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RESEARCH ARTICLE

Three-dimensional-printed bilayered scleral patches with mechano-biological synergistic activity for posterior scleral reinforcement in pathological myopia

Supplementary File

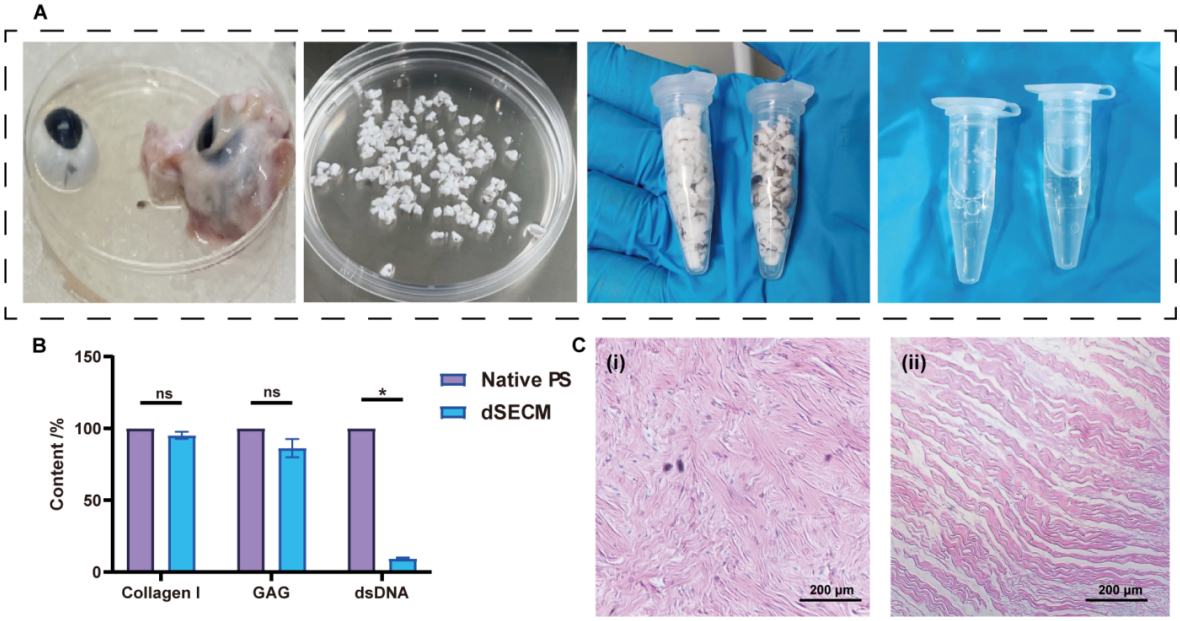

Figure S1. Decellularization efficacy and structural preservation of porcine scleral (PS) and decellularized scleral extracellular matrix (dSECM). (A) Process of dSECM extraction and decellularization. (B) Quantification of dsDNA, glycosaminoglycan (GAG), and collagen type I content in native and decellularized PS. (C) Hematoxylin and eosin staining of (i) native and (ii) decellularized porcine sclera, showing complete nuclear removal. Scale bar: 200 μm; magnification: 4×. Statistical significance was determined using a *t*-test. Statistical significance is indicated by asterisks: *$p < 0.05$.

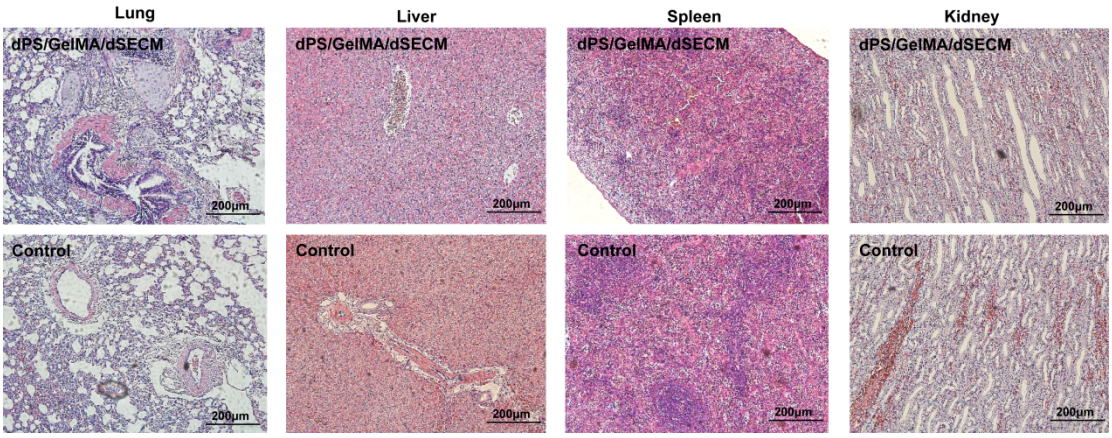

Figure S2. Hematoxylin and eosin-stained sections of major organs (liver, spleen, lung, and kidney) showed no abnormal inflammatory response at eight weeks after subcutaneous implantation of dPS/GelMA/dSECM in guinea pigs. Scale bar: 200 μm; magnification: 4×.
Abbreviations: dPS: Decellularized porcine sclera; dSECM: Decellularized scleral extracellular matrix; GelMA: Gelatin methacrylate.

