Supplementary Material (ESI) for Chemical Communications

ELECTRONIC SUPPORTING INFORMATION

MTT Assay details :

The MTT assay determines the active mitochondrial enzymes present in a cell capable of reducing MTT. It is a colorimetric assay in which only the living cell utilizes the colorless substrate, MTT, converting it to a colored (Formazon) product. In this study 2.9×10^5 cardiac fibroblast cells were seeded onto a 24- well plate; the wells either contained a sample of the 8mM choline based salt solution or the piece of the various collagen films. Coated plates were sterilized under UV and the wells were neutralized with phosphate buffer (pH 7). The cells were incubated at 37°C in a humidified atmosphere containing 5% CO_2. After 24 h incubation, the supernatant of each well was replaced with MTT diluted in serum-free medium and the plates incubated at 37°C for 4 h. After aspirating the MTT solution, acid isopropanol (500 ul of 0.04N HCl in isopropanol) was added to each well and pipetted up and down to dissolve all of the dark blue crystals and then left at room temperature for a few minutes to ensure all crystals are dissolved. Finally, absorbance was measured at 630 nm using UV spectrophotometer. Each experiment was performed at least three times. The sets of three wells for the MTT assay were used for each experimental variant. The results are expressed as mean± standard error of the mean (SEM) of the absorbance. Data were analysed by student's t-test and differences at the 95% level were considered to be significant. Although, cells were always incubated until near confluence (about 90%) before the assay were carried out, the number of cells was not exactly reproducible as values caused by each experimental sample varied.

Fluorescence Microscopy details :

L-929 cells were seeded onto the collagen materials in 96-well plates at a density of 1×10^4 cells/well. Cells were incubated at 37°C in a humidified atmosphere containing 5% CO_2. After 24 h incubation, cells were fixed in 10% neutral buffered formalin for 10 minutes, and permeabilised with ice-cold acetone for 10 minutes. The actin filaments of cells were stained with Alexa 488-Phalloidin (Invitrogen), and 4',6-diamidino-2-phenylindole (DAPI) was used to image the cell nuclei. Collagen materials were removed from well and mounted on microscope slides with Fluoromount Aqueous Mounting Media (Sigma). Cells were imaged using an Olympus IX70 inverted microscope.

