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Molecular-based nonlinear viscoelastic chemomechanical model incorporating thermal denaturation kinetics of collagen fibrous biomaterials

A. Mlyniec [a, *], K.A. Tomaszewski [b], E.M. Spiesz [c], T. Uhl [a]

[a] AGH University of Science and Technology, al. Mickiewicza 30, 30-059 Krakow, Poland
[b] Department of Anatomy, Jagiellonian University Medical College, ul. Kopernika 12, 31-034 Krakow, Poland
[c] School of Engineering and Materials Science, Queen Mary University of London, Mile End Road, London E1 4NS, United Kingdom

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ABSTRACT

In this article, we propose a constitutive model for soft collagenous biomaterials that takes into account their thermal denaturation. We created a large strain viscoelastic material model of collagenous microfibrils. The stress–strain curves from the coarse-grained simulations of collagen microfibrils have been used to calibrate the proposed rheological constitutive model. This modeling framework allows to predict viscoelastic mechanical behavior of collagenous materials for strain rates ranging from static tests to $1e9\ s^{-1}$. Moreover, we enhanced the proposed constitutive model using the kinetic theory, to calculate the influence of thermal denaturation on long term viscoelastic properties of collagen. The proposed model can be directly used in micro finite element models of collagenous biomaterials as it allows to calculate the biomechanical properties of those materials for physiologically relevant deformation rates. The model incorporates both changes of stiffness and a decrease in viscoelasticity of collagenous materials exposed to elevated temperatures (for example laser surgeries or thermal treatments). The achieved agreement with experimental data demonstrates that the molecular-based chemomechanical framework constitutes a powerful tool for prediction of stability and mechanical behavior of collagen-based biomaterials.

1. Introduction

Collagen, the most abundant fibrous protein in animals, is a biomaterial with excellent biocompatibility, low toxicity and controllable biodegradability [1]. Collagenous biomaterials are used in cosmetics, as drug carriers, artificial tissues and to accelerate wound healing [1–5]. Biomechanical properties, durability and thermal stability of collagenous biomaterials are of significant interest for material engineers, bioengineers, and physicians performing laser surgery and thermal therapy. Thermal treatment or focused laser light, generates heat, which can cause denaturation and/or degradation of collagen. Denaturation kinetics, as well as biophysical properties of collagenous materials and tissues result from the complex hierarchical structure of collagen fibrils. Collagen fibrils are composed of tropocollagens connected with each other by hydrogen bonds, as well as enzymatic and non-enzymatic crosslinks [6]. Crosslink density varies with age and with the course of specific diseases, like diabetes, affecting the biomechanics of collagenous tissues [7]. Connective tissues are rich in collagen, and become stiffer and stronger during aging [8] due to change of crosslink density. *In silico* experiments show that an excessive increase of crosslink density "leads to negative effects because the material is not capable of dissipating much energy during deformation, leading to a brittle collagen that is strong but not tough" [9]. Moreover, an increase in crosslink density alters the nanomechanical behavior of collagen, mainly at large deformations [10,11]. Increased crosslink density modifies not only tissue biomechanics, but also increases the resistance to proteolytic degradation [12] and denaturation [13,14]. Collagen denaturation is observed during thermal treatment and in diseases such as arthritis, atherosclerosis, and neoplasms [15–18]. However, this is also an issue in collagen-based tissue engineered biomaterials, which are vulnerable to local overheating during production, storage and use. Experimental investigations of collagen type I in

* Corresponding author.
E-mail address: mlyniec@agh.edu.pl (A. Mlyniec).

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rat tail tendon [19] showed that isothermal denaturation was a single reversible step, that can be described by single first-order kinetic equations. The authors proposed three kinetic models [19]: the Arrhenius model; the "absolute rate theory" model (alternatively known as "transition state theory" or "activated complex theory"); and a mathematical simple "empirical equation used by microbiologists to model the activation of microorganisms by heat" called the "D and z formulation". Moreover, the authors present the relationship between the parameters of these three models [19]. The results of isoconversional DSC analysis [20] show that thermal denaturation of collagen type I has a multi-step nature and can be described by a three state Lumry–Eyring model. The first, second and third states refer respectively to the native triple helix state, the partially unfolded state, and the denatured coil state of collagen. The first step – reversible collagen unfolding is followed by an irreversible one, leading to complete protein denaturation. Another non-isothermal kinetic analysis of collagen type I denaturation [21,22] confirmed that a Lumry–Eyring three state model most accurately describes the process of denaturation. However, the authors pointed out that at isothermal conditions one can use the two-state irreversible model [21,22].

