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A Perfusion Bioreactor for Intestinal Tissue Engineering

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Background. **Short gut syndrome is a devastating clinical problem with limited long-term treatment options. A unique characteristic of the normal intestinal epithelium is its capacity for regeneration and adaptation. Despite this tremendous capacity *in vivo*, one of the major limitations in advancing the understanding of intestinal epithelial differentiation and proliferation has been the difficulty in maintaining primary cultures of normal gut epithelium *in vitro*. A perfusion bioreactor system has been shown to be beneficial in long-term culture and bioengineering of a variety of tissues. The purpose of this study is to design and fabricate a perfusion bioreactor for intestinal tissue engineering.**

Materials and methods. **A perfusion bioreactor is fabricated using specific parameters. Intestinal epithelial organoid units harvested from neonatal rats are seeded onto biodegradable polymer scaffolds and cultured for 2 d in the bioreactor. Cell attachment, viability, and survival are assessed using MTT assay, scanning electron micrograph, and histology.**

Results. **A functional perfusion bioreactor was successfully designed and manufactured. MTT assay and scanning electron micrograph demonstrated successful attachment of viable cells onto the polymer scaffolds. Histology confirmed the survival of intestinal epithelial cells seeded on the scaffolds and cultured in the perfusion bioreactor for 2 days.**

Conclusions. **A functional perfusion bioreactor can be successfully fabricated for the *in-vitro* cultivation of intestinal epithelial cells. With further optimization, the perfusion bioreactor may be a useful in *in-vitro* system for engineering new intestinal tissue.**

Key Words: **regeneration; intestinal tissue engineering; bioreactor; short gut syndrome.**

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INTRODUCTION

Short gut syndrome is a form of intestinal failure, which results from the loss of more than two thirds of the normal jejunal-ileal length. This clinical condition afflicts both adults and children and is characterized by diarrhea, dehydration, malabsorption, and progressive malnutrition. The mainstay of therapy is the use of parenteral nutrition (PN). Although life-saving, PN is extremely expensive and associated with significant morbidity and mortality. Using the principles of tissue engineering, our laboratory has been investigating the fabrication of functional intestinal tissue using isolated cells seeded on synthetic biodegradable polymer scaffolds. Previous studies in small animal models have demonstrated the engraftment and survival of intestinal cells transplanted on tubular polymer scaffolds with regeneration of new tissue with a neomucosa and tissue morphology resembling small intestine [1–4].

One of the unique characteristics of the normal intestinal epithelium is its capacity for renewal and adaptation. Both of these processes are thought to involve intestinal stem cells residing within the crypts of the mucosa. Despite this tremendous capacity *in vivo*, one of the major limitations in advancing the understanding of intestinal epithelial proliferation and differentiation has been the difficulty in maintaining primary cultures of normal gut epithelium. A perfusion bioreactor system has been shown to be beneficial in the long-term culture and bioengineering of a variety of tissues [5–9]. A bioreactor may provide an *in vitro* environment that more closely mimics the normal physiological conditions. The purpose of this study was to design and fabricate a perfusion bioreactor for intestinal tissue engineering.

MATERIALS AND METHODS

Animals

Nonfasted 7-d old neonatal Lewis rats (Charles River Laboratories, Wilmington, MA) were used as intestine donors for isolation of

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intestinal epithelial organoid units. Animals were housed in the Animal Research Facility at the Seattle Children's Research Institute, Seattle, Washington, in accordance with National Institute of Health guidelines for the care of laboratory animals. Animals were maintained in a temperature-regulated environment (24°C) on a 12-h light-dark cycle, housed in cages with soft bedding and microisolator cover.

Polymer Scaffold Fabrication

Microporous 3-D synthetic biodegradable polymer tubes were fabricated from nonwoven sheets of polyglycolic acid (PGA) fibers (fiber diameter 15 μm; mesh thickness 2 mm; bulk density 60 mg/cm^3; porosity >96%; mean pore size 250 μm; Albany International, Albany, NY) as previously described [10]. The tubes were sprayed on the outer surface with a solution of polylactic acid (PLLA, Sigma) (5% wt/vol). The polymer tubes were sterilized with ethylene oxide, then coated with collagen Type 1 (Vitrogen; Cohesion Technologies, Palo Alto, CA). The scaffolds were rinsed with phosphate buffered saline (PBS) and Hanks balanced salt solution (HBSS) prior to cell seeding.

