte culture medium single Quote kit (Lonza, Walkersville, MD, http://www.lonza.com) containing ascorbic acid, bovine serum albumin-fatty acid free, hydrocortisone, transferrin, insulin, recombinant human epidermal growth factor, and gentamicin sulfate.

The CD133+ cells were divided into two fractions; one fraction was grown in complete molecular, cellular, and developmental biology (MCDB) medium: basal medium MCDB 131+10% heat-inactivated human AB serum, 1% -glutamine, and 1% penicillin-streptomycin + supplemented with endothelial growth medium-2 Single Quote kit (Lonza; catalog no. CC-4176) containing ascorbic acid, hydrocortisone, transferrin, insulin, recombinant human vascular endothelial growth factor, human fibroblast growth factor, human epidermal growth factor, heparin, and gentamicin sulfate to differentiate them into endothelial cells. The other fraction was grown in a commercially available smooth muscle cell medium (Gibco, Grand Island, NY, http://www.invitrogen.com) medium 231+ growth factor supplements; catalog no. S-007-25). When cells reached 90% confluence, the supernatant was removed and the cells were washed with PBS and then passaged with trypsin-EDTA.

To induce smooth muscle differentiation, the culture medium was changed to complete medium containing smooth muscle cell differentiation supplement (Gibco; catalog no. S-008-3). The medium in both cell fractions was replaced every 2–3 days. Confluent cells were passaged using trypsin-EDTA (Invitrogen).

Epithelial cells obtained from BM samples were stained with single-color immunofluorescence histology for cytokeratin 7 and EPCAM+ and EPCAM– actin. The data are expressed as means ± SEM. Statistical significance was determined by one-way analysis of variance with Dunnett’s post hoc test for cell enumeration experiment, whereas a paired t-test was used for extracellular matrix (ECM) quantification. A difference to value) of < .05 was considered significant.

RESULTS

Decellularized Small Intestine

Our initial studies demonstrated that sodium deoxycholate was unable to completely decellularize the tissues and caused greater disruption of the basement membrane and connective tissue ECM (supplemental online Fig. 2, upper panel), whereas although SDS treatment removed all cells, it produced a frail, pale tissue with leakage following two cycles of the decellularization process. Moreover, only a faint staining for collagen, fibronectin, and laminin was observed (supplemental online Fig. 2, lower panel).

Using the DMSO + Triton X-100 protocol, the median time needed to decellularize the SI segments was 72 hours. By the end of the decellularization procedure, the DSI segments maintained their gross appearance and size; however, they were white and translucent except for the mesenteric fat surrounding the tissue as compared with native tissue (Fig. 1A, 1B). Further decellularization cycles resulted in a frail, translucent tissue with leakage following two cycles of the decellularization process. Moreover, only a faint staining for collagen, fibronectin, and laminin was observed (supplemental online Fig. 2, lower panel).

The decellularized SI segments were characterized by staining with H&E and Masson’s trichrome. The sections were stained for mucins (supplemental online Materials and Methods) and with antibodies to various markers such as cytokeratin (CK) 8, CD31, and -actin.

Enumeration of Repopulation of TESI

We quantified the re-cellularization rate of the SI segments using microscopy. For the microscopic analysis, we used a commercially available high content screening platform, Olympus Scan-R (details are given in the supplemental online Materials and Methods).

Statistics

The data were expressed as means ± SEM. Statistical significance was determined by one-way analysis of variance with Dunnett’s post hoc test for cell enumeration experiment, whereas a paired t-test was used for extracellular matrix (ECM) quantification. A difference to value) of < .05 was considered significant.

Recessing of Cells in the Bioreactor

An in-house bioreactor was manufactured that facilitated a separate external and luminal medium circulation (supplemental online Fig. 1). The decellularized piece was placed into the bioreactor (5% CO2 and 95% air at 37°C and 90% humidity). At our center, we have previously isolated cells from human SI and obtained on average 5 ± 1.8 × 106 cells per cm2 of tissue [12]. Based on these observations, each seeding cycle consisted of a single injection of 12 × 106 CD133+ cultivated allogeneic endothelial cells (6-cm length; 2 × 106 cells per cm2) into the lumen of the decellularized SI, followed by 24 hours of endothelial cell medium perfusion using a constant flow of 8 ml/minute. Prior to medium perfusion, the matrix was rotated 90° every hour until all of the surfaces had been seeded with cells. After 24 hours of endothelial cell medium perfusion, EPCAM+ and differentiated smooth muscle cells (6 × 106 cells each) were added, and the procedure was followed as above. A total of three seeding cycles was performed with a 24-hour interval between each seeding.

