The Role of Stress Stimulation in Constructing Pelvic Floor Ligaments *in vitro*

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ABSTRACT

This study aims to identify the most suitable scaffold material for in vitro cultivation of pelvic floor ligaments and to investigate the effects of stress environments on ligament tissue performance and cultivation cycle. We evaluated silk, PCL, and PLA scaffold properties and compared human ligament fibroblast proliferation, morphology, and gene expression on these materials. Silk scaffolds showed superior mechanical and biological properties compared to PCL and PLA scaffolds, including larger fiber diameter, pore size, ultimate stress, and Young's modulus. Cells cultured on silk scaffolds proliferated faster, with more elongated morphology and increased expression of extracellular matrix genes like COL1 and COL3. Stress stimulation, especially at 60g, significantly accelerated cell proliferation and increased expression of key mechanical signaling pathway genes, including TGF-β, COL1, COL3, and ELN. In conclusion, silk scaffolds offer optimal conditions for in vitro pelvic floor ligament construction, and stress stimulation improves cell proliferation and extracellular matrix gene expression, enhancing ligament durability. These findings support the potential efficacy of in vitro cultivated pelvic floor ligaments in treating stress urinary incontinence (SUI).

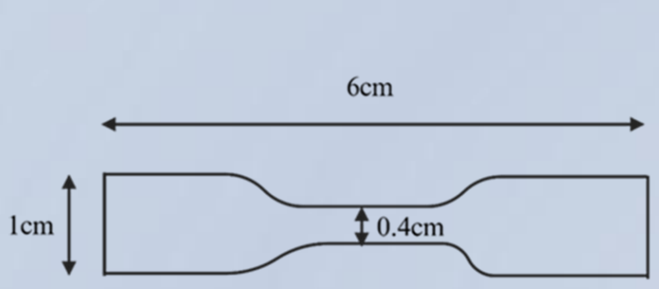
METHODS

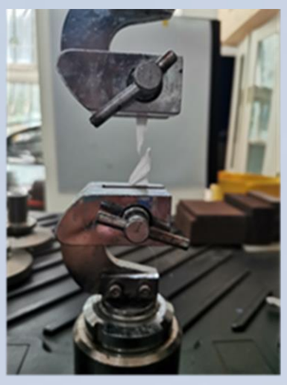
Ⅰ. Selecting of scaffold materials suitable for constructing pelvic floor ligaments in vitro (silk, PCL, PLA)

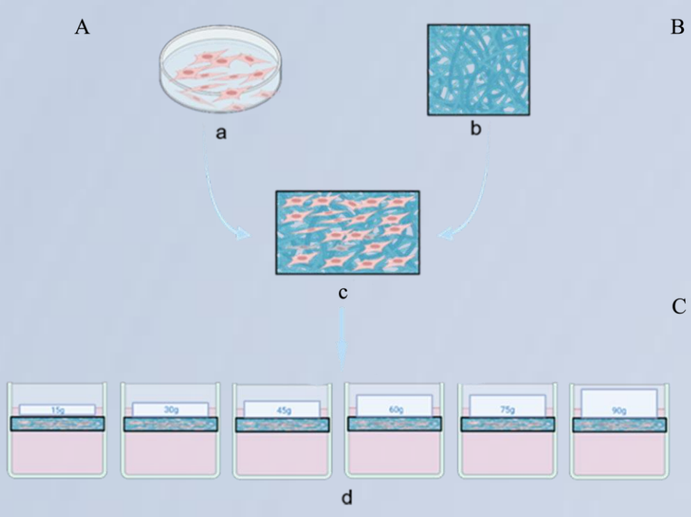
1. Preparation and sterilization treatment of materials
2. Mechanical tensile testing to determine the mechanical properties of support materials
3. Cell culture and construction of cell scaffold complexes
4. Cell proliferation on different material scaffolds
5. Cell adhesion and proliferation
6. Morphological analysis of cells on different materials
7. Determination of Young's modulus of cells
8. Reverse transcription quantitative (RT-q) PCR detection of gene expression levels in the TGF - β pathway

