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ELECTRONIC SUPPORTING INFORMATION

Chitin-Calcium Alginate Composite Fibers for Wound Care Dressings Spun from Ionic Liquid Solution

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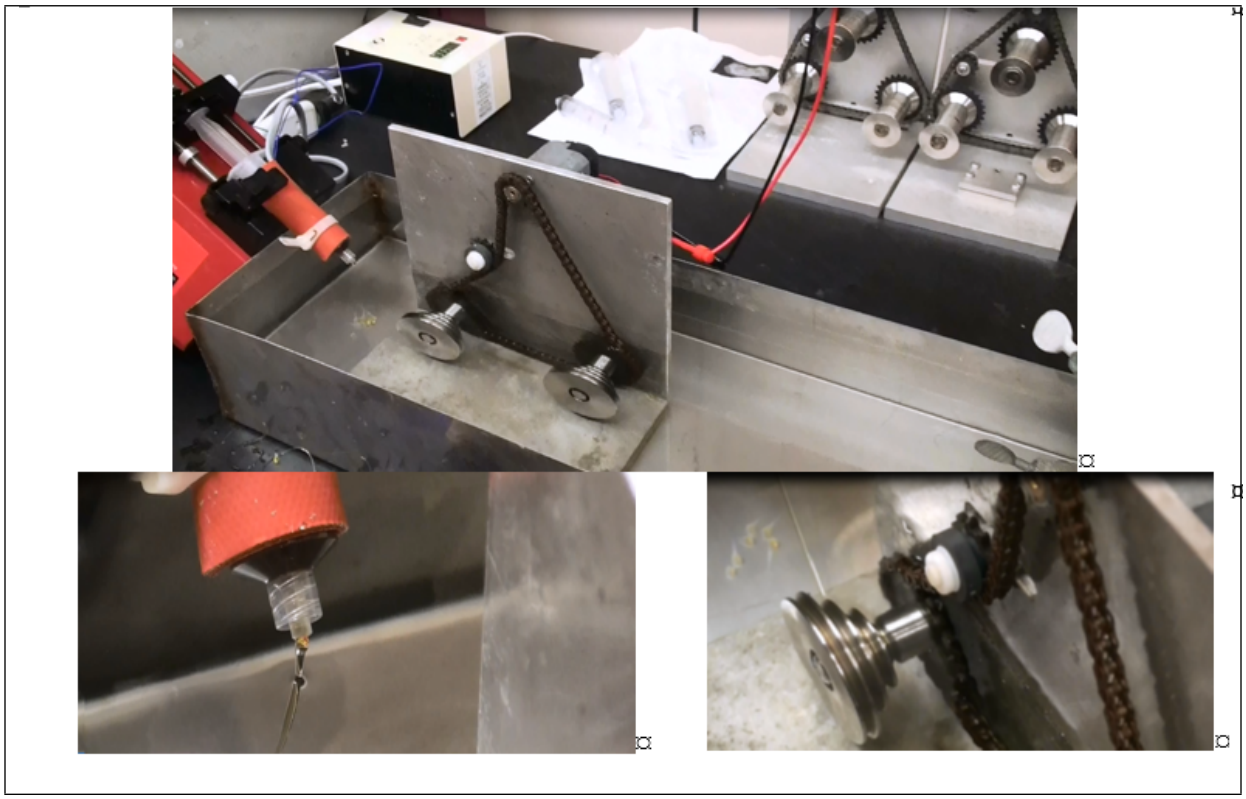


Fig S1. Fiber pulling apparatus (top) and fibers being pulled (bottom).

Table S1 The biopolymer loadings (wt%) and ratios of chitin to alginic acid dissolved in [C_2mim][OAc] studied for production of biocomposite fibers.

Chitin, wt%	Alginic Acid, wt%	Bio-polymers Ratio	Results
Effect of Biopolymers' Ratio on Fiber Quality			
1.00	2.00	0.5:1	The produced fiber is smooth with no bubbles but not strong enough. A fiber could be pulled, but could not be wrapped around the godets without breaking. Fiber also breaks when one attempts to remove it from coagulation bath.
1.50	3.00	0.5:1	The produced fiber is smooth with no bubbles but not strong enough. A fiber could be pulled but could not be wrapped around the godets without breaking.
1.50	0.75	2:1	The produced fiber is smooth with no bubbles. The fiber could be pulled but not strong enough to be wrapped around the godets without breaking.
1.75	0.88	2:1	Stronger fiber that could be pulled but still not strong enough to be wrapped around the godets without breaking.
1.75	0.583	3:1	Strong fiber that could be pulled and stretched; successfully wrapped around the godets consistently.
Effect of Biopolymers' Loading on Fiber Quality			
1.00	0.50	2:1	The solution is of low viscosity. When extruded from a syringe, the solution forms droplets and beads rather than fibers.
1.00	1.00	1:1	Solution viscosity is still low. The produced fiber is smooth with no bubbles but not strong enough. When solution is extruded into a coagulation bath, chitin gets released from solution and chitin flocs formed.
1.50	3.00	0.5:1	The produced fiber is smooth with no bubbles but not strong enough. A fiber could be pulled but could not be wrapped around the godets without breaking.
2.00	0.50	4:1	This solution was very viscous, causing the fiber to be too brittle. Fiber is not stretchable and immediately breaks when one attempts to remove it from the bath.
2.25	1.13	2:1	This solution was very viscous, causing the fiber to be too brittle. Fiber is not stretchable and cannot be wrapped around the godets.
2.50	1.25	2:1	This solution was very viscous, causing the fiber to be too brittle. Fiber is not stretchable and cannot be wrapped around the godets.
Resultant Composition of Successfully Pulled Fibers			
1.75	0.583	3:1	The fiber stretched and successfully wrapped around the godets consistently.

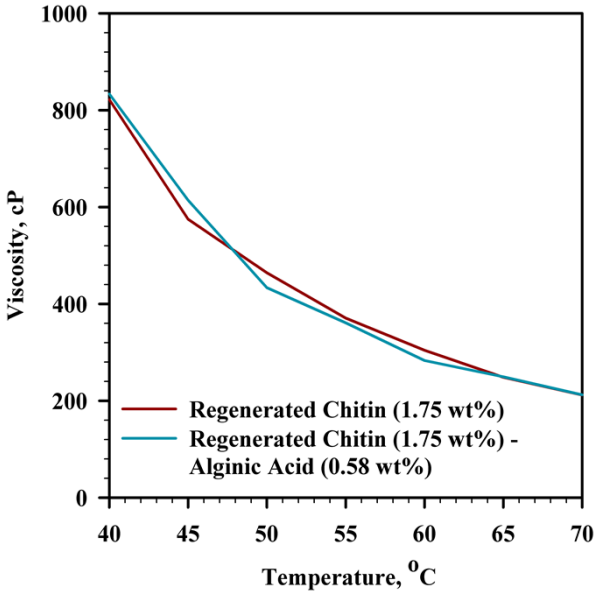


Fig. S2. Viscosity of biopolymer(s) solution in [C_2mim][OAc]: regenerated chitin (dark red), mixture of chitin and alginic acid (dark cyan).

