Biodistribution and clearance of magnetoelectric nanoparticles for nanomedical applications using energy dispersive spectroscopy

Aim: The biodistribution and clearance of magnetoelectric nanoparticles (MENs) in a mouse model was studied through electron energy dispersive spectroscopy. **Materials & methods:** This approach allows for detection of nanoparticles (NPs) in tissues with the spatial resolution of scanning electron microscopy, does not require any tissue-sensitive staining and is not limited to MENs. **Results:** The size-dependent biodistribution of intravenously administrated MENs was measured in vital organs such as the kidneys, liver, spleen, lungs and brain at four different postinjection times including 1 day, 1 week, 4 and 8 weeks, respectively. **Conclusion:** The smallest NPs, 10-nm MENs, were cleared relatively rapidly and uniformly across the organs, while the clearance of the larger NPs, 100- and 600-nm MENs, was highly nonlinear with time and nonuniform across the organs.

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It is hard to overestimate the importance of nanoparticles (NPs) for medical applications. Still at a relatively early development stage, NPs have already proven to be unsurpassed in fields of high-sensitivity imaging and high-specificity targeted drug delivery [1–4]. It is foreseen that many new lifesaving NP-based applications will emerge in the near future [5–13]. Magnetoelectric nanoparticles (MENs) are a recently introduced type of NPs in medical applications [14–20]. MENs are multiferroic NPs which display a non-zero magnetoelectric effect (ME) due to the presence of quantum mechanically coupled magnetostrictive and piezoelectric effects; as a result, they display properties which are not available in any other NP type. Like traditional magnetic NPs, they have a non-zero magnetic moment and therefore can be wirelessly controlled via magnetic fields. For example, they can be navigated through the body via application of external magnetic

field gradients and thus used for targeted drug delivery. For the same reason, MENs can also be imaged using magnetic resonance imaging or the recently introduced high-sensitivity technology of magnetic particle imaging [4,16,21,22]. Additionally, combining these functions of navigation and imaging can enable a superior image-guided targeted drug delivery, which in turn can lay a foundation for next-generation pinpoint treatment. However, unlike any traditional magnetic NPs, due to the presence of the ME effect, MENs can also provide two additional functions that make them uniquely suited for targeted drug delivery. These two functions are an externally controlled drug release off the NPs via application of an a.c. magnetic field and an externally controlled interaction between the NPs and the cellular microenvironment which allows cancer cells to be distinguished from the surrounding normal cells via application of a d.c. magnetic field

Figure 1. Secondary image versus backscattered image. On the left, both images show the secondary electron image of an area in the lungs. On the right, both images show the same area imaged by the backscattered detector. The NPs have the highest contrast in the images (the bright areas on the right images). NP: Nanoparticle.

Figure 2. Energy dispersive spectroscopy mode of scanning electron microscopy. Backscattered image (left) and EDS scans of Ba and Ti of the same area in the lungs 1 month postinjection. (A) Backscattered image overlapped with Ba EDS mapping. (B) Ba EDS mapping of the same region of lungs. (C) Ti EDS mapping of the same region of lungs. EDS: Energy dispersive spectroscopy; SEM: Scanning electron microscopy.

in a specific strength range tailored to the cancer type. In these two cases, an external magnetic field is used to control intrinsic electric fields which underlie the bond between the NP and the drug, and the interaction between the NPs and the cellular microenvironment, respectively [14–16]. To date, the most popular MENs' nanostructure has been of a spherical shape and with a coreshell composition consisting of a magnetostrictive core, for example, CoFe_2O_4 and a piezoelectric shell, for example, BaTiO_3 , with a net diameter varying from below 10 to over 500 nm. The coupled magnetostrictive and piezoelectric effects provide a nonzero ME effect on the order of 10 to over 100 $\text{mV cm}^{-1}\text{Oe}^{-1}$, depending on the quality of the interface between the two nanostructures. Through *in vitro* and *in vivo* studies, it has already been demonstrated that MENs can deliver antiretroviral therapy across the blood–brain barrier to eradicate HIV-1 virus hidden deep in the brain, wirelessly stimulate single neurons for treatment of Parkinson's disease and other neurodegenerative diseases, and provide high-specificity targeted delivery of anticancer medicine to cure ovarian and other cancers [14–26]. It has been proven that these NPs can be made biocom-