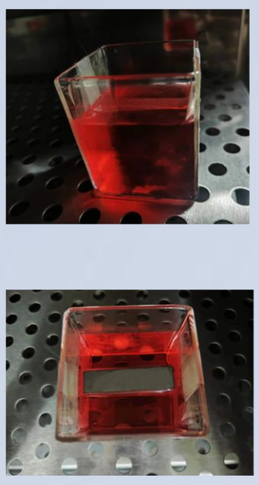
Ⅱ. The important role of stress environment on the mechanical properties and culture cycle of ligament tissue in vitro

1. Construction of extracorporeal ligaments under stress stimulation
2. Cell proliferation under different stress stimulation amplitudes
3. Transcriptomic changes before and after stress stimulation
4. Expression of extracellular matrix (ECM) genes on Silk scaffolds with different stress stimulation amplitudes



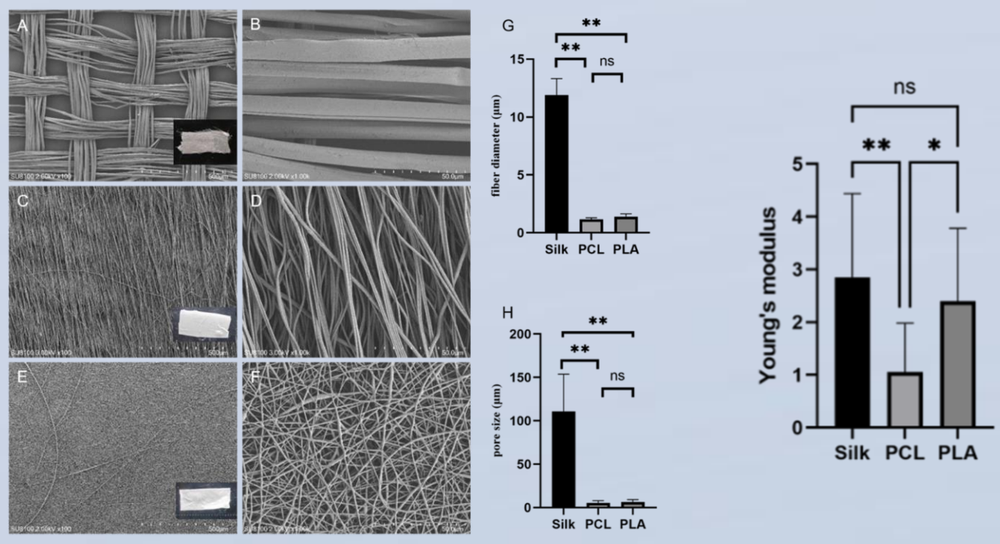




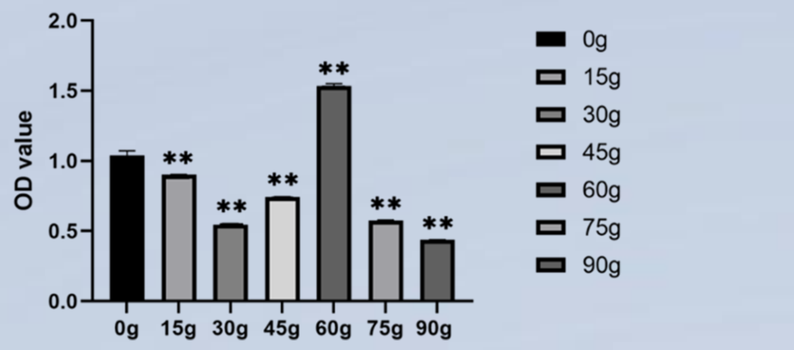


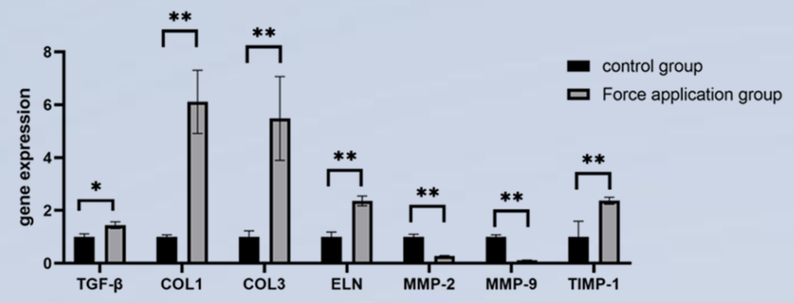
RESULTS

Ⅰ. Compared with PCL and PLA scaffolds, silk scaffolds have superior mechanical and biological properties, including larger fiber diameter (11.894 ± 0.138 μm), pore size (110.667 ± 43.036 μm), ultimate stress (4.89 ± 1.12 MPa) and Young's modulus (124.25 ± 12.01 MPa) (P<0.05). Cells cultured on silk fibroin scaffolds exhibit faster proliferation, elongated and more regularly arranged cells, and increased expression of extracellular matrix genes such as the collagen genes COL1 and COL3.



Ⅱ. Stress stimulation, especially under gravity at 60g, increased the culture efficiency by 40%, significantly accelerated cell proliferation, and increased the expression of key mechanical signaling pathway genes, TGF-β increased by 0.44 times, and extracellular matrix genes COL1, COL3 and ELN increased by 5.11, 4.48, and 2.36 times. In addition, the ratio of elastic fibers to collagen is reduced, which enhances the durability of the pelvic floor ligaments.





CONCLUSIONS

In this project, a 3D experimental platform for in vitro culture of pelvic floor ligaments stimulated by stress was constructed, and the key mechanisms of stress stimulation influence signaling pathways and extracellular matrix expression were clarified, which provided support for the fine in vitro culture of pelvic floor ligaments, which could effectively improve its mechanical properties and shorten the culture cycle. The biocompatibility, elastic modulus and stiffness of typical scaffold materials were determined, and the most suitable scaffold material for