Biomechanical properties of decellularized porcine common carotid arteries

Sylvain Roy, Paolo Silacci, and Nikolaos Stergiopulos
Laboratory of Hemodynamics and Cardiovascular Technology, Swiss Federal Institute for Technology, Lausanne, Switzerland

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Roy, Sylvain, Paolo Silacci, and Nikolaos Stergiopulos. Biomechanical properties of decellularized porcine common carotid arteries. Am J Physiol Heart Circ Physiol 289: H1567–H1576, 2005. First published May 20, 2005; doi:10.1152/ajpheart.00564.2004.—To analyze the effects of decellularization on the biomechanical properties of porcine common carotid arteries, decellularization was performed by a detergent-enzymatic procedure that preserves extracellular matrix scaffold. Internal diameter, external diameter, and wall thickness were measured by optical microscopy on neighboring histological sections before and after decellularization. Rupture tests were conducted. Inner diameter and wall thickness were measured by echo tracking during pressure inflation from 10 to 145 mmHg. Distensibility and incremental elastic modulus were computed. At 10 mmHg, mean diameter of decellularized arteries was 5.38 mm, substantially higher than controls (4.1 mm), whereas decellularized and control arteries reached the same internal diameter (6.7 mm) at 145 mmHg. Wall thickness decreased 16% for decellularized and 32% for normal arteries after pressure was increased from 10 to 145 mmHg. Decellularized arteries withstood pressure >2,200 mmHg before rupture. At 145 mmHg, decellularization reduced compliance by 66% and increased incremental elastic modulus by 54%. Removal of cellular elements from media led to changes in arterial dimensions. Collagen fibers engaged more rapidly during inflation, yielding a stiffer vessel. Distensibility was therefore significantly lower (by a factor of 3) in decellularized than in normal vessels; reduced in the physiological range of pressures. In conclusion, decellularization yields vessels that can withstand high inflation pressures with, however, markedly different geometrical and biomechanical properties. This may mean that the potential use of a decellularized artery as a scaffold for the creation of xenografts may be compromised because of geometrical and compliance mismatch.

elastic modulus; decellularized arteries; compliance mismatch; bioengineered vessel

CORONARY ARTERY BYPASS SURGERY requires the insertion of natural or artificial substitute vessels to bypass one or more blocked arteries in the heart. Natural substitute vessels are the patient’s saphenous veins or the internal mammary artery; however, these vessels may not be an option for patients who have undergone previous bypass surgery or who do not have vessels of appropriate quality (1, 24, 26, 39). Clinical experience shows that small-bore grafts made out of conventional graft material [i.e., polytetrafluoroethylene (ePTFE)] are occluded by thrombosis within and, thus, are unable to maintain long-term patency (7, 34, 47). There is a great need for the development of grafts that can be used as substitute vessels for coronary bypass surgery or other peripheral applications (20, 27, 28, 30–32, 37, 41, 45), as well as for occlusive disease of small peripheral vessels (i.e., vessels below the knee).

Implanted grafts develop a layer of disorganized protein and cell remnants, called pseudointima. At the two anastomotic ends, smooth muscle cell (SMC) proliferation leads to neointimal formation, which is usually covered by endothelial cells (5). Excessive thickening of pseudointima and neointima is believed to play a role in limiting graft patenty (43, 44). Two main biomechanical parameters have been suggested to play a role in the development of neointima: the local flow characteristics (shear stress distribution and its spatiotemporal variations), which obviously depend on the rate of flow and local geometry (23), and compliance mismatch between the graft and the host vessel, which, during cardiac pulsation, leads to excess stress concentration at the suture line and further augments flow disturbances (2, 5, 33, 40, 46).

