Acta Biomaterialia 17 (2015) 125–136

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Selective enzymatic removal of elastin and collagen from human abdominal aortas: Uniaxial mechanical response and constitutive modeling

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ARTICLE INFO

Article history:
Received 23 April 2014
Received in revised form 31 October 2014
Accepted 5 January 2015
Available online 24 January 2015

Keywords:
Human aorta
Elastase
Collagenase
Trypsin inhibitor
Damage modeling

ABSTRACT

The ability to selectively remove the structurally most relevant components of arterial wall tissues such as collagen and elastin enables ex vivo biomechanical testing of the remaining tissues, with the aim of assessing their individual mechanical contributions. Resulting passive material parameters can be utilized in mathematical models of the cardiovascular system. Using eighteen wall specimens fromnon-atherosclerotic human abdominal aortas (55 ± 11 years; 9 female, 9 male), we tested enzymatic approaches for the selective digestion of collagen and elastin, focusing on their application to human abdominal aortic wall tissues from different patients with varying sample morphologies. The study resulted in an improved protocol for elastin removal, showing how the enzymatic process is affected by inadequate addition of trypsin inhibitor. We applied the resulting protocol to circumferential and axial specimens from the media and the adventitia, and performed cyclic uniaxial extension tests in the physiological and supraphysiological loading domain. The collagenase-treated samples showed a (linear) response without distinct softening behavior, while the elastase-treated samples exhibited a nonlinear, anisotropic response with pronounced remanent deformations (continuous softening), presumably caused by some sliding of collagen fibers within the damaged regions of the collagen network. In addition, our data showed that the stiffness in the initial linear stress–stretch regime at low loads is lower in elastin-free tissue compared to control samples (i.e. collagen uncrimping requires less force than the stretching of elastin), experimentally confirming that elastin is responsible for the initial stiffness in elastic arteries. Utilizing a continuum mechanical description to mathematically capture the experimental results we concluded that the inclusion of a damage model for the non-collagenous matrix material is, in general, not necessary. To model the softening behavior, continuous damage was included in the fibers by adding a damage variable which led to remanent strains through the consideration of damage.

1. Introduction

Understanding the individual mechanical contributions of arterial wall constituents such as collagen and elastin is important for the comprehension of the overall biomechanical response, and essential for the formulation and improvement of constituent-based biomechanical models. For example, arterial tissues cleared of elastin can offer insights into common vessel wall pathologies including abdominal aortic aneurysms or the natural stiffening of the wall with age, see, e.g., [1–6]. The first biomechanical study of arterial wall constituents of human external iliac arteries was performed by Roach and Burton [7], who selectively tried to remove collagen via formic acid digestion and elastin via crude trypsin digestion containing elastase.

The soft and linear mechanical response of arterial wall tissues after removal of collagen is mostly accepted, but a more complicated picture arises regarding the removal of elastin and the associated mechanical response of the remaining tissue. Two studies [7,8] reported mechanical data of tissues cleared from elastin. Recent measurements on elastase-treated human aortic tissues, see [9], yielded a continuous softening under the same loading conditions, leading to the conclusion that the integrity of the tissue is destroyed upon removal of elastin. None of these studies, however, considered the potentially damaging effect of trypsin during the

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http://dx.doi.org/10.1016/j.actbio.2015.01.003
1742-7061/

degradation process (or used trypsin inhibitor to control its potentially damaging effect), something that the study [10] pointed out is important when using elastase in order to remove elastin.

In summary, the (highly) cross-linked elastin is very resistant to chemical attacks; considering that elastase can attack denatured collagen and also targets proteoglycan molecules [11,12], the specificity of the enzyme elastase for digesting elastin without affecting other wall constituents seems to be an open issue. Hence, one objective of the present study is the development of an experimental protocol that addresses these limitations and to investigate the individual mechanical response including potential softening behavior of the remaining tissue in the physiological and supra-physiological domain. In particular, we performed a systematic study of the influence of various amounts of trypsin inhibitor on control samples to rule out any undesired or unexpected influence of the inhibitor itself, followed by a series of tests to determine the most suited elastase concentration with trypsin inhibitor and digestion times for the removal of elastin. Mechanically, we performed uniaxial extension tests and applied a cyclic loading protocol with 10 cycles per load step until rupture.

With respect to the description of arterial tissues within a continuum mechanical framework, the papers [13,14] introduced a decoupled form of the strain energy. The isotropic matrix material, which is mainly composed of elastin, and the two families of embedded collagen fibers in the aorta (see [15,16]) are accounted for by separate contributions. This approach was extended by the consideration of dispersed fibers around a preferred direction [17]. The decoupled representation was extended by several researchers; one example is the additional term in the constitutive law accounting for smooth muscle cells [18].

