ARTERES ARTIFICIELLES AVANCEES POUR LE DEVELOPPEMENT ET L'OPTIMISATION DES DISPOSITIFS ENDOVASCULAIRES

ADVANCED ARTERIAL MIMICS FOR ENDOVASCULAR DEVICE TESTING AND OPTIMIZATION

Établissement **École polytechnique**

École doctorale **Interfaces : approches interdisciplinaires, fondements, applications et innovation**

Spécialité **biologie**

Unité de recherche **LADHYX - Laboratoire d'hydrodynamique**

Directeur de la thèse ABDUL BARAKAT (mailto:abdul.barakat@ladhyx.polytechnique.fr)

Financement du 01-10-2017 au 30-09-2020

Autre concours - Appel à projets - IDEX, Concours d'accès aux contrats doctoraux des établissements d'enseignement supérieur

Début de la thèse le **1 octobre 2017**

Date limite de candidature **29 avril 2017**

Mots clés - Keywords

atherosclerose, stents, artere artificielle, bioinginierie, suivi cellulaire, imagerie

atherosclerosis, stents, artificial artery, bioengineering, cellular tracking, imaging

Profil et compétences recherchées - Profile and skills required

Physicien ou inginieur avec interet dans le domaine d'inginierie biomedicale. Des connaissances en biologie est un plus.

Physicist or engineer with an interest in biomedical engineering. Some background in biology is a plus.

Description de la problématique de recherche Project description

See below.

Background and Significance
Heart attacks and strokes are the leading cause of mortality in the Western world and are most commonly caused by atherosclerosis. In their advanced stages, atherosclerotic plaques narrow the arterial lumen and obstruct blood flow. The most insidious pathological complication of atherosclerosis is plaque rupture and consequent thrombosis, or blood clot formation. Blood clots may either partially or completely block blood flow in a coronary artery, which could lead to a heart attack and associated tissue ischemia.

The most common treatment for atherosclerosis today is the implantation of a stent, a wire-mesh structure that is deployed on a balloon catheter and expanded at the occluded zone of the diseased artery in order to open up the blocked vessel and restore blood flow. Every year, there are approximately seven million stents deployed globally. In spite of their widespread use, stents continue to suffer from two serious complications: restenosis and thrombosis. Restenosis is a progressive and complex process whereby the vessel becomes re-occluded due to the uncontrolled proliferation of smooth muscle cells (SMCs) within the arterial wall. Thrombosis, on the other hand, involves the sudden development of a blood clot. Although relatively infrequent, this complication is fatal in ~50% of the cases when it occurs.

To expand a stent and implant it firmly in an artery, the balloon catheter used to deploy the stent is inflated at a high pressure. Thus, stent deployment causes massive damage to the endothelium, the cellular monolayer lining the inner surfaces of blood vessels. Two essential roles of a normally functional endothelium are to control the rate of proliferation of SMCs within the arterial wall and to prevent thrombosis. Stent-induced endothelial damage thus greatly increases the likelihood of both restenosis and thrombosis, and sufficiently rapid endothelial wound healing is critical to the success of a stenting procedure.

Most stents in clinical use today fall in one of two categories: bare metal stents (BMS) or drug-eluting stents (DES). DES are coated with drugs that are released into the arterial wall in a controlled fashion to inhibit SMC proliferation and hence limit restenosis, However, the same drugs that inhibit SMC proliferation also inhibit endothelial cell (EC) proliferation and thus greatly retard vascular wound healing. For both BMS and DES, there is a wide variety of stent designs depending on the manufacturer, and there is ample evidence that the

design of a stent and the dynamics of drug release from DES play critical roles in the occurrence of stent complications [1,2]. Today, testing the performance of a particular stent is performed either in rubber tubes for studies that focus on stent mechanical performance or in vivo in animals when biological responses to stent deployment are of interest. Each of these two approaches has its limitations: rubber tube experiments cannot account for the important biological responses whereas animal experiments are costly and fraught with ethical concerns. There is an urgent need for alternative systems that overcome these limitations.

