Basic fibroblast growth factor-releasing polycaprolactone scaffolds for intestinal smooth muscle regeneration

Min Lee[1], Benjamin M. Wu[1,2], and James C.Y. Dunn[1,3]
[1]Department of Bioengineering, [2]Department of Materials Science and Engineering, [3]Department of Surgery
University of California, Los Angeles, California 90095

Introduction

Intestinal tissue engineering is a promising therapy for patients with inadequate small bowel length and absorptive surface area [1]. Previous studies, however, failed to regenerate intestinal muscle layer, which is essential for functional peristalsis [2]. Basic fibroblast growth factor (bFGF) has been shown to induce the proliferation of endothelial cells, fibroblasts, and smooth muscle cells [3]. It also has been shown to promote angiogenesis and to enhance wound healing and tissue repair [4]. In this study, we developed bFGF-releasing polycaprolactone (PCL) scaffolds, and their ability to enhance smooth muscle growth was evaluated in vivo. To evaluate the efficiency of the delivery system, two methods of incorporation was assessed: incorporating bFGF into the collagen-coating or into microspheres.

Materials and Methods

PCL scaffolds were fabricated by the solvent casting and particulate leaching technique. bFGF (1 or 10 ug) was mixed with neutralized 0.025% collagen solution and coated onto PCL scaffolds. bFGF-loaded PLGA microspheres were prepared by a double emulsion solvent evaporation method. PLGA microspheres containing bFGF (1 ug) were dispersed in neutralized 0.025% collagen solution and the mixture was coated onto PCL scaffolds. Smooth muscle cells (SMC) were isolated from small intestine of GFP transgenic neonatal Lewis rats by enzymatic digestion. SMC were seeded onto bFGF-loaded PCL scaffolds and implanted into the omentum of adult Lewis rats. Constructs were harvested at 0.5, 2, 4, and 8 weeks after implantation, and GFP-positive cells were detected from tissue sections. Expression of alpha-smooth muscle actin was examined by double-immunostaining.

Results and Discussion

GFP signals in the harvested constructs were observed by fluorescence microscopy. More intense fluorescent signals were found in constructs that released bFGF. Histological sections of scaffolds showed the growth of implanted smooth muscle cell during the first 2 weeks, but the number of implanted cells decreased at 4 weeks after implantation in the absence of bFGF (Fig. 1). More GFP-positive cells were observed with 10 ug of bFGF releasing scaffolds after 4 weeks of implantation, and these cells were also positively stained for α-SMA (Fig. 2). To get a sustained release, 1 ug of bFGF was encapsulated into PLGA microspheres . At 4 weeks after implantation, a larger number of GFP-positive cells was found on the scaffolds incorporating bFGF-loaded microspheres compared with 1 ug of bFGF loaded into collagen-coating of PCL scaffolds (Fig. 3).

Conclusions

bFGF-releasing PCL scaffolds enhanced survival of implanted intestinal smooth muscle cells. This delivery system may be useful in the regeneration of small intestinal muscle layer.

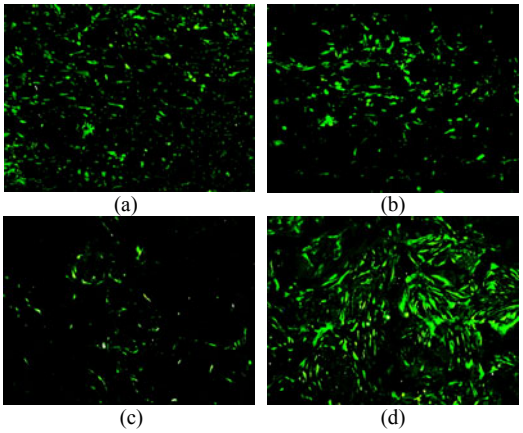

Figure 1. Immunohistochemical detection of GFP from cross sections of the harvested constructs 4 days (a,b) or 4 weeks (c,d) after implantation in the absence (a,c) or presence (b,d) of bFGF (10 ug).

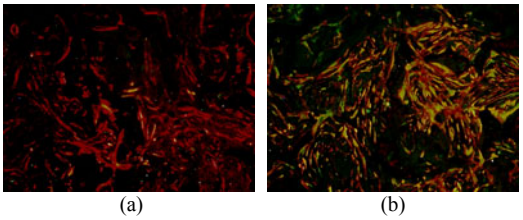

Figure 2. Immunohistochemical detection of α-SMA from cross sections of the harvested constructs 4 weeks after implantation in the absence (a) or presence (b) of bFGF (10 ug).

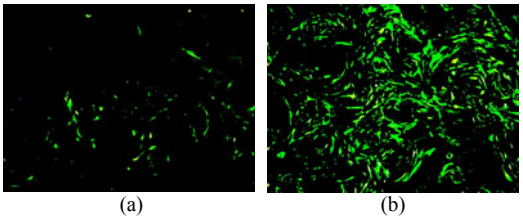

Figure 3. Immunohistochemical detection of GFP from cross sections of the harvested constructs releasing bFGF (1 ug) from collagen coating (a) or microspheres (b) of scaffolds 4 weeks after implantation

References

1. Flavio G et al. J Surg Res. 2004;120:320-325
2. Hori Y et al. J Surg Res. 2002;102:156-160
3. Baird A et al. Br Med Bull. 1989;45:438-452
4. Tsuboi R et al. J Exp Med. 1990;172:245-251