The stentable in vitro artery: an instrumented platform for endovascular device development and optimization

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Although vascular disease is a leading cause of mortality, in vitro tools for controlled, quantitative studies of vascular biological processes in an environment that reflects physiological complexity remain limited. We developed a novel in vitro artery that 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 lumenal shear stress using particle image velocimetry. The wall of the stentable in vitro artery consists of an annular collagen hydrogel containing smooth muscle cells (SMCs) and whose lumenal surface is lined with a monolayer of endothelial cells (ECs). The system has in vivo dimensions and physiological flow conditions and allows automated high-resolution live imaging of both SMCs and ECs. To demonstrate proof-of-concept, we imaged and quantified EC wound healing, SMC motility and altered shear stresses on the endothelium after deployment of a coronary stent. The stentable in vitro artery provides a unique platform suited for a broad array of research applications. Wide-scale adoption of this system promises to enhance our understanding of important biological events affecting endovascular device performance and to reduce dependence on animal studies.

1. Introduction

Although in vivo studies are the gold standard for understanding disease processes and screening new treatments, their complexity, often limited predictive capacity, prohibitive cost and ethical concerns demand alternative, novel approaches. In the specific case of vascular disease, there is a critical need for systems that enable controlled, quantitative assessment of the efficacy of new drugs as well as optimization of implantable devices [1,2]. For instance, although endovascular stents have become the primary treatment for atherosclerotic and thrombotic disease in both coronary and peripheral blood vessels [3], catastrophic complications such as late stent thrombosis (LST) and in-stent restenosis (ISR) still pose significant risk to patients [3]. Therefore, there is a clear clinical need for novel stent concepts; however, assessment of the efficacy of such concepts and optimization of their performance is both difficult and expensive using only in vivo data.

In vitro studies using two-dimensional cell culture systems have been invaluable for elucidating the complex biological mechanisms that lead to events such as LST and ISR and have provided significant insight into the extent of communication among different cell types found in the arterial wall as well as coupling between the mechanical stress field and biological endpoints. Such studies have demonstrated, for example, that altered fluid shear stress, such as that found around struts of implanted stents, perturbs the function of the endothelial monolayer lining the arterial wall [4]. Smooth muscle cells (SMCs) have been shown to change phenotype in response to loss of integrity of an adjacent endothelium, as occurs during stent deployment [5], while simultaneously regulating endothelial cell (EC) function [6]. While in vitro studies have contributed greatly to

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our understanding of specific biological mechanisms of post-
sten complications, translation of results obtained under
such idealized conditions to clinical applications remains a
challenge. Experiments performed on two-dimensional
substrates do not provide the three-dimensional micro-
environment that has been repeatedly shown to play a vital
role in regulating essential functional endpoints includ-
ing cell morphology, signalling and migration [7,8]. Indeed,
hydrogel-based three-dimensional engineered tissues have
been shown to better mimic in vitro functionality than
two-dimensional cultures [9,10].

Recently, more complex experimental in vitro platforms
have been developed in order to address these limita-
tions. Implantable tissue-engineered blood vessels (TEBV)
are being designed for use in regenerative medicine. Modern
TEBV systems are capable of mimicking physiological
response and functionality to a high degree [11,12]. For
example, a recently developed TEBV composed of collagen
hydrogel, fibroblasts or stem cells, and ECs exhibits contract-
ility, vasoactivity and inflammatory response [13]. A major
limitation of such tissue-mimicking TEBV systems is their
lack of compatibility with real-time imaging, which inhibits
quantification and assessment of dynamic cellular processes.

In parallel, there is considerable ongoing work in the de-
velopment of in vitro diagnostic tools for the study of biological
phenomena. These 'lab-on-a-chip' or 'organ-on-a-chip' devices are designed for high-resolution cellular imaging and/or
quantitative assays. These systems vary in complexity; one
recent example is a multi-layered gelatin-based microchannel
incorporating fibroblasts, SMCs, and a complete endothelium,
all of which remain viable for at least 3 days [14]. However, this
and similar micro-scale diagnostic platforms are not adapted
for investigation of macro-scale vasculature or endovascular
device implantation [14–16].

Here we present a novel platform, dubbed the ‘stentable
in vitro artery’, which addresses many of the limitations of
in vivo and previous in vitro work. Specifically, the proposed
system combines physiological dimensions, flow and cellular
components with advanced high-resolution imaging and
quantification of cellular behaviour. Moreover, the stentable
in vitro artery allows deployment of endovascular devices
used clinically, including balloons and stents, and the
measurement of the effect of these devices on the flow field
and wall shear stress (WSS). Beyond its usefulness for endo-
vascular device research, the system constitutes a versatile
platform for a variety of vascular research applications.

