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In vitro reconstruction of a tissue-engineered endothelialized bladder from a single porcine biopsy

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Abstract *Objective:* Augmentation of the urinary bladder using a tissue-engineered approach with autologous cells is a very promising technique. To prevent risks of necrosis after transplantation, the graft vascularization process could be markedly enhanced by incorporation of autologous endothelial cells in the tissue-engineered organ. The purpose of this study was to develop a separation technique to extract four bladder cell types from the same biopsy, and to prepare an endothelialized reconstructed bladder.
Materials and methods: Fibroblasts, smooth muscle cells (SMC), urothelial cells (UC) and endothelial cells (EC) were extracted from a small porcine bladder biopsy. The SMC, fibroblasts and EC were seeded on the top of the sponge and cultured for 10 days. Then, the UC were seeded on top of these cells for 15 additional days to produce a three-dimensional bladder wall.
Results: The UC and EC extracts from a single porcine biopsy were 97.2 $\pm$ 0.6% keratin 8/18-positive and 97.7 $\pm$ 0.3% PECAM-1-positive pure cells, respectively, as assessed by flow cytometry. The SMC could not be dissociated from fibroblasts, and were present as 37 $\pm$ 0.5% desmin-positive cells. UC differentiated into a urothelium characterized by umbrella cells and a laminin-positive basal membrane. The EC reorganized in the matrix to form PECAM-1-positive capillary-like tubes.

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Conclusion: This new model of tissue-engineered bladder has the main advantages of being at least 2 mm thick, autologous, and able to promote the formation of capillary-like tubes. It could be a promising alternative to the use of gastrointestinal segments to improve bladder capacity.

Introduction

For many years, gastrointestinal segments have been the tissue of choice to perform vesical augmentations. However, these surgical techniques sometimes cause severe complications such as infection, metabolic disturbances, malabsorption, perforation, increased mucus production and malignancy [1]. A promising alternative could be the reconstruction of an autologous tissue with bladder-like functional properties by tissue engineering. The use of acellular biomaterials of various compositions did not give satisfactory results. Some success has been obtained using a polymer on which two cell types, urothelial and smooth muscle bladder cells, were grown [2]. Nevertheless, the clinical application of a thick autologous tissue-engineered bladder is still limited by the slow process of complete graft vascularization, which is not fast enough to prevent necrosis or fibrosis of the central zone [3].

A similar limitation was observed after transplantation of tissue-engineered skin onto wounds. To overcome this problem, our group developed an innovative skin model, a reconstructed endothelialized skin. When human endothelial cells and fibroblasts were seeded on a biopolymer, they spontaneously formed capillary-like structures that organized into a complex three-dimensional network [4,5]. After transplantation onto mice, an inosculation process was demonstrated between this capillary network and the host's vasculature. In less than 4 days the host's capillaries were connected to the capillary-like structures in the biopolymer, while it took 15 days for the conventional neovascularization process to occur in the skin model without endothelial cells [6,7]. A similar endothelialization of a tissue-engineered bladder could achieve a comparable acceleration in the tissue's vascularization and thus help to prevent necrosis. To prepare an autologous endothelialized reconstructed bladder, we need to isolate the cells. First, urothelial cells (UC) are needed to ensure the impermeability of the tissue [8]. Secondly, smooth muscle cells (SMC) and fibroblasts are essential to give the tissue mechanical resistance and the consistency to be grafted without tearing [9]. Finally, endothelial cells (EC) are necessary to support the reconstruction of a new capillary-like network to promote rapid vascularization [4]. The extraction of UC and muscle cells (SMC and fibroblasts) is well documented [10], but extraction of EC has never before been successfully performed on this tissue [11]. In addition, these cell types must be purified from the same bladder biopsy in order to produce a completely autologous tissue. This requirement greatly increases the difficulty of the task.

This study has been performed on pigs because this animal is known to be well suited to the study of vesical regeneration and because the healing of several porcine tissues resembles the regeneration observed in humans [12]. Moreover, the surgical procedures required and size of the bladder are very similar to those in humans. After the extraction of the required cell types from a small porcine bladder biopsy, we succeeded in obtaining a tissue-engineered endothelialized autologous bladder.

Materials and methods

Bladder

The different cell types present in the bladder were extracted from a small porcine bladder biopsy, which was obtained sterilely from a slaughterhouse. The bladder was washed in phosphate-buffered saline (PBS) containing 100 U/ml penicillin, 25 μg/ml gentamicin (Sigma, Oakville, ON, Canada) and 0.5 μg/ml fungizone (Squibb, Canada). A thin strip of 5 cm × 1 cm was sterilely cut from the bladder and dissociated into two pieces longitudinally (sideways); the exterior part which does not contain the urothelium was kept in PBS with antibiotics and reserved for the extraction of smooth muscle and endothelial cells.

Extraction of urothelial cells

The inner part of the thin strip, which contains the urothelium, was cut into small pieces and incubated overnight at 4 °C in 10 ml of a solution containing 500 μg/ml thermolysin (Sigma) in a