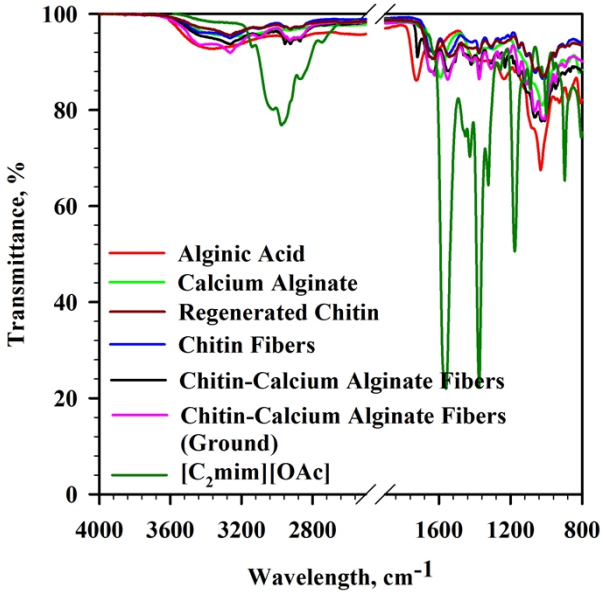


Fig. S3. FT-IR spectra of commercial alginic acid (red), commercial calcium alginate (green), regenerated chitin powder (dark red), chitin fibers (blue), whole chitin-calcium alginate fibers (black), ground chitin–calcium alginate fibers (pink), and ionic liquid [C_2mim][OAc].

Table S2 Optical microscopy images of chitin and chitin-calcium alginate fibers

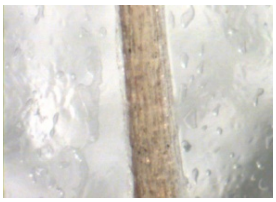
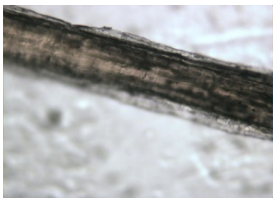
			
Chitin whole fiber	Chitin fiber cut in half, x100	Chitin-calcium alginate whole fiber	Chitin-calcium alginate fiber cut in half, x100

Table S3 SEM of chitin and chitin-calcium alginate fibers

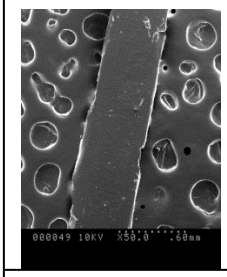
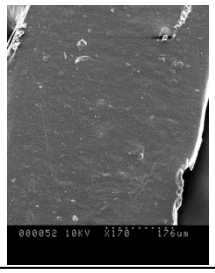
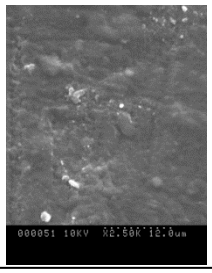
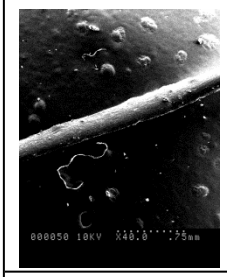
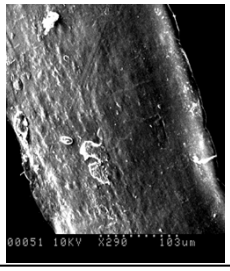
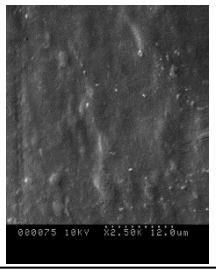
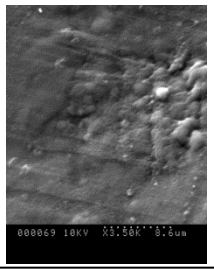
Chitin Fiber				
	x50	x 170	x 2500	x 3500
Chitin-calcium alginate fiber				
	x40	x 290	x 2500	x 3500

Table S4 TEM images of chitin and chitin-calcium alginate fibers

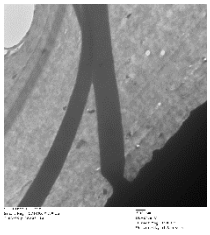
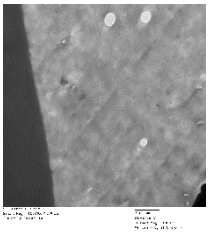
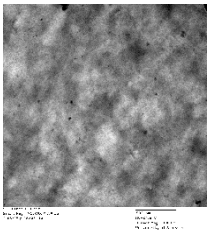
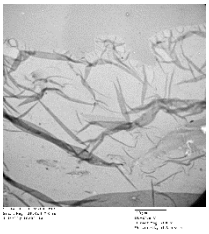
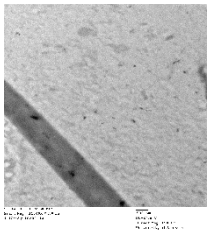
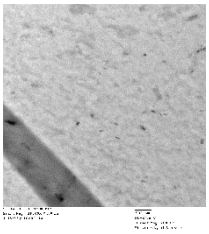
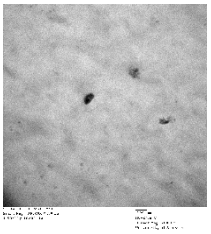
Chitin Fiber	n/d			
	x2000	x 12000	x 30000	x 50000
Chitin-calcium alginate fiber				
	x2000	x 15000	x 20000	x 70000

Fig. S4. Representative photos of chitin-covered and chitin–calcium alginate covered wound sites on Day 7 and 14. Tissue sections were stained with hematoxylin and eosin (H&E) and are shown with 40x magnification.