2. Material and methods

2.1. Cell culture

Bovine aortic ECs (gift of C. Boulanger, Georges Pompidou
Hospital, Paris, France) and bovine aortic SMCs (Tebu-Bio) were
used in this study. ECs were cultured in Dulbecco’s modified
Eagle’s medium (DMEM; Invitrogen) supplemented with 10%
fetal bovine serum (Invitrogen) and 1% penicillin/streptomycin
(Invitrogen), while SMCs were cultured in bovine SMC growth
medium (Tebu-Bio). Cell cultures were maintained in a 5% CO2
atmosphere at 37°C in an incubator; ECs in passage 7 and SMCs
in passage 6 were used. Immediately prior to each experiment,
SMCs were labelled with red fluorescent Vybrant CM-Dil (Life
Technologies) while ECs were labelled with green fluorescent
Vybrant DiO (Life Technologies) for time-lapse imaging.

2.2. Smooth muscle cell-embedded collagen hydrogel

Collagen I was obtained from rat tail tendon as described pre-
viously [17]. Briefly, tendons were extracted from rat tails,
dissolved in a solution of 0.01 mol l⁻¹ hydrochloric acid (HCl;
Sigma), and centrifuged at 30,000g and 4°C for 1 h. The super-
natant was cooled to −80°C, lyophilized, and stored at −20°C
for up to six months. In preparation for each experiment, the
lyophilized collagen was reconstituted to 12 mg ml⁻¹ in
0.01 mol l⁻¹ HCl and stored at 4°C for no more than one week.
Collagen hydrogels mimicking the arterial wall were fabrica-
ted by combining 1 ml cold acetic acid solution with 1 ml
cold neutralizing buffer containing 200 µl 10× concentrated
DMEM (Sigma), 756 µl EC culture medium, 43.5 µl of 1 mol l⁻¹
sodium hydroxide (Sigma) and 400,000 SMCs for a final pH of
8.4, collagen concentration of 6.0 mg ml⁻¹, and SMC concentra-
tion of 200,000 cells ml⁻¹ [17].

2.3. Device design and assembly

The stentable in vitro artery is represented schematically in
figure 1a and by a photograph in figure 1b. Following Buchanan
et al. [18], fluorinated ethylene propylene (FEP) tubing (inner
diameter 4.8 mm, wall thickness 0.8 mm, length 60 mm)
(Fisher) was plasma-treated to activate surface hydroxyl groups
and cross-linked with 1% polyethylenimine (Sigma) for 10 min
followed by 0.1% glutaraldehyde (Sigma) for 20 min to render
the surface hydrophilic. After treatment, the FEP was thoroughly
dried by aspiration, inserted through holes longitudinally drilled
in a 24 mm wide × 60 mm long × 10 mm high acrylic frame, and
fitted at the ends with custom-machined stainless steel sleeves
with concentric 3.0 mm ports. The neutralized collagen/SMC
solution was carefully stirred with a spatula, pipetted into the
prepared device, fitted with a 3.0 mm stainless steel pin and
allowed to polymerize at 37°C for 20 min, resulting in a hydrogel
with a compression modulus of 4600 ± 1500 Pa and average pore
diameter of 1.4 ± 0.1 µm (mean ± s.e.m.) [17]. After polymeriz-
ation, the pin was removed from the device, leaving a 3.0 mm
channel centred in the hydrogel. Finally, the channel was
seeded in multiple stages over a period of 1 h, rotating the
device between each stage, with a 1.8 million cells ml⁻¹ suspen-
sion of ECs to form a monolayer covering the channel lumen.
The in vitro artery was encased in a transparent frame for live
fluorescence imaging and quantification of biological response
to a range of mechanical and/or biochemical stimuli.

In preparation for imaging, a 24 × 60 mm glass coverslip was
sealed to the bottom of the acrylic frame using vacuum grease.
The device was filled with phosphate buffered saline (PBS) and
sealed with a matching acrylic coverslip on the top of the
frame to prevent evaporation of the PBS bath during the exper-
iment. Because the refractive index of FEP closely matches
those of PBS and cell-culture medium, this configuration permits
acquisition of undistorted transmitted light and fluorescence
images on an epifluorescence microscope.

