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Modification of Decellularisation Methods to Assess the Effects of Swelling on the Mechanical Properties of Porcine Tendon

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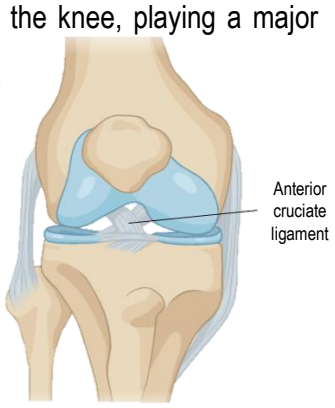
Introduction

The ACL is **the main intra-articular ligament** in the knee, playing a major role in joint stability.

When ruptured, **reconstruction is required to restore function** with 400,000 surgeries performed annually worldwide [1].

Gold standard treatments (autografts, allografts) suffer from limitations, whereas **decellularised porcine tendon** would provide an **off-the-shelf, cost-efficient option** for ACL reconstruction [2].



Tendon Hydration

During decellularisation, **PBS** is used for **washing** out cytotoxic solutes and reagents.

It also maintains tissue **hydrated**.

It **increases water content** in tendon, **swelling** the tissue [3].

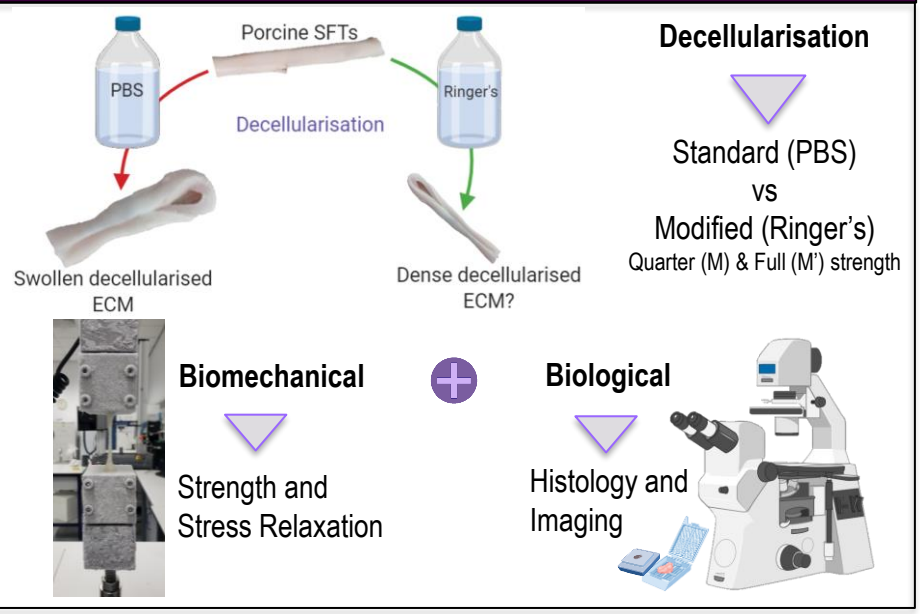
Sodium chloride-based solution with other physiological ions.

Used for **hydrating** human **tendon** [4].

Not used as **swelling-inducing** solution.

Tissue hydration and swelling could affect the mechanical properties of tendon.

Methodology



Results

Table 1. Measurements (mm) of SFTs pre-decellularisation (*i*) and post-decellularisation (*f*) (mean±95% CI). Groups that do not share the same superscript are significantly different (1-way ANOVA with Tukey post-hoc analysis). S: standard decellularisation, M: decellularisation with quarter strength Ringer's, M': decellularisation with full strength Ringer's.

	$Width_i$	$Width_f$	$Thickness_i$	$Thickness_f$
S	10.74±0.32^a	11.98±0.25^a	5.07±0.29^a	5.89±0.37^b
M	10.41±0.89^a	8.94±0.97^b	5.11±0.93^a	3.35±0.73^b
M'	10.61±0.34^a	12.02±0.40^a	4.94±0.24^a	5.96±0.29^a

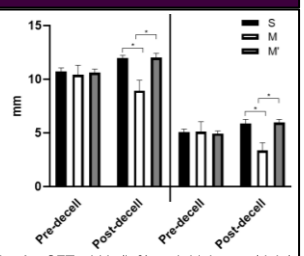


Fig. 1. pSFT width (left) and thickness (right) measurements pre- and post-decellularisation. Asterisks indicate significant difference between groups.