The relationship between mechanical load and deformation in soft tissue biomaterials is described by constitutive equations. There exist several phenomenological material models which relate energy stored in the material to the deformation gradient. Those models include microstructural ones, originally developed for rubbery materials, for example the Arruda–Boyce model [23] and its further extensions [24,25]. Another class of biomechanical models represent constitutive equations describing the non-linear anisotropic behavior of arterial tissues – these were proposed by Holzapfel, Gasser and Ogden [26]. The extension of Holzapfel–Gasser–Ogden model presented in Ref. [27] is reported to be suitable for modeling of viscoelastic anisotropic materials subjected to finite strains. Incorporating fibers anisotropy into constitutive modeling of tissues is particularly relevant for modeling the complex tissues like arterial walls [28] or hard collagenous tissues like bone [29]. Collagen fibers dispersion is particularly relevant in layered arterial walls and bones, but can be omitted in tendons because collagen fibers exhibit significant unidirectional alignment. Rheological models presented in Ref. [10] are calibrated using results of MD simulations and take into account the uniaxial mechanical behavior of collagen (including crosslinks contribution). The proposed model consists of three elements: an elastic spring; a frictional element which describes plasticity; and a "delay" element "created to account for the delayed response due to the unraveling of the telopeptide". Another constitutive model calibrated by means of *in silico* creep tests [30] describes the behavior of a collagen fibril using a staggered array of Kelvin–Voigt viscoelastic elements.

There are relatively few viscoelastic constitutive models of connective tissues in the literature, which can be used directly in larger scales finite element (FE) modeling of connective tissues [31]. Moreover, existing models neglect the influence of degradation kinetics on collagenous tissue biomechanics. It is known that denaturation starts at low temperatures but intensifies at temperatures around 50 °C, depending on the hydration level and tissue type, and considerably alters biomechanical properties. Thermal stability of collagenous tissues is a key issue for physicians performing laser surgery and thermal therapy because of the local overheating of the material. This is also an issue in collagen-based tissue engineered biomaterials and biocomposites, which are vulnerable to overheating during production, use and storage. Therefore, it is necessary to provide appropriate chemomechanical constitutive models incorporating chemical reaction kinetics to describe the influence of the overheating on material's behavior at higher spatial levels.

The objective of this study was to create a modeling framework that takes into account changes of biomechanical properties of collagen microfibrils resulting from the temperature exposure. The model proposed in this article, incorporates the changes in nonlinear viscoelastic behavior of collagen microfibrils resulting from their molecular structure and denaturation kinetics. We performed the Coarse-Grained simulations for different strain-rates, to calibrate a rheological constitutive model. The proposed computational scheme has been used to analyze the long-time influence of thermal denaturation on viscoelastic properties of collagen microfibrils.

2. Constitutive modeling of collagen microfibrils incorporating denaturation kinetics

In the following section, we have described the hierarchical structure of the proposed multiscale model of collagen microfibrils. To derive appropriate material parameters we conducted three dimensional *in silico* tensile tests. Tension of collagen microfibrils was performed for different strain rates using Coarse-Grained (CG) models calibrated by means of atomistic simulations, according to the procedure presented in our previous article [32]. The obtained results are used to calibrate the large strain viscoelastic model of collagen microfibrils. Moreover, the created model was enhanced by reaction kinetics which allows to predict the influence of denaturation on the biomechanical behavior of collagen microfibrils.

2.1. Viscoelasticity of collagen microfibrils – molecular dynamics and Coarse-Grained model

In order to understand the influence of deformation rate on biomechanics of collagen microfibrils, we carried out a series of Steered Molecular Dynamics [33] and Coarse-Grained (CG) Molecular Dynamics (MD) simulations. All simulations were carried out using the LAMMPS simulation package (http://lammps.sandia.gov) [34]. Visualization of atomistic and CG results was done using VMD software (http://www.ks.uiuc.edu/Research/vmd) [35].

The structure of a single tropocollagen molecule 1QSU, determined with 1.75 Å resolution [36] served as a starting point for the simulations of tensile and shear tests in solution. The water molecules were added to the structure using VMD software.

Atomistic simulations were carried out using AMBER Force Field (FF) parameters [37] revised to include a non-standard amino-acid (hydroxyproline), based on the work of Park et al. [38]. Performed atomistic simulations consisted of three steps: (1) heating up the system to 300 K using NVE ensemble and Berendsen thermostat during 60 ps; (2) equilibrating the system (for 10 ps) in constant temperature using Berendsen thermostat with a temperature damping parameter equal to 0.01 ps; (3) loading of the tropocollagen molecules.

Schematic representation of the atomistic tropocollagen models is shown in Fig. 1a. Tensile and shear tests in aqueous environment were performed on such modeled single tropocollagen molecules. Fully atomistic approach poses certain limitations in the time and length scales of the simulations (usually femto seconds and nanometer scales). In order to model collagen microfibrils in more physiologically relevant time and length scales we used CG MD simulations, representing the highly detailed atomistic information in a simplified, homogenized CG manner. The results of *in silico* fully atomistic experiments were used to derive the CG potentials for the mesoscale model of collagen fibrils. Each tropocollagen molecule was replaced by a CG segment with ten grains, which resulted in 43 fold reduction in number of particles relative to fully atomistic models. The intermolecular and intramolecular