2.4. Perfusion

After assembly, the in vitro artery was submitted to precondition-
ing flow using EC culture medium supplemented with 25 mM
HEPES (Gibco). First, the in vitro artery was placed in an incuba-
tor (37°C, 5% CO₂) and connected via luer adapters and silicone
tubing to a syringe pump (Harvard Apparatus) outside the incu-
bator. An initial flow rate of 0.016 ml min⁻¹, corresponding to a
WSS less than 0.1 mPa (0.001 dyn cm⁻²), was applied in order
to provide media replacement with minimal shear during endo-
thelial adhesion and spreading. Over a period of 72 h, the flow
rate was increased over three orders of magnitude in a stepwise
fashion to ultimately achieve coronary flow without damaging
the newly formed endothelium. During flow preconditioning,
the in vitro artery was removed from the incubator, transferred from the syringe pump to the pulsatile flow loop in figure 1c and mounted on an epifluorescence microscope for imaging. Pulsatile flow loop components included a pulsatile flow pump (Masterflex), peristaltic tubing (Masterflex L/S 16), media reservoir, flow resistance valves (Flow-Rite), and a pressure sensor (PendoTech) and were connected using luer adapters and silicone tubing. A temperature of 31 ± 0.5 °C was maintained in the in vitro artery by placing the media reservoir in a circulating water bath at 38 °C.

The final flow rate applied to the in vitro artery was designed for hydrodynamic similarity with the flow of blood in the human coronary artery. A time-averaged Reynolds number of 360 and a Womersley number of 16 with a pressure amplitude of 20 mmHg (2.7 kPa) (figure 1d) were achieved at a mean flow rate of 40 ml min⁻¹ and by adjusting the position of the flow resistors. Non-dimensional parameters were calculated based on the nominal channel diameter (3.0 mm), nominal pump roller frequency (3.5 Hz), and density and dynamic viscosity of culture medium at 37 °C (1000 kg m⁻³ [19] and 0.78 mPa s [18], respectively). At this flow rate, the mean shear stress on the endothelium is estimated, based on an assumption of Poiseuille flow, to be 0.2 Pa (2 dyn cm⁻²). The system was subjected to this flow condition for 18 h before stent deployment.

### 2.5. Stent deployment

After flow preconditioning, an 18 mm long bare metal stainless steel stent with 80 µm thick struts (Tsunami Gold, Terumo) was deployed in the in vitro artery. First, the balloon catheter on which the stent was mounted was connected to an inflation syringe (Merit Medical), filled with PBS, purged of air and connected to a three-way valve in the flow loop. Flow in the in vitro artery was paused. The catheter was inserted through the three-way valve to the centre of the in vitro artery and inflated to a pressure of 12 atm (1.2 MPa), overexpanding the stent to a diameter of 3.1 mm. This overexpansion was targeted in order to ensure retention within the artery during and after removal of the balloon. After deflation, the catheter was withdrawn and flow was restarted. This process can be visualized in electronic supplementary material, video S1.

### 2.6. Time-lapse imaging

Cell migration was recorded using time-lapse imaging on an epifluorescence microscope with a motorized stage (Ti-E, Nikon), 1280 × 1024 CCD camera (Retiga 1300C, Qimaging) and 4× magnification (1.66 µm pix⁻¹). Images were acquired at 1 frame per hour prior to stent deployment and at 2 frames per hour after stenting. A halogen lamp was used for phase-contrast illumination while a mercury lamp was used for fluorescence illumination, with a FITC filter for imaging ECs and a TRITC filter for imaging SMCs. At each time point, phase-contrast and fluorescence images were acquired at each position of an 18 × 4 grid with 5% overlap in x–y covering the entire in vitro artery. Each image sequence was acquired with the focal plane either in the centre of the channel or at a plane tangent to the lumen. Image acquisition was controlled through µManager, an ImageJ plugin [20]. Preliminary image processing, stitching and minor rotation to align the channel with the imaging axis were performed in FIJI, an ImageJ suite [21].

### 2.7. Quantification of endothelial wound healing

All endothelial wound healing analysis was performed in Matlab. First, two regions of interest (ROIs) of similar size were selected from the FITC (endothelial) channel, each containing a well-defined section of wound: one ROI upstream of the stent and one ROI within the stent. Whole-image shifts in the time-lapse sequence were corrected using a particle image velocimetry (PIV)-based image registration scheme. Because the wound boundary is poorly defined due to variation in individual cell position as well as heterogeneous cell fluorescence, bulk analysis was performed on profiles obtained by averaging the fluorescence along the length of the wound. Subsequently, each profile was normalized to reduce the influence of lighting variations. The speed of wound healing was computed...
robustly using parametric variation of the intensity threshold used to estimate boundary position as well as the number of frames (time points) used in linear regression for slope estimation. The normalized threshold was varied between 0.1 and 0.5 in steps of 0.05, while the number of frames used for regression was between 16 and 21 (7.5–10 h post-stenting). This parametric variation provided 54 ‘replicates’ for each wall boundary (top and bottom) within each ROI (upstream and in-stent). Comparisons were performed using ANOVA and Tukey’s post hoc test. Intermediate steps of wound healing analysis can be found in electronic supplementary material, figure S1.

