

## The Effect of Chitosan on Rat Olfactory Neuroepithelium Cells

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### Extended Abstract

Olfactory dysfunction significantly affects quality of life, alters in appetite, loses the ability to react to dangerous situations, and adversely affects psychological well-being [1]. In the general population, hyposmia varies from 13% to 18%, and anosmia from 4% to 6% [2]. To develop treatment modality for anosmia and regenerate olfactory neuroepithelium (OE), an *in vitro* culture system which can promote olfactory neuronal differentiation and expansion of progenitor cells is conducted by several researching groups. Chitosan has been demonstrated to bridge large gaps in peripheral nerves and regulate formation of neurospheres. However, whether chitosan can promote the differentiation of OE cells or regulate formation of olfactory neurospheres remains unexplored. This study evaluates the effect of chitosan on OE cells, which is a critical step in treating olfactory dysfunction and regeneration of OE. Cell sources were isolated from 17-day-old Wistar rat embryos, and then cultured on control or chitosan films for 12 days. Poly-L-lysine-co-laminin-coated was adopted as a control group. The effects of treatment were assessed by immunocytochemistry, real-time PCR, western blot and following culturing. In contrast with control groups, rat OE cells formed olfactory neurospheres on chitosan films. The percentages of the projected sphere area on chitosan films at day 12 were significantly higher. Particularly, the olfactory neurospheres contained progenitor cells, immature and mature olfactory receptor neurons (ORN), which were respectively labelled by anti-*Ascl1*, anti- $\beta$ III Tubulin and anti-olfactory marker protein (*OMP*).  $\beta$ III tubulin was clearly present throughout the neuron, in soma, dendrites, and axons. At day 6 the mRNA ratio of *Ascl1* and  $\beta$ III tubulin normalized to the internal gene *GAPDH* were significantly higher on chitosan films than on control groups. And the expression of 5-bromo-2'-deoxyuridine (*BrdU*), a proliferation marker, was also positive within olfactory neurospheres. It can explain why the diameter of spheres steadily increased during culture periods. However, at day 12, the expression level of  $\beta$ III tubulin significantly decreased on both groups, but the expression level of *OMP* was much higher on chitosan films. It means that chitosan may promote ORN to reach terminal differentiation. Notably, the distribution of mature ORNs with positive *OMP* gathered at out-layer of the spheroids. This finding indicates that spheroids may start to develop the polarity and behave like their counterpart *in vivo* [3]. Experimental results reveal that chitosan films can facilitate formation of olfactory neurospheres with expressing markers of progenitors and proliferation. Meanwhile, this study demonstrates a novel role of chitosan films in promoting differentiation of ORNs. Therefore, chitosan is a potential biomaterial for developing treatment modality of olfactory disorder in the future.

### References

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