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Epithelial internalization of superparamagnetic nanoparticles and response to external magnetic field

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Abstract

Superparamagnetic magnetite nanoparticles (MNP) coated with silica were synthesized and chronically implanted into the middle ear epithelial tissues of a guinea pig model ($n = 16$) for the generation of force by an external magnetic field. In vivo limitations of biocompatibility include particle morphology, size distribution, composition and mode of internalization. Synthesis of MNP was performed using a modified precipitation technique and they were characterized by transmission electron microscopy, X-ray diffractometry and energy dispersive spectroscopy, which verified size distribution, composition and silica encapsulation. The mechanism for internalizing 16 ± 2.3 nm diameter MNP was likely endocytosis, enhanced by magnetically force. Using sterile technique, middle ear epithelia of tympanic membrane or ossicles was exposed and a suspension of particles with fluoroscein isothiocyanate (FITC) label applied to the surface. A rare earth, NdFeBo magnet (0.35 T) placed under the animal, was used to pull the MNP into the tissue. After 8 days, following euthanasia, tissues were harvested and confocal scanning laser interferometry was used to verify intracellular MNP. Displacements of the osscicular chain in response to an external sinusoidal electromagnetic field were also measured using laser Doppler interferometry. We showed for the first time a physiologically relevant, biomechanical function, produced by MNP responding to a magnetic field.

Keywords: Nanoparticles; Middle ear; Biomechanics; Hearing; Endocytosis

1. Introduction

Superparamagnetic nanoparticles (MNP), such as magnetite, have been widely used for biomedical applications [1]. Utilization of MNP to produce forces in living cells, likewise, is not entirely a new concept [2]. Magnetic twisting cytometry was previously developed to generate torque on cells in culture to assess the role of mechanical stress during development, notably in developing pulmonary epithelium [3–5]. Biomedical applications of MNP include magnetic resonance imaging contrast enhancement, hyperthermia, intravascular targeted delivery of therapeutics, biosensors and others [1,6–9]. Targeted delivery of therapeutics requires adequate particle susceptibility to and directional control by an external magnetic field. Chronically implanted MNP for assisted biomechanical organ or tissue functions is feasible only if particle susceptibility is commensurate with directionality and strength of external magnetic fields [2]. Physiological mechanisms for cellular internalization of particles include fluid phase or receptor-mediated endocytosis, phagocytosis and non-endocytic pathways. Enhancement of MNP internalization by magnetic forces and subsequent long-

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term viability are not understood [1]. Additionally, the limits of intracellular MNP mass sufficient to exert forces without harming cells are unknown. Little is also known of intracellular trafficking following magnetically enhanced endocytosis and potential iron cytotoxicity from particle degradation [10]. Additionally, the effects of cytosolic MNP on cellular apoptotic processes are untested.

In recent years electromagnetic implantable hearing devices for patients with sensorineural hearing loss have been developed. Permanent magnets, such as neodymium–iron–boron, are surgically implanted into the middle ear, wherein the implants interact with external electromagnetic fields mechanically directly driving the ossicular chain [11]. Resulting vibrations representing acoustic input are conveyed into the inner ear (cochlea), creating the percept of sound [12]. Such "direct drive" hearing device technology has generated high fidelity, amplitude and frequency sounds. Since implanted hard magnets can generate sufficient force to produce sound, we hypothesized that comparable vibratory forces could be produced by MNP, with sufficient mass implanted in the ossicular epithelium or tympanic membrane. Perhaps, hearing amplification could serve as a model of tissue biomechanics from implanted MNP responding to an external magnetic field.

Of prospective mechanisms for internalization, we hypothesized that endocytic processes will be enhanced by forces pulling silica encapsulated MNP into the tympanic membrane and middle ear epithelium. Widder and colleagues have shown in magnetic drug targeting studies that external magnetic fields can concentrate MNP in target tissue, enhancing cell entry by endocytosis [13]. By contrast, intravascular MNP targeting of endothelial receptors must contend with opsonization by proteins and phagocytosis by the reticuloendothelial system. Hence, coatings such as polyethylene glycol (PEG) have been used to reduce macrophage recognition and uptake [1]. Silica encapsulation of MNP with negative surface charge (zeta potential) is useful for corrosion protection, colloidal dispersion and as substrate for particle functionalisation [14,15]. Silica does not substantially interfere with magnetic susceptibility [16,17]. We directed forces on silica encapsulated MNP using an external magnetic field to more effectively internalize these particles, irrespective of endocytic and non-specific mechanisms [1,18].

A requirement for MNP generating forces in cells and tissues is long-term viability. Unlike MNP for drug delivery, chronic implantation requires cell compatibility, hermetic encapsulation, and no exocytosis. Potential iron leaching and toxicity, an aspect of long-term MNP viability, has limited data. Normal intracellular iron homeostasis involves internalization of the complex of ironbound transferrin via the transferrin receptor but excessive intracellular iron ions are potentially toxic through the formation of oxygen radicals and peroxidative damage [10,19]. Intracellular iron balance could be upset if MNP were to leach iron. Nevertheless, studies on magnetic resonance imaging showed (I.V.) ferrite nanoparticles as compatible with hepatic reticuloendothelial cells and causing no hepatocellular injury [20].

We report here on a model for generation of forces in living tissues, implantation of superparamagnetic nanoparticles in the middle ear epithelium. Magnetite nanoparticles (Fe_3O_4) were synthesized by a modified Massart technique [21] and silica encapsulated for hermeticity and functionalisation. Particles were characterized using X-ray diffraction (XRD) and transmission electron microscopy (TEM) equipped with energy dispersive X-ray spectroscopy (EDS). Middle ear epithelial cell internalization was enhanced by an external magnetic field and confirmed by observing conjugated fluoroscein isothiocyanate (FITC) intracellular fluorescence under confocal microscopy. Neither the mechanisms of MNP endocytosis nor their intracellular trafficking were the focus of this study.

The ability to generate sinusoidal, vibratory forces by the interaction of MNP with an external magnetic field was also tested. The tip of an electromagnetic coil was placed 1–2 mm from either the implanted tympanic membrane or incus epithelium. One and two kHz sinusoidal vibration of the ossicular chain was produced. Thus, we concluded that intracellular MNP, implanted up to 15 days, remained viable in situ and could be used to produce ossicular vibrations consistent with the perception of sound in humans.

2. Materials and methods

2.1. Synthesis and characterization of magnetite nanoparticles (MNP)

2.1.1. Preparation of magnetic nanoparticles

Nanoparticle synthesis employed criteria for size, superparamagnetism, mass, hermetic encapsulation, substrate for linkers and viability in tissues. Minimal size optimizes ease of entry across cell membranes [21,22]. To enhance endocytosis by magnetic forces, MNP less than 30 nm diameter were sought. Magnetite less than 30–50 nm also exhibits superparamagnetism due to single domain crystalline structure. Superparamagnetism exhibits no remanence, dispromoting agglomeration that occurs with magnetized particles The spherical magnetite particles (Fe_3O_4) with relatively high magnetic susceptibility included an ~5 nm shell of silica (SiO_2). Encapsulation of the magnetite by SiO_2 prohibited corrosion and provided an anionic surface charge that promoted endocytosis [15] as well as a substrate for attachment of amines that can serve as linkers to other molecules.