patible and nontoxic [14–19]. However, before these NPs can be translated to clinical studies, it is important to understand their biodistribution and clearance rates. It can be noted that in general, a magnetic field can be applied to directly guide MENs in and out of clearance pathways. In addition, PEGylation or another surface modification can be adjusted to favorably affect the biodistribution and clearance. However, the question of biodistribution and clearance of nonfunctionalized MENs from different organs without the application of external magnetic fields needs to be answered to fully exploit the potential of MENs in medicine [27].

The current study aims to exploit backscattered electron microscopy and energy dispersive spectroscopy (EDS) based analysis to understand the biodistribution of MENs depending on their size and the time spent in the body. As a mode of scanning electron microscopy (SEM), backscattered electron microscopy along with EDS analysis provides a high-resolution compositional analysis which allows to detect individual NPs as small as 10 nm in diameter. To the best of the authors' knowledge, this study has for the first time used BEC–EDS for biodistribution measurements.

Figure 3. Scanning electron microscopy image of magnetolectric nanoparticles in the kidneys. SEM images of different magnifications showing a particle cluster in the kidneys (100-nm, 1 week postinjection). The red box indicates the magnified area. SEM: Scanning electron microscopy.

Figure 4. Energy dispersive spectroscopy distribution of magnetoelectric nanoparticles in the kidneys. Ba and Ti EDS mapping of the kidneys with 100-nm MENs a week postinjection. (A) Backscattered image of the particle overlapped with Ba EDS mapping. (B) Ba EDS mapping of the same kidney region. (C) Ti EDS mapping of the same kidney.

EDS: Energy dispersive spectroscopy; MEN: Magnetoelectric nanoparticle.

The EDS-based biodistribution analysis is site specific and does not require any chemical staining. Thus, it is not limited to MENs and can be applied to any other inorganic and organic NP type as long as the NP's average atomic weight exceeds that of the tissue [20].

Methods

All the methods used in this study were carried out on healthy adult female SCID mice (Jackson Labs) in

accordance with the approved guidelines of the Institutional Animal Care and Use Committee document # 13–045 at Florida International University.

Chemical synthesis of MENs

Details on the fabrication of toxicity-free CoFe_2O_4 - BaTiO_3 core shell MENs were described in our previous publications [14]. The 10-nm MENs required the core prepared by the coprecipitation of Co^{2+} and Fe^{3+}

Figure 6. The nanoparticle-caused reduction of the kidney glomeruli (see facing page). SEM of particles in the glomerular capillary over time. Blue boxes indicate the glomeruli and red boxes indicate particle clusters. (A) Shows clusters of 10-nm MENs in the glomerulus a week postinjection. (B) Shows a glomerulus that has shrunk after trapping a particle cluster 2 months postinjection. SEM: Scanning electron microscopy.

Figure 5. MEN discharge from the kidneys. Evolution of differently sized MENs in the kidneys over the 8-week study period. MEN: Magnetoelectric nanoparticle.

Figure 7. Magnetolectric nanoparticles summarizing distribution in the kidneys. NP distribution in the kidneys for different sizes of NPs over time.
NP: Nanoparticle.

or Fe^{2+} with NaOH. The core nanostructures for 30-, 100- and 600-nm MENs were prepared according to a polyvinylpyrrolidone (PVP) assisted hydrothermal method. First, 0.058 g of Cobalt Nitrate Hexahydrate ($\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$) and 0.16 g of Ferric Nitrate Nona-

hydrate ($\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$) were dissolved by stirring in 15 ml of distilled water. PVP, 0.2 g was dissolved in 5 ml of aqueous solution containing 0.9 g of sodium borohydride. PVP–sodium borohydride solution was added dropwise to the above solution and the mixture

Figure 8. Scanning electron microscopy image of magnetolectric nanoparticles in the liver. Different magnification images showing a particle cluster in the liver (100-nm MENs 1 month postinjection). The clusters of MENs are seen as bright patches in the images.
MEN: Magnetolectric nanoparticle.