Decellularized arteries may offer an attractive scaffold model onto which autologous endothelial cells may be seeded to produce an artificial graft with good biomechanical and antithrombogenic properties (20, 27, 32, 41). The biomechanical properties of decellularized arteries and, in particular, compliance and incremental elastic modulus have not been discussed in detail. As mentioned above, compliance is an important quantity, because compliance mismatch between the host vessel and the graft has often been related to graft failure (2, 5, 17). The goal of this study is therefore to study the effects of decellularization on graft morphology, geometry, and elastic properties. Specifically, our analysis aims to define the effect of decellularization on 1) vessel geometry, 2) structural characteristics, as expressed by the pressure-dependent compliance, and 3) effective elastic material properties, as expressed in the strain-dependent incremental elastic modulus. The above-mentioned biomechanical analysis will help in understanding the role of vascular smooth muscle (VSM), which is the main element removed during the decellularization process, on the passive structural properties of the arterial wall.

MATERIALS AND METHODS

Artery Preparation

Common carotid arteries from 29 adult pigs weighing 150–200 kg were obtained from a local slaughterhouse. Each artery was from a different animal. Within 15 min after death, a careful, sterile technique was used to dissect left common carotid arteries from a region 10–15 cm distal to the bifurcation. The arteries were immediately washed with ice-cold phosphate buffer solution (PBS) and stored in PBS on ice until they were returned to the laboratory within 30 min. On arrival, the arteries were prepared under a laminar flow hood; excess adventitial and connective tissues were removed using sterile instruments.

Artery Groups

In the first group, 12 arteries were used to assess the effect of decellularization on histological preparation. In 6 of the 12 arteries, a
A 1-cm-long ring was cut from the distal segment (Fig. 1A); in the remaining 6 arteries, a ring was cut from the proximal segment. Each ring was then cut into two pieces, and each piece was assigned to the normal or the decellularized subgroup. The cut section was labeled for precise orientation face to face, and the pieces from the normal subgroup were fixed unloaded in 4% formaldehyde and prepared for classical histology. The other subgroup was subjected to the decellularization process. The remaining midportions were discarded. A second group of five arteries were used as control for the rupture test, and another five arteries were subjected to decellularization and then used for the rupture test. A third group of 12 arteries was used for biomechanical testing. Arteries collected from the slaughterhouse underwent the complete biomechanical testing protocol (see below) within 8 h after they were harvested. Each artery was then decellularized and again underwent the same biomechanical testing to assess the effects of decellularization on mechanical proprieties.

Decellularization Protocol

Decellularized carotid arteries were subjected to a four-stage detergent-enzymatic procedure in which Triton X-100 and SDS were used to remove cellular components of untreated vessels while preserving the extracellular matrix scaffold (4, 38). Triton X-100 promoted a nondenaturing step, and SDS had a denaturing action on the cellular elements. In the first stage, arteries were placed in a hypotonic buffer containing 10.0 mM Tris (pH 8.0) supplemented with protease inhibitors under continuous shaking for 48 h at 4°C. In the second stage, hypotonic buffer was replaced by a hypertonic buffer containing 1% Triton X-100, 1.5 M KCl, and 10.0 mM Tris (pH 8.0) supplemented with protease inhibitors under the same shaking conditions. After 48 h, the vessels were rinsed several times with 0.15 M Sorensen’s phosphate buffer (pH 7.3). In the third stage, the arteries were incubated with deoxyribonuclease (0.2 mg/ml) and ribonuclease (20 μg/ml) at 37°C for 5 h. After the samples were washed with Sorensen’s phosphate buffer, a final extraction buffer containing 1% SDS and 10.0 mM Tris (pH 9.0) was added, and the specimens were incubated for 48 h at room temperature under continuous shaking. At the end of the fourth stage, decellularized arteries were extensively washed with and incubated in PBS for 48 h. The arteries were decellularized, fixed unloaded (at zero pressure and with no axial stretch) in 4% formaldehyde, and prepared for classical histology, or they were subjected to rupture test or biomechanical measurements.

