Electronic Supplementary Material (ESI) for Soft Matter.

Supplementary material

In situ and Non-cytotoxic Cross-linking Strategy for 3D Printable Biomaterials

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Hydrogel Content and Printing Data

Table S1. *Code names and the % content of the hydrogel combinations*

Code name of the Combination	*Gelatin (w/v)*	*Alginate (w/v)*	*Whey Protein Isolate (w/v)*	*MaTrp-Ru(bipyr)$_2$-MaTrp*	*GelMA (w/v)*	*Irgacure (w/v)*
GA-Whey-MATRu	11	9	2	625ppm 50µL	-	-
GA-Whey$_{(ionic\ cross\text{-}linking)}$	11	9	2	-	-	-
GA-Whey	11	9	2	-	-	-
GelMA-Alg-Whey	-	4	2	-	7	7
GA-MATRu	11	9	-	625ppm 50µL	-	-

Table S2. *Printing parameters for a scaffold by using GA-Whey-MATRu combination*

Needle Diameter (mm)	0.25
Printing Temperature (˚C)	25 - 30
Printing (XY) Speed (mms^{-1})	10 – 12
Pressure (bar)	1.0 – 2.0
Postflow & Preflow delay (s)	(-0.1) – (+0.1)
Dimensions (X x Y x Z) (mm)	15 x 15 x 2.5
Width Between Lines (mm)	1.0
Printing Time (s)	27.5
Bioink Volume (µL)	0.016

CD Spectroscopy of Whey Protein Isolate

Circular dichroism (CD) spectroscopy gives information about the secondary structures, α helix or β sheet etc., of protein [1,2]. Beside this, deformations or modifications in proteins or protein based biomaterials could be defined from changes in secondary structure via CD spectroscopy. The secondary structure of whey protein isolate was analyzed by CD spectroscopy. CD spectrum was obtained 180-380 nm wavelength interval using 1 mm of light path quartz cuvette by Chirascan CD Spectrophotometer. For analysis, 1000 ppm whey protein was prepared in deionized water.

In Figure S1, negative band at 208-222 interval and positive band at 193 nm indicated α-helix folding in whey protein isolate. β-sheet folding of isolated whey protein was seen at 220-240 nm interval. So, secondary structure of whey protein was exposed using CD spectrum.

