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Animal models for the assessment of novel vascular conduits

Michael J. Byrom, MBChB,[a,b,c] Paul G. Bannon, PhD,[a,c,d] Geoffrey H. White, FRACS,[c,d] and **Martin K. C. Ng, PhD,[b,c,d]** *Sydney, New South Wales, Australia*

The development of an ideal small-diameter conduit for use in vascular bypass surgery has yet to be achieved. The ongoing innovation in biomaterial design generates novel conduits that require preclinical assessment in vivo, and a number of animal models have been used for this purpose. This article examines the rationale behind animal models used in the assessment of small-diameter vascular conduits encompassing the commonly used species: baboons, sheep, pigs, dogs, rabbits, and rodents. Studies on the comparative hematology for these species relative to humans are summarized, and the hydrodynamic values for common implant locations are also compared. The large- and small-animal models are then explored, highlighting the characteristics of each that determine their relative utility in the assessment of vascular conduits. Where possible, the performance of expanded polytetrafluoroethylene is given in each animal and in each location to allow direct comparisons between species. New challenges in animal modeling are outlined for the assessment of tissue-engineered graft designs. Finally, recommendations are given for the selection of animal models for the assessment of future vascular conduits. (J Vasc Surg 2010;52:176-95.)

Clinical Relevance: **The limitations of autologous arterial and venous segments used in vascular surgery drives the development of novel vascular conduits which ultimately require assessment in vivo. The abundance of articles proclaiming successful development of new conduits contrasts starkly with the handful of candidates reaching clinical trials, a discrepancy due at least in part to a lack of standardised approach to the selection and utilisation of appropriate animal models. This review seeks to provide for the first time a broad comparison of the pertinent variables determining the utility of species used for vascular conduit assessment, relevant to researchers and to clinicians evaluating reports of novel conduit development.**

Animal models are used to simulate human anatomy, physiology, and pathologic processes, with extrapolation of findings to human medicine. Animals have been used for this purpose since at least the second century, when the Greek physician Galen (130-200) began his anatomic experiments on the circulation, later correctly described as a result of further animal research by William Harvey (1578-1658), and the use of animals has since continued as an important component of surgical research.[1]

The development of conduits for use in vascular bypass surgery has a much more recent history. The use of autologous (from the same individual) vein for arterial reconstruction was described in 1906, but it was not until 1949 that autologous saphenous vein was first used as a femoropopliteal bypass graft. Frozen and formalin-preserved arterial allografts (from the same species) were described in 1908, but the utility of allograft vessel transplants has been limited by thrombosis and aneurysmal degeneration.[2] Bovine arterial xenografts (from a different species) were first implanted in 1962 and human umbilical vein allografts in 1974,[3] but it is polymer prosthetics, such as polyethylene terephthalate/Dacron and expanded polytetrafluoroethylene (ePTFE), that remain the recommended alternatives in peripheral vascular bypass surgery when an autologous vessel is not available.[4,5] Prosthetic vascular conduits were first introduced in 1952 in the form of porous cloth tubes,[2] but the arrival of knitted and woven Dacron conduits in 1957 changed the management of occlusive vascular disease.[6] PTFE was unsuccessfully trialed as a textile prosthesis before ePTFE was introduced to peripheral vascular surgery in 1972. Other fields of vascular bypass surgery share an even shorter history of conduit development. For hemodialysis access surgery, the autologous subcutaneous arteriovenous fistula first reported in 1966 remains the best method for provision of long-term vascular access, whereas ePTFE is the most commonly used graft material for bridge fistulas.[7] Coronary artery bypass grafting surgery began with the internal thoracic artery as a bypass graft in 1964 and reversed saphenous vein in 1967,[8] and these remain the primary conduits used due to the rapid failure of ePTFE in this location.[9]

The ideal vascular conduit remains the Holy Grail of vascular surgery,[10,11] and the ongoing development of novel conduits reflects the limitations of autologous vessels as well as the inadequacies of currently available small-diameter vascular synthetics. Autologous saphenous vein is unsuitable in up to one-third of patients presenting with arterial disease of the lower extremity due to small vein size, varicosities, anatomic variation, previous removal, or other abnormalities,[12] and

From The Baird Institute,[a] The Heart Research Institute,[b] the University of Sydney,[c] and Royal Prince Alfred Hospital.[d]

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Reprint requests: Martin K. C. Ng, The Heart Research Institute, 7 Eliza St, Newton, NSW 2042, Australia (e-mail: martin.ng@email.cs.nsw.gov.au).

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most of the remainder demonstrate fibrotic wall change despite normal preoperative quality assessment.[13] Vein quality is an important factor in the later development of graft stenosis,[14,15] and even if good-quality vein is available, vein harvest is time-consuming and is a major contributor to postoperative morbidity (leg edema and wound complications) that occurs in 20% to 30% of infrainguinal bypass surgery patients.[16]

Synthetic vascular conduits work well for large-diameter, high-flow, low-resistance applications such as aortic replacement and aortoiliofemoral grafting, where Dacron conduits crimped and sealed during the manufacturing process remain the material of choice.[4] ePTFE has become favored for medium-diameter (6-9-mm) situations where autologous veins are unavailable or unsuitable,[3,4] but for small-diameter applications (<6 mm), such as the infragenicular and coronary vessels, the use of synthetic conduits has produced disappointing patency rates, and an ideal vascular graft for these situations is lacking.[10,11]

The continuing innovation in vascular biomaterial design aims to overcome these inadequacies and in the process generates novel conduits that ultimately require assessment in vivo. Evaluation of new conduits in preclinical animal studies is required to assess the capacity of the prosthesis to maintain physiologic function in the circulatory system and to determine the response of both the host and the prosthesis to implantation.[17] It is also a requirement for regulatory approval, as outlined in published standards for graft manufacturers recognized by the United States (U.S.) Food and Drug Administration.[17,18]

In addition to describing appropriate in vitro testing methods, these standards lay out the requirements for acquisition of data relating to the preoperative, operative, and postoperative periods of in vivo preclinical studies. Such data include the preoperative health status of the animal, implanting surgeon and method, medications given and methods of assessment used, adverse events, and the details of animal termination and specimen explant. Although not specifying which model should be used for any particular prosthesis, they state that the intended diameter and length of conduit along with the intended clinical application and the biologic characteristics of the animal should be considered in the selection of the animal model. These animal experiments precede initial clinical evaluation studies that establish the short-term safety and efficacy before general marketing.[17]

The endeavor to develop an ideal vascular conduit has so far spanned more than half a century, accumulating a wealth of published literature and covering a multitude of species, implant locations, and assessment techniques. This review provides a comprehensive summary of the comparative hematology between species, comparison of known values for hydrodynamic measures relevant to vascular conduit assessment, and an analysis of the commonly used animal models, detailing the advantages and disadvantages for each. Where possible, a direct comparison between species is given, especially regarding the performance of ePTFE, because this remains the most widely used control material in the evaluation of novel vascular prosthetics. The current era of tissue-engineered vascular grafts brings new challenges in animal modeling and a discussion of these is provided. Finally, recommendations are given for the design of future in vivo assessments. The scope of this review should prove useful to clinicians seeking knowledgeable assessment of reports of conduit development as well as to researchers in the field of vascular biomaterial design.

SEARCH METHODS

An electronic literature search of the U.S. National Library of Medicine public domain database (MEDLINE) was performed for articles published from 1950 to present using the following keywords: *animal models*, *blood vessel prosthesis*, *polytetrafluoroethylene*, *tissue engineering*, *vascular surgical procedures*, *peripheral vascular diseases*, *papio*, *swine*, *sheep*, *dogs*, *rabbits*, and *rodentia*. The references of the retrieved articles were also manually searched for any additional relevant articles. These articles were supplemented with textbooks of vascular surgery, biomaterials science, animal models, and veterinary biology.

CONSIDERATIONS IN THE SELECTION OF AN ANIMAL MODEL

Minimally acceptable criteria for the expected performance and methods of evaluation of arterial prostheses were first proposed in 1993[19] and were soon followed by published standards.[20,21] Desirable characteristics of arterial prostheses proposed at that time remain important today and include sterility, consistency, reasonable cost, low porosity, good handling (suturability, conformability, and kink resistance), infection resistance, strength, and durability. An ideal prosthesis would also demonstrate biomechanical properties that match those of the target artery and exhibit a thrombosis-resistant surface hospitable to endothelialization.[19]

No ideal animal model exists for the assessment of novel conduits against all of these performance criteria, but a number of models perform well for specific purposes.[19] This reality is reflected in the number of animal species and methods of in vivo assessment that have been used. A summary of animal models commonly used in the assessment of vascular conduits is given in Table I for large animals and Table II for small animals. Other species used to test vascular grafts include guinea pigs, hamsters, goats, macaques, and vervet monkeys.[22-26]

A number of considerations should be made when determining an appropriate animal model. This decision includes not only the animal species but also the anastomotic site, caliber and length of prosthesis, and duration of implantation. Cost, availability, ease of handling during examinations, response to anesthesia and surgery, and arterial size and flow at sites of conduit implantation are often used to guide animal selection; however, of primary importance is the need to select an animal model that correctly simulates the relevant aspect of human physiology, and species differences must be considered.

For the chosen animal, decisions regarding conduit implantation should be made with the particular objective of testing in mind. For example, short-term implantation of short prostheses in high-flow locations (eg, 4-cm carotid

Table I. Large-animal models for the assessment of vascular conduits

Species	*Site*	*Size (cm, diameter × length)*
Ex vivo conduit		
Baboon	FA-FV	0.4 × NR[62]
Sheep	CA-CA	0.5 × 4[38]
Dog	FA-FV	0.3 × 11[185]
In vivo patch		
Sheep	CA	0.8 × 5 cm[116]
Dog	CA+FA	NR[186]
In vivo conduit		
Baboon	CA	0.4 × 5-7[89]
	Ao-IA	0.4 × 5,[61] 0.4 × 7-9[90]
	FA	0.4 × 8.2[93]
Sheep	CA	0.4 × 4,[114] 0.4 × 4-5,[26] 0.4 × 5/10,[111] 0.5 × 5,[36] 0.6 × 10,[187] 0.6 × 11.5[113]
	FA	0.4 × 4,[120] 0.4 × 5/10,[111] 0.5 × NR,[122] 0.6 × 4-5[121]
	AV	CA-JV 0.6 × 10-15,[188] CA-JV 0.6 × 11-14[118]
	Other	Ao 0.7 × 3-4[189]
Pig	AV	FA-FV 0.4 × 6.5,[130] CA-JV 0.5 × 7,[129] CA-JV 0.6 × 15[128]
	Other	SA NR × 3.5,[123] SVC 0.4 × 10[40]
Dog	CA	0.3 × 4,[190] 0.4 × 6,[191] 0.45 × 6[192] (NR)[193]
	Ao	0.6 × 5,[194] 0.6 × 6,[195] 0.6 × 6-8,[196] 0.8 × 12,[180] 0.8 × 30[131]
	Ao-IA	0.4 × 5,[197] 0.5 × 10-12,[198] 0.5 × NR[133]
	AV	CA-JV 0.6 × 10,[196] FA-FV 0.6 × 30[199]
	Other	FA 0.4 × 12,[200] CA-Ao 0.6 × 45,[132] CA-Ao 0.8 × 70[41]
In vivo other		
Sheep	Ao[a,201]	
Dog	SC implant[202]	

Ao, Aorta; *AV,* arteriovenous graft; *CA,* carotid artery; *FA,* femoral artery; *FV,* femoral vein; *IA,* iliac artery; *JV,* jugular vein; *NR,* not reported; *SA,* saphenous artery (pig); *SC,* subcutaneous; *SVC,* superior vena cava.
[a]Material suspended inside bare stent.

Table II. Small-animal models for the assessment of vascular conduits

Species	*Site*	*Size (cm, diameter × length)*
In vivo conduit		
Rabbit	CA	0.15 × 1,[78] 0.15 × 2,[203] 0.2 × 1,[142] 0.2 × 1.5,[140] 0.2 × 2,[141] 0.4 × 3-4,[148] 0.5 × 2[149]
	Ao	0.2/0.3 × 1,[136] 0.4 × 2.4,[137] 0.4 × 2.4,[139] 0.4 × 4-5[39]
	Ao-IA	0.5 × 4[38]
	FA	0.1 × 1,[144] 0.15 × 1,[145] 0.15 × 2[143]
Rat	Ao	0.1 × 1,[163] 0.1 × 1,[153] 0.13 × 1,[161] 0.13 × 1,[155] 0.15 × 1,[150] 0.15 × 2/10,[151] 0.15 × NR,[152] 0.15 × NR,[176] 0.2 × 1.3,[154] 0.3 × 1[149]
	Other	FA 0.1 × 1,[157] FA-FA 0.1 × 6,[156] CA 0.4 × 1[158]
Mouse	Ao	0.07-0.13 × 1[165]
In vivo other		
Rat	IP implant	0.5 × 0.5[131]
	SC implant	0.15 × 1.2[159]

Ao, Aorta; *CA,* carotid artery; *FA,* femoral artery; *NR,* not reported; *IA,* iliac artery; *IP,* intraperitoneal; *SC,* subcutaneous.

interposition grafts) may not provide a stringent patency test but might satisfy other objectives such as safety.[19] Prostheses intended for coronary grafting are best assessed in that position due to differences in hemodynamics, graft flow, kinking, and anastomotic conditions compared with peripheral arterial locations.[27] Conduit caliber is a major determinant of graft patency and depends on native vessel diameter, the type of anastomosis (end-to-end or end-to-side), and the required flow rate through the graft. Small calibers result in high resistance and consequently low blood flow, whereas larger calibers lead to low wall shear stress, greater pseudointima thickness, and more anastomotic intimal hyperplasia (IH), with both situations leading to reduced patency.[28] If anastomotic IH is the focus of the study, a larger graft/native vessel diameter ratio may be required to simulate the shear stress conditions of clinical bypass grafts.[29]

The choice of model is also influenced by the methods used to measure outcomes and determine significance. Aortic interposition grafting generally precludes serial ultrasound assessment of patency or morphology, necessitating postmortem evaluation unless other techniques are available (eg, computed tomography angiography). Implantation of single conduits prevents paired-site evaluation with a suitable control, in contrast with bilateral graft placement (eg carotid or femoral interposition or bypass). Placement of the control conduit in the same animal reduces the effect of interindi-

vidual variability, such as the known but variable tendency for platelet aggregation in dogs, and allows paired tests of significance, reducing the number of subjects required.[30] The choice of control conduit is also important, and new prostheses should be assessed in comparison with an accepted currently used alternative, usually ePTFE for smaller conduits due to difficulties using animal vein.[19]

Technical considerations include the effects of flexion and tension. Grafts placed over a flexion crease are known to exhibit reduced patency, but carotid implants may also be affected by considerable flexion if sufficiently long.[30] Of 48 ePTFE conduits 4-mm in diameter and 5-cm in length implanted in dogs for 3 months, kinking was noted in 50% of femoral grafts and also in 17% of carotid grafts.[31] Kinking due to excessive length may be avoided by implanting conduits under mild tension similar to native vessels. In situ vessels are under longitudinal tension that changes with age and location: excised arteries retract 22% to 39% in patients aged 11 to 20 years and 13% to 25% in those aged 36 to 52 years, with retraction increasing in more peripheral locations.[32] Moderate tension after implantation does not affect patency, but in very small arteries excessive tension leads to increased suture hole bleeding, reduced native vessel diameter, and reduced patency once the elastic reserve of the vessel is exceeded.[33,34] Increased tension is normalized within 7 days of surgery by wall tissue remodeling, but these processes are unable to correct for tension below physiologic levels due to excessive conduit length.[35]

Although often not reported, the postoperative mortality of species after the implantation of vascular conduits may be significant, depending on the nature of the procedure and experience of the institution. Sheep, for instance, pose a difficulty during endotracheal intubation because the cranial lobe of the right lung arises directly from the trachea rather than from the right mainstem bronchus. Nonventilation of this pulmonary segment combined with a high risk of regurgitation without adequate preoperative fasting and intraoperative orogastric drainage may result in postoperative pneumonia, a common cause of early death after surgery in sheep.[36,37] Paraplegia may develop in rabbits after infrarenal aortic clamping for placement of aortoiliac conduits,[38] and anesthetic complications may result in an operative mortality of 30%.[39] Mortality rates of 10% to 30% have also been reported in dogs and pigs.[40,41] These figures should be included in published reports to enable more accurate calculation of the number of animals required to achieve statistical power.