Optical Microscopy

For measurements of inner and outer diameter, specimens from normal and decellularized subgroups were observed under an Olympus Plan Apo ×2/0.4 objective, and photographs were taken on an imaging microscope (model BX 41, Olympus, Tokyo, Japan) with a digital camera (Camedia digital camera C-3040 zoom, Olympus). For measurement of wall thickness, specimens from both subgroups were observed under a Zeiss A-Plan ×200/0.45 objective, and photographs were taken on a Zeiss Axioplan 2 imaging microscope with a charge-coupled device (CCD) camera (model 3CCD color video camera, Sony, Tokyo, Japan). Photographs were computerized with an image processing program (KS 400, version 3.0, Zeiss, Wetzlar, Germany). To estimate diameters, the largest and smallest elliptical axes were measured. Cross-sectional radial lines were similarly drawn for wall thickness. Sizes were estimated by comparison with a microruler image pictured under the same condition. Mean SMC density in media was estimated by counting the number of nuclei per unit area in hematoxylin-eosin-stained sections for the normal proximal and distal portions.

Rupture Test

A 5-cm-long specimen of normal or decellularized artery was mounted on an artery holder, with one end connected to a digital manometer (Ecol1, Keller, Wintherthur, Switzerland) and the other to a 50-ml syringe (Braun Melsungen, Melsungen, Germany) filled with PBS. Arteries were stressed to a longitudinal stretch ratio (λL) of 1.5 to avoid excessive bending during inflation, and pressure was increased at a rate of 25 ml/s until the artery ruptured or peak pressure was reached. Peak values from five arteries in each group were recorded.

Opening Angle and Length Measurements

Before the artery was mounted onto the cannula for biomechanical tests, a small (5-mm long) ring was cut from the artery for measurement of the opening angle. The ring was dyed with Miller’s blue so as to easily distinguish wall borders and then cut open and left to rest for 30 min. The section was photographed with a digital camera (Coolpix 950, Nikon, Tokyo, Japan). The opening angle was measured using ImageJ 1.32 software (National Institutes of Health, Bethesda, MD). The unleaded length of the remaining portion before and after decellularization was then measured using a precision ruler.

Biomechanical Test Setup

Eight arterial specimens, 4 cm in unstretched length, were mounted on an artery holder and stretched to λc = 1.3 (19). Four other specimens were mounted, and each was stretched to λc = 1.3 and 1.6. The artery was held at 37.5°C in a PBS bath. Arterial inner diameter and wall thickness from the midportion (Fig. 1) were continuously measured by a 10-MHz ultrasonic echo-tracking device (NIUS, Asulab, Marín, Switzerland). These measurements should not be confused with diameter and thickness measured on the histological sections of the proximal and distal segments. The NIUS device allows an absolute precision of 75 μm and a dynamic accuracy of ~1 μm, giving accurate data on changes in the wall cross-sectional geometry (42). The probe was positioned 10 mm above the flank of the artery, and a computer program (Non Invasive Ultra Sound NIUS 02, Asulab) recorded inner diameter and wall thickness vs. pressure over time. A syringe pump (model sp210iw, World Precision Instruments) connected to the artery increased pressure at a rate of 1 mmHg/s from 10 to 150 mmHg. Pressure was not decreased below 10 mmHg, because the echo-tracking signal was often lost. Pressure was recorded by a blood pressure transducer (BLPR, World Precision Instruments), and the signal was transmitted to the NIUS computer through a four-channel transducer-amplifier (Transbridge 4M, World Precision Instruments). Before pressure-wall thickness and pressure-diameter measurement of wall thickness, specimens from both subgroups were observed under a Zeiss A-Plan ×200/0.45 objective, and photographs were taken on a Zeiss Axioplan 2 imaging microscope with a charge-coupled device (CCD) camera (model 3CCD color video camera, Sony, Tokyo, Japan). Photographs were computerized with an image processing program (KS 400, version 3.0, Zeiss, Wetzlar, Germany). To estimate diameters, the largest and smallest elliptical axes were measured. Cross-sectional radial lines were similarly drawn for wall thickness. Sizes were estimated by comparison with a microruler image pictured under the same condition. Mean SMC density in media was estimated by counting the number of nuclei per unit area in hematoxylin-eosin-stained sections for the normal proximal and distal portions.

