ABSTRACT

Polyamidoamine cationic polymers with DNA have potential as non-viral gene delivery systems. However, they have poor in vivo distribution and a tendency to aggregate. This can be overcome by the use of homo/copolymer blends to form multicomponent DNA delivery systems (MCDS). Multicomponent DNA delivery systems have been proven to have poor transfection efficiency in vitro (Rackstraw et al 2002). Therefore, the aim of this study was to improve the uptake of the multicomponent DNA delivery systems by incorporating a targeting moiety. Folinic acid, as a novel-targeting moiety, was suggested for this work.

Formulation and screening studies for the best ratio/s of multicomponent DNA delivery systems were investigated. This was carried out using DNA with different blends of polyamidoamine homo/copolymer (MBA-DMEDA and PEGylated MBA-DMEDA, which are referred to by their batch numbers NG52 and NG54 respectively). Homo/copolymers at (2:1) and (3:1) ratios proved to have the best collective physicochemical characteristics among all of the investigated series. The investigation was carried out using ethidium-bromide (Et-Br) displacement assay, gel electrophoresis, photon correlation spectroscopy (PCS) and transmission electron microscopy (TEM).

Folinic acid-PEG-PAA-PEG-folinic and folic acid-PEG-PAA-PEG-folic acid conjugates were synthesised using the water-soluble carbodiimide coupling agent (1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide). The final molar substitution ratios (MSR) were 2:1 of folinic acid to polymer and 1.9:1 of folic acid to polymer. The physicochemical characteristics of both folinic acid and folic acid modified complexes with DNA were then investigated. The results showed no significant differences between folinic acid modified particles and the non-modified ones. However, there was a significant difference between folic acid modified particles and the non-modified ones.

Studying of the binding and the uptake of folinic and folic acid-BSA conjugates on a 791T osteosarcoma cell line were investigated. The binding of this targeting moiety could be inhibited by the presence of free folinic acid and its conjugate but not with free folic acid, which showed the specificity to this ligand. Folinic-BSA conjugates were also endocytosed by cells through non-clathrin mediated pathway. These results showed the ability of folinic acid to work as a targeting moiety with potential in vivo advantages.

In the last chapter three main topics were investigated. The first topic was to compare the transfection of NG52/54–folinic acid modified nanoparticles with both folic acid modified and the non-modified nanoparticles. The results showed the ability of folinic acid to improve the transfection of the polymer blend 40–200 times relative to a non-modified system. At the same time folinic acid modified complexes have a transfection activity similar to that of folic acid complexes. The second topic was qualitative and quantitative investigation of DNA complex trafficking using YO-YO dye. The results showed the ability of folinic acid complexes to be taken by the cells faster and to a higher level than that of folic acid and the homopolymer on its own. The third topic was the in vivo assessment of the complexes. This was carried out by comparing the pharmacokinetics, biodistribution and the transfection of folinic acid, folic acid and the non-modified nano-particles, which showed no difference in the examined parameters of the systems studied. This might be attributed to the instability of the complexes in serum.
The initial aims of the project have therefore been accomplished. A ligand has been incorporated resulting in enhanced uptake and transfection activity. Folinic acid was validated as a suitable targeting moiety in vitro showing appropriate specificity and uptake.