A Magnetic Multi-Enzyme Nanoplatform for High-Purity Collagen Preparation through Efficient Contaminant Protein Elimination

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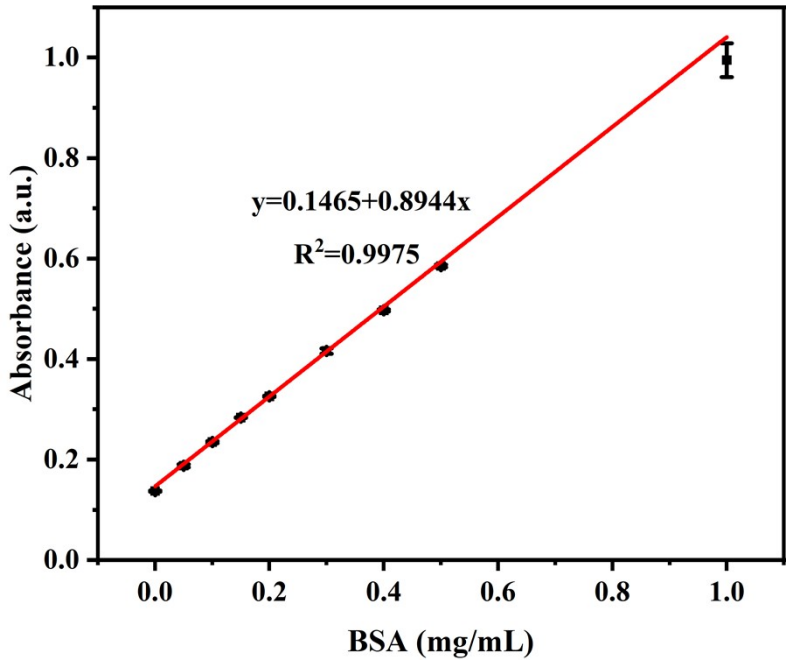

Fig. S1 BSA standard curve obtained using the BCA protein assay kit. The linear regression equation is y = 0.8944 x + 0.1465 (R^2 = 0.9975).

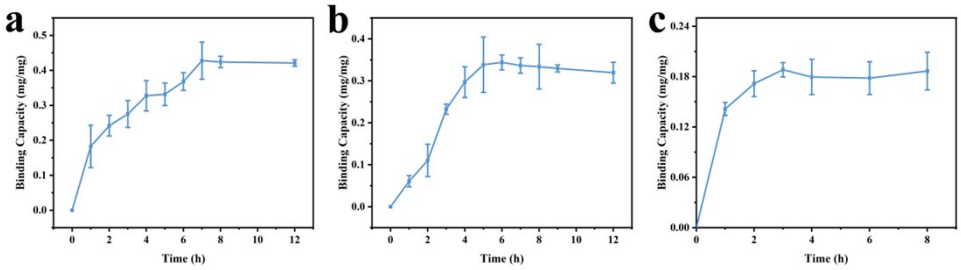

Fig. S2 Enzymatic loading on amino-functionalized magnetic beads with diameters of 0.5 μm, 1 μm, and 4 μm. (a) Trypsin loading capacity of 0.5 μm beads over various time intervals; (b) Trypsin loading capacity of 1 μm beads over time; (c) Trypsin loading capacity of 4 μm beads as a function of incubation duration.