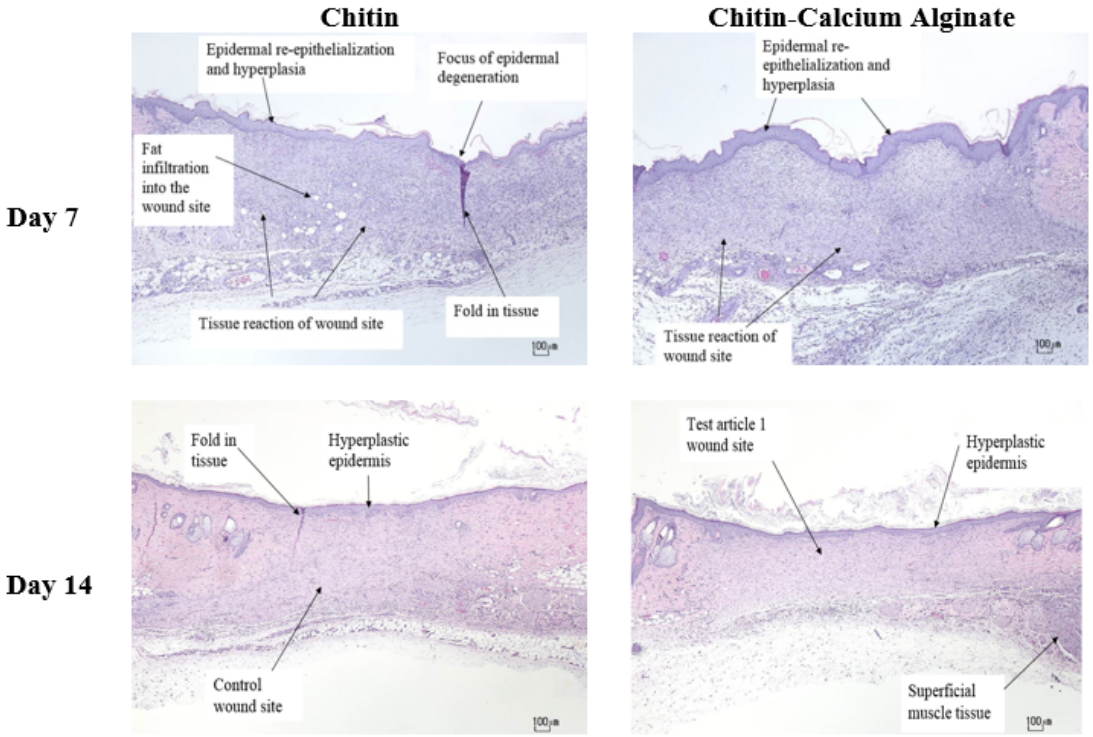


Table S5 Diameters of chitin and chitin-calcium alginate fibers (measured in inches and converted to mm.)

Fiber	#		Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Avg.
Chitin Fiber	1	mm	0.089	0.10	0.10	0.10	0.089	0.097(6)
		inches	0.0035	0.0040	0.0040	0.0040	0.0035	0.0038(2)
	2	mm	0.089	0.076	0.076	0.064	0.089	0.079(9)
		inches	0.0035	0.0030	0.0030	0.0025	0.0035	0.0031(3)
	3	mm	0.064	0.064	0.076	0.089	0.064	0.071(10)
		inches	0.0025	0.0025	0.0030	0.0035	0.0025	0.0028(4)
	4	mm	0.064	0.089	0.102	0.089	0.089	0.086(12)
		inches	0.0025	0.0035	0.0040	0.0035	0.0035	0.0034(5)
	5	mm	0.051	0.064	0.076	0.076	0.076	0.069(10)
		inches	0.0020	0.0025	0.0030	0.0030	0.0030	0.0027(4)
Chitin-calcium alginate fiber	1	mm	0.076	0.064	0.089	0.076	0.102	0.081(13)
		inches	0.0030	0.0025	0.0035	0.0030	0.0040	0.0032(5)
	2	mm	0.10	0.089	0.11	0.089	0.10	0.099(9)
		inches	0.0040	0.0035	0.0045	0.0035	0.0040	0.0039(3)
	3	mm	0.089	0.10	0.089	0.11	0.10	0.099(9)
		inches	0.0035	0.0040	0.0035	0.0045	0.0040	0.0039(3)
	4	mm	0.089	0.076	0.089	0.10	0.076	0.086(9)
		inches	0.0035	0.0030	0.0035	0.0040	0.0030	0.0034(4)
	5	mm	0.076	0.089	0.10	0.089	0.10	0.091(9)
		inches	0.0030	0.0035	0.0040	0.0035	0.0040	0.0036(4)
Average for all trials								
Chitin Fibers			mm			0.080(1)		
			inches			0.0031(4)		
Chitin-calcium alginate Fibers			mm			0.091(7)		
			inches			0.0036(5)		

Table S6 Summary data for chitin and chitin-calcium alginate fibers for ultimate stress and failure strain

Sample	Chitin Fibers	Chitin-Calcium Alginate Fibers
Width (diameter), inches	0.003 [a]	0.004 [a]
Peak Load, lbs	0.26	0.35
Peak Stress, MPa	256	216
Break Elongation, %	5.0	5.4
Yield Elongation, %	4.51	4.1
Young Modulus, GPa	8.8	6.9

[a] Five test fibers at least 30 cm long and with average diameters of 0.0031(4) inches for chitin and 0.0036(5) inches were tested. Shown here are summary results as they appear in the MTS Q-Test 25 instrument software.

Table S7 Average denier calculations of chitin and chitin-calcium alginate fibers

Fiber	Length, mm	Weight, mg	Linear Density, D, g/9,000 m	Average Linear Density, D_{ave}. g/9,000 m	D_{ave}. highest and lowest dropped, g/9000m
Chitin Fiber	30	1.20	360	401(103)	389(83)
	25.5	1.00	353		
	30	1.90	570		
	30	1.50	450		
	30	1.40	420		
	30	1.00	300		
	30	1.00	300		
	30	1.10	330		
	30	1.10	330		
	30	2.00	600		
Chitin-calcium Alginate Fiber	30	1.70	510	339(87)	330(67)
	30	1.00	300		
	30	1.20	360		
	30	0.90	270		
	30	1.20	360		
	30	1.30	390		
	30	0.80	240		
	30	0.90	270.00		
	30	0.80	240		
	30	1.50	450		

Table S8 Chitin fibers extract reactivity test – dermal observations - 0.9% Normal saline (NS)

Rabbit # 29675	**Control Scores**						**Test Scores**					
	24 h		**48 h**		**72 h**		**24 h**		**48 h**		**72 h**	
	ER	ED	ER	ED	ER	ED	ER	ED	ER	ED	ER	ED
Site 1	0	0	0	0	0	0	1	0	0	0	0	0
Site 2	0	0	0	0	0	0	1	0	0	0	0	0
Site 3	0	0	0	0	0	0	1	0	0	0	0	0
Site 4	0	0	0	0	0	0	1	0	0	0	0	0
Site 5	0	0	0	0	0	0	1	0	0	0	0	0
Total	**0**		**0**		**0**		**5**		**0**		**0**	
Rabbit # 29676	**Control Scores**						**Test Scores**					
	24 h		**48 h**		**72 h**		**24 h**		**48 h**		**72 h**	
	ER	ED	ER	ED	ER	ED	ER	ED	ER	ED	ER	ED
Site 1	0	0	0	0	0	0	0	0	0	0	0	0
Site 2	0	0	0	0	0	0	0	0	0	0	0	0
Site 3	0	0	0	0	0	0	0	0	0	0	0	0
Site 4	0	0	0	0	0	0	0	0	0	0	0	0
Site 5	0	0	0	0	0	0	0	0	0	0	0	0
Total	**0**		**0**		**0**		**0**		**0**		**0**	
Rabbit # 29680	**Control Scores**						**Test Scores**					
	24 h		**48 h**		**72 h**		**24 h**		**48 h**		**72 h**	
	ER	ED	ER	ED	ER	ED	ER	ED	ER	ED	ER	ED
Site 1	0	0	0	0	0	0	1	1	1	0	0	0
Site 2	0	0	0	0	0	0	1	1	1	0	0	0
Site 3	0	0	0	0	0	0	1	1	1	0	0	0
Site 4	0	0	0	0	0	0	1	1	1	0	0	0
Site 5	0	0	0	0	0	0	1	1	1	0	0	0
Total	**0**		**0**		**0**		**10**		**5**		**0**	

ER = Erythema; ED = Edema

Table S9 Summary results of chitin fibers extract reactivity test – dermal observations -0.9% Normal saline (NS).

