Interventional Procedure Based on Nanorobots Propelled and Steered by Flagellated Magnetotactic Bacteria for Direct Targeting of Tumors in the Human Body

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***Abstract*—Flagellated bacteria used as bio-actuators may prove to be efficient propulsion mechanisms for future hybrid medical nanorobots when operating in the microvasculature. Here, we briefly describe a medical interventional procedure where flagellated bacteria and more specifically MC-1 Magnetotactic Bacteria (MTB) can be used to propel and steer micro-devices and nanorobots under computer control to reach remote locations in the human body. In particular, we show through experimental results the potential of using MTB-tagged robots to deliver therapeutic agents to tumors even the ones located in deep regions of the human body. We also show that such bacterial nanorobots can be tracked inside the human body for enhanced targeting under computer guidance using MRI as imaging modality. MTB can not only be guided and controlled directly towards a specific target, but we also show experimentally that these flagellated bacterial nanorobots can be propelled and steered *in vivo* deeply through the interstitial region of a tumor. The targeting efficacy is increased when combined with larger ferromagnetic micro-carriers being propelled by magnetic gradients generated by a MRI platform to carry and release nanorobots propelled by a single flagellated bacterium near the arteriocapillar entry. Based on the experimental data obtained and the experience gathered during several experiments conducted *in vivo* with this new approach, a general medical interventional procedure is briefly described here in a biomedical engineering context.**

***Index Terms*—Flagellated bacteria, bacterial nanorobots, medical nanorobots, direct tumor targeting.**

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I. Introduction

MEDICAL nanorobots can be described as untethered robots often with overall dimensions in the micrometer-scale which exploit nanometer-scale components and phenomena for medical applications. As such, developments of medical nanorobots would often combine principles of robotics including closed-loop control with nanotechnology and nanomedicine to provide new medical diagnostic and interventional tools capable of reaching regions in the human body which are still inaccessible to catheterization. In particular, tumor targeting may benefit from medical nanorobotics where secondary toxicity resulting from interventions such as chemotherapy for instance, could be minimized by avoiding or at least reducing significantly the amount of toxic agents circulating through the systemic blood networks while enhancing therapeutic efficacy using lower dosages.

So far, the most advanced method to directly target tumors relies on an external permanent magnet or in some instances, an electro-magnet. Such a magnet is then typically placed as close as possible to the tumor prior to release magnetic nanoparticles as close as possible to the target using a catheter. With this approach, targeting efficacy is best when the tumor is close to the skin because of the higher field intensity towards the external magnet while a significant reduction of targeting efficacy would be observed for targets located deeper in the body. Since the approach relies on trapping the particles without navigation or trajectory control over pre-planned paths as it would be the case with a robotic platform, targeting efficacy would be further affected by the distance between the releasing site and the tumor in particular when the reachable limits of catheterization combined with complex microvasculature networks are taken into account. Therefore, a nanorobotic platform can provide an efficient interventional infrastructure for many operations in the human microvasculature and in particular for direct tumor targeting by not only providing means to manipulate magnetic carriers or nanorobots in a 3D volume but also by integrating in the closed-loop control an imaging modality allowing effective displacements along pre-planned paths from the catheterization boundaries to specific targets such as tumoral lesions. But this new approach requires new interventional

procedures and protocols taking into account the use of various types of medical micro-devices and/or nanorobots needed to improve targeting efficacy. Although many types of interventional procedures can be derived from such novel approach, a general procedure still under development and common to most interventions is briefly described here.

