External Magnetic Field Control of Intrinsic Electric Fields in Magneto-Electric Nanoparticles for Enabling Patient- and Disease-Specific Nanomedicine

Rakesh Guduru1,2, Ping Liang3, Jeongmin Hong1,2, Madhavan Nair1, and Sakhrat Khizroev 1,2,3\*

1Center for Personalized Nanomedicine, Department of Immunology, Herbert Wertheim College of Medicine, Florida International University, Miami, Florida 33199.

2Department of Electrical and Computer Engineering, Florida International University, Miami, Florida 92174.

3Department of Electrical Engineering, University of California, Riverside, CA 92521.

**Corresponding Author**

Sakhrat Khizroev

[khizroev@fiu.edu](mailto:khizroev@fiu.edu)

Tel: 305-348-3724

Fax: 305-348-3707

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**Short Abstract**

We describe the fabrication and characterization protocols used in our studies of magneto-electrical nanoparticles (MENs) for enabling Personalized Nanomedicine (PNM) from the perspective of fundamental physics and nanoengineering. This approach exploits quantum-mechanical coupling between electric and magnetic fields within MENs. Anti-Cancer and anti-HIV drugs were released on demand.

**Abstract**

The use of nanoparticles is often considered as an enabling force of personalized nanomedicine (PNM). Using nanoparticles to precisely navigate a drug through the patient’s body and control its dosage and composition as well as to detect even minute disease-caused changes in the surrounding cellular microenvironment can make personalized treatment a reality. However, the fundamental physics that underlies the nanoparticles’ characteristics in the perspective of the intrinsic interaction with the patient’s body in the aforementioned applications is poorly exploited. Our recent discovery of the unprecedented capabilities of magneto-electric nanoparticles (MENs) helps fill this gap. MENs could be used as energy-efficient and dissipation-free field-controlled nano-vehicles for targeted delivery and on-demand release of anti-Cancer and anti-HIV drugs as well as nano-stimulators for field-controlled non-invasive treatment of patients with central nervous system (CNS) disorders. Further, the intrinsic coupling between electric and magnetic forces within MENs enables molecular specificity that provides an entirely new dimension even to conventional state-of-the-art diagnostic methods such as MRI, magnetic nanoparticle imaging (MNI), and PET-CT.

Here, we present detailed fabrication protocols and characterization procedures to study and develop MENs that could be used for targeted drug delivery and on-demand release with no heat dissipation. To demonstrate the new application, we use scanning probe microscopy approaches to directly trace the kinetics of a magneto-electric action used to release AZT 5’-triphosphate (AZTTP), an anti-HIV drug, from 30-nm CoFe2O4-BaTiO3 MENs by applying a DC and low-frequency (below 1000 Hz) AC field. Finally, we present a study to employ MENs for an on-demand targeted treatment of Ovarian Epithelial Cancer with Paclitaxel (Taxol), a popular mitotic inhibitor.

**Introduction**

Researchers across the globe have long been struggling to find a better way to navigate and dispense the cargo of drugs and/or disease-specific image contrast agents to the damaged tissue or at the site of action with adequately high efficiency and 3-D navigation precision *1*. Personalized NanoMedicine (PNM) has recently emerged as a multi-disciplinary field that leverages nanotechnology to achieve these diagnostics and treatment milestones. However, in spite of its unprecedented potential, this field is at its very early stage of development and no viable PNM technologies exist today. The use of nanoparticles is often considered an essential enabling tool of PNM. Indeed, because of their unique size- and shape-dependent properties, nanoparticles promise superior applications in diverse areas such as Cancer relief, targeted drug delivery, immunoassays, functional MRI, MNI, and fluorescence imaging, and many others. For ideal medical treatment, every patient requires his or her own optimal combination of drugs and microenvironment that can be controlled at the sub-cellular level. Using nanoparticles to precisely define drug dosage and composition as well as to detect even minute disease-caused surrounding cellular and tissue changes can make such personalized treatment a reality.

