Magnetically targeted delivery through cartilage

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Weinberg Medical Physics, Inc., 12156 Parklawn Dr, 20852 North Bethesda, Maryland, USA
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In this study, we have invented a method of delivering drugs deep into articular cartilage with shaped dynamic magnetic fields acting on small metallic magnetic nanoparticles with polyethylene glycol coating and average diameter of 30 nm. It was shown that transport of magnetic nanoparticles through the entire thickness of bovine articular cartilage can be controlled by a combined alternating magnetic field at 100 Hz frequency and static magnetic field of 0.8 tesla (T) generated by 1” dia. x 2” thick permanent magnet. Magnetic nanoparticles transport through bovine articular cartilage samples was investigated at various settings of magnetic field and time durations. Combined application of an alternating magnetic field and the static field gradient resulted in a nearly 50 times increase in magnetic nanoparticles transport in bovine articular cartilage tissue as compared with static field conditions. This method can be applied to locally deliver therapeutic-loaded magnetic nanoparticles deep into articular cartilage to prevent cartilage degeneration and promote cartilage repair in osteoarthritis. © 2017 Author(s). All article content, except where otherwise noted, is licensed under a Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/). https://doi.org/10.1063/1.5006156

I. INTRODUCTION

There is no accepted medical cure for post-traumatic osteoarthritis (OA). About 10% of men and 18% of women over 60 years have OA. In OA, articular cartilage breaks down, causing pain, swelling and restricted joint motion. For such indications, it is necessary to deliver therapeutics to cartilage itself. The application of steroids and other anti-inflammatory agents are well-known therapies intended to prevent long-term cartilage degeneration. Also well-known are the many unwanted side effects associated with systemic administration of steroids. Moreover, because cartilage is avascular, it is inefficiently targeted by systemic delivery of drugs, which must first reach the synovial fluid and then diffuse through the cartilaginous extracellular matrix (ECM). Intra-articular injection of steroids has been promoted as a means of reducing the natural progression of injury to OA. Intra-articular steroids were found to have modest reduction in knee pain but not a clear protective effect in slowing cartilage erosion. Drugs need to penetrate the full depth of cartilage to reach the chondrocytes and ECM targets involved in OA- associated cartilage pathogenesis. Depending on the location of the target (cartilage vs synovium), the lack of efficacy is potentially related to the poor transport of drugs through cartilage ECM, resulting in drugs that are cleared from the joint before they can penetrate deep into the cartilage. Small compounds and large macromolecules are rapidly cleared from the joint via sub-synovial capillaries and lymphatics, respectively. Accordingly, it is worthwhile to find an efficient way to deliver drugs to the cartilage.

Drug delivery and transport kinetics depend on drug carrier size, charge, and surface-functional properties, and on the biophysical and morphological properties of the animal joint and its constituent tissues. The size of joint space and, in particular, the thickness of cartilage increase with animal
Diffusion times are proportional to the square of cartilage thickness, therefore drug carriers penetrate rapidly into 50 µm thick mouse cartilage, while in larger animals and humans they can be easily cleared from the joints before penetration. Conversely, once a drug reaches therapeutic levels inside the cartilage, the theoretical retention time also increases with cartilage thickness. The outward diffusion time is also proportional to cartilage thickness squared, and inversely proportional to the effective diffusivity of the drug and/or drug carrier inside cartilage, including the effects of binding.

Considering all mentioned above, drugs could be more effective in changing the natural course of joint disease (from injury to degenerative arthritis) if it were possible to deliver the drugs through the entire thickness of articular cartilage. The small pore size in dense joint cartilage has stymied attempted experimental methods of increasing the depth of delivery (e.g. with electric charge, ultrasound, and acoustic shock waves). However, clinical techniques to deliver drugs locally into articular cartilage are still to be developed.