II. MC-1 Bacteria for Propulsion and Steering in the Microvasculature

Tumoral lesions are difficult targets and a real challenge for medical robotics. Tumors are accessible by transiting through anarchic arteriocapillar networks stimulated by tumoral angiogenesis. Such networks are built from capillaries located near such targets with diameters that can be as small as 4-5µm. At such a scale, self-propelled micro- or nanorobots with an embedded source of propulsion based on an artificial molecular machine such as proposed in [1] and capable of sufficient thrust for practical applications in the microvasculature cannot be implemented considering actual available technologies. But embedding a natural motor through the use of flagellated bacteria could become an option for the propulsion of nanorobots, an idea first proposed and explained in [2] that also described a method for precise computer-based directional or steering control of these bacteria. Such directional control exploits magnetotaxis [3, 4] where a directional torque is induced on the intracellular magnetite nanoparticles assembled in a chain-like structure and termed magnetosomes embedded in Magnetotactic Bacteria (MTB) from low intensity local magnetic fields allowing accurate displacement control along pre-planned paths as demonstrated experimentally for the first time in [5]. Directional control which has not been demonstrated yet by any other groups is an important yet critical aspect for nanorobots especially when considering applications in the human blood vessels [6]. In particular, we have considered MTB of type MC-1 for targeting tumors for several reasons. Besides encouraging results showing biocompatibility and acceptable reactions of the immune system with the non-pathogenic MC-1 cells, the cell's diameter of approximately 2µm makes it also ideal for transiting in the smallest human's capillaries. Further studies conducted by our group also showed that the thrust force in the range of 4.0 to 4.7pN provided by the pair of flagella of each MC-1 cell translated onto an average and peak speeds of approximately 200 and 300µm/s respectively. Although presently much faster than the speeds between 4 and 5µm/s achieved recently with a synthetic approach based on the implementation of artificial flagella in the form of nanocoils being actuated by a rotating magnetic field [7], the operation in the microvasculature is limited presently unlike in the case of nanocoils to less than an hour due to the effect of the higher internal temperature of the human body with a continuous decrease of the swimming velocities. Nonetheless, even with these constraints, MTB-based actuation seems to be the most efficient method of propulsion for targeting applications in the microvasculature.

Although being more efficient when operating in small capillaries compared to any other means known so far, the use of flagellated bacteria as propulsion is not sufficient to cope with the higher blood flow rates encountered in larger diameter vessels. As such, special micro-carriers being propelled by Magnetic Resonance Propulsion (MRP), a technique developed by our group which induces a directional propulsion force on ferromagnetic cores embedded in the micro-carriers from magnetic gradients generated by a clinical MRI system as demonstrated *in vivo* in [8], must be developed. Such micro-carriers could be designed to transport such MTB-tagged robots by encapsulating them in a polymeric envelope. The controlled delivery of these micro-carriers towards pre-defined release sites constitutes the first part of the interventional procedure which is referred here to as the MRP interventional phase.

III. MRP Interventional Phase

The MRP interventional phase relies on larger size micro-carriers where higher magnetophoretic velocities can be achieved with a higher effective volume of embedded ferromagnetic material having sufficient magnetization saturation for adequate propulsion force when subjected to the maximum gradient amplitude achievable with a clinical MRI platform.

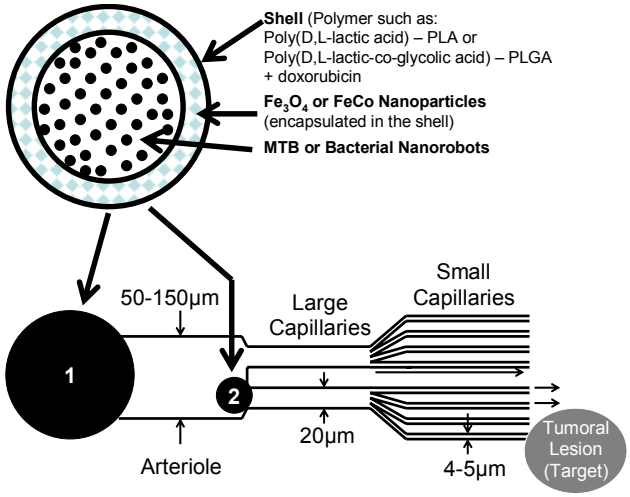

Fig. 1. Diagram of a micro-carrier with the two target embolization sites denoted as 1 and 2 in black circles where MTB-tagged nanorobots can be released. Release at the arteriole entry (site 1) is possible with a clinical MRI platform upgraded with software only whereas release at the arteriolocapillar entry (site 2) for enhanced targeting efficacy is possible by upgrading existing MRI machine with coils capable of higher magnetic gradients.

The overall size of each micro-carrier as depicted in the schematic in Fig. 1 must be large enough to encapsulate sufficient ferromagnetic material and MTB-tagged nanorobots but it must also consider vessel geometries and in particular vessel wall effects on drag force that limit the maximum achievable magnetophoretic velocity when the

size of the micro-carriers approaches the diameter of the vessel. During this initial phase of the interventional procedure, targeting aims at focusing these micro-carriers and stopping them through embolization at the arterioles entry (site 1 in Fig. 1) or at the arteriolocapillar networks entry (site 2 in Fig. 1), the latter being possible if the MRI machine has been upgraded by special gradient propulsion coils capable of several hundreds mT/m instead of the typical 40mT/m provided by each of the three orthogonal coils used for spatial encoding during MR-imaging in typical clinical MRI platforms. Hence the overall diameters and the number of these micro-carriers will depend upon the targeted embolization sites where for site 1 in Fig. 1, diameters between approximately 200 to a few hundreds micrometers are expected while for targeted site 2 in Fig. 1, diameters between approximately 20 and 50µm are envisioned.

