Video Article

Ferromagnetic Bare Metal Stent for Endothelial Cell Capture and Retention

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Abstract

Rapid endothelialization of cardiovascular stents is needed to reduce stent thrombosis and to avoid anti-platelet therapy which can reduce bleeding risk. The feasibility of using magnetic forces to capture and retain endothelial outgrowth cells (EOC) labeled with super paramagnetic iron oxide nanoparticles (SPION) has been shown previously. But this technique requires the development of a mechanically functional stent from a magnetic and biocompatible material followed by *in-vitro* and *in-vivo* testing to prove rapid endothelialization. We developed a weakly ferromagnetic stent from 2205 duplex stainless steel using computer aided design (CAD) and its design was further refined using finite element analysis (FEA). The final design of the stent exhibited a principal strain below the fracture limit of the material during mechanical crimping and expansion. One hundred stents were manufactured and a subset of them was used for mechanical testing, retained magnetic field measurements, *in-vitro* cell capture studies, and *in-vivo* implantation studies. Ten stents were tested for deployment to verify if they sustained crimping and expansion cycle without failure. Another 10 stents were magnetized using a strong neodymium magnet and their retained magnetic field was measured. The stents showed that the retained magnetism was sufficient to capture SPION-labeled EOC in our *in-vitro* studies. SPION-labeled EOC capture and retention was verified in large animal models by implanting 1 magnetized stent and 1 non-magnetized control stent in each of 4 pigs. The stented arteries were explanted after 7 days and analyzed histologically. The weakly magnetic stents developed in this study were capable of attracting and retaining SPION-labeled endothelial cells which can promote rapid healing.

Video Link

The video component of this article can be found at <http://www.jove.com/video/53100/>

Introduction

Patients implanted with vascular stents manufactured from thrombogenic materials like stainless steel, cobalt chromium, and platinum chromium – both bare metal stents (BMS) and drug eluting stents (DES) – need anti-platelet therapy to prevent thrombus formation. BMS heal rapidly, but are subject to late stage restenosis due to incomplete healing. DES require long term anti-platelet therapy due to delayed healing. Anti-platelet therapy administered to avoid thrombosis as a result of incomplete or delayed healing leads to increased bleeding risk and may not be suitable for certain patients^{1,2}. An ideal stent will heal completely and quickly thus avoiding long-term anti-platelet therapy and late stage restenosis. This complete healing can only be achieved if the stent is rapidly coated with a monolayer of endothelial cells after implantation. Coating the stents with biocompatible materials such as gold or other biopolymers has been shown to improve thrombo-resistance, but none of these techniques achieved ideal blood compatibility as may be possible by coating with endothelial cells^{3,4}.

A stent can be coated with endothelial cells post implantation by attracting circulating progenitor cells. This self-seeding technique can be achieved by utilizing ligands and antibodies. But this technique is limited by the low number of circulating endothelial progenitor cells. A promising strategy is to deliver cells directly to the stent immediately following implantation during a short period of blood flow occlusion^{3,5}. This strategy requires a technique for rapidly capturing cells and retaining them on the stent even after restoring blood flow. We have developed a technique in which a magnetic stent is used to attract and retain magnetically-labeled endothelial cells delivered post implantation. To achieve this, a functional BMS with sufficient magnetic properties to capture and retain magnetically-labeled endothelial cells is required⁶.

In this paper, we discuss the methods for designing, manufacturing, and testing a 2205 stainless steel stent. The stents were designed using CAD and FEA. The manufactured stents were magnetized using a neodymium magnet and the retained magnetic field was measured using a magneto-resistance microsensor probe. We then tested the stents for magnetically-labeled cell capture in a culture dish during our *in-vitro* experiments. Finally, the stents were tested *in-vivo* by implanting magnetic and non-magnetic stents in 4 pigs and histologically analyzing the stented arteries.

Protocol

All animal studies were approved by the Institutional Animal Care and Utilization Committee (IACUC) at Mayo Clinic.