COMPARISON OF SPECIES USED IN THE ASSESSMENT OF VASCULAR CONDUITS

The ability of animals to correctly model the human response to vascular prosthetics is determined by species differences in factors that influence conduit performance. For instance, comparative studies have shown that the clotting and fibrinolytic systems of the calf and nonhuman primates are more similar to the human than those of dogs or pigs, and interspecies differences also exist regarding neointimal formation.[42] Knowledge of these differences reduces the potential for bias in the interpretation of test results and allows an assessment of the validity of the model used.

In 1993 the Ad Hoc Committee of the Joint Councils of the Society of Vascular Surgery and the International Society for Cardiovascular Surgery, North American Chapter, recommended that the dog be used for preclinical testing of arterial grafts in most instances.[19] This was due to an apparent lack of significant spontaneous endothelialization of prosthetic surfaces (reflecting the situation seen in humans) and a tendency towards hypercoagulability, making the canine a potentially stringent challenge for conduit assessment. As a result, canine models were frequently used throughout the 1990s (see Table I).

With concern for animal rights increasing, the expense and difficulty of acquiring dogs for experimental use has also risen as the use of purpose-bred animals replaces the relatively inexpensive mongrel so that pigs and sheep are now comparatively cheap to purchase.[23,43] Recent studies have also questioned the appropriateness of canines for the assessment of endothelialization and patency, as discussed later.[29,44-47] Nonhuman primates resemble human anatomy and physiology more closely than other species, but their use is limited for many by ethical considerations, prohibitive cost, and their protected status.[23,42]

COMPARATIVE HEMATOLOGY

The acute response of the blood to materials implanted into the vascular circulation results from a complex interplay between a number of interconnected systems that include platelets, coagulation proteins, and cellular elements such as macrophages, and has been summarized elsewhere.[48] Briefly, adsorption of plasma proteins to the material surface is followed by platelet activation and attachment resulting in shape change and release of cytoplasmic granule contents. Delivery of additional platelets and proteins to the surface, influenced by the effects of blood flow and wall shear stress, results in a luminal coating of platelets and fibrinogen/fibrin that may lead to thrombotic occlusion of the conduit. These thrombotic processes are balanced by a number of regulatory mechanisms such as the fibrinolytic system. Comparisons of the coagulation systems of species therefore examine the response of platelets to surfaces (attachment/activation) and to agonists of the release reaction (eg, adenosine diphosphate, collagen, epinephrine, thrombin, ristocetin) as well as levels of coagulation cascade proteins and times to achieve coagulation end points, including thrombin, prothrombin, and activated partial thromboplastin times.

An appreciation of the comparative hematology of commonly used species is essential when determining the validity of the in vivo thrombogenicity assessment of vascular biomaterials. Relevant hemodynamic and hematologic values for commonly used animals compared with humans are given in Tables III and IV, and these animals are discussed in detail below. Owing to variability in normal values, depending on methodology, values for coagulation tests are best compared with normal control animals at the same time and in the same laboratory.[49-54] For most mam-

Table III. Hemodynamic values[a] for some commonly used species

Species	*SBP (mm Hg)*	*DBP (mm Hg)*	*MBP (mm Hg)*	*HR (beats/min)*	*CI (mL/min/kg)*	*TBV (mL/kg)*
Human[43,204]	126 ± 14	79 ± 10	95 ± 11	70 ± 14	93 ± 20	80
Baboon[55,205-208]	122	77	107 ± 16	92 ± 13	103	60-80
Pig[43,209]	127 ± 8	86 ± 7	102 ± 9	105 ± 10	99 ± 20	57
Sheep[43,209,210]	140	90	114	95 ± 24	115 ± 31	58
Dog[43,209]	126 ± 23	90 ± 20	96 ± 7	98 ± 18	88 ± 19	93
Rabbit[49,146,209,211,212]	120	80	98 ± 3	130-325	279 ± 56	56
Rat[49,209,211,212]	127 ± 7	85 ± 15	120 ± 8	250-450	441 ± 142	54

CI, Cardiac index; *DBP,* diastolic blood pressure; *HR,* heart rate; *MBP,* mean blood pressure; *SBP,* systolic blood pressure; *TBV,* total blood volume.
[a]Values are presented as mean ± standard deviation or range.

Table IV. Hematology values[a] for some commonly used species

Species	*RBC ($\times 10^9$/mL)*	*Hct (%)*	*Plt ($\times 10^9$/L)*	*WBC ($\times 10^9$/L)*	*Neut (%WBC)*	*Lymph (%WBC)*	*Fib (g/L)*
Human[43,204,211]	4.8-5.4	44.5 ± 8	265 ± 135	7.4 ± 3.4	50-70	20-40	2-4
Baboon[54,61]	4.5	36.2 ± 2	370 ± 105	7.1 ± 2.1	44	46	
Pig[43,209,211,213]	6-8	41	350 ± 150	14.8	40	50	2-4
Sheep[43,209,211,213]	10-13	36 ± 9	550 ± 250	8 ± 4	30	60	2-4
Dog[43,209,211,213]	6-8	45 ± 10	350 ± 150	11.5 ± 5.5	60	25	1-4
Rabbit[55,209]	4-7.2	42.5 ± 3	200-1000	7.5-13.5	30	55	
Rat[55]	7-10	46.4	500-1300	6-17	9-34	65-85	

Fib, Fibrinogen; *Hct,* hematocrit; *Neut,* neutrophils; *Lymph,* lymphocytes; *Plt,* platelet count; *RBC,* red blood cell count; *WBC,* white blood cell count.
[a]Values are given as mean ± standard deviation or range.

malian species, blood volume averages 6% to 8% of body weight (L/kg).[55]

Historical studies of platelet responses to biomaterials or other stimuli, as well as for other aspects of the hemostatic system such as activation of the coagulation pathway, are available for all commonly used species. Similar studies also exist for other animals, including camelidae (camels, llamas),[56] goats,[57] and guinea pigs.[58] It is important to remember that species differences are biomaterial dependent, with the response of any given species significantly affected by the material being investigated.[59] Furthermore, laboratory measures of coagulation cannot be used in isolation to predict graft performance; for instance, canines demonstrate generally superior patency for ePTFE conduits compared with sheep despite shorter coagulation times in vitro.[44,60]

Baboon. Baboons exhibit a high degree of genetic similarity with humans,[61] so it is unsurprising that the hemostatic system of humans is most closely approximated by the baboon and other Old World primates.[62-64] In a comparative study, Feingold et al[65] found that baboons demonstrate a similar fibrinogen level and thrombin time to humans, and the prothrombin time (PT) and activated partial thromboplastin time (APTT) are only slightly increased, whereas they are significantly shorter in canines. Baboon factor VIII activity is also similar to humans, and factor VIII antigen cross-reacts with human factor VIII antibodies. Baboon platelets demonstrate several similarities, including size distribution, number of dense bodies, and responsiveness to collagen and ristocetin, although with increased responsiveness to arachidonic acid and reduced responsiveness to adenosine diphosphate (ADP) and epinephrine. The baboon is also immunologically closest to humans.[66]

Sheep. Sheep have been described as possessing a coagulation system that is closer to the human than either dogs or pigs,[42] although a number of differences are notable in the assessment of the thrombogenicity of vascular prostheses. Platelet number is high compared with most species (3-8 $\times$ 10^5/mL vs 2-5 $\times$ 10^5/mL),[49] and adhesiveness is increased. Sheep also demonstrate reduced fibrinolytic activity, and together, these attributes confer a tendency toward hypercoagulability.[43,67] Compared with humans, platelet aggregation is similar with thrombin,[68] ADP,[69] and platelet-activating factor (PAF),[70] is reduced with collagen type I and ristocetin,[69] and is nearly absent with epinephrine.[68,69]

The cellular blood elements also show some differences between sheep and humans, with erythrocyte values of particular relevance. Ovine red blood cells are relatively small and the sedimentation rate is slow due to the absence of rouleau formation in ruminants.[67] This should be considered when separating sheep blood by differential centrifugation, for example, when isolating platelets, because higher centrifugation speeds may be required when sheep are used[69] than those recommended for human blood.[71] Sheep red cells are also more osmotically fragile than human red cells, with hemolysis beginning at 0.65% to 0.76% saline and complete at 0.40% to 0.45%.[67,68] Leucocytes are

predominantly neutrophils in humans, but lymphocytes form the main proportion in sheep.[68]

Tillman et al[69] studied platelet function and coagulation parameters in healthy unanesthetized sheep as well as from sheep during vascular surgery and compared these with human values. Results for PT, APTT, activated coagulation time (ACT), platelet counts, and platelet aggregation were unaffected by anesthesia and surgery, with the exception of prolongation of PT, APTT, and ACT by systemic administration of heparin, which were all fully reversed with protamine sulfate. Coagulation parameters from healthy sheep were similar to human values, as was platelet aggregation due to ADP. Ovine platelet counts were slightly higher as expected.

Pig. Like sheep, pigs have also been attributed with a coagulation system that is similar to humans,[72,73] although with a tendency towards hypercoagulability.[42] Pelagalli et al compared buffalo, horse, pig, and sheep platelets for their ability to aggregate in response to agonists[74] and to attach to immobilized autologous fibrinogen.[75] Pig platelets were more responsive to ADP than sheep, but sheep platelets demonstrated greater sensitivity to ristocetin, collagen type I, and PAF. Platelet attachment to immobilized fibrinogen both with and without activation by ADP was greatest for humans, lowest for sheep, and in between for pigs. Sheep platelets were unable to attach in the absence of ADP.

Platelet attachment to pyrolytic carbon mechanical heart valve leaflets, polyethylene, silicone rubber, and Formvar (polyvinyl formal resin) was compared between sheep, pigs, and humans.[76] Scanning electron microscopy was used to assess attachment and spreading after exposure to the materials under static conditions and found similar results in pigs and humans and a comparatively reduced platelet response in sheep. The low numbers in this study, however, limit generalization.

Dog. Despite also being attributed with a hematologic system similar to humans, the dog is known to be relatively hypercoagulable[43] and exhibits greater dissimilarities to humans than do sheep or pigs. Whittle et al[77] found sensitivity of canine platelet aggregation to ADP was low compared with humans and sheep, and inhibition of aggregation by prostaglandin D2 was weak. The fibrinolytic system is more active in the canine than in the pig.[42] Canines have a significantly shorter PT, APTT, and thrombin time than humans and may have significantly higher concentrations of fibrinogen.[65] Factor VIII activity is markedly higher than in humans or baboons, and canine factor VIII antigen fails to cross-react with human factor VIII antibodies. Platelets from dogs are slower to aggregate in response to collagen and show less aggregation to epinephrine and ADP. Sato and Harasaki[60] used a clot signature analyzer to compare platelet-mediated hemostasis, collagen-induced thrombus formation, and clotting time between human, bovine, ovine, and canine species. All three parameters were similar between sheep and humans, but distinctly shorter times were found in dogs. They concluded that, "the dog is not an ideal animal model for the evaluation of blood-surface interactions."

Rabbit and rat. The rabbit may provide a more valid model for thrombogenicity assessment than the rat due to greater similarities in hemostatic mechanisms with humans. These include a coagulation system which resembles more closely that of the human and arteries with similar thromboplastic and fibrinolytic properties.[78] Differences in platelet aggregation to agonists include relatively less sensitivity of rabbit platelets to ADP than those of humans or rats,[77] whereas rabbit platelets are more sensitive to PAF than human platelets and show a different response and release pattern. Platelets from rats do not aggregate or release when exposed to PAF.[70]

A comparison of rat and human hematology and coagulation found a number of similarities as well as differences.[79] Platelet count averaged 1109×10^9/L for rats with a mean diameter of 2 μm, which is more numerous and slightly smaller than human platelets. Compared with humans, platelet aggregation in rats was reduced or absent with collagen, ristocetin, thrombin, epinephrine, and arachidonic acid but similar with ADP. Levels of coagulation factors were mostly similar; however, PT and APTT were shorter than in humans and thrombin time was longer, with a low-grade thrombin inhibitor detected in rat plasma.

COMPARATIVE HYDRODYNAMICS OF COMMON IMPLANT LOCATIONS

Knowledge of relevant hydrodynamic values for common implant locations in animal models used for vascular conduit assessment allows informed decisions to be made about the applicability of experimental results to clinical practice. We hope that the summary of values provided here also facilitates the design of future animal studies by providing an indication of values that can be expected from various implant models (Table V). For example, 5.5-mm-diamater saphenous vein implanted as femoral artery bypass grafts in dogs[80] achieve a mean flow of 170 mL/min and a mean shear stress of 18 dynes/cm^2, similar to values for the native canine femoral artery.[28]

A number of factors should be considered when interpreting published values for vessel diameter, blood flow, and shear stress. Values within species will vary due to a number of factors, notably the size of the animal given the positive association between body weight and cardiac output or blood vessel flow.[81] This relationship is stronger for the common carotid artery than for the femoral artery,[81] at least in the canine, and is not significant in small animals such as the rat.[82] The origin of the animal may also confer an important influence: compared with mongrel dogs, racing greyhounds demonstrate a higher mean arterial pressure, higher cardiac index, lower peripheral vascular resistance, lower vascular impedance, and a femoral artery blood flow of 179 mL/min compared with 80 mL/min for mongrels.[83] The timing of assessment is crucial due to the effects of anesthesia and surgery, with increased blood flow seen, for example, after arterial clamp removal due to postischemic hyperemia.[84] Lastly, it is important to be precise regarding the anatomic location; for instance, the

Table V. Values[a] of relevant hydrodynamic variables for common implant locations

Species, location	*Diameter (mm)*	*Mean flow (mL/min)*	*Mean velocity (cm/s)*	*Mean WSS (dynes/cm²)*
Human				
CCA[214-216]	6.5, 7.0	455, 483	22.3, 24.4, 21.8[b]	7.8, 10.4[b]
CFA[217,218]	8.0, 10.2	284, 359	8.6, 10.2, 8.2[b]	2.9[b]
SFA[217,218]	6.2, 8.3	152, 196	7.5, 8.8, 7.0[b]	3.1[b]
Baboon				
CCA[98,206]	3.2[c]	61	12.6[b]	12.6[b]
IA[219,220]	2.5[d,c]	170[d]	55.0[d], 74.2[c], 57.7[b]	37.5[c], 73.9[b]
Sheep				
CCA[111,221,222]	5.6, 7.2	325	16.8[b]	8.4[b]
IA[223]	5.9			
CFA[111,122,221]	4.2, 5.0	160	16.0[b]	11.2[b]
Dog				
CCA[28,81,82]	3.0-4.0	153	26.5[b]	27.0, 24.2[b]
Abdo.Ao[85,224]	6.0-7.0, 8.0	430-580	21.9[b]	10.0[b]
IA[225-228]	3.2, 4.9, 7.4	84, 97, 105, 137	13.9, 8.4[b]	11.0, 17.8, 5.2[b]
FA[28,81-83]	4.4, 4.0-5.0	80, 97, 202	13.5[b]	14.0, 9.7[b]
Rabbit				
CCA[82,229,230]	1.9-2.1, 2.0	49.8	26.4[b]	9.1-10.3, 42.3[b]
Abdo.Ao[39,137,139]	2.5-3.0, 3.0	32-51[f]	10.7[b]	11.9[b]
FA[82,143]	1.0-1.2			
Rat				
Abdo.Ao[154,162,163]	1.0, 2.0			
Mouse				
Abdo.Ao[165]	1.0			

Abdo.Ao, Abdominal aorta; *CCA,* common carotid artery; *CFA,* common femoral artery; *FA,* femoral artery; *IA,* iliac artery; *SFA,* superficial femoral artery; *WSS,* wall shear stress.