The beginning of the MRP phase of this medical interventional procedure requires doses of ketamine to be given to maintain anesthesia. Then an infusion catheter (such as a 3 Fr tracker 18, Boston Scientific, Natick, MA) is advanced under fluoroscopic guidance into an appropriate artery over a guidewire. Using Doppler ultrasound measurements, a chart of blood flow velocity in the artery is then recorded. Once complete, the patient is placed in the MRI scanner and centered inside the MRI bore and the steering gradient coils if implemented with respect to the artery chosen to reach the selected arterioles entry site.

A high resolution micro-carriers sensitive T2* weighted MR scan of the patient is then performed. Such a scan serves as a control scan to be compared with subsequent scans that will be recorded once the targeting phase of the micro-carriers has been completed. A 3D angiographic scan of the Region Of Interest (ROI) is also performed with gadoteridol (e.g. ProHance, Bracco Diagnostics, Mississauga, Canada) in order to identify the arterial delivery route towards the targeted locations and the 3D position and extent of the tumoral area. This information is used by the control software for path planning along with data from external motion tracking sensors for patient motion compensation.

Prior to release of the micro-carriers, an option is to insert a balloon catheter to control and reduce the relatively high blood flow in the artery to maximize targeting efficacy especially through arterioles entry to reach the arteriolocapillar entry (site 2 in Fig. 1). An infusion catheter is then used as a release route for the micro-carriers. As an option to optimize the parameters of the learning control algorithm, small doses of test magnetic micro-carriers without MTB and therapeutic agent can be injected and targeted. Once system identification is considered co optimal, a dose of micro-carriers loaded with MTB can then be injected. The real-time targeting software as depicted in Fig. 2 handles the control feedback loop sequence to steer the micro-carriers along a trajectory identified by waypoints (see Fig. 2) towards a predefined embolization site (site 1 or 2 in Fig. 1). Upon efficient targeting, the real-time targeting software terminates and a high resolution micro-carriers sensitive T2* weighted MR scan of the patient is performed to record the spatial distribution of the micro-carriers and to compare it with the original scan.

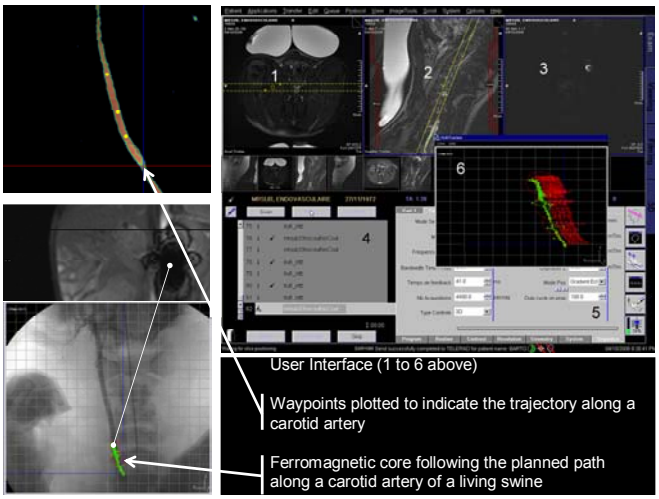

Fig. 2. An example of the user interface as seen on a computer display during the interventional procedure.

IV. Scouts Interventional Phase

Once the embolization site targeting efficacy has been confirmed with a MR scan, micro-carriers loaded with unloaded MC-1 magnetotactic bacteria referred here to as scouts are released from an infusion catheter following the same procedure as described in the previous section (Fig. 3).