There are many different types of nanoparticle systems used in medicine for drug delivery purposes alone. To mention a few, they rely on using thermally-responsive polymers, optically (UV, Visible-Wavelength, and IR) or acoustically activated materials, liposomes, electrochemical processes, and magnetic forces *2*. The unique advantages of an external magnetic field control place magnetic nanoparticle (MN) systems in a class of their own, especially for the purposes of targeted delivery. In this case, the speed of delivery is limited by the external field sources and not just by the blood circulation. For instance, as it is related to the release across the blood-brain barrier (BBB), the magnetic delivery provides a unique way to transfer drugs sufficiently fast to avoid their engulfing by the reticuloendothelial system (RES) *3*. However, all the existing delivery technologies suffer from the lack of certainty of drug release from the carrier if and when the nano-carrier reaches the target. Probably, the main stumbling block is the inability of the current nanoparticle systems to remotely control the intrinsic phenomena that define the interaction of the nanoparticles with the surrounding cells and tissues. The conventional approaches are extrinsic in their nature and often fail to adequately regulate cellular phenomena such as exocytosis of drug with intracellular vesicle, control of ionic channels, using externally controlled signals *4*. In other words, the fundamental physics that underlies the nanoparticle system characteristics in the perspective of their intrinsic interaction with the surrounding cells and tissues in the aforementioned applications is barely exploited. Revealing and controlling the interaction of nanoparticles at the nanoscale, whether it is electric field-, magnetic spin- , photon-, or phonon-triggered, is vital for enabling perfect diagnostics and/or recovery/regeneration of all the medical functions.

Our recent discovery of the unprecedented capabilities of magneto-electric nanoparticles (MENs) paves a way to fill this gap through nanotechnology approaches. Previously, we have shown that due to the intrinsic quantum-mechanically defined coupling between electric dipoles and magnetic spins within each nanoparticle, MENs could be used as energy-efficient and dissipation-free field-controlled nano-vehicles for targeted delivery and on-demand release of anti-Cancer and anti-HIV drugs as well as nano-stimulators for non-invasive treatment of patients with central nervous system (CNS) disorders such as Parkinson’s Disease, Alzheimer’s Disease, and other dementia *5*. Further, this intrinsic coupling between electric and magnetic fields within MENs provides molecular composition specificity that can enable an entirely new dimension even to the conventional diagnostic methods such as MRI, MNI, and PET-CT. In addition, the specificity allows assigning unique “passport” features to small groups of nanoparticles thus pushing the real-time health monitoring capability to a new level. Finally, because the coupling between magnetic and electric fields is achieved at the intrinsic level, ideally the described field-controlled PNM functions (delivery, drug release, CNS stimulation, diagnostics) can be accomplished with ideal (100%) efficacy and without any destructive heat dissipation (with no need to use high-power electronics as with the conventional NPs).

Here, we present detailed fabrication protocols and characterization procedures for MENs that could be used for energy-efficient field-controlled targeted drug delivery and on-demand release with no heat dissipation and unprecedented high efficacy. As examples, we discuss HIV and Ovarian Cancer models.

**Protocol:**

**1) Magneto-electric nanoparticles: synthesis and characterization**

1.1) Prepare CoFe2O4 nanoparticles by hydrothermal method described previously *6*.

1.2) Dissolve 15 ml of aqueous mixture of 0.058 g of Co(NO3)2.6H20, 0.16 g of Fe(NO3)3.9H20 and 0.2 g of polyvinylpyrrolidone in 5 ml of aqueous 0.9 g sodium borohydride at 120°C for 12 hours.

1.3) Next, prepare the precursor solution of BaTiO3 by mixing 30 ml of aqueous 0.029 g of BaCO3 and 0.1 g of citric acid with 30 ml of ethonalic solution of 0.048 ml titanium isopropoxide and 1 g of citric acid.

1.4) Prepare CoFe2O4-BaTiO3 coreshell MENs by dispersing 0.1 g of CoFe2O4 nanoparticles in the precursor solution and sonicate the mixture for 2 hours.

1.5) Dry the well-dispersed mixture at 60°C overnight while stirring continuously and later, calcinate the mixture at 780°C for 5 hours to obtain CoFe2O4-BaTiO3 coreshell MENs.

**2) Surface functionalization of the nanoparticles for maximizing the drug carrying efficiency**

Depending on the type of the drug carried, MENs need to be surface functionalized in order to maximize their drug carrying efficiency. The most popular linkers used for loading the drug molecules onto nanoparticles are PEG, PLGA, GMO, Poly-L-Lysine etc., *7,8,9,10*. In this study, GMO was used to surface functionalize the MENs.

2.1) Dissolve 100% weight ratio of the linker molecules (GMO) to the MENs in PBS buffer (pH7.4) and incubate it for 24 hours while agitating slowly.

2.2) Upon completion of the incubation process, centrifuge the solution at 14000 rpm for 10 minutes at 10°C to remove the unbound linker molecules.

2.3) Later, re-dissolve the particles in the ethyl acetate and acetone solution (50:50 vol. ratio) and centrifuge at 14000 rpm for 10 minutes to remove the excess unbound GMO (repeat this process thrice to ensure the complete removal of unbound GMO). Use the as-prepared surface functionalized MENs to further load the desired therapeutic drugs.