Rabbit #	**Control Scores (Total ER&ED)**			**Test Scores (Total ER&ED)**		
29675	0	0	0	5	0	0
29676	0	0	0	0	0	0
29680	0	0	0	10	5	0
Rabbit #	**Total / 15**			**Total / 15**		
29675	0			0.3		
29676	0			0		
29680	0			1.0		
Average (total/3)	**0**			**0.4**		
Comparative Results (average test – average control) are 0.4-0 = 0.4						

ER = Erythema; ED = Edema

Table S10 Chitin fibers extract reactivity test – dermal observations –Cottonseed oil (CSO).

Rabbit # 29675	**Control Scores**						**Test Scores**					
	24 h		**48 h**		**72 h**		**24 h**		**48 h**		**72 h**	
	ER	ED	ER	ED	ER	ED	ER	ED	ER	ED	ER	ED
Site 1	1	0	1	0	1	0	1	0	1	0	1	0
Site 2	1	0	1	0	1	0	1	0	1	0	1	0
Site 3	1	0	1	0	1	0	1	0	1	0	1	0
Site 4	1	0	1	0	1	0	1	0	1	0	1	0
Site 5	1	0	1	0	1	0	1	0	1	0	1	0
Total	**5**		**5**		**5**		**5**		**5**		**5**	
Rabbit # 29676	**Control Scores**						**Test Scores**					
	24 h		**48 h**		**72 h**		**24 h**		**48 h**		**72 h**	
	ER	ED	ER	ED	ER	ED	ER	ED	ER	ED	ER	ED
Site 1	1	0	1	0	1	0	1	0	1	0	1	0
Site 2	1	0	1	0	1	0	1	0	1	0	1	0
Site 3	1	0	1	0	1	0	1	0	1	0	1	0
Site 4	1	0	1	0	1	0	1	0	1	0	1	0
Site 5	1	0	1	0	1	0	1	0	1	0	1	0
Total	**5**		**5**		**5**		**5**		**5**		**5**	
Rabbit # 29680	Control Scores						Test Scores					
	24 h		48 h		72 h		24 h		48 h		72 h	
	ER	ED	ER	ED	ER	ED	ER	ED	ER	ED	ER	ED
Site 1	1	0	1	0	1	0	1	0	1	0	1	0
Site 2	1	0	1	0	1	0	1	0	1	0	1	0
Site 3	1	0	1	0	1	0	1	0	1	0	1	0
Site 4	1	0	1	0	1	0	1	0	1	0	1	0
Site 5	1	0	1	0	1	0	1	0	1	0	1	0
Total	**5**		**5**		**5**		**5**		**5**		**5**	

ER = Erythema; ED = Edema

Table S11 Summary results for Chitin fibers extract reactivity test – dermal observations – Cottonseed oil (CSO).

Rabbit #	**Control Scores (Total ER&ED)**			**Test Scores (Total ER&ED)**		
29675	5	5	5	5	5	5
29676	5	5	5	5	5	5
29680	5	5	5	5	5	5
Rabbit #	**Total / 15**			**Total / 15**		
29675	1.0			1.0		
29676	1.0			1.0		
29680	1.0			1.0		
Average (total/3)	**1.0**			**1.0**		
Comparative Results (average test – average control) are 1.0-1.0 = 0						

ER = Erythema; ED = Edema

Table S12 Chitin-calcium alginate fibers extract reactivity test – dermal observations -0.9% Normal saline (NS)

Rabbit # 29682	**Control Scores**						**Test Scores**					
	24 h		**48 h**		**72 h**		**24 h**		**48 h**		**72 h**	
	ER	ED	ER	ED	ER	ED	ER	ED	ER	ED	ER	ED
Site 1	0	0	0	0	0	0	0	0	0	0	0	0
Site 2	0	0	0	0	0	0	0	0	0	0	0	0
Site 3	0	0	0	0	0	0	0	0	0	0	0	0
Site 4	0	0	0	0	0	0	0	0	0	0	0	0
Site 5	0	0	0	0	0	0	0	0	0	0	0	0
Total	**0**		**0**		**0**		**0**		**0**		**0**	
Rabbit # 29685	**Control Scores**						**Test Scores**					
	24 h		**48 h**		**72 h**		**24 h**		**48 h**		**72 h**	
	ER	ED	ER	ED	ER	ED	ER	ED	ER	ED	ER	ED
Site 1	0	0	0	0	0	0	0	0	0	0	0	0
Site 2	0	0	0	0	0	0	0	0	0	0	0	0
Site 3	0	0	0	0	0	0	0	0	0	0	0	0
Site 4	0	0	0	0	0	0	0	0	0	0	0	0
Site 5	0	0	0	0	0	0	0	0	0	0	0	0
Total	**0**		**0**		**0**		**0**		**0**		**0**	
Rabbit # 29683	**Control Scores**						**Test Scores**					
	24 h		**48 h**		**72 h**		**24 h**		**48 h**		**72 h**	
	ER	ED	ER	ED	ER	ED	ER	ED	ER	ED	ER	ED
Site 1	0	0	0	0	0	0	0	0	0	0	0	0
Site 2	0	0	0	0	0	0	0	0	0	0	0	1
Site 3	0	0	0	0	0	0	0	0	0	0	0	1
Site 4	0	0	0	0	0	0	0	0	0	0	0	1
Site 5	0	0	0	0	0	0	0	0	0	0	0	0
Total	**0**		**0**		**0**		**0**		**0**		**3**	

ER = Erythema; ED = Edema

Table S13 Summary results for Chitin-calcium alginate fibers extract reactivity test – dermal observations -0.9% Normal saline (NS)

Rabbit #	**Control Scores (Total ER&ED)**			**Test Scores (Total ER&ED)**		
29682	0	0	0	0	0	0
29685	0	0	0	0	0	0
29683	0	0	0	0	0	3
Rabbit #	**Total / 15**			**Total / 15**		
29682	0			0		
29685	0			0		
29683	0			0.2		
Average (total/3)	**0**			**0.1**		
Comparative Results (average test – average control) are 0.1-0=0.1						

ER = Erythema; ED = Edema

Table S14 Chitin-calcium alginate fibers extract reactivity test – dermal observations – Cottonseed oil (CSO)

