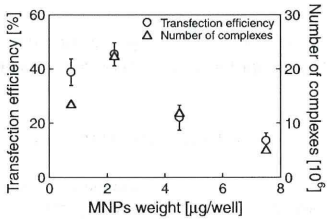

Fig. 9 The number of DNA/polyethylenimine (PEI) max/magnetic nanoparticles (MNP) complexes assessed on the basis of size distribution compared with the transfection efficiency. The trend in transfection efficiency corresponds to the numbers of complexes

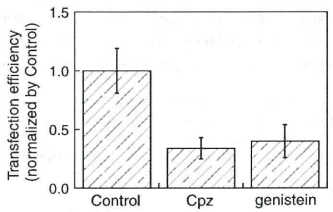

Fig. 10 Effects of inhibitors on the transfection efficiency of γ-Fe2O3 nanoparticles (control: inhibitor-free, Cpz: addition of chlorpromazine, genistein: addition of genistein). 10 μg/ml chlorpromazine or 200 μM genistein was added

With respect to receptor-mediated endocytosis, the model of endocytosis for efficient internalization has been reported (Gao et al. 2005; Decuzzi and Ferrari 2007; Lunov et al. 2011). Wrapping time, and threshold and optimal radii of particles are important factors for particle endocytosis. These factors can be represented as the function of receptor/ligand density ratio and estimated by receptor/ligand binding energy factor, bond elasticity factor, and non-specific attractive/repulsive factor at cell/particle interface (Gao et al. 2005; Decuzzi and Ferrari 2007). Moreover, wrapping time is also represented as the function of the forces acting on particle captured by receptors, which contain the elastic forces of the cellular membrane and the internal forces of receptor (Lunov et al. 2011). The optical radius of particles is estimated from wrapping time, cell lateral size, and the number of nanoparticles captured per second (Lunov et al. 2011). Optimal size of nanoparticle is up to 50 nm for efficient internalization by receptor-mediated endocytosis (Gao et al. 2005). On the other hand, in vitro test has indicated that nanoparticle with diameter up to 100–200 nm is optimal (Win and Feng 2005). Therefore, the estimation for efficient internalization must take into account the factors influenced on particle configuration, coating agent, cell type, and cultural environment.

Figure 10 illustrates the transfection rate for DNA/PEI max/MNP (γ-Fe_2O_3 of 2.25 μg) complexes in the presence of chlorpromazine as a CDE inhibitor and genistein as a CIE inhibitor. The transfection rate was decreased by both endocytic inhibitors, showing that the complexes were internalized by CDE and CIE. This result agrees with those of previous studies (Huth et al. 2004). DNA/PEI max/MNP complexes are internalized by CDE and caveolae- and flotillin-dependent endocytosis, which are classified as CIE because cellular internalization of polyplexes through these endocytic pathways has been confirmed (Huth et al. 2004; Rejman et al. 2005; Payne et al. 2007; Vercauteren et al. 2011). It is indicated that the efficient internalization of the complexes depends on the size of the complexes and other factors such as the number of complexes and the ligand/receptor interactions between PEI and PEI receptors on cell surfaces.

Conclusion

Magnetofection using DNA/PEI max/MNP complexes was studied. Transfection efficiency was enhanced using MNPs and an applied magnetic field, but it decreased with high weight of MNPs despite the increase in cell viability. DNA/PEI max/MNP complexes aggregated because of alkaline pH of the medium and the reduction in electrostatic repulsion induced by DNA binding. The sizes of the complexes increased with high weight of MNPs in the medium. Aggregation induced by high weight of MNPs inhibited cellular uptake by size-dependent endocytosis and led to a decline in the number of complexes. The decline of transfection efficiency in high weight of MNPs was due to aggregation of the complexes; therefore, it was concluded that this decline was not due to cytotoxicity.

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