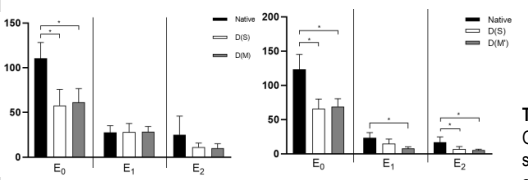


Fig. 2. Relaxation moduli (MPa) of quarter strength (left) and full strength (right) decellularised groups. Asterisks indicate significant difference between groups.

Table 2. Stress relaxation parameters (mean±95% CI). Groups that do not share the same superscript are significantly different (1-way ANOVA with Tukey post-hoc analysis).

	E_0 (MPa)	E_1 (MPa)	E_2 (MPa)	τ_1 (s)	τ_2 (s)
N	114.7±12.36^a	27.58±7.28^a	25.19±16.73^a	7.35±2.1^a	162.1±68.27^a
S	57.79±17.1^b	28±9.21^a	11.26±4.32^a	6.01±1.83^a	143.83±34.44^{a,b}
M	61.37±12.34^b	28.37±4.82^a	10.14±3.93^a	2.98±1.4^b	43.52±21.14^b
M'	69.03±11.42^b	7.94±2.32^b	5.25±1.43^b	9.48±3.27^a	122.13±66.74^{a,b}

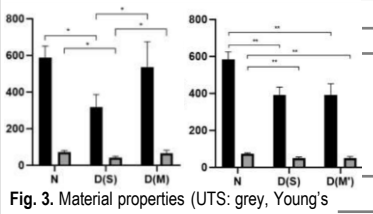


Fig. 3. Material properties (UTS: grey, Young's Modulus: black; MPa) of quarter strength (left) and full strength (right) decellularised groups. Asterisks indicate significant difference between groups.

Table 3. Strength testing parameters (mean±95% CI). Groups that do not share the same superscript are significantly different (1-way ANOVA with Tukey post-hoc analysis).

	σ_T (MPa)	ε_T (mm/mm)	E_{toe} (MPa)	E_{linear} (MPa)	UTS (MPa)	ε_{Fail} (mm/mm)
N	1.03±0.68^a	0.02±0.004^a	45.5±28.26^a	588.06±59.98^a	71.76±9.02^a	0.19±0.02^a
S	0.38±0.17^a	0.02±0.01^a	15.31±7.21^b	390.76±43.37^b	48.94±6.59^b	0.22±0.02^b
M	0.16±0.2^b	0.03±0.01^{a,b}	4.26±4.95^b	536.45±131.13^a	65.02±16.3^a	0.21±0.05^{a,b}
M'	0.45±0.26^a	0.02±0.01^a	26.05±9.69^{a,b}	390.93±61.57^b	50.55±7.61^b	0.20±0.02^{a,b}

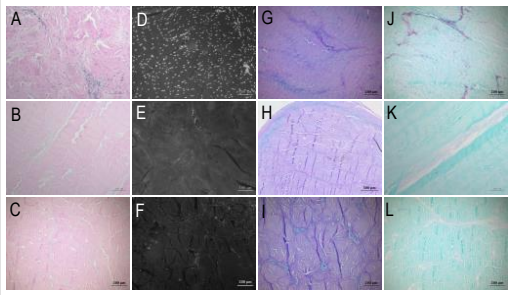


Fig. 4. Histological sections of native (A, D, G, F), standard decellularised (B, E, H, K) and modified decellularized (C, F, I, L) porcine SFTs. Stained with H&E (A-C), showing no cellular content postdecellularisation. Stained with DAPI (D-F) showing fluorescent nuclei in (D), but not (E, F). Stained with Alcian Blue (G-I) with some blue GAGs in (G) endotenon and a lesser amount in (H, I). Stained with Safranin O (J-L) with green collagen, nuclei and no GAGs in (J), and no nuclei in (K, L).

Discussion & Conclusion

Significant reduction in mechanical properties for both decellularised groups compared to native pSFTs, yet not different between decellularised groups.

Histological assessment of decellularised tissue shows significant reduction in cellular nuclei for both solutions (PBS and Ringer's).

Contradicting results in tissue dimensions for decellularised groups using different concentration of Ringer's solution. This should be further investigated using 0.9% saline solution.

Decellularised pSFTs using the standard or modified process have suitable mechanical properties to act as a graft for ACL reconstruction.

References and Acknowledgements

[1] *Acosta-Olivo, C et al. (2020). The Open Orthopaedics Journal, 13, 244.*
[2] *Herbert, A., Jones, G., Ingham, E., Fisher, J. (2015). Journal of Biomechanics, 48, 22-29.*
[3] *Safa, BN, Meadows, KD, Szczesny, SE, Elliot, DM. (2017). Journal of Biomechanics 61, 18.*
[4] *Schechtman, H. et al. (1997). Journal of Biomechanics. 30, 829-835.*

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