Targeted Delivery of Therapeutic Agents with Controlled Bacterial Carriers in the Human Blood Vessels

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Abstract—**The induction of a steering force on a ferromagnetic carrier from magnetic gradients generated by an upgraded clinical Magnetic Resonance Imaging (MRI) system has been demonstrated. But in applications such as targeted delivery of therapeutic agents to a tumor mass, the gradient strengths required in some sections of the capillary network may be technologically and practically very difficult to achieve for human considering other aspects such as the size and cooling issues of additional gradient coils embedded in the MRI bore. As such, a complementary means of propulsion in smaller vessels is investigated and consists of using Magnetotactic Bacteria (MTB) to push microbeads acting as carriers to transport therapeutic agents in the capillaries**.

Keywords—**Magnetotactic bacteria, bacterial actuation, MRI system, targeted drug delivery, capillary network.**

I. Introduction

The treatment of cancer is one of the most challenging tasks of modern medicine and secondary toxicity remains a critical issue. Although intra-arterial chemotherapy or chemo-embolization provides interesting success, the release of drug in the systemic circulation prevents high intra-tumoral drug concentration to be sustained. Hence, targeting specifically the tumor cells becomes a major goal of modern oncology. As such, providing means of carrying microparticles for specific endovascular drug delivery or radioisotopes at the site of the tumor mass would be extremely attractive. Although we have shown experimentally that a clinical Magnetic Resonance Imaging (MRI) system can propel a ferromagnetic core in the human cardiovascular network through an induced force generated by the same magnetic gradients used for MR-imaging [1-5], it becomes technological very challenging to apply this method to navigate particles in a 3D space in order to reach the tumor cells through an anarchic arteriolocapillar network stimulated by tumoral angiogenesis. Since the induction of a propulsion force decreases significantly for much smaller particles, propulsion in some sections of capillaries could require gradient strengths that may be technologically and practically not an alternative considering the size and cooling issues of an additional gradient coils system embedded in the MRI bore [6].

As such, a complementary means of propulsion in smaller vessels is investigated and consists of using Magnetotactic Bacteria (MTB) [7] to push microbeads acting as carriers to transport therapeutic agents through the capillary network. We have shown that the displacement path of a swarm of MTB could be controlled under computer software (see Fig. 1a) and that the swimming path of a single magnetotactic bacterium pushing a microbead could be modified by computer software or other means by changing the orientation of the lines of a magnetic field as depicted in Fig. 1b.

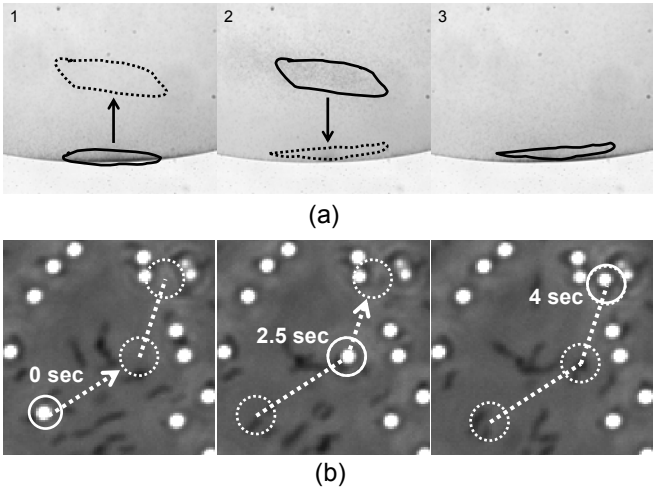

Fig. 1 – (a) Directional control of a swarm of MC-1 magnetotactic bacteria sweeping an aqueous solution at an average speed of 130 μm/s. The black arrows indicate the direction of the controlled swimming path. (b) Directional control of a 3-micrometer bead being pushed by a single *Magnetospirillum gryphiswaldense* MTB with an intentional change in the direction of the swimming path occurring after 2.5 seconds.

We also demonstrated through preliminary experimental results that magnetotactic bacteria of type MC-1 could swim efficiently in human blood for a few minutes and that a swarm of these bacteria pushing ferromagnetic microbeads could potentially be detected by MR-imaging. Although the high DC magnetic field of clinical MRI systems complicates the directional control of such bacteria compared to a platform where an X-ray system could be upgraded with peripheral permanent or electromagnets, the use of an MRI system has many advantages in term of imaging modalities and the lack of radiation.