Rabbit # 29682	**Control Scores**						**Test Scores**					
	24 h		**48 h**		**72 h**		**24 h**		**48 h**		**72 h**	
	ER	ED	ER	ED	ER	ED	ER	ED	ER	ED	ER	ED
Site 1	1	0	1	0	1	0	1	1	1	0	1	0
Site 2	1	0	1	0	1	0	1	1	1	0	1	0
Site 3	1	0	1	0	1	0	1	1	1	0	1	0
Site 4	1	0	1	0	1	0	1	0	1	0	1	0
Site 5	1	0	1	0	1	0	1	0	1	0	1	0
Total	**5**		**5**		**5**		**8**		**5**		**5**	
Rabbit # 29685	**Control Scores**						**Test Scores**					
	24 h		**48 h**				**24 h**		**48 h**			
	ER	ED		ER	ED		ER	ED		ER	ED	
Site 1	1	0	1	0	1	0	1	0	1	0	1	0
Site 2	1	0	1	0	1	0	1	0	1	0	1	0
Site 3	1	0	1	0	1	0	1	0	1	0	1	0
Site 4	1	0	1	0	1	0	1	0	1	0	1	0
Site 5	1	0	1	0	1	0	1	0	1	0	1	0
Total	**5**		**5**		**5**		**5**		**5**		**5**	
Rabbit # 29683	**Control Scores**						**Test Scores**					
	24 h		**48 h**		**72 h**		**24 h**		**48 h**		**72 h**	
	ER	ED	ER	ED	ER	ED	ER	ED	ER	ED	ER	ED
Site 1	1	1	1	1	1	0	1	0	1	0	1	0
Site 2	1	1	1	1	1	0	1	0	1	0	1	0
Site 3	1	1	1	1	1	0	1	0	1	0	1	1
Site 4	1	1	1	1	1	0	1	0	1	0	1	1
Site 5	1	1	1	1	1	0	1	0	1	0	1	0
Total	**10**		**10**		**5**		**5**		**5**		**7**	

ER = Erythema; ED = Edema; *Negative value reported as zero (0)

Table S15 Summary results for Chitin-calcium alginate fibers extract reactivity test – dermal observations –Cottonseed oil (CSO)

Rabbit #	**Control Scores (Total ER&ED)**			**Test Scores (Total ER&ED)**		
29682	5	5	5	5	5	5
29685	5	5	5	5	5	5
29683	10	10	5	5	5	7
Rabbit #	**Total / 15**			**Total / 15**		
29682	1.0			1.2		
29685	1.0			1.0		
29683	1.7			1.1		
Average (total/3)	**1.2**			**1.1**		
Comparative Results (average test – average control) are 1.1-1.2 = 0*						

Table S16 Comparison of the averages of the 7 and 14 days chitin and chitin-calcium alginate treated wound sites

	Day 7		Day 14	
Group Number	**3**	**1**	**3**	**1**
Wound Site	**Chitin**	**Chitin-calcium alginate**	**Chitin**	**Chitin-calcium alginate**
Inflammation (I)				
Polymorphonuclear Cells	1	1	1	0
Lymphocytes	1	1	1	1
Eosinophils	0	0	0	0
Mast Cells	1	1	1	1
Plasma Cells	0	0	0	0
Macrophages	3	3	1	1
Multinucleated Giant Cells	1	1	1	1
SUBTOTAL (I)	**7**	**7**	**5**	**4**
Regenerative Tissue Response (RTR)				
Neovascularization	3	3	2	2
Fibrous Connective Tissue	2	3	1	1
Blood Clot	0	0	0	0
Epidermal Scab	1	1	0	
Re-epithelialization of Wound (Migration)	3	4	4	0
Epidermal Differentiation in Re-epithelialization	3	4	4	4
Hyperplastic Epidermis	3	4	4	4
Dermal Collagen Remodeling	2	2	4	4
SUBTOTAL (RTR)	**17**	**19**	**19**	**19**
TOTAL (I + RTR)	**25**	**27**	**24**	**24**
Degenerative Tissue Response (DTR)				
Epidermal Degeneration	1	1	1	1
Abscesses	0	0	0	0
Subacute/Chronic Hemorrhage	1	1	1	1
Necrosis/Apoptosis	0	0	0	0
Ulcer	1	0	0	0
Granuloma	0	0	0	0
SUBTOTAL (DTR)	**3**	**2**	**2**	**2**
(I+RTR) - DTR	**22**	**24**	**22**	**22**
Other Tissue Changes				
Myofiber Degeneration/Necrosis	1	1	1	1
Myofiber Regeneration	1	1	1	1
Fat Infiltration	1	1	0	0
Soft Tissue Mineralization	0	0	0	0
Hair fibers in Wound	1	1	1	1
Dermal Wound Width (millimeters)	3.81	3.22	1.48	1.98
Width of Epidermal Gap (microns)	0.51	0.17	0	0

Table S17 Comparison of the averages of the 7 and 14 Day chitin and chitin-calcium alginate treated wound sites

	Average of Wound Sites			
	Chitin		**Chitin-calcium alginate**	
Group Number	**3**	**3**	**1**	**1**
Duration	**7 Days**	**14 Days**	**7 Days**	**14 Days**
Inflammation (I)				
Polymorphonuclear Cells	1	1	1	0
Lymphocytes	1	1	1	1
Eosinophils	0	0	0	0
Mast Cells	1	1	1	1
Plasma Cells	0	0	0	0
Macrophages	3	1	3	1
Multinucleated Giant Cells	1	1	1	1
SUBTOTAL (I)	**7**	**5**	**7**	**4**
Regenerative Tissue Response (RTR)				
Neovascularization	3	2	3	2
Fibrous Connective Tissue	2	1	3	1
Blood Clot	0	0	0	0
Epidermal Scab	1	0	1	0
Re-epithelialization of Wound (Migration)	3	4	4	4
Epidermal Differentiation in Re-epithelialization	3	4	4	4
Hyperplastic Epidermis	3	4	4	4
Dermal Collagen Remodeling	2	4	2	4
SUBTOTAL (RTR)	**17**	**19**	**19**	**19**
TOTAL (I + RTR)	**25**	**24**	**27**	**24**
Degenerative Tissue Response (DTR)				
Epidermal Degeneration	1	1	1	1
Abscesses	0	0	0	0
Subacute/Chronic Hemorrhage	1	1	1	1
Necrosis/Apoptosis	0	0	0	0
Ulcer	1	0	0	0
Granuloma	0	0	0	0
SUBTOTAL (DTR)	**3**	**2**	**2**	**2**
(I+RTR) - DTR	**22**	**22**	**24**	**22**
Other Tissue Changes				
Myofiber Degeneration/Necrosis	1	1	1	1
Myofiber Regeneration	1	1	1	1
Fat Infiltration	1	0	1	0
Soft Tissue Mineralization	0	0	0	0
Hair fibers in Wound	1	1	1	1
Dermal Wound Width (millimeters)	3.81	1.48	3.22	1.98
Width of Epidermal Gap (microns)	0.51	0	0.17	0

