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Original Article: Laboratory Investigation

Ex vivo construction of a novel model of bioengineered bladder mucosa: A preliminary study

Boris D Jaimes-Parra,[1,2,3] Francisco Valle-Díaz de la Guardia,[4] Miguel Á Arrabal-Polo,[5] Bernardo Herrera-Imbroda,[6] María F Lara,[6] Francisco-Javier Machuca-Santa-Cruz,[6] Antonio Campos,[1,2] Miguel Alaminos,[1,2] Pascual V Crespo[1,2] and Ingrid Garzón[1,2]

[1]Department of Histology (Tissue Engineering Group), University of Granada, [2]Biomedical Research Institute, University of Granada, [3]PhD Program in Biomedicine, University of Granada, Granada, [4]Department of Urology, Hospital San Juan de la Cruz, Jaen, [5]Urology Department, Hospital La Inmaculada, Almeria, and [6]Division of Urology and Urology Unit Research, Virgen de la Victoria Hospital, Malaga, Spain

Abbreviations & Acronyms
BMS-14 = human bladder mucosa 3-D substitutes at day 14 of development
BMS-7 = human bladder mucosa 3-D substitutes at day 7 of development
CTR = human bladder control samples
TEM = transmission electron microscopy

Correspondence: Ingrid Garzón D.D.S., Ph.D., Department of Histology, Faculty of Medicine, University of Granada, Avenida de Madrid 11, E18012 Granada, Spain. Email: igarzon@ugr.es

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Objective: To generate and to evaluate *ex vivo* a novel model of bioengineered human bladder mucosa based on fibrin-agarose biomaterials.
Methods: We first established primary cultures of stromal and epithelial cells from small biopsies of the human bladder using enzymatic digestion and selective cell culture media. Then, a bioengineered substitute of the bladder lamina propria was generated using cultured stromal cells and fibrin-agarose scaffolds, and the epithelial cells were then subcultured on top to generate a complete bladder mucosa substitute. Evaluation of this substitute was carried out by cell viability and histological analyses, immunohistochemistry for key epithelial markers and transmission electron microscopy.
Results: The results show a well-configured stroma substitute with a single-layer epithelium on top. This substitute was equivalent to the control bladder mucosa. After 7 days of *ex vivo* development, the epithelial layer expressed pancytokeratin, and cytokeratins CK7, CK8 and CK13, as well as filaggrin and ZO-2, with negative expression of CK4 and uroplakin III. A reduction of the expression of CK8, filaggrin and ZO-2 was found at day 14 of development. An immature basement membrane was detected at the transition between the epithelium and the lamina propria, with the presence of epithelial hemidesmosomes, interdigitations and immature desmosomes.
Conclusions: The present results suggest that this model of bioengineered human bladder mucosa shared structural and functional similarities with the native bladder mucosa, although the epithelial cells were not fully differentiated *ex vivo*. We hypothesize that this bladder mucosa substitute could have potential clinical usefulness after *in vivo* implantation.

Key words: bladder mucosa, cytokeratins, fibrin-agarose, tissue engineering.

Introduction

The human urinary bladder consists of an epithelium on the lumen surrounded by a collagen-rich connective tissue and muscle layer.[1] The epithelial layer or urothelium plays an important barrier role, and prevents the urine from sweeping into the body cavity. Urothelium consists of basal cells (the most undifferentiated cells), intermediate cells and umbrella cells (the most superficial and differentiated type of urothelial cells),[1] and uroplakins and tight junctions between cells are responsible to ensure the impermeability of the bladder epithelium.[2,3] Urothelial cells express several epithelial markers, such as cytokeratins, and uroplakins are considered as terminal markers of urothelial differentiation.

Surgical repair of the urinary bladder is required during the management of bladders affected by malignances, trauma, spinal cord injuries and congenital pediatric disorders, such as bladder exstrophy, and many of these conditions require the use of non-urological tissues for bladder repair or augmentation.[4] One of the most commonly used organs for bladder reconstruction is the human intestine. However, the numerous side-effects and complications derived from these techniques, and the suboptimal results often obtained, make necessary the search of alternative tissue sources for bladder repair.[5]