Supplementary Information

Materials and Methods

Differential Scanning Calorimetry (DSC)

Thermal degradation of collagen film was determined by DSC analysis using DSC Q200 (V23.10 Build 79) differential scanning calorimeter. The temperature was standardized using iridium as standard. 3mg of air dried samples of native collagen, PCSN cross linked collagen and glutaraldehyde (GTA) cross linked collagen was weighed and transferred into a standard aluminum pans. The aluminum pans were then sealed by Tzero sample press machine. Empty pan was used as a reference. The heating was set from 20 to 300ºC at the rate of 5ºC/min respectively. The denaturation temperature (Td) / melting temperature was determined as the onset value of the temperature for the occurrence of endothermic peak.

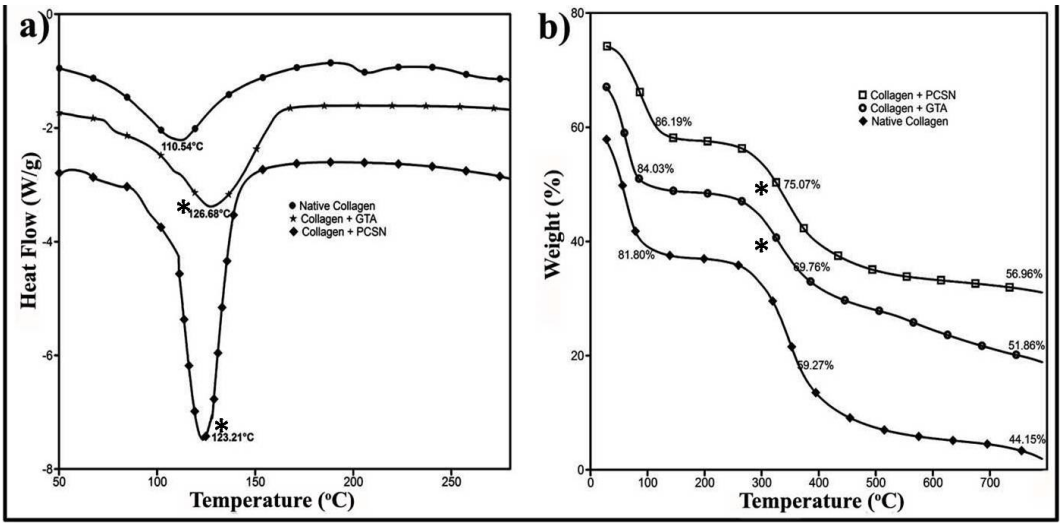
Thermo Gravimetric Analysis

Thermo gravimetric analysis was performed using TGA Q50 (V20.6 Build 31TA instrument, USA to determine the percentage of weight loss of collagen scaffolds based on its thermal decomposition temperature. The native collagen (control), PCSN and glutaraldehyde (reference) cross linked air dried collagen scaffolds was weighed (10mg) and transferred into a platinum pan with handle and the decomposition of the samples was monitored from 20ºC to 800ºC under nitrogen atmosphere at heating rate of 20ºC/min.

Results

Thermal Stability of PCSN cross-linked collagen

The thermal transition was measured using Differential scanning calorimetry, a single endothermic peak was obtained and their thermograms are shown in Figure 5a. The thermogram of native collagen showed a transition temperature at 110.54ºC and PCSN cross linked collagen had 123.21ºC which was near to that of glutaraldehyde cross-linked collagen (126.68ºC - used as a reference). The increase in the denaturation temperature was due to the extensive cross linking of the collagen with PCSN. In order to develop a good scaffold it is important that the materials used for its preparation should withstand higher temperature so that it would not get decomposed during manufacturing processes or in abnormal physiological condition. The thermal stability of collagen scaffold is determined as a measure of thermal transition (denaturation or melting) temperature Td. Td is the temperature at which the triple helical structure of collagen gets converted into random coil structures, generally it is termed as helix-coil transition which depends on degree of hydration. Denaturation temperature of PCSN cross linked collagen shifted to higher temperature when compared with native collagen due to increased inter and intra fibrillar interaction mediated by PCSN.



Supplementary Figure 1 Thermodynamic analysis. a. DSC thermograms of native collagen, glutaraldehyde and PCSN cross-linked collagen. b. Thermo gravimetric analysis of native collagen, glutaraldehyde and PCSN cross-linked collagen scaffolds. * Statistically significant compared to native collagen ($p<0.05$).

Thermogravimetric Analysis

Collagen cross linked with plumbagin caged silver nanoparticles based scaffold was further investigated by thermo gravimetric analysis. Figure 5b depicted the thermal degradation curves of native collagen, PCSN and glutaraldehyde

cross linked collagen. The thermal degradation of collagen showed a three step temperature transition as well as corresponding weight loss which accounted for the function of cross linking efficiency. In native collagen, the thermal degradation started from initial dehydration of bound water moieties at 60ºC to 90ºC, polymer denaturation was observed at 250ºC to 400ºC and the final transition was observed at above 400ºC to 700ºC which is generally considered as a decomposition phase or carbonization of polymer. Thermal degradation of native collagen was initiated at 95.50°C which is the first transition step of native collagen with the weight loss of ~20%. On the other hand, in PCSN cross linked collagen, there was a shift to higher transition temperature of 110°C and weight loss was ~14%. The second transition of collagen cross-linked with plumbagin caged nanoparticle started at 250.37°C with 25% weight loss whereas in native collagen ~40% weight loss was observed. Even at final transition (Carbonization) also the residual weight was more than 56% for PCSN cross linked collagen whereas for native collagen only 44% residual weight remained. The low degradation and higher melting temperature observed with PCSN cross-linked collagen was due to the cross-linking effect of PCSN. Further, higher amount of residual weight observed in PCSN cross linked collagen indicated the thermal stability of PCSN cross linking when compared to native collagen. Further, our thermo gravimetric analysis also showed that there was an increase in transition temperature and decrease in weight loss for PCSN cross linked collagen when compared with native collagen due to the effective cross linking induced by PCSN. The thermo gravimetric data is consistent with our DSC results. PCSN introduced inter and intra molecular linkages with collagen fibrils that increased the thermal stability of PCSN cross linked collagen.