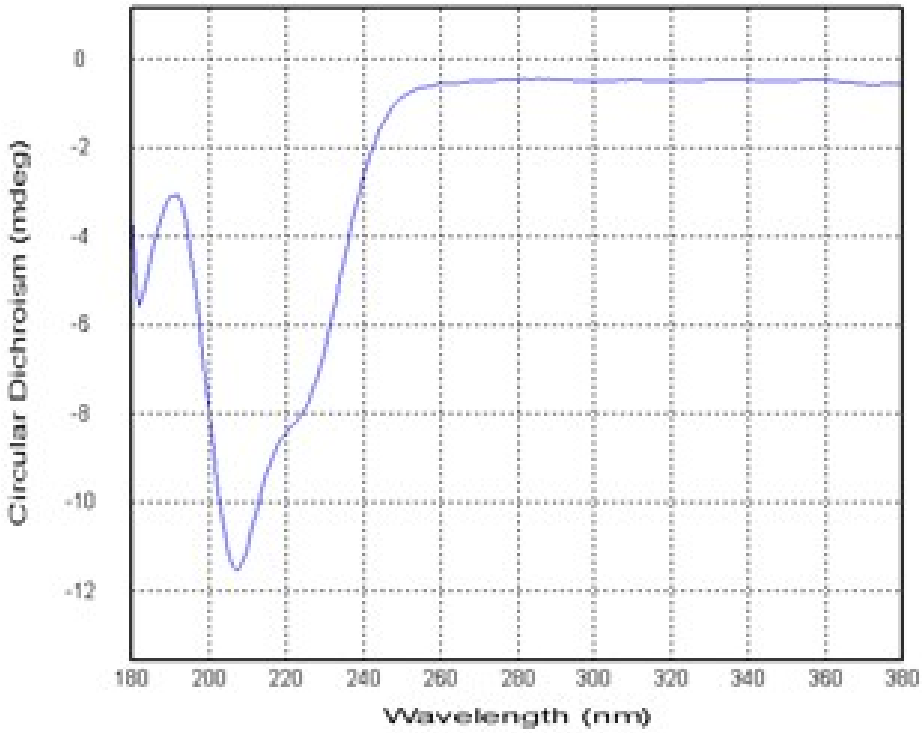

Figure S1. *CD spectra for whey protein isolate*

3D Printing of Different Structures

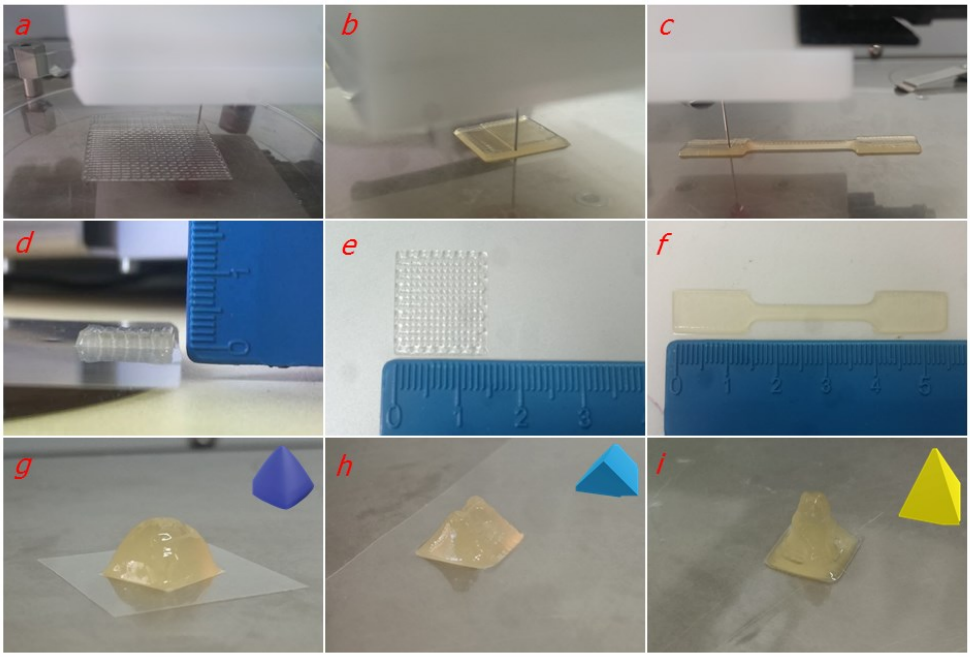

Figure S2. *GA-Whey-MATRu combination 3D printing* ***(a)*** *scaffold with 1.5 mm line width,* ***(b)*** *scaffold with 0.5 mm line width,* ***(c)*** *dog bone shaped mechanical tester* ***(d)*** *10-layer scaffold,* ***(e)*** *scaffold which has 15 mm width,* ***(f)*** *dog bone shaped mechanical tester which has 55 mm length,* ***(g, h, i)*** *different structures with CAD*