Table S1. Mass loss (%) of different materials in 14 days

Group	Condition	d0	d1	d3	d5	d7	d14
dPS	PBS	0.0 ± 0.0	0.0 ± 0.0	0.8 ± 0.0	0.0 ± 0.0	0.5 ± 0.4	1.3 ± 0.5
	Col XIV	0.0 ± 0.0	7.3 ± 0.7	19.7 ± 3.3	37.6 ± 4.8	45.3 ± 5.2	58.5 ± 5.2
GelMA	PBS	0.0 ± 0.0	12.6 ± 0.9	20.3 ± 0.5	38.3 ± 0.9	52.3 ± 2.4	73.5 ± 1.8
	Col XIV	0.0 ± 0.0	22.1 ± 0.8	49.9 ± 2.2	83.3 ± 2.1	94.7 ± 2.9	100.0 ± 0.0
GelMA/dSECM	PBS	0.0 ± 0.0	12.9 ± 0.5	26.9 ± 1.1	36.3 ± 2.2	46.1 ± 1.7	75.9 ± 2.3
	Col XIV	0.0 ± 0.0	23.1 ± 0.9	57.9 ± 1.6	74.9 ± 0.7	87.0 ± 0.7	98.3 ± 1.1
dPS/GelMA/dSECM	PBS	0.0 ± 0.0	0.2 ± 0.2	9.6 ± 0.6	14.5 ±1.9	22.8 ± 2.1	33.0 ± 6.9
	Col XIV	0.0 ± 0.0	16.1 ± 1.6	27.5 ± 1.1	39.8 ± 1.5	42.0 ± 0.9	46.4 ± 1.9

Abbreviations: Col XIV: Collagenase XIV; dPS: Decellularized porcine sclera; dSECM: Decellularized scleral extracellular matrix; GelMA: Gelatin methacryloyl; PBS: Phosphate-buffered saline.

Table S2. Two-way analysis of variance (Group × Time) results in different treatment groups and time points

Parameter	Effect	Sum of squares	df	*F*-value	*p*-value	Sig.
Diopter (D); $R^2 = 0.946$	Group	139.534	4, 600	1.751×10^2	<0.001	***
	Time	204.405	2, 600	5.133×10^2	<0.001	***
	Group × Time	84.586	8, 600	5.313×10^1	<0.001	***
ACD (mm); $R^2 = 0.736$	Group	0.054	4, 600	6.04	<0.001	***
	Time	0.556	2, 600	1.254×10^2	<0.001	***
	Group × Time	0.104	8, 600	5.85	<0.001	***
LT (mm); $R^2 = 0.649$	Group	0.037	4, 600	0.86	0.490	ns
	Time	1.801	2, 600	8.31×10^1	<0.001	***
	Group × Time	0.230	8, 600	2.65	0.010	*
VCD (mm); $R^2 = 0.894$	Group	1.240	4, 600	3.23×10^1	<0.001	***
	Time	5.456	2, 600	7.11×10^2	<0.001	***
	Group × Time	1.093	8, 600	2.97×10^1	<0.001	***
AL (mm); $R^2 = 0.885$	Group	3.994	4, 600	9.56	<0.001	***
	Time	28.853	2, 600	1.73×10^2	<0.001	***
	Group × Time	5.746	8, 600	1.72×10^1	<0.001	***

Note: *$p < 0.05$, ***$p < 0.001$; ns = no significant.
Abbreviations: ACD: Anterior chamber depth; AL: Axial length; LT: Lens thickness; VCD: Vitreous chamber depth.

Table S3. Tukey HSD post-hoc: Four-week group comparisons in different treatment groups

Parameter	Comparison	Mean diff	*p*-adj	Sig.
Diopter (D)	Control vs. FDM control	6.339	<0.001	***
	dPS/GelMA vs. FDM control	2.314	<0.001	***
	dPS vs. FDM control	2.381	<0.001	***
	HDM vs. FDM control	2.214	<0.001	***
	dPS/GelMA vs. HDM	0.100	0.996	ns
	dPS/GelMA vs. dPS	−0.067	0.999	ns
	dPS vs. HDM	0.167	0.965	ns
AL (mm)	Control vs. FDM control	1.344	<0.001	***
	dPS/GelMA vs. FDM control	0.654	<0.001	***
	dPS vs. FDM control	0.544	<0.001	***
	HDM vs. FDM control	0.143	0.624	ns
	dPS/GelMA vs. HDM	−0.512	<0.001	***
	dPS/GelMA vs. dPS	−0.110	0.852	ns
	dPS vs. HDM	−0.402	0.006	**
VCD (mm)	Control vs. FDM control	0.619	<0.001	***
	dPS/GelMA vs. FDM control	0.352	<0.001	***
	dPS vs. FDM control	0.297	<0.001	***
	HDM vs. FDM control	0.107	0.211	ns
	dPS/GelMA vs. HDM	−0.245	<0.001	***
	dPS/GelMA vs. dPS	−0.055	0.831	ns

Note: $^{**}p < 0.01$, $^{***}p < 0.001$; ns = no significant.
Abbreviations: ACD: Anterior chamber depth; AL: Axial length; dPS: Decellularized porcine skin; dPS/GelMA: Decellularized porcine skin/gelatin methacrylate; FDM: Form-deprived myopia; HDM: Human dura matter; LT: Lens thickness; VCD: Vitreous chamber depth.