Modelling the influence of re-endothelialization on in-stent restenosis

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Introduction

Recent advances in the field of endovascular stents have reduced the rates of in-stent restenosis (ISR) from 30 % (with the use of bare metal stents) to 10% (with drug eluting stents) (1). But the chance of getting a restenosis varies between patient to patient, depending on the age and health condition of the patient, vessel size, and the complexity of the developed lesion. There seems to be a link between the arterial injury due to stent deployment with the rate of restenosis (2) but there is no significant injury score information available for patient/animal models who did not develop restenosis. Such experiments are required to analyze whether the injury is the only key factor initiating this response or there may be some other unknown factors that are not understood yet. In this study, we aim to link the results obtained from a multi-scale model of ISR to identify processes that inhibit the development of restenosis.

The innermost layer of a vessel, called endothelium, consists of a mono-layer of endothelial cells (ECs). ECs play an important role in regulating the vascular tone and permeability by managing the exchange of molecules in response to physical and chemical signals (3). After injury caused by balloon angioplasty or stent deployment in percutaneous coronary intervention, the endothelium is completely denuded triggering inflammatory mechanisms like platelets formation, smooth muscle cells (SMCs) migration and proliferation, extra cellular matrix (ECM) degradation and, finally, ISR (1). ECs sense fluid stresses and regulate their effects by releasing vasodilative or vasoconstrictive enzymes to the underlying SMCs. In the absence of a healthy and intact endothelium, the balance between the vasodilators and vasoconstrictors is disturbed and a mismanagement of the vascular tone occurs that can lead to the development of plaque or restenosis (4).

The presence of an intact endothelium inhibits the proliferation of medial SMCs (5). Endothelial progenitor cells (EPC) capturing stents showed an accelerated re-endothelialization but didn't reduce neointimal growth at 28 and 90 days in the porcine coronary arteries (6). This finding hints that the presence of endothelium is not the only factor to inhibit SMC growth but there are some other mechanisms being controlled by a functional endothelium. .

A functional endothelium senses shear stress and translates that as a stimulus to produce nitric oxide (NO). NO, being a highly diffusible molecule, is generated by one of the isoforms of the nitric oxide synthase enzyme (NOS). Endothelial NOS (eNOS) is produced by the endothelial cells. The amount of eNOS released by EC depends on the availability of intracellular calcium (Ca^{2+}) that is regulated in response to shear stresses / blood borne agonists, such as thrombin, adenosine nucleotides, acetylcholine, bradykinin etc. activated pathways (3). Recent evidence (7) also suggests that fluid shear stress modulates the NO production through platelet endothelial cell adhesion molecule (PECAM-1) which directly regulates the basal eNOS activity. And, in agreement with (6), recent experiments have measured PECAM-1 expression and have shown that a regenerated endothelium does not translate into a functional endothelium (8).

Materials and Methods

A two dimensional multi-scale model of in-stent restenosis based on pig coronary artery data has been developed, involving blood flow and SMCs proliferation. Blood flow is modelled as a Newtonian incompressible fluid using a Lattice Boltzmann method. SMC proliferation is modelled using an agent based model (ABM), SMCs grow, proliferate or die based on a set of rules described in ABM. Further details about the model itself and coupling scheme is presented elsewhere (9, 10). In a previous paper, we published the first outcome of this model and compared the results with the available in-vivo and experimental data (2). In the present study, we focused on the process of re-endothelialization and its

possible role in inhibiting the development of neointimal hyperplasia. The domain was prepared by deploying a stent into a healthy artery. This deployment resulted in a complete removal of ECs and a partial rupture of internal elastic lamina (IEL). After IEL is broken, SMCs start to proliferate and give rise to neointima. ECs are not explicitly modelled in the current model. Here two scenarios have been taken into account to evaluate the growth of SMCs. Firstly (scenario 1), using experimental results (8), a probability function was used based on PECAM-1 expression, which is released by mature (functional) ECs from the 3rd day after stenting. So if endothelium is present, then the amount of Nitrite, a stable metabolite of NO, is calculated as a function of wall shear stress, using experimental results from (11). After 15 days, a 100 % functional endothelium is present as an inner most layer in the domain, regulating wall shear stress (WSS) into NO production. The NO produced then serves to inhibit the growth and proliferation of the underlying SMCs. The second scenario (scenario 2) assumes the proliferation of the SMC in response to WSS (when there is no EC) until 15 days, and from 15 day onwards, a healthy endothelium is present with in the domain, producing NO in response to WSS. NO is not modelled explicitly in this model and once generated in response to stress is assumed to quickly diffuse into SMCs.

Results & Conclusion

Results suggest an inhibition of SMC proliferation as soon as a functional endothelium is present (scenarios 1 & 2), compared to our previous results where SMC growth was inhibited by a high wall shear stress as a result of the narrowing of the vessel (figure 1). Figure 1. also demonstrates a dramatic decrease in the neointimal cell number between both scenarios, showing a very low number of cells (almost no restenosis) with the assumption of having a probability of a functional endothelium from day 3 after stenting, whereas the other scenario dictates the presence of a healthy endothelium after 15 days showing a restenosis. These results clearly hint on the importance of a fully functional endothelium by showing different neointimal cell growths. The model does not seem to produce much difference based on different deployment depths using the 1st scenario (Figure 1). However, different growth patterns were observed using the 2nd scenario (Figure 1), showing a direct relationship between the deployment depth and neointimal cell number. An increase in the deployment depth results in a faster neointimal growth and this has already been shown in our previous finding (2). Our previous published results had a limitation that similar amount of final neointima was observed for all deployment depths whereas in the current study, we captured the influence of a functional endothelium that results in different end points (Figure 1). A qualitative comparison of neointimal cell growth between both scenarios and the previous model is also shown in figure 2.