Figure 9. Energy dispersive spectroscopy distribution of magnetoelectric nanoparticles in the liver. Ba- and Ti-specific EDS mapping of the liver with 100-nm MENs a week postinjection. (A) Backscattered image overlapped with Ba EDS mapping. (B) Ba EDS mapping of the same region of liver. (C) Ti EDS mapping of the same region of liver.

EDS: Energy dispersive spectroscopy.

was stirred at 120°C until the liquid phase evaporated. CoFe_2O_4 particles were recovered, dispersed in distilled water through sonication and were washed three-times using magnetic separation. Purified CoFe_2O_4 cores were dried at 120°C for 24 h and stored at room temperature until further use. The barium titanate (BaTiO_3) shell was prepared using the citrate gel method. Briefly, the CoFe_2O_4 cores were dispersed in distilled water through sonication. Barium carbonate (BaCO_3), 174 mg, was dissolved in 60 ml DI water containing 1 g of citric acid. This solution was mixed with a 150 ml ethanolic solution of titanium (IV) iso-

propoxide (284 μl) and 6 g citric acid. The BaTi precursor solution was added to the cores and sonicated at room temperature for 1 h. The translucent yellow liquid was stirred at 70°C until the liquid phase evaporated completely. Finally, the gel was calcined at various temperatures ranging from 500 to 800°C (CMF-1100) for 5 h and cooled naturally to room temperature. The gelation temperature and the final temperature were important determinants of the crystal structure and the final size of CoFe_2O_4 - BaTiO_3 core shell MENs. We found that 600°C resulted in 30-nm MENs, 700°C for 100-nm MENs, 780°C for 200-nm MENs and 850°C

for 600-nm MENs. $\text{CoFe}_2\text{O}_4\text{-BaTiO}_3$ were submitted for further characterization. The particle size distribution was measured by a Zetasizer Nano series that uses the standard dynamic light scattering approach.

Tissue preparation

Mice were euthanized by means of CO_2 inhalation, immediately after which the tissues of interest were excised and stored in a 10% formalin solution overnight at 4°C . The tissues were cleaned under a stereomicroscope by removing hair, excess fat, etc. A small piece of the tissue was cut and transferred into phosphate-buffered saline, where it was rocked for 30 min

with the PBS being replaced three-times in 10-min intervals to remove excess fixative. Tissues were carefully dried with a Kimwipe and transferred to a plastic mold containing the optimum cutting temperature compound (OCT). Tissues were frozen either by immersing the mold directly into liquid nitrogen or in a bath of 2-methylbutane/liquid nitrogen at approximately -100°C , depending on the type of tissue being processed. Frozen tissues were transferred to a -80°C freezer for at least one night before being cut into 10 μm sections with a Leica CM3050 Cryostat. Finally, tissue slices were mounted on VWR® Superfrost® Plus microscope slides and dried on a slide warmer at

Figure 10. Magnetoelectric nanoparticles discharge from the liver. Evolution of different size MENs in the liver over the 8-week study period.
MEN: Magnetoelectric nanoparticle.

Figure 11. Magnetoelectric nanoparticles summarizing distribution in the liver. NP distribution in the liver for different sizes of NPs over time. NP: Nanoparticle.

37°C for 1–2 h. Slides were then ready to use for EDS analysis.

Excrement sample preparation

Mice under study were administered with a single bolus dose of 5 mg of glycerol monooleate (GMO)-coated MENs by means of lateral tail vein injection. Urine and feces were collected daily and stored for imaging. Urine samples are prepared by drying 1 ml of urine on copper tape in multiple layers to create a small, highly concentrated point for imaging. Feces are weighed out to 30 mg and dissolved under sonication and heat; the resulting slurry is spread in a thin layer onto copper tape for imaging. Imaging is performed via JEOL-JIB 4500 multibeam system (FIB/SEM) with a Thermo-scientific Noran system 7 for EDS confirmation of heavy elements as MENs.

Transmission electron microscopy

Phillips CM-200 200 kV transmission electron microscope with EDS option was used to obtain transmission electron microscopy images and EDS profiles.