2.8. Tracking smooth muscle cells
TRITC (smooth muscle) fluorescence image series were first registered using a similar method to that described above to correct for whole-image shifts. SMC tracking was performed in two ROIs in a 400 μm wide section of collagen hydrogel at the channel wall: one ROI upstream of the stent and one ROI adjacent to stent. In Fiji, images were binarized using a user-defined threshold (constant for all datasets) and preprocessed using an erosion/dilation sequence to regularize cells for tracking. Tracking was performed using the MTrack2 plugin, with a minimum radius of 5 μm for an object to be considered a cell and a maximum allowable speed of 50 μm h⁻¹ for accurate cell matching between frames. A custom Matlab script was used to load the results generated by Fiji/ MTrack2, compute cell velocity and persistence and perform statistical analysis. Only cells present in at least three sequential frames were considered for analysis. Cell velocity was broken into three different metrics: net velocity \( U \) parallel to the channel wall (positive \( U = \) migration in the direction of flow), net velocity \( V \) perpendicular to the channel wall (positive \( V = \) migration towards the wall) and speed, which is calculated as the total cell path length divided by total time and is, therefore, larger in magnitude than either \( U \) or \( V \). was computed from the maximum peak of the discrete Fourier transform of the mean velocity signal, and finally, phase-unwrapping was performed on the sequence of image pairs to order them within a single flow period. Because the resulting temporal resolution was higher than strictly necessary for this analysis, the effective resolution was reduced to 167 frames per period by grouping unwrapped images into bins containing six pairs each. All further PIV analysis was performed using sum-of-correlation within each group [27]. This had the effect of increasing the signal-to-noise ratio and, therefore, the accuracy of PIV measurements at each of the remaining time points.

Using the unwrapped, binned image pairs, a second run of PIV was performed to identify regions of each image where particles could not be visualized. In this experiment, this was critical due to the presence of the stent which blocked imaging in a significant portion of the channel. In this run, high-resolution two-pass PIV with small windows was performed using universal outlier detection (UOD) [28] and thresholding to flag vectors inconsistent with surrounding measurements. Vectors that were flagged in 50% or more of all image pairs in sequence were determined to be unreliable, and the corresponding positions were combined to form a mask which was applied to all future analysis steps. This mask was further modified to include masking of the region outside the channel wall.

A final PIV run was performed using the combined validation/wall mask, 64 × 128 pixel correlation regions with 75% overlap windows to 32 × 64 pixels with a Gaussian filter, two-pass robust phase sum-of-correlation, three-point Gaussian peak fitting and UOD validation to obtain velocity fields with a vector resolution of 14.8 μm × 29.6 μm × 3 ms in \( X \), \( Y \) and \( T \), respectively. Each vector field was shifted to move peak flow to the initial time point, enabling the measurements for each imaging position to finally be stitched into a single, coherent vector field covering a 16.65 × 1.85 mm section of the channel.

2.10. Wall shear stress measurement
Five ROIs along the channel wall were selected for WSS measurement. Four ROIs beginning 25 μm downstream of strut struts and extending an additional 280 μm downstream were analysed. All regions were 550 μm high and vertically centred over the channel wall. For comparison, WSS was also measured in a control ROI with the same dimensions. The boundary layer development length for (steady) laminar flow is \( L = 0.05 \times R_e \times D \); for a Reynolds number \( R_e \) of 360 and strut thickness \( D \) of 80 μm, this length is 1.4 mm. Therefore, the downstream ROI was placed 2 mm downstream of the stent in order to provide measurements representative of the undisturbed flow field.

In order to identify the channel wall position, the brightfield image was sharpened using the Matlab function graydiffweight, filtered and binarized. The profile of the wall was smoothed using a median filter, and subpixel wall positions were identified by fitting with a cubic spline function [29]. In order to calculate the WSS for each ROI, vector fields were first filtered using proper orthogonal decomposition with 95% energy recovery. Subsequently, thin plate spline radial basis functions were computed for a 5 × 5 vector grid at each wall position (9–10 positions per stent ROI, 63 positions for the downstream ROI) and used to obtain an enhanced estimation of the velocity gradient tangential to the wall at each time during the cycle (167 time points) [30]. The time-varying WSS was finally averaged for all wall positions within each ROI. Comparisons between time-averaged WSS for each ROI were performed using ANOVA and Tukey’s post hoc test.