The total period of bioreactor culture was 2 weeks. The recellularized SI segments were characterized by staining with H&E and Masson’s trichrome. The sections were stained for mucins (supplemental online Materials and Methods) and with antibodies to various markers such as cytokeratin (CK) 8, CD31, and -actin.
was not detected in DSI. A large amount of the ECM proteins was preserved in the decellularized ECM during the decellularization process. The decellularized ECM was rich in collagen (2116 ± 0.5 ng/mg) and soluble elastin (6,446 ± 2.8 ng/mg) ECM wet weight. A small amount of sulfated GAG (58.8 ± 0.01 ng/mg) ECM wet weight was also found. The quantitative assay showed that extracellular matrix collagen (significantly $p < .05$) and elastin were enhanced, whereas the amount of GAG was decreased after decellularization (Fig. 11).

Assessment of the mechanical properties of the DSI matrix showed a progressive increase in tensile strength with increasing decellularization cycles (Fig. 2A). We found that after two cycles of DMSO + Triton X-100, the decellularization process did not greatly influence the force that the DSI could withstand before breaking compared with the native SI (Fig. 2A). No clear differences in tensile extension at the maximum load of the DSI and native SI samples were seen. However, sample stiffness increased slightly, although not significantly, with further treatment cycles (Fig. 2A).

Growth Factors Produced by DSI

Using the technology of Luminex, we found several residual growth factors present in the DSI (Fig. 2B). Interestingly, most of the growth factors tested were still present, albeit at lower concentrations in the DSI. In general, a three- to fourfold decrease in the concentrations of the growth factors was found in the DSI as compared with normal SI.

Histochemical Staining of Decellularized Small Intestine

H&E staining of DSI showed absence of nuclei, and the SI tissue retained its histoarchitecture with intact villi but no nuclei. In
addition, several blood vessels were also detected (Fig. 2C). Furthermore, Masson’s trichrome staining showed the presence of blue collagen fibers but no black nuclei, whereas staining for mucins showed no expression of these proteins (supplemental online Fig. 3A–3C).

Characterization of Bone Marrow-Derived Stem Cells

EPCAM+ Cells

Following isolation, the magnetically isolated EPCAM+ cells were counted and viability tested by trypan blue exclusion method. On average, approximately 2–3 × 10^6 EPCAM+ cells were isolated from 7–8 × 10^6 BM mixed cell population, and the viability was 96%. The EPCAM+ cells grew in several clusters and formed a monolayer within 10 days. They showed typical epitheloid morphology. The EPCAM+ cells could be maintained with stable morphology for five or six passages. Immunofluorescence staining with anti-keratin antibodies CK7 and CK18 demonstrated a positive reaction in the form of cytoplasmic strands in the cultured cells (Fig. 3A).

CD133+ Endothelial Cells

The number of CD133+ cells obtained was approximately 1 × 10^6 from 10 × 10^6 BM mixed cells, and the viability was 98%. The CD133+ cells grown in endothelial cell medium grew as numerous clusters and formed a monolayer within 3 days. They showed typical cobblestone morphology and could be maintained for five or six passages. The cells stained positive for the endothelial cell markers CD31 and von Willebrand factor using immunofluorescence (Fig. 3B).

CD133+ Smooth Muscle Cells

Differentiated CD133+ SMC grew as elongated, spindle-shaped cells and could be maintained for 8–10 passages with stable morphology. Immunofluorescence staining showed that these cells were positive for α-smooth muscle cell actin and vimentin (Fig. 3C).