Scanning electron microscopy & energy dispersive spectroscopy

JEOL-JIB 4500 multibeam system (FIB/SEM) with

a Thermo-scientific Noran system 7 was used to obtain SEM images and carry out the EDS analysis. As described below, the EDS analysis was performed in the mode with a backscattered electron detection (BED).

In this study, four different average MENs' sizes, 10, 30, 100 and 600 nm, respectively, were tracked through five vital organs of interest (spleen, kidneys, liver, lungs and brain) over the course of 1 day, 1 week, 4 and 8 weeks. A single dose of MENs was administered intravenously through an injection in the caudal vein in a healthy mice. Then, their organs, urine and feces were collected at different times for EDS–SEM quantification of MENs content.

As with any SEM mode, an EDS image can be accompanied by a secondary electron detection (SED) or BED. Because SED is dominated by the sample's topography, which is mostly tissue, it is challenging to spatially detect NPs as small as 10 nm with this approach. On the other hand, BED provides an image contrast based on the atomic number and thus is more suitable for imaging MENs on the background of tissues mostly made of relatively light organic elements [28]. For comparison, two SEM images obtained with SED and BED approaches, respectively, are shown in Figure 1. Consequently, the current EDS-

Figure 12. Scanning electron microscopy image of magnetoelectric nanoparticles in the spleen. Different magnification images showing a particle cluster in the spleen (100-nm MENs 1 month postinjection). MNP: Magnetoelectric nanoparticle.

based biodistribution analysis was conducted with the BED approach, for example, as shown in [Figure 2](#).

Details of the fabrication of toxicity-free MENs have been presented in a previous publication [15]. When in the blood, nonfunctionalized MENs are negatively charged due to the colloidal chemistry caused by the interplay of chemical and electrical forces (with ζ potential of approximately -45 mV). However, this charge not only strongly depends on the microenvironment (becoming less negative with the reduction of the pH level) but also can be significantly varied and even reversed through both surface functionalization and field application. To simplify the analysis, this study focuses on the nonfunctionalized NPs. In this study, an equivalent dose of approximately 5 mg of MENs is administrated intravenously through the caudal vein injection. Five vital organs including the liver, lungs, spleen, kidneys and brain, respectively, are collected at the four postinjection time intervals under study. In addition, urine and feces are collected daily for imaging. The cover slips with collected organ tissues are imaged with SEM. The EDS is particularly

important to differentiate MENs from the normally occurring elements of the biological tissues. Indeed, minerals with relatively heavy elements such as, iron and calcium also display high-contrast profiles in SEM images, similar to those from heavy elements making up inorganic NPs such as MENs. Only through the detailed compositional analysis provided by EDS, can the key elements making up MENs be distinguished from iron, calcium and other heavy elements present in tissues.

Results

Imaging of the kidneys

It is well known that for the NPs to be rapidly excreted (within approximately 24 h) through the renal clearance pathway, they need to be smaller than approximately 10 nm in diameter [29–32]. Otherwise, they get filtered out by the kidneys and other organs. Since all the NP sizes under study are above this threshold, they are not expected to be cleared through the renal pathway and should therefore be retained for longer time in the blood. Typical SEM images of different mag-

nifications showing a cluster of 100-nm MENs in the glomerular region of the kidneys of a mouse 1 week postinjection are shown in **Figure 3**. The EDS mapping of two key elements making up MENs, Ba and Ti, respectively, are shown in **Figure 4**. EDS images for 10, 30, 100 and 600 nm MENs for 1 week, 4 and 8 weeks postinjection demonstrate the evolution of the NP discharge from the kidneys depending on the NP size, as shown in **Figure 5**.

It is believed that although after MENs enter the kidneys they can move to different parts of the organs, eventually they still get discharged [29]. Based on the images, given enough time after administrating MENs, the main parts of the kidneys that trap and accommodate the NP clusters are the glomeruli. The glomeruli act as the pressure valves to maintain the blood flow constant by changing their size [33]. It is

likely that the glomeruli that intake NPs grow smaller over time due to the increased pressure caused by the NPs in that site, as shown in **Figure 6**. Such an effect was most pronouncedly observed for 10-nm MENs.