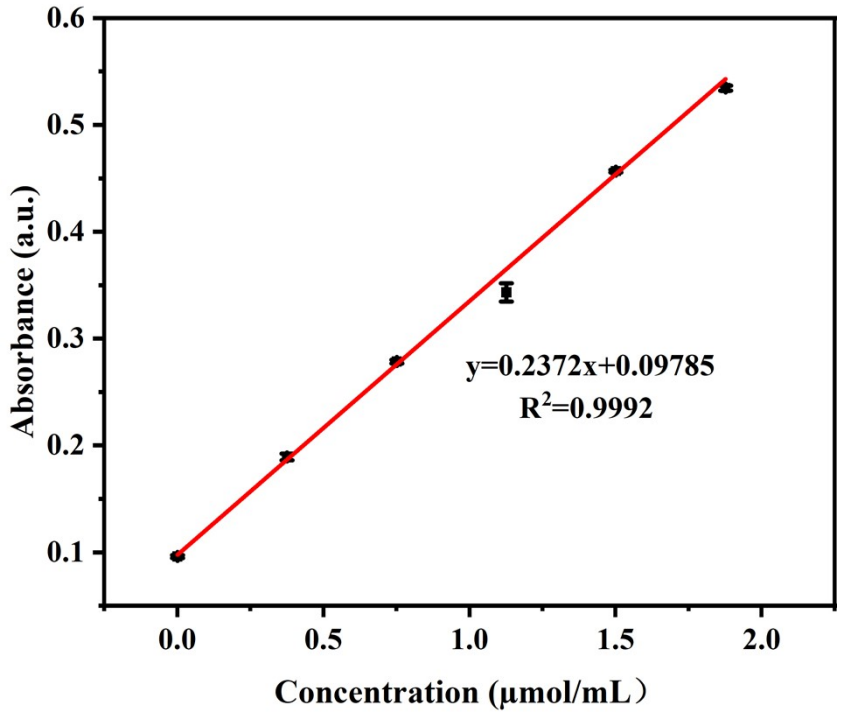

Fig. S3 Standard curve of tyrosine: $y = 0.2372x + 0.0978$, $R^2 = 0.9992$.

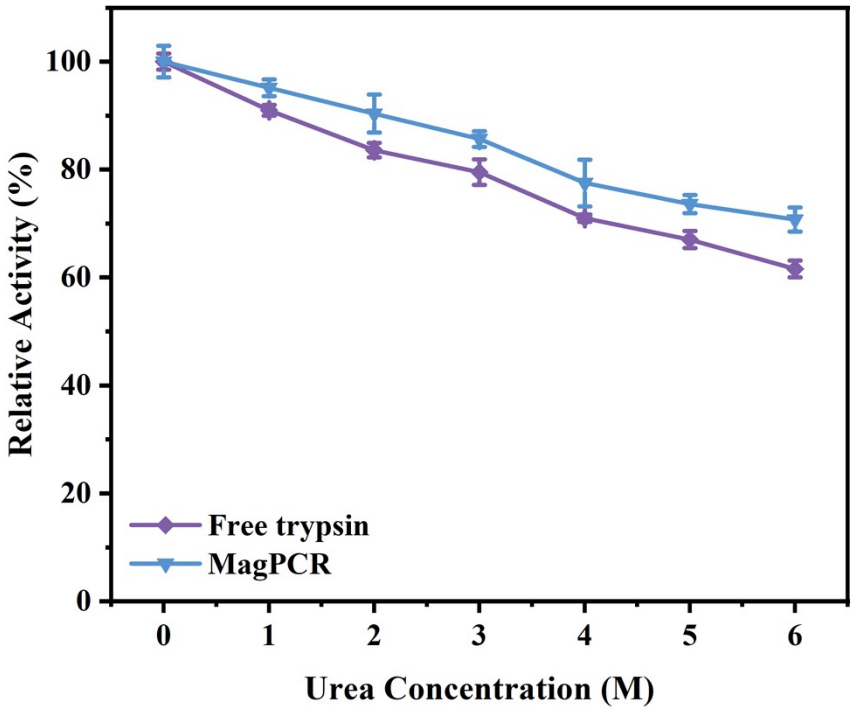

Fig. S4 Relative enzymatic activities of MagPCR and free trypsin under varying urea concentrations.

