Video Article

Implantation of a Carotid Cuff for Triggering Shear-stress Induced Atherosclerosis in Mice

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Abstract

It is widely accepted that alterations in vascular shear stress trigger the expression of inflammatory genes in endothelial cells and thereby induce atherosclerosis (reviewed in [1] and [2]). The role of shear stress has been extensively studied *in vitro* investigating the influence of flow dynamics on cultured endothelial cells [1,3,4] and *in vivo* in larger animals and humans [1,5,6,7,8]. However, highly reproducible small animal models allowing systematic investigation of the influence of shear stress on plaque development are rare. Recently, Nam et al. [9] introduced a mouse model in which the ligation of branches of the carotid artery creates a region of low and oscillatory flow. Although this model causes endothelial dysfunction and rapid formation of atherosclerotic lesions in hyperlipidemic mice, it cannot be excluded that the observed inflammatory response is, at least in part, a consequence of endothelial and/or vessel damage due to ligation.

In order to avoid such limitations, a shear stress modifying cuff has been developed based upon calculated fluid dynamics, whose cone shaped inner lumen was selected to create defined regions of low, high and oscillatory shear stress within the common carotid artery [10]. By applying this model in Apolipoprotein E (ApoE) knockout mice fed a high cholesterol western type diet, vascular lesions develop upstream and downstream from the cuff. Their phenotype is correlated with the regional flow dynamics [11] as confirmed by *in vivo* Magnetic Resonance Imaging (MRI) [12]: Low and laminar shear stress upstream of the cuff causes the formation of extensive plaques of a more vulnerable phenotype, whereas oscillatory shear stress downstream of the cuff induces stable atherosclerotic lesions [11]. In those regions of high shear stress and high laminar flow within the cuff, typically no atherosclerotic plaques are observed.

In conclusion, the shear stress-modifying cuff procedure is a reliable surgical approach to produce phenotypically different atherosclerotic lesions in ApoE-deficient mice.

Video Link

The video component of this article can be found at http://www.jove.com/video/3308/

Protocol

1. Preparing the shear stress modifier (cuff)

1. The shear stress modifier consists of two longitudinal halves of a cylinder with a cone shaped lumen. The half shells are made of thermoplastic polyetherketone produced by a plastic casting procedure. The cast elements are sent out while still connected to the runner. Hence, the half shells have to be cut off before usage. Each cast contains half shells of different sizes ranging from 150 µm - 300 µm (lowest inner diameter at downstream end).
2. The cuff preparation should be performed under a surgical microscope.
3. Hold the cuff half shell with blunt forceps and gently cut it off from the cast by using a sharp scalpel. This procedure will lead to half shell precursors which have to be further processed.
4. Place the half shell precursor on an even non-slippery plate; fix it with blunt forceps and cut it exactly along the preformed incision in order to remove the closed end. This will yield the cuff half shell ready for use.
5. Control the half shells under the microscope and gently remove any remaining sharp edges. The conical inner lumen is essential for inducing different qualities of shear stress within, upstream and downstream of the implanted cuff. A groove on the outer surface of the half shells running perpendicular to the inner lumen serves as a guiding for the thread which finally sticks the half shells together to form the functional cuff.
6. Store the cuff half shells, classified according to size, in 70% ethanol.

