available at www.sciencedirect.com
journal homepage: www.europeanurology.com

European Association of Urology

Reconstructive Urology

Laparoscopy in Ureteral Engineering: A Feasibility Study

Hervé Baumert [a,b,*], *Mehrak Hekmati* [b], *Irene Dunia* [c], *Dhouha Mansouri* [d], *Walid Massoud* [a], *Vincent Molinié* [d], *E. Lucio Benedetti* [c], *Bernard Malavaud* [e]

[a] *Department of Urology, Paris Saint Joseph Hospital Trust, Paris, France*
[b] *IMM Research, Paris, France*
[c] *Biologie Cellulaire, Institut Jacques Monod, CNRS, Université Paris 6, Paris, France*
[d] *Department of Pathology, Paris Saint Joseph Hospital Trust, Paris, France*
[e] *Department of Urology, University Hospital, Toulouse, France*

Article info

Article history:
Accepted January 4, 2008
Published online ahead of print on January 14, 2008

Keywords:
Tissue engineering
Ureter
Cell culture
Scaffold
Omentum
Laparoscopy

Abstract

Objective: We recently bioengineered a ureter substitute from a seeded scaffold implanted by open surgery in the omentum. In view of the development of laparoscopy in the treatment of benign conditions of the ureter, obtaining a ureter substitute by minimally invasive techniques would be a desirable objective. However, conflicting results about the biological impact of carbon dioxide insufflation on the microcirculation of intra-abdominal organs prompted us to investigate first whether the results obtained by open surgery, in terms of vascular supply and maturation, could be reproduced laparoscopically.

Materials and methods: Bladder full-thickness tissue was harvested laparoscopically from three pigs for urothelial and smooth muscle cell primary cultures subsequently used to seed a small intestinal submucosa (SIS) matrix. After 2 wk, the in vitro seeded constructs were shaped around silicone drains and transferred laparoscopically into the abdomen for omental maturation. Three weeks later, the constructs were harvested for histological, immunohistochemical, and electron microscopic analysis.

Results: The laparoscopic procedures were performed successfully in all animals. After omental maturation, the constructs were vascularized and comprised of a well-differentiated multilayered urothelium with umbrella cells, over connective tissue and smooth muscle cells, with no evidence of fibrosis or inflammation. Electron microscopic analysis showed characteristics of a terminally differentiated urothelium.

Conclusion: As shown by conventional microscopy, immunochemistry, and electron microscopy, carbon dioxide insufflation does not impact cell growth and differentiation. These findings validate the laparoscopic approach for omental maturation of ureter substitutes.

* Corresponding author. Department of Urology, Hôpital Saint Joseph, 185 Rue Losserand, 75014 Paris, France. Tel. +33 662 874 791; Fax: +33 144 123 245.
E-mail address: baumertherve@yahoo.fr (H. Baumert).

0302-2838/$ – see back matter doi:10.1016/j.eururo.2008.01.012

1. Introduction

We recently proposed to take advantage of the omental blood supply to circumvent the ischemic damage driven by the in vivo transfer of ex vivo generated substitutes of the urinary tract and introduced the concept of ''omental bioreactor'' [1,2], where the construct was implanted in the greater omentum by an open approach. The recent introduction of the laparoscopic approach in reconstructive surgery for omental harvest [3,4] prompted us to consider it for ureter construct maturation. The laparoscopic approach would have the potential to avoid the specific complications of the open route in omental harvest [5], such as hernia and wound infection, and would reduce peritoneal adhesions [6] that could hinder secondary ureter replacement with the matured construct. However, conflicting results about the biological impact of carbon dioxide insufflation on the microcirculation of intra-abdominal organs prompted us to investigate whether the results obtained by open surgery, in terms of vascular supply and maturation, could be reproduced laparoscopically [2].

2. Materials and methods

After Animal Care Committee approval, all animal procedures were conducted according to the Institute of Laboratory Animal Research guide for the care and use of laboratory animals [7]. We used three large-white female pigs weighing between 50 to 60 kg (GAEC Beets, Saint Germain des Prés, France). The procedure comprised four separate steps, laparoscopic partial cystectomy, primary cultures, sandwich coculture of the small intestinal submucosa (SIS) scaffold, and laparoscopic transfer of the construct for omental maturation, before sacrifice and histology.

2.1. *Laparoscopic tissue harvesting*

Three ports were inserted in the supine position, one 10-mm port for the optic in the midline, one 12-mm port on the right lower flank to permit endo-GIA insertion (Universal Endo GIA 12/45, Autosuture, Elancourt, France), and one 5-mm port in the left lower flank (Fig. 1A). The bladder dome was identified and an approximately 3 × 2-cm oblong full-thickness specimen was harvested (Fig. 1B–D) and retrieved through the 12-mm port. After resection of the stapling line, the specimen was immediately immersed in DMEM culture medium before further processing.

2.2. *Autologous urothelium and smooth muscle cells primary cultures*

Primary cultures were produced as described previously [1,2]. Briefly, the urothelial layer was gently scraped away from the bladder wall (Fig. 1E) and incubated in serum-free keratinocyte growth medium (keratinocyte SFM, Invitrogen, Cergy Pontoise, France) supplemented with epidermal growth factor and pituitary extract.

Smooth muscle fragments digested with collagenase IV (200 units/ml; Sigma Aldrich, St. Louis, Missouri, USA) were cultured in a 1:1 mixture of DMEM and Ham's F12 supplemented with 10% foetal calf serum (GIBCO, Grand Island, NY, USA). The medium was replaced every 2 days, and cells were passaged when confluent.

2.3. *Cell seeding on small intestinal submucosa scaffold*

Three confluent 25-cm^2 plates of each cell type were processed for seeding onto one 7 × 5-cm SIS monolayer patch (Cook Medical, Limerick, Ireland), with approximately 2×10^5 cells of each type per square centimetre. The urothelial cells were first seeded on the rough aspect of the graft. When the urothelial layer had covered more than 80% of the rough aspect of the patch (2 days), it was turned over and seeded with smooth muscle cells on its smooth aspect, as previously described by Zhang et al. as a ''sandwich coculture'' [8]. Patches were then kept in an incubator for another 6 days before laparoscopic transfer. The production of cell cultures and seeding of the scaffold required 2 wk.

2.4. *Laparoscopic transfer of the construct for omental maturation*

The seeded scaffold was shaped around a 20-Fr silicone drain (5 cm long), with the urothelial layer positioned towards the drain. Each end of the seeded scaffold was fixed to the silicone drain with transfixion sutures to avoid retraction during the in vivo maturation.

The transfer was performed in the pig used for primary cultures. In the supine position, three ports were positioned (two 10-mm ports, one 5-mm port) in the right flank (Fig. 2A). The abdomen was insufflated to 12 mmHg with carbon dioxide, and the omentum was identified and mobilized to the right flank. The pneumoperitoneum was exsufflated before inserting the scaffold through the medial 10-mm port to avoid the cultured cells from being flushed away by carbon dioxide backflow when opening the port valve. Insufflation was then resumed, and care was taken not to traumatize the construct during its transfer to the omentum, which was wrapped and fixed around the construct by three interrupted 3/0 prolene sutures (Fig. 2B–E). Three weeks later, the same port locations were used to evaluate the aspect of the abdominal cavity and the construct before open harvest for histological studies.

2.5. *Histological and immunohistochemical analyses*

After resection, each specimen was fixed in 10% formalin solution and serially sectioned and submitted in entirety. For histological analysis, 5-μm tissue sections from paraffin-embedded blocks were obtained for routine haematoxylin and eosin (H&E) staining.