A NOVEL CHITOSAN BASED GENE DELIVERY SYSTEM FOR TRANSGENESIS

Asst.Prof.Dr. M.Koray GÖK[A*], Assoc.Prof.Dr. Erdal CEVHER[B], Dr. Kamber DEMİR[C], Prof. Dr. Serhat PABUCCUOĞLU[C], Prof. Dr. Saadet (ÖZGÜMÜŞ) PABUCCUOĞLU[A]

A: Istanbul University, Faculty of Engineering, Department of Chemical Engineering, Subdepartment of Chemical Technologies, 34320 Avcılar-ISTANBUL
B: Istanbul University, Faculty of Pharmacy, Department of Pharmaceutical Technology, 34116 Beyazıt-ISTANBUL
C: Istanbul University, Faculty of Veterinary Medicine Department of Reproduction and Artificial Insemination, 34320 Avcılar -ISTANBUL

*Corresponding author Phone Number:+904737070/17669; Email: mkgok@istanbul.edu.tr

CONTENT

This study contains the synthesis and characterization of novel biocompatible modified chitosan and nanoparticles (nMChi), their cytotoxicity tests and their *in vitro* application for transgenesis.

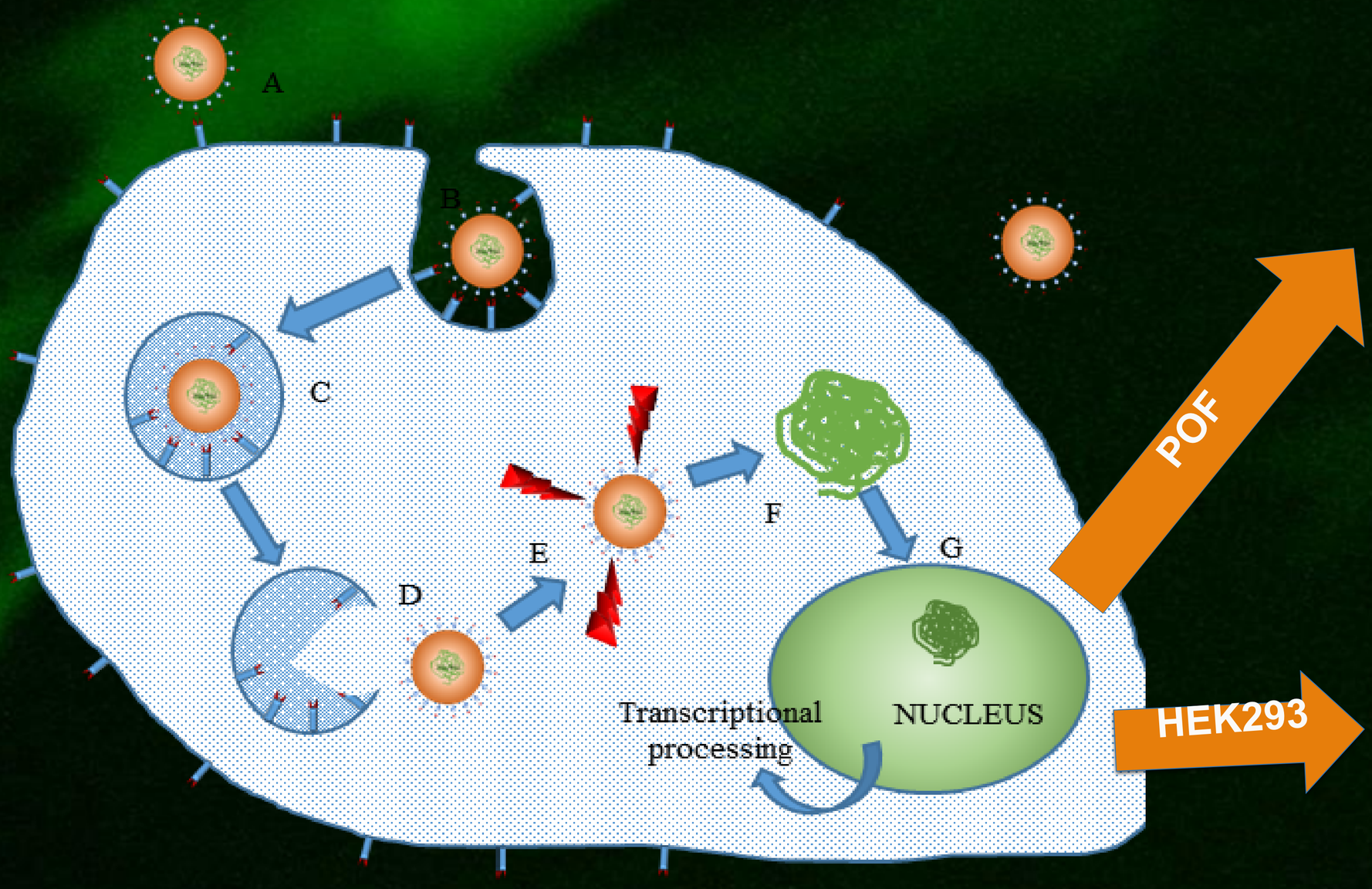
Keywords: Transgenesis, polymeric non-viral vector, chitosan, transgenic cationic polymers

