

Glycated collagen – a 3D matrix system to study pathological cell behavior

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SUPPLEMENTARY INFORMATION:

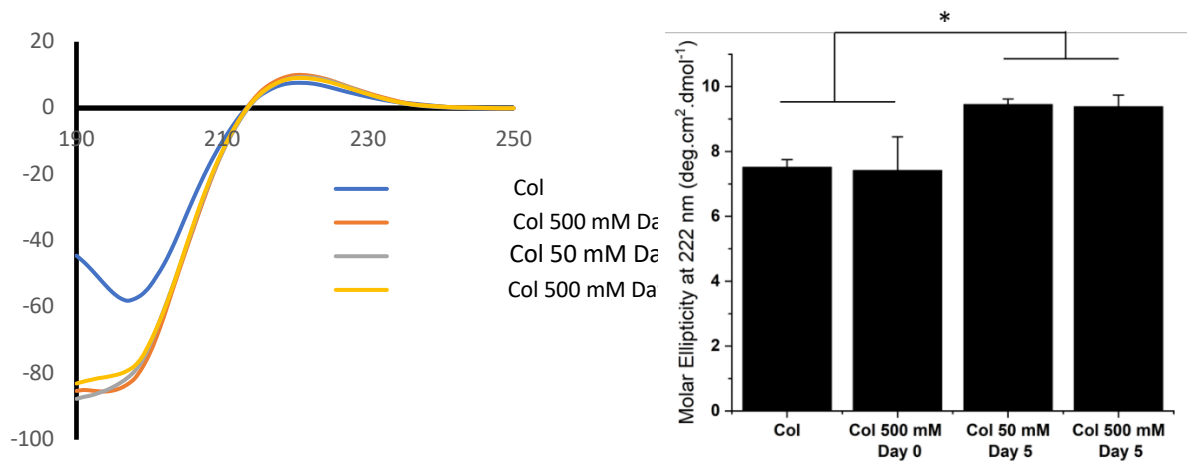


Figure S1. Characterization of unfiltered glycosylated collagen. CD spectra of unglycosylated collagen (Col) and that of collagen incubated with 50 mM (Col 50 mM Day 5) or 500 mM of glucose for 0 days (Col 500 mM Day 0) or 5 days (Col 500 mM Day 5) (left); Bar chart quantifying positive molar ellipticity as observed through CD spectroscopy for glycosylated and unglycosylated collagen(right). * $p < 0.05$.

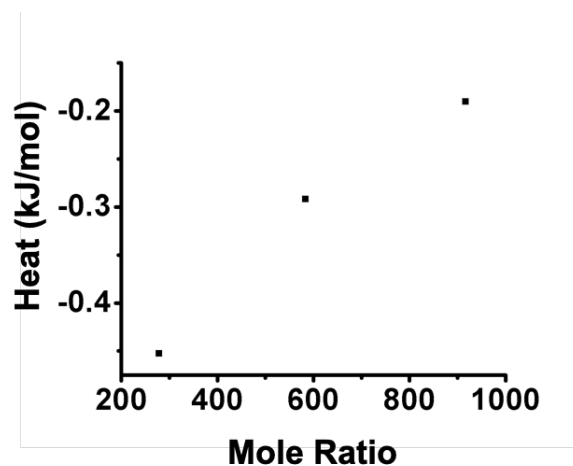


Figure S2. Isothermal titration calorimetric measurements showing the amount of heat released due to interaction of collagen with sugar when three injections of glucose (1M) were titrated with collagen successively, each at an interval of 3 hours.

