Supplementary Materials

Plant Recombinant Human Collagen Type I Hydrogels for Corneal Regeneration

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Supplementary Tables

Table S1. Method of *in vivo* biocompatibility grading of H&E slides from rat sub-cutaneous implantation at three months post-operation.

Cell Type/Response	**Score**				
	0	**1**	**2**	**3**	**4**
Polymorphonuclear cells	0	Rare, 1-5/phf	6-20/phf	>21/phf	packed
lymphocytes	0	Rare, 1-5/phf	6-20/phf	>21/phf	packed
Plasma Cells	0	Rare, 1-5/phf	6-20/phf	>21/phf	packed
Macrophages	0	Rare, 1-5/phf	6-20/phf	>21/phf	packed
Giant Cells	0	Rare, 1-5/phf	6-20/phf	>21/phf	packed
Necrosis	0	Minimal	Mild	Moderate	Severe
Neovascularisation	0	1-3 focal capillaries	4-10 capillaries with supporting fibroblastic structure	>10, or broad band of capillaries with supporting structure	Numerous capillaries with supporting fibroblastic structure
Band of Fibrosis	0	Narrow	Moderate	Thick	Extensive
Fatty Infiltrate	0	Minimal fat associated with fibrosis	Layering of fat and fibrosis	Elongated and broad accumulation of fat cells at implant site	Extensive fat surrounding the whole implant

PHF: Per high power field (400x)

Under the conditions of this study, the test sample was considered as a non-irritant (0,0 up to 2,9) slight irritant (3,0 up to 8,9) moderate irritant (9,0 up to 15,0) severe irritant (> 15)

Table S2. Antibodies used for immunohistochemistry

Antibody	**Dilution**	**Specificity**	**Reference**
Anti-ΔNp63	1:100	Delta Negative p63 isotype	NBP2-29467, Novus Bio, Littleton, Colorado
Anti-Coll-IV	1:100	Collagen type IV	Ab6586, Abcam, Cambridge, UK
Anti-KRT14	1:100	Cytokeratin 14	Ab9220, Abcam
Anti-Laminin	1:25	Laminin	Ab11575, Abcam
Anti-KRT3	1:100	Cytokeratin 3	Ab68260, Abcam
Anti-E-cad	1:100	E-cadherin	Ab1416, Abcam
Anti-INTB4	1:50	Integrin-β4	Ab110167, Abcam
Anti-Syndecan-1	1:100	Plasma Cells	Ab34164, Abcam
Anti-CD68	1:200	Macrophages	Ab955, Abcam
Anti-CD3	1:100	Lymphocytes	Ab16669, Abcam
Anti-Myeloperoxidase	1:100	Granulocytes	Ab9535, Abcam
Anti-vWF	1:500	von Willebrand Factor	Ab6994, Abcam
Anti-αSMA	1:50	Smooth muscle cells	Ab7817, Abcam
Donkey anti-Rabbit IgG (H+L), Cy3 conjugate	1:1000	Secondary antibody	711-165-152, Jackson ImmunoResearch (JI), Suffolk, UK
Donkey anti-Mouse IgG (H+L), Biotin conjugate	1:100	Secondary antibody	715-065-151, JI
FITC conjugated Streptavidin	1:100	Tertiary antibody	016-010-084, JI
Goat anti-rabbit IgG (H+L), Alexa Fluor 488 conjugate	1:1000	Secondary antibody	A-11008, Thermo Fisher Scientific
Goat anti-rabbit IgG (H+L), Alexa Fluor 594 conjugate	1:1000	Secondary antibody	A-11012, Thermo Fisher Scientific
Goat anti-Mouse IgG (H+L), Alexa Fluor 488 conjugate	1:1000	Secondary antibody	A-11029, Thermo Fisher Scientific
Goat anti-Mouse IgG (H+L), Alexa Fluor 594 conjugate	1:1000	Secondary antibody	A-11005, Thermo Fisher Scientific

Table S3. Microbial susceptibility of HAM versus aligned and random RHCI hydrogels.