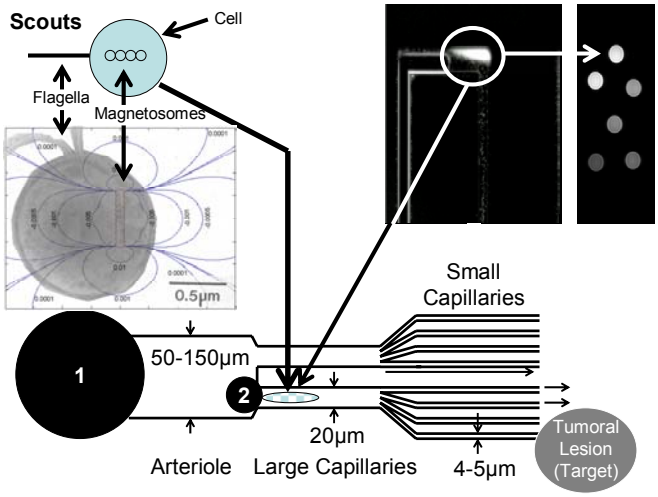

Fig. 3. Concept of scouts in the form of self-propelled MTB being controlled by computer where the chain of magnetosomes embedded in each bacterium causes a local distortion of the magnetic field of the MRI system which allows an agglomeration of MTB after being released from the micro-carriers to be tracked and then feedback controlled using MRI towards a desired target (upper right of the figure showing how an agglomeration of MC-1 bacteria seen under an optical microscope is seen using MRI shown as circles of various intensity corresponding to various concentrations of bacteria).

Since small vessels and capillaries cannot be imaged using any modern medical imaging modalities including MRI,

scouts are used to identify and to confirm an appropriate route or steering sequence to reach a tumor since we showed that an agglomeration of MTB can be visible using MRI. The idea of using unloaded MTB is to prevent ahelp level of toxicity in the systemic circulation in case of unsuccessful targeting sequences where MTB would miss the tumoral lesion. The concept of scouts using self-propelled MTB is described in Fig. 3. The chain of magnetosomes causes a local magnetic field distortion similar to contrast agents used for MRI allowing tracking inside the body and hence closed-loop directional control towards a tumors.

The targeting efficacy can be evaluated by measuring the concentration of scouts in the tumoral region by the level of intensity obtained by MRI. Because the steering of ferromagnetic micro-carriers becomes much less effective beyond the arteriolocapillar entry due to a limit of magnetic gradient amplitudes that can be generated for a human (higher gradients are possible on smaller animals) coupled with the smaller quantity of magnetic material in smaller carriers, bacteria are ideally released at the arteriolocapillar entry (site 2 shown by a black circle with the number 2 in Fig. 3) for enhanced targeting efficacy. The releasing mechanisms are still under investigation but include approaches relying on time biodegradable polymer and techniques used in hyperthermia where nanoparticles can be heated to melt polymer. As for guiding the MTB, hyperthermic-based release is performed outside the MRI bore where the magnetic field intensity is negligible.

V. Direct Targeting Interventional Phase

This is the final phase where loaded MTB are released from the micro-carriers at a pre-determined embolization site following the procedures described in previous sections as depicted in Fig. 4.

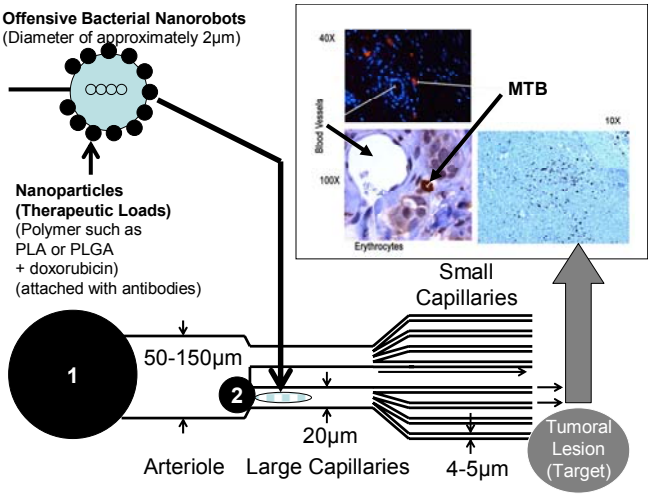

Fig. 4. Concept of offensive bacteria loaded with therapeutic agents with experimental results showing microscopy images of MTB in a tumor.

The therapeutic load is embedded in nanoparticles which are attached using antibodies already developed by our group. Embolization caused by the micro-carriers helps to reduce the blood flow rate in parts of the microvasculature and hence the directional control of MTB for enhanced targeting efficacy. Although further studies and experiments need to be done, the efficacy of MTB for targeting tumors is shown in Fig. 4 where MTB have been guided *in vivo* through the interstitial region of tumors in mice.

VI. Conclusion

A novel interventional procedure for direct tumor targeting has been briefly described with preliminary experimental results. The results show that although more investigation is required, directly targeting of tumors with magnetotactic bacteria controlled by computer while avoiding toxic agents in the systemic circulation is possible.

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