*In vitro* and *in vivo* visualization and trapping of fluorescent magnetic microcapsules in a blood stream

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ABSTRACT: Remote navigation and site specific targeting of biologically active compounds is one of the current challenges in development of drug delivery systems. Modern methods of micro- and nanofabrication allow us to make particles and capsules bearing cargo to deploy and possess magnetic properties to be externally addressed. In this work we explore multilayer composite magnetic microcapsules as targeted delivery systems under natural conditions of living organism involving *in vitro* and *in vivo* studies.. Herein, we demonstrate magnetic addressing of fluorescent composite microcapsules with embedded magnetite nanoparticles in a blood flow environment. First, the visualization and capture of the capsules at defined blow flow by magnetic field was shown *in vitro* in artificial glass capillary employing wide-filed illumination laser microscope. Afterwards, the capsules were visualized and successfully trapped *in vivo* on externally exposed rat mesentery microvessels. Histological analysis showed that capsules infiltrate small mesenteric vessels whereas large vessels preserve the microcirculation. The effect of magnetic field, including capsule retention at site upon its switching off, capsule preferential localization in bifurcation areas of vasculature is under discussion. The research outcome demonstrates that microcapsules can be effectively addressed in a blood flow what makes them promising delivery system with remote navigation by magnetic field.

1. Introduction

Nowadays, the development of multimodal drug delivery systems is an emerging area of research. However, the design of intelligent stimuli responsive, in particular with multiple functions, delivery systems remains a challenge. During the last two decades, a number of systems were introduced as potential drug carriers.[1-4](#_ENREF_1) Among them, polymeric multilayer microcapsules[5](#_ENREF_5) produced by the layer-by-layer (LbL) assembly[6](#_ENREF_6) technique are envisaged as one of the most promising candidates for targeted delivery and remote controlled release of encapsulated compounds. The significant benefits of using polyelectrolyte microcapsules are their high loading capacity and adjustability of physicochemical properties. Capsule size in the micrometer range allows for efficient loading of various materials into the capsule’s cavity, which is an important prerequisite for the delivery applications. Capsule assembly by stepwise deposition allows for utilization of macromolecules as building blocks with different reactive chemical groups. Incorporation of various charged species, such as inorganic nanoparticles, brings multifunctionality and responsibility to various external stimuli.[7-10](#_ENREF_7)

For instance, the presence of magnetite nanoparticles in the microcapsule shell makes them responsive to an external magnetic field that is promising for remote navigation and release of encapsulated agents. It was shown that behavior of magnetic microcapsules in permanent magnetic field governed by the field homogeneity. Under uniform magnetic field, capsules are aligned with the lines of magnetic field strength.[11-13](#_ENREF_11) On the other hand, under non-uniform magnetic field capsules tend to move to the area with the highest magnetic field strength that enables controlled motion and localization of the capsules in the desired place with a high precision. This fact is generally employed to demonstrate the magnetic properties of microcapsules with incorporated magnetic nanoparticles by simple separation applying permanent magnet.[14-16](#_ENREF_14) As a recent practical example, magnetic capsules were attached to the gate of a pH-sensitive field effect transistor by a permanent magnetic field to form a bioreceptor on its surface.[17](#_ENREF_17) Furthermore, it was shown that magnetic microcapsules can be triggered to release the encapsulated cargo under alternate magnetic field due to mechanical and/or thermal effects of oscillating field.[18-22](#_ENREF_18)

