Magnetic Exosomes as Magneto-Mechanical Actuators to Treat Breast Cancer
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Abstract
One of the most recent strategies that are being used for cancer therapy is focused on magneto-mechanical actuation, which has the potential to overcome certain side effects reported in traditional strategies (e.g., chemotherapy). Among the different biocompatible magnetic nanomaterials, superparamagnetic iron oxide nanoparticles (SPIONs) are widely used as smart magnetic actuators. Intracellular SPIONs can be activated when exogenous alternating super-low frequency magnetic fields are applied, which can induce changes in cell function and cytoskeletal disruption and subsequent selective death of cancer cells. Even though some SPIONs are FDA approved, they exhibit a low rate of accumulation in tumors, which is one of the main obstacles in further developing this therapeutic strategy. In this study, we developed magnetic exosomes that can accumulate at a high rate in targeted areas under alternating current (AC) magnetic field exposure. Exosomes isolated from human blood via column separation technique are decorated with star-like nitro-dopaamine PEGylated (ND-PEG) SPIONs (sND-PEG SPIONs). Due to the vital role of magnetic field actuated sND-PEG SPIONs in this strategy, their biophysics-chemical and magnetic properties are investigated via dynamic light scattering (DLS), nanoparticle tracking analysis (NTA), transmission electron microscopy (TEM), vibrating sample magnetometry (VSM), Fourier-transform infrared spectroscopy (FTIR), and X-ray powder diffraction (XRD). TEM data confirmed the size (~30 nm) and morphology (star) of sND-PEG SPIONs. DLS and NTA data showed a hydrodynamic size of ~120 nm for these nanoparticles in phosphate buffer saline (PBS), and colloidal stability of suspensions in cell culture media. The concentration of sND-PEG SPIONs in exosomes is studied by inductively coupled plasma mass spectrometry (ICP-MS). Proliferation rate and live viability studies on breast cancer cells exposed to sND-PEG SPIONs for up to 72 hr indicated the nanoparticles are biocompatible even at 400 µg/ml. Then the efficiency of magnetic exosomes as magneto-mechanical actuators is evaluated for breast cancer treatment. For this purpose, intracellulare uptake, biodistribution and clustering, and cytoskeleton disruption are studied on three breast cancer cells (MCF-7, MDA-231, and LCC-6) and one normal breast cell (MCF-10A) in the presence and absence of magnetic fields. Our findings indicate that cell death can be selectively enacted upon cancer cells while leaving healthy cells intact. This piece of research represents a step forward to advance this field and could serve as a platform technology in cancer theranostics and remotely controlled nanomedicines.

Introduction
Magneto-mechanical actuation (MMA) is one of the most recent strategies used in cancer therapy. The active component in MMA is based on superparamagnetic iron oxide nanoparticles (SPIONs). Modifying the size and surface of SPIONs facilitates selective internalization into cancer cells. Intracellular SPIONs can be activated when exogenous alternating super-low frequency magnetic fields are applied, which can induce cytoskeletal disruption and subsequent death of cancer cells without causing any damage to healthy cells. Magneto-mechanical actuation (MMA) of SPIONs plays thus a critical role and assessing their bio-physicochemical properties and structure/activity relationship might shed more light to optimize the efficacy of remotely controlled therapies. High filtration of SPIONs by main organs (liver, spleen, kidney, ) and consequently low rate of accumulation in tumor is of the main obstacles for developing this technology. For overcoming this hindrances, antistropic-morphology ND-PEG SPIONs were converted exosome to magnetic actuator which can accumulate in targeted area under AC magnetic field.

Methods
Star-like ND-PEG SPIONs were synthesized by thermal decomposition method. Exosome was isolated from human blood and then by using Thermo Fisher electroporation system, ND-PEG SPIONs decorated exosome. Concentration of Fe was measured by ICP-MS. Physicochemical properties such as morphology, size distribution, agglomeration state, and zeta potential in cell culture media were evaluated by transmission electron microscopy (TEM) and dynamic light scattering. Fourier transform infrared (FTIR) spectroscopy was used to confirm the chemical bonding between PEG with magnetite. MDA-MB-231 (mammary gland adenocarcinoma), MCF-7 (human breast ductal carcinoma) and MCF10A (human non tumorigenic mammary gland cells) were purchased from ATCC. In vitro cytotoxicity and cell viability of ND-PEG SPIONs were assessed on normal and breast cancer cells with/without magnetic field exposure via standard Presto-Blue and Live & Dead assays. ICP-MS and confocal microscopy were used to measure cellular uptake and cytoskeleton disruption, respectively. Biocompatibility, cellular uptake, and cytoskeleton disruption in presence and absence of exogeneous magnetic fields were assessed on breast cancer cell lines.

Results
Our preliminary data reveal that the physicochemical properties and surface passivation with magnetic exosome can significantly influence their MMA capabilities for magnetic therapy. In vitro results confirmed that the response of star-like ND-PEG SPIONs have high biocompatibility and can kill the cancer cells under magnetic field. However, some questions about the leading cause remain unanswered, and is being currently investigated by the authors. Further advancement in this field will allow precise tuning of magnetic exosome for applications in cancer theranostics and remotely controlled nanomedicines.

Discussion
TEM and DLS data confirmed the star-like morphology of ND-PEG SPIONs with hydrodynamic size of 30 and 175 nm, respectively. Cell proliferation and viability results indicated that ND-PEG SPIONs are highly biocompatible up to 400 µg/ml. Size, morphology and surface modification of SPIONs are important as a collective ‘passive’ property in nanomedicines that influences cellular uptake. The morphology of SPIONs affects the cytoskeleton extravasation and accumulation/clustering of SPIONs. Therefore, under magnetic field cells proliferation dropped for cancer cells.

Conclusions

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