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Growth Factors Adsorbed on Polyglycolic Acid Mesh Augment Growth of Bioengineered Intestinal Neomucosa

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Production of tissue engineered small intestine (TESI) has been limited by the relatively large amount of native tissue required to generate neomucosa. The influence of growth factors and three-dimensional (3D) extracellular matrices on TESI has been studied both *in vitro* and *in vivo*, and positive growth effects on tissue mass and differentiation were noted. The present study investigates the impact of single doses of glucagon-like peptide-2 (GLP-2), hepatocyte growth factor (HGF), or holo-transferrin adsorbed onto a polyglycolic (PGA) mesh scaffold using a rat small-intestinal organoid transplant model. In Experiment I, intestinal organoids were seeded onto PGA mesh discs, suspended in either Matrigel ($n = 8$) or a vehicle control ($n = 8$), and implanted into syngenic recipients. In Experiment II, GLP-2 ($n = 8$), HGF ($n = 8$), or transferrin ($n = 8$) were adsorbed onto PGA mesh discs. Intestinal organoids were then suspended in Matrigel and seeded onto each growth factor-loaded PGA disc or onto control discs without growth factors ($n = 12$). In addition, organoids were suspended in vehicle and seeded onto control discs ($n = 12$). All discs were implanted into syngenic recipients. After 4 wk, histologic analysis of the samples revealed significantly greater neomucosal surface area (3.62 ± 0.33 mm^2 *versus* 0.92 ± 0.11 mm^2, $P < 0.0001$) and cyst diameter (2.83 ± 0.14 mm *versus* 2.06 ± 0.07 mm, $P < 0.0001$) in groups treated with Matrigel compared with vehicle controls. The addition of holo-transferrin to the scaffolds further augmented neomucosal surface area (9.11 ± 0.66 mm^2 *versus* 3.01 ± 0.22 mm^2, $P < 0.01$), whereas that of GLP-2 stimulated the formation of increased numbers of cysts (8.88 ± 0.46 *versus* 4.18 ± 0.25, $P < 0.01$). These data suggest that Matrigel and growth factors adsorbed to polymer scaffolds can be used to manipulate the morphology of TESI. Published by Elsevier Inc.

Key Words: **tissue engineering; growth factors; scaffolds; Matrigel; small intestine; neomucosa.**

INTRODUCTION

Massive loss of small bowel leads to short bowel syndrome (SBS) with significant morbidity, including malnutrition, diarrhea, electrolyte abnormalities, profound dehydration, and failure to thrive [1–3]. Current therapeutic options for SBS are limited and include total parenteral nutrition (TPN), bowel lengthening procedures, and small bowel transplantation [3], all of which carry significant morbidity and mortality.

Creation of tissue-engineered small intestine (TESI) may be a means of generating functional intestinal absorptive area, thereby avoiding the complications of current therapeutic options. The smallest transplantable mucosal units known to date are intestinal "organoids." These are clusters of 20–40 cells isolated from intestinal mucosa crypts. The clusters contain the putative intestinal stem cells and other cells that comprise the stem cell niche. Transplantation of these intestinal organoids has been shown to generate TESI tissues that resembles native intestine in both structure and function in rats and mice [4–8]. Furthermore, TESI has been demonstrated in rats to reverse clinical malabsorption syndromes [7, 9]. Recently, we have been the first to report the successful generation of intestinal neomucosa using organoid transplantation in a large animal model [10]. All described models of intestinal organoid transplantation are limited by a major problem

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that must be addressed in order to translate their success into a clinical application. Currently, large numbers of organoids are needed to generate even a modest amount of neomucosa. Attempts at propagating organoids *in vitro* to amplify organoid mass have not yet succeeded even though methods to propagate individual mucosal stem cells have recently been described [11, 12]. By optimizing the conditions that augment neomucosal growth and architecture, we may increase the amount of bioengineered tissue generated from a specific number of seeded cells.

Many epithelial growth factors have been shown to promote intestinal epithelial growth both *in vitro* cell culture systems and *in vivo* native intestinal adaptation models [13–26]. Despite this extensive body of literature, the effects of growth factors on tissue-engineered neomucosa have been rarely studied.