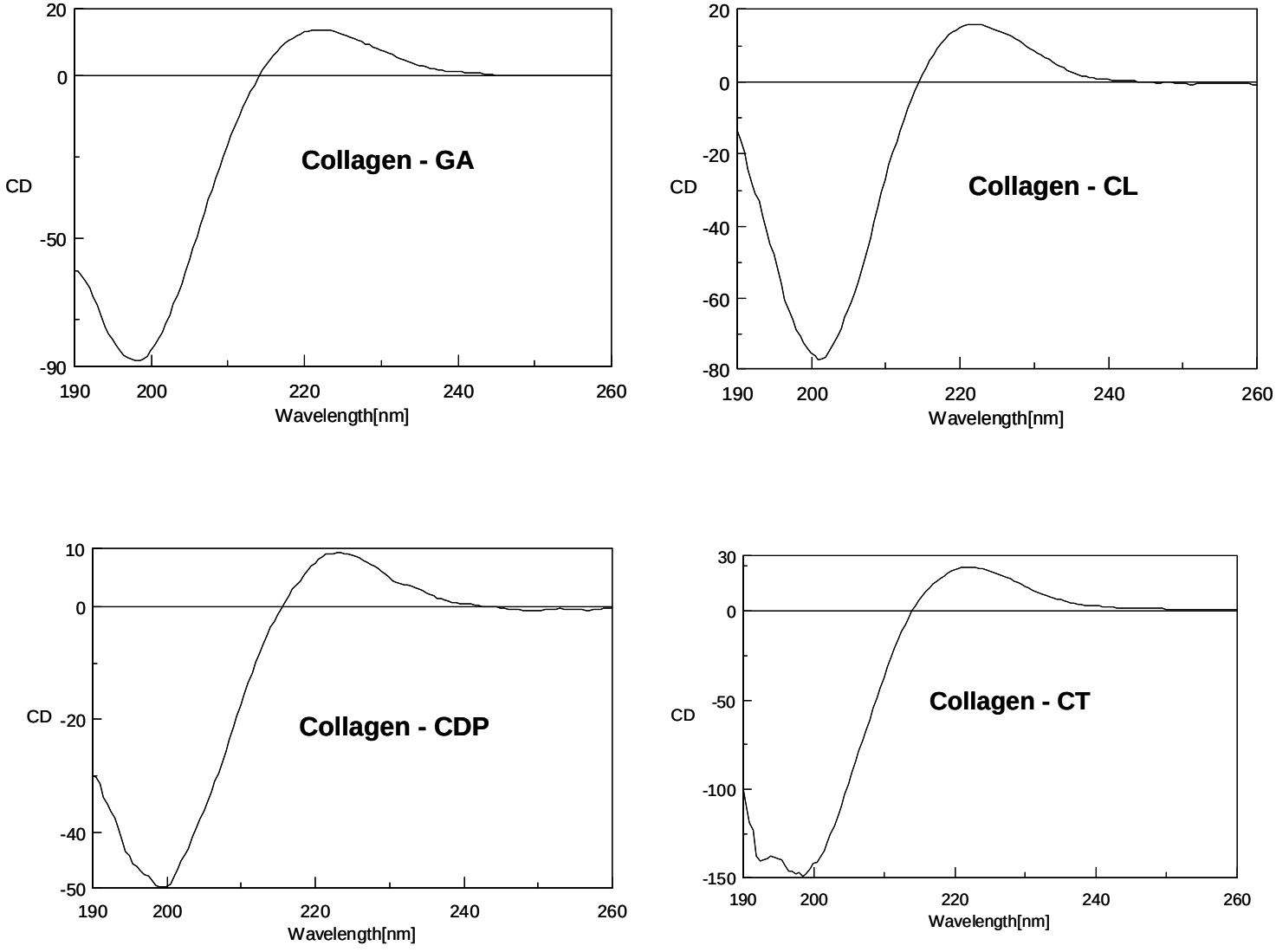
ESI- Table 1 : IR data of Collagen based biomaterials crosslinked GA and with various ionic liquids

S.NO	Specimen used	Amide I (cm-1)	Amide II (cm-1)	Amide III (cm-1)	Amide A (cm-1)
1*	Native collagen	1626	1552	1232	3304
2	Collagen treated with GA	1632	1552	1240	3349
3*	Collagen treated with Cr(III)	1645	1556	1234	3341
4	Collagen treated with CL	1627	1546	1239	3357
5	Collagen treated with CDP	1642	1550	1239	3322
6	Collagen treated with CLu	1631	1550	1240	3326
7	Collagen treated with CT	1664	1549	1240	3318

* indicates literature information (Usha et al ref 29 of manuscript)