Magnetic nanoparticles (MNPs) have been the subject of extensive research investigations for their biomedical applications more than a decade now; e.g. drug delivery for tumors, contrast agents, magnetic hyperthermia, magnetic separation, and very recently tissue engineering and bone regeneration. Magnetic drug targeting pursues therapeutics attached to magnetically responsive carriers while magnetic fields are used to direct therapeutics to disease locations, thereby increasing the local concentration of drug load and minimizing the systemic drug dose. We have developed methods of propelling drug-loaded magnetic particles through living tissues in vivo. Examples have included the delivery of steroids into the inner ear through the round-window membrane, and cancer-fighting drugs (cyclopamine, temazolamide) into tumors. Here, we demonstrate a new opportunity for this technology as a novel approach to treat OA by magnetically-driven drug delivery deep into articular cartilage, which, to the best of our knowledge, is the first approach to magnetically deliver drugs to the entire thickness of cartilage.

## II. EXPERIMENTAL DETAILS

Knee joints from 1- to 2-year-old steers were obtained from a local slaughterhouse 6-10 h post-mortem. Cartilage explants were extracted from joints immediately and were kept in phosphate-buffered saline (PBS) for a few minutes until the test was started. The whole thickness of cartilage including all different structural zones; i.e. superficial, middle, deep, and calcified zones, which was around 2-3 mm was maintained for the experiments. MNPs were purchased from TurboBeads, LLC (Zürich, Switzerland). The metallic spherical particles were made of Co with primary particle size distribution of 20-60 nm and mean particle size of 30 nm, dispersed in water with a loading of 30 mg/ml. The particles’ surface was coated with several carbon layers in an onion-type arrangement with the whole thickness of 2 nm, functionalized with Poly Ethylene Glycol (PEG) (giving about 5 PEG units), while carboxy functionalities were introduced at the end of the PEG chains. The particles were highly magnetic exhibiting a high saturation magnetization (>150 emu/g) together with a low (<100 Oe) coercivity. The synthesis and coating methods as well as characterizations were described in details in Grass et al. papers. The experimental magnetic set-up consisting of an alternating magnetic (AC) field generator (oscillator), a permanent NdFeB magnet and the sample positioned between electromagnets and the permanent magnet is shown in Figure 1. The magnetic actuation apparatus consisted of two pairs of Helmholtz coils. Signals were generated using Matlab and amplified via audio amplifiers. MNPs were wiggled with an AC magnetic field at frequency of 100Hz and coil current of 3 A for 10, 30 and 40 minutes. The electromagnetic coils generated an AC magnetic field in the plane of the sample (x-axis) and the permanent magnet generated a static field gradient perpendicular to the plane of sample and parallel to the thickness of cartilage (y-axis). Samples were subsequently imaged with our home-built MRI system, in a spin-echo sequence (where particles are visible as signal losses). Hereafter, the surface of cartilage in touch with magnetic solution is called particle side and that of close to permanent magnet is called magnet side. The experiments were performed with static field and combination of both AC and static magnetic fields. Control sample was kept in contact with magnetic solution while there was no magnetic field applied to the sample.
III. RESULTS AND DISCUSSION

The magnetofection experiments were performed as shown in table I. Frequency and current were kept constant at 100 Hz and 3 A, respectively. The magnetic delivery experiments were done either in the presence of static magnetic field alone or combination of both static and AC magnetic fields. The directions of magnetic fields are shown in Figure 1.

Figure 2a–e show the optical microscopy images of cartilage specimens taken from their magnet side. Under no magnetic field in control sample, particles remained suspended in magnetic solution (Fig. 2a). With applying only static magnetic field, particles mostly remained on particles side and were not pulled to the other side. As can be seen in Figure 2b, particles can be only observed in two spots on the magnet side of sample with only static magnetic field applied for 40 minutes. By applying both static and alternating magnetic fields at the same time, particles were transported from particle side to the magnet side. Transport of particles was increased by 15 orders of magnitude through cartilage matrix when the field was alternated for 10 minutes as compared to a static field alone. By applying both AC and static magnetic fields for 40 minutes (Fig. 2e), transport of particles increased by 3 orders of magnitude comparing to sample 2 in which the field was only alternated for 10 min. Figure 2f illustrates particles density on magnet sides of samples. It demonstrates that by extending the time that field was alternated, the number of particles travelling through the cartilage thickness and reaching the opposite side of cartilage, i.e. magnet side, increased significantly.

