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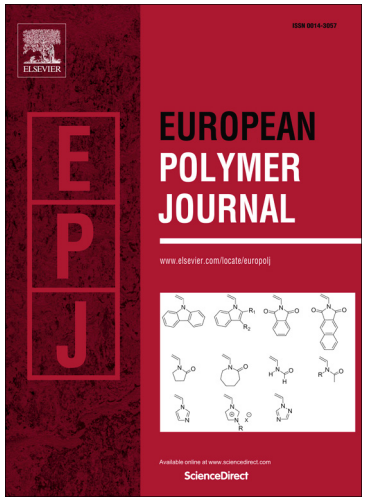


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# PENETRATION AND TOXICITY OF CHITOSAN AND ITS DERIVATIVES

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**Abstract**

Chitosan (CH) is a highly attractive biopolymer with multiple reactive groups which can be used to develop targeted nanoparticles. Positively and negatively charged chitosan derivatives can be used to obtain nanoparticles with different properties. The aim of this work was to determine the role of charge, hydrophobicity and solubility at neutral pH in CH cell penetration and cytotoxicity. We showed that positively charged unmodified CH do not penetrate the cells and is not toxic. Quaternization of CH (CHq) led to the increase in solubility, cell penetration, and cytotoxicity manifested by reactive oxygen species production, cell cycle arrest and inhibition of cell proliferation. Modification of CHq with hydrophobic residues significantly diminished cytotoxic activity however preserved cell penetration. Negatively charged CH derivative succinylchitosan (CHs) penetrated the cells highly efficiently and demonstrated the stimulation of cell proliferation. Thus, quaternized hydrophobic CH derivatives can be recommended to develop positively charged delivery systems, while any negatively charged chitosans are safe to design nanoparticles.

***Key words:*** *chitosan, chitosan derivatives, trypan blue, cellular uptake, flow cytometry, confocal microscopy*

## 1. Introduction

Chitosan (CH) - deacetylated derivative of chitin, a natural polysaccharide is one of the most widely studied polymers for the development of delivery systems for bioactive substances of different nature (proteins, peptides, nucleic acids, antitumor agents, and others) [1,2]. Over the past decade, the number of publications in this area increased more than 10 times, not only because of popular trends, but also because of such CH properties as biodegradability and biocompatibility, as well as the possibility of a variety of chemical modifications providing polymers with the desired characteristics. This resulted in the accumulation of a considerable amount of data on CH derivatives and submicron carriers [3–5]. Nevertheless, the results obtained by different research groups are fragmented and cannot be compared directly due to the diversity of chitosan derivatives, cell lines, methods of particle formation used and so forth. Toxicity and intracellular transport of such systems are also poorly characterized.

Low toxicity was shown for CH with different molecular weight (MW) and deacetylation degree (DD) as well as for succinyl derivatives (CHs) and CH based nanoparticles [6–9]. At the same time Loh at al. demonstrated that CH nanoparticles are toxic to hepatic cells and compromise cell membrane integrity evidenced by the registration of alanine transaminase and CYP3A4 enzyme leakage [10]. Moderate toxicity of positively charged CH was also shown by MTT cytotoxic assay by us and by many other groups [6,11–14] A large number of studies is devoted