Pseudomonas aeruginosa strain	Material	**CFU**			Average number of CFU/mL ± SEM
ATCC 15442 (1,9 x 10^8 CFU/mL)	HAM	5,4 x 10^7	9,0 x 10^7	1,0 x 10^7	5,1 ± 2,3 x 10^7
	RHCI random	2,2 x 10^5	2,0 x 10^5	0,9 x 10^5	1,7 ± 0,4 x 10^5
	RHCI aligned	4,1 x 10^5	3,4 x 10^5	3,0 x 10^5	3,5 ± 0,3 x 10^5
	TSB	3,4 x 10^{10}	3,5 x 10^{10}	3,7 x 10^{10}	3,5 ± 0,1 x 10^{10}
	PBS	8,3 x 10^7	5,3 x 10^7	8,1 x 10^7	7,2 ± 1,0 x 10^7
ATCC 9027 (8,9 x 10^8 CFU/mL)	HAM	9,0 x 10^7	2,1 x 10^8	3,1 x 10^8	2,0 ± 0,6 x 10^8
	RHCI random	0,2 x 10^6	1,6 x 10^6	2,9 x 10^6	1,5 ± 0,8 x 10^6
	RHCI aligned	7,0 x 10^6	5,9 x 10^6	5,1 x 10^6	6,0 ± 0,6 x 10^6
	TSB	2,3 x 10^{10}	4,5 x 10^{10}	3,6 x 10^{10}	3,4 ± 0,6 x 10^{10}
	PBS	4,0 x 10^7	3,7 x 10^7	1,7 x 10^7	3,1 ± 0,7 x 10^7

CFU: Colony Forming Unit; HAM: Human Amniotic Membrane; TBS: Tryptic Soy Broth; PBS: Phosphate Buffered Saline

Table S4. Results of in vivo biocompatibility grading of HE slides.

Parameter	**RHCI**					**HAM**				**Fibrin**			**Sham**	
Implant present (P) or absorbed (A)	P	P	P	P	P	A	A	A	A	A	A	A	A	A
PMN cells	0,13	0,1	0	0,1	0	/	1,27	0,73	1,07	0,46	0,4	0,47	0,1	0,47
Lymphocytes	2,73	2,33	3,13	1,87	2,07	/	1,6	1,47	1,33	0,4	0,87	1,4	0,85	0,8
Plasma Cells	0,2	0,1	0	0,13	0	/	0,1	0,1	0,1	0	0,1	0,13	0	0
Macrophages	1,87	2,13	2	1,33	1,87	/	1,47	0,87	1,93	0,86	0,6	1,2	1,85	1,4
Giant Cells	0	0	0	0	0	/	0	0	0	0	0	0	0	0
Necrosis	0	0	0	0	0	/	0	0	0	0	0	0	0	0
Neovascularisation	1,73	1,27	1,33	1,27	1	/	1,6	1,6	1,8	0,93	0,87	1,2	0,85	1,53
Fibrosis	1,53	1,87	1,87	1,33	1,27	/	4	4	4	4	4	4	4	4
Fatty Infiltrate	0	0,1	0	0	0	/	0,1	0,1	0	0,4	0,47	0,13	0	0
No. sites /slide examined	3	3	3	3	3	3	3	3	3	3	3	3	3	3
PMN: polymorphonuclear; /: quality of sample insufficient for grading														

Supplementary Figures

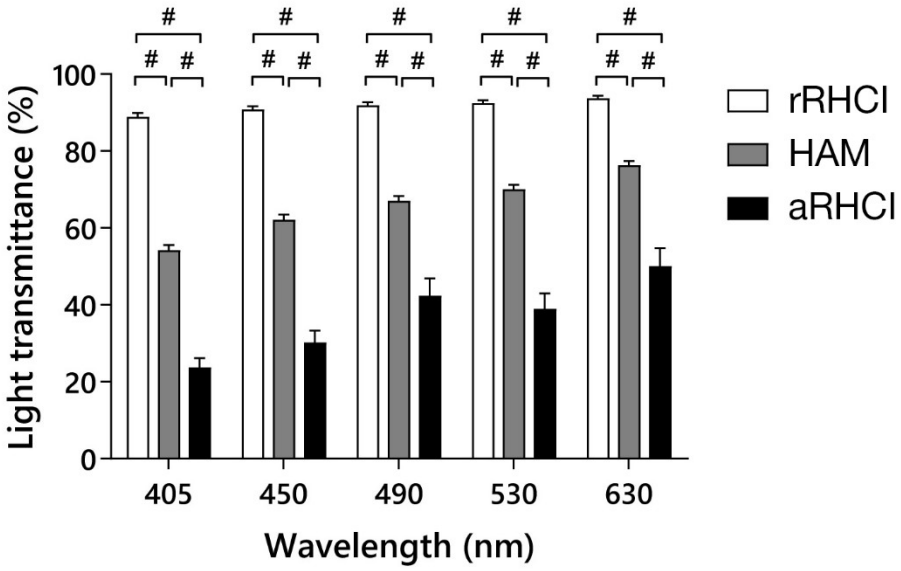

Fig. S1. Light transmittance of collagen hydrogels and amnion (HAM) at various wavelength. The rRHCI hydrogels showed highest light transmittance, whereas aRHCI showed lowest transmittance of any of the tested substrates. #p<0.01.