Preliminary Results and Motivation for Proposed Studies

The cardiovascular bioengineering group in the host laboratory (LadHyX) has recently developed a novel stentable in vitro artery mimic that can serve as a powerful tool for endovascular device development and optimization [2]. The system exhibits a number of unique features distinguishing it from tissue-engineered or organ-on-a-chip constructs, most notably that it allows deployment of endovascular devices including stents, quantitative real-time tracking of cellular responses and detailed measurement of flow velocity and luminal shear stress using particle image velocimetry. The in vitro artery mimic consists of an annular collagen hydrogel containing embedded SMCs and lined with a monolayer of ECs on the luminal surface. Steady or pulsatile flow can be produced in the artery and sustained for several days to weeks. Within the proof-of-concept model that has been developed, flow patterns matching those in the human coronary artery were measured using particle image velocimetry, and stent-induced flow disturbance was demonstrated [2]. Furthermore, following implantation of a stent, EC migration to heal stent-induced wounds and SMC activity within the collagen matrix were tracked and quantified [2].

The current version of the in vitro artery mimic has several important limitations. One limitation is that the wall consists of a single layer made of an annular collagen hydrogel containing embedded SMCs and lined with a monolayer of ECs on the luminal surface. In vivo, arteries have multiple distinct layers with each layer containing specialized cells. A second limitation of the current system is that it does not contain any plaques or lesions that protrude into the lumen, which is obviously not the case for diseased arteries in which stents are deployed. Finally, while quantitative tracking of cellular movement has been demonstrated in the present system, the critical processes of restenosis and thrombosis have not yet been captured. The present Ph.D. project aims to overcome these limitations.

Proposed Studies

The goal of the proposed Ph.D. project is to further develop and optimize the in vitro artery mimic in order to render it more representative of the in vivo situation. To this end, the project has three specific aims.

Aim 1: Develop a multi-layer arterial wall. In order to better mimic human arteries, a multi-layer artery mimic will be developed. The structure of medium to large human arteries includes an additional cell-free layer of extracellular matrix between the endothelium and the SMC-containing tissue (the basement membrane portion of the tunica intima), as well as a porous membrane between these two layers (the internal elastic lamina [IEL]). These additional layers will be produced in the artery mimic via a multi-step casting process. The annular SMC-containing collagen hydrogel will be fabricated according to the current protocol. Subsequently, the lumen will be treated with a high-density collagen solution, forming a dense protein layer representative of the IEL. A parametric study of collagen polymerization will be performed in order to identify the conditions necessary to create a porous membrane representative of the human IEL. A layer mimicking the basal lamina will be produced in the artery mimic by injection and polymerization of a second collagen hydrogel, cell-free and with mechanical properties matched to the basement membrane. Once the multi-layer artery is developed, its response to stent deployment in terms of endothelial wound healing, SMC activity, and stent-induced flow disturbance will be studied, and the results will be compared to those obtained in the present single-layer system.

Aim 2: Produce arterial stenosis. Because stents are deployed into atherosclerotic arteries that contain significant stenosis, it is essential to develop the ability to produce in vitro mimics with irregular luminal geometries. To this end, two approaches are envisioned. In the current system, the arterial lumen is created by inserting a constant-diameter metallic pin into the unpolymerized collagen hydrogel and then subsequently polymerizing the gel and removing the pin to expose the lumen. The first approach for producing luminal stenosis consists of replacing the constant-diameter pin used in the current system by a pin with a profiled shape representing the desired stenosis shape. Because removing a single solid pin is likely to perturb the stenosed geometry, we propose to use a pin that consists of two half-pins that can be screwed into one another. Once the hydrogel is fully polymerized, the pin will be unscrewed and each half-pin pulled out separately from the two ends of the mimic. If the idea of a profiled metallic pin proves problematic, a second possible approach involves the 3D printing of glassy sugar made of sucrose, glucose and dextrans as has been described elsewhere [3]. The printed sugar scaffold will have the appropriate shape to create the desired luminal constrictions. The collagen hydrogel will be poured over the sugar scaffold, and this scaffold will subsequently be dissolved once the hydrogel has fully polymerized. Once arterial stenoses are produced, their impact on EC wound healing, SMC activity, and arterial flow field will be quantitatively studied.

Aim 3: Elicit restenosis and thrombosis. Restenosis and thrombosis are the two most serious complications associated with stent implantation. We wish to develop the capability of observing these events in our artificial artery mimic. Restenosis involves the uncontrolled proliferation of SMCs due to stent-induced EC damage and arterial wall injury and inflammation and subsequent SMC migration into the lumen. It has already been demonstrated that deploying a stent in the present artery mimic damages ECs similar to what occurs in vivo [2]. We will measure the effect of this damage on SMC proliferation. Furthermore, we will generate a gradient of inflammatory cytokines that we hypothesize will then drive SMC migration from the arterial wall into the lumen. To investigate thrombosis, we will need to perform experiments using whole blood as the working fluid. The occurrence of thrombosis will be visualized directly using microscopy. The host laboratory has extensive experience in generating thrombi in vitro. Once we have succeeded in producing restenosis and thrombosis in the arterial mimic, we will investigate the flow conditions and stent designs that lead to these complications.