II. Maximum Magnetic Gradient Strength

Navigation in much smaller diameter blood vessels differs compared to larger blood vessels. The blood velocity in smaller diameter blood vessels decreases from more than 1 m/s in the aorta to less than 1 mm/s in the capillaries with an increase of the shear rates. The Reynolds number also decreases significantly from up to 10,000 in the aorta down to 0.001 in the capillaries and the microdevice will also experience an increase of the drag coefficient. In the smallest vessels such as capillaries, the blood becomes inhomogeneous and as such, it will operate in the presence of the Fahraeus-Lindqvist effect which tends to reduce blood viscosity. It is obvious that a clinical MRI system cannot provide sufficient gradient amplitudes to propel a ferromagnetic core in capillaries and as such, upgrading the MRI system with of special gradient coils is essential.

This particular application would yield the following estimate of maximum gradient strength for a "body-size" shielded gradient coil of outer diameter 65 cm (upper limit to fit in the MRI bore), inner diameter 50 cm (to accommodate human torso), linear region length (in the Z axe) of 35 cm (typical field-of-view (FOV)), inductance of 8.4 mH: gradient efficiency of 0.75 mT/m/A. The Siemens Sonata MRI gradient drivers (presently used by our group and corresponding to standard clinical MRI system specifications) are capable of 500A output, although to be on the conservative side, that due to the unusually high inductance of this coil, we may assume a lower peak current such as 400A (notice that propriety gradient drivers could be designed to deal with high inductance outputs, but standard MRI drivers have been considered here). Therefore, the maximum gradient strength for this coil would be 300mT/m. On the other hand, considering the construction tolerances, this coil would still be too large to fit in the "standard" Sonata system without removing the body RF coil, an option which is obviously not desirable. Furthermore, this coil has a wire density that is much higher than the conventional Sonata imaging coils. Furthermore, the peak power dissipation in such a coil could be extremely high, although it is not clear at the present time what the thermal limitations of this coil would be. Hence, a very conservative maximum magnetic gradient strength would be half that first estimate, i.e. 150mT/m leading to (because of the high inductance) a minimum rise time of this gradient coil of approximately 2ms. In order to conform to FDA's slew rate limit of 20 T/m/s, the rise time should be increased to 2.7ms to safely avoid nerve stimulation in humans. This would set some limits on how quickly we could pulse on and off the propulsion gradients for steering purpose, potentially affecting targeting efficacy to some degrees. Nevertheless, as 2.7ms is only 10% of Siemens' real time system refresh rate and as it can be easily included in the propulsion theoretical model, the targeting efficacy should not be affected to a great extent. Although targeting tumors in other regions outside the torso (e.g. the head) would simplify coil design dramatically through a reduction of space constraints, and potentially yielding higher gradient strengths, the human torso has been considered here as a worst case analysis.

III. Parameters Affecting Maximum Induced Force

The induced force does not only depend on the maximum gradient strengths but on other aspects as well and in particular, the material used for the magnetic carrier. For instance, the torque and the propulsion force on the ferromagnetic core or bead that can be induced depends as depicted in Eq. 1 and Eq. 2 not only on the size of the ferromagnetic bead but also on the choice of the ferromagnetic material.

$$\vec{\tau} = \vec{m} \times \vec{B} = V_{ferro} \vec{M} \times \vec{B}, \tag{1}$$

$$\vec{F}_{magnetic} = \vec{m} \cdot \nabla \vec{B} = (V_{ferro} \cdot \vec{M}) \cdot \nabla \vec{B}. \tag{2}$$

In the above equations, τ is to the magnetic torque (N·m), $\vec{F}_{\text{magnetic}}$ is the magnetic force (N), $\vec{m}$ is the magnetic moment of the ferromagnetic body (A·m^2), $\vec{M}$ is the magnetization of the material (A/m), V_{ferro} is the volume of the ferromagnetic body (m^3), $\vec{B}$ is the magnetic induction (T), and $\nabla \vec{B}$ is the gradient or spatial variation of the magnetic induction (T/m). Furthermore, the cumulative effect of maximum blood flow, drag, buoyancy, and the gravitational force or weight for larger devices may vary the induced force required for proper displacement. Here, an MRI system is used to generate the magnetic gradients and as such, the technique is referred here to as Magnetic Resonance Propulsion* (MRP) [3], providing advantages such as availability in a clinical environment and the integrated imaging modality required for displacement control.

A few options may be available to increase the region within the human body accessible to this technology. First, the percentage of the volume of the microdevice occupied by the ferromagnetic material may be increased beyond 50%

* US Patent No. 10/417,475

provided that enough volume remains for supporting the medical task. Second, the shape of the microdevice can be modified to reduce the drag force but the DC magnetic field of the MRI system and the orientation of the blood vessels used to reach the target must be taken into account. The orientation of the device will remain fixed along the lines of the DC magnetic field (typically 1.5 T) of the clinical MRI system and as such, the drag force could become larger in blood vessels with different orientations with potential damages for the vessel walls caused by friction for sharper shapes.