Mechanical Test Demonstration of Printed Structures

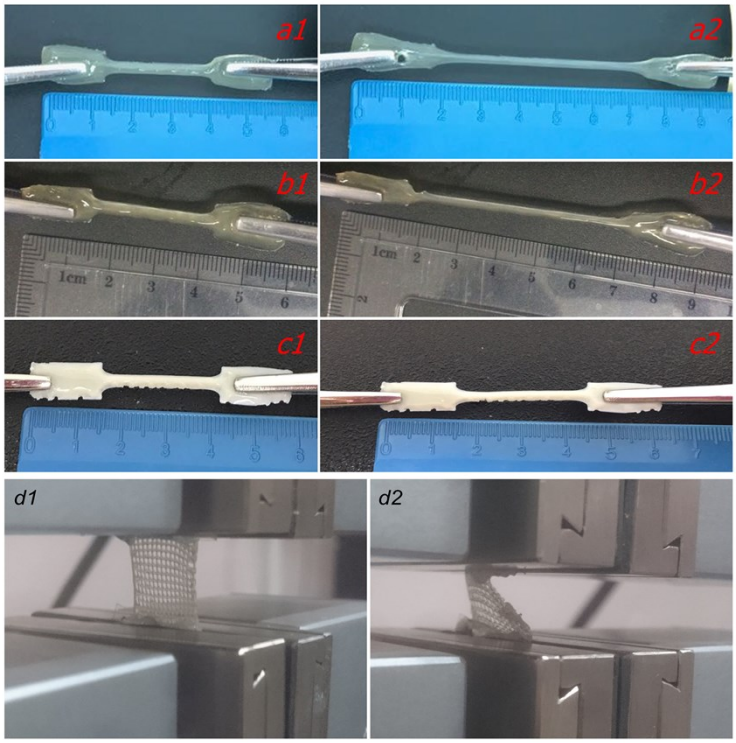

Figure S3. *Dog bone shaped mechanical testers' elongation (a1) GA-Whey intact, (a2) GA-Whey ruptured, (b1) GA-Whey-MATRu intact, (b2) GA-Whey-MATRu ruptured, (c1) GA-Whey*$_{(ionic\ cross\text{-}linking)}$ *intact, (c2) GA-Whey*$_{(ionic\ cross\text{-}linking)}$ *ruptured, and (d1) GA-Whey-MATRu scaffold intact, (d2) GA-Whey-MATRu scaffold ruptured*

Protein Release

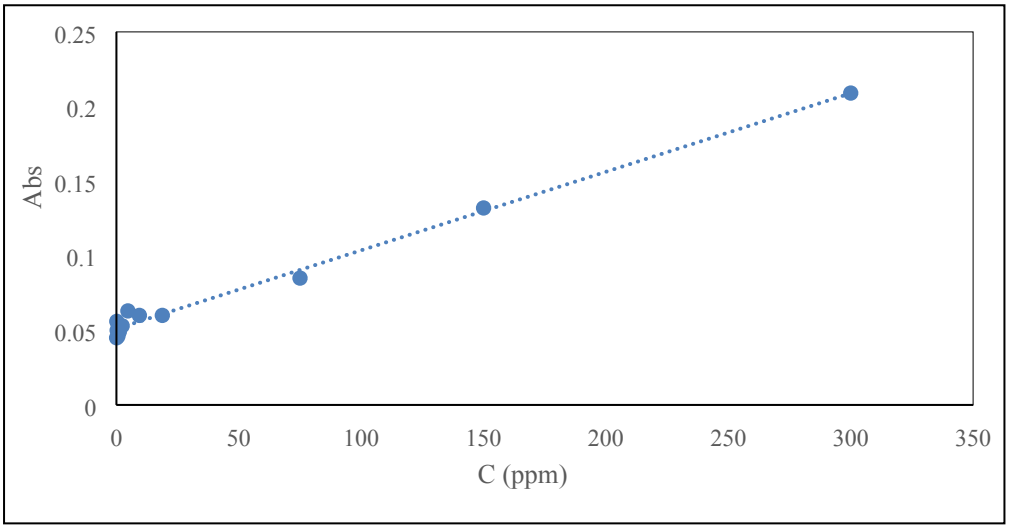

Figure S4. *Calibration graph of GA-Whey-MATRu scaffold's protein release*

Table S3. *Protein release data of GA-Whey-MATRu and GA-Whey$_{(ionic\ crosslinking)}$ scaffolds*

DAYS	GA-Whey-MATRu (ppm)	GA-Whey$_{(ionic\ crosslinking)}$ (ppm)
0	0.4267 ± 0.8554	0.76 ± 0.0566
1	9.8333 ± 2.0052	4.0417 ± 1.4731
2	10.4348 ± 0.0869	3.9783 ± 1.5065
3	11.5757 ± 1.2376	2.9995 ± 1.6714
4	12.5714 ± 0.9086	3.8571 ± 1.4142
7	12.9333 ± 1.9553	6.65 ± 2.4749

