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Corresponding Author:	Feng-Xun Li Ludong University YanTai, ShanDong CHINA
Corresponding Author's Institution:	Ludong University
Corresponding Author E-Mail:	ldulifengxun@163.com
First Author:	Feng-Xun Li
Other Authors:	Zhen-Zhe Li Rui Jiang Mei-Ling Zhang
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TITLE:

A Collection of Biomaterials and Biomechanical Experiments and Analysis Methods

AUTHORS:

Feng-Xun Li^{1*}, Zhen-Zhe Li^{2*}, Rui Jiang^{2*}, Mei-Ling Zhang^{3*}

¹Ulsan Ship and Ocean College, Ludong University, Shandong, P.R. China

²College of Mechanical and Electrical Engineering, Wenzhou University, Zhejiang, P.R. China

³School of Pharmacy, Wenzhou Medical University, Zhejiang, P.R. China

CORRESPONDING AUTHORS:

*Feng-Xun Li (ldulifengxun@163.com)

*Zhen-Zhe Li (a13868659593@163.com)

*Rui Jiang (jrui@wzu.edu.cn)

*Mei-Ling Zhang (meiling308@163.com)

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EDITORIAL TEXT:

As an important interdisciplinary research field, biomaterials and biomechanics reveal the relationship and mechanism 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 of the human pancreas and 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 effect of mechanical force on organization mainly includes gene expression, cell differentiation, tissue remodeling^{1,2,3} and changes in the extracellular matrix (ECM)^{4,5,6}. Understanding the tissue response to mechanical forces^{7,8,9} will help the development of the field of tissue engineering and theoretical models. Kolel et al.¹⁰ proposed 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 discussed 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 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 the role of the biological process under more physiological 3D conditions and contributed to the field of tissue engineering.

Small animal imaging plays an important role in guiding the research of 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^{11,12}. Yang et al.¹³ reported 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 were 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 WIM images. By comparing 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 the islet isolation^{14,15,16}. Tamburrini et al.¹⁷ proposed a new, detergent-free decellularization method that creates less ECM damage and can preserve critical components of 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 the current acellular methods cannot quantify the residual Triton X-100 on ECM post-decellularization and the feasibility of expanding the manufacturing process in cGMP environment, 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 pancreas from donors with BMI < 30 were generally considered suitable for decellularization.

89
90 The use of absorbable biomaterials to induce regeneration directly *in vivo* is an attractive
91 strategy^{18,19,20,21,22}, but this kind of biomaterials can cause an inflammatory reaction after
92 implantation, which is also the driving force for subsequent resorption and regeneration of new
93 tissue. Both the inflammation and regeneration process are determined by the tensile and shear
94 stress. Koch et al.²³ described in detail the use of a bioreactor for decoupling tensile and shear
95 stresses on tubular scaffolds. They suggest that the application of this tubular bioreactor system
96 will help to study their individual and combined effects in mechanics. This bioreactor
97 systematically evaluates the contributions of the shear stress and cyclic stretch on inflammation
98 and tissue regeneration in tubular resorbable scaffolds. Also, this makes it possible to standardize
99 inflammation and regeneration capabilities under the influence of tubular stents under well-
100 controlled mechanical loads. The key steps of this method are discussed in detail: construction
101 and setup of the bioreactor, preparation of the scaffold and cell inoculation, application and
102 maintenance of stretch and shear flow, sample collection and analysis. This system can test a
103 variety of tissue-engineered vascular grafts (for example, synthetic or natural sources, different
104 microstructures, different porosity). In order to effectively decouple the application of shear
105 stress and tension, the key concepts used in bioreactor are as follows. First, separate the shear
106 stress and stretch control using different pump systems. Second, stimulate the scaffolds in an
107 inside-out manner with computationally driven dimensions. The flow is applied on the outer
108 surface of the tubular scaffold using a flow pump, while the silicone tube is expanded using a
109 separate strain pump, and the circumferential tension of the support is expanded on it using a
110 separate strain pump. This method can also be used for a large number of analysis on vascular
111 construction. The results indicate the unique effects of different combinations of shear and
112 tension on the growth and remodeling of tissue-engineered vascular grafts (TEVGs) structure. The
113 insights obtained through the collection *in vitro* platform are helpful to optimize the newly
114 developed design parameters for *in situ* TEVG.

115
116 Posture and balance control can be divided into static and dynamic states^{24,25,26,27}. Among them,
117 dynamic balance ability refers to the body's ability to control and adjust the body's center of
118 gravity and posture in motion²⁸. From the perspective of sports biomechanics, the main body to
119 maintain dynamic balance is the ankle joint of the lower limbs. If the hip joint is the main body to
120 maintain balance, it is easy to fall. Zhou et al.²⁹ analyzed the changes of lower limb biomechanics
121 at the gait termination caused by unexpected stimulus. A motion analysis system and a plantar
122 pressure platform are used to collect the motion data of lower limb movement *in situ*. This
123 method can be used in biomechanics research, a virtual reality system, robot remote control,
124 animation production, sports training, ergonomics research, interactive games, etc.