A summarizing dependence of the MENs' distribution in the kidneys depending on the NP size and the postinjection time is shown in **Figure 7**.

Imaging of the liver

It is believed that the noncontinuous endothelia in the liver have fenestrations with diameters ranging from 50 to 180 nm in humans and 50 to 280 nm in rodents [34–37]. As a result, NPs in this range may get accumulated in the liver. A typical cluster of 100-nm MENs in a region in the liver 1 month postinjection is shown in **Figure 8**. The EDS mapping of two key elements making up MENs, Ba and Ti, respectively are

Figure 13. Energy dispersive spectroscopy distribution of magnetoelectric nanoparticles in the spleen. Ba- and Ti-specific EDS mapping of the spleen with 100-nm MENs 1 week postinjection. **(A)** Backscattered image overlapped with Ba EDS mapping. **(B)** Ba EDS mapping of the same region of spleen. **(C)** Ti EDS mapping of the same region of spleen.

EDS: Energy dispersive spectroscopy; MEN: Magnetoelectric nanoparticle.

Figure 14. Magnetoelectric nanoparticles' discharge from the spleen. Evolution of different size MENs in the spleen over the 8-week period of the study.
MEN: Magnetoelectric nanoparticle.

shown in Figure 9. The evolution of the NP discharge from the liver depending on the NP size is shown in Figure 10. A summarizing dependence of the MENs' distribution in the liver depending on the NP size and the postinjection time is shown in Figure 11.

Imaging of the spleen

The spleen is believed to filter out NPs with a diameter above 200 nm due to its interendothelial cell slits with a pitch of approximately 200 nm. The ability of the spleen to filter out the NPs increases as the NP size increases [35,38,39]. A typical cluster of 100-nm MENs in a region in the spleen 1 month postinjection is shown in Figure 12. The EDS mapping and the evolution of

the NP discharge from the liver depending on the NP size are shown in Figures 13 & 14, respectively. A summarizing dependence of the MENs' distribution in the spleen depending on the NP size and the postinjection time is shown in Figure 15.

Imaging of the lungs

Capillaries in the lungs are known to trap NPs in the micron size range [40]. A typical cluster of 100-nm MENs in a region in the lungs 1 month postinjection is shown in Figure 16. The EDS mapping and the evolution of the NP discharge from the lungs depending on the NP size are shown in Figures 17 & 18, respectively. A summarizing dependence of the MENs' distribu-

Figure 15. Magnetoelectric nanoparticles summarizing distribution in the spleen. NP distribution in the spleen for different sizes of NPs over time.
NP: Nanoparticle.

tion in the lungs depending on the NP size and the postinjection time is shown in [Figure 19](#).

Imaging of the brain

Compared with the other organs under study, the brain doesn't show any significant accumulation of MENs. The number of nanoparticles found in the brain is orders of magnitude smaller and therefore difficult to quantify. Nevertheless, relatively rare and small clusters of nanoparticles still can be found in the brain. [Figure 20](#) shows different magnification images

of a cluster of 600-nm MENs in the cerebellum region in the brain. MENs clusters can be consistently found in the cortex, as qualitatively illustrated in [Figure 21](#). It can be noted that the cortex contains the types of cells that can potentially become malignant, for example, astrocytes. These cells give origin to the most aggressive tumors in the brain, for example, astrocytoma and glioblastoma multiforme [36,37,38]. Hence, the consistent appearance of MENs in the cortex gives them an advantage for being used as drug carriers for intracellular delivery into brain tumors.

Figure 16. Scanning electron microscopy image of magnetoelectric nanoparticles in the lungs. Different magnification images showing a particle cluster in the lungs (100-nm MENs 1 month postinjection).
MEN: Magnetoelectric nanoparticle.

Figure 17. Energy dispersive spectroscopy distribution of magnetoelectric nanoparticles in the lungs. Ba- and Ti-specific EDS mapping of the lungs with 100-nm MENs 1 week postinjection. **(A)** Backscattered image overlapped with Ba EDS mapping. **(B)** Ba EDS mapping of the same region of lungs. **(C)** Ti EDS mapping of the same region of lungs.

