Ultrasonic monitoring of artificial tissue mechanical properties in biorreactor

Guillermo Rus, Nicolas Bochud, Juan Melchor, Laura Peralta,
Juan Chiachio, Manuel Chiachio, Antonio Gómez, and Juan Antonio Marchal

Dpt. Structural Mechanics, University of Granada, 18071 Granada, Spain.
{grus,nbochud,jmelchor,lperalta,jchiachio,mchiachio}@ugr.es
http://www.ugr.es/~grus

Abstract. Quantitative control of tissue processes in bioreactors is an open problem in tissue engineering, aimed at creating artificial tissues and organs. To standardize and optimize the process, it is necessary to control most of the parameters that may vary its effectiveness. We propose to monitor the changes that may suffer the matrix during the process using mechanical parameters. To ensure the viability of this protocol, a bioreactor has been designed. The proposed methodology consists of three elements: an (1) experimental setup based on ultrasound-tissue interactions monitored in real-time, a (2) computational model that simulates the ultrasound-tissue interactions, and a (3) model-based inverse problem to reconstruct the evolution of the mechanical parameters.

1 Introduction

The rational principles of continuum mechanics are proposed together with a formal probabilistic formulation to address the problem of characterizing mechanical properties of tissue samples based on noninvasive and non-ionizing ultrasonic measurements.

Some researchers are recently investigating the acoustic (elastic) properties of cells and soft tissue at the microscale, and envisaging the potential of ultrasound as a technique to provide real-time online assessments for non-destructive tissue characterization. Brand *et al.* [1] explored changes in the acoustic properties of cells when exposed to chemotherapy for monitoring treatment response, using high-frequency ultrasound spectroscopy and scanning acoustic microscopy. Hattori *et al.* [2] developed a novel system for evaluating articular cartilage, measuring the acoustic properties of the articular cartilage by introducing an ultrasonic probe into the knee joint under arthroscopy, which successfully predicts the histological findings of degenerated cartilage. Rice *et al.* [3] used ultrasound data to construct cross-sectional B-scan images for qualitative observations of evolving constructs used in tissue engineering.

It is known that dispersive and viscous properties of tissue are strongly sensitive to tissue changes and easily unveil deeper dimensions of its micro and macrostructure. Static or slow viscoelastic mechanical constitutive laws and their values were reported by Bader *et al.* for skin [4], and by Ahuja for various internal tissues [5]. At audible frequency dynamics, a linear viscoelastic model was proposed by Pereira *et al.* [6] to fit the experimental observations. There are many types of uncertainty involved in the modeling of interaction between ultrasonic waves and tissue, such as excitation, material viscosity, and material heterogeneity. In this paper, multiple models of ultrasound-tissue interaction are proposed, implemented and contrasted against experimental observations. All assume homogeneous media with varying moduli and energy-dissipation forms that are expressed as attenuation models.

To provide a rational basis on the model choice, model-class selection algorithms have attracted substantial interest for selecting the most plausible class of models among some specified model classes, based on system measurements. Some recent developments and civil engineering applications of Bayesian model class selection have been carefully reviewed by Yuen [7]. A probabilistic model reconstruction inverse problem is proposed based on the concept of joint probability of prior information about observation and probabilistic information introduced by the model between model parameters and observations, as put forth by Tarantola *et al.* [8]. The model-class selection is formulated following Beck *et al.* [9]. Finally, a simple formulation of the joint probability is proposed, from which either the inverse problem or the model-class selection can be derived just by extracting specific marginal probabilities, thus unifying all the approaches.

2 Methodology

The proposed methodology consists of four elements: An novel (1) experimental setup based on ultrasound-tissue interactions is monitored in real time, a (2) set of alternative models that simulate the ultrasound-tissue interaction is numerically solved by the transfer matrix formalism, and a (3) stochastic model-class selection formulation is used to rank which of the models are more plausible, and (4) to reconstruct the evolution of the relevant mechanical parameters during the culture reaction time.

2.1 Experimental setup

A biorreactor with a specifically designed 1 [MHz] ultrasonic transmitter and receiver in transmission setup was manufactured for real-time measurement of mechanical and geometrical properties of porous scaffold layers of tissue culture. The monitoring scaffold dish is connected to the electronic setup detailed in Fig 1.

The scaffold consists in a 3D-printed porous structure of polylactic acid biocompatible with a volume fraction of 0.67. Human chondrocyte cells were cultivated in standard incubator conditions.