Analysis of TESI Segments

In general, the gross morphology of the recellularized TESI was very similar to the native tissue (supplemental online Fig. 4A).
with intact villi (supplemental online Fig. 4B) resembling the original SI segment. H&E staining revealed well-formed recellularized TESI (Fig. 4A). The TESI demonstrated a muscularis (arrowheads), abundant villi, and crypts (arrows). Masson's trichrome staining showed the presence collagen fibers (blue), several nuclei (black), and abundant connective tissue and muscle fibers (red) and several blood vessels expressing endothelial cells (Fig. 4B, arrows). In addition, staining for mucus showed the clear presence of secretory epithelial cells, goblet cells that stained pink/magenta, indicating the presence of neutral mucus (Fig. 4C).

Immunofluorescence staining with antibodies for the three cell types used for recellularization showed positive staining for CK18, an epithelial cell marker (Fig. 5A, green); CD31, an endothelial cell marker in the blood vessels (Fig. 5B, green); and α-smooth muscle cell actin (Fig. 5C, green). DAPI staining of various sections provided further proof of the presence of cells in the recellularized SI sections (Fig. 5D).

**Engraftment**

Table 1 presents the number of cells quantified at the various time points. We found that the number of detected cells increased with increasing time points. Supplemental online Figure 4C represents a gallery of images used for enumeration of the cells at 2 weeks of recellularization.

**DISCUSSION**

The present work demonstrates the first step toward development of recellularized human small intestine matrix for possible future clinical transplantation. We show that decellularized human small intestine segments, nature’s scaffold, serve as biologically active blueprints and modulators of TESI. We present a technique for efficient in vitro recellularization of the graft, which maintains cell viability and allows cell attachment. Furthermore, we demonstrate that the decellularized TESI samples during and after recellularization showed progressive increase in the amount of cell infiltrate in the matrix, including a morphologically intact regenerated mucosa, intact villi, and crypts lined by columnar epithelium with goblet cells and blood vessels lined with endothelium and abundant smooth muscle cells lining the muscularis.

The rationale for use of the detergents in the present decellularization protocols was based on the decellularization agent’s effectiveness and destabilization of ECM [13–16]. Detergents that are strongly ionic or hydrophobic or zwitterionic are commonly used.
and may be effective against one or two of the protein-protein interactions or protein-lipid interactions but not all. Therefore, a mixture of detergents will be required for efficient removal of cellular material from the grafts. The use of sodium deoxycholate and SDS in our initial experiments did not give satisfactory results; we therefore decided to try other chemicals and chose DMSO, which is a very common organic solvent used for dissolving lipophilic substances [17]. At the same time, DMSO is known to be cytotoxic at high concentration [18, 19]. We therefore combined the actions of DMSO together with Triton X-100 to achieve a gentle and efficient decellularization protocol for SI. Here, we report for the first time the use of DMSO for decellularization of SI segments. We found that treatment with DMSO/H11001 Triton X-100 did not significantly compromise the tensile strength of the SI walls and successfully preserved the three-dimensional architecture, vasculature, and native matrix composition of the SI tissue.

We characterized the DSI segments with regard to the retention of the major structural extracellular matrices and found that collagen type I, fibronectin, and laminin were still present after decellularization, indicating that the process did not have an effect on the major ECM scaffold composition. It has long been known that growth factors are present in ECM scaffolds [20–22]. In fact, growth factors and glycosaminoglycans have been correlated with in vivo constructive remodeling of biologic scaffolds [23–25]. We therefore analyzed the presence of residual growth factors in the DSI and found that several angiogenic growth factors, such as FGF-2, VEGF, angiopoietin, were still present after the decellularization process. It is likely that the retention of these important growth factors induced better cell attachment and migration and favored the growth of endothelial, epithelial, and smooth muscle cells during the recellularization process.