2. Implanting the cuff around the right carotid artery

1. ApoE knockout mice should be around 10 weeks of age and at a weight of at least 20 grams. If plaque development should be investigated under high cholesterol diet, starting such a western type diet 4 weeks prior the cuff implantation is recommended.
2. The whole surgical procedure should be performed under at least semi-sterile conditions: Wear a surgical gown, mask and cap and sterile gloves. Sterilize the instruments for 30 seconds in a bead instrument sterilizer and place them on a sterile opaque sheet. Beware! Instruments have to cool down before use!
3. Place the mouse in an anesthesia induction chamber filled with 3% isoflurane until it is completely anesthetized. Verify depth of anesthesia with response to toe pinch. Alternatively, anesthesia can be performed with intraperitoneal (i.p.) injection of Ketamine (80 mg/kg) and Xylazine (10 mg/kg) or alternate anesthetics approved by the IACUC. For i.p. injection, hold the mouse in supine position and inject the anesthetic in the left lower quadrant of the abdomen.
4. Place the mouse on a heated surgical plate, in supine position. In order to prevent the eyes of running dry moisten them with eye salve (e.g. Bepanthen eye and nose lotion). Spread the fore- and hind-paws and tape them down to the plate. If performing isoflurane anaesthesia, the narcotic gas flow (2% isoflurane) has to be supplied via a small rodent mask.
5. Remove the hair between the mandible and the sternum by either applying depilatory agent (e.g. Pilca Med) or by using a fine electric shaver (e.g. Wella Contura). Shaving has to be performed with caution in order to avoid irritating the skin. If using a depilatory agent, give it 1-2 min for penetrating the hair, thereafter, gently rub until all hair and depilatory agent is removed.
6. Have ready previously prepared cuff half shells of different sizes (two of each size) and a 2.5 cm-long piece of 6-0 silk suture for interconnecting the half shells at the site of carotid occlusion.
7. Disinfect the operating field with liberal amount of Betadine. Use sharp small scissors to open the skin and the underlying fascia of the neck by a 4-5mm medial incision starting from the top of the sternum (*manubrium*).
8. Expand the opening, shift the right parotid gland aside and insert a surgical spreader. Then bluntly dissect into the deep, just left of the trachea (from the surgeons view), where the right sternomastoideus muscle crosses the right omohyoid muscle, until you are able to identify the pulsating right common carotid.
9. Using very fine angled or curved forceps (e.g. Dumont #5/45), dissect the right common carotid artery by gently removing the surrounding connective tissue. Separate the carotid from the vagus nerve - the white, stringy object running directly along the carotid - as this step is necessary to completely expose the vessel. Be careful neither to harm the vagus nerve nor a branch of the left internal jugular vein, which is also in close connection to the carotid.
10. In order to choose the right cuff size, compare the diameter of the exposed carotid with the inner-diameter of the cuff half shells: The largest width of the cuffs lumen should meet the outer diameter of the carotid.
11. Now, carefully put the tip of the forceps under the carotid, open the forceps, thread the piece of 6-0 silk suture under the carotid and form a loop. Between the loop and the carotid place one cuff half shell beneath the carotid. The side of largest narrowing has to be downstream.
12. Place the second cuff half shell within the loop on top of the carotid.
13. Gently tighten the suture loop using suturing forceps and node the thread. By doing this the functioning shear stress modifier is formed. For the precise fitting of the cuff it is essential that the suture is running exactly within the preformed groove on the outer surface of the cuff.
14. Move the right parotid gland back in its original position, approximate and close the skin either using a small amount of 6-0 prolene suture or, alternatively, you can use wound clips.
15. Inject a single dose of 5 mg/kg Carprofen (e.g. Rimadyl) subcutaneously to provide prophylactic pain treatment and place the mouse in a warming chamber until it recovers. Normally, this takes 30-60 min when using Ketamine/Xylazine anesthesia. When using isoflurane inhalation anaesthesia the recovery period is substantially shorter (10-20 min).
16. In our experience, animals regain normal unstressed activity within the first 24 hours after cuff implantation with the intervention being performed by an experienced surgeon. However, if the animal appears to be distressed even one day post-surgery, repeat the analgesic treatment and consult with veterinary staff.

3. Explanting the cuff and the carotid arteries

1. For histological analysis the carotid arteries have to be harvested at the end of the observation period. Before starting explantation, the animal has to be killed according to IACUC guidelines.
2. If the cuff half shells are intended for reuse, carefully remove all tissue fragments and the connecting suture from the plastic elements, dissecting from the carotid arteries, wash and store the half shells in 70% alcohol solution. One should keep in mind that the cuff is embedded in connective tissue adhesions after several weeks of implantation and that one has to dissect the cuff with caution in order not to harm the vessel walls.
3. Alternatively, the cuff can be explanted and embedded together with the carotid artery. The plastic material of the cuff is resistant to normal fixation solutions and solvents used for paraffin embedding. Also, the embedded samples can be cut using a normal microtome equipped with normal blades.