[a]Values are presented as mean or range.

[b]Calculated from average values for diameter and flow. Viscosity of blood = 0.04 Poise.

[c]At 30 days after implantation of heparin-coated stent, calculated from luminal + neointimal area.

[d]After bilateral placement of aortoiliac prosthetic grafts.

[e]At 4 days after placement of an external expanded polytetrafluoroethylene wrap around the iliac artery.

[f]At implantation of 3.6/4.0-mm diameter interposition graft.

canine aortic diameter is typically 40% greater in the thorax than in the abdomen.[85]

LARGE-ANIMAL MODELS FOR THE ASSESSMENT OF NOVEL VASCULAR CONDUITS

Large domestic animals have generally been used for the evaluation of cardiac and vascular biomaterials because of a closer resemblance to human anatomic and physiologic characteristics than that obtained from models in other mammals such as rabbits or rats.[43] Commonly used species include the baboon, sheep, pig, and dog, accommodating conduits mostly 3 to 6 mm in diameter although up to 8 mm in the canine aorta.

Baboon (primate, nonhuman). As outlined earlier, the baboon provides the closest approximation to humans for the assessment of blood compatibility of vascular surfaces. The baboon is also frequently used in the assessment of endothelialization of prosthetic conduits, usually in the iliac position. Despite a higher purchase cost, the overall expense may be balanced in some countries by maintenance costs below that for more difficult species such as pigs.[23,49]

The effect of modifying the luminal surface of vascular prosthetics on acute thrombogenicity is conveniently studied by imaging implanted conduits for the attachment of radiolabeled compounds. This method has been shown to be suitable across all commonly used animal models.[86,87] Jordan et al[62] recently assessed the acute thrombogenicity of a novel "elastin-mimetic" coated ePTFE conduit inserted in a baboon arteriovenous shunt. Using autologous ^{111}Indium-labelled platelets and ^{125}Iodine-labelled fibrin, evidence for the use of recombinant elastin polymers as nonthrombogenic coatings was greatly strengthened by the demonstration of minimal attachment to the treated surface over 60 minutes at 100 mL/min blood flow.

As with thrombogenicity, the use of animals for the assessment of endothelialization is complicated by species differences. The incomplete endothelialization of ePTFE that characterizes human implants has also been attributed to baboons;[63] however, others have found comparatively rapid healing of arterial synthetics placed in baboons as well as pigs and dogs.[88] Clowes et al[89,90] implanted ePTFE conduits in baboon carotid and iliac arteries and showed 60% of conduits 7 to 9 cm in length fully endothelialized after 12 months, with endothelialization occurring at 0.2 mm/d. These researchers demonstrated in a further investigation the importance of ePTFE porosity on facilitating endothelialization through transmural ingrowth: a 60-μm internodal distance resulted in complete endothelial coverage in baboons, whereas a 30-μm distance limited coverage

to the perianastomotic regions.[91] Despite the genetic similarity between baboons and humans, these results failed to translate successfully in human clinical trials using 60-μm ePTFE reinforced with an outer wrap.[92]

Nevertheless, the use of baboons has contributed significantly to the development of endothelial cell (EC) seeding to improve the long-term patency of synthetic conduits. Zilla et al[93] implanted ePTFE conduits coated in vitro with autologous ECs into baboon femoral arteries and demonstrated retention of a nonthrombogenic endothelial surface after 4 weeks. They went on to develop and clinically apply in vitro endothelialized ePTFE conduits in >130 patients, achieving a 7-year primary patency rate for infrainguinal grafts of 63%.[94]

The use of baboons has also advanced our understanding of anastomotic IH through studying the healing response to implanted vascular conduits as well as other forms of vascular injury. Late failure of vascular conduits occurs as a result of stenosis and occlusion from IH, which occurs most commonly around the distal anastomosis. Contributing events include compliance mismatch, turbulent flow, and altered wall shear stress leading to endothelial injury, platelet activation, cellular proliferation, and the deposition of extracellular matrix.[14,95-97]

A number of models are available for the study of IH and restenosis after arterial insult, although these often use nonsurgical methods to generate localized endothelial and mural injury, most commonly by inflating and withdrawing a Fogarty balloon catheter. These methods have been used to experimentally produce complex lesions, similar to those seen in humans, in nonhuman primates and pigs. Sheep, dogs, rabbits, and rats have also been used to study IH and restenosis after stent placement. Like the response to vascular grafting, stenosis after stent implantation occurs due to IH, whereas constrictive remodeling (loss of area within the external elastic lamina) predominates after balloon angioplasty.[42] Not surprisingly, the same species have also been used to assess IH after implantation of vascular conduits, including baboons, rhesus monkeys, pigs, sheep, dogs, rabbits, and rats.[36] Lin et al[98] demonstrated in baboons that carotid stenting using a heparin-coated balloon-expandable stent reduced IH compared with uncoated stents. Chen et al[61] demonstrated reduced IH at the distal anastomosis of ePTFE aortoiliac bypass grafts in baboons after 4 weeks of exposure to a perianastomotic heparin infusion. More recently, Zilla et al[99] assessed midgraft-IH in vein grafts to the baboon femoral artery and showed reduced IH in the presence of a constrictive external nitinol mesh. These authors have also compared the response of baboon and sheep models to xenogenic tissue using porcine stentless bioprosthetic heart valves and found a stronger acute xenograft response in the primate compared with more calcification and foreign body-type reaction in the sheep.[66]

Sheep (ovine). The sheep is one of the most widely used animal models for the evaluation of implanted cardiovascular devices and has served as the gold standard for models of bioprosthetic heart valve research for decades.[66,76] Numerous advantages include being relatively low cost and an easy animal to manage in the laboratory as well as presenting a stable size and suitable anatomy for the implantation and monitoring of conduits 4 to 6 mm in diameter as carotid or femoral artery implants.[23,42] Measures of blood coagulation in sheep undergoing vascular surgery are similar to those found in humans, but these animals do exhibit a number of hematologic differences along with a tendency to hypercoagulability, as outlined earlier. Sheep can be anticoagulated with heparin and warfarin, although at generally higher doses than those used for humans; however, the response to clopidogrel is modest, and aspirin fails to inhibit platelet aggregation.[100-102]

Carotid artery interposition. The ovine carotid artery is readily accessible and similar in diameter to human peripheral arteries. In addition, implantation of conduits in the neck is well tolerated, with minimal postoperative morbidity.[23,36,103] Neurologic complications as a result of graft implantation or subsequent occlusion seem rare despite temporary bilateral common carotid artery (CCA) occlusion during insertion or permanent loss of patency postoperatively, in keeping with the known anatomy and physiology of ovine cerebral blood flow.

Unlike most other species, the basilar artery in the sheep has only tenuous connections with the vertebrals, which instead communicate with the distal CCAs by a well-developed occipitovertebral anastomosis. From here, blood flows cranially through the external carotid artery on each side to eventually reach the circle of Willis.[104] Blood from each CCA is unilaterally distributed to the cephalic area, but occlusion of one side results in bilateral distribution due to flow through the available anastomoses.[105] Occlusion of one or both CCAs causes increases in blood flow and pressure in the remaining CCA or vertebral arteries, or both.[106,107] Unilateral CCA occlusion is well tolerated, but temporary bilateral occlusion results in changes in the amplitude and frequency in the electroencephalogram of anesthetized sheep, or swaying and ataxia without loss of consciousness in awake sheep. Clamping both CCAs after previous ligation of both occipitovertebral anastomoses invariably results in abolition of the electroencephalogram, as does clamping of both external carotid arteries.[108] Surprisingly, sheep generally recover ≤24 hours from even this complete ischemia so long as it lasts no longer than 12 minutes, beyond which they exhibit blindness, ataxia, and death.[109] Sequential occlusion due to stepped loss of patency postoperatively may be better tolerated due to enlargement of the remaining vessels and development of collaterals.[110]

Dunn et al[111] found preimplantation flows in the carotid to be twice that seen in the femoral artery despite the similarity in size (325 ± 108 vs 160 ± 70 mL/min). Despite use of lignocaine to reduce arterial spasm, postimplantation flows were also more profoundly reduced in the carotid (236 ± 47 mL/min) than the femoral (153 ± 60 mL/min) after insertion of 4-mm diameter conduits. This may be attributed to a slightly greater size mismatch in the carotid position or to greater spasm in the more muscular

carotid artery. Lundell et al[87,112,113] investigated the effect of blood flow by implanting bilateral carotid conduits and reducing flow to 25 mL/min on one side, demonstrating greater platelet and fibrinogen uptake and reduced patency for conduits exposed to reduced flow.

To provide a useful model for the assessment of patency, control conduits need to exhibit an occlusion rate sufficient to allow a significant patency difference to be demonstrated within a practical timeframe. Unmodified ePTFE conduits have shown 50% patency at 1 month for 5-mm-diameter carotid implants,[36] dropping to 33% (5 mm)[36] or 0% (4 mm)[114] after 3 months.

In addition to the assessment of conduit thrombogenicity and patency, sheep have frequently been used as a model for anastomotic IH. Taylor et al[115] and Simoni et al[37] implanted ePTFE conduits as carotid artery grafts in sheep and showed a reduction of IH with systemic heparin administration postoperatively. Ao et al[116] and Hawthorne et al[117] used patches of synthetic graft materials placed in the carotid artery to compare the hyperplastic response to the various polymers and develop a new methodology for IH assessment. Ueberrueck et al[72] implanted polyester conduits as sheep carotid bypass or pig aortic interposition grafts and assessed IH at 3 months. They found no difference between species, although the differences in anastomotic geometry, animal maturity, and possible size mismatch for these 8-mm-diameter conduits are important uncontrolled confounding factors. In addition to patches and arterial grafts, arteriovenous grafts between the carotid artery and jugular vein have been used in sheep to model the IH seen at the venous anastomosis of hemodialysis access grafts, with a thick neointima developing within 4 weeks.[118]

Femoral artery. Despite a slightly smaller size and a lower blood flow, ePTFE conduits implanted in the femoral position have shown similar patency rates to the carotid. Christenson et al[119] inserted 4-mm ePTFE conduits in the femoral artery and showed 50% patency at 1 month and 33% at 3 months. Like the carotid, the femoral position is suitable for the assessment of acute thrombogenicity by the measurement of conduit radioactivity in response to uptake of ^{111}Indium-labelled autologous platelets. Peak attachment occurs about 60 to 90 minutes after restoration of blood flow, with little activity seen at 3 or 4 days after implantation, despite adjustment for decay.[119,120] Researchers have also found this location convenient to assess anastomotic IH in response to femoral artery bypass grafts and the effect of using vein cuffs, demonstrating reduced IH at the vein-artery anastomosis.[121,122]

Pig (porcine). Human cardiovascular anatomy and physiology is closely represented in the pig, with their nearly identical coronary vasculature making pigs ideal for the study of ischemia-reperfusion and coronary stent technology.[43] The arterial morphology of the pig is also similar, but their arteries are smaller and show greater fragility.[42,43] Other advantages include ready availability and low purchase cost. In some respects, the pig offers a similar model to the sheep for the assessment of vascular conduits, with similarities in platelet function and coagulation to humans and a propensity for calcification of biomaterial implants.[68]

In contrast to sheep and dogs, however, swine are infrequently used for testing of vascular conduits. Disadvantages include greater difficulties in handling, less tolerance of anesthesia, and maintenance costs may be higher.[42] A recent comparison study[72] found that operative time was similar for sheep carotid and pig aortic implants and costs were comparable; however, sheep afforded easy assess for ultrasound imaging at follow-up, which was not possible in pigs. In addition, complete endothelialization was found in all polyester conduits after 3 months in pigs but only perianastomotic endothelialization was seen in sheep, which represents more closely the situation seen in humans.

The main disadvantage, however, is the rapid growth inherent in commonly available strains of swine, with a typical 25-kg pig weighing about 100 kg 8 weeks later.[23] Using this growth to their advantage, Robotin-Johnson et al[40] implanted grafts of small intestine submucosa with a mean diameter 11.7 mm and length 9.9 mm into the thoracic superior vena cava of piglets to assess the ability of the grafts to grow with the animal over 3 months. During this time, the piglets' weight increased 575%, from a mean 10.3 to 59.2 kg, and the grafts increased to a mean diameter of 19.5 mm and length of 15.8 mm (unresected superior vena cava controls: 19.5-mm diameter, 23.8-mm length), demonstrating the potential of this material as a cardiovascular substitute in growing children. Niklason et al[123] used Yucatan minipigs, which like sheep show little adult growth.[23,124] The minipigs received autologous tissue conduits grown in vitro then implanted as interposition conduits into the saphenous artery, a branch of the femoral artery supplying the distal hind limb, and demonstrated their utility during a 4-week period.

The pig is currently the species of choice for the in vivo evaluation of vascular balloon injury, stenting, and restenosis, particularly for coronary studies but also for more peripheral vessels.[43] As in humans, there is a proportional relationship between vascular injury and neointimal response that is most marked in swine, intermediate in baboons and rabbits, and almost flat in dogs.[42] The pig femoral artery has been used to study IH after balloon angioplasty[125] as well as the use of vein interposition cuffs to reduce the intimal hyperplastic response to ePTFE bypass grafts.[126]

Recently, this animal has been used to investigate the particular problem of IH at the venous anastomosis of arteriovenous grafts used for hemodialysis access, with potential applicability to prosthetic conduit-induced IH in general. This problem has long been recognized clinically in humans where the known stimuli at the distal anastomosis of compliance mismatch, turbulent flow, and altered wall shear stress are exaggerated by the presence of high flow through the conduit (typically 1.0-1.5 L/min) as well as repetitive puncture resulting in ongoing endothelial injury, platelet activation, and myointimal proliferation.[127]

Therapeutic strategies attempting to reduce IH affecting carotid artery-jugular vein ePTFE grafts in swine in-

clude transduction of the venous limb to reduce smooth muscle cell (SMC) proliferation[128] and coating ePTFE grafts with anti-CD34 antibodies intended to capture circulating endothelial progenitor cells.[129] Roy-Chaudhury et al[130] placed ePTFE conduits as femoral arteriovenous grafts in pigs, showing almost complete abolition of luminal stenosis by wrapping the graft-vein anastomosis with a paclitaxel-loaded wrap.[130]

Dog (canine). Experimental surgery using canines has an extensive history, and as animal models they possess a number of advantages. Dogs are usually familiar with humans, making them easy to handle, and mongrel dogs are readily available and inexpensive. Canine cardiovascular physiology is similar to that of humans, dogs possess low body fat, and vascular access is relatively straightforward.[43] Peripheral arteries are available in diameters from 3 to 5 mm, and the aorta can accommodate interposition grafts up to 8 mm diameter. Dogs are also tolerant of prolonged anesthesia.[42]

Increasingly, however, disadvantages are reducing the favored status of dogs for the in vivo assessment of novel vascular conduits. It has long been known that platelet reactivity in the dog is variable and not reliably measured in vitro, and the screening and classification of subjects by platelet aggregometry is imperfect. The use of bilateral implants with a test and control conduit in each animal is therefore highly recommended to reduce the effects of inter-individual variations in thrombogenicity in all animal models but especially in canines.[30] Also apparent is the changing mood of society towards the use of canines for research, and the cost of using dogs is rising sharply as a consequence.[42,43] The viscoelastic properties of canine arteries are different from those in humans, being more elastic overall and showing less increase in wall stiffness in the more peripherally situated arteries. The carotid artery in the dog demonstrates a particular dissimilarity, being muscular like the femoral artery of the dog and human rather than elastic like the human carotid.[32] The tendency toward hypercoagulability has been highlighted, but further scrutiny of comparative studies draws attention to the dissimilarities between human and canine hemostatic systems and emphasizes the greater similarity shown by sheep or pigs and especially nonhuman primates such as baboons.