GelMA Synthesis and Characterization

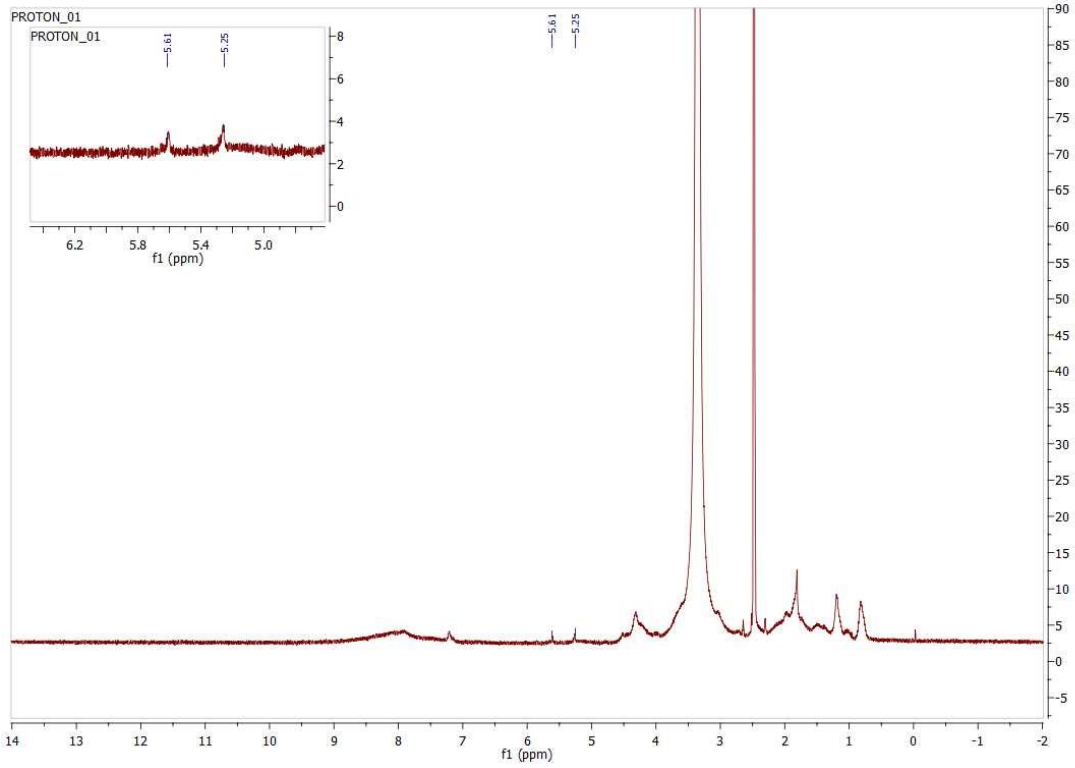

Figure S5. *H-NMR spectra of GelMA*

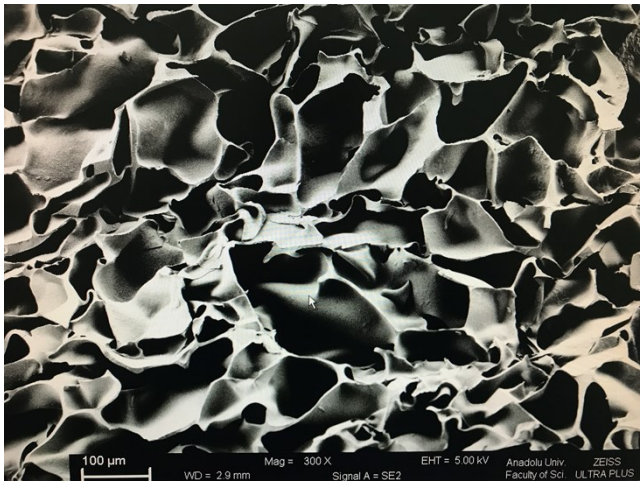

Figure S6. *SEM micrograph of GelMA*

References

[1] L. Whitmore, B.A. Wallace, Protein secondary structure analyses from circular dichroism spectroscopy: Methods and reference databases, Biopolymers. 89 (2008) 392–400. doi:10.1002/bip.20853.

[2] A.J. Miles, B.A. Wallace, Circular dichroism spectroscopy of membrane proteins, Chem. Soc. Rev. 45 (2016) 4859–4872. doi:10.1039/C5CS00084J.