**Supplementary Information**

# Development of tissue engineered ligaments with tita- nium spring reinforcement

Richard L. Williams,[a‡] Anqi Wang,[a‡] Neeraj Jumbu,[a‡] Jennifer Z. Paxton,[b‡] Edward T. Davis,[c] Martyn A. Snow,[c] Alastair C. Ritchie,[d] Carina B. Johannson,[e] Rachel L. Sammons,[e] and Liam M. Grover[*a]

[*] Corresponding author: Prof. Liam Grover, email: l.m.grover@bham.ac.uk

[a] School of Chemical Engineering, University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK.

[b] Centre for Integrative Physiology, Hugh Robson Building, The University of Edinburgh, 15 George Square, Edinburgh, EH8 9XD, UK

[c] The Royal Orthopaedic Hospital NHS Foundation Trust, Bristol Road South, Birmingham B31 2AP, UK.

[d] The School of Dentistry, College of Medical and Dental Sciences, University of Birmingham, St Chad's Queensway, Birmingham, B4 6NN, UK.

# SUPPLEMENTARY INFORMATION

## S2.2 Titanium substrates – initial screening of mechanical properties

Table S1: Physical dimensions of the different Ti springs screened to decide the best spring type to use in reinforcing the tissues-engineered ligaments.

| | TiS0 | TiS25 | TiS50 | TiS100* |
|---|---|---|---|---|
| **Coil diameter /mm** | 0.55 | 0.55 | 0.55 | 0.55 |
| **Wire diameter /μm** | 86 | 86 | 86 | 86 |
| **Coil gap /μm** | 0 | 25 | 50 | 100 |

*TiS100 was selected for experiments with the TEL.

The mechanical properties of the TiSs with various gap sizes were investigated using an environmental mechanical testing machine (Instron, Microtester) using a 10N loading cell. The spring was extended at a speed of 1 mm/min, and load-extension (L-E) values were recorded. The L-E curve can be divided into two parts: a linear part representing an elastic deformation, and a subsequent non-linear part where the spring loses its elasticity and deforms inelastically. The stiffness of the spring was calculated from the slope of the linear region of the L-E graph. Derived mechanical properties of the different springs are presented in Table S2 below.

Table S2: Physical dimensions of the different Ti springs screened to decide the best spring type to use in reinforcing the tissues-engineered ligaments.

| Spring type | TiS0 | TiS25 | TiS50 | TiS100 |
|---|---|---|---|---|
| ***k* (N/m)** | 29 | 83 | 40 | 58 |
| **$F_{max}$[1] (N)** | 0.09 | 0.19 | 0.13 | 0.18 |
| **Δl[2] (mm)** | 3.14 (60.7%) | 2.43 (59.5%) | 3.26 (59.25%) | 3.15 (60.58%) |
| **Max compression[3] (mm)** | 0 | 2.7 | 4.4 | 6.4 |

1. $F_{max}$ is the load when the spring starts to deform inelastically.
2. Δl is the extension when the spring starts to deform inelastically. The initial length before deformation is 8 mm for TiS0, TiS50 and TiS100, 6 mm for TiS25.
3. Max compression is a theoretical value of maximum compression of a 12 mm-long spring, calculated by adding all the gaps together. 12 mm is the length used in the present ligament model.

The high inelastic deformation thresholds for the springs (~50% extension of original length) is much higher than what would be expected of an actual ligament (~2.5%) and no creep/relaxation behavior was observed in the springs while under cyclic load testing within their strain elastic limit. This ensured that the springs themselves would not fail while under the mechanical loading regimes experienced by the ligaments over time. The TiS100 was selected on the basis that it facilitated the largest degree of compression while still having one of the highest elastic deformation limits of the different springs.

## S2.3 Formation of tissue-engineered ligaments (TEL) with titanium spring (TiS) reinforcement

S2.3.1 Manufacture of brushite anchors with embedded titanium springs.