4. Representative Results

The cuff has to be placed always around one of the two common carotid arteries of an animal (Fig. 1A, 1B) - the contralateral side serves as a control. On the image presenting the two half shells of the cuff (Fig. 1B) the conical shape of the inner lumen is visible. This conical shape is essential for establishing the three regions with distinct flow dynamics. Typical shear stress patterns induced by the cast calculated from Doppler measurements [11] and the corresponding flow velocities based on phase-contrast velocity MR imaging [12] are given in Figure 2.

When the cuff is implanted in ApoE knockout mice fed a western type diet the altered flow dynamics provoke shear stress induced atherosclerotic plaque deposition (Fig. 3): Upstream of the conical constriction low laminar shear stress leads to massive development of atherosclerotic plaques of a more vulnerable phenotype, characterized by lipid cores close to the central lumen covered only by a thin fibrous cap (Fig. 3A1). The conical inner lumen of the cuff leads to an increase in flow velocity. Nearly no plaque deposition is observed in this area. Directly downstream of the site of the bottleneck the immediate broadening of the artery results in an area of vortices and oscillatory flow parameters, which causes less extended plaque development of a more stable phenotype (i.e. less lipid cores which are localized more close to the media).

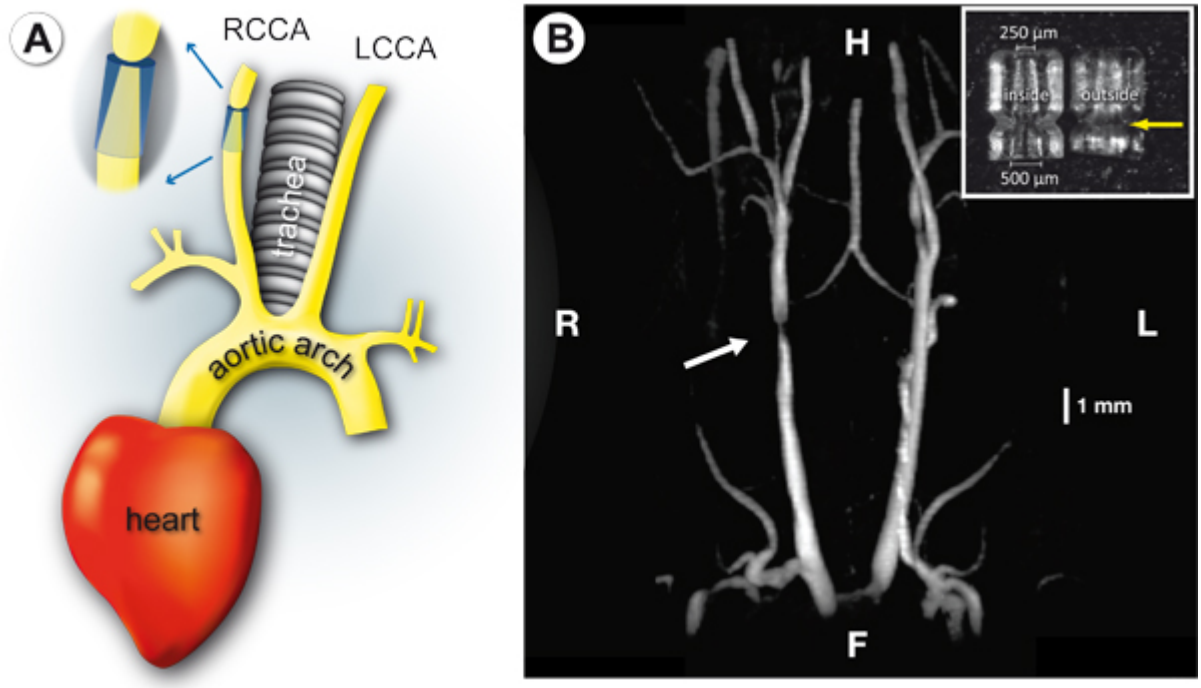

Figure 1. The shear stress modifier - assembly and placement. (A) Schematic drawing of the implanted cuff: The cuff is placed around the right common carotid artery (RCCA) - the contralateral side (LCCA = left common carotid artery) serves as control. (B) In vivo MRI angiography in a mouse: In the maximum intensity projection of a three-dimensional time-of-flight MR angiography the conical constriction induced by the cast (white arrow) is visible (for details see [12]). (H=head, F=feet, R=right, L=left). In the upper right corner a macroscopic view on the inner lumen and the outer surface of the cuff half shells is given. When assembled, two half shells form a conical cylinder which modifies the flow dynamics within the vessel in a defined way. To ensure the right fit, a groove on the outer surface of the cuff (yellow arrow) serves as a guiding for the linking suture.