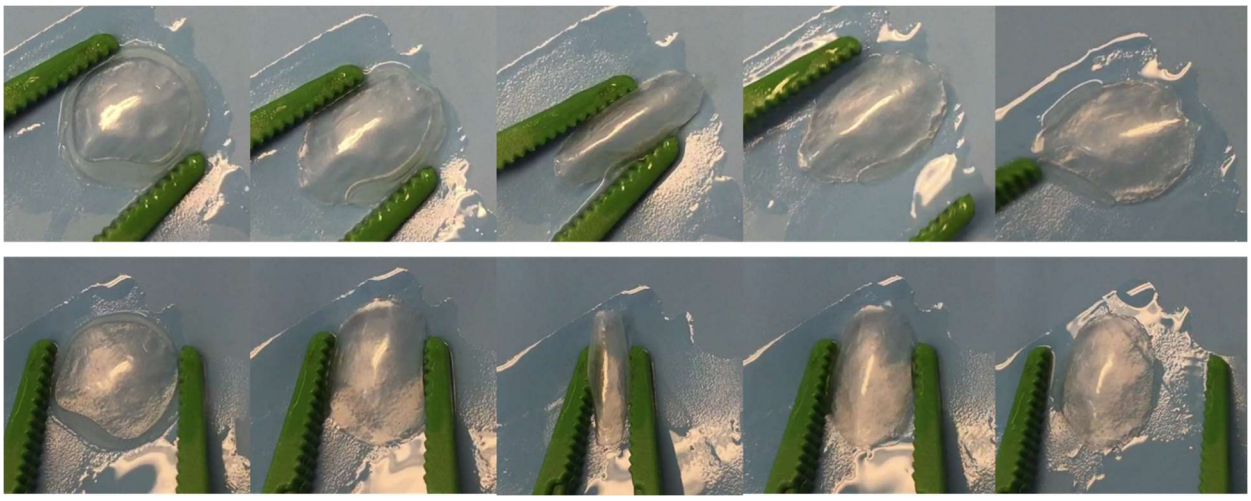

Fig. S2. Still images from a video showing that RHCI hydrogels are robust and pliable, and retains its shape after repeated manipulation. Conversion from video to frame images was performed using the VLC media player 3.0.11 (VideoLAN, Paris, France).

