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# Encapsulation of Magnetotactic Bacteria for Targeted and Controlled Delivery of Anticancer Agents for Tumor Therapy

Fatemeh Afkhami, Samira Taherkhani, Mahmood Mohammadi, and Sylvain Martel, *Senior Member, IEEE*

*NanoRobotics Laboratory, Department of Computer and Software Engineering, and Institute of Biomedical Engineering, École Polytechnique de Montréal (EPM), Montréal, Canada*
*E-mail: sylvain.martel@polymtl.ca*

***Abstract*- We showed that magnetotactic bacteria (MTB) have great potentials to be used as microcarriers for targeted delivery of therapeutic agents. Indeed, magnetotaxis inherent in MTB can be exploited to direct them towards a tumor while being propelled by their own flagellated molecular motors. Nonetheless, although the thrust propelling force above 4 pN of the MC-1 MTB showed to be superior compared to other technologies for displacement in the microvasculature, MTB becomes much less efficient when travelling in larger blood vessels due to higher blood flow. In the latter case, a new technique developed by our group and referred to as Magnetic Resonance Navigation (MRN), has been successfully applied in larger vessels using synthetic microcarriers nut proved to be less efficient in the microvasculature due mainly to technological constraints. These findings called for the need to integrate both approaches by encapsulating MTB in special MRN-compatible microcarriers to be release in the vicinity of microvascular networks where they becomes more effective for targeting purposes in tumoral lesions. In this study Magnetococcus strain MC-1 were encapsulated in giant vesicles. The survival of the encapsulated bacteria was monitored. The release of bacteria from giant vesicles was also studied in different time intervals and conditions.**

## I. INTRODUCTION

Targeted delivery of anticancer agents to cancerous cells is believed to be the best way to treat a cancer with high specificity to the cells and minimal systemic toxicity. However penetration of drugs deep into tumors is a major concern in targeted therapy. Tumor hypoxic regions cannot be targeted by chemotherapy since the cells in these areas are not rapidly growing. Investigations on bacterial growth within tumor tissue *in vitro* have shown that bacteria can accumulate in tumor tissues by chemotaxis suggesting the possibility of bacterial-based therapeutics for cancer treatment [1]. Penetration of bacteria in deep regions of tumors where tumor cells are distant from functional blood vessels may overcome the current limitation of drug delivery to hypoxic and quiescence areas of tumors.

Flagellated Magnetotactic Bacteria (MTB) [2] are superior candidate for the delivery of anticancer agents into inaccessible tumor regions. They can be used as micro-nanorobots with a directional autonomous propulsion system. In this bacterial robotic system, autonomous propulsion is performed by two bundles of flagella. The direction of movement of the bacteria is controlled by applying a directional magnetic field of a relatively low intensity which is capable of influencing the orientation of a chain of nano-scale organelles named magnetosomes. These organelles act as a navigational magnetic compass needle embedded in each bacterial cell and allow us to control the displacement of the bacteria very accurately (Figure 1).

Exploiting these bacteria opens many potential applications including targeted drug transport through the blood vessels. Computer-controlled bacterial movement can be used for targeted delivery in capillaries of the human blood network including the angiogenesis network (tumor blood vessels). Therapeutic agents can be attached to the bacteria and delivered to the desired sites. However, the high flow rate in large blood vessels overcomes the thrust force of MTB and remains an issue when navigating in larger vessels [3, 4].

In [5, 6], we have demonstrated the feasibility of navigating microscale devices in blood vessels of living animals using a new method referred to as Magnetic Resonance Navigation (MRN). Typical MRN operations relies on an upgraded clinical MRI scanners where propulsive forces are induced on the magnetic carriers along a planned trajectory in the blood vessels. Although the techniques is efficient in larger blood vessels, it becomes much less appropriate in the microvasculature due to technological limits at providing sufficient magnetic gradient to induce sufficient propelling force on microcarriers of less than a few tens of micrometers in diameter. As such, encapsulating MTB in special MRN compatible microcarriers to be released near the microvasculature could potentially enhance tumor targeting and hence, therapeutic efficacy while minimizing secondary toxicity by avoiding or at least reducing systemic circulation of toxic agents.