2.11. Computational flow model
The experimentally measured flow and WSS were compared with a computational fluid dynamics (CFD) model of the
in vitro artery in order to assess the validity of the values obtained. The CFD simulations were performed using the commercial finite-element multi-physics code COMSOL (v. 5.2a). The model geometry consists of a 3 mm diameter straight cylinder, representing the fluid within the in vitro artery, with cutouts corresponding to the struts of the deployed stent. The strut geometry for the simulations was simplified as a repeating diamond structure, including connecting struts while neglecting the rotation between diamond elements. Only a 7.5 mm long section of the stent was modelled in order to reduce computational time. Entrance and exit sections of 1.5 mm and 2.5 mm, respectively, were added in order to ensure developed flow within the stent. Struts were assumed to be embedded within the arterial wall to 50% of their height; therefore, the strut height in the model geometry was set to 40 μm. A further reduction in computational time was achieved by simulating only a 1/6 radial section of the fluid. The fluid geometry was meshed using a combination of prism and tetrahedral elements, with a total of 883,000 elements in the model. The time-averaged WSS over the entire lumenal surface was calculated using the described mesh and compared with the results using a mesh containing 10 times more elements; this difference was only 3%, confirming mesh independence. The model geometry and a representative section of the mesh are shown in figure 6a.

For the numerical model, the fluid was assumed to be Newtonian with the density and dynamic viscosity of culture medium reported above (1000 kg m⁻³ and 0.78 mPa s, respectively). A pulsatile flow with parabolic profile was imposed at the inlet, using the centreline velocity profile obtained from PIV measurements (figure 5f), while a zero-pressure condition was imposed at the outlet. A no-slip condition was imposed on the lumenal surface containing stent struts, while symmetry boundary conditions were imposed on the lateral walls. The spatial distribution of the flow field was computed implicitly using the generalized minimal residual nonlinear finite-element solver, and the temporal evolution was computed implicitly using a time-dependent solver with a temporal resolution of 5 μs. Simulations were performed over five pulsatile cycles and results were taken from the last, in order to ensure a time-periodic solution.

Because the stent geometry in the numerical model was idealized, it was not possible to evaluate WSS at the same ROIs as in the experimental measurements. Therefore, WSS from the numerical simulation was measured at four manually selected ROIs (I–IV) of the same length as those in the experimental measurements, located at various positions relative to stent struts, in addition to a downstream ROI placed identically to that of the experimental analysis. ROI positioning is indicated in figure 6a.

2.12. Supplementary immunostaining and live/dead imaging
In order to visualize cellular actin networks as well as cell survival after stent implantation, two additional in vitro arteries were constructed and preconditioned up to the point at which they would normally be transferred from the incubator to the pulsatile flow loop and mounted on the epifluorescence microscope for imaging. At this time, the first in vitro artery (for immunofluorescence, IF) was fixed with 4% paraformaldehyde (Alfa Aesar), permeabilized with 0.1% Triton X-100 (Sigma) in Tris-buffered saline (TBS; Sigma), incubated with a blocking buffer containing 5% bovine serum albumin (Sigma) and 0.01% Triton X-100 in TBS, stained with 1:100 Alexa Fluor 594 phalloidin (Life Technologies) for visualization of actin filaments and 1:1000 DAPI (Sigma) for visualization of cell nuclei, and rinsed thoroughly with blocking buffer before imaging.

The live/dead in vitro artery was implanted with a 14 mm long bare metal stent (Qualimed) overexpanded to 3.1 mm according to the same protocol as described in the section ‘Stent deployment’. Immediately after stent deployment, the in vitro artery was flushed with PBS, incubated with 8 μM calcein AM (Life Technologies) (to stain live cells) and 8 μM ethidium homodimer-1 (Life Technologies) (to stain dead cells) in EC culture medium, and rinsed thoroughly with PBS before imaging.

For both the IF and the live/dead in vitro arteries, fluorescence images were acquired on the epifluorescence microscope described previously using a Hamamatsu Orca Flash-4.0 camera and A × 10 or 20 × magnification.

3. Results
The in vitro artery incorporates a dense collagen hydrogel embedded with aortic SMCs, a 3 mm diameter central channel lined with aortic ECs, and perfusion by cell culture medium with a physiologically relevant pulsatile waveform (figure 1). The dimensions and dimensionless flow parameters (Reynolds and Womersley numbers) mimic those found in coronary arteries in vivo. After 3 days of initially steady and ultimately pulsatile flow preconditioning, the endothelium forms a continuous monolayer and the SMCs elongate along collagen fibres within the hydrogel. This can be seen in images of labelled live cells (figure 2) as well as fixed samples stained for cell nuclei and actin (electronic supplementary material, figure S2, video S2).
As occurs in vivo [31], insertion of the balloon catheter and deployment of the stent in the in vitro artery resulted in significant damage to the endothelial monolayer; specifically, a 600 μm wide swath of endothelium both upstream of and within the stent was completely sheared away (figure 3a). The vast majority of both SMCs and ECs in the in vitro artery remained viable (electronic supplementary material, figure S3). In the following sections, we describe endothelial wound healing, SMC motion and fluid dynamic shear stress alterations after deployment of the coronary stent.