2.2 Propagation and numerical models

The experimental system is idealized by a mathematical model of the propagation and interaction of the transmitted ultrasonic waves with all the parts of

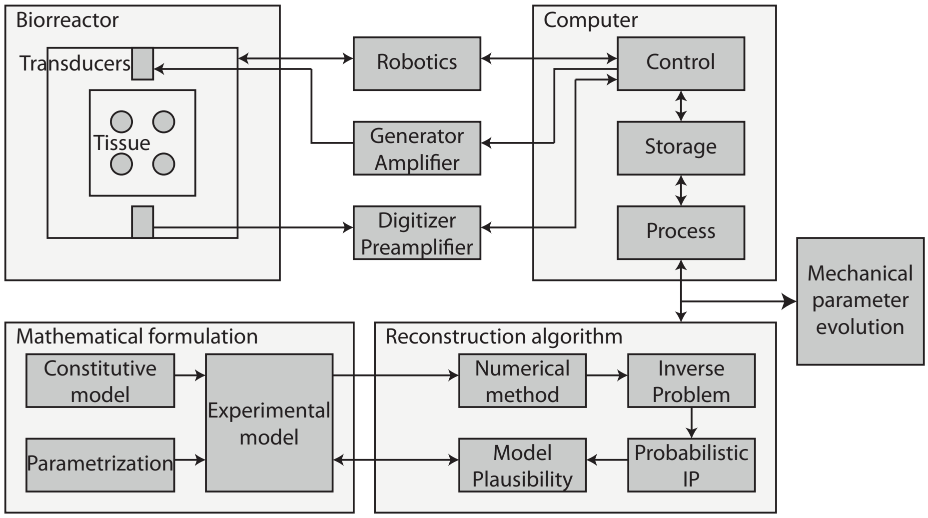

Figure 1. Schematic experimental and electronic setup.

the system until they are received by the sensor. The relevant ultrasonic paths along the biorreactor material (PMMA, polymetylmetacrilate) and the scaffold culture are illustrated in Fig 1. The bulk modulus K of all traversed materials is related to Young's modulus E and compressional waves speed c_p.

Several models are tested to idealize the removal of energy by dissipation or radiation. Three alternative damping models are used, viscous, hysteretic, proportional to integer time derivatives of the particle movement, and based on their fractional time derivatives. The viscoelastic d^{vis}, hysteretic d^{hys} (left) and fractional time derivative damping (right) are defined by [11, 12],

$$M^*(\omega) = M^0 \left(1 - i\omega d^{vis} - i d^{hys}\right) \qquad M^*(\omega) = M^0 \frac{1 + b(i\omega)^\beta}{1 + a(i\omega)^\alpha} \tag{1}$$

The mathematical model described above is approximated by a semi-analytical model of the wave interactions within multilayered materials based on the transfer matrix formalism (TMF) [13] (see [14]).

2.3 Probabilistic inverse problem

Following the probabilistic formulation of the model reconstruction inverse problem established by Tarantola *et al.* [8], the solution is not a single-valued set of model parameters $\mathcal{M}$. On the contrary, the solution is provided by probability density functions (PDF) $p(\mathcal{M})$ of the values of the model parameters $\mathcal{M}$ within the manifold $\mathfrak{M}$ of possible values. The probability density is assigned the sense of plausibility of the model values to be true.

Statistical inference theory is used to incorporate to the *a priori* information about the measured observations $\mathcal{O}$, the model parameters $\mathcal{M}$ and the model class $\mathcal{C}$, the information of idealized relationship between them $\mathcal{O} = \mathcal{O}(\mathcal{M})$ computed by a numerical model pertaining to a model class $\mathcal{C}$. The former are defined by the probability densities to prior (labeled ^{0}) data $p^0(\mathcal{O})$, $p^0(\mathcal{M})$ and $p^0(\mathcal{C})$ respectively, whereas the additional information about relationship (labeled ^{m}) between observations and model provided by the model class $\mathcal{C}$ is given by the PDF $p^m(\mathcal{O}, \mathcal{M}|\mathcal{C})$. The *a posteriori* probability $p(\mathcal{O}, \mathcal{M}, \mathcal{C})$ of the hypothetical model $\mathcal{M}$ is obtained jointly with the observations $\mathcal{O}$ and class $\mathcal{C}$,

$$p(\mathcal{O}, \mathcal{M}, \mathcal{C}) = k_1 \frac{p^0(\mathcal{O}, \mathcal{M}, \mathcal{C}) p^m(\mathcal{O}, \mathcal{M}, \mathcal{C})}{\mu(\mathcal{O}, \mathcal{M}, \mathcal{C})} \tag{2}$$