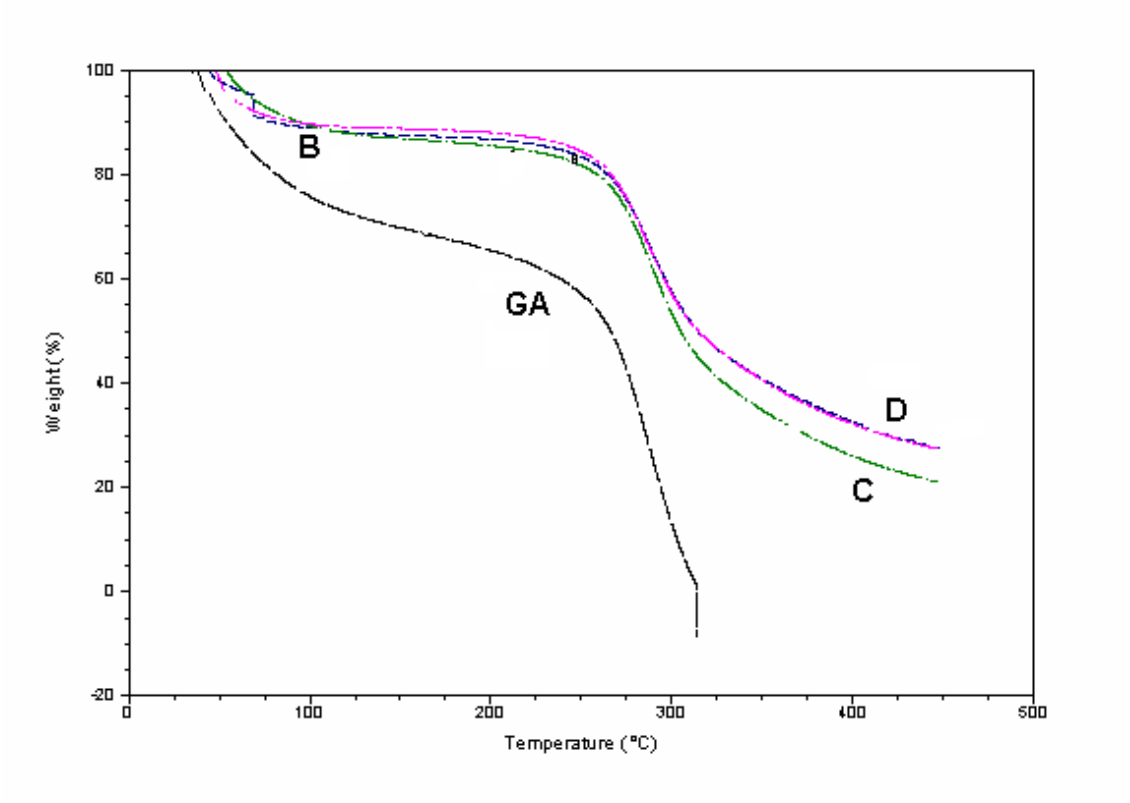
The results of IR Spectroscopy indicate that the ionic liquid crosslinked biomaterials retained the triple helical structure of native collagen and the characteristic Amide II (N-H) bending vibrations were observed at around 1550 cm^{-1}. Similarly Amide I (C=O stretching) and Amide A (N-H stretching) signatures were also seen respectively at 1632-1664 and 3318-3350 cm^{-1}. Moreover a shift (to higher frequencies) in the Amide A band indicates the crosslinking and this trend has been observed in all samples crosslinked with IL, GA and Cr(III). This shift may stem from coordinate or H-bonding interactions of the amide nitrogen, hence in the present case where all of the anions are capable of multiple H- bonds, it seems likely that this is the origin of the cross-linking effect.

ESI -Fig 1: CD Spectra of Collagen based biomaterials crosslinked GA and with various ionic liquids ;



The Chiro – optical properties of collagen based biomaterials, (crosslinked with GA and other ionic liquids) give information on the conformational behaviour, were determined by Circular Dichroism (CD) measurements. The CD spectra were recorded at 25 ^{0}C in the far UV region (190 – 250 nm) under nitrogen and the results demonstrate that crosslinked collagen samples exhibit a minimum at 198 nm and a maximum at 230 nm with a cross over at 215 nm indicating the characteristics of triple helical structure of native collagen and therefore in all these samples the secondary structure is retained.

ESI -Fig 2: TGA curves of Collagen based biomaterials crosslinked GA and with various ionic liquids ; B= CDP ; C= CLu ; D = CT



Thermogravimetric Analyser (TGA) studies were carried out to study the effect of thermal stability of collagen samples crosslinked with GA and other ionic liquids. The results show that collagen samples crosslinked with ionic liquids exhibit higher thermal stability compared to GA crosslinked sample. This shows better crosslinking efficiency (as evidenced by IR results suggesting strong coordinate / H-bonding interactions of the amide nitrogen) of the ionic liquids over GA crosslinked sample.