1. Design and Analysis of a 2205 Stainless Steel Stent

1. Designing a bare metal stent using CAD
 1. Make an extruded hollow cylinder by selecting on 'extruded boss/base' feature with the wall thickness equal to the stent strut thickness.
 2. Design a stent pattern on a different sketch plane tangential to the extruded cylinder. Make the width of the flat pattern to match the circumference of the extruded hollow cylinder.
 3. Transfer the flat pattern design onto the hollow cylinder using the wrap feature.
 4. Save the part in its native format and also in ACIS format to be exported for FEA.
2. Finite element analysis for stent models
 1. Import the solid geometry saved in ACIS format into the part module of the FEA software for further analysis.
 2. Model 2 analytical cylinders coaxially to the stent in the part modeler of the FEA software. The outer cylinder has an initial diameter larger than the diameter of the stent to simulate the crimper and the inner cylinder has an initial diameter of 1 mm to simulate a balloon for inflation.
 3. Double click on the 'instances' tree item of the assembly modeler to assemble the above said parts in relative positions.
 4. Use the mesh module of the FEA software, specify the element type as 20-node hexahedral element with reduced integration, specify the element size, and mesh the stent.
 5. Specify frictionless rigid contact pairs between the stent and the two cylinders respectively in the 'interaction properties' of the model tree.
 6. Assign elasto-plastic stress-strain behavior of 2205 stainless steel to the stent model.
 7. Define boundary conditions to firstly crimp the outer cylinder to 1 mm which simulates the crimping of the stent. Remove the outer cylinder to simulate the relaxation of the crimped stent. Expand the inner cylinder to 3 mm to simulate expansion and finally, remove the inner cylinder to simulate recoil of the stent.
 8. Define the simulation parameters including the number of processors and amount of RAM allocated in the 'Analysis' model tree item and run the simulation.
 9. Once the simulation is complete, open the result file (filename.odb) and post-process the results to study the principal strains and iteratively improve the stent design to achieve a principal strain of 20% which is less than the failure limit of the material.

2. Stent Fabrication and Testing for Crimping and Expansion

1. Stent fabrication
 1. Obtain the 2205 stainless steel tubes by gun drilling and precision grinding bar stock material at a precision machining company such as Action Precision Products in Pioneer, OH.
 2. Transfer the precision ground tubes and the flat pattern stent design to a stent cutting company such as Laserage Technology Corporation in Waukegan, IL for laser cutting and electropolishing.
 3. Passivate the surface of the electropolished stents by submersing them in a strong acid (50% HCl) for 10 min followed by a base (10% NaHCO₃) for another 10 min. CAUTION: handle chemicals with proper protective equipment and under a fume hood. Finally, wash the stents with ethyl alcohol and deionized water. This process is called acid pickling.
2. Testing of manufactured stent for crimping and expansion
 1. Crimp the stent onto a trifold balloon using a hand held crimping tool. Hold the stent and the trifold balloon in the crimping tool. Press the handle to radially deform the stent to be crimped on the balloon.
 2. Inspect the crimped stent using a microscope for uniform crimping and any signs of failure in the structure due to plastic deformation.
 3. Expand it to the designed diameter of 3 mm by pressurizing the trifold balloon with water. Examine the expanded stents for microscopic fractures and uniform expansion.

3. Characterization of Stent for Retained Magnetic Field

NOTE: Cylindrical magnet of 2 inch diameter and 1 inch height was used in this study. The poles of the magnet are aligned along the axis. The surface magnetic flux density of the magnet is approximately 1 T.

1. Magnetize the stents diametrically or axially using a strong neodymium magnet. Hold the stent close to the strong magnet for approximately 1 min for magnetization.
2. Hold the stent on one of the flat faces with its diameter along the magnetic field lines to be magnetized diametrically or hold the stent next to the cylindrical surface with its axis along the magnetic field lines to magnetize it axially. Retained magnetic field of the stent was found to be stable for at least 24 hr, but use the stent as soon as possible after magnetization.
3. Mount the stents individually onto glass mandrels and then mount the glass mandrels in the precision chuck of the magnetic probing fixture. Magnetic microsensor probe can be precisely positioned close to the stent without touching the surface using the XYZ stages assembly of the magnetic probing fixture (**Figure 4**).