Table S18 Histopathology of 7 day chitin treated wound sites

Animal Number	**7021**		**7022**		**7023**		**7025**		**7031**		**Average of Wound Sites**
Group Number	**3**										
Wound Site	**LT**	**RT**	**LT**	**RT**	**LT**	**RT**	**LT**	**RT**	**LT**	**RT**	
Inflammation (I)											
Polymorphonuclear Cells	1	1	1	1	1	1	0	1	1	1	1
Lymphocytes	1	1	1	1	1	1	1	1	2	2	1
Eosinophils	0	0	0	0	0	0	0	0	0	0	0
Mast Cells	1	1	1	1	1	1	1	1	1	1	1
Plasma Cells	0	0	0	0	0	0	0	0	0	0	0
Macrophages	3	4	3	3	3	3	3	3	3	4	3
Multinucleated Giant Cells	1	1	1	1	1	1	1	1	1	1	1
SUBTOTAL (I)	**7**	**8**	**7**	**7**	**7**	**7**	**6**	**7**	**8**	**9**	**7**
Regenerative Tissue Response (RTR)											
Neovascularization	3	4	2	2	3	3	3	3	3	3	3
Fibrous Connective Tissue	3	3	1	1	2	2	3	3	3	3	2
Blood Clot	0	0	0	0	0	0	1	0	0	0	0
Epidermal Scab	1	1	0	0	1	1	1	0	1	1	1
Re-epithelialization of Wound (Migration)	2	2	4	4	4	3	4	4	4	3	3
Epidermal Differentiation in Re-epithelialization	2	2	4	4	2	3	4	4	4	2	3
Hyperplastic Epidermis	2	2	4	4	2	3	4	4	4	2	3
Dermal Collagen Remodeling	1	1	3	3	2	2	1	1	1	1	2
SUBTOTAL (RTR)	**14**	**15**	**18**	**18**	**16**	**17**	**21**	**19**	**20**	**15**	**17**
TOTAL (I + RTR)	**21**	**23**	**25**	**25**	**23**	**24**	**27**	**26**	**28**	**24**	**25**
Degenerative Tissue Response (DTR)											
Epidermal Degeneration	1	1	2	0	2	0	1	1	1	2	1
Abscesses	0	0	0	0	0	0	0	0	0	0	0
Subacute/Chronic Hemorrhage	1	0	1	1	1	1	1	1	1	1	1
Necrosis/Apoptosis	0	0	0	0	0	0	0	0	0	0	0
Ulcer	2	3	0	0	0	2	0	0	0	1	1
Granuloma	0	0	0	0	0	0	0	0	0	0	0
SUBTOTAL (DTR)	**4**	**4**	**3**	**1**	**3**	**3**	**2**	**2**	**2**	**4**	**3**
(I+RTR) - DTR	**17**	**19**	**22**	**24**	**20**	**21**	**25**	**24**	**26**	**20**	**22**
Other Tissue Changes											
Myofiber Degeneration/Necrosis	1	0	0	1	0	0	1	0	1	1	1
Myofiber Regeneration	1	1	1	1	1	1	1	1	1	1	1
Fat Infiltration	1	0	1	1	1	1	0	1	1	1	1
Soft Tissue Mineralization	0	0	0	0	0	0	0	0	0	0	0
Hair fibers in Wound	1	0	1	1	0	1	1	1	1	1	1
Dermal Wound Width (millimeters)	3.57	3.60	1.86	3.86	5.18	4.23	3.82	4.30	3.54	4.12	3.81
Width of Epidermal Gap (microns)	1.13	2.32	0	0	0	1.37	0	0	0	0.25	0.51

LT = Left Side RT = Right Side

Table S19 Histopathology of 14 day chitin treated wound sites

Animal Number	**7026**		**7027**		**7028**		**7029**		**7030**		**Average of Wound Sites**
Group Number	**3**										
Wound Site	**LT**	**RT**	**LT**	**RT**	**LT**	**RT**	**LT**	**RT**	**LT**	**RT**	
Inflammation (I)											
Polymorphonuclear Cells	1	0	0	1	1	0	0	0	1	1	1
Lymphocytes	1	1	1	1	1	1	1	1	1	1	1
Eosinophils	0	0	0	0	0	0	0	0	0	0	0
Mast Cells	1	1	1	1	1	1	1	1	1	1	1
Plasma Cells	0	1	0	1	0	0	0	0	0	0	0
Macrophages	1	1	1	1	1	1	2	1	3	1	1
Multinucleated Giant Cells	0	1	1	1	0	0	1	1	1	0	1
SUBTOTAL (I)	**4**	**5**	**4**	**6**	**4**	**3**	**5**	**4**	**7**	**4**	**5**
Regenerative Tissue Response (RTR)											
Neovascularization	2	2	2	2	2	2	2	2	2	2	2
Fibrous Connective Tissue	1	1	1	1	1	1	1	1	1	1	1
Blood Clot	0	0	0	0	0	0	0	0	0	0	0
Epidermal Scab	1	0	0	0	0	1	0	0	1	0	0
Re-epithelialization of Wound (Migration)	4	4	4	4	4	4	4	4	4	4	4
Epidermal Differentiation in Re-epithelialization	4	4	4	4	4	4	4	4	4	4	4
Hyperplastic Epidermis	4	4	4	4	4	4	4	4	4	4	4
Dermal Collagen Remodeling	4	4	4	4	4	4	4	4	4	4	4
SUBTOTAL (RTR)	**20**	**19**	**19**	**19**	**19**	**20**	**19**	**19**	**20**	**19**	**19**
TOTAL (I + RTR)	**24**	**24**	**23**	**25**	**23**	**23**	**24**	**23**	**27**	**23**	**24**
Degenerative Tissue Response (DTR)											
Epidermal Degeneration	0	0	1	1	0	1	1	1	1	1	1
Abscesses	0	0	0	0	0	0	0	0	0	0	0
Subacute/Chronic Hemorrhage	1	1	1	1	1	1	1	1	1	1	1
Necrosis/Apoptosis	0	0	0	0	0	0	0	0	0	0	0
Ulcer	0	0	0	0	0	0	0	0	0	0	0
Granuloma	0	0	0	0	0	0	0	0	0	0	0
SUBTOTAL (DTR)	**1**	**1**	**2**	**2**	**1**	**2**	**2**	**2**	**2**	**2**	**2**
(I+RTR) - DTR	**23**	**23**	**21**	**23**	**22**	**21**	**22**	**21**	**25**	**21**	**22**
Other Tissue Changes											
Myofiber Degeneration/Necrosis	1	1	1	1	1	1	1	1	1	1	1
Myofiber Regeneration	1	1	1	1	1	1	1	1	1	1	1
Fat Infiltration	0	1	0	0	0	1	1	0	0	0	0
Soft Tissue Mineralization	0	0	0	0	0	0	0	0	0	0	0
Hair fibers in Wound	0	1	1	1	0	0	0	1	1	0	1
Dermal Wound Width (millimeters)	1.64	0.71	2.42	1.14	1.69	0.41	1.44	1.59	2.25	1.54	1.48
Width of Epidermal Gap (microns)	0	0	0	0	0	0	0	0	0	0	0