where $\mu(\mathcal{O}, \mathcal{M}, \mathcal{C})$ is the noninformative density function and k_1 is a normalization constant. The probabilistic model definition is given by its probability density function, which, after a number of assumptions regarding independency of processes and uniformity of prior information, is obtained by the marginal probability,

$$p(\mathcal{M})\big|_{\mathcal{C}=\mathcal{C}_i} = k_3 \int_{\mathfrak{O}} p^0(\mathcal{O}) p^m(\mathcal{O}|\mathcal{M}, \mathcal{C}) d\mathcal{O} \tag{3}$$

where k_3 is a normalization constant that replaces the dropped uniform distributions, and is needed for $p(\mathcal{M})\big|_{\mathcal{C}=\mathcal{C}_i}$ to fulfill the theorem of total probability.

The goal is to find the probability $p(\mathcal{C})$, understood as a measure of plausibility of a model class $\mathcal{C}$ [15]. It can be derived as the marginal probability of the posterior probability $p(\mathcal{O}, \mathcal{M}, \mathcal{C})$,

$$p(\mathcal{C}) = \int_{\mathfrak{O}} \int_{\mathfrak{M}} p(\mathcal{O}, \mathcal{M}, \mathcal{C}) d\mathcal{M} d\mathcal{O} = \tag{4}$$

$$= k_1 p^0(\mathcal{C}) \int_{\mathfrak{O}} \int_{\mathfrak{M}} \frac{p^0(\mathcal{O}) p^0(\mathcal{M}) p^m(\mathcal{O}|\mathcal{M}, \mathcal{C})}{\mu(\mathcal{O})} d\mathcal{M} d\mathcal{O} \tag{5}$$

2.4 Evolution search algorithms

The minimization of $\tilde{p}(\mathcal{M})$ for monitoring the evolution of the culture is carried out by two sequential algorithms: When an initial guess is not available, genetic algorithms are used as a full-range random search technique [16]. Since the change between consecutive measurements of the process is expected to be small, the BFGS-algorithm is employed thereon as a local search based on Hessian update [17], assisted by finite differentiation and line search.

3 Results

A sample of signals recorded by the ultrasound-monitored petri dish, without and with specimen respectively, every 60 seconds is shown in Fig 2. No clear evolution is detectable by bare visual inspection of the signals.

The estimation of Occam's factor, as well as the certainty metric $\overline{\sigma}$ are summarized in Table 1. The most plausible model class is shown to be 2, involving

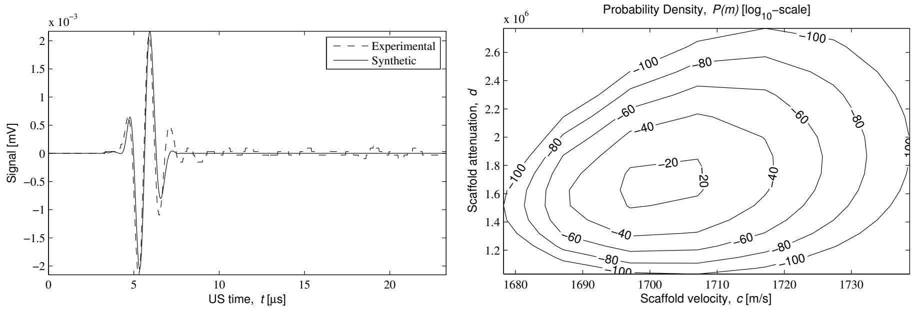

Figure 2. Left: Signal sample: Sequence of signals with specimen every 60 seconds. Right: Example of map of probabilistic inverse problem solution: plausibility value for each set of mechanical properties.

Model class	Hysteretic	Viscous	Fractional time derivative
$p(\mathcal{C})$ [%]	37.42	43.72	18.86
Certainty [log_{10}]	2.22	1.66	1.82
Model size	2	2	4

Table 1. Plausibility of model classes.

K^{tissue}, viscoelastic damping and temperature correction. It is closely followed by classes 1 (hysteretic). The evolution of the relevant reconstructed mechanical parameters during the reaction process is shown in Fig. 3 for two of the aforementioned model classes.

4 Conclusions

A computational technique to determine in real time the energy release and other mechanical parameters noninvasively during tissue growth ir presented by combining the solution of a probabilistic inverse problem, applying genetic search algorithms, and using a semi-analytical model of the interaction between ultrasonic waves and tissue. The proposed model-class selection and its subjacent class plausibility have enabled to rank the models according to their compatibility with the observations. The resulting trade-off between model simplicity and fitting to observations demonstrates that the viscous and hysteretic damping models, combined with the excitation signal correction, are feasible to characterize the complex evolution of the reaction process.

