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Chitosan disrupts urinary bladder urothelium regardless of the differentiation stage of superficial urothelial cells

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Urinary bladder epithelium called urothelium presents one of the most resistant biological barriers composed of superficial, intermediate, and basal urothelial cells (UCs) [1]. This low permeability barrier is the result of high resistance tight junctions (TJ), a specialized apical plasma membrane with uroplakins and surface glycans, and hindered apical endocytosis [1, 2, 3, 4]. To improve drug absorption across the urothelium, paracellular transport pathways modulated by chitosan were studied. So far the effect of chitosan was studied only on normal urinary bladders with highly differentiated superficial UCs [5, 6]. The effect of chitosan on partially or low differentiated UCs that could be found at the surface of urinary bladder in the case of continuous infections or urothelial carcinomas is still unknown. In order to determine whether the effect of chitosan depends on the differentiation stage of superficial UCs, we applied chitosan to partially and highly differentiated urothelial models *in vitro*.

Partially and highly differentiated urothelial models were established by culturing UCs in UroM medium supplemented with fetal bovine serum or physiological concentration of calcium [7]. The effects of 15 minutes treatment with chitosan (0.05% (w/v), pH 4.5) on partially and highly differentiated urothelial models were analysed by monitoring molecular, ultrastructural, and physiological changes.

The transepithelial resistance (TER) measurements revealed complete loss of TER in both urothelial models, which shows that chitosan has the effect on partially and highly differentiated UCs. Furthermore, in order to explore the cause of this quick loss of TER, we performed detailed molecular and ultrastructural analyses. The results showed that reduction of TER in both urothelial models was due to opening of TJs and necrosis of partially and highly differentiated UCs "Figure 1.".

Data presented here show clear evidence that chitosan causes TJs disruption at the molecular level, desquamation of UCs, and consequently complete loss of TER in partially and highly differentiated urothelial models. According to these results, chitosan is a promising tool for ridding the infected UCs and removing urinary bladder superficial transitional cell carcinoma.

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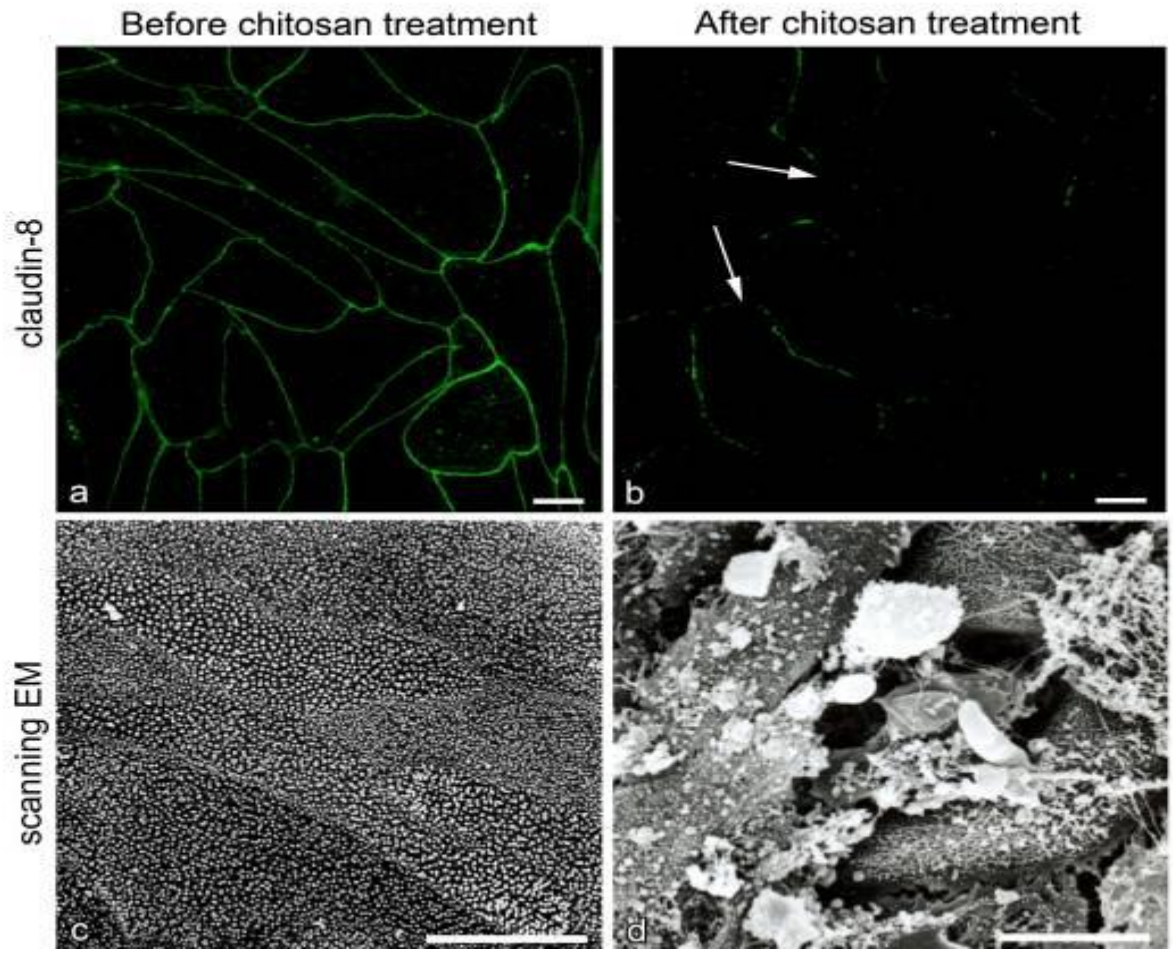

Figure 1. TJs and cell surface of partially differentiated urothelial models before and after chitosan treatment. Immunolabeling and scanning electron microscopy (EM) of chitosan treated urothelial models revealed disrupted TJs, claudin-8 localization in discontinuous lines (arrows b) and desquamation of necrotic UCs. Bars: 10 µm (a-d).