EDS: Energy dispersive spectroscopy; MEN: Magnetoelectric nanoparticle.

Size-dependent biodistribution

Having the data for the distribution of MENs in the above studied vital organs, the biodistribution of the NPs in the whole body can be mapped at least in a first order approximation. Since the number of NPs in the brain is relatively low, this organ was not taken into consideration in the summarizing analysis. The biodistribution charts for 10-, 30-, 100- and 600-nm MENs are shown in [Figure 22A–D](#), respectively.

Discussion

This EDS-based biodistribution study on mice showed

how intravenously administrated MENs in the size range from 10 to 600 nm would travel through the kidneys, liver, spleen, lungs and the brain. It should be noted that the NPs were detected according to the described EDS-based compositional analysis, so all the conclusions are based on the assumption that specific elements, particularly Ba and Ti, uniquely represent NPs. It took more than a day for the NPs to reach their highest numbers in these organs. The relatively long-term circulation of MENs under study could be explained by a lack of rapid renal clearance for NPs of these sizes and properties. It is worth noting that

this prolonged circulation half-life would be favorable for multiple medical treatments, such as that of metastasized tumors through the enhanced permeability and retention effect [44,45]. However, this particular study focused on healthy mice only and thus there was no tumor vasculature through which the NPs could extravasate. Instead, within a week after the administration, MENs became present in all the above organs. Most of these trapped MENs were cleared in the time span of 4–8 weeks postinjection, depending on the NP size and the particular organ. It is likely that resident macrophages in these organs significantly contributed to this systemic clearance process. A strong dependence on the size could be observed. For example, despite the

equivalent administration doses, the concentrations of 10-, 30-, 100- and 600-nm NPs found in all these organs 1 week postinjection, the time at which the concentration values reached their highest peaks, were proportional to approximately 19, 37, 46 and 21 au, respectively. The observation that the smallest NPs, that is, 10-nm MENs, represented the smallest fraction in this comparison could be explained by the fact that some of these 10-nm NPs were cleared through the renal pathway within the first week postinjection. Indeed, the NP size had a dispersion of approximately 50%, so it is likely that a significant portion of the 10-nm MENs rapidly underwent the kidney filtration because their actual size could be as small as 5 nm.

Figure 18. Magnetoelectric nanoparticles' discharge from the lungs. Evolution of different size MENs in the lungs over the 8-week study period.
MEN: Magnetoelectric nanoparticle.

Figure 19. Magnetolectric nanoparticles' summarizing distribution in the lungs. NP distribution in the lungs for different sizes of NPs over time. NP: Nanoparticle.

Figure 20. Scanning electron microscopy imaging of magnetolectric nanoparticles in the brain. Different magnification SEM images of a section of the brain (cerebellum) showing a cluster of 600-nm MENs 1 week postinjection. MEN: Magnetolectric nanoparticle; SEM: Scanning electron microscopy.

Figure 21. Magnetolectric nanoparticles in the brain. Qualitative illustrations showing measured localized regions of 600-nm MENs in the brain over the 8-week period.
MEN: Magnetolectric nanoparticle.

This observation is in agreement with the fact that only the smallest NPs were found in urine samples. Further, the measurements showed that over 90% of the 10-nm MENs found in all these organs 1 week postinjection were cleared within the subsequent 7-week period of the study. For comparison, the equivalent clearance rates were 86, 85 and 61% for 30-, 100- and 600-nm MENs, respectively. The smaller the trapped NPs were, the faster they were cleared from these organs. A

special observation could be made regarding the largest NPs, that is, 600-nm MENs. The peak number of the trapped 600-nm MENs 1 week postinjection was almost as small as it was for the smallest NPs, that is, 10-nm MENs. This finding might imply that many 600-nm MENs either remained in the circulation for a relatively long period of time or were discharged through the liver relatively fast. A similar conclusion of an improved hepatic uptake of this size range NPs,

Figure 22. Biodistribution charts (cont. on facing page). Biodistribution charts for (A) 10-nm MENs, (B) 30-nm MENs, (C) 100-nm MENs and (D) 600-nm MENs for 24 h and, 1, 4 and 8 weeks postinjection. MEN: Magnetoelectric nanoparticle.