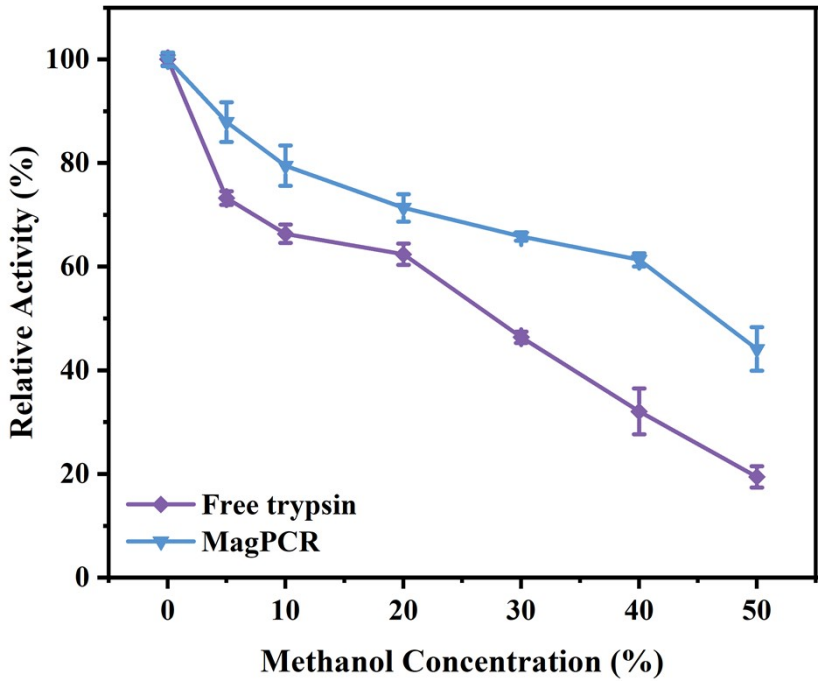

Fig. S5 Relative enzymatic activity of MagPCR and free trypsin under varying methanol concentrations.

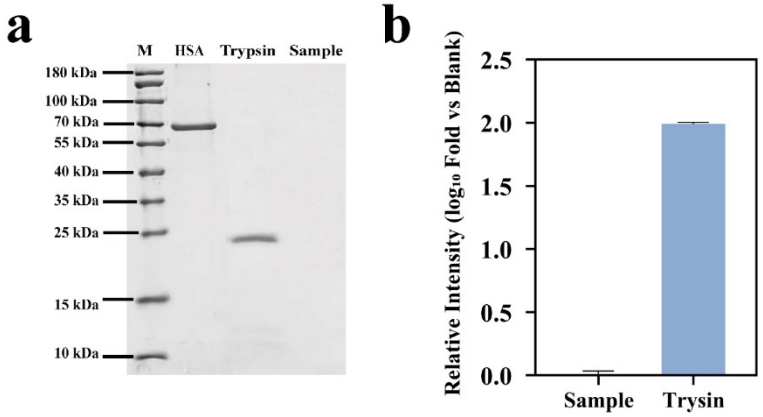

Fig. S6 Residual enzyme contamination analysis of MagMEN-purified samples. (a) Electrophoretic bands of human serum albumin (HSA), trypsin, and Mag-Try-treated HSA. (b) Gray-scale analysis of electrophoretic bands performed using ImageJ software.