At present time, these microcapsules are intensively studied regarding their potential for biomedical applications that involves several research trends. The basics of targeted delivery treatment are closely related to interactions between vesicles and cells, thus cellular uptake, cytotoxicity and degradability of microcapsules attract much attention.[23-28](#_ENREF_23) Furthermore, the mechanical properties of microcapsules were also shown to effect on the uptake and cells functions.[29](#_ENREF_29) In turn, *in vivo* investigations are mostly devoted to biodistribution and biodegradation of microcapsules. A number of recent works are focused on intratumoral injection of microcapsules with encapsulated drug and responsive (or even multiresponsive) to different external stimuli.[30-35](#_ENREF_30) All these research directions may be relevant to microcapsules application for cancer therapy (e.g. injection of capsules, uptake by cancer cells, triggered release of encapsulated drug, and capsules degradation) and are of great importance to turn the microcapsules to useful delivery system. However, not many efforts were made to find out the possibility of microcapsules remote navigation in a natural environment of living body that is essential for cancer therapy since the delivery vesicles not always can be directly injected into the tumor. Eventually, most of the targeted delivery systems are implied to be administrated through injection into a blood or lymphatic vessels that allows direct access to internal organs avoiding skin or gastrointestinal tract barrier. Therefore, it is essential to investigate the behavior of the capsules injected in a blood vessel under natural conditions of the organism and the ability to trap the capsules in the whole blood stream. From this point, the non-uniform magnetic field seems to be perspective for accumulation of magnetic microcapsules, for instance, injected in a blood vessel feeding a tumor.

Thus, the aim of this work was to fabricate fluorescent magnetic microcapsules to fulfill the two major requirements. First, in order to carry out the real-time *in vivo* observation of the capsules behavior they have to be well visible in a whole blood environment. On the other hand, for remote controlling of capsule navigation the magnetic nanoparticles should be embedded into the shell. Therefore, one have to combine bright fluorescence and magnetic responsiveness in a single capsule avoiding a fluorescence quenching. Glass capillary filed blood flow with capsules has been used as *in vitro* model while *in vivo* experiments were done on rat mesentery microvessels exposed externally for visualization and magnetic addressing.

2. Materials and methods

2.1. Materials

Calcium chloride (dihydrate), sodium carbonate (anhydrous), iron(II) chloride (tetrahydrate), iron(III) chloride (hexahydrate), ethylenediaminetetraacetic acid disodium salt (EDTA, dihydrate), poly(allylamine hydrochloride) (PAH, MW 17500 Da), poly(sodium 4-styrenesulfonate) (PSS, MW 70000 Da), rhodamine B isothiocyanate (RITC), bovine serum albumin (BSA, lyophilized powder) were purchased from Sigma-Aldrich. Millipore Milli Q water (18.2 MΩ∙cm-1) was used as an aqueous medium during all sets of experiments.

2.2. Synthesis of Fe3O4 nanoparticles

Magnetite Fe3O4 nanoparticles were synthesized by chemical precipitation of iron(II) and iron(III) salts in alkaline medium as described by Massart.[36](#_ENREF_36) The synthesis was carried out with a homemade automated reactor setup that provides one with a precise control over amount of reacting agents in the chamber and preserve an inert atmosphere during all over the synthesis.[37](#_ENREF_37) Upon preparation, the particles were stabilized with 0.1M citric acid and afterwards dialyzed in water for 4 days. The resulted colloid had the concentration of 3 mg/ml with the mean particles size of 12 nm and zeta-potential of -30 mV.

2.3. Preparation of RITC-conjugated BSA

First, 160 mg of BSA was dissolved in 40 ml of 0.1M PBS buffer (pH=8). Afterwards, the solution of RITC in ethanol (5 mg/ml) was prepared. 40 ml of BSA solution was added to 5 ml of RITC alcoholic solution under gentle stirring and further the mixture was stirred under 4°C in the dark for 12 hours. Finally, freshly prepared RITC-conjugated BSA was dialyzed for 3 days in deionized water.

2.4. Microcapsules preparation

The microcapsules were LbL assembled on freshly prepared CaCO3 cores. The calcium carbonate templates were prepared by common co-precipitation of 1M CaCl2 (0.615 ml) and 1M Na2CO3 (0.615 ml) salts with addition of 2.5 ml RITC-BSA solution.[38](#_ENREF_38), [39](#_ENREF_39) Afterwards, several bilayers of PAH and PSS (both from 1 mg/ml aqueous solutions) were assembled on the cores. In order to make the capsules responsive to magnetic field a layer of Fe3O4 nanoparticles (0.5 mg/ml) was added instead of one of PSS layers as well as additional RITC-BSA layers were added to the shell to increase the capsule fluorescence. Finally, the template cores were dissolved with 0.2M EDTA (pH 7.3) to form hollow microcapsules. The resulted microcapsules suspension had a concentration of about 2.45∙108 ml-1.

2.5. Confocal microscopy

Fluorescent properties of as-prepared microcapsules was studied with Leica TCS SP8 X inverted confocal microscope (Leica Microsystems). The emission was excited by 552 nm laser and the detection band was 565 to 700 nm.

2.6. Scanning electron microscopy (SEM)

SEM measurements were performed with a MIRA II LMU (TESCAN) microscope at an operating voltage of 30 kV. A drop of microcapsules suspension was placed on Si substrate and dried under ambient conditions. Afterwards, the samples were attached to a carbon tab glued to an aluminum sample holder and then sputtered with gold in a vacuum.

2.7. Optical measurements

Optical visualization of the microcapsules was performed with a homemade optical setup that was built using LOMO microscope base equipped with bright-field condenser with white LED light source. The fluorescence was excited by 532 nm laser (65 mW, Lascompany) that was reflected by a dichroic mirror to a 10x/0.27 infinity corrected long work distance objective. In order to get a wide-field illumination the laser was focused by a two-lens-system on the back focal plane of the objective. The fluorescence signal from the sample passed the dichroic mirror and through a tube lens (f = 200) came to a CMOS detector (acA2040-180km, Basler Inc.) with the mounted fluorescence emission colored glass filter (OC-14, LOMO). The laser power was 24.5 mW at the output of the optical system. The scheme of the setup is shown on Figure S1 in the Supporting Information.

2.8. Magnets and magnetic field measurements

For in vitro magnetic trapping a permanent magnet was employed. A sharp steel plate that acted as a magnetic concentrator was attached to the magnet in order to localize the magnetic field area.

In vivo magnetic trapping was carried out with a homemade electromagnetic setup made of two electromagnets (Figure 1). The magnets were assembled of a copper wire wounded on a spool and a steel core. The cores had a sharp tip from the one side to effectively concentrate the magnetic field in a targeted area. Each electromagnet had a separate power supply that allowed to turn them on with a different polarity so that the tips formed north and south poles respectively. Additionally, the cores were coupled from the other side with a steel link to increase the magnets mutual effectivity.



Figure 1. Schematic overview of the electromagnetic setup that was employed for *in vivo* trapping of the microcapsules in rat mesenteric vessels.

The magnetic field of the homemade magnets employed for microcapsules trapping was measured with FH 51 gauss-/teslameter (Magnet-Physik Dr. Steingroever GmbH, Germany). For permanent magnet magnetic field was measured along magnetic dipole axis. In order to estimate the magnetic field distribution in the gap of electromagnet both transverse and axial components were measured and total field was calculated. The measurements were carried out for several planes in the gap.

2.9. *In vitro* experiments

*In vitro* detection and trapping of microcapsules was performed by pumping the mixture of microcapsules suspension and rat blood through a glass tube. In particular, 400 μl of a whole rat blood was mixed with 100 μl of microcapsules suspension and pumped through a glass tube previously rinsed with heparin (Biochemi, Austria 25000 ED/5ml). The tube had a rectangular cross section with the inner size of 2680 × 200 μm. The volume flow rate of the mixture was controlled with IPC Ismatec peristaltic pump (Cole-Parmer GmbH, Germany) and set on 200 μl/min. When the tube was completely filled with the mixture a permanent magnet was put to the tube wall. A sharp steel plate that acted as a magnetic concentrator was attached to the magnet in order to localize the magnetic field area.

2.10. *In vivo* experiments

In vivo visualization and trapping of the microcapsules were carried out on rat mesentery microvessels. All procedures were performed in accordance with the “Guide for the Care and Use of Laboratory Animals”.[40](#_ENREF_40) The experimental protocol was approved by the Committee for the Care and Use of Laboratory Animals at Saratov State Medical University (Protocol H-147, 17.04.2001). All experiments with animals were performed using the general anesthesia (Zoletil mixture, 50 µL, 40 mg/kg (Virbac SA, Carros, France) and 2% Rometar, 10 µL, 10 mg/kg (Spofa, Czech Republic) via intraperitoneal injection. The study was carried out on adult male white mongrel rats with an average weight of 200 – 250 g. The jejunum with mesentery was taken out through a small abdominal incision and placed on a glass slide The suspension of microcapsules was injected into a branch of superior mesenteric artery (diameter of about 0.5 mm) through a thin polyethylene tubing (0.28 mm inner diameter (ID), 0.61 mm outer diameter (OD), Portex, Smiths Medical International Ldt., UK) with stainless steel needle on the end (OD 0.33 mm). The needle was firmly fixed to avoid vessel damage by a sharp steel edge. Before implantation, the tubing was filled with the isotonic (0.15 M) NaCl aqueous solution. Prior to injection, the capsules were transferred to 0.15 M NaCl aqueous solution as well and diluted 3-fold to prevent circulation blocking due to their aggregation. The suspension was injected partially by a portion of 0.1 ml. To enhance the probability of capsules detection, the total volume of the injection was 0.5 ml.

For *in vivo* trapping, two electromagnets were set up in a close proximity to the selected mesentery region. The gap between the tips was set to 2 mm. Magnetic field was turned on just before the capsules injection. Both of the magnets were run at 0.5 A and 25 V.

2.12. Histology

Histological analysis was carried out to determine the topography of microcapsules in mesentery tissue and vessels. For histological study, the animals were sacrificed at the end of the experiments and the regions of interest of the mesentery tissue were fixed in 10% (volume) formalin for 24 h. The formalin fixed specimens were embedded in a paraffin, sectioned (5 µm), and stained with hematoxylin and eosin. Further, the histological sections were evaluated by a light microscopy.

3. Results and discussion

3.1. Microcapsules preparation



Figure 2. Sketch of a fluorescent magnetic microcapsules shell structure (a) and confocal fluorescent (b) and scanning electron microscopy (c) images of the microcapsules.

Following the aim of the work, the beginning of the study was devoted to preparation of fluorescent magnetic microcapsules. Figure 2a shows the structure of the microcapsule shell. RITC-conjugated BSA was employed as a fluorescent agent for two reasons. The first one is because rhodamine is a very common organic dye for *in vivo* fluorescent labeling. In turn, BSA is widely used as a model macromolecule for capsulation experiments. Additionally, BSA isoelectric point in aqueous medium is under pH = 4.7, thus, at neutral pH values BSA is negatively charged and can be LbL assembled with positively charged polyelectrolytes. Therefore, one can use two types of encapsulation to bring as much fluorescence as possible to distinguish the capsules from the blood autofluorescence. Magnetite nanoparticles were embedded into the shell to make the capsules responsive to remote navigation by magnetic field. Magnetite nanoparticles were shown to combine well established magnetic properties, stability, biocompatibility, and thus are perfectly suited for biological and medical applications.[41](#_ENREF_41) However, one has to consider that magnetite nanoparticles quench fluorescence of adjacent fluorophores.[42](#_ENREF_42), [43](#_ENREF_43) Therefore, the layer of magnetic nanoparticles and fluorescent RITC-BSA core and layers were separated by several polyelectrolyte bilayers to avoid quenching. Confocal microscopy image in Figure 2b clearly shows a fluorescent emission from the designed microcapsules. The shape of the microcapsules was studied by SEM. Figure 2c shows SEM image of the microcapsules that are typically collapsed after drying. It is seen that the capsules are quite uniform with an average diameter of 3 μm.

3.2. *In vitro* trapping

The first set of experiments was dedicated to *in vitro* investigations in order to check whether the capsules are visible in a whole blood and can be trapped by the magnet in the blood stream.



Figure 3. Sketch of experiment on *in vitro* trapping of fluorescent magnetic microcapsules capsules in a blood stream (a) and magnetic field distribution (with error bars) of applied permanent magnet inside the capillary (b).

Figure 3a shows the scheme of experiment on *in vitro* magnetic trapping of the microcapsules. The magnet was attached to the one side of the capillary that gives a non-uniform magnetic field in the inner capillary area proved by magnetic field measurements (Figure 3b).



Figure 4. *In vitro* visualization of the microcapsules moving in a -rat blood stream without magnet (a) and with a permanent magnet in 30, 60 and 90 s after application of magnetic field (b-d). Trapping of the capsules is well seen as white areas in the vicinity of a glass capillary wall.

Figure 4 shows the fluorescent images of glass capillary section captured at excitation by 532 nm laser light. An arrow designates the direction of the blood stream. The white spots all over the flow area clearly indicate the microcapsules due to their fluorescence. A large white spot on the capillary wall corresponds to microcapsules aggregate formed by the capsules trapped with magnetic field. Accumulation of the capsules nearby a magnetic tip is a clear evidence of their magnetic response that corresponds to already known behavior of magnetic capsules in a non-uniform magnetic field. It was shown previously, that the capsules containing superparamagnetic iron oxide nanoparticles can be magnetically separated by permanent magnet and tend to move to the area with the highest magnetic field lines density.[14-16](#_ENREF_14) According to the measured lateral distribution of magnetic field inside the capillary (Figure 3b) the capsules accumulated exactly in the highest magnetic field area where it reached the value of 200 mT.

Furthermore, the amount of trapped capsules can be controlled by the time of magnetic field exposure as it is shown on time-lapsed fluorescent images in Figure 4. After 30 sec under magnetic field the capsules formed an aggregate with the thickness of 40 μm and lateral size of 476 μm (Figure 4b) and in 90 sec the aggregate had grown up to 63 μm of thickness and 661 μm along capillary wall (Figure 4c). These data allow one to estimate an approximate number of trapped capsules and follow the dynamics of their accumulation. According to SEM images, the average capsules size is about 3 μm that corresponds to the area of 7 μm2. In turn, the area of capsules aggregates can be roughly approximated as a sum of patterns of rectangles and right-angle triangles. Therefore, one can calculate that it was trapped about 2700 capsules in first 30 sec, about 2800 capsules in 60 sec, and about 4500 capsules in 90 sec of magnetic exposure (Figure 5).



Figure 5. Estimated number of the microcapsules trapped by magnetic field and capsules released after magnetic field was turned off.

However, after the magnet was removed from the capillary the capsules were almost completely washed from the wall by the blood flow. Figure 6 shows the dynamics of capsules removal. After 10 sec without magnetic field the area of capsules spot decreased drastically and only about 2100 capsules remained on the wall (Figure 6a) and in another 40 sec only two small capsules spots containing 637 capsules can be distinguished (Figure 6c). Although, some capsules can be seen on the wall even after 70 sec of blood circulation (Figure 6d) most of the capsules left the surface.



Figure 6. Removing of the capsules from the capillary wall by the blood flow after the magnet was detached.

Thus, magnetic field can effectively trap and retain the microcapsules but the capsules do not attach to the capillary wall. However, some single capsules and small aggregates still may remain on the wall after the magnet was removed due to possible incomplete coverage of the surface with heparin that led to capsules adsorption.

*In vitro* targeting of microcapsules by magnetic field in a flow channel was previously shown by Zebli et al.[13](#_ENREF_13) The capsules were successfully trapped in the area of permanent magnet application that was further indirectly confirmed by increased internalization of the capsules by the cells due to high local concentration of the capsules. However, conversely to our study the capsules were trapped in the cell medium (i.e. not in the blood) and the trapping was not observed in a real time.

To sum up, the capsules with designed structure are well visible in a whole blood and can be effectively caught in the blood stream by local applied non-uniform magnetic field of permanent magnet and further removed by the blood stream after the magnet is put off.

3.3. *In vivo* trapping

*In vitro* experiments in a glass tube demonstrated that capsules are well visible in a blood and can be localized in the desired area by magnetic field. However, this system is rather artificial and cannot present an appropriate model on capsule behavior in blood flow in real life systems due to specific complex internal conditions. Therefore, the next step of the study was devoted to investigation of *in vivo* trapping of the capsules injected into the blood circulatory system. In order to find out how real biological system might respond capsule flow and external localization the adequate experimental system to monitor the effect on *in vivo* trapping have to be sought.



Figure 7. Photo of a rat mesentery indicating the branches of superior mesenteric artery, the injection site, and region of interest for *in vivo* visualization and trapping of the capsules.

Our *in vivo* experiments on visualization and trapping of the capsules in the blood flow were carried out on mesenteric microvessels: arterioles, venules, and capillaries. The capsules were injected directly into the branch of superior mesenteric artery suppling the blood to the vessels of interest so as to improve the probability of capsules detection (Figure 7). A rat mesentery is commonly employed as a model tissue for the study of blood circulation and microcirculatory dysfunctions as blood flow in mesenteric microvessels can be well visualized by optical systems.[44-47](#_ENREF_44) Furthermore, jejunal mesenteric windows are easy to reach and can be spread enough to set a magnet on the region of interest. For *in vivo* experiments, a pair of coupled electromagnets were employed that were placed on top and bottom of mesentery tissue analogous to a magnetic trap concept that is used for cell probing.[48](#_ENREF_48), [49](#_ENREF_49) Permanent magnet was substituted to electromagnets as some surgical manipulations had to be performed after the magnet set to a desired place and therefore switchable magnetic field is required. Magnetic field in the gap between the magnets can be considered as an “onion-like” with the highest magnetic field lines density nearby the magnetic tips. This was confirmed by the measurements of magnetic field distribution in between the poles (Figure S2 in Supporting Information). The highest magnetic field was in the area close to magnetic tips and reach the magnitude of about 0.6 T. Thus, the capsules should be concentrated in the area nearby the magnetic tips.



Figure 8. *In vivo* visualization and trapping of the microcapsules in mesentery vessels; (a-c) trapping of the capsules in the Y-branched microvessel: (a) image of the microvessel captured in a white light transmission mode, (b) the same image taken at excitation by 532 nm laser light, and (c) merged white light and fluorescent images; (d-f) examples of *in vivo* trapping of the capsules in bends and bifurcations of mesentery microvessels (yellow lines on the (f) denote the microvessel surrounded by a fat tissue).

Figure 8 shows the images of the microcapsules trapped by magnetic field in a rat mesentery microvessel. Figure 8a is an image of Y-branched mesentery microvessel captured in a white light transmission mode. A white glow around the microvessel is due to transmission by fat tissue. Figure 8b is the same image taken at excitation by 532 nm laser light. One can clearly see the fluorescence from the microcapsules trapped by the magnet. Figure 8c is a merged white light and fluorescent image. It is remarkable that unlike *in vitro* trapping the capsules remain on the vessel wall after magnetic field was turned off. Further *in vivo* experiments demonstrated that capsules are generally trapped by magnetic field in the bends and bifurcations of blood vessels (Figure 8d-f) that can be attributed to the blood turbulence[50](#_ENREF_50) slowing down the capsules and facilitation their mechanical infiltration into the vessel wall. This seems to be promising with respect to magnetic targeted delivery of microcapsules to a tumor as growth of cancer tissue is always attended with high vascularization due to development of new angiogenic vessels.[51](#_ENREF_51) Tumor neovasculature tends to behave different to healthy blood vessels and generally have convoluted and excessive branching with disturbed blood flow[52](#_ENREF_52), [53](#_ENREF_53) that appears to be preferable for *in vivo* magnetic trapping.



Figure 9. Typical histological slice of a rat mesentery after magnetic trapping of the capsules: (a) a vein wall marked with yellow lines; (b) erythrocytes; (c) a small capillary filled with the microcapsules; (d) agglomeration of the microcapsules; (e) microcapsules penetrated through the endothelium into the vein wall.

Further, a histological analysis of the mesentery tissue was carried out to find out the accumulation and distribution of the microcapsules trapped by magnetic field. Figure 9 shows a typical image of taken histological slices. One can clearly distinguish a lengthwise section of large vein, vein walls (a), erythrocytes (b), and small capillaries (c). Histological analysis shows accumulation of microcapsules along the walls of large vessels yet preserving the vein lumen (d). However, the capsules were also found to fill some small mesenteric vessels (c) although the number of blocked vessels was insignificant. Insert in Figure 9 shows an enlarge view of the vein wall (e). It is seen that a small part of capsules penetrated through the endothelium into the blood vessel wall.

Significantly, the magnetic trapping did not block the blood flow in large vessels that was confirmed by histology (Figure 9) and was clearly seen during live-time observations (Supporting Video at http://pubs.acs.org.). This can be attributed to inhomogeneity of magnetic field in the gap between the magnets that attracts the capsules to the top and bottom walls of the vessel. However, the fact that blood circulation retained in most of the vessels is of great importance as disturbed microcirculation particularly in large vessels give rise to myocardial infarction,[54](#_ENREF_54) acute stroke,[55](#_ENREF_55) and various types of embolisms. On the other hand, considering a potential cancer therapy, partly or full blocking of tumor-associated angeogenic neovasculature by microcapsules trapped by magnetic field could be an additional way to beat the disease.[56](#_ENREF_56), [57](#_ENREF_57) Thus, further research should be carried out to find out the impact of blocking of certain capillaries on tissue state.

4. Conclusions

We have developed the technique for fluorescent microcapsules visualization in vivo in a whole blood stream and their retention with magnetic field in the site of interest. *In vitro* experiments on glass tube the experiments demonstrated the possibility to catch the capsules in a blood stream by locally applied non-uniform permanent magnetic field. Removal of magnetic field results on detachment of most of the capsules from glassy wall of capillary. *In vivo* experiments carried out on mesenteric vessels of white mongrel rats demonstrated that capsules could be successfully trapped by magnetic field in a bend of a blood vessel. The capsules remained on the bend of the vessel after magnetic field turned off. Histological analysis showed that capsules infiltrate into small mesenteric vessels. While the large veins remained unblocked for blood passage. Although, the capsules could form large aggregates the blood flow remains intact that was clearly seen during real-time *in vivo* observations. Furthermore, the histology slices demonstrated that under magnetic field capsules can penetrate through the endothelium into the vessel walls during the time of experiment (30 – 40 min).

The provided experimental data demonstrate a potential to further exploration of multifunctional capsules as remotely controlled delivery systems in biological liquids. External magnetic field has been found appropriate to retain capsules in blood flow. These capsule based delivery systems are promising for external navigation to site of importance to ensure a local effect where it is much needed such as in a cancer treatment (e.g. different kinds of intestine cancer) where injected of drug delivery systems guided by magnetic field can be localized into the blood vessels feeding the tumor. Obviously the application is not limited to deliver to tumor site and can be explored for delivering to other organs where the magnet is appropriate for retention.

ASSOCIATED CONTENT

Supporting information

Scheme of the homemade optical setup employed for *in vivo* and *in vitro* visualization of fluorescent magnetic microcapsules in the blood stream; magnetic field distribution in the gap of electromagnet; movie of trapping of fluorescent magnetic microcapsules in rat mesentery microvessels. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

The manuscript was written through the equal contribution of all of the authors. All authors approved the final version of the manuscript

Notes  
The authors declare no competing financial interests.

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**TOC graphic**