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Biomechanical Properties of Decellularized Arteries

Histology of arterial sections. Arterial wall cross-sectional area \( A_w \) was calculated from histological sections using an elliptical geometry approximation

\[
A_w = \pi \cdot \frac{(d_{oa} \cdot d_{ia} - d_{oa} \cdot d_{ia})}{4}
\]

where \( d_{oa} \) is the major outer axis, \( d_{ia} \) is the minor outer axis, \( d_{oa} \) is the major inner axis, and \( d_{ia} \) is the minor inner axis.

Histological sections of normal and decellularized arteries are shown in Figs. 2–4. Mean wall thickness before and after decellularization was 810 (SD 100) and 519 (SD 25) \( \mu m \) for proximal and 635 (SD 90) and 305 (SD 36) \( \mu m \) for distal artery rings, respectively. Removing cellular elements from intima and media resulted in 36% and 52% thickness reduction for proximal and distal segments. For the proximal segment, mean outer diameter was 5.54 (SD 0.41) and 5.27 (SD 0.42) mm for normal and decellularized arteries, respectively, and mean inner diameter was 3.92 (SD 0.35) and 4.23 (SD 0.45) mm, respectively. For the distal segment, mean outer diameter was 3.16 (SD 0.29) and 3.52 (SD 0.25) mm for normal and decellularized arteries, respectively, and mean inner diameter was 1.89 (SD 0.38) and 2.9 (SD 0.22) mm, respectively (Table 1).

Mean SMC density was 2,000 and 3,000 nuclei/mm² for proximal and distal segments, respectively; decellularization reduced cross-sectional area to 7.72 (SD 0.51) and 3.09 (SD 0.52) mm² for proximal and distal segments, respectively, which represented 38% and 36% reductions in cross-sectional area, respectively. Mean SMC density was ~2,000 and 3,000 nuclei/mm² for proximal and distal portions, respectively.

Rupture Tests

Peak pressure before rupture was 2,970–3,350 mmHg and mean pressure was 3,124 (SD 158) mmHg for normal arteries. For decellularized arteries, pressure was 1,950–2,910 mmHg, and mean pressure was 2,338 (SD 245) mmHg. Ruptures in the arterial wall most generally occurred along the longitudinal axis in the form of ~4-mm-long cracks. Until rupture occurred, the arteries remained watertight, and no leaks were noticed through the wall.

Opening Angle and Length

The mean opening angle was 26 (SD 16) and 69 (SD 8) degrees for decellularized and normal arteries, respectively. Mean unloaded length was 41.12 (SD 1.3) and 46.06 (SD 3.0) mm for normal and decellularized arteries, respectively, representing a mean increase of 12%.

Biomechanical Tests

Pressure-wall thickness and pressure-diameter recordings were fitted using Eqs. 2 and 3 for pressures of 10–145 mmHg for all measurements. These limits allowed good ultrasound images to be obtained.
signaling throughout the pressurization spectrum, without zero-pressure wall flutter and over the physiological pressure range. Mean inner diameter at 10 mmHg was significantly higher for decellularized than for normal arteries: 5.38 (SD 0.46) vs. 4.1 (SD 0.49) mm (Fig. 5). Pressure inflation led to a much higher increase in diameter in the normal arteries, and at 150 mmHg both groups reached the same diameter: 6.71 (SD 1.01) and 6.74 (SD 0.83) mm for normal and decellularized arteries, respectively, which represented diameter increases of 64% for normal and 25% for decellularized arteries. Consequently, the slope of the pressure-diameter curve was steeper for normal than for decellularized arteries, which showed a markedly flat curve at >100 mmHg (Fig. 5).

Mean wall thickness was initially measured at 669 (SD 100) and 649 (SD 96) µm for normal and decellularized arteries and decreased to 453 (SD 65) and 542 (SD 101) µm, respectively, which represented 32% and 16% radial thinning, respectively. Normal arterial wall thickness decreased quasi-linearly at >70 mmHg, whereas decellularized arterial wall thickness progressed along a flatter slope and was almost horizontal at >90 mmHg. At that point, thickness was 558 µm and was further reduced only by 16 µm over the next 55 mmHg (Fig. 6).