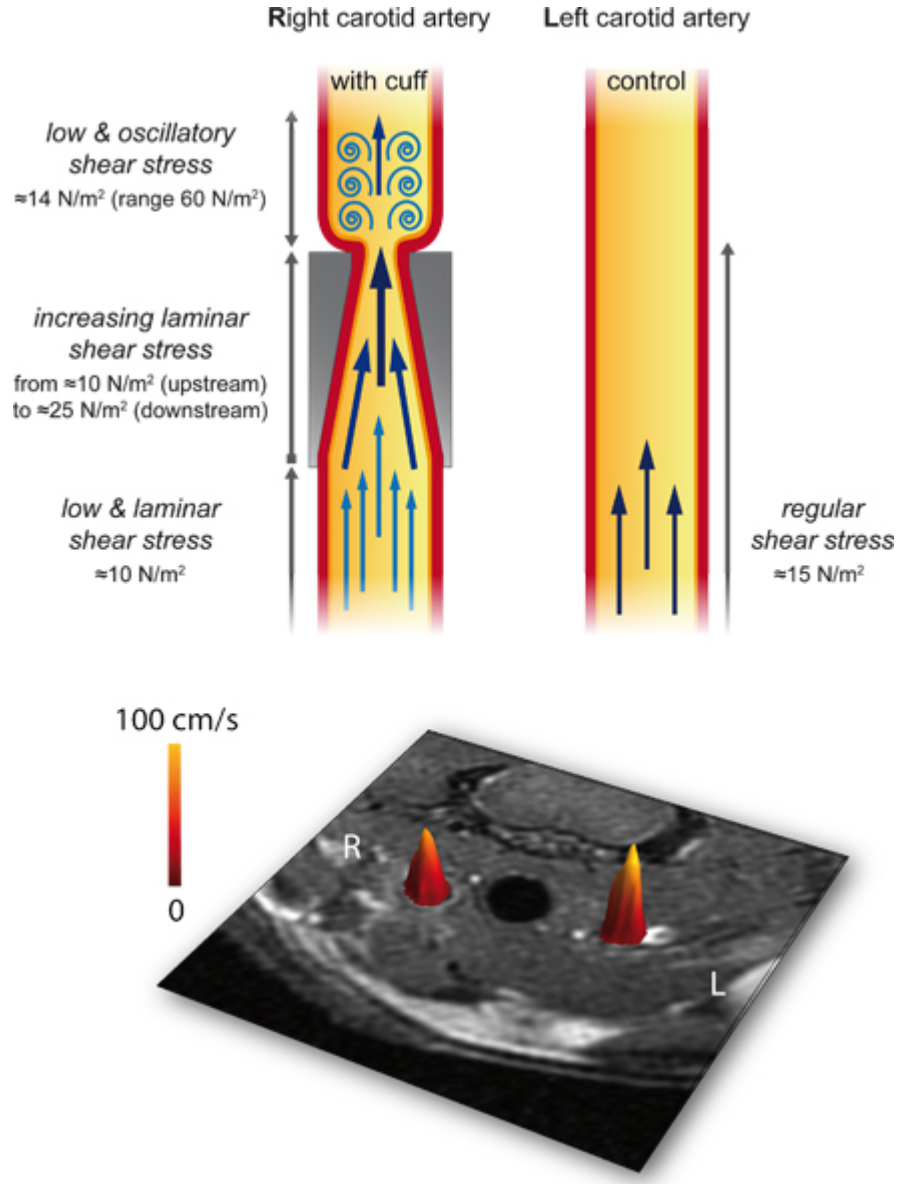

Figure 2. Regions of different flow dynamics and shear stress. Implantation of the cuff alters flow dynamics and subsequently shear stress in the constricted carotid artery in a defined manner. In the upper schematic illustration the different flow properties are indicated and the local approximate values for shear stress are given (based on Doppler measurements [11]). Below, corresponding flow velocities for a single animal measured by MRI upstream of the implanted cuff are shown in a T1-weighted cross-sectional MRI-image of the neck (*phase-contrast velocity imaging*, for details see [12]).