We have hypothesized that a healthy and functional endothelial layer is necessary for the control of the neointimal formation. Once endothelium is functionally present and regulates vasodilators, it can aid to stop the SMC hyperplasia by keeping them in a quiescence state via NO release. Our results do not try to claim that endothelium presence is the only factor required to inhibit restenosis. There is a need to promote a fully functional endothelium that is considered to inhibit the growth of the underlying cells. So despite of the urgent need to recover the endothelial layer, it is much more important to identify the mechanisms that make this monolayer of EC fully functional in order to promote proper cellular

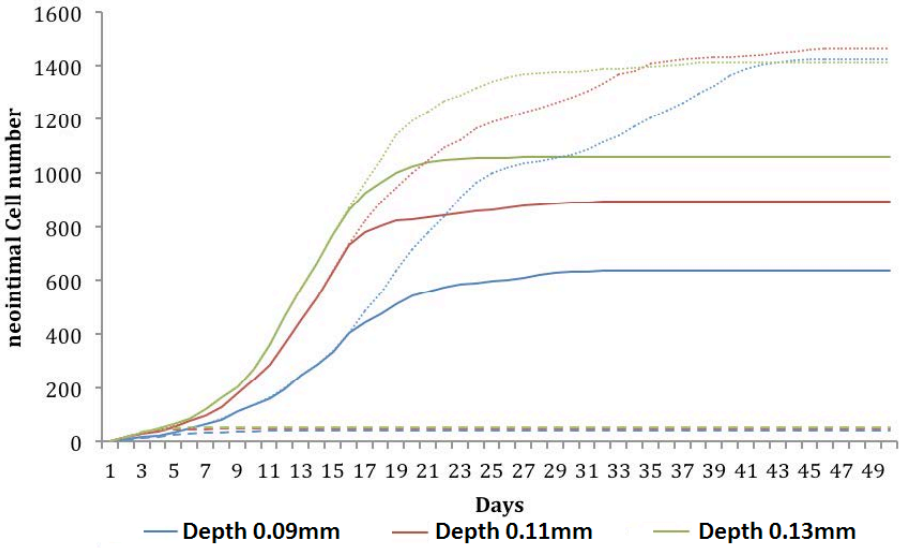

Figure 1. Neointimal cell number as a function of time using three different deployment depths for each scenario. Dashed line, Scenario-1; Solid line, Scenario-2; Dotted line, previous model.

signalling generated in response to physical and chemical stimuli.
The main objective of this study was to obtain different amounts of neointima based on the different injury scores, which was the main flaw of our previous model, but it also points towards an answer to the basic question, which is why 70% of patients do not develop restenosis and why the remaining 30% tend to suffer ISR. This model allows us to estimate the overall growth of SMC by tuning just one parameter, which is availability of a fully functional endothelium.

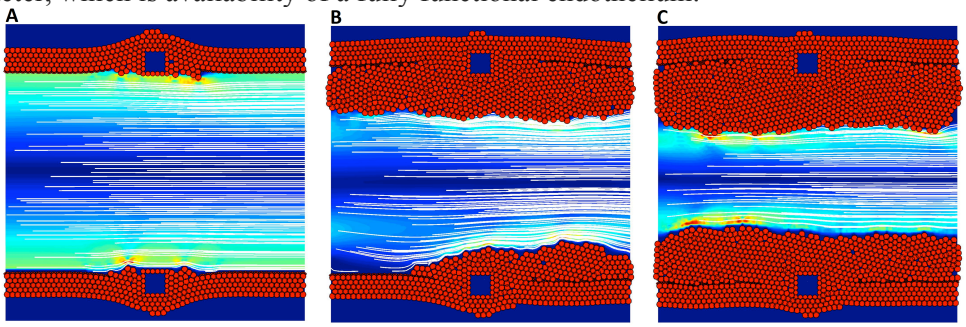

Figure 2. Neointimal growth after 50 days post stenting, showing SMC growth and flow streamlines. (A) NO along with EC probability interpolated from (8), (B) NO effective after 15 days (C) previous model results (2).

A hypothesis based on our results dictates that a presence of a functional endothelium may be the only regulator that controls the growth of the underlying tissue. But the existence of this functional layer seems to be completely patient or animal specific where blood borne agonists and hormonal or genetical properties of individuals may play an important role. More clinical or experimental investigations are required to obtain animal or patient specific time frames, showing a presence of a healthy endothelium. This will allow us to validate our current findings but acquiring such information is far from trivial based on the available knowledge or techniques. To conclude, an ISR response, showing a qualitative agreement with the porcine data (2), has been observed assuming the presence of a functional endothelium after 15 days. Moreover, if a functional endothelium appears much earlier, no ISR is observed, but just some neointima covering the struts as observed in the histological data (unpublished data).

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