Elegant work has been performed by Mertsching et al. [26] and Schanz et al. [27] using an acellular porcine small-bowel segment for tissue engineering. They succeeded in generating a decellularized porcine jejunal scaffold with preserved functional tubular structures of the capillary network in the matrix, which is a prerequisite for a functional bioartificial tissue. Other intestinal tissue engineering studies have isolated organoid units from neonatal rat intestine [28, 29]. However, these approaches are limited with regard to human clinical applications. Unless patient's autologous cells are harvested, long-term postoperative immunosuppression will be necessary to prevent rejections. Alternatives such as isolating sufficient numbers of viable stem cells before surgery may be difficult or even impossible to achieve in patients with diseased or damaged intestine. Therefore, the goal of this study was to develop a decellularized SI scaffold conducive to cellular repopulation by a cell source that may one day make SI transplantation clinically feasible. As a possible solution to the problem, here we investigated the use of BM-derived stem cells for tissue engineering small intestine. In previous studies, it has been demonstrated that BM-derived cells contribute to the regeneration of damaged intestinal epithelium as epithelial cells [30, 31], although this is controversial [32].
nevertheless decided to test the hypothesis. We isolated three different populations, epithelial, endothelial, and smooth muscle cells, with relative ease from the BM. The cells could be expanded in vitro without difficulties and found to express the necessary associated cell surface markers. We also succeeded in developing an in-house bioreactor that helped in the efficient recellularization of the SI. Histological analysis of the recellularized scaffolds revealed that the scaffolds were capable of supporting cell attachment and migration. We detected a morphologically intact regenerated mucosa, intact villi, and crypts lined with goblet cells and blood vessels lined with endothelium, indicating that all three cell types had participated in the recellularization process.

Although promising, our study has several limitations. The current model relied on intraluminal decellularization, a strategy that may not be optimal for construction of a successful graft. Decellularization via the vascular arcades would be far more efficient, as shown by Mertsching et al. [26] and Totonelli et al. [33]. We have not addressed important issues of functionality. Functional peristalsis of the luminal contents in TESI will require a neuromuscular layer. Although we successfully demonstrated the presence of actin in the muscularis mucosa, it will be necessary to seed the DSI with other important cells such as nerve cells, mesenchymal cells, and enterocytes. In the future, it might be possible to harvest small quantities of organoid units from the remnant small bowels of patients that could then be expanded in vitro. A complete TESI may then be possible using a mixture of bone marrow- and small bowel-derived cells. Such studies are ongoing in our laboratory.

**CONCLUSION**

This is the first report demonstrating that human BM-derived cells can be efficiently used for TESI. Our data established foundations for in vitro SI tissue engineering with DSI scaffolds and formed the basis for methods that may be necessary for eventual in vivo transplantation for patients with small-bowel syndromes. Extensive recellularization experiments with a range of cell populations will be necessary to assess the full potential of these scaffolds and understand the usefulness of different cell lineages for recellularization strategies. In addition, functional characterization of the TESI will be very important. Findings from this study serve as initial steps toward the development of future engineered SI constructs and will set the stage for preclinical studies in nonhuman primates.

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**Figure 5.** Immunofluorescence staining of the various cell types in tissue-engineered small intestine was further examined by immunofluorescence for the presence of nuclei and other cell markers. (A–C): Immunofluorescence staining with antibodies for the various cell types used for recellularization showed positive staining for CK18, an epithelial cell marker (arrows) (A), CD31, an endothelial cell marker in the blood vessels (arrows) (B), and α-actin (arrows), a smooth muscle marker, which stained the walls of the villi (C). (D–F): 4′,6-Diamidino-2-phenylindole staining of various sections further confirmed the presence of cells in the recellularized tissue segments. Magnification, ×60.

**Table 1.** Enumeration of cell numbers in tissue-engineered small intestine after recellularization with bone marrow cells

| Sample                  | Total number of cells per cm³ | p value |
|-------------------------|-------------------------------|---------|
| Normal SI               | 1.40 ± 0.32                   | <.01    |
| 2 days postrecellularization | 0.46 ± 0.11                   | <.01    |
| 5 days postrecellularization | 0.83 ± 0.07                   | <.05    |
| 7 days postrecellularization | 0.93 ± 0.05                   | >.05    |
| 14 days postrecellularization | 0.93 ± 0.05                   | >.05    |

All p values were calculated in comparison with normal control. Abbreviation: SI, small intestine.
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AUTHOR CONTRIBUTIONS

P.B.P., P.B.C., and H.B.: collection and assembly of data analysis and interpretation, manuscript writing; V.K.K.: collection and assembly of data analysis and interpretation; S.A.: collection and assembly of data, data analysis and interpretation; D.B.: provision of study material; G.H.: provision of patient material, manuscript writing; M.O.: manuscript writing, financial support, final approval of manuscript; S.S.-H.: conception and design, data analysis and interpretation, manuscript writing, financial support.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

The authors indicate no potential conflicts of interest.

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