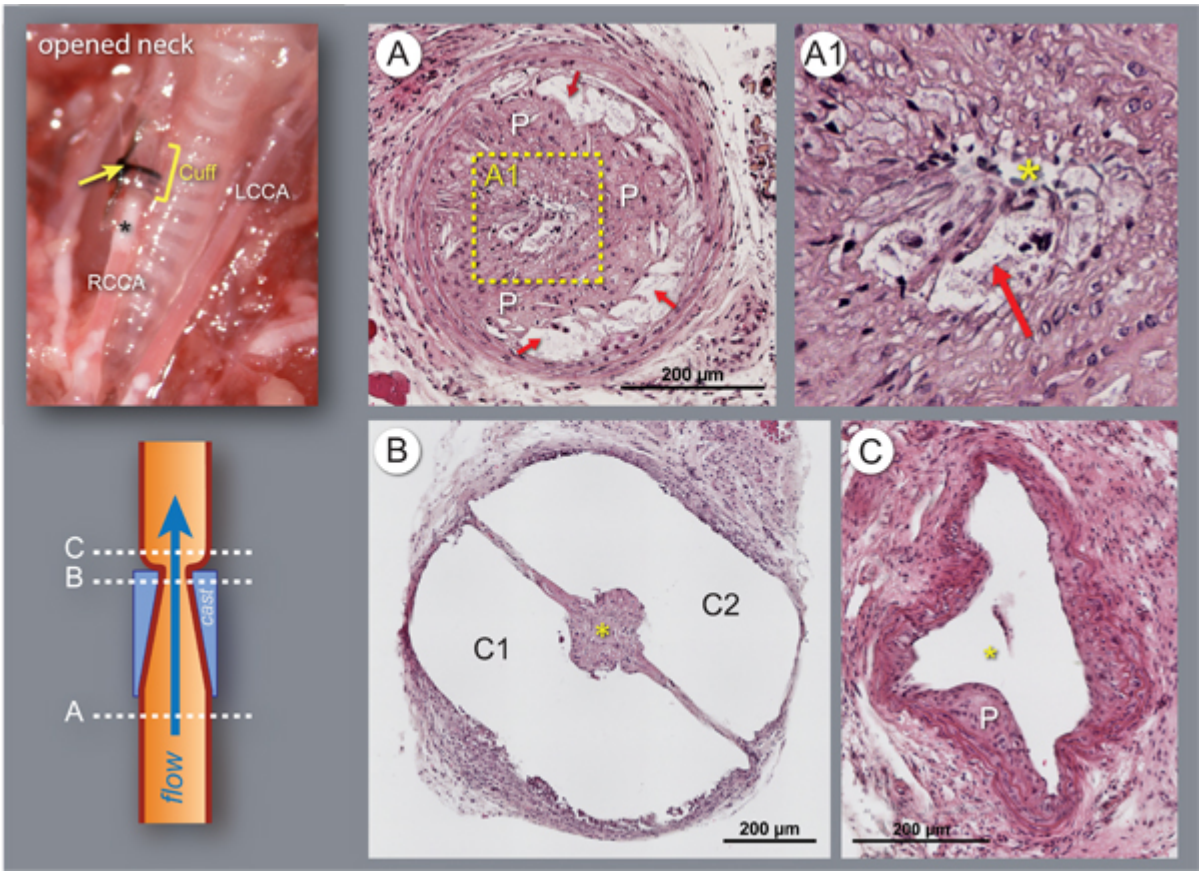

Figure 3. Shear stress induced plaque development in ApoE knockout mice. The upper left corner shows the macroscopic view on the opened neck and exposed carotid arteries of an ApoE knockout mouse under high fat (western type) diet 8 weeks after implantation of a shear stress modifier around the right common carotid artery (RCCA). The opaque white region of the vessel (*) upstream of the cuff corresponds to a site of extensive atherosclerotic plaque deposition. The yellow arrow indicates the thread which combines the two cuff halves. In contrast, there are no signs of plaque deposition in the left common coronary artery (LCCA).

(A)-(C) Representative HE stained cross-sections of the right common carotid artery of an ApoE knockout mouse under western type diet 8 weeks after implantation of the cuff. The schematic illustration gives the corresponding planes (dotted lines) where the sections were located. (A) Upstream of the cuff atherosclerosis leads to massive plaque deposition. Large lipid cores (red arrows), which in part are located close to the central lumen (detail A1) characterize the vulnerable character of these plaques. (B) At the plane of the cuff bottleneck nearly no plaque development is obvious, whereas directly downstream of the cuff (C) oscillatory flow leads to moderate plaque development of a more stable type (i.e. less or no lipid cores). (C1, C2 = half shells of the cuff, P = plaques, asterisk = vessel lumen)

Discussion

In order to minimize experimental variation it is recommended to work with animals of nearly the same age and with the same diet history. A recently published investigation demonstrates that the shear stress modifier applied in wildtype mice might be a good model for the investigation of endothelial dysfunction and early inflammatory responses induced by altered flow dynamics [13]. However, for the enquiry of atherosclerotic plaque development transgenic hyperlipidemic mouse models (e.g. the ApoE knockout mouse) are required. Progression and extent of plaque deposition in each mouse model depend on the type of diet used. In general, the higher the cholesterol/fat content of the diet, the more rapid the progression of the disease is.