The compliance curve for normal arteries was maximal [0.2 (SD 0.076) mm²/mmHg] at 70 mmHg and decreased thereafter to 0.11 (SD 0.075) mm²/mmHg at 150 mmHg. In contrast to the inverted U-shaped curve for normal arteries, compliance in decellularized arteries decayed quasi-exponentially from 0.19 (SD 0.044) mm²/mmHg at 10 mmHg to 0.37 (SD 0.019) mm²/mmHg at 150 mmHg. Over the entire range of physiological pressures (70–150 mmHg), compliance of decellularized arteries was 50% lower than that of normal arteries (Fig. 7).

Similar trends are observed in the distensibility curves, except for maximal distensibility in the normal artery group, which occurred at subphysiological pressures [7.97 × 10⁻³ (SD 1.57 × 10⁻³) mmHg⁻¹ at 30 mmHg]. At 100 mmHg, distensibility was 0.0045 (SD 0.0016) and 0.00142 (SD 0.00047) mmHg⁻¹ in decellularized and normal arteries, respectively (P < 0.001; Fig. 8). Distensibility is normalized to compliance and related to wave speed and relative diameter.
inflation for a given pressure rise; therefore, it is a better indicator of the "compliance mismatch." On the basis of distensibility, relative stiffening was greater (by a factor of >2) for decellularized than for normal arteries over the entire physiological pressure range.

Mean hoop stress ($\sigma_0$) and Hudetz incremental elastic modulus were plotted against $\lambda_0$ (Fig. 9). Circumferential stretch-incremental elastic modulus curves differed significantly between the two groups, with the wall material in the decellularized artery group being substantially stiffer (Fig. 9B). At 10
mmHg, incremental elastic modulus was essentially the same in both groups: 522 (SD 111) and 506 (SD 65) mmHg for normal and decellularized arteries, respectively. In normal arteries, the incremental modulus remained relatively constant for stretch ratios up to 1.2 and increased slowly thereafter; in decellularized arteries, the incremental modulus increased substantially and continuously from a stretch ratio just above 1, indicative of an early and substantial collagen fiber engagement.

Hudetz incremental elastic modulus in normal and decellularized arteries was plotted against \( \lambda_0 \) for \( \lambda_c = 1.3 \) and 1.6. The effects of circumferential and longitudinal stretch were qualitatively similar for normal and decellularized arteries (Fig. 10). Longitudinal stretching led to a decrease in the apparent incremental elastic modulus.

**DISCUSSION**

**Effects of Decellularization on Arterial Geometry and Structure**

The decellularization process leads to profound changes in arterial geometry and structure: a significant increase in inner diameter followed by a significant decrease in wall thickness (Fig. 4, Table 1). Calculation of cross-sectional area based on histological data shows that decellularization leads to a net decrease in wall cross-sectional area of \( \sim 37\% \). The dimensional data from histological studies do not reflect the diameter and thickness as measured in the “intact” artery during the biomechanical tests. Close inspection of the pressure-diameter (Fig. 5) and pressure-thickness (Fig. 6) curves shows that, although at very low pressure (i.e., 10 mmHg) inner diameter is indeed higher in decellularized than in normal arteries, normal arteries are not thicker than decellularized arteries, as anticipated by the histological results. Furthermore, the segment length, on average, increased in decellularized arteries by 12%. This leads to an apparent paradox: during the inflation experiments in which the artery was pressurized and submerged in a physiological solution, the wall volume was significantly higher in decellularized than in normal arteries. To explain this seemingly paradoxical result, we hypothesize that the void created by the removal of cells in decellularized arteries is filled with the bathing solution and, furthermore, that the structural changes resulting from decellularization lead to an effective void volume that is somewhat higher than the volume of the cells that have been removed. Also, the incompressibility of the decellularized arteries is not compromised; i.e., wall volume is conserved during the pressure inflation. This can be seen in Figs. 5 and 6, where a large increase in diameter of normal arteries during inflation from 10 to 150 mmHg was accompanied by an analogous large decrease in thickness, such that wall cross-sectional area \( A_w = \pi(r_2^2 - r_1^2) \) was conserved. The same holds for decellularized arteries, where the relative changes in diameter and thickness during Table 1. *Morphometric data of arteries*