**3) Conjugating the therapeutic drugs with the MENs**

Non-functionalized and functionalized MENs are used to conjugate the therapeutic drugs. In-this study, the most widely used anti-HIV drug (3′-Azido-3′-Deoxythymidine-5′-Triphosphate, AZTTP) and anti-cancer drug (paclitaxel) are used. AZTTP drug is directly conjugated on the MENs surface without functionalization whereas paclitaxel drug is conjugated onto MENs functionalized with GMO.

3.1) Load AZZTP drug with MENs at 1: 10 weight ratio (drug: MENs) in PBS buffer solution by incubating it for 3 hrs. Load paclitaxel with GMO-MENs at 1:10 weight ratio in MPBS (30% methanol and 70% PBS solution) by incubating for 3 hrs. (Note: the weight ratios for each drug to the MENs were previously determined by a binding isotherm plot.)

3.2) Centrifuge the incubated solution at 14000 rpm for 10 minutes at 10°C.

3.3) Isolate the supernatant and measure the absorbance at the appropriate wavelength (see note) to determine the binding efficiency.

Note: The absorbance value for AZTTP is 267nm and for Paclitaxel is 230nm. Figures 1 and 2 show the standard calibration plots at varying drug concentration for AZTTP and Paclitaxel, respectively.

3.4) Use calibration plots to determine the amount of drug in each sample.

3.5) Calculate the drug loading efficiency with the following formula: Drug loading percentage = (Absorbance of total amount of drug used – absorbance of drug used in the supernatant after incubating the drug and the MENs for a specific incubation time) x 100%.

1. **Remote DC and AC field triggering for on-demand drug release**

4.1) After isolating the supernatant for drug loading measurements, re-suspend the precipitate of MENs conjugated with the drug in the MPBS solution and centrifuge at 14000 rpm for 10 minutes at 10°C.

4.2) Re-disperse the precipitate in 190μl of the TE buffer for AZTTP-MENs and 1ml of MPBS buffer for Paclitaxel-GMO-MENS respectively (Note: The amount of buffer used was previously calibrated for these drugs based on the drug absorption maxima.)

4.3) Subject the dispersed solution to a magnetic field of varying field strength and frequency using a low-field Helmholtz pair connected to the function generator.

4.4) After the field treatment, centrifuge the solution and separate the supernatant as described in step 3.

4.5) Measure the absorbance of the supernatant as described in step 3. Determine the amount of drug in the supernatant from calibration plots.

4.6) Calculate the absorbtion maximum of the solution spectrophotometrically using the formula: Percentage of drug release = (absorbance of supernatant after magnetic field treatment)/(absorbance of supernatant after incubating the particles with drug) ×100%.

**Note: Drug binding and release can be confirmed using various established experimental methods.** Qualitative analysis of the drug release process can be confirmed through Fourier Transform Infra-Red (FTIR) and atomic force microscopy (AFM) at key stages of the process. Use UV-Vis spectrophotometry to determine quantitative results.

**Representative Results**

Qualitative results

*FTIR analysis:*

FTIR analysis of the drug binding and release process was confirmed by performing the measurements at three key stages, 1) before loading the drug on MENs, 2) when the drugs are attached to the MENs surface, 3) after releasing the drugs by remote 44 Oe AC magnetic field strength and 100-Hz frequency. The FTIR results for AZTTP-MENs are shown in Figure 3. These results indicate 30% weaker absorbance of bound samples when compared to unbound particles from 1750 to 1250 cm-1.

*AFM imaging analysis:*

To visualize the drug binding and the release process, AFM imaging was performed at the molecular level in tapping mode. The sample preparation process is elaborated in the discussion section. The prepared samples were glued to a metallic sample puck and mounted onto the AFM stage to obtain both topography and phase contrast images. All the images were performed with a 512 × 512 scan resolution at a scan rate of 0.5 Hz.

Figure 4 shows the binding and release process of AZTTP drug at three different stages. AFM images clearly show MENs before and after release of the drug look similar (Figure 4a and 4d).

Quantitative results

The quantitative results of drug loading and release percentage of both AZZTTP and Paclitaxel were obtained by determining the absorption maxima at different stages of the process. Once the absorption maxima of AZTTP (267nm) and Paclitaxel (230nm) values were determined, the amount of drug present in the solution was calculated from the spectrophotometric drug absorption calibration plot. From the Figure 5, it can be seen that 24% of the drug was loaded onto nanoparticles by incubating AZTTP with the MENs in the Tris-EDTA (TE) buffer (pH 7.4) for 3 hours. The Paclitaxel drug loading percentage for the same incubation time was 14.7%, but GMO surface functionalized MENs increased the drug loading percentage to 47.8%. When the drug bounded MENs were subjected to the field treatment to trigger the drug release, it was found that 44-Oe field at 1000 Hz results in maximum AZTTP drug release (89.3%) and for Paclitaxel, 66-Oe field and 0 Hz results in maximum release (97.6%). Figure 6 summarizes the drug release percentage results.