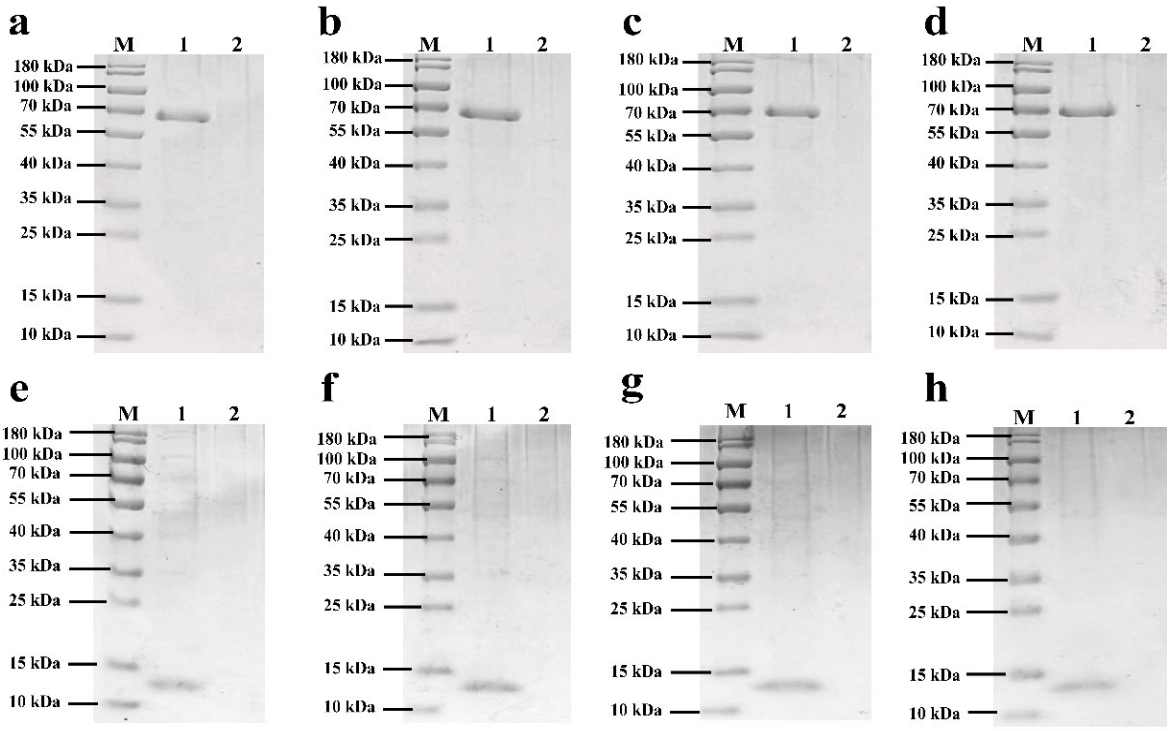

Fig. S7 Enzymatic digestion of human serum albumin and bovine hemoglobin using MagMEN. SDS-PAGE analysis of human serum albumin (a) and bovine hemoglobin (e) treated with Mag-Try. Lanes 1 and 2 represent samples digested with Mag-Try for 0 hours and 24 hours, respectively. SDS-PAGE analysis of human serum albumin (b) and bovine hemoglobin (f) treated with Mag-Pep. Lanes 1 and 2 represent samples digested with Mag-Pep for 0 hours and 24 hours, respectively; SDS-PAGE analysis of human serum albumin (c) and bovine hemoglobin (g) after Mag-Pap digestion. Lanes 1 and 2 represent samples digested with Mag-Pap enzyme for 0 hours and 24 hours, respectively; SDS-PAGE analysis of human serum albumin (d) and bovine hemoglobin (h) after Mag-Bro digestion, with lanes 1 and 2 representing samples digested for 0 hours and 24 hours, respectively.

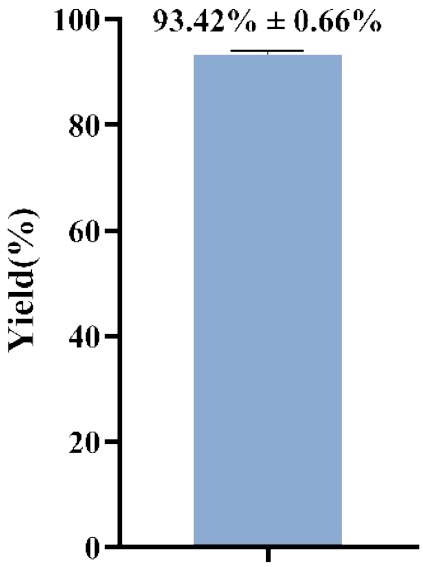

Fig. S8 MagMEN's purification efficiency for type I collagen.