4. Measure the baseline reading of the magnetic microsensors far away from the stent and then measure the retained magnetic field at the stent's surface by positioning the probe using the XYZ stages of the magnetic probing fixture.

4. Magnetic Cell Capture Studies

1. Obtaining cells, labeling with SPION and staining with fluorescent dye
 1. Derive the endothelial outgrowth cells (EOC) from porcine peripheral blood as described in^{5,7}. Culture in a T-75 flask until approximately 80% confluent (5×10^6 to 8×10^6 cells).
 2. Synthesize SPIONs as 10 nm diameter magnetite (Fe_3O_4) core surrounded by 50 nm thick poly(lactic-co-glycolic acid) (PLGA) shell as described in^{8,9}.
 3. Incubate the derived EOC with SPION at a concentration of 200 $\mu\text{g}/\text{ml}$ of cell culture medium for 16 hr at 37 °C.
 4. Aspirate the cell culture medium gently. Gently wash the cells by adding 10 ml of phosphate-buffered saline (PBS) to the flask, rocking, and aspirating the PBS.
 5. Stain the cells with fluorescent dye (CM-Dil) for visualizing during experiments. This is done per the manufacturer's instructions by adding the dye to 10 ml of cell culture medium at a concentration of 5 $\mu\text{l}/\text{ml}$ and incubating with the cells for 30 min at 37 °C.
 6. Wash the cells with PBS as in step 4.1.4 and incubate with 3 ml of 0.25% trypsin-EDTA solution for 5 min at 37 °C to lift the cells from the flask.
 7. Transfer the cell suspension to a 15 ml conical tube, top off with PBS, and centrifuge at 500 x g for 5 min to form a cell pellet.
 8. Re-suspend the cell pellet in PBS at a concentration of $1\text{--}2 \times 10^6$ cells/ml and mix thoroughly by pipetting in and out of the conical tube several times.
2. *In-vitro* cell studies
 1. Design and fabricate (e.g., 3D printing) a simple fixture to hold the stent just above the surface of a glass coverslip.
 2. Demagnetize a stent using an electromagnetic degausser or magnetize a stent diametrically or axially using a strong neodymium magnet.
 3. Pipette the SPION-labeled EOC suspended in PBS into the dish containing the axially magnetized or diametrically magnetized or non-magnetized control stents. Image the stents with EOC suspended in PBS immediately for fluorescence using an inverted fluorescence microscope.

5. In-vivo Animal Studies

1. Stent implantation
 1. Draw peripheral blood from 4 healthy Yorkshire pigs – weighing approximately 50 kg – 3 weeks prior to stent implantation respectively and culture EOC as described in^{5,7}.
 2. Administer anti-platelet medication starting 3 days prior to surgery (aspirin 325 mg and clopidogrel 75 mg daily).
 3. On the stent implantation day, anesthetize the pigs with intramuscular Telazol, Xylazine, and Atropine (5/2-3/0.05 mg/kg respectively) as stated in the applicable institutional animal care and use guidelines.
 4. Intubate and place the pig on inhalation of 1-2.5% Isoflurane anesthesia.
 5. Shave the ventral neck region of the pig and conduct the procedure in general sterile conditions.
 6. Implant 1 magnetized and 1 non-magnetized stent into the right coronary artery (RCA) using standard cardiac catheterization technique.
 1. Catheterization of animals should be performed by a trained interventional cardiologist. Access the right carotid artery with a 9 French sheath.
 2. Cannulate the target coronary artery and inject iodinated contrast dye to obtain fluoroscopic images.
 3. Place a 0.014 inch standard coronary guidewire in the artery. Advance the balloon and stent using this guide wire and deploy the stent in a 3-3.5 mm diameter vessel.
 7. Occlude the blood flow within the RCA proximal to the implanted stents using an over the wire balloon and deliver approximately 2×10^6 autologous EOC labeled with SPION suspended in 4 ml of PBS via the central catheter over a 2 min period.
 8. Restore blood flow to the RCA after 2 min of additional occlusion.
 9. Transfer the animal to the recovery room and closely monitor the animal until it has regained consciousness.
 10. Continue to administer anti-platelet medication (aspirin 325 mg and clopidogrel 75 mg) post operatively until sacrifice.
2. Stent explant and histology
 1. Euthanize the animals 7 days after surgery by first anesthetizing the animal as explained previously and then administer intravenously a lethal dose of sodium pentobarbital (100 mg/kg) as per applicable institutional animal care and use guidelines.
 2. Surgically harvest the stented arterial segments. Fix the explanted arteries in 10% formalin buffer for a minimum of 30 min. Leave the samples in formalin buffer for further histological analysis.
 3. Outsource the fixed sample to facilities capable of performing histology with metal stents. During this processing, the samples are embed in methylmethacrylate, cross-sectioned, and analyzed histologically using Mallory's staining technique with Prussian blue stain for iron particles.