The objective of this study was then to encapsulate MTB in a controlled release pattern and carry the capsules containing MTB (with higher thrust force compared to a single bacterium) to the vicinity of the capillary network

where they could be released and guided toward the desired area. As such, assessing the development and optimization of MTB encapsulation process to obtain MRN (MRI navigable) MTB microcapsules with controlled sizes and release process was the main goal of this study.

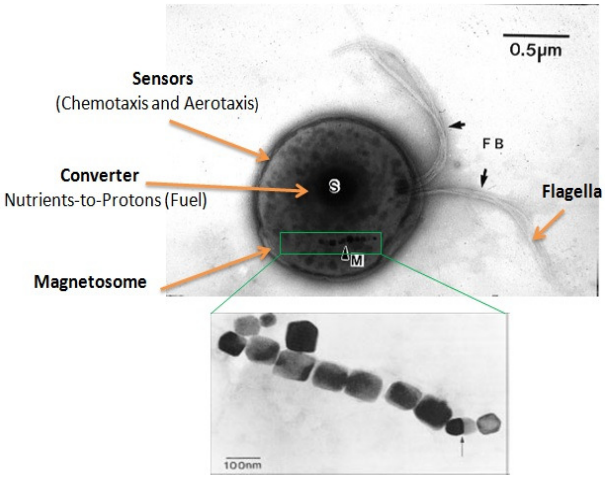



Figure 1. MC-1 MTB and its chain of magnetosomes (bottom), (Martel, S. et al, 2009).

## II. EXPERIMENTAL SECTION

### A. *Bacterial culture*

Magnetococcus sp. MC-1 was grown in microaerobic condition on chemoheterolithotrophic liquid medium in a dark chamber at room temperature. Iron-enrichment of the medium was done using 50□m of ferrus sulfate heptahydrate $FeSO_4$-$7H_2O$.

### B. *Encapsulation of MTB in giant unilamellar vesicles*

The encapsulation of MTB was carried out in giant vesicles with a bilayered membrane and a size in the range of 50-100 □m. Here we described encapsulation of MC-1 bacteria in giant vesicles formed from an initial w/o emulsion stabilized with phospholipid using a microfluidic device. A dried lipid film of phospholipid either PC (L-α-Phosphatidylcholine, SIGMA), DOPC (1,2-dioleoyl-*sn*-glycero-3-phosphocholine, Avanti Polar Lipids) or DOPC: CH, (CH: Cholestrol, SIGMA) was made by evaporating the organic solvent of the phospholipid solution under nitrogen flow and further dried under vacuum for about 15-20 minutes. Lipid film was then dispersed in mineral oil by ultrasonication for 60 min at 50°C followed by immediate vortexing. The final concentration of lipid in oil was 4-8 mM. Bacteria make the aqueous phase and they were grown until they reach to the desired concentration for encapsulation $10^6$-$10^7$/ml, maintaining in their own media and were encapsulated without centrifugation.

Water droplets dispersed in oil (micelles) were obtained from microfluidic channels by adjusting the water and oil flow rate. An oil/water interface was made to form the second monolayer of the membrane bilayer. To set an oil/water interface, an aqueous solution was placed at the bottom of small vial followed by adding phospholipid-containing oil on top of the aqueous solution. Phospholipids were aligned between the upper oil phase and the lower aqueous phase. Micelles containing water phase (bacterial culture) which are heavier than oil normally pass through oil/water interface and assemble phospholipids from the interface to form a monolayer and then complete the bilayer. To accelerate precipitation of vesicles, the vial was slightly centrifuged.

### C. *Microfluidic device fabrication*

A T-junction microchannel device was fabricated to perform the experiments. The channels with 150 □m diameter were patterned on a polymethyl methacrylate (PMMA) sheet (100×70×2 mm) using an end mill. After patterning the T-junction, another PMMA sheet was placed on top of the first one to seal the microfluidic device. Sealing was performed by high-pressure thermal sealing technique (Figure 2).