Expected Contributions

At the end of the proposed research, we expect to have gained significant quantitative insight into the performance of stents, as well as the impact of arterial wall structure and of plaques on this performance. The ability to capture restenosis and thrombosis in this model system is expected to greatly enhance our understanding of the physicochemical factors governing these complications. Indeed, the results of this research will set the stage for the systematic implementation of novel stent designs that will minimize the incidence of stent-

related complications. Beyond the field of stenting, the research is also expected to establish the in vitro artery mimic as a powerful and highly versatile platform that can be adapted for use in a host of other vascular investigations such as toxicity testing of cardiovascular drugs, dynamic monitoring of leukocyte interaction with the arterial wall during inflammation, and assessment of the effects of nano-materials on the arterial wall.

Project Timeline
It is anticipated that Aim 1 can be completed within 9 months: 4 months for developing a multi-layer version of the artery mimic and 5 months for the ensuing experiments. Aim 2 will require 9 months for the development of stenoses and running the associated experiments. Aim 3 is the most challenging part of the project and is expected to require 15 months. The final 3 months will be dedicated to the final preparation of the dissertation.

Thématiques /Domaine /Contexte

biomedical engineering

cardiovascular bioengineering; tissue and cellular engineering.
Despite their widespread use, stents continue to suffer from two serious complications: restenosis, a process by which blockage re-develops within a few months of stent deployment, and thrombosis (blood clotting) which, though rare, is often fatal when it occurs. There is evidence that the design of stent struts as well as the dynamics of drug release from DES play critical roles in the likelihood of occurrence of stent complications. Today, testing the performance of a particular stent design is conducted either in rubber tubes on in vivo in animals. Rubber tube experiments are useful from a mechanical standpoint but provide no information on the biological responses to stent deployment. In contrast, in vivo animal studies can provide detailed biological information but are complicated, expensive, and raise ethical concerns. Therefore, there is a critical need for alternative systems that enable controlled, quantitative assessment of biological responses to stent deployment. Such systems promise to be invaluable for understanding the performance of stents as well as for optimizing this performance.

Objectifs

The goal of the proposed Ph.D. thesis project is to continue the development of an in vitro artery mimic that we have recently developed and to use this mimic to study important open questions in the field of cardiovascular stenting.

Précision sur l'encadrement

Weekly one-on-one meetings, weekly group meetings, publications, conference presentations, visits to other laboratories, summer schools.

Conditions scientifiques matèrielles (conditions de sécurité spécifiques) et financières du projet de recherches

The project will be conducted in a highly interdisciplinary group that combines engineers, physicists, biologists, and medical doctors. The experiments will be conducted within the cardiovascular bioengineering group at Ecole Polytechnique.

Funding for this project needs to be secured through a doctoral fellowship.

Objectifs de valorisation des travaux de recherche du doctorant : diffusion, publication et confidentialité, droit à la propriété intellectuelle,...

The candidate will publish his/her results in top bioengineering international journals and will present the findings at international conferences. Depending on the results, the system can be used as a basis for innovation/startup activity.

Collaborations envisagées

Envisaged collaborations with colleagues at the University of Glasgow and at Strathclyde University.

Ouverture Internationale

The group is highly international with members hailing from 8 different countries. The candidate will have a chance to present his/her work at international conferences, He/she will also visit several international laboratories.

Références bibliographiques

1. https://sites.google.com/site/cardiovascularbiotech/coronary-artery-disease-essay.
2. Antoine EE, Cornat FP, Barakat AI. The stentable in vitro artery: an instrumented platform for endovascular device development and optimization. Journal of the Royal Society Interface 13: 20160834, 2016.

3. Miller JS, Stevens KR, Yang MT, Baker BM, Nguyen DT, Cohen DM, Toro E, Chen AA, Galie PA, Yu X, Chaturvedi R, Bhatia SN, Chen CS. Rapid casting of patterned vascular networks for perfusable engineered three-dimensional tissues. Nature Materials 11: 768-774, 2012.

Dernière mise à jour le 4 avril 2017