To understand how magnetic propulsion works in our experiments, it is important to know the mechanism of magnetic forces and how they work. The magnetic force on spherical particles is given by: \( \mathbf{F} = \nabla (\mathbf{M} \cdot \mathbf{B}) \), where \( \mathbf{M} \) is particles’ magnetic moment and \( \mathbf{B} \) is the total magnetic field applied which is given by \( \mathbf{B} = B_{\text{static}} + B_{\text{AC}} \). The NdFeB permanent magnet generates significant and strong field gradients along the y axis, i.e. cartilage thickness. The average field at the sample location was measured to be around \( B_{\text{static}}y \approx 0.8T \). Hence, the static magnetic force on PEG-coated MNPs along y-axis is approximately given by: \( F_{\text{static}}^y \approx M_s \frac{\partial B_{\text{static}}}{\partial y} \). The main AC force on particles mainly originates from electromagnets in our set-up and along the x-axis. This force is approximately given by: \( F_{\text{AC}}^x = M_s \frac{B_{\text{AC}}}{B_{\text{static}}} \frac{\partial B_{\text{static}}}{\partial x} \). Since in our set-up, the static field gradients along x and y axis are about the same, the ratio of AC to static force is given by \( \frac{B_{\text{AC}}}{B_{\text{static}}} \). In our experiments, this ratio is in the

| Experiment | Frequency (Hz) | Current (A) | Time (min) | Static B field | Oscillating B field |
|------------|----------------|-------------|------------|----------------|---------------------|
| Control    | 100            | 3           | 120        | -              | -                   |
| 1          | 100            | 3           | 10         | ✓              | -                   |
| 2          | 100            | 3           | 10         | ✓              | ✓                   |
| 3          | 100            | 3           | 30         | ✓              | -                   |
| 4          | 100            | 3           | 30         | ✓              | ✓                   |
| 5          | 100            | 3           | 40         | ✓              | -                   |
| 6          | 100            | 3           | 40         | ✓              | ✓                   |
FIG. 2. Improved particle transport with dynamic magnetic field. Black arrows point to particles in optical images of magnet side of cartilage slice in (a) control sample, (b) sample subjected to only static magnetic field for 40 minutes, (c) sample subjected to combination of static and alternating magnetic fields for 10 minutes, (d) sample subjected to combination of static and alternating magnetic fields for 30 minutes, and (e) sample subjected to combination of static and alternating magnetic fields for 40 minutes. (f) particle density for each sample calculated in particles/mm².

FIG. 3. MRI of cartilage specimen for which magnetic field was alternated for 40 minutes showing penetration of MNPs from particle side to magnet side.

order of 10⁻⁶. This proves that the AC force applied on MNPs is much smaller than the static force. Consistent with the reports of MacDonald et al.²⁶ and Mair et al. experiments,²⁷ the dynamic field component increases the velocity of MNPs by only decreasing the effective local viscosity of the tissue/viscous fluid around the particles.

Samples were imaged with our home-built MRI system. Figure 3 shows the sagittal image of cartilage sample that field was alternated for 40 minutes with the presence of gradient field. Particles are seen as black spots due to the lack of signals. It can be clearly seen that MNPs were pulled through the entire thickness of cartilage.

IV. CONCLUSION

Magnetic drug delivery was performed for the first time for cartilage tissue in our experiments. PEG-coated MNPs were transported through the entire thickness of cartilage by applying static and alternating magnetic fields simultaneously. The results of this study showed that the application of an alternating magnetic field can greatly improve the transport of MNPs through the very dense tissue by wiggling the particles and thus reducing the local viscosity of tissue around particles. The MNPs transport increased by extended application of oscillating magnetic field. While more investigation has to be done on attaching different drugs to the MNPs and delivering them to articular cartilage, our results demonstrate the potential of this approach to achieve more effective delivery of therapeutics to the entire thickness of cartilage.