<table>
<thead>
<tr>
<th>Artery Type</th>
<th>OD, mm</th>
<th>ID, mm</th>
<th>Wall Thickness, mm</th>
<th>Wall Cross-Sectional Area, mm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>3.16 (SD 0.29)</td>
<td>1.89 (SD 0.38)</td>
<td>635 (SD 90)</td>
<td>5.00 (SD 0.64)</td>
</tr>
<tr>
<td>Decellularized</td>
<td>3.52 (SD 0.25)</td>
<td>2.9 (SD 0.22)</td>
<td>305 (SD 36)</td>
<td>3.09 (SD 0.52)</td>
</tr>
<tr>
<td>Proximal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>5.54 (SD 0.41)</td>
<td>3.92 (SD 0.35)</td>
<td>810 (SD 100)</td>
<td>12.07 (SD 1.97)</td>
</tr>
<tr>
<td>Decellularized</td>
<td>5.27 (SD 0.42)</td>
<td>4.23 (SD 0.45)</td>
<td>519 (SD 25)</td>
<td>7.72 (SD 0.51)</td>
</tr>
</tbody>
</table>

Values are mean (SD); \( n = 6 \).
inflation were much less pronounced. Calculation based on the pressure-diameter-thickness data showed that the relative volume variation during inflation did not surpass 1% for normal and decellularized arteries. To the best of our knowledge, this is the first demonstration of incompressibility in decellularized arteries, despite large-scale changes induced by decellularization in their wall architecture (i.e., voids created by, e.g., destructed VSM cells and reorganization of the extracellular matrix).

The changes in arterial dimensions discussed above cannot be the mere result of cell removal; rather, they are caused by profound structural changes within the wall provoked by the decellularization process. Let us consider, for example, the diameter increase after decellularization. If the main constituent elements of the wall (i.e., elastin, collagen, and SMC) were to be structurally independent, removal of SMC under no load would not produce a change in diameter (9). Hence, it is reasonable to assume that the diameter increase can be related to the structural links between SMC and elastin, as well as the existence of residual stresses in the wall. Physical links among SMC, elastin, and collagen are well documented in the vascular physiology literature (9). Furthermore, there is substantial evidence that residual stresses are related to the elastin and VSM components of the arterial wall (18, 48). Our hypothesis is that, under no load, elastin and SMC are connected, such that elastin sheaths and fibers are prestressed. The exact three-dimensional configuration of the links between SMC and elastin is not exactly known; however, on the basis of our results, it is inferred that release of the prestress by decellularization leads to a radial expansion of the vessel. Because a substantial part of elastin fibers (elastic laminae) is oriented circumferentially, we conclude that prestress is primarily compressive in these fibers. Visual inspection of elastin bands in Figs. 2 and 3 clearly shows the markedly wavy appearance of elastic laminae in normal arteries, which is indicative of compressive stresses, whereas waviness is much less evident in decellularized arteries.

The changes in decellularization induced in the opening angle confirm that decellularization releases internal pre-stresses. The opening angle characterizes the degree of opening of an arterial ring after a radial cut and indicates the existence of residual stresses within the arterial wall (15, 48). As a first approximation and on the basis of simple geometric analysis, the larger the opening angle, the higher the level of residual strains in the wall. If the opening angle is close to zero, residual stresses are minimal. Residual stresses were substantially reduced in the decellularized artery group (see RESULTS). Combining the above observations, we may conclude that residual stress, apparently linked to the state of prestress of elastic fibers, is significantly reduced by disruption of the structural integrity of VSM.

