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Nanomechanical characterization of heterogeneous and hierarchical biomaterials and tissues using nanoindentation: The role of finite mixture models

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ABSTRACT

Mechanical characterization of biological tissues and biomaterials at the nano-scale is often performed using nanoindentation experiments. The different constituents of the characterized materials will then appear in the histogram that shows the probability of measuring a certain range of mechanical properties. An objective technique is needed to separate the probability distributions that are mixed together in such a histogram. In this paper, finite mixture models (FMMs) are proposed as a tool capable of performing such types of analysis. Finite Gaussian mixture models assume that the measured probability distribution is a weighted combination of a finite number of Gaussian distributions with separate mean and standard deviation values. Dedicated optimization algorithms are available for fitting such a weighted mixture model to experimental data. Moreover, certain objective criteria are available to determine the optimum number of Gaussian distributions. In this paper, FMMs are used for interpreting the probability distribution functions representing the distributions of the elastic moduli of osteoarthritic human cartilage and co-polymeric microspheres. As for cartilage experiments, FMMs indicate that at least three mixture components are needed for describing the measured histogram. While the mechanical properties of the softer mixture components, often assumed to be associated with Glycosaminoglycans, were found to be more or less constant regardless of whether two or three mixture components were used, those of the second mixture component (i.e. collagen network) considerably changed depending on the number of mixture components. Regarding the co-polymeric microspheres, the optimum number of mixture components estimated by the FMM theory, i.e. 3, nicely matches the number of co-polymeric components used in the structure of the polymer. The computer programs used for the presented analyses are made freely available online for other researchers to use.

1. Introduction

It is well established that the mechanical properties of tissues [1–5] and biomaterials [6–9] are important for proper functioning of both. It is therefore important to be able to characterize the mechanical properties of tissues and biomaterials not only to understand their functional behavior but also to diagnose any potential pathological conditions of tissues or to fine-tune the mechanical properties of synthetic biomaterials for their intended function.

Biological tissues and biomaterials are often made of several phases and components that make them highly heterogeneous [10–12]. Moreover, these heterogeneous structures are often made at different scales, giving rise to certain hierarchical patterns [13–16] of organization that enable tissues and biomaterials to perform certain functions. Furthermore, the macroscopic shapes of biological tissues are often complex [17–19]. From constitutive modeling perspective, description of the mechanical behavior of biological tissues and biomaterials often requires the use of time-dependent [20–23], anisotropic [24–28], and nonlinear [29–33] constitutive equations. Mechanical characterization of such complex structures at the various relevant scales is a challenging task. Nanoindentation using atomic-force microscopy (AFM) or nanoindentation devices [34,35] has been recently used for characterization of the mechanical properties of biological tissues [36–40] and synthetic biomaterials [41–45]. Since the size of probes is in the nano-scale to micro-scale range, the distribution of the mechanical properties of tissues and biomaterials as well as their constituents could be mapped at both nano- and micro-scales.

It has been shown that characterization of heterogeneous and hierarchical structures, such as the ones found in biomaterials and tissues, requires repetitive nanoindentation measurements such that a histogram of the mechanical properties can be established [46]. The frequency peaks found in such a histogram are often assumed to be representing the mechanical properties of the different constituents of the tissue or biomaterial at the nano- or micro-scale [47].

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Since the measurements plotted in such histograms originate from very complex multi-component, heterogeneous, and hierarchical structures, interpretation of such histograms requires application of statistical clustering theories. Study of recent literature shows that more often than not the complexity of such statistical distributions is neglected when interpreting the histograms. In this study, a flexible clustering theory from advanced statistics, namely finite mixture theory, has been proposed for analysis of the histograms obtained from nanomechanical characterization of biomaterials and tissues.

2. Materials and methods

The finite mixture theory is first briefly introduced, and the application of the Gaussian mixture theory for analysis of the histograms of nanomechanical properties of tissues and biomaterials is discussed. Then, the Gaussian mixture theory is applied for analysis of the cartilage nanomechanical properties measured using nanoindentation. The codes used for this case study are written in Matlab and are made freely available online so that the readers could quickly use the proposed techniques in their own research problems.