that is, approximately 500 nm, was made in a study by Alexis *et al.* [46]. They argued that the relatively large surface area of these NPs resulted in an improved surface absorption of proteins, which in turn intensified opsonization during the hepatic uptake. It could be noted that only 61% of all the 600-nm MENs found in all the organs 1 week postinjection were cleared from the organs within the 7-week observation period after the 1-week postinjection mark, compared with 90% of the 10-nm MENs. The smallest NPs, 10-nm MENs, were cleared relatively rapidly and uniformly

across the organs. These NPs could be found in both urine (during the first week) and feces throughout the period of observation. Compared with the 10-nm MENs, the 30-nm MENs found in the liver represented the majority 4 weeks postinjection. These NPs could be detected only in feces. The clearance of the larger NPs, that is, 100- and 600-nm MENs, was highly nonlinear with time and nonuniform across the organs. It could be noted that, while the number of these MENs decreased in the lungs during the period of the study, it increased in the liver (for both 100-

and 600-nm MENs) and in the spleen (for 600-nm MENs) for the period from 4 to 8 weeks postinjection. These NPs were detected in feces during this period of time.

Future perspective

The current biodistribution study paves a way to many new potential medical applications of magnetoelectric NPs. Unlike any other NPs, MENs display unique properties which allow to use of magnetic fields for an external control of intrinsic electric fields at the subcellular level; in turn, the intrinsic electric fields are responsible for high-specificity targeted drug delivery and release on demand as well as wireless electric stimulation of individual cells. In

the future, due to these properties, MENs will enable so-called pinpoint treatment of some of the most devastating diseases such as cancer, HIV/AIDS and neurodegenerative diseases including Parkinson's and Alzheimer's disease, Autism and others. Last but not the least, the EDS-based biodistribution approach can be successfully extended to understand the cellular level clearance mechanisms of any other inorganic and organic NPs.

Financial & competing interests disclosure

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No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Summary points

Background

- Due to the presence of a nonzero magnetoelectric effect, unlike any other nanoparticle type, magnetoelectric nanoparticles (MENs) provide unique properties making them ideally suitable for the emerging fields of precision medicine and personalized nanomedicine, for example, for treatment of cancer, HIV/AIDS and neurodegenerative diseases. Understanding the biodistribution and clearance rates is the stumbling block which needs to be overcome for unlocking all these applications.
- This paper exploits the backscattered electron detector and energy dispersive spectroscopy (EDS) mode of scanning electron microscopy to study the biodistribution and the clearance of inorganic MENs in a mouse model.
- Through an elemental compositional analysis, the EDS approach allows to detect NPs in tissues with the spatial resolution of SEM, does not require any tissue-sensitive staining and is not limited to MENs.

Methods

- The MENs' configuration under study had a spherical core-shell nanostructure made of the CoFe_2O_4 magnetic core and the BaTiO_3 piezoelectric shell.
- The nanoparticle size varied from 10 to 600 nm through hydrothermal and coprecipitation methods.
- Through the EDS analysis, the biodistribution of intravenously administered MENs, depending on their size, was measured in vital organs including the kidneys, liver, spleen, lungs and brain at four different postinjection times including 1 day, 1 week, 4 and 8 weeks, respectively.

Results & conclusion

- Compared with the number of MENs in all the organs 1 week postinjection, within the subsequent 7 weeks of the study, the measured net clearance rates were approximately 90, 86, 85 and 61% for 10-, 30-, 100- and 600-nm MENs, respectively.
- The smallest NPs, that is, 10-nm MENs, were cleared relatively rapidly and uniformly across the organs, while the clearance of the larger NPs, that is, 100- and 600-nm MENs, was highly nonlinear with time and nonuniform across the organs.
- While the number of the larger MENs decreased in the lungs during the period of the study, it increased in the liver (for both 100- and 600-nm MENs) and in the spleen (for 600-nm MENs) for the period from 4 to 8 weeks postinjection. These NPs were detected in feces during this period of time.

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