pelvic floor ligament culture, Silk, was screened out. Breaking through the traditional mechanical stimulation mode and simulating the human pelvic floor stress mode, the optimized stress environment for in vitro culture of pelvic floor ligament was obtained, and the in vitro culture efficiency could be improved by about 40% by applying 60g pressure. The key mechanism of stress stimulation to improve the culture efficiency of ligaments was clarified, that is, the stress environment promoted the expression of TGF-β, a mechanical signaling pathway, and increased the expression of extracellular matrices such as COL1, COL3 and ELN, which play a key role in the service function of the pelvic floor ligament, by several times, so that the pelvic floor ligament could meet the performance requirements of the service.

REFERENCES

[1] Chapple C R, Cruz F, Deffieux X, et al. Consensus Statement of the European Urology Association and the European Urogynaecological Association on the Use of Implanted Materials for Treating Pelvic Organ Prolapse and Stress Urinary Incontinence[J]. Eur Urol, 2017, 72(3): 424-431.

[2] Schmid F A, Williams J K, Kessler T M, et al. Treatment of Stress Urinary Incontinence with Muscle Stem Cells and Stem Cell Components: Chances, Challenges and Future Prospects[J]. Int J Mol Sci, 2021, 22(8)

[3] Liu S, Al-Danakh A, Wang H, et al. Advancements in scaffold for treating ligament injuries; in vitro evaluation[J]. Biotechnol J, 2024, 19(1): e2300251

[4] Wang X, Xie L, Crane J, et al. Aberrant TGF-β activation in bone tendon insertion induces enthesopathy-like disease[J]. J Clin Invest, 2018, 128(2): 846-860.

[5] Altman G H, Horan R L, Martin I, et al. Cell differentiation by mechanical stress[J]. Faseb j, 2002, 16(2): 270-2

[6] Semitela Â, Carvalho S, Fernandes C, et al. Biomimetic Graphene/Spongin Scaffolds for Improved Osteoblasts Bioactivity via Dynamic Mechanical Stimulation[J]. Macromol Biosci, 2022, 22(1): e2100311.

[7] Nöth U, Schupp K, Heymer A, et al. Anterior cruciate ligament constructs fabricated from human mesenchymal stem cells in a collagen type I hydrogel[J]. Cytotherapy, 2005, 7(5): 447-55.