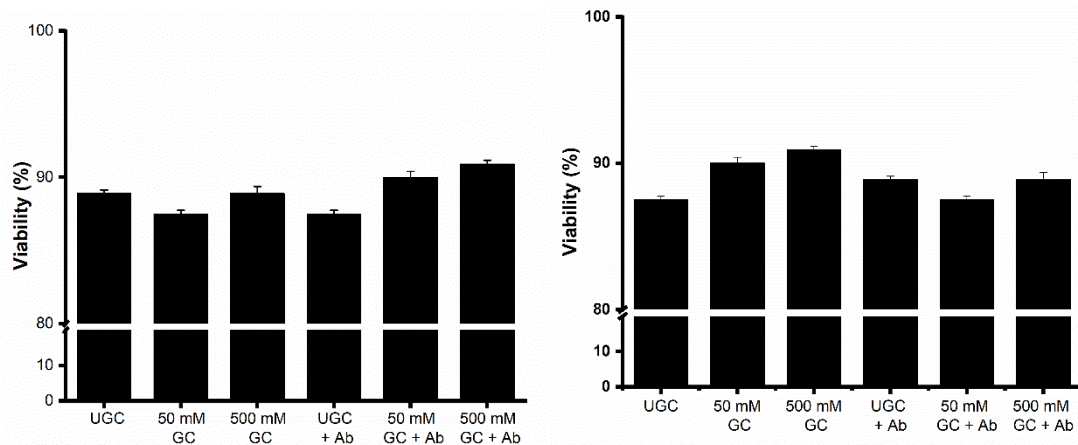


Figure S3. Viability of cells seeded on 2D (left) and in 3D (right) collagen matrices. Percentage viability of untreated cells on unglycated collagen (UGC), 50 mM glycated collagen (50 mM GC) and 500 mM glycated collagen (500 mM GC); and of RAGE neutralized cells on unglycated collagen (UGC + Ab), 50 mM glycated collagen (50 mM GC + Ab) and 500 mM glycated collagen (500 mM GC + Ab).

Conditions	Viable Cell count after 48 hours	
	2D	3D
UGC	26,600	23,300
50 mM GC	23,333	30,000
500 mM GC	26,666	33,330
UGC + anti-RAGE	23,300	26,600
50 mM GC + anti-RAGE	30,000	24,000
500 mM GC + anti-RAGE	33,330	26,660

Table S1. Count of viable cells seeded on 2D and in 3D collagen matrices. Conditions depict untreated cells on unglycated collagen (UGC), 50 mM glycated collagen (50 mM GC) and 500 mM glycated collagen (500 mM GC); and of RAGE neutralized cells on unglycated collagen (UGC + Ab), 50 mM glycated collagen (50 mM GC + Ab) and 500 mM glycated collagen (500 mM GC + Ab).

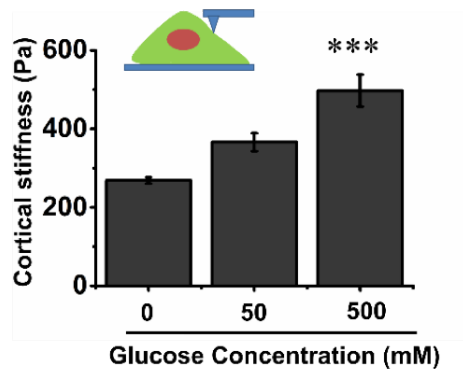


Figure S4. AFM measurements for cortical stiffness of cells cultured on glycated and unglycated collagen gels.

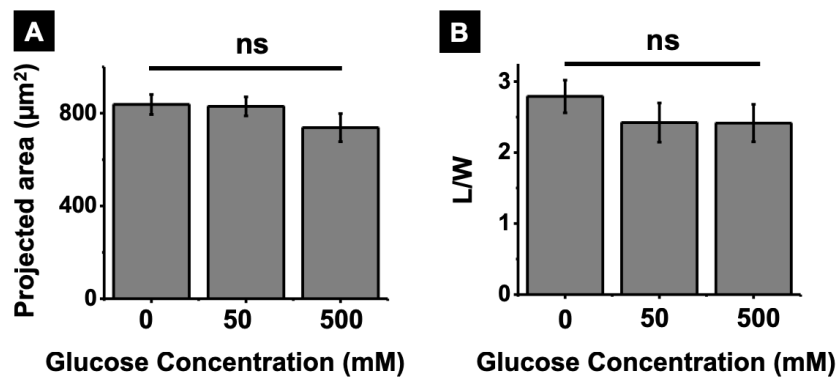


Figure S5. Effect of excess glucose on cell morphology. Graphical representation of (A) cell spread area and (B) elongation (L/W ratio) calculated from phase contrast images of cells cultured on 2D petri plates in presence of 0 mM, 50 mM and 500 mM glucose. ns $p > 0.05$.

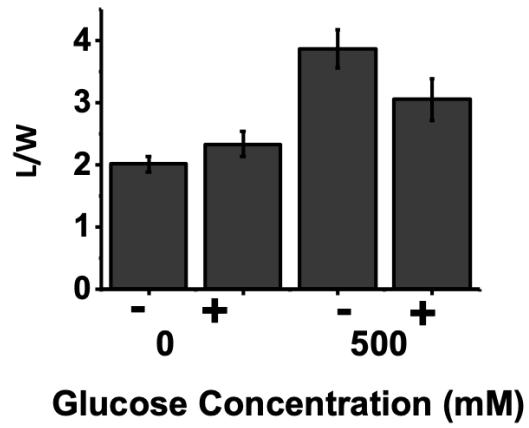


Figure S6. Effect of RAGE neutralization on elongation of cells. Graphical representation of elongation (L/W ratio) of cells untreated (-) or treated (+) with anti-RAGE antibody and then cultured on collagen matrix, unglycated or glycated with 500 mM glucose.