

# A Collection of Biomaterials and Biomechanical Experiments and Analysis Methods

Feng-Xun Li<sup>1</sup>, Zhen-Zhe Li<sup>2</sup>, Rui Jiang<sup>2</sup>, Mei-Ling Zhang<sup>3</sup>

<sup>1</sup> Ulsan Ship and Ocean College, Ludong University <sup>2</sup> College of Mechanical and Electrical Engineering, Wenzhou University <sup>3</sup> School of Pharmacy, Wenzhou Medical University

## Corresponding Authors

**Feng-Xun Li**      **Zhen-Zhe Li**      **Rui Jiang**      **Mei-Ling Zhang**  
 Idulifengxun@163.com    13868659593@163.com    rui@wzu.edu.cn    meiling308@163.com

## Citation

Li, F.X., Li, Z.Z., Jiang, R., Zhang, M.L. A Collection of Biomaterials and Biomechanical Experiments and Analysis Methods. *J. Vis. Exp.* (173), e62998, doi:10.3791/62998 (2021).

## Date Published

July 13, 2021

## DOI

10.3791/62998

## URL

jove.com/video/62998

## Editorial

As an important interdisciplinary research field, biomaterials and biomechanics reveal the relationships and mechanisms between the structure, performance, and function of biomaterials through experimental research and theoretical analysis, which is connected to materials science, mechanics, bioengineering, mathematics, etc. In order to improve the experimental methods of biomaterials and biomechanics, this JoVE Methods Collection discusses the corresponding experimental methods and their respective characteristics in two categories: tissue mechanical properties and bionics mechanics. The tissue mechanics observations include a 3D hydrogel stretching method, a dual raster-scanning photoacoustic method, a detergent-free decellularization method of the human pancreas, and a decoupling method of tensile and shear stresses on tubular scaffolds. The bionics mechanics observations include a data collection method of lower limb movement and postural control for patients with ankle instability.

The effects of mechanical force on organization mainly include gene expression, cell differentiation, tissue remodeling<sup>1,2,3</sup>, and changes in the extracellular matrix

(ECM)<sup>4,5,6</sup>. Understanding the tissue response to mechanical forces<sup>7,8,9</sup> will help the development of the field of tissue engineering and theoretical models. Kolei et al.<sup>10</sup> propose a method for stretching 3D hydrogels, which allows static or cyclic uniaxial strain during a confocal microscope. They molded a fibrin gel with a hole about 2 mm in diameter on 0.5 mm thick silicon rubber strips, and then uniaxial stretching was performed under live confocal microscopy. Finally, they discuss the possibility of embedding cells in hydrogels and exposing them to controlled external stretching. The stretching system used in this protocol consists of 3D printing components and low-cost electronic components. The system has the following unique features compared with other existing methods. First, the system allows the uniaxial stretching of thick 3D soft hydrogels, and the whole hydrogel has Z-uniform deformation. Secondly, because of the simplicity of 3D printing and the low cost of this equipment, it is easy to build this kind of stretching equipment in other labs. Third, the geometry and size of the sample can be freely operated according to the user. This method has improved the ability to study external forces on

biological process under more physiological 3D conditions and has contributed to the field of tissue engineering.

Small-animal imaging plays an important role in guiding the research on human homologous diseases and seeking effective treatment methods. Photoacoustic imaging (PAI) is a noninvasive imaging technology that combines the advantages of optical imaging and ultrasonic imaging<sup>11,12</sup>. Yang et al.<sup>13</sup> report a dual raster-scanning photoacoustic imager (DRS-PAI), and through the acoustic coupling scanning of different parts of mice, the vascular images in WIM and RIM modes are collected, respectively. The advantage of DRS-PAI is that WIM and RIM are integrated in one system and provide high-resolution wide-field vascular visualization of real-time blood dynamics. The real-time imaging mode (RIM) can reveal the characteristics of respiration or pulse by measuring the displacement of vasculature along the depth direction. Additionally, RIM can quantitatively measure the specific area of the WIM images. By comparing the images, the details of local changes can be accurately revealed. This method can be easily applied to various fields of biomedical basic research.