IV. Controlled Bacterial Actuation

To reduce the magnetic gradient amplitude to facilitate the implementation of additional coils in the MRI bore while minimizing problems related with heat dissipation, the concept of steering gradients is considered instead of propulsion gradients. Propulsion gradients would require excessive gradient amplitudes in the order of several T/m which is technologically and within the context of this application, not a valid option. In the concept of steering force, an angular force is induced on the ferromagnetic particles being propelled by the capillary flow itself. Hence, angular magnetic gradient forces generated by the additional inner coils within the clinical MRI system must be synchronized adequately to steer the ferromagnetic microparticles towards the tumoral region.

Although exhaustive calculations and simulations can be done, the following simple analysis is intended to provide a comparison between bacterial actuation and the use of the magnetic gradients within the feasibility of developing coils geometries for humans for the purpose of steering microparticles in capillaries. The analysis assumes particles of various sizes containing 50% volume of Permendur ((49%Fe 49%Co), corresponding to the highest magnetization at saturation (2.45T) and hence, maximum induced forces. A volume ratio of 50% instead of 100% of Permendur has been selected to be more realistic since entrapping magnetic nanoparticles into other sustained-release polymeric drug carrier systems such as in microparticles formulated from poly-DL-lactide-*co*-glycolyde (PLGA) or polylactides (PLL) or in dendrimers and other polymers results in significant loss in magnetization (~40-50%). To compute the corresponding drag forces on the particles, the analysis uses proven models from Francis and Kehlenbeck that adjust Stokes law in cylindrical vessels under conditions extending from purely-laminar to fully-turbulent flow (low Reynolds number hydrodynamics), and which take into account the retarding effect of the blood vessel walls. The data were computed using the dynamic viscosity of blood (37 ^{o}C) of 3.5e-3 Pa S.

Fig. 2 indicates that a single MC-1 magnetotactic bacterium may be more effective at steering a 50% volume Permendur bead with a diameter of less than 3 micrometers. This would correspond as depicted in Fig. 3, to a transversal speed of 18μm/s. The transversal speed of the particles for steering purpose is the necessary speed to cross diagonally the blood vessel during the time period that it is subjected to the axial blood flow.

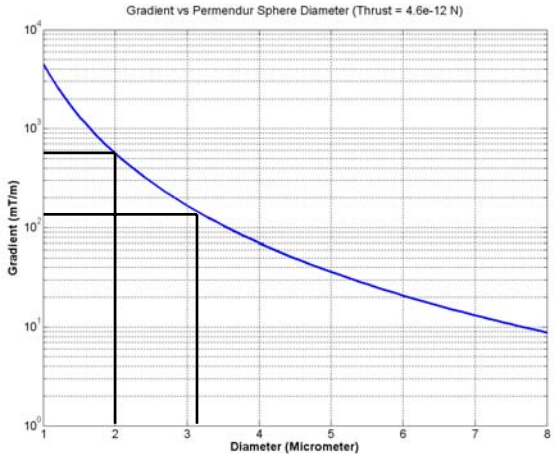

Fig. 2 – Magnetic gradients required to a force equivalent to a thrust of 4.6e-12 N provided by a MC-1 magnetotactic bacteria, on a 50% volume Permendur microbead of various diameters. With a maximum gradient strength of 150mT/m, the results indicate that pushing the bead in blood with a single bacterium attached to it, may be more effective when the diameter of the bead is less than approximately 3 micrometers. In the smallest capillaries, a ferromagnetic bead with a diameter of 2 micrometers would be the most effective providing the best trade-off between a maximum volume of ferromagnetic material and a reduction of the retarding effect caused by the capillary walls. With a bead with a diameter of 2 micrometers, an extremely high 600mT/m gradient strength would be required to provide the same steering force as a single MC-1 bacterium.