3.1. Endothelial wound healing post-stenting
Following stent deployment, ECs migrated to fill damaged regions, including the large wound created by the stent and balloon catheter (figure 3b; electronic supplementary material, video S3). We quantified this wound healing by tracking the position of the endothelial front both upstream of and within the stent, using a robust algorithm implemented in Matlab (figure 3c). The endothelial front advanced at a nearly constant rate for the first 10 h post-stent, after which healing slowed to the end of the experiment. The overall rate of endothelial wound healing, defined as the average speed of endothelial front migration during the first 7.5–10 h post-stenting (detailed in the electronic supplementary material), was measured to be 15.4 ± 0.8 μm h⁻¹. The two opposing wound fronts healed at different apparent rates (13.7 ± 0.5 μm h⁻¹ for the top wound front versus 17.1 ± 1.0 μm h⁻¹ for the bottom wound front, p < 0.0001), a difference that is consistent with the positions of the fronts on the channel lumen relative to the imaging plane: the wound front with lower apparent velocity (top) is farther from the in-focus plane of the in vitro artery than the faster wound front (bottom) and, therefore, has a significant out-of-plane component that is not quantifiable in two-dimensional images. More importantly, the presence of the stent had no impact on the rate of endothelial wound healing (15.5 ± 0.4 μm h⁻¹ within the stent versus 15.3 ± 0.4 μm h⁻¹ upstream of the stent, p = 0.9927).

3.2. Smooth muscle mobility within the collagen gel
In addition to endothelial wound healing, migration of SMCs (figure 4a; electronic supplementary material, video S4) can be visualized and quantified in the in vitro artery. We examined SMC migration in the hydrogel in regions both upstream of and immediately adjacent to the stent. Qualitatively, SMCs could be classified as either migratory or stationary, with migratory cells elongating and following short, random paths within the hydrogel (figure 4a). No apparent preferential migration towards the stent was identified. Quantitative analysis produces results in agreement with the qualitative observations (figure 4b). With 191 cells tracked in the upstream region and 428 tracked in the stent-adjacent region, both populations followed near-normal distributions of net velocity in

Figure 3. Endothelial wound healing in the stentable in vitro artery. (a) Stent deployment wounds the endothelium upstream of and within the stent. From top to bottom: phase-contrast, endothelium, smooth muscle, overlay of endothelium (green) and smooth muscle (red). The three lower frames show the region indicated by the white dashed box in the top image. Scale bar, 1 mm for all frames. Flow is from left to right. Images were acquired at a plane tangent to the channel lumen for time-lapse imaging for endothelial migration. (b) (i) Endothelial cells migrate to fill the wound in the region of interest highlighted by the red box on the phase-contrast image in panel (a). Frames shown are (top to bottom): 0, 5, 10 and 15 h post-stent. (ii) The mean intensity profile for each frame is used to estimate the rate of endothelial wound healing. Scale bar, 500 μm. (c) Wound size, calculated as the distance between the top and bottom wound front positions, has a similar magnitude and temporal profile for the regions upstream of and within the stent.
both the $x$- and $y$-directions ($U$ and $V$, respectively), with means near zero. Cell speed followed a skewed distribution reminiscent of a log-normal and was also similar in both regions albeit slightly increased upstream of the stent, with mean ± s.e.m. (median) speeds of $6.2 \pm 0.3$ (5.3) $\mu$m h$^{-1}$ upstream of the stent and $5.3 \pm 0.2$ (4.3) $\mu$m h$^{-1}$ adjacent to the stent as well as a small minority of cells moving at speeds up to 20 $\mu$m h$^{-1}$ in both regions. Cell persistence, defined as the ratio of the net displacement to total path travelled by each cell, followed a nearly random distribution for both regions, consistent with the qualitative observation that SMC migration in the isotropic hydrogel was random and not directed.

### 3.3. Alterations in wall shear stress downstream of stent struts

Because ECs are highly sensitive to shear stress and flow perturbation, and as stent struts can induce significant perturbation of the flow field in the vicinity of the stent, high-resolution near-wall flow measurement is an important factor in understanding endothelial response to stenting [32]. By combining the in vitro artery with a synchronized camera and a pulsed laser, we were able to quantify flow alteration by the deployed stent in a large region of the artery including a significant portion of the stent as well as a downstream section (figure 5a). High-resolution velocity fields (figure 5b) were obtained using PIV, an optical technique for quantitative flow measurement well-adapted for quantification of arterial flow [33]. While it appears that an assumption of Poiseuille flow (fully developed parabolic profile) could be used to estimate WSS at the time of peak flow (figure 5b(i)), it is evident that a parabolic fit does not accurately capture near-wall velocity at the time of minimum flow (figure 5b(ii)). The WSS experienced by the endothelium was calculated from the PIV measurements at a resolution comparable with the size of a single cell (14.6 $\mu$m).