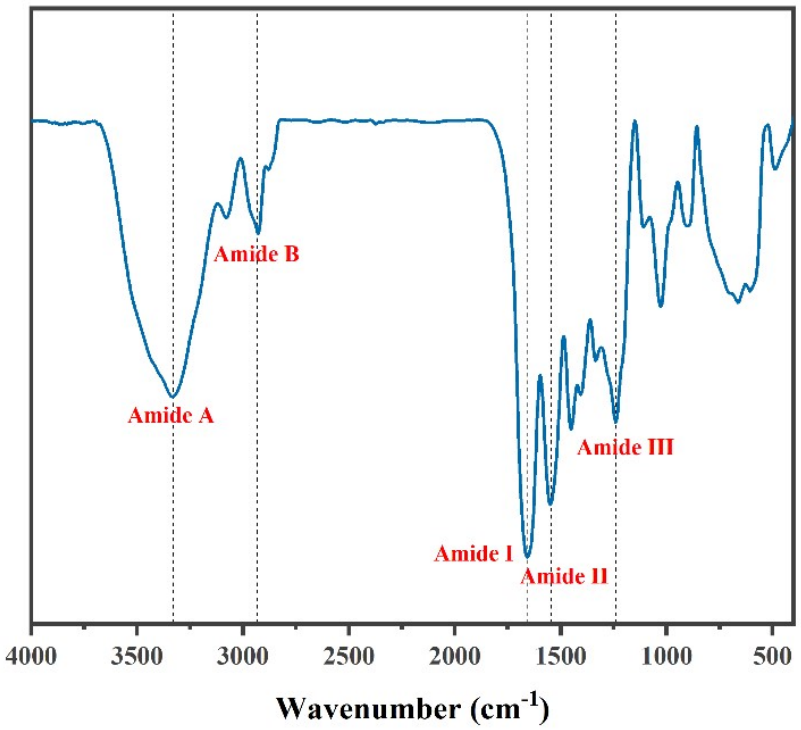

Fig. S9 FTIR spectrum of MagMEN purified collagen.