Two syringe pumps were connected to the microfluidic device for pumping two phases with different flow rates. Oil phase was pumped into the channels at 20 □l/min and the aqueous phase (containing encapsulated materials such as bacterial culture or microbead) was pumped at 2 □l/min flow rate.

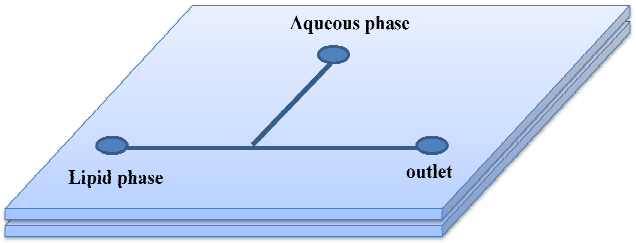



Figure 2. Schematic design of microfluidic channels used for performing the experiments

In order to estimate the required time for release of bacteria from the liposomes at the desired location, release rate was studied with encapsulation of microbeads in different conditions. Polystyrene microbead with 1□m diameter were encapsulated in PC, DOPC and DOPC:Cholestrol based liposomes. One group of liposomes was incubated at room temperature with or without serum and the other group was incubated at 37°C with or without serum, FBS (Fetal Bovine serum, Sigma). Samples were taken at different time intervals and microbeads released from liposomes were counted under a microscope.

## III. RESULTS AND DISCUSSION

Giant round-shape unilamellar vesicles were fabricated with diameters ranging between 50-150 □m (Figure 3). Giant unilamellar vesicles offer the feasibility of encapsulation of MTB in an aqueous solution [7] (e.g. bacterial media in

which bacteria are maintained in optimum condition for viability and metabolic activity). Observation of the encapsulated bacteria under microscope showed that bacteria remained viable and active for 2.5 hours. After this time, bacterial motility decreased. This period of activity is long enough for the bacteria to act as a drug carrier *in vivo*. Being viable and active at the moment of release is critical for the bacteria to be directed to the vicinity of the tumor and to deliver therapeutic agents.

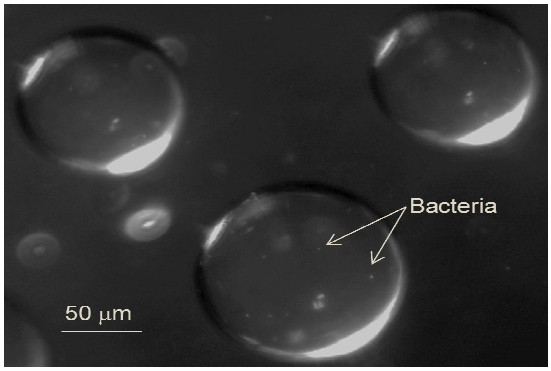



Figure 3. Magnetotactic bacteria MC-1 encapsulated in giant DOPC vesicles

The demanding growth condition of MC-1 makes it a challenging agent to be encapsulated in current bacterial encapsulation methods that normally involve in mixing the bacteria with a polymer and gelation process. Phospholipid molecules that form the bilayer have a hydrophilic head group and a hydrophobic tail group. The head groups of inner layer face the aqueous phase of inside the vesicle and head group of external layer face the exterior of the vesicle. The hydrophobic tails of phospholipids make a surface away of water. This environment is suitable for MC-1 bacteria that need to be encapsulated in liquid environment preferably in their own media without immobilization within a polymer. For preparing giant vesicles, several methods have been reported including lipid film hydration, electroformation, w/o transfer method, double emulsion via microfluidic techniques, fusion of small vesicles and possibly a combination of different techniques. Choosing the most suitable preparation method depends very much on the application and the condition used. For encapsulating MC-1 bacteria within the giant vesicles, there are many chemical and physical constraints affecting viability and activity of the bacteria that do not leave many options in the methods of preparation. We already tried to mix MC-1 bacteria with polymer to encapsulate them with gelation process; however they did not survive upon mixing with polymer. Different polymers and various concentrations generated the same results. That might be due to their need to liquid media for survival and growth. The method applied in this study is based on using a microfluidic device to obtain monodispersed droplets of water in oil and then transferring through an oil/water interface to complete the liposomes. This method is fast and simple and does not require any detergent or high temperature that may have a negative effect on the bacteria.

