Controlled-release and magnetic resonance imaging of doxorubicin-conjugated magnetic nanoparticles from 3D poly(propylene fumarate) scaffolds

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Introduction

Implantable 3D polymer scaffolds have drawn enormous attention from various disciplines because of their unique properties[1]. Poly(propylene fumarate) (PPF) scaffolds[2] have several advantages (e.g., biocompatibility, sufficient mechanical strength, large porous surface area, and long decomposition time with biodegradability) that make them favorable for developing bone substitute materials with a capacity for *in vivo* controlled release of cancer drugs. However, several hurdles must be overcome in the development of drug-dispensing PPF scaffolds. Since PPF is extremely hydrophobic, it might be difficult to load most water-soluble drug molecules. In addition, loaded drugs might often be degraded during the scaffold fabrication, a procedure that involves heating and desalinization. In this study, we used super paramagnetic iron oxide (SPIO) and manganese oxide (MnO) nanoparticles (nps) as carriers for the anti-cancer drug, doxorubicin, and measured the kinetics of the release from the PPF scaffold surface using MRI. Because of the persistent release of drugs in the vicinity of a malignancy, these macroporous PPF scaffolds could be used for many biomedical applications, including MR-guided implantation, as drug-carrying vehicles, and as a tumor treatment.

Materials and Methods

SPIO (30nm) and MnO (60nm) nanoparticles with amine groups on their surfaces were prepared using silane chemistry and a surface exchange method[3]. A JEM 2100 transmission electron microscope (JEOL, Tokyo, Japan) was used to characterize the nanoparticles. One mg/ml of each nanoparticle batch was dispersed in PBS, then 10ul of an anti-cancer drug molecule, doxorubicin (1mmol), was mixed into the nanoparticles to coat the surface through electrostatic adsorption. Ten pieces of PPF scaffolds, 6.3 mm in diameter and 5 mm thick, were prepared[4]. One hundred ul of drug-coated nps were either sprayed on or mixed in the scaffolds, and then placed in 2ml tubes containing PBS or cell culture media. MR images were obtained at different time points using an 11.7T Bruker Avance system equipped with a 15 mm birdcage RF coil. T1 and T2 relaxation were measured using a modified MSME protocol. All data processing was performed using custom-made codes in Matlab (ver. R2009a, Mathworks, Natick, MA). Scaffolds carrying drug nanoparticles were also incubated onto a monolayer of PC12 cells. After 48h incubation, a cytotoxicity assay was performed to assess the released doxorubicin drug efficacy using a Celltiter Blue assay (Promega, Madison, WI).

Results and Discussions

SPIO (30nm) and MnO (60nm) nanoparticles were both well-dispersed in water and coated with a porous silica shell (Figure 1A). An SEM image clearly showed electrostatic attachment of nanoparticles and their aggregates on the PPF porous scaffold (Figure 1B). Since the porous structure of PPF scaffolds can trap water molecules inside and the scaffold itself provides a short T2 relaxation contrast, the scaffold is clearly visible on an MR image (Figure 1C). PPF scaffolds carrying drug-coated nanoparticles were incubated for 48h at physiological conditions, during which time the drug-coated nanoparticles were released. Since iron oxide (T2 relaxation shortening) and manganese oxide (T1 relaxation shortening) nanoparticles are MR contrast agents, the release of drug nanoparticles can be monitored by the MR contrast change in the media containing the scaffold. Figure 2, panel A, shows T2 shortening in the media where doxorubicin-coated iron oxide nanoparticles were released from the scaffold. Panel B shows T1 shortening in the solution where doxorubicin-coated manganese oxide particles were released from the scaffold. R1 and R2 shortening were proportional to the amount of magnetic nanoparticles present in the solution, which were released from each scaffold at different time points (Figure 3A) and showed different kinetics for mixed and sprayed particles. Released nanoparticles carrying doxorubicin were still effective after their 48h incubation with PC12 cells. Figure 3, panel B, summarizes a drug efficacy test using a cell viability assay that showed that the doxorubicin-coated iron oxide nanoparticles retained their anti-cancer activity.

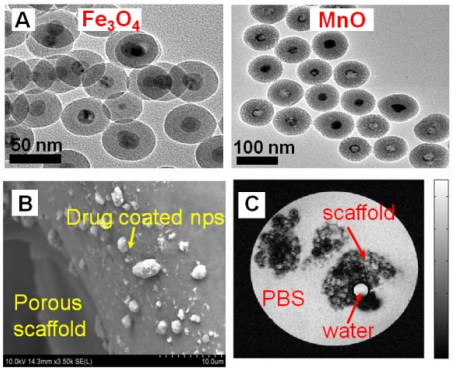

Figure 1. Nanoparticles' size and shape, PPF scaffold surface, MRI of PPF scaffold in PBS solution.

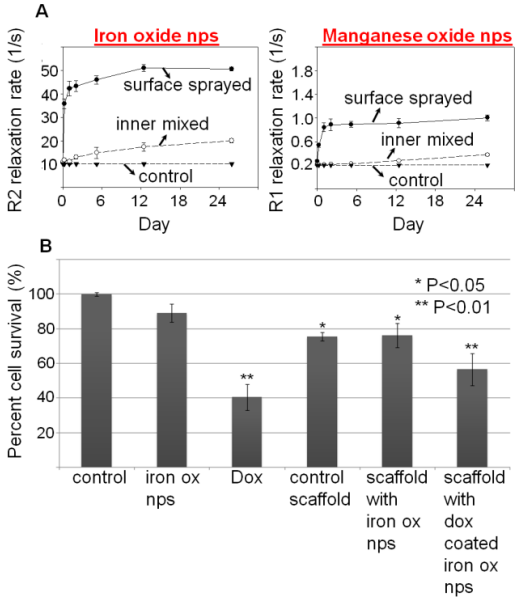

Figure 3. Released drug nanoparticle quantification using MRI, doxorubicin efficacy on PC 12 cells

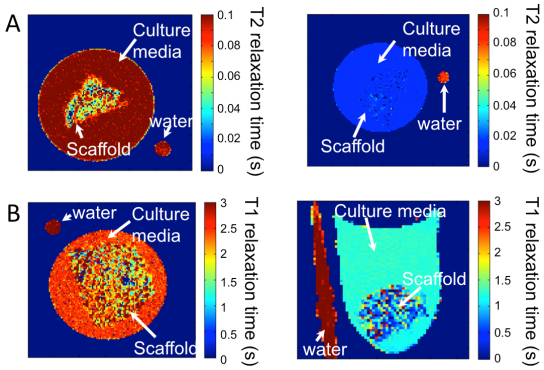

Figure 2. MRI of PPF scaffold and released nanoparticles (MRI was taken after 48h incubation).

Conclusion

PPF scaffolds were imaged with MRI. Doxorubicin-coated magnetic nanoparticles were successfully attached to the scaffolds and released into solution in a timely manner. We monitored the releasing profile and quantified the amount of released particles by measuring MRI contrast changes. Released drug-coated nanoparticles retained their cancer cell-treating efficacy.

References

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