The increasing demand for islets puts forward higher requirements for islet isolation<sup>14,15,16</sup>. Tamburrini et al.<sup>17</sup> propose a new, detergent-free decellularization method that creates less ECM damage and can preserve critical components of the pancreatic ECM. The decellularization method avoids the use of classic ionic and nonionic chemical detergents. Moreover, the decellularization of tissue in an orbital vibrator rather than the injection of detergent through a vascular system greatly promotes the simplicity, consistency, and feasibility of decellularization technology, thus increasing the production of ECM for translation. As current acellular methods cannot quantify the residual Triton X-100 on the

ECM post-decellularization and the feasibility of expanding the manufacturing process in the cGMP environment is low, they also studied the feasibility of obtaining decellularization by mechanical vibration rather than perfusion of the whole pancreas. Data on the quantification of collagen and glycosaminoglycans show a trend consistent with previous experience. Some limitations were found by using this protocol. Therefore, human pancreases from donors with BMI <30 are generally considered suitable for decellularization.

The use of absorbable biomaterials to induce regeneration directly *in vivo* is an attractive strategy<sup>18,19,20,21,22</sup>, but this kind of biomaterial can cause an inflammatory reaction after implantation, which is also the driving force for subsequent resorption and regeneration of new tissue. Both the inflammation and regeneration process are determined by the tensile and shear stress. Koch et al.<sup>23</sup> describe in detail the use of a bioreactor for decoupling tensile and shear stresses on tubular scaffolds. They suggest that the application of this tubular bioreactor system will help to study their individual and combined effects in mechanics. This bioreactor systematically evaluates the contributions of shear stress and cyclic stretch to inflammation and tissue regeneration in tubular resorbable scaffolds. Also, this makes it possible to standardize the inflammation and regeneration capabilities under the influence of tubular stents under well-controlled mechanical loads. The key steps of this method are discussed in detail: the construction and setup of the bioreactor, the preparation of the scaffold and cell inoculation, the application and maintenance of stretch and shear flow, the sample collection, and the analysis. This system can test a variety of tissue-engineered vascular grafts (for example, synthetic or natural sources, different microstructures, different porosity). In order to effectively decouple the application of shear stress and tension, the

key concepts used in the bioreactor are as follows. First, the shear stress and stretch control are separated using different pump systems. Second, the scaffolds is stimulated in an inside-out manner with computationally driven dimensions. The flow is applied on the outer surface of the tubular scaffold using a flow pump, while the silicone tube is expanded using a separate strain pump, and the circumferential tension of the support is expanded using a separate strain pump. This method can also be used for a large number of analyses on vascular construction. The results indicate the unique effects of different combinations of shear and tension on the growth and remodeling of tissue-engineered vascular graft (TEVG) structure. The insights obtained through the collection of the *in vitro* platform are helpful to optimize the newly developed design parameters for *in situ* TEVGs.

Posture and balance control can be divided into static and dynamic states<sup>24,25,26,27</sup>. Among them, dynamic balance ability refers to the body's ability to control and adjust the body's center of gravity and posture in motion<sup>28</sup>. From the perspective of sports biomechanics, the main body to maintain dynamic balance is the ankle joint of the lower limbs. If the hip joint were the main body to maintain balance, it would be easy to fall. Zhou et al.<sup>29</sup> analyze the changes in lower limb biomechanics at the gait termination caused by an unexpected stimulus. A motion analysis system and a plantar pressure platform are used to collect the motion data of lower limb movement *in situ*. This method can be used in biomechanics research, virtual reality systems, robot remote control, animation production, sports training, ergonomics research, interactive games, etc.