Early studies in dogs used large vessels such as the thoracic or abdominal aorta, which can accommodate conduits 6 to 8 mm in diameter. Expansion into other territories began in the late 1960s,[43] leading to implantation of smaller conduits as CCA interposition (3- to 5-mm) or aortoiliac bypass (4- to 5-mm) grafts generally 4 to 6 cm in length. To perform a more demanding assessment of a length more closely resembling clinical use, Marois et al[131] inserted 30-cm-long conduits as thoracoabdominal aortic bypass grafts in adult mongrel dogs for up to 6 months. The flow rate is high in this position, and the authors recommend that the model be used primarily for the evaluation of surface thrombogenicity and healing rather than as a patency challenge, with all test conduits remaining patent in this study. Randall et al[132] were able to assess conduits 45 cm in length by dividing and anastomosing the distal CCAs, then implanting a 6-mm-diameter conduit from this common lumen to the distal aorta, with patency again remaining high out to 12 months. Conduits 60 to 70 cm in length have also been implanted subcutaneously in dogs.[41]

Compared with sheep, control conduits such as ePTFE and Dacron implanted in canines have shown relatively high patency rates, questioning the belief that dogs provide a stringent assessment of conduit patency. At 4 months, 86% of 4-mm-diameter ePTFE and Dacron conduits placed as aortoiliac bypass grafts in dogs remained patent,[84] while 75% of 5-mm ePTFE grafts remained patent after 6 months in the same location.[133] Wilson et al[31] found an overall 3-month patency of 77% across forty-eight 4-mm ePTFE conduits inserted as carotid or femoral interposition grafts, and noted that in their previous canine studies, few thrombotic occlusions occurred ≤3 months after implantation and almost none thereafter. In a direct comparison, Ortenwall et al[44] placed 4-mm ePTFE conduits as CCA interposition grafts in both sheep and dogs and assessed their patency at 2 and 5 weeks. For grafts seeded with autologous ECs, patency was 75% and 83% in dogs at 2 and 5 weeks respectively, but 0% and 11% for sheep. More importantly for unseeded control grafts, patency was also 75% and 83% for dogs but only 40% and 0% for sheep.

Possible explanations for these species differences in patency include differences in flow, coagulation, and/or conduit healing. Despite generally being somewhat larger, sheep have demonstrated similar values to their canine counterparts for carotid and femoral blood flow (Table V). Both species have been described as being relatively hypercoagulable, but shorter coagulation times have been seen in dogs compared with sheep or humans,[60] which would be expected to lead to reduced patency in dogs. On the other hand, canine models generally use aspirin to reduce the known problem of platelet aggregation[31,44] and this may contribute to increased patency.

A major contributing factor, however, is the rapid "spontaneous" endothelialization of vascular prosthetics that has been demonstrated to occur in dogs.[29,45-47] ePTFE and Dacron conduits explanted from humans show ECs over the perianastomotic regions but these never extend more than a short distance over the luminal surface of the prostheses, which instead is covered with fibrin or collagen (mostly type III), or both.[134] As a result, endothelialization in humans must rely on transmural tissue ingrowth or blood-borne sources due to the conduit lengths implanted in typical bypass grafts.[29] Dogs, meanwhile, can quickly endothelialize synthetic conduits by all three mechanisms.[29,88,135] Zilla et al[29] emphasized the importance of selecting the appropriate model for the assessment of prosthetic vascular grafts, particularly in regard to the assessment of endothelialization. Species is the major factor determining the rate of transanastomotic endothelialization (along with graft surface and dimensions and also animal senescence), with dogs able to cover in 3.5 weeks the ingrowth distance that humans would take more than a year to accomplish.[29] The baboon, dog, pig, and calf

are all able to form a complete tissue lining over the inner aspect of arterial prostheses, in marked contrast to humans.[47,90]

In summary, the preferential use of dogs in the preclinical assessment of arterial grafts can no longer be supported in light of studies challenging the assumptions of low patency and lack of spontaneous endothelialization attributed to prosthetic vascular conduits implanted in the canine. The increasing reluctance of society to the use of dogs for animal experimentation is also a contributing factor. Sheep, meanwhile, demonstrate the same qualities that led to the original recommendation to use dogs for vascular conduit assessment: a relatively high occlusion rate and restriction of endothelialization to the perianastomotic regions. This greater ability to correctly model the human response to vascular conduits, combined with the other advantages previously stated, means that sheep are the large animal of choice for the assessment of novel vascular conduits. Nonhuman primates such as the baboon offer greater similarity for thrombogenicity assessment but the restrictions surrounding their use limit their availability.

SMALL-ANIMAL MODELS FOR THE ASSESSMENT OF NOVEL VASCULAR CONDUITS

Small animals are cheaper to purchase and maintain than larger alternatives and they are easier to handle. Their size, however, presents a technical challenge for the surgical implantation of vascular conduits.[23] Rabbits and rats are the predominant small animals used, generally accommodating small diameter (2- to 5-mm) conduits in rabbits and very small (<2-mm) diameter conduits in rats.

Rabbit (leporine). Rabbits provide a useful model for the assessment of small-diameter conduits, being able to accommodate a range of conduit sizes across different locations: aortoiliac bypass (up to 5-mm diameter),[38] aortic interposition (3-4 mm),[39,136-139] carotid interposition (generally 2 mm)[78,140-142] and femoral interposition (1-1.5 mm),[143-145] with lengths up to 4 or 5 cm in most locations. For carotid and femoral procedures, other advantages include simple anesthesia and the availability of bilateral implantation.[140]

Carotid artery interposition. Carotid artery grafting is convenient in the rabbit. Up to 5 cm of carotid artery can be exposed bilaterally through a midline incision accommodating either straight grafts up to 3 cm or loop grafts up to 10 cm in length. Bilateral conduits can be placed, and patency is easily assessed by palpating the ipsilateral central ear artery or by Doppler ultrasound assessment.[140,142] Longitudinal tension is similar to humans, with CCAs removed from rabbits retracting 38%.[35] Like sheep, rabbits tolerate CCA occlusion due to graft insertion or postoperative loss of patency without signs of neurologic injury, even after bilateral occlusion for 30 minutes.[78,140] The rabbit brain receives blood flow from the common carotid and vertebral arteries by the circle of Willis, allowing supply through anastomotic connections to continue despite isolated occlusion of arterial inflow. Ligation of the internal carotid artery, for example, results in doubling of contralateral internal carotid artery flow to maintain total cerebral blood flow.[146,147] With experience, CCA grafting in the rabbit results in a postoperative survival of >90%, with mortality generally due to anesthetic complications.[140]

Cassel et al[140] inserted 2-mm ePTFE grafts (n = 158) in the carotid position and found two distinct patterns of occlusion: a failure rate of 11.2% in the first 2 weeks due to thrombotic occlusion, then patency remained stable out to 16 weeks, beyond which progressive patency loss was seen due to development of IH most marked at the distal anastomosis. Autologous carotid artery grafts meanwhile maintained 100% patency out to 32 weeks. These results closely simulate the clinical response to chronic ePTFE grafts seen in humans.[140]

The rabbit carotid model has also been shown to approximate the endothelial response to prosthetic conduits seen in humans. Endothelialization has been found limited to the perianastomotic regions of 2-mm ePTFE grafts in the rabbit CCA after study at 3 weeks[142] and at 8 weeks.[141] The wide variation in patency of these studies, however, serves to highlight the difficulties in finding consensus among examples of animal models: 20% at 3 weeks and 100% at 8 weeks, respectively.

Other positions. Insertion of grafts 3 to 5 mm in diameter in the rabbit generally requires placement in the aortic[39,136-139] or aortoiliac[38] position, although size-mismatched conduits up to 5 mm diameter have been placed in the CCA.[148,149] The disadvantage of aortic interposition grafting is the lack of an intra-animal control. Nordestgaard et al[136] reported 3-month patencies for ePTFE in this position of 82% for 3-mm conduits and only 24% for 2-mm conduits, possibly due to under-sizing resulting in restricted blood flow. Acute thrombogenicity can be assessed using ^{111}Indium-labelled autologous platelets[39] and patency assessed by hind limb paralysis with a sensitivity of 94% and specificity of 89% for aortic graft thrombosis.[136] Aortoiliac grafts are amenable to follow-up with Doppler or duplex ultrasound or even contrast-enhanced computed tomography.[38] Like the carotid, ePTFE grafts in the aortic position show only rare endothelialization, even after 6 months.[139]

The femoral artery in the rabbit can only accommodate conduits 1 to 1.5 mm in diameter, the same as the rat aorta, but with the potential for bilateral graft placement. Reported 1-month patency for ePTFE in this position includes 92% (1.5-mm diameter)[143] and 25% (1.0-mm diameter).[144]

Rat/mouse (murine). The small size of these rodents generally limits their use for the assessment of novel conduits beyond the assessment of very small grafts in the aortic (1- to 2-mm diameter)[150-155] or femoral (1-mm diameter)[156,157] positions, although size-mismatched grafts up to 4 mm in diameter have been used.[158] As well as being restricted in caliber, their length also rarely exceeds 1 to 2 cm, which is problematic because re-endothelialization from the anastomoses occurs easily in 1-cm-long grafts and a length of at least 2 cm is required to provide a sufficient

challenge.[82] To overcome this limitation, O'Brien et al[156] implanted ePTFE conduits 1 mm in diameter and 6 cm in length as femoral artery crossover grafts in rats, achieving 80% patency at 4 weeks and demonstrating only perianastomotic endothelialization. Rats have also been used to evaluate the compatibility of vascular conduit biomaterials outside of the circulation, with conduit segments used as both subcutaneous[159] and intraperitoneal[131] implants to assess the healing response of the host animal.

The main concern when using rats to model human responses to very small conduits is the generally high patency rates seen for ePTFE grafts in contrast to clinical results,[78,136] and the call to move away from rats for the patency assessment of short graft segments was made 30 years ago.[160] Reports of patency are best provided in comparison with an acceptable control such as ePTFE, with Pektok et al[154] recently reiterating 100% patency at 6 months for 2-mm ePTFE in the rat aortic position. If no control conduit is evaluated, the expected high patency in this model should be stated, with examples of studies using ePTFE available in Table II. The model has continued to find use in the evaluation of vascular conduits when high patency is desired, such as the assessment of intimal proliferation and other aspects of graft healing. Aspects of inquiry have included SMC seeding of biodegradable polyurethanes,[161] EC seeding of ePTFE,[158] use of mechanical anastomotic devices,[162,163] and systemic immunosuppression to reduce intimal proliferation.[164] Like many species, however, pharmacologic strategies that look promising in rat models of IH often fail to achieve clinical success in human trials.[42]

Despite the significant technical challenge, mice have also been used for the assessment of vascular conduits. Lopez-Soler et al[165] successfully implanted four types of conduit in the abdominal aorta of severe combined immunodeficiency mice to determine their utility as tissue-engineering scaffolds for xenogenic cells such as human ECs. Conduits 0.7 to 1.3 mm in diameter were found to be matched in caliber to the native aorta, and the model allowed follow-up of patency with duplex ultrasound imaging or microcomputed tomography, with 1 month patency of 100% for all conduit types.

DIRECT COMPARISON OF CONDUIT PERFORMANCE BETWEEN SMALL-ANIMAL MODELS

Lidman et al[82] implanted ePTFE conduits in the carotid and femoral arteries of dogs and rabbits, and in the femoral arteries of rats, to determine their patency after 2 weeks compared with autogenous vein grafts. Citing the large variability in patency both within and between species found in previous studies, they investigated whether consistent results could be obtained. Only rabbit carotid artery grafts (1.8-mm diameter graft to 2.0-mm artery) demonstrated an acceptable patency rate of 83%. Dog carotid (2.0-mm graft to 3- to 4-mm artery; patency, 0%) and femoral (3.0-mm graft to 4- to 5-mm artery; patency, 6%) implants failed compared with vein graft controls (100% patency both locations), although with an appreciable size discrepancy, as did rabbit femoral grafts (1.0-mm graft to 1.0- to 1.2-mm artery; patency, 8%; vein graft control, 89% patency). ePTFE in the rat femoral artery also performed poorly (1.0-mm graft, 20% patency), although somewhat better than the other species and with no autogenous control. Attributing the good results of the rabbit carotid implant to the higher flow rate in that location, the authors recommended this model be used for future assessment of very small vascular conduits.

Concerned with the excessively high rates of patency for ePTFE prostheses implanted in rats, van der Lei et al[78] implanted ePTFE and polyurethane conduits (1.5 mm × 1 cm) into rabbit carotid arteries and assessed their patency for up to 6 weeks compared with arterial autografts. Earlier studies by the same group had found almost 100% patency for both prostheses when implanted in the rat aorta. In the rabbit, both prosthetics quickly lost patency, with 0% open to flow beyond 1 week, whereas autografts remained 100% patent during 2 weeks of follow-up. Rabbits were again recommended as a more suitable model than rats, being able to demonstrate the thrombogenicity of microarterial prostheses closer to the clinical situation.

Campbell et al[149] developed a novel autologous vascular graft by implanting a silicone elastomer rod in the peritoneal cavities of rats and rabbits for 2 weeks, then removing and inverting the tissue tube to create a "neovessel" that was implanted into the rabbit CCA (5-mm diameter × 2-cm length) or rat aorta (5 mm × 1 cm) for up to 4 months. Both animals demonstrated development of a tube resembling the architecture of a blood vessel, with 4-month patency of about 70% in both models.

The selection of a small animal model can generally be summarized as a choice between rabbits and rats. Greater limitations in the diameter and length of conduit able to be accommodated in rats, combined with a lack of bilateral graft placement and a high overall patency rate for ePTFE, make the rabbit more favored for the assessment of very small vascular conduits.

NEW CHALLENGES IN THE ASSESSMENT OF TISSUE-ENGINEERED VASCULAR CONDUITS

The field of vascular conduit design is increasingly moving from the development or modification of polymeric biomaterials into the incorporation of tissue elements to create tissue-engineered vascular grafts. The first generation of these grafts were ePTFE conduits endothelialized in vitro and resulted in patencies matching vein grafts in human trials.[94] Current techniques include directed tissue ingrowth into scaffolds that aim to mimic the extracellular environment to achieve an off-the-shelf conduit capable of rapid endothelialization in vivo[166] and the use of wholly autologous living tissue-engineered grafts.[167] These approaches to the bioengineering of vascular conduits create a number of new challenges in their assessment, including novel outcome measures, a greater impact of host factors regarding tissue ingrowth and healing, and the likely sus-

ceptibility of these living-tissue grafts to degeneration through atherosclerotic disease.

