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Negative Poisson's ratios in tendons: An unexpected mechanical response

Ruben Gatt^{a,}*, Michelle Vella Wood^a, Alfred Gatt^{b,g}, Francis Zarb^c, Cynthia Formosa^{b,g}, Keith M. Azzopardi ^a, Aaron Casha ^d, Tonio P. Agius ^e, Pierre Schembri-Wismayer ^d, Lucienne Attard ^f, Nachiappan Chockalingam ^{g,b}, Joseph N. Grima ^{a,h}

^a Metamaterials Unit, Faculty of Science, University of Malta, Msida MSD 2080, Malta

^b Department of Podiatry, Faculty of Health Sciences, University of Malta, Msida MSD 2080, Malta

^c Department of Radiography, Faculty of Health Sciences, University of Malta, Msida MSD 2080, Malta

^d Department of Anatomy, Faculty of Medicine & Surgery, University of Malta, Msida MSD 2080, Malta e Department of Physiotherapy, Faculty of Health Sciences, University of Malta, Msida MSD 2080, Malta

^f Department of Orthopaedics, MaterDei Hospital, Msida MSD 2090, Malta

^g Faculty of Health Sciences, Staffordshire University, Science Centre, Leek Road ST4 2DF, UK h Department of Chemistry, Faculty of Science, University of Malta, Msida MSD 2080, Malta

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ABSTRACT

Tendons are visco-elastic structures that connect bones to muscles and perform the basic function of force transfer to and from the skeleton. They are essential for positioning as well as energy storing when involved in more abrupt movements such as jumping. Unfortunately, they are also prone to damage, and when injuries occur, they may have dilapidating consequences. For instance, there is consensus that injuries of tendons such as Achilles tendinopathies, which are common in athletes, are difficult to treat. Here we show, through in vivo and ex vivo tests, that healthy tendons are highly anisotropic and behave in a very unconventional manner when stretched, and exhibit a negative Poisson's ratio (auxeticity) in some planes when stretched up to 2% along their length, i.e. within their normal range of motion. Furthermore, since the Poisson's ratio is highly dependent on the material's microstructure, which may be lost if tendons are damaged or diseased, this property may provide a suitable diagnostic tool to assess tendon health.

Statement of significance

We report that human tendons including the Achilles tendons exhibits the very unusual mechanical property of a negative Poisson's ratio (auxetic) meaning that they get fatter rather than thinner when stretched. This report is backed by in vivo and ex vivo experiments we performed which clearly confirm auxeticity in this living material for strains which correspond to those experienced during most normal everyday activities. We also show that this property is not limited to the human Achilles tendon, as it was also found in tendons taken from sheep and pigs. This new information about tendons can form the scientific basis for a test for tendon health as well as enable the design of better tendon prosthesis which could replace damaged tendons.

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1. Introduction

In view of their biological importance, the biomechanical properties of tendons have been the subject of intensive research in recent years, particularly following the early work by Rigby et al.

⇑ Corresponding author. E-mail address: ruben.gatt@um.edu.mt (R. Gatt).

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[\[1\]](#page--1-0) in the mid-twentieth century. Early research described animal models such as rat and horse tendons, with later studies also considering a number of human tendons, particularly those susceptible to injury, such as energy-storing tendons $[2]$. Most of these studies have focused on their stiffness, where it was reported that tendons appear to adapt their properties in response to the mechanical demands placed on them. Over a period of time, they become stronger and stiffer when subjected to increased stress

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(by repeated strenuous activities), and weaker and less stiff when the stress is reduced [\[3\]](#page--1-0). In addition, other factors such as ageing, pregnancy, mobilisation and immobilisation, comorbidities (examples of which can be diabetes mellitus, connective tissue disorders, renal disease), and pharmacologic agents (steroids, non-steroidal anti-inflammatory drugs) are known to affect the biomechanical properties, in particular the stiffness, of tendons [\[4\].](#page--1-0) For instance, Stenroth and co-workers [\[5\]](#page--1-0) reported that a relationship between muscle thickness and cross-sectional area exists, with the older population showing increased tendon thickness and smaller muscle size. This hints at altered tendon performance as compared to different muscle size, and suggests tendon compensation for optimised locomotion in daily activities.

From a structural perspective, tendons are hierarchical structures: triple helices of tropo-collagen form fibres, which in turn form fibrils, fascicles and eventually tendons. They display a wave-form or crimped structure when relaxed [\[1\]](#page--1-0), with most recent studies describing it as helical $[6,7]$. Stretching tendons at low strains results in the disappearance of this crimping [\[8\]](#page--1-0). This initial deformation corresponds to the 'toe-region' of the stress– strain curve and is followed by the 'elastic region', a zone in which, due to further stretching, the fibres and fascicles slide against each other, eventually returning to their original shape when the load is released. Stretching beyond this range results in permanent deformation [\[9\]](#page--1-0). In vivo, tendons usually deform within the toe-region [\[10,11\],](#page--1-0) with the exception of the energy-storing tendons, which are known to undergo higher strains [\[12\]](#page--1-0). This is one of the reasons for the low incidence of injuries to positional tendons when compared to energy storing tendons [\[13\]](#page--1-0).