Four ROIs downstream of stent struts were examined and compared with an ROI far enough downstream of the stent (2 mm) to be representative of the undisturbed flow field (figure 5a). The time-varying WSS within each ROI was estimated from the near-wall velocity field (figure 5c). The WSS profiles in all ROIs (figure 5d(i)) were qualitatively similar to the centreline flow velocity profile (figure 5d(ii)) as well as the driving pressure wave in the in vitro artery (figure 1d). The mean time-averaged WSS in all stent ROIs was significantly lower than the mean WSS in the downstream ROI ($p < 0.0001$) except stent ROI 3 ($p = 0.6341$), and all stent ROIs had significantly different mean WSS from one another ($p < 0.0001$) except ROI 1 versus ROI 4 ($p = 0.9537$) (figure 5e).

The numerical simulation reveals WSS profiles and values similar to the experimental data (figure 6). The time-varying WSS is nearly synchronized with the centreline velocity and follows a similar profile (figure 6c). The time-averaged WSS is lower within the stented region than in the downstream ROI (figure 6d). The primary difference between the results of experiment and simulation is in the magnitude of WSS. The downstream time-averaged WSS in the simulated in vitro artery is identical to that predicted by theory, while the WSS measured experimentally using PIV is less than a third of that value, most probably due to vessel curvature, flow asymmetry and the presence of three-dimensional flow patterns.

### 4. Discussion

The in vitro artery provides a versatile platform for quantitative dynamic studies of cellular behaviour in a configuration mimicking blood vessels. In this paper, we have studied the response of ECs and SMCs to the implantation of a stent in an in vitro artery designed to mimic a coronary artery. The results obtained are coherent with observations in vivo, as discussed below, and demonstrate the potential of this novel platform for future work in cardiovascular research.

The state of the endothelium in the in vitro artery before, during, and after stent implantation was carefully studied. Interestingly, large patches of endothelium as well as scattered cells remained viable within the stent immediately after stent deployment. This observation is consistent with the findings of a recent study that compared in vivo histology with...
computational models of re-endothelialization in order to understand mechanisms of endothelial repair after stenting [34]. Tahir et al. performed computations contrasting the scenarios of random seeding of ECs within the stent with the case permitting EC growth from only outside the stented section and found that the random seeding model was more consistent with in vitro endothelial growth patterns. Based on this study, it appears that EC viability patterns within the stented portion of our in vitro artery succeed in mimicking important aspects of in vivo conditions.

The in vitro artery was designed to achieve flow similarity with human coronary flow by matching Reynolds and Womersley numbers because these two dimensionless parameters determine the structure of the flow field within the vessel and its evolution over the pulsatile cycle. The design also includes a constraint of non-scalable geometry, as endo-vascular devices are not scalable. A notable consequence of matching Reynolds and Womersley numbers for flow similarity is that the WSS within the in vitro artery is not matched to that present in the coronary artery in vivo. More specifically, for Poiseuille flow in a cylindrical vessel, \( \text{WSS} = 4\mu Q/\pi R^3 \), where \( \mu \) is the dynamic viscosity, \( Q \) is the volumetric flow rate, and \( R \) is the vessel radius. The Reynolds number in a cylindrical vessel is \( Re = 2\mu Q/\pi R \), where \( \rho \) is the fluid density. Therefore, Reynolds number and vessel diameter matching between the in vitro artery and in vivo conditions leads to the WSS in the in vitro artery scaling by the square of the ratio of the viscosities of blood and cell culture medium.

As the viscosity of the culture media used in this study was 0.78 mPa s while the viscosity of blood is 3.5 mPa s [35], the WSS in the in vitro artery is expected to be approximately 20 times lower than in vivo. Accordingly, the PIV-measured mean downstream WSS of 0.06 Pa (0.6 dyn cm\(^{-2}\)) in the in vitro artery scales to approximately 1.2 Pa (12 dyn cm\(^{-2}\)) using blood viscosity, which is a close match with in vivo measurements of 0.33–1.24 Pa (3.3–12.4 dyn cm\(^{-2}\)) in the human coronary artery as reported by Doriot et al. [35]. Because in vitro WSS varies strongly spatially within arteries due to vessel curvature [36] and in vitro WSS measurements are subject to significant uncertainty themselves due to the low resolution of velocity measurements and assumptions of Poiseuille flow [35,37], this agreement between in vivo and in vitro data is more than satisfactory. Furthermore, the numerical simulation reproduces the flow and WSS patterns obtained experimentally even under the idealized conditions assumed for the CFD computations.