Perfusion Bioreactor Design and Fabrication

The design for the perfusion bioreactor was adapted from the previously reported system [6]. The bioreactor consisted of a multichannel peristaltic pump, culture medium reservoir, oxygenation and gas exchange unit, air trap, and cell-polymer construct housing unit (Fig. 1). The culture medium was pumped at a flow rate of 1.5 mL/min from a 100 mL reservoir through the oxygenation and cell-polymer housing units and recirculated back to the reservoir. The adequacy of the oxygenation and gas exchange unit was assessed by measuring pH, PO_2, and PCO_2 of the culture medium with varying incubator CO_2 concentrations using a Bioprofile 400 Analyzer (NovaBiomed, Waltham, MA). The culture medium analysis was performed in duplicate (CO_2 10% and 15%) or triplicate (CO_2 5%) studies. The bioreactor circuit was modified using three-way stopcocks to allow the addition of a dynamic cell-seeding loop in continuity with the culture loop as well as sampling sites for culture medium analysis. The culture medium was collected distal to the cell-polymer housing unit for analysis. The entire circuit was sterilized by autoclave or ethylene oxide gas and maintained at 37°C with 8% CO_2 supplementation during cell seeding and culture. The bioreactor circuit was primed initially with PBS then with culture medium prior to loading with the cell-polymer constructs.

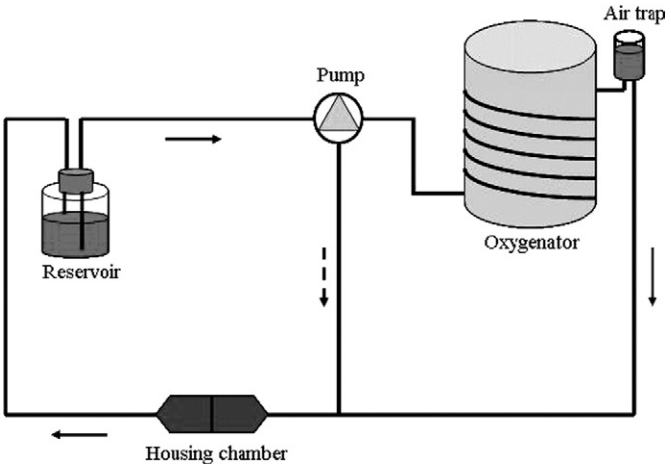

FIG 1. Schematic diagram of the perfusion bioreactor. Dashed arrow depicts the seeding loop. Solid arrows depict the bioreactor loop.

Cell Isolation

Intestinal epithelial organoid units were harvested from nonfasted neonatal Lewis rats using an enzymatic dissociation technique as described by Evans [11]. Briefly, under isoflurane inhalational anesthesia, the entire length of small intestine was harvested, stripped of its mesentery, and placed in HBSS on ice. The intestines were flushed with cold HBSS, split open and cut into 2- to 3-mm fragments. The intestinal fragments were further washed with HBSS, sharply minced into <1 mm^3 pieces, and then enzymatically dissociated (0.1 mg/mL dispase, neutral protease Type 1; Roche Applied Science, and 300 U/mL collagenase type 2, (Worthington) at room temperature on an orbital shaking platform at 80 rpm for 25 min. After mechanical agitation, the intestinal epithelial organoid units were further purified by centrifugation in a solution of Dulbecco's modified Eagle's medium, 2.5% heat-inactivated fetal calf serum, and 2% sorbitol (Sigma Chemical Co., St. Louis, MO) at 300 rpm for 2 min. The resulting pellet was resuspended in culture medium and counted.

Perfusion Bioreactor Culture

The tubular polymer scaffolds were placed into the cell-polymer housing unit under sterile conditions after the circuit had been primed with PBS and culture medium. The intestinal epithelial organoid units were resuspended in culture medium and dynamically seeded through the seeding loop of the bioreactor circuit as previously described [6]. After the seeding period, the three-way stopcocks were turned to exclude the seeding loop, and the reservoir bottle containing the cell suspension was replaced with fresh warmed culture medium. The cell-polymer constructs (n = 4) were cultured under flow conditions for 2 d. Prior to harvest, the cells were incubated in 1% 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT; Sigma Chemical Co., St. Louis, MO) solution for an additional 12 h to assess general metabolic activity and distribution of cells within the scaffolds. The constructs were then harvested and processed for scanning electron microscopy and histology.

RESULTS

Polymer Scaffold Fabrication

Highly porous tubular 3-D polymer scaffolds were fabricated with dimensions of 5 mm o.d., 2 mm i.d., and 10 mm length (Fig. 2). The devices were >95% porous with a mean pore size approximately 250 μm. The outer coating of PLLA provided structural integrity to the polymer tubes as previously reported [10].

Perfusion Bioreactor Design and Fabrication

A functional perfusion bioreactor culture system was successfully designed and manufactured (Fig. 3). Analysis of the culture medium under flow conditions demonstrated adequate oxygenation of the medium within the circuit. There was good correlation between culture medium pH and PCO_2 levels with changes in incubator CO_2 concentration demonstrating proper function of the gas exchange unit (Fig. 4).

Intestinal Epithelial Organoid Unit Isolation

Approximately 1.4 to 1.7 $\times$ 10^5 organoid units were harvested from each 7-d old neonatal Lewis rat. The organoid units were resuspended in culture medium at