In Figs. 2 and 3, a proximal and a distal carotid artery segment are compared. The more-distal carotid artery segment (Figs. 3 and 4) is more muscular, i.e., has a higher percentage of SMC volume; consequently, the decellularization effects are more pronounced. Indeed, after decellularization, diameter increases more at low pressures in the distal arteries, and the volume change and decrease in elastin fiber waviness are also more pronounced in the distal arteries. This also leads to the conclusion that the relative SMC content in a given artery will be...
a factor that determines the biomechanical properties of the arterial graft after decellularization.

Effects of Decellularization on Elastic Properties

The pressure-diameter curve of normal carotid arteries exhibits the typical S-shape of an elastic conduit artery (Fig. 5). The early part of the curve is convex to the pressure axis, which means that compliance and distensibility are increasing with pressure (Figs. 7 and 8). This pressure-diameter response is characteristic of quasi-linear elastic materials and, in the case of arterial mechanics, has been identified with the arterial response to low inflation pressures (25, 36, 50). At low inflation pressures, the load is borne almost exclusively by the elastin fibers, which exhibit a quasi-linear elastic response (36, 50). As inflation pressure continues to increase, collagen fibers will start to engage, resulting in a progressively stiffer vessel wall (36). In a qualitative sense, the concave part of the pressure-diameter curve is dominated by the elastin fibers, whereas, beyond the inflexion point, the convex part of the curve is dominated by collagen fibers. The point at which collagen fibers begin to engage in a significant manner corresponds to the peak of the distensibility curve, which, in our case and for the normal arteries, is 30 mmHg (Fig. 8). The continuous recruitment of collagen fiber beyond the point of peak distensibility limits the further distension of the artery (Fig. 5) and leads to a quasi-exponential decrease in distensibility (Fig. 8).

In contrast to normal arteries, decellularized arteries do not exhibit an S-shaped pressure-diameter curve (Fig. 5); consequently, their distensibility decreases monotonically with pressure (Fig. 8). Analysis of the normal arteries leads us to conclude that, in the decellularized arteries, collagen starts to engage at very low pressures, thereby limiting the elastic response of the artery and yielding a substantially stiffer vessel even at low inflation pressures. At the physiological pressure of 100 mmHg, decellularized arteries were less distensible (by a factor of 3) than normal arteries. This conclusion is further supported by the graph of the incremental elastic modulus-circumferential stretch ratio (Fig. 9B), which clearly shows that the elastic modulus of decellularized arteries increases significantly and exponentially with circumferential extension. The increase in elastic modulus occurs even at very low stretch ratios, which means that collagen fibers engage immediately on pressurization. In contrast, the fairly constant elastic modulus exhibited by normal arteries over a wide range of stretch ratios (1 < λ_b < 1.3) reflects the quasi-linear elastic properties of elastin, which is essentially the sole structural element of the wall that bears load at these low circumferential stretch ratios. For λ_b > 1.3, collagen begins gradually to bear load, leading to progressive stiffening of the wall and, thus, a gradual increase in the elastic modulus (Fig. 9A). At zero pressure, the elastic modulus of the normal and decellularized arteries is equal, as indeed anticipated by the above analysis; at zero pressure, only elastin contributes to the elastic properties of the wall, and the elastic properties of elastin fibers are assumed not to be affected by decellularization.

The pressure-diameter and pressure-thickness data shown in Figs. 5 and 6, as well as the derived compliance, distensibility, and incremental elastic modulus (Figs. 7, 8, and 9, respectively), were derived from experiments in which the axial stretch ratio was fixed (λ_z = 1.3). However, elastic properties are likely to differ significantly when the axial stretch ratio changes, and these effects may be different in normal and decellularized arteries. Additional experiments were thus performed in four arteries to examine the effect of axial stretch on circumferential stiffness. Increase in axial stretch induced a decrease in incremental elastic modulus in normal and decellularized arteries (Fig. 10). This may seem paradoxical and unexpected; however, we previously reported the very same phenomenon in the same kind of arteries (49). At that time, we concluded that the apparent softening with the increase in axial stretch is likely the result of vessel orthotropy. On the basis of our findings, we may further state that the apparent effects of stretch are qualitatively similar in decellularized and normal arteries.

