

Supporting Information

Antibacterial and Safe Chitosan-Graphene Hydrogel Film: A Promising Nanotherapeutics for *Staphylococcus aureus* Wound Infection

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#Both have equal contributions.

Table S1. Percentage weight loss of chitosan and CGrAP film measured from TGA

Material	Weight loss (%)
Chitosan	65.21
CGrAP nanocomposite hydrogel film	58.23

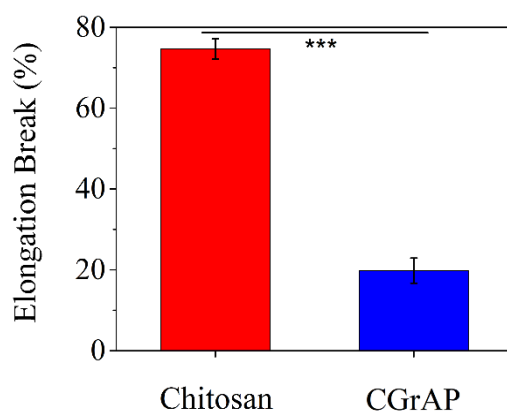


Fig. S1. Elongation at Break of chitosan and CGrAP nanocomposite hydrogel films. Data represents an average of five independent experiments; \pm SD shown by error bar (***) p value <0.01).

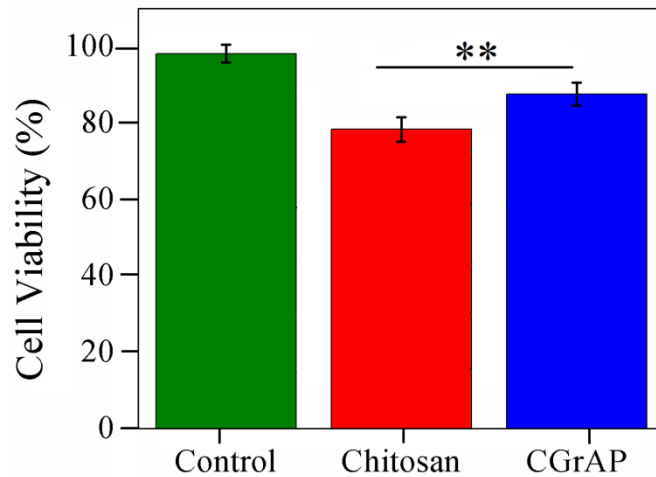


Fig. S2. MTT assay on L929 fibroblast cells on treatment with chitosan and CGrAP nanocomposite hydrogel film. (** p value < 0.02).

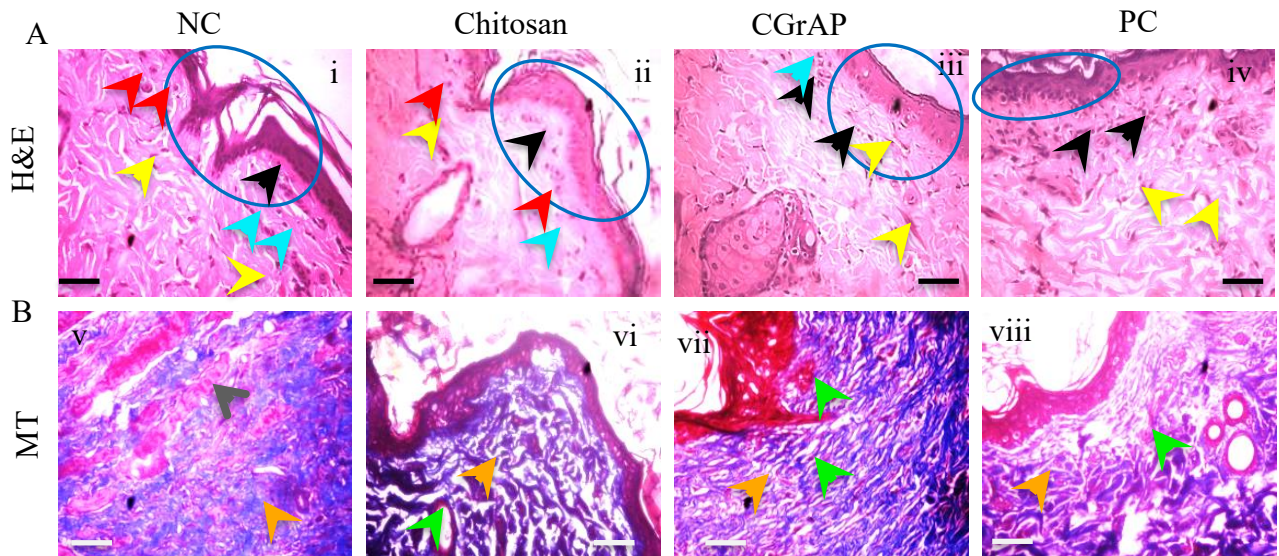


Fig. S3. Histological (H&E) images (A) and Masson's Trichrome (B) of the skin tissue at 14th day after treatment with NC (i and v); Cs (ii and vi); CGrAP nanobiocomposite hydrogel film (iii and vii) and PC (iv and viii) respectively in *S. aureus* infected Wistar rat model. (Scale bar 100 μ m). (Arrow indicates: red- neutrophils; blue- lymphocytes; black-epithelial cells; yellow- fibroblast cells; green arrow- blood vessels; orange arrow-collagen; dark grey arrow- damaged blood cells dark blue circle marks the regeneration of epidermal layer).