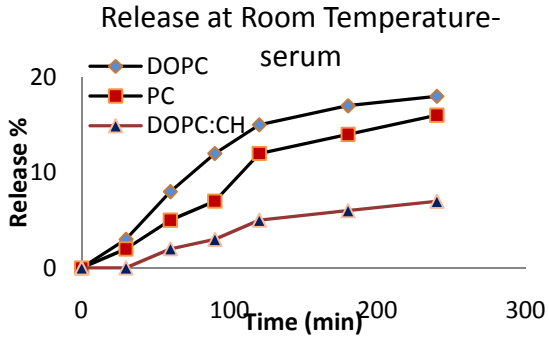
The release study showed that retention of materials in giant vesicles at room temperature is considerably high. The effects of serum in the media and lipid combination applied in vesicles preparation are relatively low at room temperature (Figure 4). In the presence of serum the release of microbeads from vesicles at 37°C were dramatically increased. There was not a significant difference between PC and DOPC- based liposomes. However, the addition of cholesterol to the lipid combination significantly increased the stability of giant vesicles at 37°C in the presence of serum (Figure 5). Presence of serum promoted the release rate of all types of liposomes. However, cholesterol clearly makes more stable vesicles when they were incubated with serum.

The interaction of lipid vesicles with lipoproteins and serum components affect the retention of encapsulated bacteria. In case of encapsulating bacteria, the rate and extent of release of bacteria can be controlled by manipulating lipid composition [8]. For shorter release time, the phospholipids with unsaturated fatty acyl chain can be used. In this project we intend to release the bacteria within less than 30-40 minutes after being injected in the body, therefore the release rate has to be performed in a controlled manner. In the vicinity of a targeted tumor, almost all bacteria have to be released and ready to penetrate as deep as possible inside the tumor. In case of relying on serum-induced leakage, the designed liposomes cannot be made very stable since the release rate would be very long. However sonosensitive liposomes can be designed to release the content upon exposure to ultrasound. Some specific phospholipids, e.g. DSPE, cholesterol and some polymers such as PEG for instance, make very stable and also sonosensitive liposome [9] that can be ruptured in a few minutes after applying ultrasound. There are other controlled release methods such as applying photosensitive, pH sensitive or thermosensitive vesicles by using specific polymers in designing vesicles that upon UV exposure, heat exposure or changing pH, the content of vesicles can be released. However, there might be potential harm on bacterial survival by using these methods.

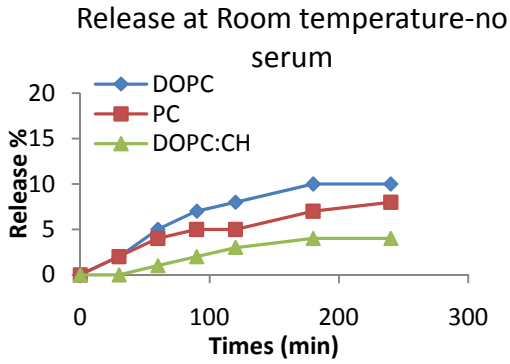
## IV. CONCLUSION

In this study we have shown a suitable method for encapsulating MTB MC-1 bacteria in spite of many chemical and physiological constraints. Encapsulation of MC-1 is of great importance due to the potential application in drug delivery system and other biomedical purposes. The capsules of bacteria can be used as a drug carrier that can be navigated and directed to the desired location. As the serum promotes the release, the capsule can be engineered to be ruptured at the right time. To fully develop this system for therapeutic purposes, more experiments are required including designing a sosnosensitive liposome, navigating capsules in animal

models and applying ultrasound to release the bacteria at the desired location.