Chronic ankle instability (CAI) is one of the most common sports injuries, characterized by persistent pain and swelling of the ankle, the ankle giving way, and self-reported

disability, which seriously affects the postural stability of the patients<sup>30,31</sup>. CAI could be improved through kinesiology taping. Yin et al.<sup>32</sup> analyze the contributions of vision, proprioception, and vestibular sensation in maintaining postural stability by using computer dynamic posturography. Sensory organization tests (SOT), unilateral stance (US), limit of stability (LOS), the motor control test (MCT), and adaptation tests (ADT) were conducted and measured. These measurements provide a new method for observing the process of coordinating the three sensory systems and regulating muscle activation to maintain postural stability.

Combined with multidisciplinary comprehensive research, the advanced measuring equipment and methods promote the development and evolution of biomaterials and biomechanics. Although this article discusses different testing methods, it is necessary to integrate different research methods and ideas in various fields as an interdisciplinary and comprehensive research direction in order to accelerate the research of biomaterials and biomechanics and promote the communication between researchers in different fields.

## Disclosures

The authors have nothing to disclose.

## Acknowledgments

This study was supported by Wenzhou Municipal Science and Technology Bureau (Y2020069).

## References

1. Bleuel, J., Zucke, V., Bruggemann, G. P., Niehoff, A. Effects of cyclic tensile strain on chondrocyte metabolism: a systematic review. *Plos One*. **10**, 0119816 (2015).

2. Pennisi, C. P., Olesen, C. G., de Zee, M., Rasmussen, J., Zachar, V, Uniaxial cyclic strain drives assembly and differentiation of skeletal myocytes. *Tissue Engineering Part A*. **17**, 2543-2550 (2011).
3. Grodzinsky, A. J., Levenston, M. E., Jin, M., Frank, E. H. Cartilage tissue remodeling in response to mechanical forces. *Annual Review of Biomedical Engineering*. **2**(1), 691-713 (2000).
4. Ban, E. et al. Mechanisms of plastic deformation in collagen networks induced by cellular forces. *Biophysical Journal*. **114** (2), 450-461 (2018).
5. Storm, C., Pastore, J. J., MacKintosh, F. C., Lubensky, T. C., Janmey, P. A., Nonlinear elasticity in biological gels. *Nature*. **435**, 191-194 (2005).
6. Muiznieks, L. D., Keeley, F. W. Molecular assembly and mechanical properties of the extracellular matrix: a fibrous protein perspective. *Biochimica et Biophysica Acta*. **1832**, 866-875 (2012).
7. Livne, A., Bouchbinder, E., Geiger, B. Cell reorientation under cyclic stretching. *Nature Communications*. **5**, 3938 (2014).
8. Xu, G. K., Feng, X. Q., Gao, H. Orientations of cells on compliant substrates under biaxial stretches: a theoretical study. *Biophysical Journal*. **114** (3), 701-710 (2017).
9. Chagnon-Lessard, S., Jean-Ruel, H., Godin, M., Pelling, A. Cellular orientation is guided by strain gradients. *Integrative Biology*. **9** (7), 607-618 (2013).
10. Kolel, A. et al. Controlled strain of 3D hydrogels under live microscopy imaging. *Journal of Visualized Experiments*. (166) (2020).
11. Li, L. et al. Single-impulse panoramic photoacoustic computed tomography of small-animal whole-body dynamics at high spatiotemporal resolution. *Nature Biomedical Engineering*. **1** (5), 0071 (2017).
12. Jeon, S., Kim, J., Lee, D., Baik, J. W., Kim, C., Review on practical photoacoustic microscopy. *Photoacoustics*. **15**, 100141 (2019).
13. Yang, F., Wang, Z., Yang, S. Dual raster-scanning photoacoustic small-animal imager for vascular visualization. *Journal of Visualized Experiments*. (161) (2020).
14. Korpos, E. et al. The peri-islet basement membrane, a barrier to infiltrating leukocytes in type 1 diabetes in mouse and human. *Diabetes*. **62** (2), 531-542 (2013).
15. Daoud, J., Petropavlovskaja, M., Rosenberg, L., Tabrizian, M. The effect of extracellular matrix components on the preservation of human islet function in vitro. *Biomaterials*. **31** (7), 1676-1682 (2010).
16. Paraskevas, S., Maysinger, D., Wang, R., Duguid, T. P., Rosenberg, L., Cell loss in isolated human islets occurs by apoptosis. *Pancreas*. **20** (3), 270-276 (2000).
17. Tamburrini, R. et al. Detergent-free decellularization of the human pancreas for soluble extracellular matrix (ECM) production. *Journal of Visualized Experiments*. (163) (2020).
18. Wang, J. et al. Ex vivo blood vessel bioreactor for analysis of the biodegradation of magnesium stent models with and without vessel wall integration. *Acta Biomaterials*. **50**, 546-555 (2017).
19. Wolf, F. et al. VascuTrainer: a mobile and disposable bioreactor system for the conditioning of tissue-