In the 1980s, Thompson *et al.* first described the augmentation of neomucosal growth after exposure to luminal factors and systemically administered urogastrone, a peptide that shares an intestinal receptor with epidermal growth factor (EGF) [27, 28]. This work led to additional studies on the administration of growth factors on TESI. Ramsanahie *et al.* found that systemic administration of glucagon-like-peptide 2 (GLP-2) increased neomucosal growth and sodium-dependent glucose cotransporter-1 (SGLT1) expression in a segment of TESI that was anastomosed to the native intestine in rats [29]. This same group demonstrated cyst viability at 20 wk without growth factor supplementation [30]. However, Lloyd *et al.* demonstrated prolonged neointestinal cyst longevity by directly adding hepatocyte growth factor (HGF) to the organoid suspension [31]. Another study performed by Rocha *et al.* investigated the effect of sustained-release vascular endothelial growth factor (VEGF) on tissue-engineered intestine. By using microsphere-encapsulated VEGF, they found increased vascularity and augmented intestinal epithelial proliferation in their samples of TESI [32].

Matrigel, an extract from the Englebreth-Holm-Swarm tumor, is a commercially available substrate composed of basement membrane proteins and is commonly used as a 3-D extracellular matrix (ECM) both *in vitro* and *in vivo*. It is well known for its supportive effects on epithelial morphogenesis and differentiation of endothelial cells [33]. The use of an artificial extracellular matrix has been demonstrated to enhance enterocyte growth and to induce differentiation of intestinal epithelia [34–37]. Recently, Laschke *et al.* demonstrated that Matrigel contains growth factors that could be used to improve vascularization of tissue engineering bioscaffolds [38].

Many epithelial growth factors including EGF, insulin-like-growth factor 1 (IGF-1), GLP-2, HGF, and transferrin have been shown to promote intestinal epithelial cell growth in both *in vitro* cell culture systems and *in vivo* models [14–17, 20, 25].

However, for many growth factors, well-documented dose-response studies or investigations comparing different modes of administration and their effects on mucosal growth do not exist. In the tissue engineering literature, growth factors are given systemically, added to the organoids at implantation, or locally released from microspheres. In contrast, adsorption of growth factors to the scaffold material has never been investigated. The main aims of this study were (1) to demonstrate that mucosal growth factors that are adsorbed to PGA scaffolds have significant biological effects and (2) to search for evidence of a synergistic effect between growth factors and Matrigel. We empirically selected three well-established growth promoters of intestinal mucosa in concentrations that we estimated to be biologically effective based on the available evidence in the literature.

GLP-2, a trophic hormone, plays an important role in the regulation of cell proliferation, apoptosis, nutrient absorption, and motility by up-regulating SGLT-1 [29, 39–41]. Its intestinotrophic properties and effects on mucosal absorption have been well documented; consequently, the use of GLP-2 has been translated into a potential therapy for inflammatory bowel disease and SBS [20, 42–45]. Results from clinical studies have revealed increased small intestinal villus height, crypt depth, and mitotic index [46, 47].

HGF was first identified in 1984 as a stimulant for hepatocyte DNA synthesis in rats [48]. However, after further investigation, its expression was detected outside of the liver in several organs, including the small intestine [49, 50]. In cell culture models, HGF was found to stimulate the proliferation and migration of intestinal epithelial cells and to promote tubular cell formation and growth [33]. *In vivo* studies using HGF have revealed an increase in intestinal epithelial cell mass and gut function [23, 51].

Transferrin is a carrier glycoprotein that carries ferric iron in the plasma, lymph, and cerebrospinal fluid [52]. It has been suggested that the transferrin receptor supplies iron to neonatal epithelial cells; thus the use of transferrin in intestinal cell culture has been routinely adopted to induce epithelial proliferation [14]. Transferrin receptors have been identified within small intestinal crypts, and are predominantly localized on the basal, lateral, and intracellular membranes of intestinal epithelial cells [53]. Studies in rats have demonstrated increased concentrations of transferrin receptors in actively dividing cells of the crypts, with lower levels found at the well-differentiated villus tips [54]. This correlation between transferrin receptor distribution and intestinal cell proliferation and migration was also supported by the finding of diminishing transferrin-bound iron uptake along the crypt-villus axis [55].