Representative Results

Iterative stent design based on FEA (**Figure 1**) showed a stent which can crimp and expand with a principal strain of 20% which is less than the 30% ultimate strain. Crimping and expansion test (**Figure 2**) showed no signs of fracture. Pictures of the deformed stent showed good

agreement with FEA calculated deformations and also microscopy pictures showed no fractures (**Figure 3**). As expected from the retained magnetic field measurements (**Figures 4 & 5**), SPION-labeled cells were preferentially attracted to bent segments in axially magnetized stents and more uniformly attracted to straight segments in diametrically magnetized stents (**Figure 6**). Histology images showed iron staining near the stent struts proving EOC attraction and retention to the stent during the 7 day implantation period (**Figure 7**).

Figure 1. Stent modeling and analysis flow chart. The schematic shows the computer aided modeling and finite element analysis showing a step-by-step process applied to a 2205 stainless steel stent. Modified from *Uthamaraj et al. 2014*⁶ with re-print permission.

Figure 2. Stainless steel stent crimping and expansion. Laser cut and electropolished stent a) as-cut, b) crimped onto a trifold balloon catheter, and c) expanded to 3 mm using the trifold balloon. Modified from *Uthamaraj et al. 2014*⁶ with re-print permission.

Figure 3. Microscopic inspection of stent. Light microscopy was used to image the expanded stent which was compared to FEA simulation.

Figure 4. Magnetic probe measurement stage setup. The XYZ stages and rotational stages were assembled for positioning the stents and magnetic probe during magnetic field measurements.

Figure 5. Magnetic field measurement regions on a stent and the measurement values. The image shows the measured retained magnetic fields of 2205 stents in axially magnetized and diametrically magnetized configurations. Modified from *Uthamaraj et al. 2014*⁶ with re-print permission.

Figure 6. In-vitro cell capture studies. Fluorescence microscopy images of 2205 stainless steel stents showing cell capture in (A) non-magnetized stent, (B) diametrically magnetized stent and (C) axially magnetized stent. Modified from *Uthamaraj et al. 2014*⁶ with re-print permission.

Figure 7. Images of histological cross sections of stented coronary artery segments of (A) magnetic stent with blue iron staining near the strut and (B) non-magnetic control stent showing no blue staining near the strut. The samples were stained using Mallory's staining technique with iron particles stained with Prussian blue stain. The "*" symbol indicates stent strut locations. Modified from *Uthamaraj et al. 2014*⁶ with re-print permission.