**Figures Captions**

Figure 1: AZTTP drug absorption maxima at different amount of drug in 1ml of PBS buffer.

Figure 2: Paclitaxel drug absorption maxima at different amount of drug in 1ml of MPBS buffer.

Figure 3: FTIR measurements at three different stages: (i) MENs only, in black color, (ii) AZTTP bound to MENs, in red color and (iii) MENs after AZTTP released by AC-field treatment, in gray color.

Figure 4: AFM images at different stages of the release process: (a) MENs and (b) AZTTP chains before the attachment, (c) AZTTP-MEN nanoformulations as a result of the binding process, (d) MENs after the drug release by a 44-Oe AC field at 1000 Hz.

Figure 5: AZTTP and Paclitaxel drug loading percentage after three hours of incubation.

Figure 6: AZTTP and Paclitaxel drug release percentage after subjecting the drug bounded MENs to varying magnetic fields at three different frequencies.

**Discussion**

Below, we discuss the most important aspects one should bear in mind while conducting the above experiments on field-controlled delivery and on-demand release of the drug carried by MENs. These aspects mostly relate to the use of field and power sources according to the described protocols and procedures.

MENs display all the properties that are displayed by the conventional MNs. Therefore, MENs can replace MNs in any existing application, e.g., as drug nano-carriers or contrast enhancement agents in MRI. In addition, unlike the conventional MNs, MENs show an unprecedented capability that can pave a way for making PNM a reality. They have a relatively strong non-zero magneto-electric (ME) effect because of the quantum-mechanical coupling between the magnetic spin and the electric dipole. As a result, energy-efficient and dissipation-free remote control of the intrinsic charge distribution in the MENs (and consequently, control of the bonding force between the MENs and the drug) can be enabled via application of an external magnetic field. Because of the intrinsic ME effect, even if the drug is strongly bonded to the MEN carriers (as required for high-efficacy delivery), it can be fully released at the target location via application of a local magnetic field with a strength above a certain threshold defined by the ME effect. For comparison, the drug release process using the conventional MNs (often, superparamagnetic nanoparticles) is not controlled at the same fundamental level but instead, is based on an irreversible extrinsic process that is triggered by an external AC magnetic field and typically results in relatively high energy dissipation. This irreversibility and the resulting heat dissipation is either due the use of temperature-sensitive intermediate materials together with MN, or due to a mechanical deformation at very high frequencies (often above hundreds of kilohertz), or due to another extrinsic process *11, 12, 13*. On the contrary, the MEN-triggered release process is achieved at the intrinsic level and thus is dissipation free and extremely energy efficient. The release with MENs can be triggered by an AC magnetic field at a relatively low frequency (below 100 Hz) and even at a DC field provided the field strength is above a certain threshold value, with power consumption in the sub-watt range. Figure 5 summarizes the requirements on the field’s strength and frequency.

In the fabrication of MENs, it is critical to keep the nanoparticles relatively small (~ 30 nm) and maintain relatively high magneto-electric coefficient (100 V cm-1 Oe-1) and saturation magnetization (~100 emu/cc). The properties were measured using local SPM techniques and B-H-loop magnetometer.

To conduct the described AFM measurements, nanoparticles were dispersed on the silicon wafer and care was taken to obtain well-dispersed particles without aggregation. To obtain the uniformly dispersed nanoparticles on the silicon wafer, nanoparticles of choice were first taken in chloroform (1mg of particles in 600 μl of chloroform). Later, 100 μl of the solution was mixed with 200 μl of isopropanol and the solution was centrifuged at 3500 rpm for 3 minutes. Supernatant was discarded and 200 μl of isopropanol was added (to the pellet) and the solution was re-centrifuged. The pellet was then mixed with 200 μl of chloroform. This final solution was used to prepare the samples for AFM imaging.

To conduct the described FTIR measurements, one drop of the desired nanoparticle solution (0.5 mg per 100 μl of TE buffer) was placed on a pre-cleaned silicon wafer and the wafer was air-dried overnight. Once the particle solution was completely dried, FTIR measurements were carried out.

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**Disclosures:**

The authors declare that they have no competing financial interests.

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