INTRODUCTION

Gene therapy not only aims treatment diseases but it also provides the recombinant genetic material transport to the nucleus whereby the gene expression, which activates or deactivates the protein synthesis, takes place. The carrier systems, which deliver gene for the transgenesis or gene therapy, are called vectors. The well targeted, nontoxic gene carrier systems, which can carry the gene to the nucleus, are needed. The vectors are mainly divided into two groups as nonviral and viral vectors. The non-viral vector systems still need to be well developed for the effective gene transfer. These systems are divided into two groups as lipophilic and polymeric vectors. The most important advantage of polymeric vectors is the enhancement of their efficacy by allowing various modifications which improve the genetic material release characteristics of the polymers to the targeted site. The natural polymers generally used in gene delivery are the poly(aminoacids), polyornithine etc., chitosan, dextran, gelatin and their modified derivatives. Chitosan is one of the prominent nonviral gene carriers in gene therapy.

METHOD

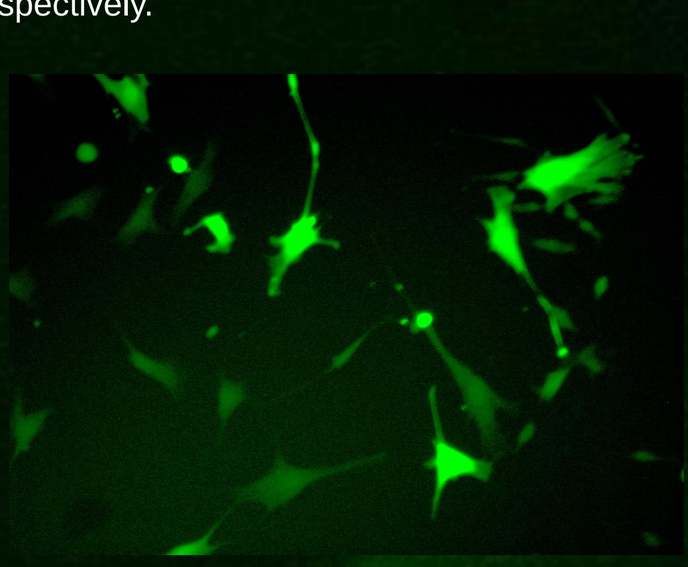
In the study, the synthesis of MChi was carried out via modification for increasing the end groups amount which increase the transfection efficiency, on the chitosan molecule. The complex formation of nMChi with pDNA (i.e. green fluorescence protein gene; eGFP) in different ratios (w/w) are carried out via ionic interactions. Primer Ovine Fibroblasts (POF) and Human Embryonic Kidney (HEK293) Cells were used for the transfection efficiency tests. The transfection efficiency of the nanoparticle was analyzed by determining of the green fluorescent illuminated cell ratio under 460-480 nm fluorescence light by using Olympus IX71 inverted microscope.



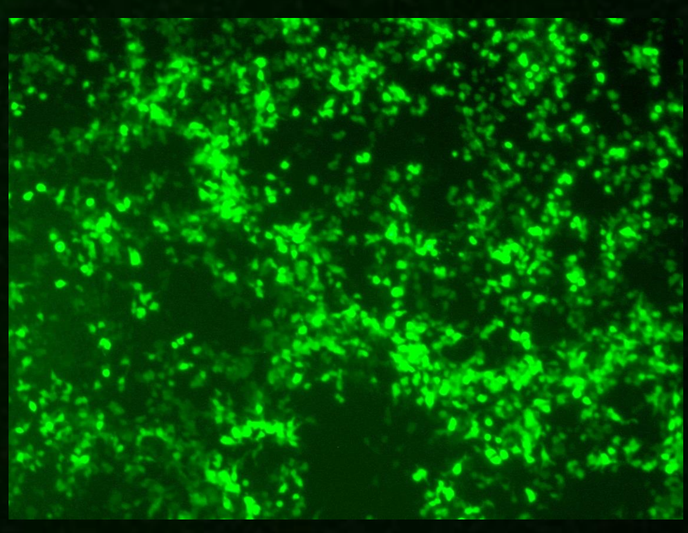
RESULTS AND DISCUSSION

According to all results, the transfection efficiencies of nMChi were assayed in HEK293 and POF cells. The transfection efficiency of nMChi formulation is the highest in HEK293 and POF cells.

The transfection efficiency of nMChi formulation is the highest in HEK293 and POF cells. In the case of where the cell number was 20.000, the polymer:pDNA ratio was 5:1 to 7:1, the amount of pDNA was 0,5 to 2 µg and the degree of transfection efficiency was 95% and 20% for HEK293 and POF cells, respectively.



It was concluded that nMChi synthesized in this study can be used in gene transfer to human HEK293 or primer ovine fibroblast cells.



This work was supported by Scientific Research Projects Coordination Unit of Istanbul University, Project Numbers 31781 and 7383. Nanoparticles formulation which was developed to be a patent applied the Turkish Patent Institute (09.09.2014-2014/10605) and European Patent Office (09 September 2014–EP14184168)