A few studies have also investigated the Poisson's ratio of tendons, both experimentally [\[2,14–19\]](#page--1-0) and numerically [\[20,21\].](#page--1-0) The Poisson's ratio [\[22\]](#page--1-0) is a fundamental material property in its own accord and describes the change in size of a system in a direction perpendicular to an applied stress. Mathematically, this is defined as the negative of the ratio of the transverse strain to axial strain. Since most materials get thinner (negative strain) when uniaxially stretched (positive strain), one could wrongly assume that the Poisson's ratio is always positive. Nevertheless, it is well known that a negative Poisson's ratio, i.e. the property of getting wider rather than thinner when stretched (auxetic behaviour), is permitted by the classical theory of elasticity, with the range of permissible Poisson's ratio for isotropic materials (i.e. having the same properties in all directions) being $-1 \leq v \leq 0.5$ [\[23\].](#page--1-0) This range is even wider for non-isotropic materials. Negative Poisson's ratio has in fact been found in a wide variety of materials including graphene $[24]$, metals $[25]$, foams $[26]$, zeolites $[27]$, silicates $[28]$ and even biological materials such as arteries [\[29\]](#page--1-0) and skin [\[30\].](#page--1-0) Auxeticity in such materials can result in several beneficial features, ranging from enhanced resistance to indentation to the natural ability to form dome shaped surfaces [\[31\]](#page--1-0). Here it must be noted that unlike stiffness, the Poisson's ratio is a two-dimensional property and in anisotropic materials, its sign and magnitude may depend not only on the direction of stretching, but also on the orthogonal direction being measured. Unfortunately, in view of the complexity associated with studying the Poisson's ratios, studies reporting this property in tendons have been limited in number $[2,14-19]$, and normally make various assumptions, which may have resulted in incomplete reporting of the Poisson's ratio. For example, in most ex vivo studies on the Poisson's ratios of tendons it had been assumed that the tendon exhibits transverse isotropy, with reported values typically ranging between 0.4 and 4.3 $[2,14-17]$ when the Poisson's ratio is measured in the elastic region.

In this paper we show, through both ex vivo and in vivo studies, that healthy tendons of both human and animal origin have a negative Poisson's ratio when stretched along their length, in the plane

Fig. 1. Figure showing the different parameters used to define the various tendon dimensions in this study. Note that the xy plane is equivalent to the coronal plane (this being the plane where auxeticity was measured), the yz plane is equivalent to the sagittal plane (this being the plane where conventional Poisson's ratio was measured), and the xz plane is equivalent to the axial plane.

of the width of the tendon, i.e. for example the coronal plane in the case of the Achilles tendon (see Fig. 1).

2. Materials and methods

2.1. Ex vivo experiments

Ex vivo experiments were carried out on a number of tendons: human, pig and sheep in origin. The human tendons were obtained from cadavers donated to the Anatomy Department of the University of Malta, whilst sheep and pig tendons were obtained from a local abattoir. More specifically, in the case of the human samples, the mechanical properties at room temperature of the Achilles and Peroneus brevis tendons, obtained from human fresh frozen cadaveric tissue, were measured in this study. Two samples were tested, due to the difficulty of acquiring such tissues. In the case of the animal samples, the mechanical properties of the deep flexor tendon were measured on five samples from each species. These samples were obtained and tested within 24 h of the animal death, and were kept in a refrigerator at 5° C until the tests were carried out.

The tendons were first dissected to remove connective tissue and surrounding sheaths. The ends of the tendons were wrapped with nylon cord prior to clamping, with the aim of preventing the tendon from expanding laterally within the clamp on tightening. Additionally, the size of the clamp itself was just large enough to fit the tendon and nylon wrapping with no space for further expansion. The clamps used were as those used by Fessel et al. [\[32\]](#page--1-0). The Poisson's ratios of the tendons were tested using a tensile loading machine (Testometric, UK) having a 100 kg F load cell (S/N 31,931), equipped with a duly calibrated camera videoextensometer (Messphysik, Germany). Before the actual test phase, a pre-conditioning step was applied. This involved stretching the tendon until a force of 1 N was reached, holding this force for 30 s and then returning to 0 N. The cycle was repeated for a total of 10 times. Tests were carried out using strain rate control at a rate of 5 mm/min. Measurements, through video extensometry, were taken for the length (l) and width (t) of the tendons, which were appropriately marked for the Messphysik pattern recognition software, as shown in [Fig. 2.](#page--1-0) The pattern recognition protocol

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