2.1. Finite mixture theory

If the frequency values found in a histogram of the measured mechanical properties are divided by the total number of measurements, the histogram is transformed to a discrete representation of a probability distribution function (PDF). The PDF demonstrates the probability of measuring specific values of mechanical properties when performing nanoindentation on the surface of tissue or biomaterial. The scatter found and the presence of several peaks in the PDF of the measured nanomechanical properties are due to two reasons: 1. Variation in the mechanical properties of the same constituent of the tissue or biomaterial and 2. The presence of several constituents that the nanoindentation probe may hit during the nanoindentation experiments. The finite mixture theory enables us to objectively separate both above-mentioned effects. As a consequence we could:

- Calculate the mean and standard deviations of the mechanical properties of the different constituents of the tissue or biomaterial
- Objectively estimate the number of constituents being presented by the PDF.
- Calculate the weight of every constituent in the measured PDF.

In the finite mixture theory, it is assumed that any PDF, $f(x)$, can be presented as a combination of m weighted statistical distributions [48]:

$$f(x) = \sum_{i=1}^{m} w_i f_i(x). \quad (1)$$

The weighting factors w_i are called *mixing proportions*, are nonzero ($0 \le w_i \le 1$), and sum to one:

$$\sum_{i=1}^{m} w_i = 1. \quad (2)$$

The distributions $f_i(x)$ are called *component densities* and the distribution $f(x)$ is called an m-component finite mixture density [48].

In the case of the nanomechanical properties of tissues and biomaterials, the number of components m describes the number of different types of constitutes that are hit by the nanoindenter tip during the nanoindentation experiments. If we could assume that the variations in the mechanical properties of one single constitute of the tissue or biomaterial are normally distributed, component densities $f_i(x)$ in Eq. (1) could be replaced by Gaussian distributions $N(\mu_i,\sigma_i)$:

$$f(x) = \sum_{i=1}^{m} w_i N(\mu_i, \sigma_i) \quad (3)$$

where μ_i and σ_i respectively represent the mean and standard deviation of the measured mechanical property of the constituent i of the tissue or biomaterial under study. The mixing proportions w_i show the contribution of the mechanical properties of every constituent to the overall distribution. If the nanoindentation measurements are uniformly distributed over the surface of the tissue or biomaterial, mixing proportions measure the probability that the nanoindentation probe hits a specific constituent during the experiments.

Once the nanomechanical properties are measured using nanoindentation for a large number of points on the surface of the tissue or biomaterial and the histogram and corresponding distribution are established, one needs to use a fitting procedure to fit Eq. (3) to the established distribution and estimate the mixing proportions, the mean values, and the standard deviations of the measured mechanical properties of different constituents of the tissue or biomaterial. Different techniques have been proposed for estimating the parameters of the finite mixture models including "graphical methods, method of moments, minimum-distance methods, maximum likelihood, and Bayesian approaches" [48]. Maximum likelihood (ML) is probably the most widely used technique for that purpose and was used in the current study too.

2.2. Estimating the number of components in the mixture model

The number of components in the finite mixture model can be determined in two ways. First, one could use a priori information regarding the expected number of constituents present on the surface of the tissue or biomaterial. The advantage of this technique is its simplicity, but it might induce certain bias when interpreting the measurement results. That is particularly important given the fact that there is often not much information available regarding the nanomechanical properties of the different constituents of tissues and biomaterials. For example, it is often assumed that the histogram of the measured nanomechanical properties of cartilage is composed of two major components: one component representing the mechanical properties of the collagen network and the other representing the mechanical properties of the Glycosaminoglycans (GAGs) [47]. It is, however, not clear whether there is only one type of GAG species with a specific range of mechanical properties or there might be different GAG species present with different and perhaps overlapping ranges of mechanical properties.

Alternatively, one could use statistical techniques to estimate the number of components or constituents present in the distribution of the mechanical properties of a tissue or biomaterial. The main advantage of this technique is that no a priori knowledge about the number of present constituents is required and that the estimation of the mechanical properties will be objective, thereby ensuring that no bias is induced when interpreting the measurement results.

Several criteria have been proposed for objective determination of the optimum number of mixture components. Three of the most important examples of such criteria are Silhouettes [49,50], Davies-Bouldin [51], and Calinski-Harabasz [52] criteria. As is clear from the references provided for the above-mentioned criteria, these criteria have been known for decades in advanced statistics texts and are therefore only briefly described here. In the Calinski-Harabasz criterion, the number of components is determined based on the assumption that optimal clustering, i.e. optimal separation of one probability distribution into several Gaussian distributions, results in clusters that have relatively large 'variance' between themselves while the variance within the clusters is relatively small. The Davies-Bouldin works on a similar basis but this time using the inter-cluster and intra-cluster 'distances'. In the Silhouette criterion, every point within a cluster is compared with all