- engineered vascular grafts. *Annals of Biomedical Engineering*. **46** (4), 616-626 (2018).
20. Ramaswamy, S. et al. A novel bioreactor for mechanobiological studies of engineered heart valve tissue formation under pulmonary arterial physiological flow conditions. *Journal of Biomechanical Engineering*. **136** (12), 121009 (2014).
  21. Vanerio, N., Stijnen, M., de Mol, B. A. J. M., Kock, L. M. An innovative ex vivo vascular bioreactor as comprehensive tool to study the behavior of native blood vessels under physiologically relevant conditions. *Journal of Engineering and Science in Medical Diagnostics and Therapy*. **2** (4) (2019).
  22. Kural, M. H., Dai, G., Niklason, L. E., and Gui, L. An ex vivo vessel injury model to study remodeling. *cell Transplantation*. **27** (9), 1375-1389 (2018).
  23. Koch, S. E. et al. A multi-cue bioreactor to evaluate the inflammatory and regenerative capacity of biomaterials under flow and stretch. *Journal of Visualized Experiments*. (166) (2020).
  24. Gribble, P. A., Hertel, J., Denegar, C. R. Chronic ankle instability and fatigue create proximal joint alterations during performance of the star excursion balance test. *International Journal of Sports Medicine*. **28** (3), 236-242 (2006).
  25. Herrington, L., Hatcher, J., Hatcher, A., McNicholas, M. A comparison of star excursion balance test reach distances between ACL deficient patients and asymptomatic controls. *The Knee*. **16** (2), 149-152 (2009).
  26. Olmsted, L. C., Hertel, J. Influence of foot type and orthotics on static and dynamic postural control. *Journal of Sport Rehabilitation*. **13** (1), 54-66 (2004).
  27. Kahle, N. L., Gribble, P. A. Core stability training in dynamic balance testing among young, healthy adults. *Athletic Training & Sports Health Care*. **1** (2), 65-73 (2009).
  28. Lieberman, D. E. et al. Foot strike patterns and collision forces in habitually barefoot versus shod runners. *Nature*. **463** (7280), 531-535 (2010).
  29. Zhou, H., Cen, X., Song, Y., Ugbohue, U. C., Gu, Y. Lower-limb biomechanical characteristics associated with unplanned gait termination under different walking speeds. *Journal of Visualized Experiments*. (162) (2020).
  30. Doherty, C. et al. The incidence and prevalence of ankle sprain injury: a systematic review and meta-analysis of prospective epidemiological studies. *Sports Medicine*. **44**, 123-140 (2013).
  31. Vuurberg, G. et al. Diagnosis treatment and prevention of ankle sprains: update of an evidence-based clinical guideline. *British Journal of Sports Medicine*. **52** (15), 956 (2018).
  32. Yin, L., Lai, Z., Hu, X., Liu, K., Wang, L. Evaluating postural control and lower-extremity muscle activation in individuals with chronic ankle instability. *Journal of Visualized Experiments*. (163) (2020).