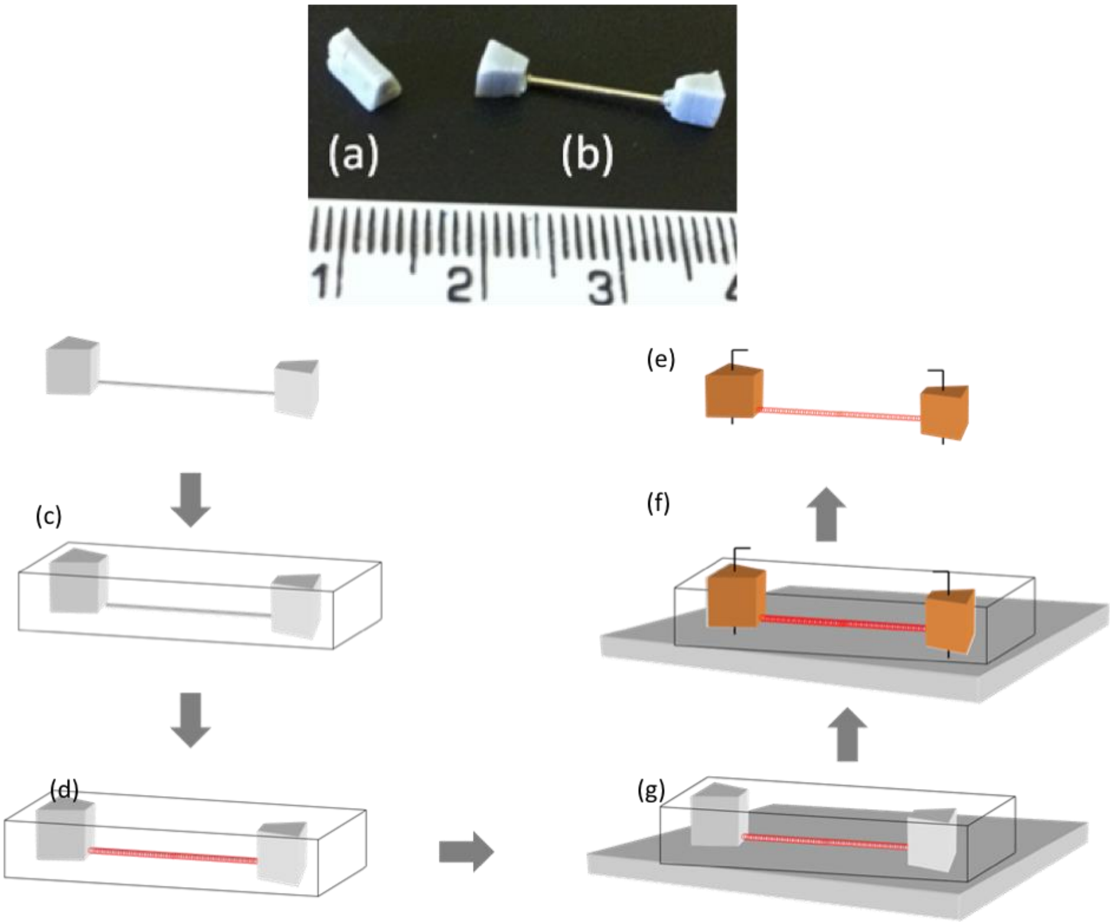



Figure S1. A schematic drawing to show the preparation of brushite cement-spring skeleton for the titanium reinforced TEL.

## S2.3 Formation of tissue-engineered ligaments (TEL) with titanium spring (TiS) reinforcement

### S2.4.1 Mechanical properties of titanium springs.

The spring was extended at a speed of 1 mm/min until the deformation reached 0.5 mm, and then the load was removed at the same speed until the spring returned to its original length. This cycle was repeated 10 times, before the spring was extended again to 0.5 mm and held for 3 min.

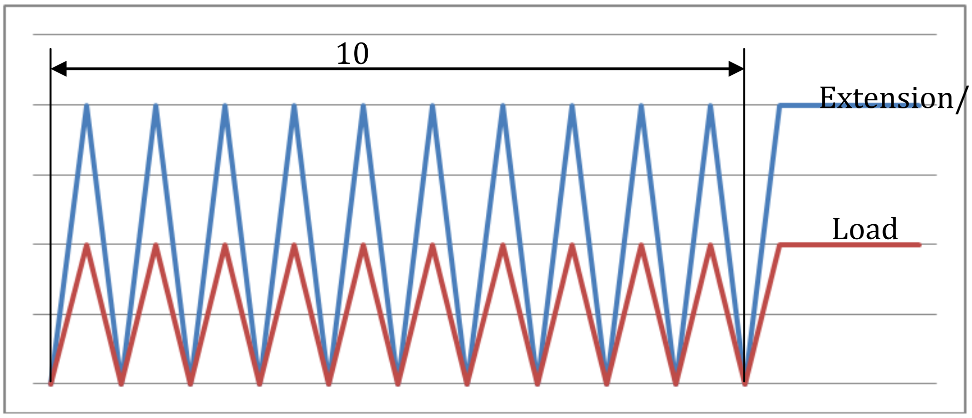
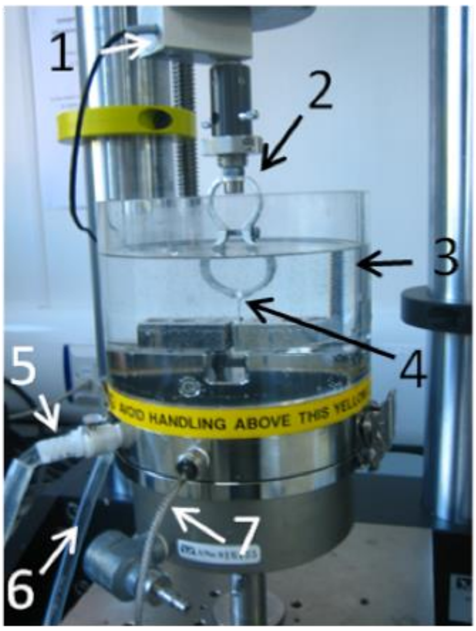



Figure S2: Diagram of the cyclic loading regime used in the cycling testing experiments.

### S2.4.2 Mechanical properties evaluation of tissue engineered ligaments, with and without springs.




1. 10 N loading cell.
2. A custom-built clamp.
3. PBS water bath.
4. TEL specimen.
5. Liquid inlet.
6. Liquid output.
7. Thermocouple.

Figure S3: Diagram showing the Instron Microtester with custom grips to attach to the TELs while submerged in a bath of PBS kept at 37°C.