125
126 Chronic ankle instability (CAI) is one of the most common sports injuries, characterized by
127 persistent pain and swelling of the ankle, giving away and self-reported disability, which seriously
128 affects the postural stability of the patients^{30,31}. CAI could be improved through kinesiology taping.
129 Yin et al.³² analyzed the proportion of vision, proprioception, and vestibular sensation in
130 maintaining postural stability by using computer dynamic posturography. Sensory organization
131 tests (SOT), unilateral stance (US), Limit of stability (LOS), motor control test (MCT) and adaptation
132 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 discussed 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.

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The authors have nothing to disclose.

REFERENCES:

1. Bleuel, J., Zaucke, 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).

- 177 13. Yang, F., Wang, Z., Yang, S. Dual raster-scanning photoacoustic small-animal imager for
178 vascular visualization. *Journal of Visualized Experiments*. (161) (2020).
- 179 14. Korpos, E. et al. The peri-islet basement membrane, a barrier to infiltrating leukocytes in
180 type 1 diabetes in mouse and human. *Diabetes*. **62** (2), 531–542 (2013).
- 181 15. Daoud, J., Petropavlovskaja, M., Rosenberg, L., Tabrizian, M. The effect of extracellular
182 matrix components on the preservation of human islet function in vitro. *Biomaterials*. **31** (7),
183 1676–1682 (2010).
- 184 16. Paraskevas, S., Maysinger, D., Wang, R., Duguid, T. P., Rosenberg, L., Cell loss in isolated
185 human islets occurs by apoptosis. *Pancreas*. **20** (3), 270–276 (2000).
- 186 17. Tamburrini, R. et al. Detergent-free decellularization of the human pancreas for soluble
187 extracellular matrix (ECM) production. *Journal of Visualized Experiments*. (163) (2020).
- 188 18. Wang, J. et al. Ex vivo blood vessel bioreactor for analysis of the biodegradation of
189 magnesium stent models with and without vessel wall integration. *Acta Biomaterials*. **50**, 546–
190 555 (2017).
- 191 19. Wolf, F. et al. VascuTrainer: a mobile and disposable bioreactor system for the
192 conditioning of tissue-engineered vascular grafts. *Annals of Biomedical Engineering*. **46** (4), 616–
193 626 (2018).
- 194 20. Ramaswamy, S. et al. A novel bioreactor for mechanobiological studies of engineered
195 heart valve tissue formation under pulmonary arterial physiological flow conditions. *Journal of*
196 *Biomechanical Engineering*. **136** (12), 121009 (2014).
- 197 21. Vanerio, N., Stijnen, M., de Mol, B. A. J. M., Kock, L. M. An innovative ex vivo vascular
198 bioreactor as comprehensive tool to study the behavior of native blood vessels under
199 physiologically relevant conditions. *Journal of Engineering and Science in Medical Diagnostics and*
200 *Therapy*. **2** (4) (2019).
- 201 22. Kural, M. H., Dai, G., Niklason, L. E., and Gui, L. An ex vivo vessel injury model to study
202 remodeling. *cell Transplantation*. **27** (9), 1375–1389 (2018).
- 203 23. Koch, S. E. et al. A multi-cue bioreactor to evaluate the inflammatory and regenerative
204 capacity of biomaterials under flow and stretch. *Journal of Visualized Experiments*. (166) (2020).
- 205 24. Gribble, P. A., Hertel, J., Denegar, C. R. Chronic ankle instability and fatigue create proximal
206 joint alterations during performance of the star excursion balance test. *International Journal of*
207 *Sports Medicine*. **28** (3), 236–242 (2006).
- 208 25. Herrington, L., Hatcher, J., Hatcher, A., McNicholas, M. A comparison of star excursion
209 balance test reach distances between ACL deficient patients and asymptomatic controls. *The Knee*.
210 **16** (2), 149–152 (2009).
- 211 26. Olmsted, L. C., Hertel, J. Influence of foot type and orthotics on static and dynamic postural
212 control. *Journal of Sport Rehabilitation*. **13** (1), 54–66 (2004).
- 213 27. Kahle, N. L., Gribble, P. A. Core stability training in dynamic balance testing among young,
214 healthy adults. *Athletic Training & Sports Health Care*. **1** (2), 65–73 (2009).
- 215 28. Lieberman, D. E. et al. Foot strike patterns and collision forces in habitually barefoot versus
216 shod runners. *Nature*. **463** (7280), 531–535 (2010).
- 217 29. Zhou, H., Cen, X., Song, Y., Ugbohue, U. C., Gu, Y. Lower-limb biomechanical characteristics
218 associated with unplanned gait termination under different walking speeds. *Journal of Visualized*
219 *Experiments* (162) (2020).
- 220 30. Doherty, C. et al. The incidence and prevalence of ankle sprain injury: a systematic review

221 and meta-analysis of prospective epidemiological studies. *Sports Medicine*. **44**, 123–140 (2013).
222 31. Vuurberg, G. et al. Diagnosis treatment and prevention of ankle sprains: update of an
223 evidence-based clinical guideline. *British Journal of Sports Medicine*. **52** (15), 956 (2018).
224 32. Yin, L., Lai, Z., Hu, X., Liu, K., Wang, L. Evaluating postural control and lower-extremity
225 muscle activation in individuals with chronic ankle instability. *Journal of Visualized Experiments*.
226 (163) (2020).
227