Decellularized Artery as an Artificial Graft Scaffold

Biomechanical analysis has shown that decellularized arteries are larger, thinner, and stiffer than native vessels. One may thus pose the question of compliance mismatch. Compliance mismatch has been proposed as one of the main causes of graft failure due to induced flow perturbations as well as the high distribution of stresses on the suture line provoked by the differential extension of the less-compliant graft with respect to the compliant host vessel (5, 17, 23, 33, 40, 43, 44, 46). It has been shown in previous studies that endothelial hyperplasia could be related to compliance mismatch at the anastomosis (5, 17, 33). One may address, to some extent, this question by appropriate choice of the original vessel, which, depending on its wall composition, after decellularization may yield acceptable levels of wall distensibility in the physiological pressure range; however, the criteria for choice of the original vessel are not clear, and further research is needed to evaluate the applicability of this approach. Despite their clear increase in stiffness, decellularized arteries remain much more compliant than artificial vessels (e.g., Dacron and ePTFE grafts) used clinically. In that respect, decellularized artery-based grafts may offer a certain biomechanical advantage (10). Furthermore, longitudinal stretch seemingly affects the biomechanical properties; thus one may adapt the longitudinal stretch during implantation to optimize compliance. Our preliminary results seemingly suggest that augmenting axial stretch would increase compliance. We may speculate that the appropriate axial stretch ratio would be 1.5–1.7; however, this needs to be rigorously tested in animal experiments. In addition to improved compliance over ePTFE grafts, decellularized arteries showed excellent rupture properties at pressures >2,000 mmHg. This would place decellularized artery-based grafts on the same level as de novo tissue-engineered vascular grafts, which could withstand rupture pressure on the order of 1,000–2,000 mmHg, thus allowing for safe clinical use (10, 20, 27, 32). Some studies have been done on the chemical treatment of decellularized vessels to increase tissue stability against chemical and enzymatic degradation. For instance, glutaraldehyde or polyepoxide cross-linking has been used to stabilize the extracellular matrix (10, 38). Unfortunately, these chemical treatments were aggressive, inasmuch as remnants of such substances were detectable several weeks after extensive washout (38). Glutaraldehyde affected compliance by dramatically increasing vessel stiffness (10). To avoid such a complication,
we have limited the chemical treatments to a multistep-detergent-enzymatic procedure.

Study Limitations

This study was conducted on left common porcine carotid arteries only. The results and conclusions apply to this vessel, and there is no evidence that the observations could be extended to other arteries without further consideration. Wall structure differs considerably between arteries: it depends strongly on vessel location, function, and size. We have limited our investigation to porcine carotid arteries because they are easy to dissect and because the inner diameter was in the range of the potential graft vessel needs. Many efforts are made to produce small-diameter vessels for lower limb vascular surgery. For this reason, we deliberately focused on small arteries of similar size. Furthermore, longitudinal stretch was set to a predetermined value that remained constant throughout all biomechanical measurements. Changing longitudinal stretch will most probably slightly modify the results, but the general conclusions should remain the same.

The goal of the present study was to provide a simple biomechanical description of the effect of decellularization on the structural and elastic properties of the artery segment. Our approach does not provide a thorough and rigorous stress analysis, as could have been done with the development of three-dimensional models of the constitutive behavior of normal and decellularized vessels. We simply compared the overall response of the normal and decellularized vessels under restricted but, nevertheless, physiologically relevant conditions. These data, although not complete, provide useful information about the effects of decellularization.

In summary, decellularization of common carotid arteries modified structural and elastic properties of these vessels. Removing smooth muscle from media resulted in inner diameter enlargement; however, on the basis of echo-tracking measurements, wall thickness remained almost the same. Elastic modulus was significantly increased in decellularized arteries compared with normal arteries. Early engagement of collagen fibers leads to a stiffer vessel, which was, however, able to withstand high pressure before breaking. Compliance mismatch between decellularized artery-based vascular grafts and recipient vessels may still be an important problem that needs to be addressed to avoid long-term complications.

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REFERENCES