Damage-induced softening is mostly modeled in a continuum damage mechanics framework. An attractive approach for the description of anisotropic damage in collagenous tissues that avoids the use of damage tensors is given in [19], where the discontinuous damage is considered to occur in the collagen fibers. This approach was later extended in [20] to additionally account for remanent strains in the collagen fibers and saturating behavior of the stress hysteresis under fixed maximum load levels. Therein, the damage variable was modified to also increase during reloading paths. Inclusion of these effects provided good agreement of the model response with experimental data of the media of a human carotid artery [20]. Other approaches for the modeling of anisotropic damage in soft fibrous tissues [21,22] numerically integrate several discrete fiber orientations over a unit sphere to determine the macroscopic fiber stresses, while a one-dimensional damage variable is associated to each of the integration directions. Such an approach of summing over many discrete fiber directions, initially proposed by [23], is, however, computationally more expensive. In [24] remanent strains after overstretching of the tissue are described within a finite plasticity framework under the assumption of remaining deformations in the fibers. In [25] remanent deformations in the fibers are modeled within an alternative continuum damage mechanics framework, where reduction terms are included in the structural tensors.

An alternative way to describe damage is referred to as pseudo-elasticity [26]. Such an approach was used in, for example, [27] to model damage in soft collagenous tissues. As a first micromechanically-motivated damage model, Rodríguez et al. [28] considered a stochastically distributed waviness and subsequent rupture of the individual fibers as a driver for damage evolution, in which damage is also assumed to occur in the matrix material. Examples of further models that consider damage in the fibers as well as in the matrix include [29,30,18]. In [31,32] damage is modeled in the matrix *and* in the fibers, and an additional softening variable is introduced, which yields remanent strains of the composite material (matrix and fibers). In [32] the damage variable is composed of a discontinuous as well as of a continuous contribution, whereby the continuous part describes the preconditioning behavior with a fixed maximum load level. In addition to hysteresis and remanent strains of the composite material, the study [33] modeled stress relaxation and creep of remanent strains. Stress relaxation therein explains the effect whereby a reduced amount of stress is required in a reloading path to obtain the same deformation state as in the primary loading path. To account for the latter effect, the damage evolution must include continuous characteristics. Creep of remanent strains explains the effect of reducing remanent strains, when the material is kept in a state of zero stress after unloading. In the present study, the model as documented in [20], which includes a saturating damage evolution also in the reloading paths and thereby also accounts for reducing remanent strains, is considered, and adjusted to the experimental results.

2. Material and methods

2.1. Soft biological tissues

Eighteen abdominal aortic specimens were harvested within 24 h of death (55 ± 11 years, mean $\pm$ s.d., 9 females ranging from 28 to 72 and 9 males ranging from 49 to 67). Precautions were taken not to include samples showing cardiovascular related pathologies like atherosclerotic lesions of type IV or higher [34]. The use of autopsy material from human subjects was approved by the Ethics Committee of the Medical University Graz.

All samples were stored in a phosphate buffered saline (PBS) solution (pH: 7.3) and tested within 12 h after receiving. Prior to testing, the aortic specimens were first cleaned from surrounding adipose and connective tissue, and anatomic al landmarks (such as branching of the common iliac arteries) were used to ensure consistent sample locations. Next, the intima was removed and circumferential and axial strips (dog bone shaped with a gage width of 4 mm) were cut out from the media-adventitia composite. Finally, the strips were carefully dissected into the media and the adventitia, and the thickness was measured using a video extensometer; for details regarding dissection tissue preparation for uniaxial tensile tests see [35,36].

2.2. Biochemical treatment

Based on the work in [10], one goal was to develop a well-suited experimental protocol for digesting only elastin in human abdominal aortic tissues from different patients, by using elastase in combination with a trypsin inhibitor. First, we wanted to rule out any potential influence of the inhibitor itself upon the mechanical response of the tissue. We performed cyclic uniaxial extension tests on adjacent tissue strips, where the individual strips were previously submerged in 37 °C physiological solution for 3 h, containing increasing concentrations of the trypsin inhibitor 4-(2-Aminoethyl)benzenesulfonyl fluoride hydrochloride (Sigma–Aldrich, MO, USA), ranging from 200 to 2400 μg/ml PBS.

Next we tested different combinations of purified elastase concentrations (Source: porcine pancreas. Specific activity: 10 U/mg. Manufacturer: Worthington Biochemical, NJ, USA) and associated treatment times (without trypsin inhibitor), based on the work by [1,37]. Given that tissue samples from different subjects vary in thickness and elastin content (which decreases with age), our focus was to determine the minimum elastase concentration and treatment time that would ensure proper enzymolysis of elastin for a broad spectrum of sample morphologies. Through standard histological evaluations using Elastica van Gieson (EVG) staining we found that 3 h in a physiological solution at 37 °C with an