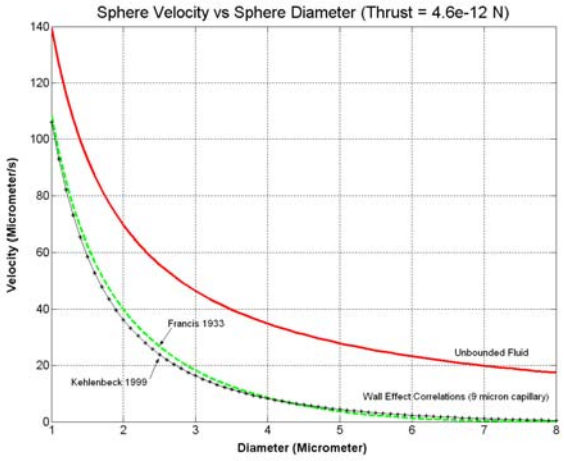

Fig. 3 – The graph shows the corresponding transversal (steering) speeds showing the retarding effects in a capillary vessel having a diameter of 18 micrometers. With a thrust equivalent to the MC-1 bacterium, the steering speed of a 3-μm bead pushed by a single MC-1 bacterium would be 18μm/s (from models taking into account retarding effects from the blood vessel walls), indicating that such steering force is likely to be sufficient to navigate in capillaries since the largest capillaries in human are in the order of 18μm with an average of 1 mm between connecting capillaries.

Although previous experiments in water showed that a single *Magnetospirillum gryphiswaldense* MTB could move

a bead of 3µm, 10µm, and 100µm in diameter with a minimum average speed of ~16.3, 4.9, and 0.49µm s^{-1} respectively, corresponding from Stokes' law to a thrust of ~0.5 pN per MTB, preliminary experiments done with MC-1 MTB indicate a thrust of ~4.6 pN per MTB in blood.

The directional control of MTB remains a critical aspect for their potential integration in such carriers. Each magnetotactic bacterium has a chain of magnetosomes which are membrane-based nanoparticles of a magnetic iron that acts like a compass and enables the MTB to orient and to swim along the lines of a magnetic field [8-10]. Hence, by modifying the direction of the lines of the magnetic field, steering a microbead being pushed by a single magnetotactic bacterium can be envisioned. Unfortunately, the 1.5 T DC magnetic field found in typical clinical MRI systems makes such directional control of the MTB performed inside the MRI bore extremely difficult. Although directional control of MTB could be done relatively easily on a X-ray platform with magnetic field lines oriented by external permanent or electromagnets, the use of an MRI system would be attractive due to the lack of radiation, the good image contrast for soft tissues, and the possibility of combining induced force through magnetic gradients with the pushing force of a bacterium. Since only 0.1 to 0.5 gauss is typically sufficient to induce a torque on the chain of magnetosomes to orient the MTB, a potential short term solution is to perform directional control by bringing the patient outside the MRI bore by mechanical means during directional control with external coils and by bringing him back inside the MRI bore for tracking and imaging purposes. By repeating such sequence and adjusting the swimming direction of the MTB taking into account the effect of the DC field in the MRI bore and the reaction time of the MTB, a sufficient level of directional control for targeting purpose, although not ideal, may be possible.

V. Conclusions

The use of special devices propelled in the human blood vessels could allow or improve many medical tasks such as on-site delivery of MRI contrast agents, highly localized drug delivery for chemotherapy, thermal treatment of tumors at selected sites, and carriers for biosensing applications, to name but a few applications. Such potential impact is due mainly to the fact that various remote locations within the human body become accessible through the human blood circulatory system. Because such blood circulatory network is composed of arteries, veins and capillaries having different diameters and blood flow velocities, the overall size of a particular device and the method to propel, control, and track such device will hence be dependent upon the pathways being considered to reach the target.

During the vascular phase of growth, the tumor size is highly regulated by the capillary network around it. Nutrients are supplied to the tumor via this network, and it provides the initial route for invading cancer cells to escape from the primary tumor and form metastases. Moreover, it is through the vascular network that chemotherapeutic drugs are delivered to the tumor. It is well established that the highly interconnected vascular structures around a tumor cause relatively low rates of drug delivery to the tumor itself, with the vast majority of drug simply by-passing the tumor and returning to the parent vessel. It is therefore by using the same capillary networks for steering the carriers that targeting efficacy to the tumor could potentially be improved. The development of gradient coils capable of inducing sufficient steering force to navigate a ferromagnetic carrier in the capillaries may be very difficult, especially when applied to the human torso. As such, additional force provided by magnetotactic bacteria under computer directional control for ferromagnetic carriers with overall dimensions in the 1-3 micrometers range may be envisioned to help reaching the target. This is particularly stimulating considering the facts that MC-1 MTB could swim very effectively in blood up to a few minutes, enough to help reaching a target, and that preliminary tests on biocompatibility are very promising. Nonetheless, more issues need to be resolved. A major issue is to counteract the effect of the DC magnetic field for the directional control of MTB in the MRI bore. A second major issue is the development of larger carriers potentially made of a larger concentration of ferromagnetic material for maximum induced force necessary for arteriole entry and made of biocompatible dissolving polymers for arteriolocapillar entry in order to carry the bacterial carriers to the capillaries.

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