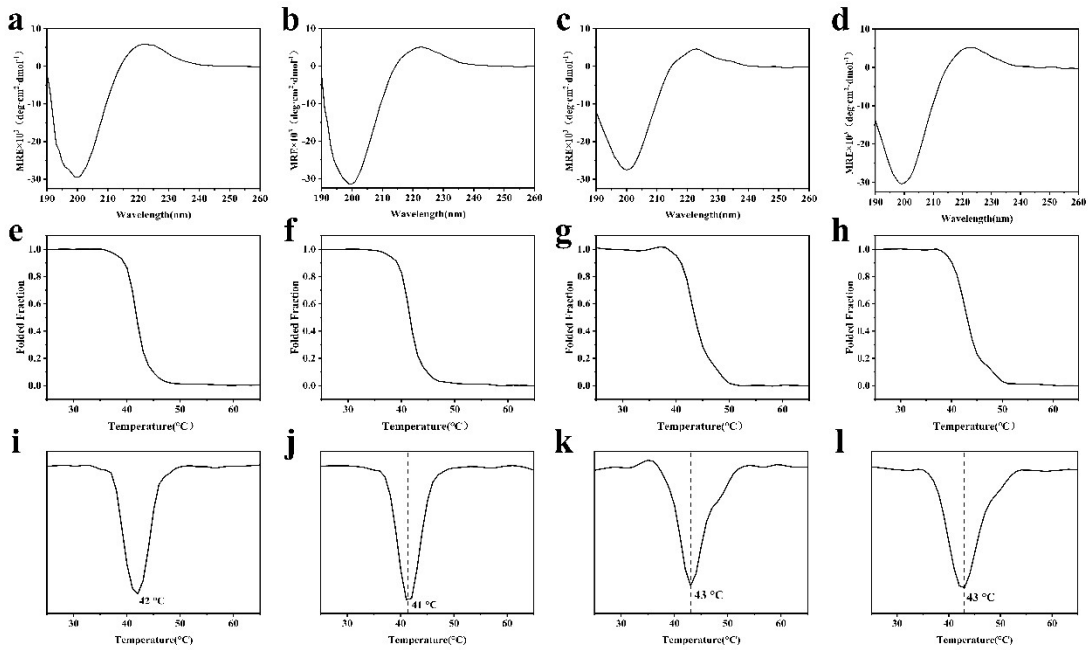

Fig. S10 The triple helix structure of collagen purified by MagMEN. Circular dichroism spectrum (a), thermo-variation curve (e), and first-derivative curve (i) of collagen purified by Mag-Try; Circular dichroism spectrum (b), thermo-variation curve (f), and first-derivative curve (j) of collagen purified by Mag-Pep; Circular dichroism spectrum (c), thermo-variability curve (g), and first-derivative curve (k) of Mag-Pap purified collagen; Circular dichroism spectrum (d), thermo-variability curve (h), and first-derivative curve (l) of Mag-Bro purified collagen.

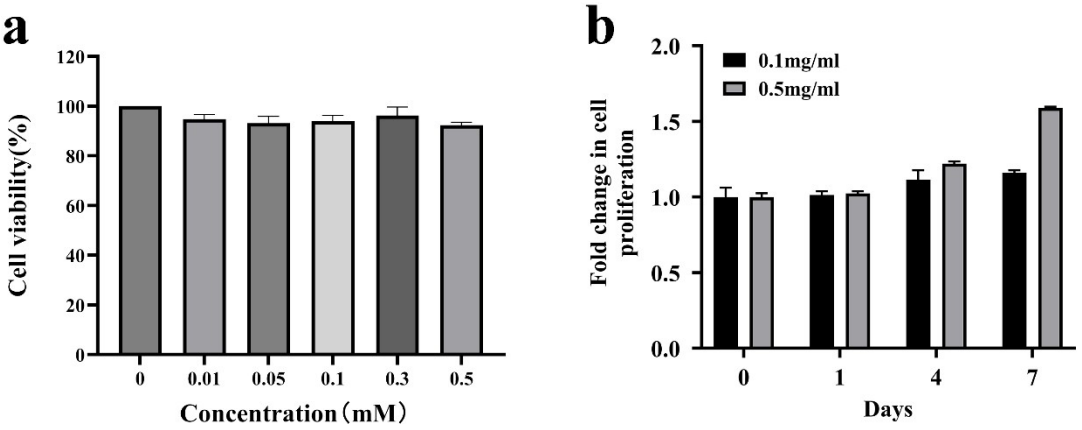

Fig. S11 Biocompatibility assessment of MagMEN-purified collagen. (a) CCK-8 assay to evaluate cytotoxicity of MagMEN at different concentrations on mouse-derived fibroblasts. (b) CCK-8 assay to detect cell viability of mouse-derived fibroblasts treated with MagMEN at different concentrations at 0, 1, 4, and 7 days.

Table S1. Enzyme loading capacity under different pH conditions: pH 7.5, pH 8.0, pH 8.5, pH 9.0.

pH	Experimental Results (mg/mg)	Standard deviation (mg/mg)
7.5	0.161 ± 0.024	0.0672
8.0	0.179 ± 0.009	0.0249
8.5	0.217 ± 0.032	0.0892
9.0	0.233 ± 0.039	0.1103

Table S2. Kinetic parameters of free trypsin, Mag-Try(0.5), Mag-Try(1), and Mag-Try(4).

Trypsin	v_{max} (μmol/mg·min)	SD (μmol/mg·min)	K_m (g/L)	SD (g/L)
Free Trypsin	1.898 ± 0.045	0.078	2.482 ± 0.172	0.298
Mag-Try(0.5)	0.868 ± 0.025	0.043	3.265 ± 0.212	0.367
Mag-Try(1)	0.867 ± 0.021	0.036	2.888 ± 0.150	0.260
Mag-Try(4)	1.350 ± 0.015	0.026	2.534 ± 0.080	0.139