(A)




(B)

Figure 4. Comparison of release profile of PC-, DOPC- and DOPC:CH based giant liposome at room temperature in different giant liposome with (A) or without FBS (B)

## V. ACKNOWLEDGMENT

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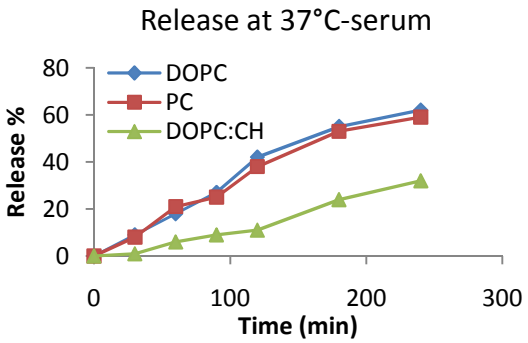
## VI. REFERENCES

1. Kasinskas,R.W. & Forbes,N.S. Salmonella typhimurium lacking ribose chemoreceptors localize in tumor quiescence and induce apoptosis. *Cancer Res.* **67**, 3201-3209 (2007)

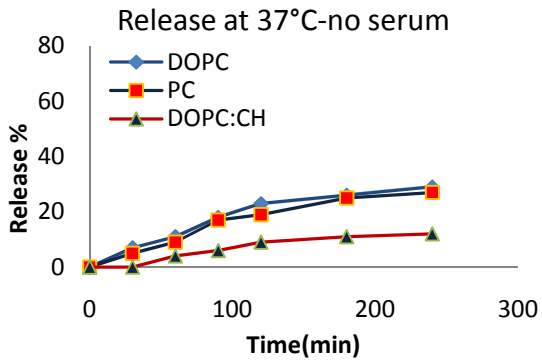
2. Blakemore,R. Magnetotactic bacteria. *Science* **190**, 377-379 (1975)

3. Martel,S., Felfoul,O., Mohammadi,M., & Mathieu,J.B. Interventional procedure based on nanorobots propelled and steered by flagellated magnetotactic bacteria for direct targeting of tumors in the human body. *Conf. Proc. IEEE Eng Med. Biol. Soc.* **2008**, 2497-2500 (2008)

4. Martel,S., Mohammadi,M., Felfoul,O., Lu,Z., & Pouponneau,P. Flagellated Magnetotactic Bacteria as Controlled MRI-trackable Propulsion and Steering Systems for Medical Nanorobots Operating in the Human Microvasculature. *Int. J. Rob. Res.* **28**, 571-582 (2009)




(A)




(B)

Figure 5. Comparison of release profile of PC-, DOPC- and DOPC:CH based giant liposome at 37°C in different giant liposome with (A) or without FBS (B)

5. Martel S., Mathieu J.-B., Felfoul O., Chanu A., Aboussouan É., Tamaz S., Pouponneau P., Beaudoin G., Soulez G., Yahia L'H., and Mankiewicz M., "Automatic navigation of an untethered device in the artery of a living animal using a conventional clinical magnetic resonance imaging system," *Applied Physics Letters*, vol. 90, 114105, March 12, 2007

6. Pouponneau, P., Leroux, J.-C., Soulez G., Gaboury L., and Martel S., "Co-encapsulation of magnetic nanoparticles and doxorubicin into biodegradable microcarriers for deep tissue targeting by vascular MRI navigation," *Biomaterials*, Vol. 32, Issue 13, pp. 3481-3486, May 2011

7. Funakoshi,K., Suzuki,H., & Takeuchi,S. Formation of giant lipid vesicle like compartments from a planar lipid membrane by a pulsed jet flow. *J. Am. Chem. Soc.* **129**, 12608-12609 (2007)

8. Allen,T.M. & Cleland,L.G. Serum-induced leakage of liposome contents. *Biochim. Biophys. Acta* **597**, 418-426 (1980)

9. Evjen T.J., Nilssen E.A., Rognvaldsson S., Brandi M., Fossheim S.L. Distearoylphosphatidylethanolamine-based liposomes for ultrasound-mediated drug delivery. *European Journal of Pharmaceutics and Biopharmaceutics.* **75** (3), 327-33 (2010)