Novel end points for outcome assessment. The study of tissue-engineered vessels introduces a number of novel end points for assessment in addition to previous outcome measures that focus on graft patency. These include the retention of cells seeded in vitro when implanted into animals, assessment of graft mechanical properties and the effect of remodeling, and characterization of tissue elements incorporated over time.

ECs seeded onto the conduit lumen in vitro may not withstand arterial shear stress in vivo, necessitating assessment of EC retention. Such studies have been used to demonstrate the superiority of prolonged EC culture under simulated flow conditions compared to static culture overnight before animal implantation.[111,168] However, the availability of cellular markers across species is a significant limiting factor in the use of immunochemistry to identify cell types.[169] EC identification through immunolabeling of von Willebrand factor, for example, is available for baboons, pigs, dogs, rabbits, rats, and mice, but assessment of EC retention in sheep requires alternative methods.[125,168,170-174]

Cell culture techniques have extended beyond EC seeding to develop vascular grafts that are predominantly or wholly tissue-engineered, requiring demonstration of satisfactory mechanical properties in vitro and assessments of remodeling and long-term performance in vivo. Early attempts using molds of SMCs and type I collagen to create tubes subsequently coated with ECs before use required external Dacron mesh support to allow even venous implantation, with remodeling gradually progressing during a 6-month period.[175] Niklason et al,[123] in contrast, cultured bovine or porcine SMCs seeded onto a biodegradable polyglycolic acid scaffold in a pulsatile bioreactor system for 8 weeks and demonstrated burst strengths superior to native vein, although implantation in minipigs did not extend beyond 4 weeks.

L'Heureux et al[176] recently reported their results using "sheet-based tissue engineering" to develop completely autologous living tissue-engineered blood vessels derived from human fibroblasts and ECs without use of a polymeric support. Initial implants as xenografts in immunosuppressed dogs showed a massive immune response at 14 days, leading to further testing in nude mice and highlighting the difficulty faced when assessing living tissue in animal models.[176] Nevertheless these conduits attained mechanical properties matching native human conduits and when implanted as arteriovenous shunts for hemodialysis access in humans demonstrated increased compliance during a 6-month period to approach that of the internal mammary artery, without evidence of dilatation or aneurysm formation.[167]

Assessment of the structural durability of tissue-engineered vessels is complicated by fibroblast migration and collagen deposition at the external surface of the vessel, potentially masking graft wall remodeling and weakening that would otherwise lead to aneurysm or rupture. This encapsulation is a feature of ruminants and is also seen in swine, but is more uncommon in primate models.[3,123]

Implantation of acellular conduit scaffolds for subsequent population through invasion of host tissues represents a promising approach for the development of an off-the-shelf commercial product able to achieve the ideal tissue-engineered autologous arterial graft.[177] Consisting of either decellularized connective tissue or porous polymer tubes, these grafts must also demonstrate retention of satisfactory mechanical properties once implanted. In addition, they require the identification and quantification of cellular elements arriving through migration and proliferation as well as extracellular elements such as collagen and elastic fibers deposited or remodeled by the developing tissues.[148,178] Grafts thought to closely resemble arterial vessels on initial inspection can later be found to be less suitable after more detailed characterization of their constituent tissues.[149,179]

Beyond the effect of species—influence of other host factors. The increasing use of tissue engineering in the development of novel conduits brings greater emphasis to the influence of host factors other than species, including those of senescence and implant location. These effects are well described for EC growth but are also seen in other aspects of graft healing. Before discussion of the effects of senescence and location, it is important to briefly outline some of the challenges faced in the use of animal models to simulate endothelialization of vascular conduits, as recently reviewed by Zilla et al.[29]

Endothelialization can occur through luminal ingrowth from the anastomoses, transmural ingrowth of capillaries from perigraft tissues, or through hematogenous seeding of ECs or their precursors. Given the lengths of conduit required for peripheral vascular surgery (generally 40-60 cm) the relevance of transanastomotic endothelialization is limited, although shorter lengths (10-20 cm) are used for coronary artery grafting. The use of animal models to study conduits mostly 4 to 7 cm in length (Table I) may therefore lead to premature midgraft healing beyond short periods of implantation, preventing a true representation of the clinical situation.

To separate these mechanisms of endothelialization, multicomponent grafts have been implanted in canines consisting of a central test segment anastomosed between two isolation segments to prevent transanastomotic endothelialization. The central segment can also be made impervious to tissue ingrowth to separate the effects of transmural and hematogenous endothelialization of the test prosthesis.[135,180] Other approaches include assessment after short implantation periods of up to 2 weeks.[181]

Recognition of these separate mechanisms to achieve endothelial graft coverage has led to targeted approaches, including coating ePTFE with antibodies designed to capture circulating endothelial progenitor cells[129] or implantation of ePTFE with increased porosity to allow transmural ingrowth.[141] However, therapeutic modulation of conduit pore size is additionally complicated by a number of factors. The relationship between porosity and graft

healing is strongly influenced by material-specific characteristics such as the actual dimensions available for ingrowth and cellular orientation, such that 30-μm ePTFE is unable to support transmural endothelialization.[29] Second, even those materials with sufficient interstitial space for ingrowth may show inhibition by another biologic mechanism most likely arising from the accumulation of fibrinogen and fibrin on the luminal surface.[29,166] Finally, high-porosity materials (>45 μm) enable fibrovascular infiltration, reducing the compliance of novel elastic conduits over time and potentially impairing their performance.[97]

These limitations in the assessment of endothelialization in animals are then compounded by the effects of senescence and location. Although generally referring to animal maturity, senescence may also refer to the "in vitro age" of cultured cells. In vitro methods of seeding conduits with autologous ECs before implantation require expansion of ECs by culture; however, the proliferative and synthetic capacity of ECs is strongly influenced by the number of subcultures made (passages), with ECs becoming senescent at approximately passage 10.[174]

The age of the animal is equally important for endothelialization in vivo, where again older animals demonstrate a reduced capacity for endothelialization of vascular synthetics, a fact that must be remembered when implants in senescent models such as canines are compared with the juvenile models often seen, for example, in calves, sheep, and swine.[29]

The importance of implant site has received little attention but has been investigated in canines. Knitted Dacron conduits implanted as retropleural (thoracic aorta) interposition, retroperitoneal (abdominal aorta) interposition, or subcutaneous (carotid-femoral) bypass grafts were compared after periods of implantation of up to 3 years.[182-184] Grafts implanted in the thoracic aorta exhibited firm adherence of perigraft tissues to the conduit exterior and were associated with rapid formation of a confluent endothelialized lumen within 8 to 16 weeks. Retroperitoneal grafts exhibited greater variation in healing, with a loosely adherent outer capsule associated with perianastomotic endothelial growth only, whereas firmly encapsulated grafts demonstrated healing comparable to thoracic grafts. Subcutaneous grafts, however, showed slow and limited endothelialization, with a luminal surface composed mostly of fibrin despite the formation of a thin well-attached outer capsule, results similar to those seen in axillofemoral grafts explanted from humans. These studies highlight the importance of implant site on graft healing and demonstrate the need to compare results obtained from similar implant locations.

Overall, it is clear that animal models for the assessment of novel conduits using tissue-engineering methods must take into account not only the variation in healing response between species but also the strong influence of animal senescence and anatomic implant location, complicating the extrapolation of results seen in healthy animals to diseased humans.

Atherosclerosis of tissue-engineered vessels. The creation of artificial blood vessels incorporating a living intima and media raises the possibility of synthetic grafts susceptible to degeneration by atherosclerosis in the same manner as native vessels. Assessment of these conduits is therefore enhanced by the use of species able to replicate the development of this disease as seen in humans.

Atherosclerosis occurs naturally in humans, in some nonhuman primates (including chimpanzees and various monkeys), and in pigs, whereas other species such as rabbits are useful because they are highly responsive to dietary cholesterol manipulation and develop fatty lesions quickly, although the lesions themselves are much more fatty and macrophage-rich. Dogs and rats are not good models because they require a significant change in diet to produce a vascular lesion, and ovine models are not used. Guinea pigs are unique in their striking similarity to humans in terms of cholesterol and lipoprotein metabolism.[42]

Nonhuman primates provide an important model due to their close phylogenetic relationship to humans and similarities in lipid metabolism and plaque deposition, although the overall severity of lesions seen in primates is less than that seen in humans. Macaque species, particularly rhesus and cynomolgus monkeys, are commonly used, and a genetic strain of baboons has also been developed. Pigs are another popular model of atherosclerosis due to similarities to humans in terms of diet, cardiovascular system, lipid metabolism, and development of atheromatous lesions, although the use of swine for vascular conduit studies entails particular difficulties, as previously described.[49]

Other common models of atherogenesis often use animals genetically altered through inbreeding of a spontaneous mutation (eg, Watanabe heritable hyperlipidemic rabbit) or as a result of genetic modification, including insertion of exogenous genes (eg, transgenic rat and rabbit models) and selective deletion or replacement of endogenous genes (eg, knockout murine models). Although these modifications render species such as mice susceptible to atherogenesis, the addition of lipid-enriched Western-type diets greatly accelerate the rate of lesion development. Mice deficient in apolipoprotein E (generated by selective inactivation of the apoE gene) are hypercholesterolemic and spontaneously develop atherosclerotic lesions strikingly similar to those in humans, even on regular chow. Low-density lipoprotein receptor-knockout mice exhibit a more modest lipoprotein abnormality that is greatly increased by the use of a cholesterol-rich diet. However, although mice are the most widely used experimental model of atherosclerosis, the use of mice would severely limit the size of conduit able to be implanted. Transgenic rabbit models expressing human genes bridge the gap between the laboratory mouse and larger domesticated mammals and are able to accommodate a variety of conduit sizes, as previously mentioned.[42]

In conclusion, the assessment of novel tissue-engineered vascular conduits provides new challenges to animal modeling and emphasizes the importance of careful con-

sideration when selecting an appropriate model. Many elements influence the performance of vascular grafts, including:

- species factors, such as coagulation and healing, including endothelialization, IH, remodeling, and encapsulation;
- host factors, such as senescence and inter-individual variation in species effects, for example, variable platelet aggregation in dogs;
- local factors, including implant location (eg, subcutaneous, retroperitoneal) and hydrodynamics (eg, blood flow, wall shear stress);
- study factors, including duration of implant and selection of method used (eg, anastomotic technique); and
- conduit factors, including diameter, length, porosity, and suitability to tissue incorporation.

The relative importance of these individual determinants depends on the study objective and the conduit material and should be considered in the design of animal experiments to assess novel vascular conduits.

RECOMMENDATIONS

The following recommendations are given for the design of future studies that seek to use animal models for the assessment of novel vascular conduits:

1. Among the commonly available species, sheep are the large animal of choice for the assessment of vascular conduits 4 to 6 mm in diameter. Although previously recommended, the canine demonstrates greater dissimilarities and variability in hemostatic mechanisms as well as a less stringent test of conduit patency and a propensity for the rapid endothelialization of vascular prosthetics.
2. Baboons provide the closest approximation for thrombogenicity studies for those with access to this species. In addition, baboons provide an ideal model for the assessment of tissue-engineered conduits due to the greater availability of cellular markers, reduced fibrous encapsulation of foreign implants, and their inherent susceptibility to atherosclerosis.
3. Rabbits are the small animal of choice for conduits 1 to 4 mm in diameter, enabling bilateral implantation of longer conduits and having a greater similarity than rats to humans in coagulation, endothelialization, and patency. The use of rats for the patency assessment of short graft segments, especially in the absence of a clinically relevant control, should be avoided.
4. Implantation of test conduits with a suitable control in the same animal and in the same position (carotid or femoral) is recommended. By doing so, the effects of interindividual variability in thrombogenicity and other determinants of conduit performance, including animal senescence and graft location, is reduced.

SUMMARY

The search for the ideal vascular conduit to overcome the limitations of currently available small-diameter grafts generates candidate materials that ultimately require assessment in vivo. The selection of the animal model to be used is most importantly determined by the validity of the assumption that the model correctly simulates the response to the prosthesis that would be expected in the clinical situation. Knowledge of the comparative hematology between species, the comparative hydrodynamics between implant locations, and species differences for other determinants of conduit performance such as endothelialization and anastomotic IH enables the reader or researcher to assess the validity of this assumption and hence the applicability of results to clinical practice. Recommendations for the initial assessment of novel vascular conduits designed for peripheral vascular surgery are for insertion as bilateral carotid or femoral implants into sheep for conduits 4 to 6 mm in diameter and into rabbits for smaller conduits. Current interest in the development of tissue-engineered conduits may result in the greater availability and use of baboons and other nonhuman primates.

AUTHOR CONTRIBUTIONS

Conception and design: MB, MN
Analysis and interpretation: MB
Data collection: MB
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Critical revision of the article: MB, MN, PB, GW
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REFERENCES

1. Toledo-Pereyra LH. The ethics of surgical research. J Invest Surg 2003;16:119-21.
2. Tabbara M, White RA. Biologic and prosthetic materials for vascular conduits. In: Veith FJ, Hobson RW 2nd, Williams RA, Wilson SE, editors. Vascular surgery: principles and practice. 2nd ed. New York: McGraw-Hill; 1994. p. 523-32.
3. Edwards GA, Roberts G. Development of an ovine collagen-based composite biosynthetic vascular prosthesis. Clin Mater 1992;9: 211-23.
4. Bezuidenhout D, Zilla P. Vascular surgery: biomaterials. In: Buschow KHJ, editor. Encyclopedia of materials: science and technology. Amsterdam: Elsevier; 2001. p. 9513-8.
5. Norgren L, Hiatt WR, Dormandy JA, Nehler MR, Harris KA, Fowkes FG, et al. Inter-Society Consensus for the management of peripheral arterial disease (TASC II). J Vasc Surg 2007;45(suppl S):S5-67.
6. Johnston K, Kalman P, Baird R. Aortoiliofemoral occlusive disease. In: Veith FJ, Hobson RW 2nd, Williams RA, Wilson SE, editors. Vascular surgery: principles and practice. 2nd ed. New York: McGraw-Hill; 1994. p. 409.
7. Bennion RS, Williams RA, Wilson SE. Principles of vascular access surgery. In: Veith FJ, Hobson RW 2nd, Williams RA, Wilson SE, editors. Vascular surgery: principles and practice. New York: McGraw-Hill; 1994. p. 1025-35.
8. Kouchoukos NT, Blackstone EH, Doty DB, Hanley FL, Karp RB. Kirklin/Barratt-Boyes cardiac surgery. 3rd ed. Salt Lake City: Churchill Livingstone; 2003.