Acknowledgments. Spanish Ministerio de Economia for project DPI2010-17065, Spanish Junta de Andalucia for projects P11ŋCTS-8089 and GGI3000IDIB, and the European Union for program 'Programa Operativo FEDER de Andalucía 2007-2013'.

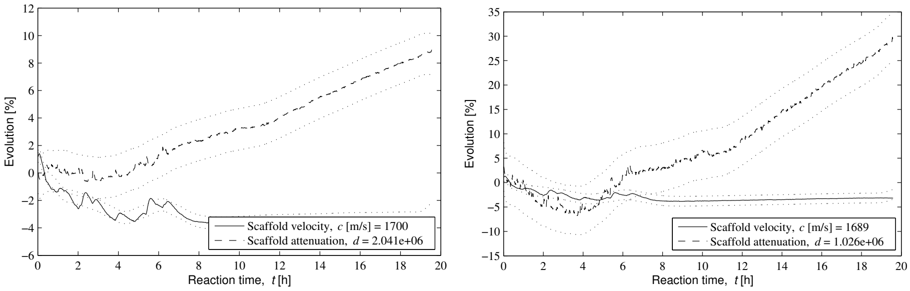

Figure 3. Evolution of model parameters during reaction. Viscoelastic (left) and Hystertic damping model (right).

REFERENCES

1. S. Brand, E. C. Weiss, R. M. Lemor, and M. C. Kolios. High frequency ultrasound tissue characterization and acoustic microscopy of intracellular changes, *Ultrasound in Medicine & Biology*, Vol. **34**(9), pp. 1396–1407, (2008).
2. K. Hattori, K. Ikeuchi, Y. Morita, and Y. Takakura. Quantitative ultrasonic assessment for detecting microscopic cartilage damage in osteoarthritis, *Arthritis Res. Ther.*, Vol. **7**, pp. R38–R46, (2005).
3. M. A. Rice, K. R. Waters , and K. S. Anseth. Ultrasound monitoring of cartilaginous matrix evolution in degradable PEG hydrogels, *Acta Biomaterialia*, Vol. **5**, pp. 152–161, (2009).
4. D. L. Bader and P. Bowker. Mechanical characteristics of skin and underlying tissues in vivo, *Biomaterials*, Vol. **4**(4), pp. 305–308, (1983).
5. A. S. Ahuja. Tissue as a voigt body for propagation of ultrasound, *Ultrasonic Imaging*, Vol. **1**(2), pp. 136–143, (1979).
6. J. M. Pereira, J. M. Mansour, and B. R. Davis. Dynamic measurement of the viscoelastic properties of skin, *Journal of Biomechanics*, Vol. **24**(2), pp. 157–162, (1991).
7. K-V. Yuen. Recent developments of Bayesian model class selection and applications in civil engineering, *Structural Safety*, Vol. **32**, pp. 338–346, (2010).
8. A. Tarantola. *Inverse Problem Theory*, SIAM, (2005).
9. J. L. Beck and K-V. Yuen. Model selection using response measurements: Bayesian probabilistic approach, *Journal of Engineering Mechanics*, Vol. **2**, pp. 192–203, (2004).
10. R. Ortega, A. Téllez, L. Leija, and A. Vera. Measurement of Ultrasonic Properties of Muscle and Blood Biological Phantoms, *Physics Procedia*, Vol. **3**, pp. 627–634, (2010).
11. N. M. M. Maia, J. M. M. Silva, and A. M. R. Ribeiro. On a general model for damping, *Journal of Sound and Vibration*, Vol. **218**(5), pp. 749–767, (1998).
12. L. Gaul. The influence of damping on waves and vibrations, *Mechanical Systems and Signal Processing*, Vol. **31**(1), pp. 1–30, (1999).
13. N. Cretu and G. Nita. Pulse propagation in finite elastic inhomogeneous media, *Computational Materials Science*, Vol. **31**, pp. 329–336, (2004).
14. N. Bochud, G. Rus Probabilistic inverse problem to characterize tissue-equivalent material mechanical properties. *IEEE TUFFC*, 2012, 59(7):1443-56

15. R. T. Cox. The algebra of probable inference, *Johns Hopkins University Press, Baltimore*, (1961).
16. D. Goldberg. Genetic Algorithms in search, optimization and machine learning, *Addison-Wesley Publishing Company, Reading, Massachussets*, (1989).
17. J. E. Dennis and J. J. Moré. A characterization off superlinear convergence and its application to Quasi-Newton methods, *Mathematics of Computation*, Vol. **28**(126), pp. 549–560, (1974).