
THE UROTHELIUM CELL CULTURE ON THE STARCH SCAFFOLD

TOMASZ DREWΑ*

Department of Tissue Engineering, Copernicus University, Tissue Engineering Department, Chair of Medical Biology 24 Karlowicza Str., 85-092 Bydgoszcz, Poland

Abstract: The etiology of the interstitial cystitis (IC) is believed to be a dysfunction of the bladder glycosaminoglycan (GAG) protective layer of the urothelial cells. Management of interstitial cystitis (IC) is pure empirical. The aim of this study was to establish primary rabbit urothelial cells (PRUCC) affinity to complex sugar scaffold in vitro. The primary rabbit urothelial cells culture (PRUCC) was set up. The cells were cultured in Jouan IG 150 incubator at temperature of 37°C and humidified atmosphere containing 5% of CO₂. The multiwell culture plates were covered with potato starch. PRUCC was seeded on the starch scaffold. On the 7th day the starch was removed. Culture analysis and photo documentation was done under inverted microscope. There was no disruption of the monolayer under the starch and the cells morphology was comparable to the rabbit urothelial cells in culture. No inhibitory effect on the PRUCC was observed. The colonies of the urothelial cells were formed over the starch grains. The author postulate that urothelial cells have affinity to the complex sugar compounds. There is strong evidence that sugar complex compounds could be used in the treatment of the IC.

Keywords: urothelium, in vitro model, complex sugar, interstitial cystitis

The etiology of the interstitial cystitis (IC) is unknown. Management of interstitial cystitis is purely empirical and experimental. No pathogens or triggering factors were found, but the most consistent findings involve some dysfunction of the bladder glycosaminoglycan (GAG) protective layer and a high number of activated bladder mast cells. There is no effective therapy even through intravesical administration of dimethylsulfoxide (DMSO) or oral pentosanpolysulfate (PPS) have had variable success (1,2). There are only a few papers connected to the in vitro experimental management of IC (3).

The aim of this study was to establish primary rabbit urothelial cells (PRUCC) affinity to complex sugar scaffold in vitro.

MATERIAL AND METHODS

Cells

The primary rabbit urothelial cells culture (PRUCC) was set up from a wall fragment taken from a rabbit bladder. For this experiment a young male rabbit weighing 1.5 kg was used. The animal had free access to food and water and maintained 24-h rhythm of day and night. After the animal was sacrificed with ketamine (25 mg/kg of body weight i.m.) and scoline (50 mg/kg of body weight) a well fragment size 5x5 mm from the rabbit’s bladder was taken. The tissue was then placed in PBS (Phosphate Buffered Saline, Biomed-Lublin, Poland), rinsed several times and then cut into 1 mm³ fragments. The fragments suspended in cultured medium were incubated for two hours in enzymatic bath containing 200 U/mL collagenase. The multiwell culture plates were covered with potato starch. PRUCC was seeded on the starch scaffold. On the 7th day the starch was removed. Culture analysis and photo documentation was done under inverted microscope. There was no disruption of the monolayer under the starch and the cells morphology was comparable to the rabbit urothelial cells in culture. No inhibitory effect on the PRUCC was observed. The colonies of the urothelial cells were formed over the starch grains. The author postulate that urothelial cells have affinity to the complex sugar compounds. There is strong evidence that sugar complex compounds could be used in the treatment of the IC.

Sugar (starch) scaffold

The multi-well Corning culture plates (Costar, MA, USA) with growth surface of 1.7 cm²/well were covered with insoluble potato starch (Sigma, Germany). On the 2nd day, when the sedimentation
process of the starch was finished the PRUCC was seeded on the starch scaffold. The density of seeded cells was ca. $1.5\times10^4/cm^2$. On the 7th day the starch was removed. Culture morphology analysis after starch removing was done under an inverted microscope (Nikon Eclipse TS100, Japan) equipped with digital camera (Nikon E5400, Japan).

RESULTS

Potato starch scaffold has built the flat layer on the bottom of the multiwell plate. The PRUCC cells sank into the starch layer and left the regular pattern well visible under inverted microscope (Figure 1). On the 7th day when the starch scaffold was removed the monolayer of the rabbit urothelial cells could be observed. There were no disruption of the monolayer culture under the starch and the cells morphology was comparable to the rabbit urothelial cells in culture (4). No inhibitory effect on the PRUCC was observed. The remaining yellowish starch grains were visible over the urothelial layer (Figure 2).

After two weeks of culture under the starch scaffold three dimensional (3D) colonies of the urothelial cells were observed in several places. Those colonies were formed over the starch grains. It was obvious that the cells were actively joined to the starch scaffold and spontaneously built the 3D structures (Figure 3).

DISCUSSION

Potato starch was chosen for the experiment, because it was not an energy supplement in the cell culture. The starch was stable in the medium solution during the whole experiment. One of the theories believed that the etiology of IC is connected with molecular event, which have taken place within the urothelium layer or strictly in the cellular membranes of the urothelial cells. The proliferation rate of explanted bladder epithelial cells from patients with IC was significantly lower than that of control cells, indicating an intrinsic abnormality in IC cell proliferation. It was shown on the population of IC patients that membrane permeability was disrupted (5). Information on the nature of the glycoconjugates of the bladder epithelium and lectins that may interact with the exogenous instilled glycoconjugates is very limited (6,7). There was a suspicion that re-establishing of the glicocalix of the urothelial layer was one of the key points of IC management. In this study it was proven that urothelial cells had affinity to sugar particles in in vitro experiment. The starch was not toxic to urothelial cells. This observation is supported by previous experiments, where urothelial cells were exposed to silicone, latex and lidocaine (4,8). The urothelial monolayer was not disrupted by starch scaffold after one week of in vitro culture. Even more, after two week of culture it

Figure 1. The potato starch scaffold was formed over the bottom of multiwell plates. The regular pattern of sinking urothelial cells can be noticed (white arrow, magnif. 30x, inverted microscope).

Figure 2. One week of the PRUCC culture (magnif.100x, inverted microscope). Remaining yellowish starch particles can be visible (white arrow). The urothelial culture (background) is growing under the starch grains.

Figure 3. Two weeks of PRUCC culture on the starch scaffold (magnif. 200x, inverted microscope). 3D colony of the PRUCC can be visible (black arrow). The urothelial cell (white arrow) has been actively incorporated into the growing colony.
was observed some kind of „chemotactic action” of the starch grains. The urothelial cells moved to the starch seeds and started to grow on the starch scaffold. The author postulates that urothelial cells have high affinity to the complex sugar compounds. The rebuilding of the glicocalix layer was postulated in several clinical trials concerning intravesical IC management (9,10). These observations support a hope that sugar complex compounds could be used in the intravesical treatment of the interstitial cystitis (IC).

REFERENCES


Received: 27.02.2006

Erratum to „Topoisomerase II alpha expression and cytotoxicity of anthracyclines in human neoplast cells”
In scheme 1, page 16, in the structural formula of Doxorubicin the atom of oxygen is missing: The corrected formula of Doxorubicin is given below: