Locally targeted drug delivery to magnetic stents for therapeutic applications

Z.G. Forbes[1], D.S. Halverson[2], G. Fridman[1], B.B. Yellen[2], M. Chorny[3], G. Friedman[2], K.A. Barbee[1]
[1]Drexel University, School of Biomedical Engineering, Science, and Health Systems, Philadelphia, PA, USA
[2]Drexel University, Electrical & Computer Engineering Department, Philadelphia, PA, USA
[3]Children's Hospital of Philadelphia, Philadelphia, PA, USA

Abstract— **The ability to safely and effectively deliver high dosages of drugs to specific sites in the human body is fundamental to the advancement of drug delivery research. Drugs with proven effectiveness under in vitro investigation often reach a major roadblock under in vivo testing due to a lack of an effective delivery strategy. In addition, many clinical scenarios require delivery of agents that are therapeutic at the desired delivery point, but otherwise systemically toxic. This paper proposes a method for targeted drug delivery by applying high magnetic field gradients within the body to an injected superparamagnetic colloidal fluid carrying a drug, without relying on magnetic coils held external to the body to provide the force. The experimental flow model presented gives insight into the use of magnetic carriers for site-specific delivery of therapeutic agents.**

Keywords— **drug delivery, magnetic targeting, stent**

I. Introduction

The intention of this research is to provide both the method and the devices for capturing drug, cells, or other therapeutic agents attached to, or encapsulated within, colloidal magnetic particles, magnetic liposomes, or ferrofluids, at selected sites in a subject. The method and devices are based on using two independent sources of magnetic field. One is a source for producing strong, but highly localized magnetic field gradients to pull the magnetized drug carriers toward the selected site within the subject. The other is a source of relatively uniform, but far reaching magnetic field needed to magnetize the drug carriers deep within the subject.

The combination of these two magnetic sources allows for optimization of the capture efficiency of drug at locally selected sites within the human body. These magnetic field sources and methods for targeted agent delivery are useful in preventive medicine, and treatment of injury and disease, particularly in applications requiring repeatable and highly concentrated dosing to the tissue surrounding the device without systemic levels of drug toxicity being introduced to the rest of the body.

A. Stent Background

Stents are commonly used in a variety of biomedical applications. For example, stents are routinely implanted in patients to keep blood vessels open in the coronary arteries, to keep the esophagus from closing due to strictures of cancer, to keep the ureters open for maintenance of kidney drainage, and to keep the bile duct open in patients with pancreatic cancer. Such stents are usually inserted percutaneously under radiological guidance. Stents comprise a tube made of metal or polymer, in a wide range of physiologically appropriate diameters and lengths [1-3].

General stent design varies in the number of intersections and interstrut area, the in-strut configuration, and the metal-to-artery ratio. The two different expansion principles for stents are balloon-expansion and self-expansion, and the design types can be categorized into five types: ring, tubular, multi-design, coil, and mesh [1-3]. Stents have been routinely used over the last thirteen years in percutaneous transluminal coronary angioplasty (PTCA), a procedure for the treatment of severe, symptomatic coronary stenosis [1].

B. Restenosis

In-stent restenosis (the re-closing of the vessel) remains a major limitation, particularly in coronary stenting. Restenosis is generally considered a local vascular manifestation of the biological response to injury. The injury as a result of catheter insertion consists of denudation of the intima (endothelium) and stretching of the media (smooth muscle). The wound-healing reaction consists of an inflammatory phase, a granulation phase, and a remodeling phase. The inflammation is characterized by growth factor and platelet activation, the granulation by smooth muscle cell and fibroblast migration and proliferation into the injured area, and the remodeling phase by proteoglycan and collagen synthesis, replacing early fibronectin as the major component of extracellular matrix [1-2].

Coronary stents comprise mechanical scaffolding that almost completely eliminates recoil and remodeling. However, neo-intimal growth or proliferation is still a problem. Neo-intimal proliferation occurs principally at the site of the primary lesion within the first 6 months after implantation, a major checkpoint for patient health post-surgery [19]. Neo-intima forms during the first week after PTCA and the progress is well under way after 4 weeks, with continued progression over the following months [2] This neo-intima is an accumulation of smooth muscle cells within a proteoglycan matrix that narrows the previously enlarged lumen. Its formation is triggered by a series of molecular events including leukocyte infiltration, platelet activation, smooth muscle cell expansion, extracellular matrix elaboration, and re-endothelialization [19].

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C. Current Treatment Options

There are several treatment options for restenosis in practice or under investigation. Local drug delivery provides limited systemic release, thereby reducing the risk of systemic toxicity [1-3]. Techniques for local drug delivery that have been described include, but are not limited to, direct coating of the stent with drug, coating of the stent with a drug-containing biodegradable polymer, and hydrogel/drug coating. Biodegradable stents have also been described [1-3].

Problems with these technologies, include damage to the polymer layer during implantation (causing portions to break off and cause clot formation), the inability to deliver effective concentrations, one-time dosage limitations, and, in the case of biodegradable stents, mechanical compromise. An additional concern with the polymer-coated drug-eluting stents is limitation of the growth of the cell layer necessary to cover the stent and prevent the bare metal from coming in long contact with the blood, thereby leading to clot formation [19].

Recently the FDA has approved the TAXUS ™ Express 2™ Coronary Stent (Boston Scientific, Boston, MA) which releases the drug paclitaxel, and in early 2003 the CYPHER™ Sirolimus-eluting Coronary Stent (Cordis, Miami, FL) was approved. The U.S. Food and Drug Administration (FDA) has announced that the agency is warning physicians about blood clots and other side effects associated with the Cypher stent, manufactured by Cordis Corporation, a subsidiary of Johnson & Johnson. The device has been linked to more than 60 deaths and 290 cases of thrombosis (Issued by the FDA on October 29, 2003).

D. Magnetically Targeted Drug Delivery

In general, methods and devices proposed in the past to deliver magnetizable drug or agent-containing magnetic carriers to specific locations in the body rely on a single source of magnetic field to both magnetize the carriers and to pull them by magnetic force to the specific location [4-8]. These single magnetic fields for attracting magnetically susceptible therapeutic agents can be applied by sources external to the body, or by an internal implant.. This single source capture method is at odds with the underlying physics of magnetic particle capture, which depends on the simultaneous imposition of very strong far-reaching magnetic fields and strong spatial magnetic field gradients. The purpose of the far-reaching field is to increase the magnetic moment of individual drug-containing particles in the vicinity of the field to the point of magnetic saturation.

Far-penetrating fields are most typically generated with large magnetic sources. However, the force on a magnetized particle also depends on the production of strong magnetic field gradients, which are most easily generated with very small magnetic sources. Thus, the ability to simultaneously produce far-penetrating magnetic fields that have strong magnetic field gradients is very difficult to accomplish with a single source .

E. Proposed Design

A model for inducing highly localized phase transformations at defined locations in the vascular system by applying 1) external uniform magnetic fields to an injected superparamagnetic colloidal fluid for the purpose of magnetization and 2) using implants with patterned magnetic material to create high magnetic field gradients was described [22]. The use of these magnetizable implants in drug delivery was also described previously by the authors [17].

The proposed design provides a magnetizable implant, preferably a stent, for targeting of magnetic agents to a selected site of implantation of the magnetizable implant in a subject through the creation of high field gradients as well as independent means for creating relatively uniform magnetic field for magnetizing the magnetic agents. The two magnetic sources are independently produced in order to improve capture efficiency and uniformity of captured coating, as well as to allow for miniaturization of the implant.

II. Methodology

To determine the legitimacy of the idea of successful of capture magnetic colloid by an implant in the cardiovascular system, multiple computational models were developed. The focus was on a limiting-case scenario, to determine the latitude of the technology. The first model designed was based around capture of particles in flow onto a placed 'anchored' particle, as seen in the literature [17]. The mathematics and logic used for these models were the main influence on the idea of a magnetically segmented stent or implant, which have also been modeled. The idea of high, localized magnetic field gradients is present in both designs, but accomplished in slightly different manners. Results of computational modeling provided adequate starting points for design of in vitro experimental flow models for testing the functionality and effectiveness of magnetic particle capture at high flow rate. A project-specific flow chamber was designed by the authors and machined at Drexel University.

A. Flow Chamber Components

1) Materials: A parallel plate flow chamber (PPFC) was selected as the best system for applying a laminar flow of particles to a magnetically patterned chip or mesh contained within the chamber. The PPFC to be used consists of three main layers: a 4" glass wafer with a magnetic island pattern, a layer of poly(dimethyl siloxane) (PDMS) with a cut-out channel, and a 6mm thick abrasion-resistant cast acrylic

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coverpiece. The cover-piece contains two reservoirs (flow in / flow out) that connect to the pump and the waste receptacle, respectively. In addition there is one vacuum connection, which feeds a milled out loop around the circumference of the piece. This top piece mainly serves to assure correct parallel plate flow, and to seal the top of the channel firmly to the PDMS layer.

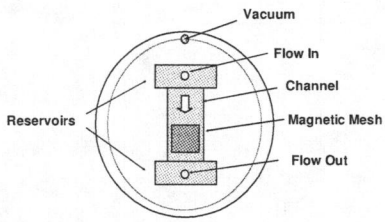
The PDMS layer itself is glued atop of a glass wafer using a thinly applied micro-layer of PDMS, and then as mentioned is held to the top coverpiece by vacuum. This method for attaching the PDMS layer has been tested for stability, endurance, leakage, as well as its ability to withstand sterilization procedures and has been successful in all tests. At the end of the channel, a 2 x 2 cm magnetically electroplated stainless steel mesh is also attached by a thin layer of PDMS at the end of the actual channel space. A description of the preparation and design of the mesh is included in section B. A diagram of the flow-setup can be seen in the Figure 1. A variable flow chemical pump (Fisher Scientific) draws in solution containing the magnetic particles and outputs the particles into the mouth of the PPFC. Flow chamber output is released into a waste bottle.

2) Design parameters: Although in the model the variable parameter is often the vessel diameter, since the focus here is on a flow chamber scenario the characterization will be somewhat different. The design of the flow chamber is based on an average sized coronary artery of diameter 3mm. The parameters of the channel in the flow chamber are Height: $2r$, Width: $18r$, Pattern Width $14r$ ($2r$ clearance on each side), and Pattern Length: 2cm, where r is the radius of the average coronary artery: 1.5mm.

The goal in designing the pattern dimensions was to simulate an 'unrolled' stent (of diameter 3mm and length 2cm) and to continue with the limiting-case scenario analysis, channel height was set at $2r$ instead of r in order to have the maximum flow velocity occurring in the center of the channel. Since each wafer/PDMS-gasket combination is single use, numerous other configurations are possible.

In addition, the pattern is aligned towards the end of the channel, to ensure many channel diameters worth of distance from the entering reservoir to minimize entrance effects at the pattern itself, and to hence obtain steady parallel plate flow. It is important to note that while the actual channel itself is around 4cm in length, the full cutout length in the PDMS is 6.5 cm, allowing for space at either end of the channel at which the reservoir feeds or empties the channel.

The reservoir dimensions are the same at the entrance and exit, and are sized at approximately 5 times the cross-sectional area of the flow channel to ensure appropriate flow parameters and speed in the channel itself. As calculated from resting blood flow rates in the coronary artery of 1.25 ml/sec, an approximately 5.0 cm/s flow velocity for the channel will used in the experiments (which can be increased or decreased without modification of the flow chamber itself, and adjusted for different channel heights).



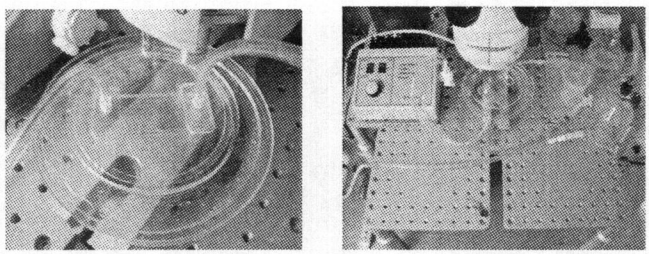


Fig 1a, 1b, 1c. 1a shows an overhead view diagram of the chamber with magnetic mesh fixed to the channel floor. 1b shows an up close view of a connected chamber. 1c shows the complete experimental setup with pump, chamber, and catch beaker. The setup is aligned over a magnetization coil, and under a Leica microscope for imaging of captured particles.

B. Magnetic Mesh Preparation

To simulate a stent-like surface as a plane instead of a circular cross-section, stainless steel wire mesh (wire diameter 140μm, square strut spacing at 400μm) was electroplated with Cobalt-Nickel alloy at a thickness of approximately 5μm. This material was chosen to combine the strong magnetic properties of Cobalt with the plating uniformity of Nickel while reducing the stress on the plated material with additives such as Saccharin and NaCl. This combination allows for the creation of a coating layer that can bend and flex without cracking or detaching from the mesh, which is critical for use in stents [20]. Using information from Myung's work a bath with higher Cobalt content was used to create a harder magnetic material [21].

Electrodeposition was performed in a 2.5cm by 2.5cm by 1.25cm flow cell with continual circulation of bath solution from a reservoir heated to 55 degrees Celsius that was agitated by gentle bubbling. A Princeton Applied Research 363 potentiostat provided an -800mV voltage with respect to an Ag/Cl reference electrode giving a plating rate of approximately one micron per minute. This resulted in strongly coatings that have a brilliant aspect, smooth surfaces and are unaffected by flexing of the mesh.

A scanning electron microscopy (SEM) image of the used mesh can be seen in Figure 2.

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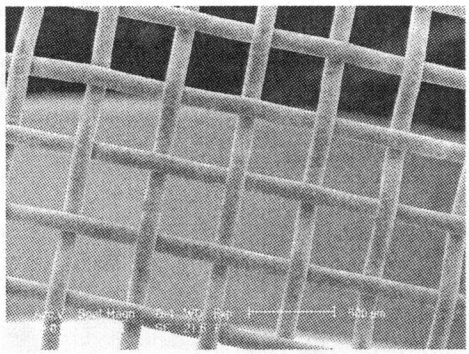


Fig. 2. SEM image of a woven , stainless steel mesh electroplated with Cobalt-Nickel alloy for provision of strong magnetic field gradients

C. Particle preparation

Particles obtained for the first flow experiments are superparamagnetic polystyrene particles (about 20% by weight magnetite), which are about twice the density of water. The beads are obtained from Spherotech, and are 2μm in diameter, and nile red fluorescent. Additional experiments have just been performed with 350nm diameter red fluorescent biodegradable magnetic nanoparticles..

From the original particle solution, data on approximately how many particles are present in a "perfectly" suspended solution is available, and can be used to estimate how many particles are introduced into the flow chamber. In each experiment the particles were diluted 1:5 from their original concentration (10^8 beads/ml for 2 μm diameter, 10^{12} beads/ml for 350 nm diameter). The fluorescence of the particles is necessary for identification of particle capture on a magnetic surface for both qualitative and quantitative analysis.

D. Flow Experiments

Of numerous conducted experiments, presented here are data from one set of experiments from each of the two listed particle sizes, each at the same flow velocity of approximately 5 cm/s (adjusted from channel parameters and volumetric flow output from the flow pump). These two flow experiments were conducted in phosphate buffered saline. In each case, a set of two control experiments were performed: flow of non-magnetic particles across a magnetic mesh, and flow of magnetic particles across a non-magnetic mesh.

The flow chamber was placed on the microscope stand just above a power supply controlled magnetic coil. The chamber was connected to vacuum, flow in from the pump, and flow out to the waste beaker. After vacuum sealing the chamber, a volume of saline was introduced to wet the channel and flush all air bubbles out. At this point all of the particle dilution (in 10 ml) was introduced in one passage through the chamber, followed by a 50 ml volume of saline. The purpose of the flushing is to prevent any part of the particle solution from being present at rest over the magnetic mesh, assuring all capture occurs under flow. In each case, a measured 150 Gauss uniform field was applied, in order to magnetize the particles for capture by the magnetized mesh.

E. Data Analysis

After experiments were complete, all imaging was conducted fluorescently, in some cases with an additional white light used for backlighting. All results presented here are based solely on qualitative analysis. Other flow experiments involve magnetic patterning of islands directly onto the wafer itself. Magnetic patterning of islands of glass wafers is accomplished with the use of photolithography and metal deposition chambers in the University of Pennsylvania clean room facilities in the micro-fabrication laboratory.

In these cases data analysis will be performed first by imaging under fluorescent microscope by counting of individual particles for experiments at extremely low particle concentrations, average intensity of fluorescence over particular areas, distribution of fluorescence along the length and width of the pattern, and statistical analysis of these variables from one experiment to another. Capture relationships between flow velocity, particle size, particle concentration, and channel size must be established for each pattern, inter-related between patterns, and ultimately the capture efficiency of each pattern will be compared.

For the experiments presented here, the limitations of the three dimensional structure directed analysis to be purely qualitative with the intent of determining the capability of capture of magnetic particles onto the magnetically plated mesh at various particle sizes under high flow.

III. Results

A. Flow Experiment Results

Magnetically plated mesh had excellent capture success. Figure 3 shows two characteristic images of captured 2μm diameter superparamagnetic particles, No images are presented from control experiments, as no particles were brought down in either control scenario.

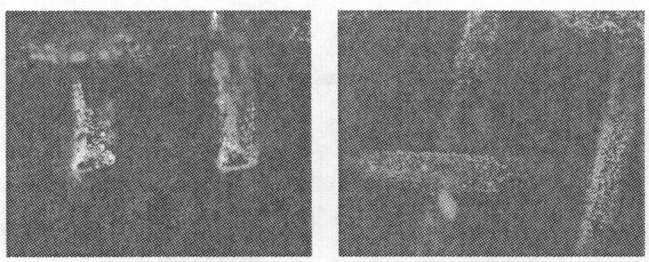


Fig 3. Images of captured 2μm magnetic particles on electroplated mesh, captured at 5 cm/s flow velocity in parallel plate flow. (Left) shows high accumulations of particles at the edge of the mesh, where large gradients accumulate. (Right) shows extremely well spread coverage by captured particles in a characteristic image consistent with the majority of the mesh.

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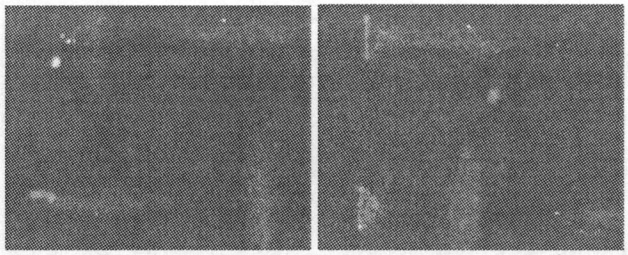


Fig 4. Images of captured 350 nm magnetic particles on electroplated mesh, captured at 5 cm/s flow velocity in parallel plate flow. (Left) shows extremely well spread coverage by captured particles in a characteristic image consistent with the majority of the mesh. (Right) shows high accumulations of particles at the edge of the mesh, where large gradients accumulate.

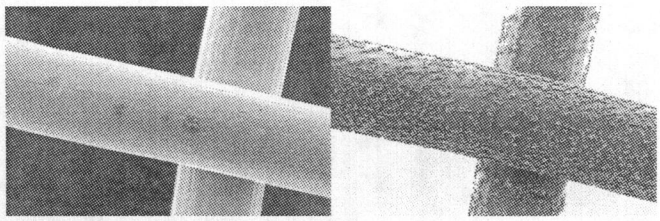


Fig. 5. SEM images of magnetic mesh wire intersections. (Left) shows mesh unexposed to 2μm magnetic particles. (Right) shows mesh successful in capture of 2μm magnetic particles.

Magnetically plated mesh also had success in capture of 350nm diameter biodegradable superparamagnetic particles. Figure 4 shows characteristic images of captured particles from these flow experiments. Again, no control images are presented. Some perspective is limited by the field of view due to the weaving arrangement of the wires in the mesh.

Figure 5 shows SEM images of magnetic mesh with and without captured 2μm diameter superparamagnetic particles. SEM images were taken in order to gain better understanding of whether particles were accumulating significantly at the wire intersection points on the mesh. It can be seen below that they are well spread across the entire surface of the mesh, even in the corners of intersections.

IV. Discussion

The ability to capture magnetic particles in flow has been studied extensively in the past. Friendlaender and co-authors have previously studied trajectory and accumulated aggregates of paramagnetic and diamagnetic particles flowing past a magnetizable surface [11-13]. However, their studies were based on relatively macroscopic magnetic anchor materials. In biological systems, it is frequently not possible to embed macroscopic objects in blood vessel walls. Prior models by the authors, by contrast, analyze particle trajectory and aggregate stability attracted to anchors of equivalent physical characteristics (ie. size, shape, magnetic properties) as the injected drug-containing magnetic particles [17]. Additional two-dimensional computational models have been designed to predict capture of 1-10 particles from varying starting points, by "islands" of ferromagnetic material, oriented along an implant in a channel (to simulate an implant scenario).

In all magnetic drug delivery systems, the magnet serves two purposes; first, to magnetize the drug, and secondly to provide magnetic field gradients to capture the drug. Although large magnets held near or implanted in the body provide strong magnetic fields to magnetize the drug, these large magnets inherently produce weak magnetic field gradients. Micron-sized magnets provide very strong magnetic field gradients, however their fields are short-range and they may not by themselves efficiently magnetize the drug.

This magnetic drug delivery system is novel in that the steps of magnetizing the drug and providing the magnetic field gradients to capture the drug are accomplished separately. By using micron-sized magnetic implants in combination with long-range magnetic fields, it is possible to both magnetize the drug and create strong magnetic field gradients for more efficient capture and localization of the drug.

It may be possible to accomplish both of these steps simultaneously in a single implant. One goal is to attempt to capture particles on a magnetic pattern without first magnetizing the particles with an external field, but so far it has been demonstrated that the presence of a 100-300 Gauss, reasonably uniform magnetic field will be sufficient in magnetizing the particles for capture by the implant-produced gradients. Magnetic material can be applied to a stent or other implant so as not to interfere with the normal function of the device. Thus it is possible to add an additional drug-delivering capability to cardiovascular implants. In addition, superparamagnetic and weakly ferromagnetic implants are typically more biocompatible, as they are composed of magnetite and other FDA approved magnetic materials.

Theoretical modeling, physical flow experiments, and flow experiments in the presence of a cellular boundary layer, should provide the relationships and theoretical basis for magnetic/magnetizable implant design. This can be accomplished by analyzing the relationships between parameters of the magnetic pattern (size, orientation, spacing), and the efficiency of capture. Whereas some magnetic patterns designed have been adapted to match strut parameters and spacing of coronary stents, a computational model provides the ability to investigate a range of pattern geometries, many of which would not work on current stent designs.

Based on model data one can pattern wafers with those varied island geometries, and analyze their capture efficiency as well. In that respect, this project has the capability to develop the design principles necessary for

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applying the technique to any clinical application which could be aided by magnetic targeting. New stent designs and endovascular implants are being used and considered for a wide range of applications outside of coronary stenting, many of which would have a different set of restrictions on particle size, magnetic island size and spacing, and flow velocity. By making the goal of the project the establishment of relationships between variable parameters, and not setting a particular percentage capture yield or coverage requirement, there is flexibility to examine numerous scenarios that can be then fitted to dosage and drug-loading curves for particular pathologies.

V. Conclusion

Flow experiments have thus far confirmed computational model predictions on the capability to capture superparamagnetic particles at small to moderate diameters without the reliance on large external magnetic gradients to direct particles to a site. Other experiments in progress involve capture on directly patterned surface in the presence of cellular monolayers, in order to evaluate interactions between cells, particles, and magnetic substrates. Particles will also be labeled with gene vectors to evaluate the capability to successfully transfer molecules from particles across cell membranes

Acknowledgment

This work was supported in part by the National Science Foundation under Grant NSF 9984276.

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