PRIZE AWARD: Best Basic Science Abstract

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DEVELOPMENT OF A NEW MODEL FOR STUDYING UROTHELIAL-AFFERENT CROSS-TALK

Hypothesis / aims of study

The epithelial lining of the bladder, the urothelium was traditionally viewed as a passive barrier; however it is now apparent that it is a much more dynamic structure which plays an active role in bladder physiology. The urothelium possesses an array of ion channels and receptors, normally associated with neurotransmission and it releases a host of signalling molecules (such as ATP and NO) in response to chemical and mechanical stimulation. Together with the close proximity of sensory nerve terminals, the urothelium is in the ideal location to both monitor and influence neural afferent activity. This has led to the concept that bladder afferent transmission is a multifactorial process involving cross-talk between the urothelium and the sensory nerves. There have been many studies examining the physiological properties of both the urothelium and the sensory nerves; however there is currently no model available which allows the cellular communication between the urothelium and the afferent terminal to be directly examined.

Microfluidic chambers could provide a unique opportunity for studying this interaction. Microfluidic chambers contain two isolated compartments separated by micro-channels. The microchannels allow axonal growth of neurons from one compartment into a second compartment enabling cell- terminal communication between two cell populations to occur (either by direct interaction or paracrine signalling). The aim of this project was to develop a model for directly studying urothelial-afferent cross talk using the microfluidic chamber system and to examine the effect of the urothelium on neurite outgrowth.

Study design, materials and methods

Urothelial cells and DRG neurons from 12 week old adult male mice were used in this study. Animals were sacrificed according to UK laws and regulations.

For collection and dissociation of urothelial cells, bladders were excised, dissected and pinned into a sterile sylguard coated petri dish with the urothelium on the top and incubated at room temperature for 2-3 hours with MEM media containing 2.5mg/ml dispase, 15mM HEPES and 1:100 penicillin/streptomycin. Cells were collected by gentle scraping under the microscope and dissociated in 0.025% trypsin EDTA at 37^{0}C for 5-15 minutes. Cells were centrifuged at 1500 RMP for 5 minutes and resuspended in keratinocyte media (5µl, KSFM). For collection and dissociation of DRG neurons, skin muscle and surrounding tissue was removed from the posterior aspect of the vertebra. Vertebral bone was cut away exposing the spinal cord and the DRGs on both sides. DRGs were collected and placed into ice cold DMEM-F12 media containing 1:100 penicillin/streptomycin. DRGs were transferred to a pre-warmed enzyme mix containing dispase II (0.1mg/ml) and collagenase IX (0.06mg/ml) and incubated for 1 hour at 37ºC. Cells were triturated vigorously, layered on to 4mls of media containing BSA (15%) and centrifuged at 1500 RPM for 10 minutes. Cells were washed and resuspended in KSFM media (5µl)

Microfluidic chambers were prepared following manufactures directions. 60mm diameter cover glasses (VWR) were coated with collagen IV and left to dry. Collagen was ashed and the microfluidic chamber was placed on top and sealed with vacuum grease around the sides. 5µl DRG cell suspension was plated in one chamber and 5µl urothelial cell suspension was plated in the other (figure1 A and B). Cells were incubated at 37ºC for 2 hours and then flooded with KSFM containing 1:100 penicillin/streptomycin and 100ng/ml NGF. Cells were maintained for 6 days at 37ºC. To examine the effect of urothelial cells on DRG neurite outgrowth, cells were fixed in 4% PFA, permeabilise with saponin. Neurofilaments were stained with a neurofilament antibody (shown in red) and urothelial cells were stained with phallodin-FITC (in green).

Table 1 Culture conditions tested

Parameters	Outcome	
	DRGs	Urothelial
Coverglass coating		
Poly orthhine 0.5mg/ml	✓	×
Collagen IV 0.5mg/ml	✓	✓
Collagen IV 1m/ml	✓	✓
Collagen/poly orthine mix	✓	×
Culture conditions		
NGF 20ng/ml	✓	✓
NGF 100ng/ml	✓	✓
DMEM-F12	✓	×
KSFM	✓	✓
DMEM-F12 and KSFM mix	✓	×
Dissociation procedure		
Percoll step	×	-
BSA (15% and media)	✓	-