9. Chard RB, Johnson DC, Nunn GR, Cartmill TB. Aorta-coronary bypass grafting with polytetrafluoroethylene conduits. Early and late outcome in eight patients. J Thorac Cardiovasc Surg 1987;94:132-4.
10. Conte MS. The ideal small arterial substitute: a search for the Holy Grail? FASEB J 1998;12:43-5.
11. Kakisis JD, Liapis CD, Breuer C, Sumpio BE. Artificial blood vessel: the Holy Grail of peripheral vascular surgery. J Vasc Surg 2005;41: 349-54.
12. Veith FJ, Moss CM, Sprayregen S, Montefusco C. Preoperative saphenous venography in arterial reconstructive surgery of the lower extremity. Surgery 1979;85:253-6.
13. Giannoukas AD, Labropoulos N, Stavridis G, Bailey D, Glenville B, Nicolaides AN. Pre-bypass quality assessment of the long saphenous vein wall with ultrasound and histology. Eur J Vasc Endovasc Surg 1997;14:37-40.
14. Davies AH, Magee TR, Sheffield E, Baird RN, Horrocks M. The aetiology of vein graft stenoses. Eur J Vasc Endovasc Surg 1994;8: 389-94.
15. Wilson YG. Vein quality in infrainguinal revascularisation: assessment by angioscopy and histology. Ann R Coll Surg Engl 1998;80:3-15.
16. Ricotta JJ. Vascular conduits. In: Rutherford RB, editor. Vascular surgery. 6th ed. Philadelphia: Elsevier Saunders; 2005.
17. Association for the Advancement of Medical Instrumentation. Cardiovascular implants—tubular vascular prostheses. American National Standard 2004; ANSI/AAMI/ISO 7198:1998/2001/(R)2004.
18. United States Food and Drug Administration. Recognized consensus standards. Cardiovascular implants—tubular vascular prostheses. http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfStandards/Detail.cfm?ID=14711. Accessed: May 4, 2009.
19. Abbott WM, Callow A, Moore W, Rutherford R, Veith F, Weinberg S. Evaluation and performance standards for arterial prostheses. J Vasc Surg 1993;17:746-56.
20. Association for the Advancement of Medical Instrumentation. Cardiovascular implants—vascular graft prostheses. American National Standard 2000; ANSI/AAMI VP20:1994.
21. International Organization for Standardization. Cardiovascular implants—tubular vascular prostheses 1998; ISO 7198.
22. Boerboom LE, Olinger GN, Bonchek LI, Gunay II, Kissebah AH, Rodriguez ER, et al. The relative influence of arterial pressure versus intraoperative distention on lipid accumulation in primate vein bypass grafts. J Thorac Cardiovasc Surg 1985;90:756-64.
23. Rashid ST, Salacinski HJ, Hamilton G, Seifalian AM. The use of animal models in developing the discipline of cardiovascular tissue engineering: a review. Biomaterials 2004;25:1627-37.
24. Richardson RL Jr, Pate JW, Wolf RY, Ledes C, Hopson WB Jr. The outcome of antibiotic-soaked arterial grafts in guinea pig wounds contaminated with E. coli or S. aureus. J Thorac Cardiovasc Surg 1970;59:635-7.
25. Menger MD, Hammersen F, Messmer K. In vivo assessment of neovascularization and incorporation of prosthetic vascular biografts. Thorac Cardiovasc Surg 1992;40:19-25.
26. Kaushal S, Amiel GE, Guleserian KJ, Shapira OM, Perry T, Sutherland FW, et al. Functional small-diameter neovessels created using endothelial progenitor cells expanded ex vivo. Nat Med 2001;7:1035-40.
27. Tomizawa Y, Noishiki Y. Regarding "Evaluation and performance standards for arterial prostheses." J Vasc Surg 1995;21:542-3.
28. Binns RL, Ku DN, Stewart MT, Ansley JP, Coyle KA. Optimal graft diameter: effect of wall shear stress on vascular healing. J Vasc Surg 1989;10:326-37.
29. Zilla P, Bezuidenhout D, Human P. Prosthetic vascular grafts: wrong models, wrong questions and no healing. Biomaterials 2007;28: 5009-27.
30. Jones DN, Rutherford RB, Ikezawa T, Nishikimi N, Ishibashi H, Whitehill TA. Factors affecting the patency of small-caliber prostheses: observations in a suitable canine model. J Vasc Surg 1991;14:441-8; discussion 8-51.
31. Wilson GJ, MacGregor DC, Klement P, Weber BA, Binnington AG, Pinchuk L. A compliant Corethane/Dacron composite vascular prosthesis. Comparison with 4-mm ePTFE grafts in a canine model. ASAIO J 1993;39:M526-31.
32. Learoyd BM, Taylor MG. Alterations with age in the viscoelastic properties of human arterial walls. Circ Res 1966;18:278-92.
33. Chow SP, Huang CD, Chan CW. Microvascular anastomosis of arteries under tension. Br J Plast Surg 1982;35:82-7.
34. Decherd ME, Calhoun KH. The effect of tension on patency of rat femoral artery anastomoses. Arch Facial Plast Surg 2003;5:83-5.
35. Jackson ZS, Gotlieb AI, Langille BL. Wall tissue remodeling regulates longitudinal tension in arteries. Circ Res 2002;90:918-25.
36. Sardelic F, Fletcher JP, Ao PY, Bilous M. Comparison of fluoropolymer passivated Dacron and polytetrafluoroethylene grafts in a sheep model. Cardiovasc Surg 1994;2:237-41.
37. Simoni G, Galleano R, Civalleri D, Decian F, Desalvo P, Ceppa P, et al. Pharmacological control of intimal hyperplasia in small diameter polytetrafluoroethylene grafts. An experimental study. Int Angiol 1996; 15:50-6.
38. Tillman BW, Yazdani SK, Lee SJ, Geary RL, Atala A, Yoo JJ, et al. The in vivo stability of electrospun polycaprolactone-collagen scaffolds in vascular reconstruction. Biomaterials 2009;30:583-8.
39. Babatasi G, Massetti M, Bara L, Mazmanian M, Samama MM, Khayat A. Graft thrombosis in small diameter vascular prosthesis: a laboratory model. Int J Angiol 1997;6:118-23.
40. Robotin-Johnson MC, Swanson PE, Johnson DC, Schuessler RB, Cox JL. An experimental model of small intestinal submucosa as a growing vascular graft. J Thorac Cardiovasc Surg 1998;116:805-11.
41. Brophy CM, Ito RK, Quist WC, Rosenblatt MS, Contreras M, Tsoukas A, et al. A new canine model for evaluating blood prosthetic arterial graft interactions. J Biomed Mater Res 1991;25:1031-8.
42. Konya A, Wright KC, Gounis M, Kandarpa K. Animal models for atherosclerosis, restenosis, and endovascular aneurysm repair. In: Conn PM, editor. Sourcebook of models for biomedical research. Totowa, NJ: Humana Press; 2008. p. 369-84.
43. Bianco RW, Grehan JF, Grubbs BC, Mrachek JP, Schroeder EL, Schumacher CW, et al. Large animal models in cardiac and vascular biomaterials research and testing. In: Ratner BD, Hoffman AS, Schoen FJ, Lemons JE, editors. Biomaterials science: an introduction to materials in medicine. 2nd ed. San Diego, CA: Academic Press; 2004. p. 379-95.
44. Ortenwall P, Bylock A, Kjellstrom BT, Risberg B. Seeding of ePTFE carotid interposition grafts in sheep and dogs: species-dependent results. Surgery 1988;103:199-205.
45. Dixit P, Hern-Anderson D, Ranieri J, Schmidt CE. Vascular graft endothelialization: comparative analysis of canine and human endothelial cell migration on natural biomaterials. J Biomed Mater Res 2001;56:545-55.
46. Bull DA, Hunter GC, Holubec H, Aguirre ML, Rappaport WD, Putnam CW. Cellular origin and rate of endothelial cell coverage of PTFE grafts. J Surg Res 1995;58:58-68.
47. Berger K, Sauvage LR, Rao AM, Wood SJ. Healing of arterial prostheses in man: its incompleteness. Ann Surg 1972;175:118-27.
48. Ratner BD, Hoffman AS, Schoen FJ, Lemons JE. Biomaterials science: an introduction to materials in medicine. 2nd ed. San Diego, CA: Academic Press; 2004.
49. Gross DR. Animal models in cardiovascular research. 2nd rev ed. Dordrecht, Boston: Kluwer Academic; 1994.
50. Kaneko JJ, Harvey JW, Bruss M. Clinical biochemistry of domestic animals. 6th ed. Amsterdam, Boston: Academic Press/Elsevier; 2008.
51. Dukes HH, Reece WO. Dukes' physiology of domestic animals. 12th ed. Ithaca, NY: Cornell University Press; 2004.
52. Duncan JR, Prasse KW, Mahaffey EA. Veterinary laboratory medicine: clinical pathology. 3rd ed. Ames, IA: Iowa State University Press; 1994.
53. Thrall MA. Veterinary hematology and clinical chemistry. Philadelphia: Lippincott Williams & Wilkins; 2004.
54. Feldman BV, Zinkl JG, Jain NC, Schalm OW. Schalm's veterinary hematology. 5th ed. Philadelphia: Lippincott Williams & Wilkins; 2000.
55. Hrapkiewicz K, Medina L. Clinical laboratory animal medicine: an introduction. 3rd ed. Ames, IA, Oxford: Blackwell; 2007.
56. Lewis JH. Comparative hematology—studies on Camelidae. Comp Biochem Physiol A 1976;55:367-71.

57. Lewis JH. Comparative hematology: studies on goats. Am J Vet Res 1976;37:601-5.
58. Lewis JH. Comparative hematology: studies on guinea-pigs (Cavia porcellus). Comp Biochem Physiol Comp Physiol 1992;102:507-12.
59. Grabowski EF, Didisheim P, Lewis JC, Franta JT, Stropp JQ. Platelet adhesion to foreign surfaces under controlled conditions of whole blood flow: human vs rabbit, dog, calf, sheep, pig, macaque, and baboon. Trans Am Soc Artif Intern Organs 1977;23:141-51.
60. Sato M, Harasaki H. Evaluation of platelet and coagulation function in different animal species using the xylum clot signature analyzer. ASAIO J 2002;48:360-4.
61. Chen C, Lumsden AB, Hanson SR. Local infusion of heparin reduces anastomotic neointimal hyperplasia in aortoiliac expanded polytetrafluoroethylene bypass grafts in baboons. J Vasc Surg 2000;31:354-63.
62. Jordan SW, Haller CA, Sallach RE, Apkarian RP, Hanson SR, Chaikof EL, et al. The effect of a recombinant elastin-mimetic coating of an ePTFE prosthesis on acute thrombogenicity in a baboon arteriovenous shunt. Biomaterials 2007;28:1191-7.
63. Harker LA, Kelly AB, Hanson SR. Experimental arterial thrombosis in nonhuman primates. Circulation 1991;83(6 suppl):IV41-55.
64. Lewis JC, White MS, Prater T, Porter KR, Steele RJ. Three-dimensional organization of the platelet cytoskeleton during adhesion in vitro: observations on human and nonhuman primate cells. Cell Motil 1983;3:589-608.
65. Feingold HM, Pivacek LE, Melaragno AJ, Valeri CR. Coagulation assays and platelet aggregation patterns in human, baboon, and canine blood. Am J Vet Res 1986;47:2197-9.
66. Trantina-Yates A, Weissenstein C, Human P, Zilla P. Stentless bioprosthetic heart valve research: sheep versus primate model. Ann Thorac Surg 2001;71(5 suppl):S422-7.
67. Blunt MH. Cellular elements of ovine blood. In: Blunt MH, editor. The blood of sheep: composition and function. New York: Springer-Verlag; 1975. p. 29-44.
68. Didisheim P. Comparative hematology in the human, calf, sheep and goat: relevance to implantable blood pump evaluation. ASAIO J 1985;8:123-7.
69. Tillman P, Carson SN, Talken L. Platelet function and coagulation parameters in sheep during experimental vascular surgery. Lab Anim Sci 1981;31:263-7.
70. Slattery CW, Beaumont DO. Sheep platelets as a model for human platelets: evidence for specific PAF (platelet activating factor) receptors. Thromb Res 1989;55:569-76.
71. Rodrigues M, Sinzinger H, Thakur M, Becker W, Dewanjee M, Ezekowitz M, et al. Labelling of platelets with indium-111 oxine and technetium-99m hexamethylpropylene amine oxime: suggested methods. International Society of Radiolabelled Blood Elements (ISORBE). Eur J Nucl Med 1999;26:1614-6.
72. Ueberrueck T, Tautenhahn J, Meyer L, Kaufmann O, Lippert H, Gastinger I, et al. Comparison of the ovine and porcine animal models for biocompatibility testing of vascular prostheses. J Surg Res 2005; 124:305-11.
73. Gross DR. Thromboembolic phenomena and the use of the pig as an appropriate animal model for research on cardiovascular devices. Int J Artif Organs 1997;20:195-203.
74. Pelagalli A, Lombardi P, d'Angelo D, Della Morte R, Avallone L, Staiano N. Species variability in platelet aggregation response to different agonists. J Comp Pathol 2002;127:126-32.
75. Pelagalli A, Belisario MA, Tafuri S, Lombardi P, d'Angelo D, Avallone L, et al. Adhesive properties of platelets from different animal species. J Comp Pathol 2003;128:127-31.
76. Goodman SL. Sheep, pig, and human platelet-material interactions with model cardiovascular biomaterials. J Biomed Mater Res 1999;45: 240-50.
77. Whittle BJ, Moncada S, Vane JR. Comparison of the effects of prostacyclin (PGI2), prostaglandin E1 and D2 on platelet aggregation in different species. Prostaglandins 1978;16:373-88.
78. van der Lei B, Robinson PH, Bartels HL, Wildevuur CR. Microarterial grafting into the carotid artery of the rabbit: some considerations concerning species-dependent thrombogenicity. Br J Plast Surg 1989; 42:59-64.
79. Lewis JH, Van Thiel DH, Hasiba U, Spero JA, Gavaler J. Comparative hematology and coagulation: studies on rodentia (rats). Comp Biochem Physiol A 1985;82:211-5.
80. Kalra M, Miller VM. Early remodeling of saphenous vein grafts: proliferation, migration and apoptosis of adventitial and medial cells occur simultaneously with changes in graft diameter and blood flow. J Vasc Res 2000;37:576-84.
81. Kover G, Tost H. Investigations on the circulation of anesthetized dogs. Acta Physiol Hung 1991;78:309-22.
82. Lidman DH, Faibisoff B, Daniel RK. Expanded polytetrafluoroethylene as a microvascular graft: an experimental study. J Microsurg 1980;1:447-56.
83. Cox RH, Peterson LH, Detweiler DK. Comparison of arterial hemodynamics in the mongrel dog and the racing greyhound. Am J Physiol 1976;230:211-8.
84. Greisler HP, Tattersall CW, Henderson SC, Cabusao EA, Garfield JD, Kim DU. Polypropylene small-diameter vascular grafts. J Biomed Mater Res 1992;26:1383-94.
85. White KC, Kavanaugh JF, Wang DM, Tarbell JM. Hemodynamics and wall shear rate in the abdominal aorta of dogs. Effects of vasoactive agents. Circ Res 1994;75:637-49.
86. Christenson JT, Arvidsson D, Thorne J, Olsson PI, Norgren L, Strand SE. A comparison of two methods of labelling autologous platelets with 111In-oxine in five different species. Eur J Nucl Med 1983;8: 389-92.
87. Lundell A, Bergqvist D, Lindblad B. The uptake of platelets, fibrinogen and leucocytes in ePTFE vascular grafts in relation to blood flow—an experimental study in sheep. Eur J Vasc Endovasc Surg 1993;7:698-703.
88. Sauvage LR, Berger KE, Wood SJ, Yates SG 2nd, Smith JC, Mansfield PB. Interspecies healing of porous arterial prostheses: observations, 1960 to 1974. Arch Surg 1974;109:698-705.
89. Clowes AW, Gown AM, Hanson SR, Reidy MA. Mechanisms of arterial graft failure. 1. Role of cellular proliferation in early healing of PTFE prostheses. Am J Pathol 1985;118:43-54.
90. Clowes AW, Kirkman TR, Clowes MM. Mechanisms of arterial graft failure. II. Chronic endothelial and smooth muscle cell proliferation in healing polytetrafluoroethylene prostheses. J Vasc Surg 1986;3: 877-84.
91. Golden MA, Hanson SR, Kirkman TR, Schneider PA, Clowes AW. Healing of polytetrafluoroethylene arterial grafts is influenced by graft porosity. J Vasc Surg 1990;11:838-44; discussion 45.
92. Kohler TR, Stratton JR, Kirkman TR, Johansen KH, Zierler BK, Clowes AW. Conventional versus high-porosity polytetrafluoroethylene grafts: clinical evaluation. Surgery 1992;112:901-7.
93. Zilla P, Preiss P, Groscurth P, Rosemeier F, Deutsch M, Odell J, et al. In vitro-lined endothelium: initial integrity and ultrastructural events. Surgery 1994;116:524-34.
94. Meinhart JG, Deutsch M, Fischlein T, Howanietz N, Froschl A, Zilla P. Clinical autologous in vitro endothelialization of 153 infrainguinal ePTFE grafts. Ann Thorac Surg 2001;71(5 suppl):S327-31.
95. Sottiurai VS, Sue SL, Feinberg EL 2nd, Bringaze WL, Tran AT, Batson RC. Distal anastomotic intimal hyperplasia: biogenesis and etiology. Eur J Vasc Surg 1988;2:245-56.
96. Trubel W, Schima H, Moritz A, Raderer F, Windisch A, Ullrich R, et al. Compliance mismatch and formation of distal anastomotic intimal hyperplasia in externally stiffened and lumen-adapted venous grafts. Eur J Vasc Endovasc Surg 1995;10:415-23.
97. Sarkar S, Salacinski HJ, Hamilton G, Seifalian AM. The mechanical properties of infrainguinal vascular bypass grafts: their role in influencing patency. Eur J Vasc Endovasc Surg 2006;31:627-36.
98. Lin PH, Chronos NA, Marijianowski MM, Chen C, Conklin B, Bush RL, et al. Carotid stenting using heparin-coated balloon-expandable stent reduces intimal hyperplasia in a baboon model. J Surg Res 2003 Jun 1;112:84-90.
99. Zilla P, Human P, Wolf M, Lichtenberg W, Rafiee N, Bezuidenhout D, et al. Constrictive external nitinol meshes inhibit vein graft intimal hyperplasia in nonhuman primates. J Thorac Cardiovasc Surg 2008; 136:717-25.