Discussion

We developed a magnetic stent which can function as a bare metal stent and can attract SPION-labeled endothelial cells. In previous studies involving magnetic stents, researchers have used nickel coated commercial stents and coils or meshes made of magnetic materials due to the unavailability of a ferromagnetic stent^{5,10-14}. Other groups have also used the paramagnetic nature of commercially available 304-grade stainless steel stents for targeting nanoparticle loaded endothelial cells³. Nickel coatings may be allergenic to the patients receiving the stents and the paramagnetic stents need an external magnetic field to attract and retain magnetic nanoparticles^{3,5}. Hence, designing and developing a functional ferromagnetic stent is important for cell delivery applications as well as other clinical applications^{10,15-20}. The duplex nature of the material chosen for this study – 2205 stainless steel – makes it weakly ferromagnetic. In addition, 2205 stainless steel has a lower ultimate strain of 30% when compared to other stainless steels used to make stents such as 316L stainless steel (70%)^{6,21,22}.

Based on this novel application of 2205 stainless steel, the protocol presented in this study explains the methods to design, manufacture, and test a weakly magnetic stent. First, a simple stent design pattern was developed using an existing stent pattern as a guide. Results from the FEA simulations suggested that material needed to be added to the bent segments of the stent to achieve a maximum principal strain of 20% which is less than the ultimate strain of the material. The final stent design had a strut thickness of 90 μm . Second, the manufactured stents were magnetized and their retained magnetic fields were measured. The retained magnetic field strength of the 2205 stainless steel stent depends upon the orientation of the applied magnetic field⁶. Axially magnetized stents showed preferential cell capture at the bent segments of the stent while diametrically magnetized stents showed a more uniform cell capture along the struts. This was also confirmed by the magnetic measurement experiments conducted on the stent²³. Magnetized stents showed a retained magnetic field in the range of 100-750 mG compared to a maximum of 10 mG for control, non-magnetized stents. Finally, the large animal implantation studies showed that the BMS manufactured from 2205 stainless steel can be used to attract and retain SPION-labeled endothelial cells even when blood flow is restored post-implantation. Histology showed the presence of blue iron staining near the stent struts of the magnetized stent, thus proving cell capture and retention after 7 days of implantation.

CAD and FEA used in our study can be applied for proper design and analysis of similar balloon expandable stents. In the current protocol, steps 1.2.5, 1.2.6, and 1.2.7 are critical for setting up the boundary conditions and material property assignment and are required for properly designing a stent. Resulting magnetized 2205 stainless steel stents implanted in large animals showed cell capture and retention. Steps 5.1.7 and 5.1.8 are also critical to achieve proper cell seeding on magnetized stents. In addition, the introduction of cells to the magnetic stent implant site during a 2 min occlusion is unique to our presented study.

The stents developed in the current study were able to rapidly endothelialize and withstand short term implantation, but it is unclear if the stents can withstand long-term implantation. To date, ferromagnetic materials have not been extensively studied to understand their limitations for clinical applications. However, our 7 day pig implantation data showed that 2205 stainless steel had good blood and tissue compatibility. The methods presented in this study do not address the techniques for advanced mechanical testing of the stents such as fatigue testing or long-term interaction of the magnetic material with the blood²⁴⁻²⁸. In addition, the weak ferromagnetic nature of 2205 stainless steel was able to capture magnetically-labeled cells, but a novel material with stronger magnetic properties may improve cell capture. Further research is also needed to study the biocompatibility and long term safety of ferromagnetic materials. The endothelial outgrowth cells used in this study were obtained by following a previously published protocol which showed how to isolate and characterize the endothelial outgrowth cells^{5,7}. The current study was also limited by the small number of animals.

In summary, rapid endothelialization of stents has been limited to date because of the unavailability of optimal delivery devices and poor adhesion of endothelial cells. The ferromagnetic stents developed in this study have the advantage of functioning as a BMS while also providing enough retained magnetic field to capture magnetically-labeled endothelial cells. As a part of our continuing studies of long term implantation effects, the stents need to undergo more rigorous mechanical and biocompatible testing. The stent developed in this study shows great promise as a functional ferromagnetic stent capable of endothelial cell capture and retention and the methods presented in this study can be used for future stent development and testing.

Disclosures

The authors declare that they have no competing financial interests.

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