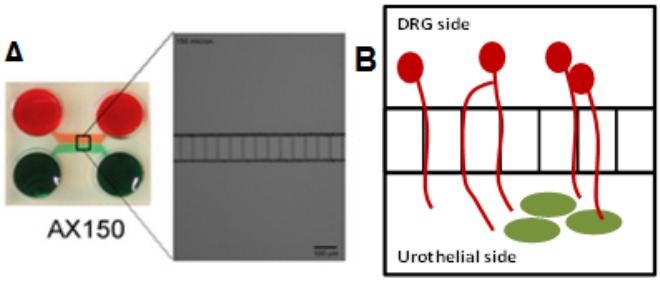

Figure 1- Microfluidic chambers

A) Microfluidic chamber, highlighting the isolation of the two components. B) Schematic diagram showing the co-culture of DRGs and urothelial cells. In these experiments DRG neurons were cultured in one chamber and urothelial cells were cultured in the other chamber. After 6 days in culture the DRGs sent neural projections through the microchannels to the urothelial chamber.

Results

A number of protocols and methodologies were tested to identify the optimum conditions for the dissociation and co-culture of urothelial cells and DRGs in microfluidic chambers (table 1). DRG neurons when plated alone exhibited little axonal branching through the microchannels compared to when DRG and urothelial cells were co-cultured (2.5 ± 2.5 vs 54.5 ± 38.5 branches through the microchannels (figure 2C). Representative images are shown in figure 2A and B. In co-culture experiments afferent terminals were seen in close proximity to urothelial cells suggesting local communication between the two cell types (figure 2D).

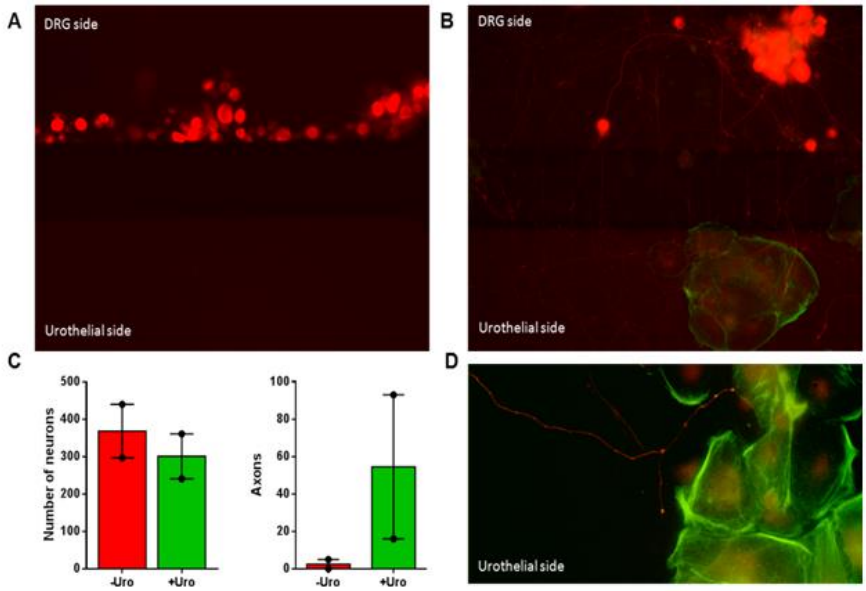

Figure 2 Development of a model to study urothelial-afferent cross-talk

A) Representative image of a monoculture (DRGs only - shown in red, 6 days). B) Representative image of a co-culture (DRGs - shown in red and urothelial cells - shown in green, 6 days). C) Data showing that the same numbers of neurons were plated in both monocultures and co-cultures, but that in co-culture experiments the number of projections through the microchannels was dramatically increased. D) Representative image showing connections between urothelial cells (in green) and a neuron (in red).

Interpretation of results

This model will have unique benefits for studying direct cell-to-cell interactions in future studies. Interestingly we found that the presence of urothelial cells significantly increased neurite outgrowth from the DRGs suggesting that the urothelium releases a diffusible neurotrophic factor. Many neurotrophic factors have been shown to be released by the urothelium and future studies will be directed at identifying which factor(s) are involved.

Concluding message In this study we have successfully developed a novel model to study direct urothelial afferent communication, future studies are required to characterise the nature of urothelial afferent interactions using calcium imaging and transmitter release assays

Disclosures

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