100. Park SG, Kim SC, Choi MJ, Lee HS, Min BG, Cheong J, et al. Heparin monitoring in sheep by activated partial thromboplastin time. Artif Organs 2003;27:576-80.
101. Connell JM, Khalapyan T, Al-Mondhiry HA, Wilson RP, Rosenberg G, Weiss WJ. Anticoagulation of juvenile sheep and goats with heparin, warfarin, and clopidogrel. ASAIO J 2007;53:229-37.
102. Spanos HG. Aspirin fails to inhibit platelet aggregation in sheep. Thromb Res 1939;72:175-82.
103. Margovsky AI, Meek AC, Lord RS. Acute platelet deposition after carotid endarterectomy in sheep: vein patch compared with gelatin-sealed Dacron and polytetrafluoroethylene patch closure. J Vasc Surg 1996;24:200-6.
104. Baldwin BA. The anatomy of the arterial supply to the cranial regions of the sheep and ox. Am J Anat 1964;115:101-7.
105. Baldwin BA, Bell FR. The anatomy of the cerebral circulation of the sheep and ox. The dynamic distribution of the blood supplied by the carotid and vertebral arteries to cranial regions. J Anat 1963;97: 203-15.
106. Baldwin BA, Bell FR. Blood flow in the carotid and vertebral arteries of the sheep and calf. J Physiol 1963;167:448-62.
107. Baldwin BA, Bell FR. The effect on blood pressure in the sheep and calf of clamping some of the arteries contributing to the cephalic circulation. J Physiol 1963;167:463-79.
108. Baldwin BA, Bell FR. The effect of temporary reduction in cephalic blood flow on the EEG of sheep and calf. Electroencephalogr Clin Neurophysiol 1963;15:465-75.
109. Terlecki S, Baldwin BA, Bell FR. Experimental cerebral ischaemia in sheep. Neuropathology and clinical effects. Acta Neuropathol (Berl) 1967;7:185-200.
110. May ND. Experimental studies of the collateral circulation in the head and neck of sheep (Ovis aries). J Anat 1968;103:171-81.
111. Dunn PF, Newman KD, Jones M, Yamada I, Shayani V, Virmani R, et al. Seeding of vascular grafts with genetically modified endothelial cells. Secretion of recombinant TPA results in decreased seeded cell retention in vitro and in vivo. Circulation 1996;93:1439-46.
112. Lundell A, Bergqvist D, Leide S, Lindblad B, Ljungberg J. The effect of a thromboxane receptor antagonist on acute ePTFE arterial graft thrombogenicity—an experimental study in sheep. Eur J Vasc Endovasc Surg 1991;5:321-6.
113. Matthiasson SE, Bergqvist D, Lundell A, Lindblad B. Effect of dextran and enoxaparin on early ePTFE graft thrombogenicity in sheep. Eur J Vasc Endovasc Surg 1995;9:284-92.
114. James NL, Schindhelm K, Slowiaczek P, Milthorpe B, Graham AR, Munro VF, et al. In vivo patency of endothelial cell-lined expanded polytetrafluoroethylene prostheses in an ovine model. Artif Organs 1992;16:346-53.
115. Taylor A, Fletcher JP, Ao PY. Inhibition of fibro-intimal hyperplasia in a polytetrafluoroethylene vascular graft with standard heparin and low molecular weight heparin. Aust N Z J Surg 1996;66:764-7.
116. Ao PY, Hawthorne WJ, Vicaretti M, Fletcher JP. Development of intimal hyperplasia in six different vascular prostheses. Eur J Vasc Endovasc Surg 2000;20:241-9.
117. Hawthorne WJ, Ao PY, Vicaretti M, Fletcher JP. New methodology for assessment of intimal hyperplasia of vascular prostheses. ANZ J Surg 2002;72:623-7.
118. Kohler TR, Kirkman TR. Dialysis access failure: a sheep model of rapid stenosis. J Vasc Surg 1999;30:744-51.
119. Christenson JT, Thulesius O, Owunwanne A, Nazzal M. Forskolin impregnation of small calibre PTFE grafts lowers early platelet graft sequestration and improves patency in a sheep model. Eur J Vasc Surg 1991;5:271-5.
120. Nazzal M, Owunwanne A, Christenson JT. Direct platelet effect of low molecular weight dextran in small calibre PTFE grafts. Eur J Vasc Endovasc Surg 1991;5:169-72.
121. Cabrera Fischer EI, Bia Santana D, Cassanello GL, Zocalo Y, Crawford EV, Casas RF, et al. Reduced elastic mismatch achieved by interposing vein cuff in expanded polytetrafluoroethylene femoral bypass decreases intimal hyperplasia. Artif Organs 2005;29:122-30.
122. Trubel W, Schima H, Czerny M, Perktold K, Schimek MG, Polterauer P. Experimental comparison of four methods of end-to-side anastomosis with expanded polytetrafluoroethylene. Br J Surg 2004;91: 159-67.
123. Niklason LE, Gao J, Abbott WM, Hirschi KK, Houser S, Marini R, et al. Functional arteries grown in vitro. Science 1999;284:489-93.
124. Panepinto LM, Phillips RW. The Yucatan miniature pig: characterization and utilization in biomedical research. Lab Anim Sci 1986;36: 344-7.
125. Tian W, Kuhlmann MT, Pelisek J, Scobioala S, Quang TH, Hasib L, et al. Paclitaxel delivered to adventitia attenuates neointima formation without compromising re-endothelialization after angioplasty in a porcine restenosis model. J Endovasc Ther 2006;13:616-29.
126. Kissin M, Kansal N, Pappas PJ, DeFouw DO, Duran WN, Hobson RW 2nd. Vein interposition cuffs decrease the intimal hyperplastic response of polytetrafluoroethylene bypass grafts. J Vasc Surg 2000; 31:69-83.
127. Swedberg SH, Brown BG, Sigley R, Wight TN, Gordon D, Nicholls SC. Intimal fibromuscular hyperplasia at the venous anastomosis of PTFE grafts in hemodialysis patients. Clinical, immunocytochemical, light and electron microscopic assessment. Circulation 1989;80: 1726-36.
128. Luo Z, Akita GY, Date T, Treleaven C, Vincent KA, Woodcock D, et al. Adenovirus-mediated expression of beta-adrenergic receptor kinase C-terminus reduces intimal hyperplasia and luminal stenosis of arteriovenous polytetrafluoroethylene grafts in pigs. Circulation 2005 Apr 5;111:1679-84.
129. Rotmans JI, Heyligers JMM, Verhagen HJM, Velema E, Nagtegaal MM, de Kleijn DPV, et al. In vivo cell seeding with anti-CD34 antibodies successfully accelerates endothelialization but stimulates intimal hyperplasia in porcine arteriovenous expanded polytetrafluoroethylene grafts. Circulation 2005;112:12-8.
130. Kelly B, Melhem M, Zhang J, Kasting G, Li J, Krishnamoorthy M, et al. Perivascular paclitaxel wraps block arteriovenous graft stenosis in a pig model. Nephrol Dial Transplant 2006;21:2425-31.
131. Marois Y, Chakfe N, Guidoin R, Duhamel RC, Roy R, Marois M, et al. An albumin-coated polyester arterial graft: in vivo assessment of biocompatibility and healing characteristics. Biomaterials 1996;17:3-14.
132. Randall RD Jr, Walley BD, Meredith JH. Comparison of polytetrafluoroethylene (PTFE) and Dacron as long, small-diameter arterial grafts in dogs. Am Surg 1982;48:622-7.
133. Brothers TE, Stanley JC, Burkel WE, Graham LM. Small-caliber polyurethane and polytetrafluoroethylene grafts: a comparative study in a canine aortoiliac model. J Biomed Mater Res 1990;24:761-71.
134. Urayama H, Kasashima F, Kawakami T, Kawakami K, Watanabe Y. An immunohistochemical analysis of implanted woven Dacron and expanded polytetrafluoroethylene grafts in humans. Artif Organs 1996; 20:24-9.
135. Shi Q, Wu MH, Hayashida N, Wechezak AR, Clowes AW, Sauvage LR. Proof of fallout endothelialization of impervious Dacron grafts in the aorta and inferior vena cava of the dog. J Vasc Surg 1994;20:546-56; discussion 56-7.
136. Nordestgaard AG, Buckels JA, Wilson SE. A laboratory model for the evaluation of thromboembolic complications of small diameter vascular prostheses. Br J Exp Pathol 1986;67:839-49.
137. Greisler HP, Endean ED, Klosak JJ, Ellinger J, Dennis JW, Buttle K, et al. Polyglactin 910/polydioxanone bicomponent totally resorbable vascular prostheses. J Vasc Surg 1988;7:697-705.
138. Ishii Y, Kronengold RT, Virmani R, Rivera EA, Goldman SM, Prechtel EJ, et al. Novel bioengineered small caliber vascular graft with excellent one-month patency. Ann Thorac Surg 2007;83:517-25.
139. Ishii Y, Sakamoto SI, Kronengold RT, Virmani R, Rivera EA, Goldman SM, et al. A novel bioengineered small-caliber vascular graft incorporating heparin and sirolimus: excellent 6-month patency. J Thorac Cardiovasc Surg 2008;135:1237-45; discussion 45-6.
140. Cassel WS, Mason RA, Campbell R, Newton GB, Hui JC, Giron F. An animal model for small-diameter arterial grafts. J Invest Surg 1989;2: 181-6.
141. Contreras MA, Quist WC, Logerfo FW. Effect of porosity on small-diameter vascular graft healing. Microsurgery 2000;20:15-21.

