6th International Conference on Advanced Nanomaterials 20 – 22 July 2015, Aveiro, Portgual

Enhanced Apoptosis/Necrosis in MCF-7 Cells by siRNA delivery with chitosan nanoparticles

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INTRODUCTION

RNA interference (RNAi) is a regulatory and natural mechanism in most eukaryotic cells. It is based on regulating specific gene expressions and defencing the organism against various pathogens by gene silencing function of the double stranded RNAs (dsRNAs). The RNAi mediated cancer medicine has become a novel strategy additionally to the conventional therapies in recent years3. Small interfering RNA (siRNA) duplex consists of 19-23 nucleotide and plays a key role in RNAi pathway. This macromolecule has anticancer potential by inhibiting the production of various specific oncogene targets associated with tumor growth, cell proliferation and metastasis4. Although siRNA has a powerful effect on downregulation of specific harmful genes in various diseases such as cancer, its active role in clinic still remains limited due to the low stability in human body. Its rapid decomposition by nucleases results in lack of efficiency and therefore, Food and Drug Administration (FDA) has not approved siRNA included drug formulations against several diseases5. For effective siRNA delivery, a protector drug vesicle that overcomes the drawbacks of siRNA is necessary. Thus far, various nanoformulation approaches have been studied excessively as siRNA carriers. Chitosan nanoparticles are widely used as polymeric delivery systems due to their unique properties such as high biocompatibility, biodegradability and low toxicity. In this study, we synthesized chitosan nanoparticles as in vitro tranfection agent for effectively delivery of ABCE1 and eRF3 siRNAs to enhance the apoptosis and necrosis rate of MCF-7 breast cancer cells.

EXPERIMENTAL

Chitosan nanoparticles were generated via ionic gelation by using sodium tripolyphosphate (TPP). 0.25% (w/v) chitosan solution in acetic acid (1% v/v) having the pH of 4.5 and TPP solution (0.25% w/v) (making final chitosan/TPP mass ratio 3:1) were used to obtain the nanoparticles in optimal size and surface charge. To prepare siRNA loaded chitosan nanoparticles 0.25% (w/v) TPP solution containing siRNA (0.25 µg/mL) was mixed with chitosan solution. The morphology of prepared nanoparticles were characterized by DLS and SEM. Double staining apoptosis/necrosis analysis were carried out after treating the cells with siRNAs alone and encapsulated with chitosan nanoparticles.

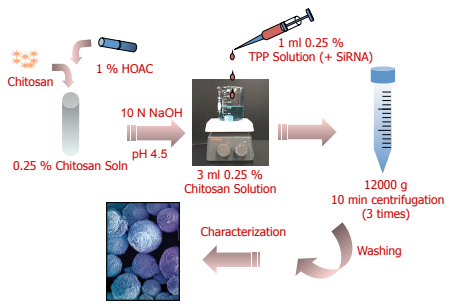
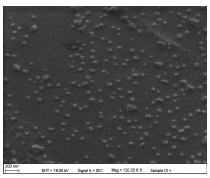


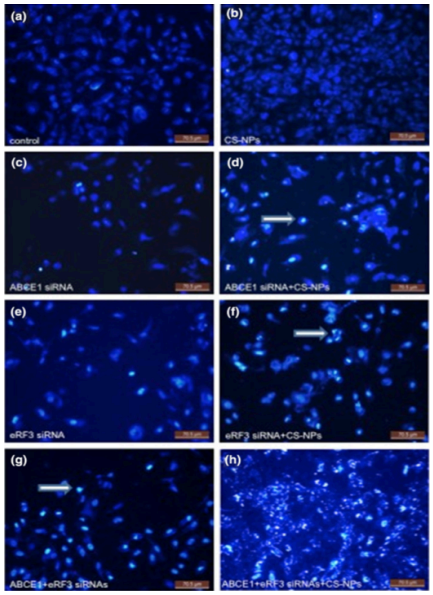
Figure 1. Preparation procedure of siRNA loaded chitosan nanoparticles



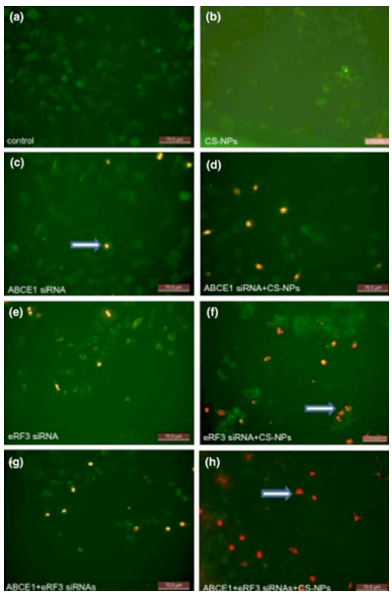
Chitosan nanoparticles were produced between the size range of 110 and 230 nm and with the zeta potential of approximately +27 mV .

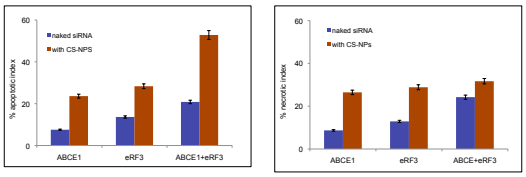
RESULTS AND DISCUSSION

Apoptotic cell images obtained from the double staining using Hoechst 33342 dye



Necrotic cell images obtained from double staining using PI





Apoptotic–necrotic percentage indices obtained as a result of double-staining test a % apoptotic index of MCF-7 cells transfected with naked siRNAs and siRNAs encapsulated with CS-NPs, b % necrotic index of MCF-7 cells interacted with naked siRNAs and siRNAs encapsulated with CS-NPs

CONCLUSION

In conclusion, this work showed that using chitosan nanocarriers as ABCE1 and eRF3 siRNAs delivery tools increased the anticancer effects of siRNAs resulting in enhanced apoptosis/necrosis in MCF-7 cells. Furthermore, ABCE1 and eRF3 genes might serve as target oncogenes, and silencing these genes could be promising option for cancer therapy.

ACKNOWLEDGMENTS

Our study was financially supported by Hacettepe University, Scientific Research Projects Coordination Unit (Grant number 011A601003).

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