Gene expression profiles in primary duodenal chick cells following transfection with avian influenza virus H5 DNA plasmid encapsulated in silver nanoparticles

ABSTRACT

In order to develop a systemically administered safe and effective nonviral gene delivery system against avian influenza virus (AIV) that induced cytokine expression, the hemagglutinin (H5) gene of AIV, A/Ck/Malaysia/5858/04 (H5N1) and green fluorescent protein were cloned into a coexpression vector pIRES (pIREGFP-H5) and formulated using green synthesis of silver nanoparticles (AgNPs) with poly(ethylene glycol) and transfected into primary duodenal cells taken from 18-day-old specific-pathogen-free chick embryos. The AgNPs were prepared using moderated temperature and characterized for particle size, surface charge, ultraviolet-visible spectra, DNA loading, and stability. AgNPs and AgNP-pIREGFP-H5 were prepared in the size range of 13.9 nm and 25 nm with a positive charge of +78 ± 0.6 mV and +40 ± 6.2 mV, respectively. AgNPs with a positive surface charge could encapsulate pIREGFP-H5 efficiently. The ultraviolet-visible spectra for AgNP-pIREGFP-H5 treated with DNase I showed that the AgNPs were able to encapsulate pIREGFP-H5 efficiently. Polymerase chain reaction showed that AgNP-pIREGFP-H5 entered into primary duodenal cells rapidly, as early as one hour after transfection. Green fluorescent protein expression was observed after 36 hours, peaked at 48 hours, and remained stable for up to 60 hours. In addition, green fluorescent protein expression generally increased with increasing DNA concentration and time. Cells were transfected using Lipocurax in vitro transfection reagent as a positive control. A multiplex quantitative mRNA gene expression assay in the transfected primary duodenal cells via the transfection reagent and AgNPs with pIREGFP-H5 revealed expression of interleukin (IL)-18, IL-15, and IL-12â.

Keyword: Silver nanoparticles; Avian influenza; Hemagglutinin; Transfection; Primary cells