LT = Left Side RT = Right Side

Table S20 Histopathology of 7 day chitin-calcium alginate treated wound sites

Animal Number	**7001**		**7002**		**7003**		**7004**		**7005**		**Average of Wound Sites**
Group Number	**1**										
Wound Site	**LT**	**RT**	**LT**	**RT**	**LT**	**RT**	**LT**	**RT**	**LT**	**RT**	
Inflammation (I)											
Polymorphonuclear Cells	0	1	1	1	1	1	1	1	0	0	1
Lymphocytes	1	1	1	1	2	1	1	2	1	1	1
Eosinophils	0	0	0	0	0	0	0	0	0	0	0
Mast Cells	1	1	1	1	1	1	1	1	1	1	1
Plasma Cells	0	1	1	0	0	0	0	0	0	0	0
Macrophages	3	3	3	4	3	4	3	4	3	3	3
Multinucleated Giant Cells	0	1	1	1	1	1	1	1	1	1	1
SUBTOTAL (I)	**5**	**8**	**8**	**8**	**8**	**8**	**7**	**9**	**6**	**6**	**7**
Regenerative Tissue Response (RTR)											
Neovascularization	3	3	3	3	3	4	3	4	3	3	3
Fibrous Connective Tissue	2	3	2	3	2	4	3	3	2	1	3
Blood Clot	0	0	0	0	0	0	0	0	0	0	0
Epidermal Scab	0	2	1	1	0	1	0	1	1	3	1
Re-epithelialization of Wound (Migration)	4	2	4	4	4	3	4	4	4	4	4
Epidermal Differentiation in Re-epithelialization	4	2	4	4	4	2	4	4	4	4	4
Hyperplastic Epidermis	4	2	4	4	4	2	4	3	4	4	4
Dermal Collagen Remodeling	2	1	2	1	2	1	1	1	3	3	2
SUBTOTAL (RTR)	**19**	**15**	**20**	**20**	**19**	**17**	**19**	**20**	**21**	**22**	**19**
TOTAL (I + RTR)	**24**	**23**	**28**	**28**	**27**	**25**	**26**	**29**	**27**	**28**	**27**
Degenerative Tissue Response (DTR)											
Epidermal Degeneration	0	2	0	2	0	1	0	2	0	0	1
Abscesses	0	0	0	1	0	0	0	0	0	0	0
Subacute/Chronic Hemorrhage	1	1	1	1	1	0	1	1	1	1	1
Necrosis/Apoptosis	0	0	0	0	0	1	0	0	0	0	0
Ulcer	0	2	0	0	0	1	0	1	0	0	0
Granuloma	0	0	0	0	0	1	0	0	0	0	0
SUBTOTAL (DTR)	**1**	**5**	**1**	**4**	**1**	**4**	**1**	**4**	**1**	**1**	**2**
(I+RTR) - DTR	**23**	**18**	**27**	**24**	**26**	**21**	**25**	**25**	**26**	**27**	**24**
Other Tissue Changes											
Myofiber Degeneration/Necrosis	0	0	1	1	0	1	1	1	1	0	1
Myofiber Regeneration	1	1	1	1	1	1	1	1	1	1	1
Fat Infiltration	1	1	0	1	1	0	0	1	0	1	1
Soft Tissue Mineralization	0	0	0	0	0	0	0	0	0	0	0
Hair fibers in Wound	1	1	1	1	1	1	1	1	1	1	1
Dermal Wound Width (millimeters)	3.49	2.95	3.78	3.46	3.35	3.15	3.64	3.91	2.37	2.14	3.22
Width of Epidermal Gap (microns)	0	0.96	0	0	0	0.75	0	0	0	0	0.17

LT = Left Side; RT = Right Side

Table S21 Histopathology of 14 day chitin-calcium alginate treated wound sites

Animal Number	**7006**		**7007**		**7008**		**7009**		**7010**		**Average of Wound Sites**
Group Number	**1**										
Wound Site	**LT**	**RT**	**LT**	**RT**	**LT**	**RT**	**LT**	**RT**	**LT**	**RT**	
Inflammation (I)											
Polymorphonuclear Cells	0	0	0	0	0	1	0	0	0	1	0
Lymphocytes	1	1	1	1	1	1	1	1	1	1	1
Eosinophils	0	0	0	0	0	0	0	0	0	0	0
Mast Cells	0	1	1	0	1	1	1	1	1	1	1
Plasma Cells	0	0	1	0	0	0	0	0	0	0	0
Macrophages	1	1	2	1	1	1	1	2	2	1	1
Multinucleated Giant Cells	0	1	1	1	1	1	1	1	1	1	1
SUBTOTAL (I)	**2**	**4**	**6**	**3**	**4**	**5**	**4**	**5**	**5**	**5**	**4**
Regenerative Tissue Response (RTR)											
Neovascularization	2	2	2	3	2	2	2	2	2	1	2
Fibrous Connective Tissue	1	1	1	1	1	1	1	1	1	1	1
Blood Clot	0	0	0	0	0	0	0	0	0	0	0
Epidermal Scab	0	0	0	0	0	0	0	4	0	0	0
Re-epithelialization of Wound (Migration)	4	4	4	4	4	4	4	4	4	4	4
Epidermal Differentiation in Re-epithelialization	4	4	4	4	4	4	4	4	4	4	4
Hyperplastic Epidermis	4	4	4	4	4	4	4	4	4	4	4
Dermal Collagen Remodeling	4	4	4	4	4	4	4	4	4	4	4
SUBTOTAL (RTR)	**19**	**19**	**19**	**20**	**19**	**19**	**19**	**23**	**19**	**18**	**19**
TOTAL (I + RTR)	**21**	**23**	**25**	**23**	**23**	**24**	**23**	**28**	**24**	**23**	**24**
Degenerative Tissue Response (DTR)											
Epidermal Degeneration	0	0	2	1	1	0	1	0	0	0	1
Abscesses	0	0	0	0	0	0	0	0	0	0	0
Subacute/Chronic Hemorrhage	1	1	1	1	1	1	1	1	1	1	1
Necrosis/Apoptosis	0	0	0	0	0	0	0	0	0	0	0
Ulcer	0	0	0	0	0	0	0	0	0	0	0
Granuloma	0	0	0	0	0	0	0	0	0	0	0
SUBTOTAL (DTR)	**1**	**1**	**3**	**2**	**2**	**1**	**2**	**1**	**1**	**1**	**2**
(I+RTR) - DTR	**20**	**22**	**22**	**21**	**21**	**23**	**21**	**27**	**23**	**22**	**22**
Other Tissue Changes											
Myofiber Degeneration/Necrosis	0	1	0	1	0	1	0	1	1	1	1
Myofiber Regeneration	1	1	1	1	1	1	1	1	1	1	1
Fat Infiltration	0	0	1	0	0	0	0	1	0	0	0
Soft Tissue Mineralization	0	0	0	0	0	0	0	0	0	0	0
Hair fibers in Wound	0	1	1	1	1	1	1	1	1	1	1
Dermal Wound Width (millimeters)	1.54	2.55	2.74	2.45	2.26	2.30	1.41	1.58	2.27	0.65	1.98
Width of Epidermal Gap (microns)	0	0	0	0	0	0	0	0	0	0	0