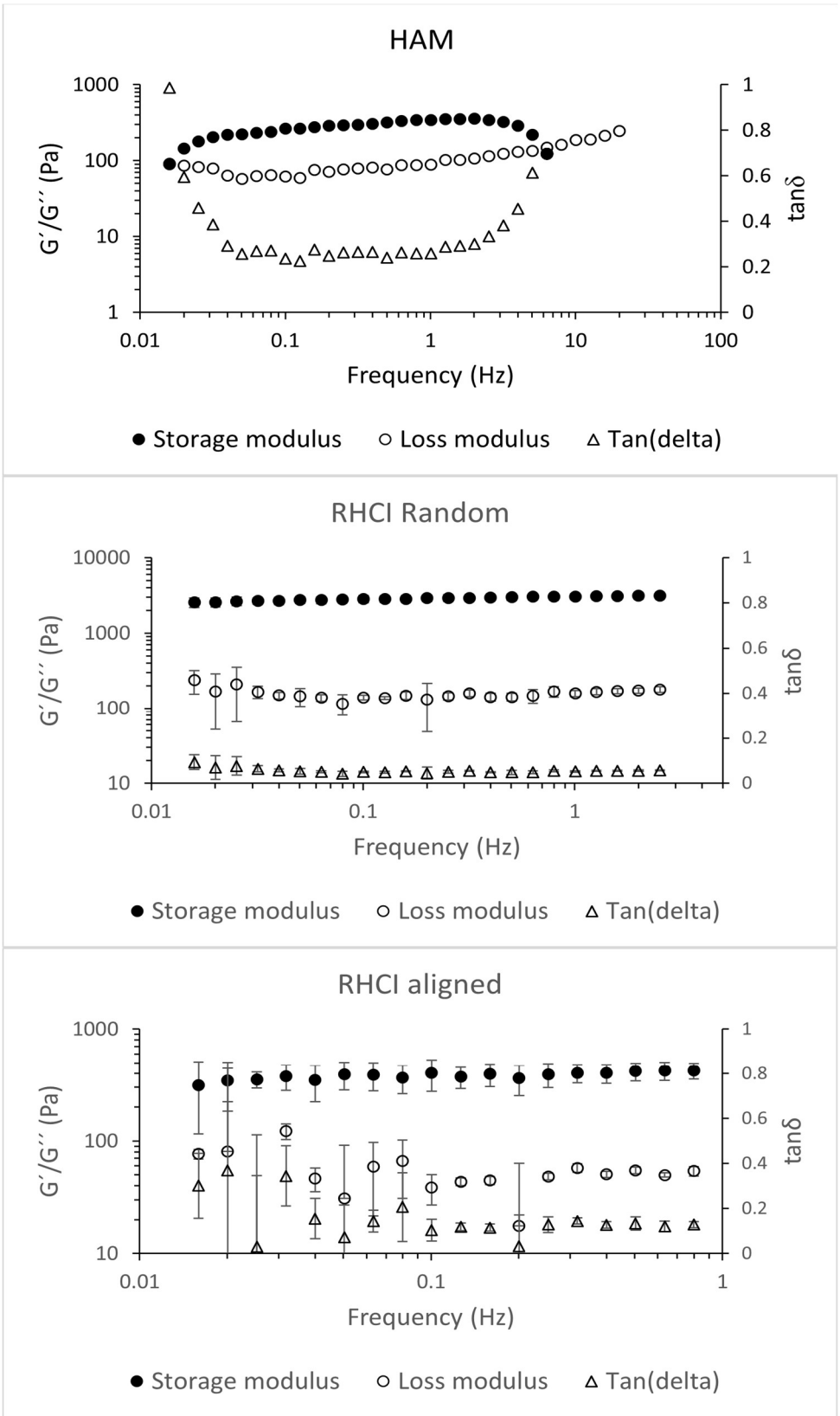

Fig. S3. Oscillatory rheology for aligned and random RHCI gels, and for amniotic membrane.

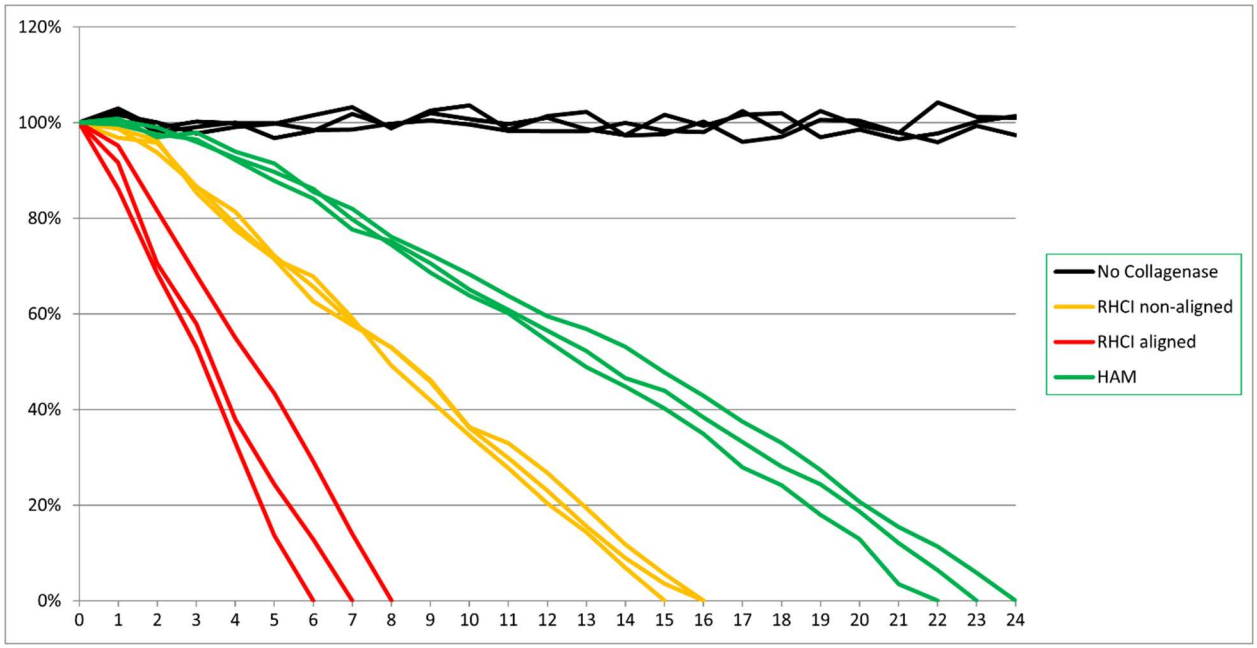

Fig. S4. *In vitro* collagenase degradation of RHCI hydrogels and human amniotic membrane (HAM). aRHCI, rRHCI and HAM degraded within 8 hrs, 16 hrs and 24 hrs respectively. HAM in Tris-HCl without the addition of type I collagenase served as a control. N=3 samples per group.