Development and characterization of injectable platelet lysate–silk fibroin hydrogels as candidate biomaterials for canine intradiscal application

Supplementary File

S1. Hysteresis energy calculation script developed using MATLAB (R2023a, The MathWorks, Inc., Natick, MA, USA)

This supplementary file contains the MATLAB code used to calculate the hysteresis energy (J/m^3) for each loading–unloading cycle during cyclic compression of silk fibroin (TSF) and platelet lysate–TSF (PL–TSF) hydrogels. Hysteresis energy for each cycle was defined as the difference between the strain–energy integrals of the loading and unloading phases:

$$E_{hysteresis} = \left[\int_{\varepsilon=0}^{\varepsilon_{max}} \sigma_{load}(\varepsilon)d\right] - \left[\int_{\varepsilon=\varepsilon_{max}}^{0} \sigma_{unload}(\varepsilon)d\varepsilon\right] \quad \text{(S1)}$$

where: σ = stress (kPa), ε = engineering strain

Numerical integration was performed using MATLAB (trapz function). The full script used for each cycle is shown below.

- Step 1: Energy under loading curve
  E_load = trapz(strain_load, stress_load);
- Step 2: Energy under unloading curve
  E_unload = trapz(strain_unload, stress_unload);
- Step 3: Hysteresis energy (kJ/m^3)
  E_hysteresis_kJ = E_load - E_unload;
- Step 4: Convert to J/m^3
  E_hysteresis_J = E_hysteresis_kJ × 1000

S2. Cycle-wise hysteresis energy during 50 cycles of 20% strain compression

Silk fibroin 2.5% hydrogels dissipated a total of 1,985.30 J/m³, whereas PL–TSF 2.5% hydrogels dissipated 887.10 J/m³ over 50 cycles. The cycle-wise hysteresis energy values for each loading cycle are summarized in Table S1.

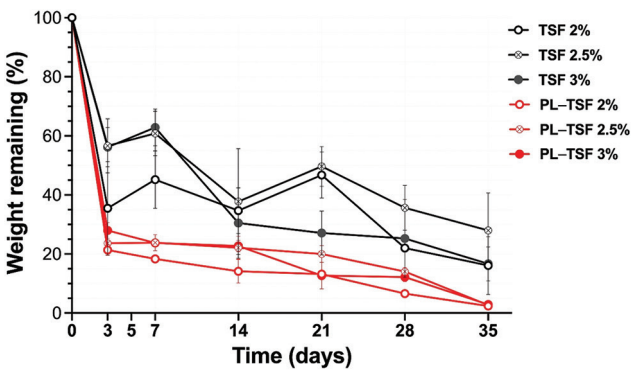

Figure S1. Weight remaining (%) of silk fibroin (TSF) and platelet lysate–TSF hydrogels measured at days 0, 3, 5, 7, 14, 21, 28, and 35 under enzymatic degradation conditions.

S3. *In vitro* degradation data

The raw degradation profile of TSF and PL–TSF hydrogels under enzymatic degradation conditions is shown in Figure S1.

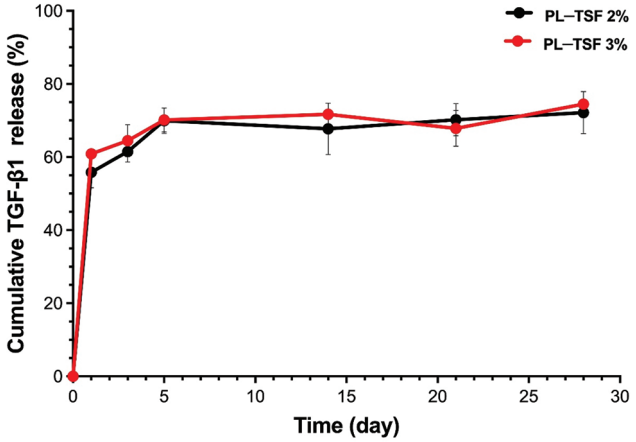

Figure S2. Raw cumulative transforming growth factor-β1 release (%) from platelet lysate–silk fibroin (PL–TSF) hydrogels (2% and 3%) over 28 days.

Table S1. Hysteresis energy (J/m^3) per cycle for PL–TSF 2.5% and TSF 2.5% hydrogels

Cycle	PL-TSF 2.5%	TSF 2.5%
1	103.3	156.00
2	40.3	69.50
3	33.9	61.00
4	29.7	56.60
5	28.9	58.00
6	27.1	54.70
7	24.8	53.50
8	22.6	49.60
9	20.7	45.50
10	21.6	47.20
11	21.2	44.10
12	19.6	41.60
13	16.5	41.00
14	16.1	41.50
15	16.8	40.80
16	15.6	36.60
17	14.6	38.70
18	14.4	35.90
19	16.7	36.60
20	14.4	37.50
21	13.3	38.30
22	15.5	37.60
23	13.9	36.30
24	15.6	35.80
25	15.9	36.20
26	14	35.50
27	12.2	35.50
28	14.3	35.90
29	13.8	32.30

(Cont'd...)

S4. Raw cumulative TGF-β1 release curve

The raw cumulative TGF-β1 release profile of PL–TSF hydrogels is shown in Figure S2.

Table S1. *(Continued)*

Cycle	PL-TSF 2.5%	TSF 2.5%
30	12	30.20
31	11.3	31.10
32	12.9	31.20
33	12.9	32.10
34	11.1	28.00
35	11.3	29.20
36	10.8	30.00
37	10	27.00
38	11.5	32.10
39	11.3	31.60
40	10.1	26.30
41	11.7	29.70
42	11.4	30.20
43	10.8	27.30
44	9.2	27.70
45	12.3	30.50
46	13.4	30.10
47	12.1	28.50
48	11.4	28.80
49	11.6	26.60
50	10.7	27.80

Abbreviations: PL–TSF: Platelet lysate–silk fibroin; TSF: Silk fibroin.

Table S2. One-phase decay model parameters for the degradation profiles of TSF and PL–TSF hydrogels

Formulation	Y_0	Plateau	K	Half-life (days)	Tau	R^2
TSF 2%	99.99	32.84	1.024	0.677	0.977	0.837
TSF 2.5%	96.98	38.05	0.235	2.950	4.256	0.858
TSF 3%	94.28	20.06	0.124	5.576	8.044	0.917
PL–TSF 2%	99.91	10.57	0.672	1.032	1.489	0.978
PL–TSF 2.5%	99.96	16.33	0.780	0.889	1.283	0.953
PL–TSF 3%	99.78	14.14	0.562	1.233	1.779	0.957

Notes: Y_0: Initial value (day 0); Plateau: Residual value at end of study; k: Decay rate constant; Tau=1/k; R^2=Goodness of fit.
Abbreviations: PL–TSF: Platelet lysate–silk fibroin; TSF: Silk fibroin.