During cuff implantation the surgeon should try to minimize tissue damage and manipulation, for this will lower inflammatory responses due to injury. Minimizing injury is especially important when inflammatory processes in the course of atherosclerosis are in the focus of the study. In order to estimate the degree of post-surgical inflammation it is highly recommended to implant non-constrictive control cuffs in some animals. The control cuff should be a cylinder made of the same material, but with a continuous, non-constrictive inner diameter.

It is also essential to always place the cuff at the same position of the carotid artery and to choose always the same side, otherwise flow velocities are not reproducible. The surgeon has to take great care of the right fit of the cuff when combining the two cuff half shells, because improper fit results in a not readily formed conical inner lumen which in turn leads to unpredictable flow parameters.

A great advantage of the shear stress modifier is that it creates three well defined regions with characteristic and reproducible patterns of flow velocities within the same vessel (Fig. 2, Table 1). In a hyperlipidemic environment each of these flow patterns cause plaque deposition of a characteristic phenotype (Fig. 2) resembling vulnerable and stable plaques in humans.

Thus, the presented mouse carotid cuff is a valuable *in vivo* model for the investigation of shear stress induced plaque phenotypes (*stable and unstable*). Furthermore, it might be also an ideal small animal model for the development of new molecular imaging probes, designed to early identify sites of atherosclerosis even before progressed plaque deposition leads to stenosis or plaque rupture, the initial event giving rise to life-threatening cardiovascular events like thrombus formation and myocardial infarction.

Disclosures

The authors have nothing to disclose.

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