142. Sparks SR, Tripathy U, Broudy A, Bergan JJ, Kumins NH, Owens EL. Small-caliber mesothelial cell-layered polytetraflouroethylene vascular grafts in New Zealand white rabbits. Ann Vasc Surg 2002;16:73-6.
143. Watanabe K. Microarterial prostheses of expanded polytetrafluoroethylene. J Microsurg 1980;2:11-21.
144. Demiri EC, Iordanidis SL, Mantinaos CF. Experimental use of prosthetic grafts in microvascular surgery. Handchir Mikrochir Plast Chir 1999;31:102-6.
145. Tsukagoshi T, Yenidunya MO, Sasaki E, Suse T, Hosaka Y. Experimental vascular graft using small-caliber fascia-wrapped fibrocollagenous tube: short-term evaluation. J Reconstr Microsurg 1999;15: 127-31.
146. Scremin OU, Sonnenschein RR, Rubinstein EH. Cerebrovascular anatomy and blood flow measurements in the rabbit. J Cereb Blood Flow Metab 1982;2:55-66.
147. Lee JS, Hamilton MG, Zabramski JM. Variations in the anatomy of the rabbit cervical carotid artery. Stroke 1994;25:501-3.
148. Huynh T, Abraham G, Murray J, Brockbank K, Hagen PO, Sullivan S. Remodeling of an acellular collagen graft into a physiologically responsive neovessel. Nat biotechnol 1999;17:1083-6.
149. Campbell JH, Efendy JL, Campbell GR. Novel vascular graft grown within recipient's own peritoneal cavity. Circ Res 1999;85:1173-8.
150. van der Lei B, Nieuwenhuis P, Molenaar I, Wildevuur CR. Long-term biologic fate of neoarteries regenerated in microporous, compliant, biodegradable, small-caliber vascular grafts in rats. Surgery 1987;101: 459-67.
151. Okoshi T, Soldani G, Goddard M, Galletti PM. Very small-diameter polyurethane vascular prostheses with rapid endothelialization for coronary artery bypass grafting. J Thorac Cardiovasc Surg 1993;105: 791-5.
152. Lee YS, Park DK, Kim YB, Seo JW, Lee KB, Min BG. Endothelial cell seeding onto the extracellular matrix of fibroblasts for the development of a small diameter polyurethane vessel. ASAIO J 1993;39: M740-5.
153. Kidd KR, Patula VB, Williams SK. Accelerated endothelialization of interpositional 1-mm vascular grafts. J Surg Res 2003;113:234-42.
154. Pektok E, Nottelet B, Tille JC, Gurny R, Kalangos A, Moeller M, et al. Degradation and healing characteristics of small-diameter poly(epsilon-caprolactone) vascular grafts in the rat systemic arterial circulation. Circulation 2008;118:2563-70.
155. Hong Y, Ye SH, Nieponice A, Soletti L, Vorp DA, Wagner WR. A small diameter, fibrous vascular conduit generated from a poly(ester urethane)urea and phospholipid polymer blend. Biomaterials 2009; 30:2457-67.
156. O'Brien CJ, Harris JP, May J. Evaluation of a clinically useful length of 1 mm diameter PTFE as a microvascular graft. Br J Plast Surg 1986; 39:103-8.
157. Harris JR, Seikaly H. Evaluation of polytetrafluoroethylene micrografts in microvascular surgery. J Otolaryngol 2002;31:89-92.
158. Kobayashi H, Kabuto M, Ide H, Hosotani K, Kubota T. An artificial blood vessel with an endothelial-cell monolayer. J Neurosurg 1992; 77:397-402.
159. Zilla P, Weissenstein C, Bracher M, Zhang Y, Koen W, Human P, et al. High glutaraldehyde concentrations reduce rather than increase the calcification of aortic wall tissue. J Heart Valve Dis 1997;6:502-9.
160. Daniel RK. Experimental microvascular polytetrafluoroethylene grafts: 6-month patency. Plast Reconstr Surg 1985;76:753.
161. Yue X, van der Lei B, Schakenraad JM, van Oene GH, Kuit JH, Feijen J, et al. Smooth muscle cell seeding in biodegradable grafts in rats: a new method to enhance the process of arterial wall regeneration. Surgery 1988;103:206-12.
162. Lanzetta M, Owen ER. Long-term results of 1 millimeter arterial anastomosis using the 3M precise microvascular anastomotic system. Microsurgery 1992;13:313-20.
163. Lanzetta M, Owen ER. Use of the 3M precise microvascular anastomotic system in grafting 1-mm diameter arteries with polytetrafluoroethylene prostheses: a long-term study. J Reconstr Microsurg 1993;9: 173-81.
164. Walpoth BH, Pavlicek M, Celik B, Nicolaus B, Schaffner T, Althaus U, et al. Prevention of neointimal proliferation by immunosuppression in synthetic vascular grafts. Eur J Cardiothorac Surg 2001;19:487-92.
165. Lopez-Soler RI, Brennan MP, Goyal A, Wang Y, Fong P, Tellides G, et al. Development of a mouse model for evaluation of small diameter vascular grafts. J Surg Res 2007;139:1-6.
166. Merzkirch C, Davies N, Zilla P. Engineering of vascular ingrowth matrices: are protein domains an alternative to peptides? Anat Rec 2001;263:379-87.
167. Konig G, McAllister TN, Dusserre N, Garrido SA, Iyican C, Marini A, et al. Mechanical properties of completely autologous human tissue engineered blood vessels compared to human saphenous vein and mammary artery. Biomaterials 2009;30:1542-50.
168. Muller-Glauser W, Zilla P, Lachat M, Bisang B, Rieser F, von Segesser L, et al. Immediate shear stress resistance of endothelial cell monolayers seeded in vitro on fibrin glue-coated ePTFE prostheses. Eur J Vasc Surg 1993;7:324-8.
169. Doyle B, Caplice N. Letter regarding article by Rotmans et al, "In vivo cell seeding with anti-CD34 antibodies successfully accelerates endothelialization but stimulates intimal hyperplasia in porcine arteriovenous expanded polytetrafluoroethylene grafts." Circulation 2005; 112:e359; author reply e-60.
170. Sun Y, Wang J, Li H, Han X. Found in inflammatory zone 1 induces angiogenesis in murine models of asthma. Lung 2008;186:375-80.
171. Lutty GA, Merges C, Grebe R, Prow T, McLeod DS. Canine retinal angioblasts are multipotent. Exp Eye Res 2006;83:183-93.
172. Yamaoka T, Yonemitsu Y, Komori K, Baba H, Matsumoto T, Onohara T, et al. Ex vivo electroporation as a potent new strategy for nonviral gene transfer into autologous vein grafts. Am J Physiol Heart Circ Physiol 2005;289:H1865-72.
173. Kelly DJ, Zhang Y, Gow RM, Itescu S, Gilbert RE. Cells expressing the stem cell factor receptor, c-kit, contribute to neoangiogenesis in diabetes. Diab Vasc Dis Res 2005;2:76-80.
174. Shi Q, Aida K, Vandeberg JL, Wang XL. Passage-dependent changes in baboon endothelial cells—relevance to in vitro aging. DNA Cell Biol 2004;23:502-9.
175. Hirai J, Matsuda T. Venous reconstruction using hybrid vascular tissue composed of vascular cells and collagen: tissue regeneration process. Cell Transplant 1996;5:93-105.
176. L'Heureux N, Dusserre N, Konig G, Victor B, Keire P, Wight TN, et al. Human tissue-engineered blood vessels for adult arterial revascularization. Nat Med 2006;12:361-5.
177. Daly CD, Campbell GR, Walker PJ, Campbell JH. Vascular engineering for bypass surgery. Expert Rev Cardiovasc Ther 2005;3:659-65.
178. Izhar U, Schwalb H, Borman JB, Hellener GR, Hotoveli-Salomon A, Marom G, et al. Novel synthetic selectively degradable vascular prostheses: a preliminary implantation study. J Surg Res 2001;95:152-60.
179. Cebotari S, Walles T, Sorrentino S, Haverich A, Mertsching H. Guided tissue regeneration of vascular grafts in the peritoneal cavity. Circ Res 2002;90:e71.
180. Bhattacharya V, McSweeney PA, Shi Q, Bruno B, Ishida A, Nash R, et al. Enhanced endothelialization and microvessel formation in polyester grafts seeded with CD34(+) bone marrow cells. Blood 2000;95:581-5.
181. Onuki Y, Kouchi Y, Yoshida H, Wu MH, Shi Q, Sauvage LR. Early presence of endothelial-like cells on the flow surface of porous arterial prostheses implanted in the descending thoracic aorta of the dog. Ann Vasc Surg 1997;11:604-11.
182. Durante KR, Wu HD, Sauvage LR, Shi Q, Wechezak AR, Coan DE, et al. Implant site: a determinant of completeness of arterial prosthesis healing in the dog and possibly in humans. Ann Vasc Surg 1990;4:171-8.
183. Hayashida N, Han MT, Wu MH, Shi Q, Wechezak AR, Sauvage LR. Differential effect of the retropleural and retroperitoneal environments on healing of the inner wall of porous fabric prostheses in the thoracic and abdominal aorta of the same dog. Ann Vasc Surg 1995;9:369-77.
184. Wu MH, Shi Q, Kouchi Y, Onuki Y, Ghali R, Yoshida H, et al. Implant site influence on arterial prosthesis healing: a comparative study with a triple implantation model in the same dog. J Vasc Surg 1997;25:528-36.
185. Hergenrother RW, Yu XH, Cooper SL. Blood-contacting properties of polydimethylsiloxane polyurea-urethanes. Biomaterials 1994;15: 635-40.

186. Benzel EC, McMillan R, Fowler MR, Landreneau MD, Kesterson L, Payne DL. Histological comparison of autogenous canine fascia lata, Gore-Tex, lyophilized human fascia lata, and autogenous canine vein for vascular patch graft material in a canine arteriotomy model. Neurosurgery 1992;31:108-13.
187. Grabenwoger M, Fitzal F, Sider J, Cseko C, Bergmeister H, Schima H, et al. Endothelialization of biosynthetic vascular prostheses after laser perforation. Ann Thorac Surg 1998;66(6 suppl):S110-4.
188. Kenney DA, Tu R, Peterson RC. Evaluation of compliant and noncompliant PTFE vascular prostheses. ASAIO Trans 1988;34:661-3.
189. Shum-Tim D, Stock U, Hrkach J, Shinoka T, Lien J, Moses MA, et al. Tissue engineering of autologous aorta using a new biodegradable polymer. Ann Thorac Surg 1999;68:2298-304; discussion 305.
190. Niu S, Kurumatani H, Satoh S, Kanda K, Oka T, Watanabe K. Small diameter vascular prostheses with incorporated bioabsorbable matrices. A preliminary study. ASAIO J 1993;39:M750-3.
191. Campbell JB, Glover JL, Herring B. The influence of endothelial seeding and platelet inhibition on the patency of ePTFE grafts used to replace small arteries—an experimental study. Eur J Vasc Endovasc Surg 1988;2:365-70.
192. He H, Shirota T, Yasui H, Matsuda T. Canine endothelial progenitor cell-lined hybrid vascular graft with nonthrombogenic potential. J Thorac Cardiovasc Surg 2003;126:455-64.
193. Conklin BS, Richter ER, Kreutziger KL, Zhong DS, Chen C. Development and evaluation of a novel decellularized vascular xenograft. Med Eng Phys 2002;24:173-83.
194. Akiyama N, Esato K, Fujioka K, Zempo N. A comparison of CORVITA and expanded polytetrafluoroethylene vascular grafts implanted in the abdominal aortas of dogs. Surg 1997;27:840-5.
195. Clarke DR, Lust RM, Sun YS, Black KS, Ollerenshaw JD. Transformation of nonvascular acellular tissue matrices into durable vascular conduits. Ann Thorac Surg 2001;71(5 suppl):S433-6.
196. Stimpson C, White R, Klein S, Shors E. Patency and durability of small diameter silicone rubber vascular prostheses. Biomater Artif Cells Artif Organs 1989;17:31-43.
197. Laredo J, Xue L, Husak VA, Ellinger J, Greisler HP. Silyl-heparin adsorption improves the in vivo thromboresistance of carbon-coated polytetrafluoroethylene vascular grafts. Am J Surg 2003;186:556-60.
198. Werkmeister JA, Edwards GA, White JF, Casagranda F, Hunt JA, Williams DF, et al. In vivo evaluation of modified mandrel-grown vascular prostheses. J Biomed Mater Res 1999;47:316-23.
199. Wilson GJ, MacGregor DC, Bridgeman J, Weber BA, Binnington AG, Pinchuk L. A Corethane/polyester composite vascular prosthesis for vascular access. Comparison with expanded polytetrafluoroethylene grafts in a canine model. ASAIO J 1995;41:M728-34.
200. Shindo S, Takagi A, Whittemore AD. Improved patency of collagen-impregnated grafts after in vitro autogenous endothelial cell seeding. J Vasc Surg 1987;6:325-32.
201. Yavuz K, Geyik S, Pavcnik D, Uchida BT, Corless CL, Hartley DE, et al. Comparison of the endothelialization of small intestinal submucosa, dacron, and expanded polytetrafluoroethylene suspended in the thoracoabdominal aorta in sheep. J Vasc Interv Radiol 2006;17:873-82.
202. Drasler WJ, Wilson GJ, Stenoien MD, Jenson ML, George SA, Dutcher RG, et al. A spun elastomeric graft for dialysis access. ASAIO J 1993;39:114-9.
203. Gherardini G, Haegerstrand A, Matarasso A, Gurlek A, Evans GR, Lundeberg T. Cell adhesion and short-term patency in human endothelium preseeded 1.5-mm polytetrafluoroethylene vascular grafts: an experimental study. Plast Reconstr Surg 1997;99:472-8.
204. Ganong WF. Review of medical physiology. 19th ed. Stamford, CT: Appleton & Lange; 1999.
205. Makris A, Thornton C, Thompson J, Thomson S, Martin R, Ogle R, et al. Uteroplacental ischemia results in proteinuric hypertension and elevated sFLT-1. Kidney Int 2007;71:977-84.
206. Werchan PM, Schadt JC, Fanton JW, Laughlin MH. Total and regional cerebral blood flow during recovery from G-LOC. Aviat Space Environ Med 1996;67:751-8.
207. Ilsar R, Chawantanpipat C, Chan K, Waugh R, Hennessy A, Celermajer DS, et al. The measurement of pulmonary flow reserve in high order primates. Clin Exp Pharmacol Physiol 2009;36:797-802.
208. Fife WP. Cardiac output and regional blood flow in the anesthetized baboon. SAM-TR-66-50. Tech Rep SAM-TR 1966:15.
209. Ruckebusch Y, Phaneuf L-P, Dunlop R. Physiology of small and large animals. Philadelphia: B.C. Decker; 1991.
210. Stephenson R. Cardiovascular physiology. In: Cunningham JG, Klein BG, editors. Textbook of veterinary physiology. 4th ed. St. Louis, MO: Saunders; 2007. p. 185,8.
211. Reece WO. Functional anatomy and physiology of domestic animals. 3rd ed. Baltimore: Lippincott Williams & Wilkins; 2005.
212. Sheeler P, Barber AA. Comparative hematology of the turtle, rabbit and rat. Comp Biochem Physiol 1964;11:139-45.
213. Akers RM, Denbow DM. Anatomy and physiology of domestic animals. Ames, Iowa: Blackwell Publishing; 2008.
214. Hirata K, Yaginuma T, O'Rourke MF, Kawakami M. Age-related changes in carotid artery flow and pressure pulses: possible implications for cerebral microvascular disease. Stroke 2006;37:2552-6.
215. Sui B, Gao P, Lin Y, Qin H, Liu L, Liu G. Noninvasive determination of spatial distribution and temporal gradient of wall shear stress at common carotid artery. J Biomech 2008;41:3024-30.
216. Tortoli P, Morganti T, Bambi G, Palombo C, Ramnarine KV. Noninvasive simultaneous assessment of wall shear rate and wall distension in carotid arteries. Ultrasound Med Biol 2006;32:1661-70.
217. Holland CK, Brown JM, Scoutt LM, Taylor KJ. Lower extremity volumetric arterial blood flow in normal subjects. Ultrasound Med Biol 1998;24:1079-86.
218. Hussain ST, Smith RE, Wood RF, Bland M. Observer variability in volumetric blood flow measurements in leg arteries using duplex ultrasound. Ultrasound Med Biol 1996;22:287-91.
219. Zierler BK, Kirkman TR, Kraiss LW, Reiss WG, Horn JR, Bauer LA, et al. Accuracy of duplex scanning for measurement of arterial volume flow. J Vasc Surg 1992;16:520-6.
220. Min S-K, Kenagy RD, Jeanette JP, Clowes AW. Effects of external wrapping and increased blood flow on atrophy of the baboon iliac artery. J Vasc Surg 2008;47:1039-47.
221. Mangell P, Lanne T, Sonesson B, Hansen F, Bergqvist D. Regional differences in mechanical properties between major arteries—an experimental study in sheep. Eur J Vasc Endovasc Surg 1996;12:189-95.
222. Margovsky A, Parsson H, Chao A, Lord RSA. A comparative thrombogenicity study of heparin soaked fluoro-passivated polyester and ePTFE patches in sheep. Eur J Vasc Endovasc Surg 2002;23:39-43.
223. Cejna M, Virmani R, Jones R, Bergmeister H, Losert U, Xu Z, et al. Biocompatibility and performance of the Wallstent and several covered stents in a sheep iliac artery model. J Vasc Interv Radiol 2001;12:351-8.
224. Walter JP, McGahan JP, Lantz BM. Absolute flow measurements using pulsed Doppler US. Work in progress. Radiology 1986;159:545-8.
225. Snow HM, Markos F, O'Regan D, Pollock K. Characteristics of arterial wall shear stress which cause endothelium-dependent vasodilatation in the anaesthetized dog. J Physiol (Lond) 2001;531:843-8.
226. Snow HM, McAuliffe SJ, Moors JA, Brownlie R. The relationship between blood flow and diameter in the iliac artery of the anaesthetized dog: the role of endothelium-derived relaxing factor and shear stress. Exp Physiol 1994;79:635-45.
227. Young MA, Vatner SF. Blood flow- and endothelium-mediated vasomotion of iliac arteries in conscious dogs. Circ Res 1987;61:II88-93.
228. Bagshaw RJ, Cox RH, Campbell KB. Sodium nitroprusside and regional arterial haemodynamics in the dog. Br J Anaesth 1977;49:735-43.
229. Calasso M, Parmeggiani PL. Carotid blood flow during REM sleep. Sleep 2008;31:701-7.
230. Sho E, Nanjo H, Sho M, Kobayashi M, Komatsu M, Kawamura K, et al. Arterial enlargement, tortuosity, and intimal thickening in response to sequential exposure to high and low wall shear stress. J Vasc Surg 2004;39:601-12.

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