On the anisotropy of skeletal muscle tissue under compression

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ABSTRACT

This paper deals with the role of the muscle fibres and extracellular matrix (ECM) components when muscle tissue is subjected to compressive loads. To this end, dissected tissue samples were tested in compression modes which induced states of fibres in compression (I), in tension (II) or at constant length (III), respectively. A comparison of the stress responses indicated that the tissue behaviour is significantly different for these modes, including differences between the modes (I) and (III). This contradicts the paradigm of many constitutive models that the stress response can be decomposed into an isotropic part relating to the ECM and an anisotropic fibre part the contribution of which can be neglected under compression. Conversely, the results provide experimental evidence that there is an anisotropic contribution of the fibre direction to the compressive stress. Interpreting these results in terms of recent microscopical studies, potential connections between the observed behaviour and the structure of muscle ECM are established.

1. Introduction

Fibre-reinforced materials are used in many fields of engineering. They combine favourable characteristics of the fibres and a matrix material in which the fibres are embedded. The basic idea of fibre-reinforced materials also applies to a large variety of soft biological tissues, where fibres are surrounded by an extracellular matrix (ECM). This includes skeletal muscle tissue, the striking feature of which is the ability of force generation based on a large number of contractile muscle fibres embedded in a composition of connective tissues forming the ECM, blood vessels and nerves. Despite the comparatively small amount of ECM, which accounts for only 1–10% of the muscle dry weight [41], it has several important functions [15,39,52]. There is high consensus that it plays a central role in force transmission [41,26,42] as it ensures the integrity and proper functioning of the entire muscle [38,57,58,36,26].

Muscle ECM can be categorised into three types: the *endomysium* is located around the single muscle fibres, the *perimysium* packages groups of muscle fibres, also known as fascicles, and the *epimysium* surrounds the whole muscle. The endomysium is composed of collagen fibre types I, III, IV, VI and XII [46,47], and represents up to 1.2% of the muscle dry weight [61]. It surrounds the muscle fibres, penetrates between them and thus forms a honeycomb-like structure [62,55,56]. As the endomysium is located

nearest to the muscle fibres, it is a highly important element for lateral force transmission [74,61], as it has been also demonstrated by applying numerical models [67,68]. The perimysium surrounds the muscle fascicles and is formed by a collagen network reaching up to 4.6% of muscle dry weight [61]. The fibres in this network vary in composition but contain a great part of collagen type I and to a smaller extent type III, VI and XII [46,47]. According to Passerieux et al. [58], the collagen network shows four hierarchical levels of organisation that seem to play a major role in force transmission: (i) a regular lattice of interwoven longitudinal and inclined circumferential fibres; (ii) a collagen plexus where circumferential fibres meet; (iii) a sub-plexus at each end of the lattice branches (so-called perimysial junctional plates); and (iv) a honeycomb arrangement of tubes composed of collagen cables connecting the tendons. For a more detailed description of the perimysium see e.g. Refs. [26,38,57,58]. Finally, the epimysium characterises the outermost sheath and surrounds the whole muscle. Following Nishimura et al. [55], the epimysium of bovine semitendinosus muscle consists of two layers: while the fibres of the inner layer run in parallel to the muscle axis, the fibres of the outer layer lie in two plies of highly oriented fibres at approximately $\pm 55°$ to the muscle fibre axis [60,61]. In contrast, more recently, Gao et al. [24,25] characterised the structure of the epimysium of rat tibialis anterior muscles and detected a third, middle layer consisting of very fine interwoven collagen fibres. Since separation of the epimysial layer from the muscle is comparatively simple, the mechanical behaviour can be characterised in a straightforward

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manner by applying standard tension tests [62,24]. These experiments revealed the classical exponential-type stress–strain curves known from several other collagenous tissues.

In general, the complex microstructure and small dimensions of the ECM layers complicate direct measurements so that, to date, there is only limited experimental data on the mechanical behaviour of the ECM that can shed light on the mechanisms of force transduction. One method is based on decellularisation, i.e. the removal of everything but the ECM from the tissue by a combination of physical, chemical and enzymatic processes and subsequent mechanical testing of the remainder [19,27]. By this means, Gillies et al. [27] conducted tension experiments on ECM samples of murine tibialis anterior muscle fascicles delivering non-linear exponential-like stress–strain curves. A less destructive, indirect method to characterise ECM was recently presented by Meyer and Lieber [53] based on the difference between the mechanical responses of single fibres and a fascicle. To this end, the authors recorded stress–length curves for single muscles fibres, muscle fascicles (connected by ECM) and a group of unconnected fibres. Their results demonstrate that ECM is much stiffer than muscle fibres and exhibits highly non-linear behaviour.

Considerably more data is available for the mechanical behaviour of skeletal muscle at the tissue level. This includes data from whole muscle experiments, where the muscle tissue itself and the adjacent aponeurosis–tendon complex at the proximal and distal ends are tested jointly [13,20,12]. Regardless of whether the muscle is activated or not, the results of these experiments can only deliver average responses and hardly allow to estimate the contribution of the ECM.