LT = Left Side; RT = Right Side

Table S22 KEY FOR TABLES 7 - 10: Severity Scores (per wound site):

	Score				
	0 (Absent)	**1 (Minimal)**	**2 (Mild)**	**3 (Moderate)**	**4 (Marked)**
Inflammation: *Polymorphonuclear cells* *Lymphocytes* *Plasma Cells* *Eosinophils* *Macrophages*	Absent	Rare, 1-5 per 40x field	6-10 per 40x field	Heavy Infiltrate	Packed
Multinucleated Giant Cells	Absent	Rare, 1-5 per 40x field	6-10 per 40x field	Heavy Infiltrate	Sheets
	Score				
	0 (Absent)	**1 (Minimal)**	**2 (Mild)**	**3 (Moderate)**	**4 (Marked)**
Neovascularization	Absent	Minimal capillary proliferation, (focal, 1-3 capillary buds), or small blood vessels (venules, and / or arterioles)	Groups of 4-7 capillaries with supporting fibroblastic structures	Broad band of capillaries with supporting structures	Extensive band of capillaries with supporting fibroblastic structures
	Score[b]				
	0 (Absent)	**1 (Minimal)**	**2 (Mild)**	**3 (Moderate)**	**4 (Marked)**
Fibrous Connective Tissue *Blood Clot* *Epidermal Scab* *Re-epithelialization* *Epidermal Differentiation* *Hyperplastic Epidermis* *Dermal Collagen Remodeling* *Abscesses* *Subacute/Chronic Hemorrhagec* *Necrosis/Apoptosis* *Ulcer* *Granuloma* *Myofiber Degeneration/Necrosis* *Myofiber Regeneration* *Fat Infiltration* *Soft Tissue Mineralization* *Hair Fibers in Wound*	Absent	> 0 to 25% of the dermal/epidermal area within the wound site is involved	> 25 to 50% of the dermal/epidermal area within the wound site is involved	> 51 to 75% of the dermal/epidermal area within the wound site is involved	> 76 to 100% of the dermal/epidermal area within the wound site is involved

Necrosis/Apoptosis consists of nuclear cellular debris from degenerating inflammatory cells. Percentages of tissue component(s) are estimates. Acute hemorrhage is not graded. Acute hemorrhage is generally due to rupture of new blood vessels within the tissue reaction from tissue handling.

Table S23 Measurement of dermal wound width of chitin treated wound sites

Animal Number	Group	Wound Site	Meas. 1 (µm)	Meas. 2 (µm)	Meas. 3 (µm)	Mean (mm)	Average of Mean Values
Day 7							
7021	3	LT	3967	3358	3370	3.57	3.81
		RT	3865	3461	3473	3.60	
7022		LT	1887	1790	1887	1.86	
		RT	4197	3648	3732	3.86	
7023		LT	5244	5034	5252	5.18	
		RT	4625	4112	3949	4.23	
7025		LT	4257	3690	3515	3.82	
		RT	4685	4118	4088	4.30	
7031		LT	3961	3443	3226	3.54	
		RT	4774	3912	3683	4.12	
Day 14							
7026	3	LT	1724	1483	1718	1.64	1.48
		RT	777	601	753	0.71	
7027		LT	2647	2116	2502	2.42	
		RT	1404	922	1084	1.14	
7028		LT	1802	1615	1645	1.69	
		RT	349	397	481	0.41	
7029		LT	1551	1320	1446	1.44	
		RT	1736	1482	1537	1.59	
7030		LT	2357	2061	2333	2.25	
		RT	1507	1368	1748	1.54	

LT = Left Side; RT = Right Side

Table S24 Measurement of dermal wound width of chitin-calcium alginate treated wound sites

Animal Number	Group	Wound Site	Meas. 1 (µm)	Meas. 2 (µm)	Meas. 3 (µm)	Mean (mm)	Average of Mean Values
Day 7							
7001	1	LT	3841	3346	3268	3.49	3.22
		RT	4046	2404	2411	2.95	
7002		LT	4420	3660	3268	3.78	
		RT	3811	3346	3219	3.46	
7003		LT	3720	3195	3147	3.35	
		RT	3599	2985	2852	3.15	
7004		LT	4432	3406	3087	3.64	
		RT	4245	3835	3660	3.91	
7005		LT	3141	1633	2327	2.37	
		RT	2484	1905	2019	2.14	
Day 14							
7006	1	LT	1965	1175	1476	1.54	1.98
		RT	3081	2417	2152	2.55	
7007		LT	3008	2604	2592	2.74	
		RT	2526	2405	2429	2.45	
7008		LT	2508	2236	2049	2.26	
		RT	2429	2224	2254	2.30	
7009		LT	1706	1259	1277	1.41	
		RT	1597	1537	1603	1.58	
7010		LT	2435	2206	2158	2.27	
		RT	668	487	807	0.65	

LT = Left Side; RT = Right Side

Table S25 Measurement of epidermal gap width of chitin treated wound sites

Animal Number	Group	Wound Site	Meas. 1 (µm)	Meas. 2 (µm)	Meas. 3 (µm)	Mean (mm)	Average of Mean Values
Day 7							
7021	3	LT	1160	1124	1099	1.13	0.51
		RT	2350	2317	2299	2.32	
7022		LT	0	0	0	0	
		RT	0	0	0	0	
7023		LT	0	0	0	0	
		RT	1389	1386	1347	1.37	
7025		LT	0	0	0	0	
		RT	0	0	0	0	
7031		LT	0	0	0	0	
		RT	267	244	243	0.25	
Day 14							
7026	3	LT	0	0	0	0	0
		RT	0	0	0	0	
7027		LT	0	0	0	0	
		RT	0	0	0	0	
7028		LT	0	0	0	0	
		RT	0	0	0	0	
7029		LT	0	0	0	0	
		RT	0	0	0	0	
7030		LT	0	0	0	0	
		RT	0	0	0	0	

LT = Left Side; RT = Right Side

Table S26 Measurement of epidermal gap width of chitin-calcium alginate treated wound sites

Animal Number	Group	Wound Site	Meas. 1 (μm)	Meas. 2 (μm)	Meas. 3 (μm)	Mean (mm)	Average of Mean Values
Day 7							
7001	1	LT	0	0	0	0	0.17
		RT	1017	918	943	0.96	
7002		LT	0	0	0	0	
		RT	0	0	0	0	
7003		LT	0	0	0	0	
		RT	745	747	752	0.75	
7004		LT	0	0	0	0	
		RT	0	0	0	0	
7005		LT	0	0	0	0	
		RT	0	0	0	0	
Day 14							
7006	1	LT	0	0	0	0	0
		RT	0	0	0	0	
7007		LT	0	0	0	0	
		RT	0	0	0	0	
7008		LT	0	0	0	0	
		RT	0	0	0	0	
7009		LT	0	0	0	0	
		RT	0	0	0	0	
7010		LT	0	0	0	0	
		RT	0	0	0	0	

LT = Left Side; RT = Right Side