The presence of reduced WSS in regions between stent struts in the in vitro artery (obtained both experimentally and numerically) is consistent with the conclusions of Charonko et al., who measured WSS around stent struts in a silicone
tube [29], and Seo et al., who simulated arterial flow around stent struts in an idealized geometry [38]. Although the overall differences in measured time-averaged WSS among the different ROIs are small (figure 5e), the time-dependent WSS results demonstrate that these differences are considerably larger during the period of flow acceleration than during deceleration and that variations in WSS temporal gradients are even more pronounced (figure 5d). Such variation could contribute to significant alterations in endothelial behaviour, as ECs are thought to respond to both time-averaged WSS as well as temporal gradients in WSS [39]. Finally, the mean WSS measured within each ROI is three to five times lower than the WSS estimated by assuming ideal flow conditions (Poiseuille flow) or from numerical simulation. The Poiseuille flow estimate of WSS assumes axisymmetric flow; however, under the flow conditions in the in vitro artery this axisymmetric mode is likely unstable [40]. This result demonstrates the importance of measurement of WSS rather than estimation based on idealized analytical or numerical models.

The stentable in vitro artery certainly is not without its limitations. Owing to the design objective of transparency for brightfield and fluorescence imaging, the seeding density of SMCs in the hydrogel is far lower than in vivo. Therefore, interactions between SMCs and ECs and biochemical cues are likely to be reduced in magnitude and perhaps complexity. Additionally, the rigid FEP housing of the in vitro artery (necessary for imaging while maintaining an axisymmetric hydrogel) inhibits changes in lumen diameter as occurs during vasodilation or vasoconstriction. There are other limitations that can and will be addressed in future work. Currently, inflammatory and other biological factors found in blood are absent from the perfusion media. This deficiency may explain the accelerated endothelial healing rate in the in vitro artery compared with in vivo data. Conversely, the sub-physiological temperature within the in vitro artery likely has an inhibitory effect on the endothelial migration rate while increasing cellular proliferation [41,42]. Future studies will introduce biological factors and temperature variation and examine their effects on cellular response to endothelial wounding. Indeed, an advantage of the in vitro artery is that such factors can be individually controlled in order to provide a more mechanistic understanding of their role in regulating EC and SMC responses. The effect of the reduction in WSS in the in vitro artery caused by viscosity mismatch between cell culture

![Figure 6. Computational model of the flow field in the stentable in vitro artery. (a) Model geometry indicating regions of interest (ROIs) in which wall shear stress (WSS) was calculated. Except for the downstream ROI, these ROIs do not correspond to the ROIs in figure 5. Scale bar, 1 mm. Inset: sample of mesh used for computation. (b) Velocity fields and longitudinally averaged velocity profiles resulting from the numerical simulation. As in the experimental results (figure 5b), the velocity profile is clearly not parabolic at the time of minimum flow. (c) The WSS in the simulation ROIs follows a similar pattern to the experimental data, with all ROIs within the stent presenting lower WSS throughout the cycle than the downstream ROI. Here, the greatest deviations from the downstream WSS occur for ROIs closest to stent struts. The WSS profiles for ROI I and ROI IV overlap nearly identically despite the difference in position. (d) The time-averaged WSS in the simulated in vitro artery is lower near and between stent struts than in the downstream region, validating the experimental measurements.](http://rsif.royalsocietypublishing.org/doi/fig/10.1098/rsif.2015.0834/fig6)
medium and blood is unknown but may be important for endothelial response. In future studies, the viscosity of the cell culture medium can be increased to match the viscosity of blood through the addition of thickening, although it would be important to first establish that such agents have a minimal effect on endothelial and SMC function. Finally, the current design of the in vitro apparatus incorporates a single layer of hydrogel containing SMCs. However, the system can be easily adapted for bi- or multi-layer configurations and additional cell types such as fibroblasts, as was done by Hasan et al. [14]. Despite these limitations, the in vitro apparatus can already serve as a robust platform for comparative research for applications such as informed design of stent geometry or drug release profiles.

We believe that the in vitro apparatus provides a model system within which the biological performance of endovascular devices can be tested prior to proceeding to animal trials. Indeed, we hope that a key additional benefit of this system will be to reduce the use of animals in vascular research. The short time scale of endothelial wound healing post-wounding—of the order of one day—contributes to the utility of the in vitro apparatus for such research. By virtue of its integration of an advanced in vitro tissue platform with automated data processing modules for quantification of cell behaviour and flow parameters, the in vitro artery described here is not only ideal for device assessment and optimization but can also be adapted for use in a host of vascular investigations including toxicity testing of cardiovascular drugs, dynamic monitoring of leukocyte interaction with the arterial wall during inflammation and assessment of the effects of nanomaterials on the arterial wall.

Authors’ contributions. E.E.A. designed and carried out experiments, analysed the data and wrote the manuscript. F.P.C. performed numerical simulations, analysed the data and wrote the manuscript. A.I.B. conceived of the study, analysed the data and wrote the manuscript. All authors gave final approval for publication.

Competing interests. We declare we have no competing interests.

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