Table S2. Draize grading scale.

Erythema Formation		Edema Formation	
No erythema	0	No edema	0
Very slight erythema	1	Very Slight Edema	1
Slight erythema	2	Slight Edema	2
Moderate to severe erythema	3	Moderate to severe edema	3

Table S3. Body weight during acute toxicity

Parameters	Control		CGrAP Film	
	1 st Day	14 th Day	1 st Day	14 th Day
Body Weight (Prior to test perform)	205 ± 2.3 g	220 ± 3.3 g	210 ± 2.5 g	226 ± 2.9 g

Table S4. Physical characteristics during acute toxicity

Parameters	Saline		CGrAP Film	
	1 st Day	14 th Day	1 st Day	14 th Day
Behavioral pattern				
First 24 h	Normal	Normal	Normal	Normal
Skin Toxicity				
Changes in skin				
Blanching	Absent	Absent	Absent	Absent
Cyanosis	Absent	Absent	Absent	Absent
Erythema	Absent	Absent	Absent	Absent
Itching	Absent	Absent	Absent	Absent
Changes in Fur				
Falling in Fur	Absent	Absent	Absent	Absent
Discoloration	Absent	Absent	Absent	Absent
Piloerection	Absent	Absent	Absent	Absent
Changes in Eyes				
Ptosis	Absent	Absent	Absent	Absent
Exophthalmos	Absent	Absent	Absent	Absent
Redness	Absent	Absent	Absent	Absent
Pupil Dilated	Absent	Absent	Absent	Absent
Pupil Constricted	Absent	Absent	Absent	Absent
Salivation				
Viscid	Absent	Absent	Absent	Absent
Watery	Absent	Absent	Absent	Absent

Table S5. Behavioral pattern during acute toxicity

Parameters	Saline		CGrAP Film	
	1 st Day	14 th Day	1 st Day	14 th Day
Behavioral Pattern				
Restlessness	Absent	Absent	Absent	Absent
Grooming	Absent	Absent	Absent	Absent
Lying flat on Belly	Absent	Absent	Absent	Absent
Lying flat on Back	Absent	Absent	Absent	Absent
Lying flat on side	Absent	Absent	Absent	Absent
Respiration				
Depression	Absent	Absent	Absent	Absent
Stimulation	Absent	Absent	Absent	Absent
Failure	Absent	Absent	Absent	Absent
Central Nervous System				
Defecation	Absent	Absent	Absent	Absent
Urination	Absent	Absent	Absent	Absent
Squatting	Absent	Absent	Absent	Absent
Ataxic Gait	Absent	Absent	Absent	Absent
Timidity	Absent	Absent	Absent	Absent
Writhing	Absent	Absent	Absent	Absent
Tremors	Absent	Absent	Absent	Absent
Paresis of hind limb	Absent	Absent	Absent	Absent
Paresis of forepaws	Absent	Absent	Absent	Absent
Twitches	Absent	Absent	Absent	Absent
Convulsion	Absent	Absent	Absent	Absent
Clonic	Absent	Absent	Absent	Absent
Tonic	Absent	Absent	Absent	Absent
Rolling & Jumping	Absent	Absent	Absent	Absent

Table S6. Blood profile test

Differential Count	Saline	CGrAP film
Neutrophils (%)	10.4 ± 1.2	10.1 ± 1.3
Lymphocytes (%)	86.2 ± 2.2	87.7 ± 1.7***
Eosinophils (%)	1.3 ± 0.02	1.3 ± 0.01
Monocytes (%)	1.43 ± 4.6	1.47 ± 8.9***
Basophils (%)	0.01 ± 0.001	0.01 ± 0.001
Haemoglobin (g/dl)	13.12 ± 1.2	14.4 ± 2.1***
Haematocrit (%)	39.2 ± 4.6	36.1 ± 4.1***
Platelet Count (lakhs/cu mm)	6.80 ± 0.413	7.59 ± 0.213***
Biochemistry		
Urea (mg/dl)	19.5 ± 4	18.4 ± 4***
Creatinine (mg/dl)	0.55 ± 0.012	0.57 ± 0.003***
Liver Function Test		
SGOT (U/L)	119.11 ± 4.5	126.15 ± 5.3 ***
SGPT (U/L)	33.66 ± 3.1	30.83 ± 2.3***
Alkaline phosphatase (U/L)	72.11 ± 3.4	75.43 ± 2.6 ***
T. Protein (mg/dl)	6.6 ± 0.1	7.5 ± 1.7***
Albumin (mg/dl)	3.4 ± 0.4	3.7 ± 1.6***
T. Bilirubin (mg/dl)	0.8 ± 0.002	0.9 ± 0.006***
D. Bilirubin (mg/dl)	0.1 ± 0.04	0.2 ± 0.04***
Lipid Profile		
Total cholesterol (mg/dl)	114.5 ± 4.7	112.5 ± 7***
Triglyceride (mg/dl)	81.56 ± 12	78.98 ± 14***
HDL cholesterol (mg/dl)	52.67 ± 3	49.55 ± 5 ***
LDL cholesterol (mg/dl)	48.79 ± 2.6	46.64 ± 1.6***
VLDL cholesterol (mg/dl)	15.6 ± 3.77	17.8 ± 3.69***

***p value < 0.002 with respect to control