More local results are obtained from studies on dissected tissue samples, which comprise muscle fibres and parts of ECM. Typical testing set-ups include compression [49,50,16,51,54,11], shear [75,54] and tension [54,2,72]. The latter type of study is preferably used to identify the material parameters of a constitutive model to establish a relation between stress and strain in the tissue. Since the fibre arrangement is approximately unidirectional on a local level, skeletal muscle is widely considered as a transversely isotropic material. For characterisation of the anisotropic properties, the samples are thus typically loaded parallel, perpendicular and with a predefined angle to the fibre orientation [49–51,11].

Although some skeletal muscle models break with this idea [37,9,10,22], the free energy function, and thus the stress, are classically decomposed into active and passive parts [40,6,63,8,17, 30,7,69]. In the majority of these decoupled models, the passive contribution of the energy function is related to an isotropic part associated with a matrix material and a transversely isotropic part characterising the passive fibres. Subject to mechanical loads, both the isotropic and anisotropic parts are assumed to be responsible for load transmission in tension, whereas the compressive resistance is often solely attributed to the isotropic matrix and the fibre contribution is set to zero [63,17,30,69]. This assumption is in line with the widespread idea that fibres in soft tissues cannot bear substantial compressive loads. While this view may serve as a good approximation for some types of fibres, its general applicability seems questionable, particularly if the fibres are embedded in a matrix. Based on experimental results by Schinagl et al. [66], evidence for the contrary was discussed by Wu and Herzog [77] with respect to articular cartilage, arguing that the local compressive modulus was particularly high where the fibre and loading directions are in parallel.

With regard to skeletal muscle, the meaning of the "fibre contribution" in tissue-scale anisotropic constitutive models needs particular attention. The structurally organised ECM mentioned above may interact strongly with the fibres during deformation and load transfer so that the anisotropic response is jointly generated by fibres, i.e. muscle cells, and the surrounding ECM. During

the preparation of this manuscript, Gindre et al. [28] proposed a first structural constitutive model that accounts for such interaction in terms of endomysial collagen fibres wrapping around the muscle fibre. These collagen fibres contribute to the stress in tension and, as a result of the assumed constant cylinder volume, also in compression, thereby maintaining anisotropy.

To the best of the authors' knowledge, there exists no experimental study designed to approve or disprove whether the neglect of the anisotropic contribution under compression in constitutive models for skeletal muscle tissue is admissible or not. The classically used unconstrained uniaxial compression experiments on tissue samples are generally incapable of resolving this issue as the fibres are always either compressed or extended as a result of the lateral expansion. To overcome this limitation, we tested dissected muscle tissue samples in semi-confined compression modes, which allowed us to induce states of fibres in compression (I), in tension (II) or at constant length (III), respectively. The main hypothesis on which we built our conclusion states that, if the contribution from the fibre direction is negligible, there is no difference between modes (I) and (III) since the fibres are not stretched in either case. However, a comparison of the stress responses indicated that the tissue behaviour is significantly different for all three modes, including significant differences between modes (I) and (III). To the best of the authors' knowledge, neither this important characteristic nor the response of skeletal muscle tissue in pure shear compression, sometimes referred to as planar compression, has been reported before.

The paper is organised as follows: a description of the experimental set-up and the experimental results are provided in Sections 2 and 3, respectively, followed by a critical discussion in Section 4, with concluding remarks presented in Section 5.

2. Materials and methods

The general scheme of the mechanical experiments is illustrated in Fig. 1. Cubic tissue samples were placed between two parallel plates and compressed along the vertical direction, e_1. While the samples can freely deform along the lateral direction, e_2, the plates pose constraints which prevent an extension in the e_3 direction. Mounting the specimens such that the muscle fibres are parallel to the vectors e_1, e_2 or e_3, the muscle fibres are compressed (mode I), stretched (mode II) or kept constant in length (mode III), respectively. To assess the effect of fibre length, we used three different sets of cubes, with edge lengths of 4, 8 and 12 mm. The realisation of these experiments poses high demands on the geometry of the samples to ensure that they just fit between the plates but are neither pre-compressed nor contactless. The preparation technique to obtain precise sample geometries as well as the custom-made compression device are described in the following.

2.1. Sample preparation

Hind legs ($n = 7$) of five female domestic pigs (*Sus scrofa domestica*) with an average weight of 168 ± 4.0 kg (mean ± SD) were obtained from a slaughterhouse immediately after animal sacrifice. After the legs had been transported in a cooling box to the laboratory, the biceps femoris muscles were excised and stored at 1 °C in a refrigerator wrapped in physiological saline-soaked cloths. Immediately after removal of the muscles, cubic samples with three characteristic edge lengths of 4, 8 and 12 mm were cut and stored at 1 °C in physiological saline solution. Alginate, commercially available as an impression material, was used to stabilise the tissue during cutting with a utility cutter. To avoid rigor mortis [44,76], the muscles/samples were cooled at 1 °C throughout the