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Review article

Sample, testing and analysis variables affecting liver mechanical properties: A review

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ABSTRACT

Given the critical role of liver mechanics in regulating cell response and directing the development of tissue fibrosis, accurately characterising its mechanical behaviour is of relevance for both diagnostic purposes as well as for tissue engineering and for the development of in-vitro models. Determining and quantifying the mechanical behaviour of soft biological tissues is, however, highly challenging due to their intrinsic labile nature. Indeed, a unique set of values of liver mechanical properties is still lacking to date; testing conditions can significantly affect sample status and hence the measured behaviour and reported results are strongly dependent on the adopted testing method and configuration as well as sample type and status. This review aims at summarising the bulk mechanical properties of liver described in the literature, discussing the possible sources of variation and their implications on the reported results. We distinguish between the intrinsic mechanical behaviour of hepatic tissue, which depends on sample variables, and the measured mechanical properties which also depend on the testing and analysis methods. Finally, the review provides guidelines on tissue preparation and testing conditions for generating reproducible data which can be meaningfully compared across laboratories.

Statement of Significance

Soft tissue mechanics is widely investigated, but poorly understood. This review identifies and discusses sample and testing variables which can influence the mechanical behaviour of hepatic tissue and consequently the measured mechanical properties. To encourage the biomaterial community towards more standardized testing of soft tissues and enable comparisons between data from different laboratories, we have established new testing methods and experimental recommendations for sample preparation and testing. The review could be of wide interest to scientists involved in biomaterials research because it addresses and proposes guidelines for several issues related to the mechanical testing of soft tissues whose implications have not been considered together before.

Contents

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1. Introduction

Mechanical cues are relevant in the liver since several pathologies, including fibrosis and non-alcoholic fatty liver disease, significantly affect hepatic stiffness at different length scales (whole organ, regional and cellular levels). Extracellular matrix (ECM) stiffness, one of the principal biomechanical cues of the cellular micro-environment, is known to be critical in regulating cell behaviour as well as in directing the development of tissue fibrosis [1,2]. Both parenchymal and non-parenchymal cells exhibit mechano-sensitivity, changing their behaviour as a function of liver stiffness. For instance, hepatocytes remain differentiated and growth arrested when cultured on soft gels, whereas they spread, proliferate and adopt a dedifferentiated phenotype on stiff supports [3–5]. Mechanical stiffness also contributes to driving the myofibroblastic differentiation of portal fibroblast [6] and hepatic stellate cells (HSCs) [7]. Moreover, hepatic stem and progenitor cells have also been shown to exhibit mechano-sensitivity and alter their behaviour on the basis of substrate stiffness [8]. Accurately characterising liver mechanical behaviour is thus relevant for both diagnostic purposes as well as for tissue engineering and in-vitro applications.

Quantifying and modelling the mechanical properties of materials is essential for understanding, monitoring and predicting their performance and responses under specific loading conditions. Ideally, mechanical characterisation via constitutive modelling requires the definition and control of both geometric and environmental testing boundary conditions as imposed by theory. Although structural materials have been well characterised for decades using various testing methods, there is still a scarcity of reliable and reproducible data for highly hydrated and degradable soft materials like hydrogels and biological non load-bearing tissues (e.g. liver, kidney and brain), mainly due to testing challenges related to their shape, softness and labile nature. These materials are typically biphasic, with a solid network that is fully swollen and surrounded by liquid media. As a consequence, their mechanical behaviour is generally viscoelastic, with the solid network responsible for elasticity, while the network mobility as well as the contribution of water and other molecules give rise to viscosity [9]. Moreover, environmental conditions and pre-load can significantly affect the status of these materials (e.g. they may degrade, change water content over time, or deform irreversibly under small pre-loads) and hence their resultant mechanical properties [10]. Indeed, the identification of a suitable experimental testing method that does not alter the native material behaviour before or during testing is crucial to obtain repeatable results that can be mathematically modelled to derive a unique and meaningful set of constitutive parameters. Despite the number of methods and results published in the literature, there is still no consensus on the optimum testing and analysis framework to characterise soft hydrated materials, which may degrade or alter over time.

Several methods and models based on direct measurements on tissue samples (e.g. rheological, compressive or indentation tests) or image-based techniques (e.g. magnetic resonance or ultrasound-based elastography) are reported in the literature to characterise the mechanical behaviour of biological tissues either ex-vivo or in-vivo. Although in-vivo testing maintains the tissue in its natural state, it has many limitations, such as accessibility, ill-defined boundary conditions, ethical issues in using animals and potential risks to human subjects. Numerous studies characterising tissue behaviour in-vivo have been described, with datasets often limited to small deformations. Moreover, interpreting in-vivo data is challenging due to difficulties in obtaining appropriate alignment between the instrument and tested specimen, the presence of physiological noise and the inability to account for and control the internal condition of the organ [11–13]. On the other hand, ex-vivo experiments are preferable when developing new testing devices, protocols and tissue models, enabling easier and more direct testing procedures with better control of boundary conditions, in addition to being less ethically problematic than in-vivo measurements [14–18]. However, although there are many studies and methods published in the literature, a unique set of values of liver mechanical properties is still lacking and reported results are strongly dependent on several factors (many of them are outlined in Fig. 1), such as the adopted testing method and configuration as well as sample type and status.

The aim of this review is to summarise the major sources of variation affecting liver mechanical properties, grouping them in "testing and analysis variables" and "sample variables", and discussing their implications on reported data. In addition, we provide general testing guidelines for ex-vivo mechanical characterisation to allow the generation of repeatable and un-biased results. The adoption of these simple guidelines should enable more meaningful comparisons between different samples (e.g. biological tissues and engineered substrates) and laboratories, and should lead to a range of standard and acceptable values for liver mechanical properties that depend only on sample variables.

We distinguish between "mechanical behaviour" and "mechanical properties", as schematised in Fig. 2. In particular, the former term refers to the intrinsic material mechanical response which, in principle, cannot be known *a priori* and depends on sample variables only (e.g. sample type and source, pathophysiological condition). The first step to characterise material mechanics is to test the sample's mechanical behaviour, obtaining raw data (e.g. force-displacement curves) to analyse. It follows that, in addition to sample variables, the measured "mechanical behaviour" is also dependent on testing variables (e.g. testing condition and method chosen). Finally, a given analysis model (e.g. purely elastic, linear or non-linear viscoelastic) has to be chosen to derive the material "mechanical properties", i.e. the "final numbers" describing the material mechanics. According to these definitions, material