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Iron(II)-catalysed tyrosinase crosslinked hyaluronic acid hydrogel for the controlled release of human antibodies

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Supporting Information

A. The effects of iron(II) on tyrosinase crosslinked hydrogels determined by rheology

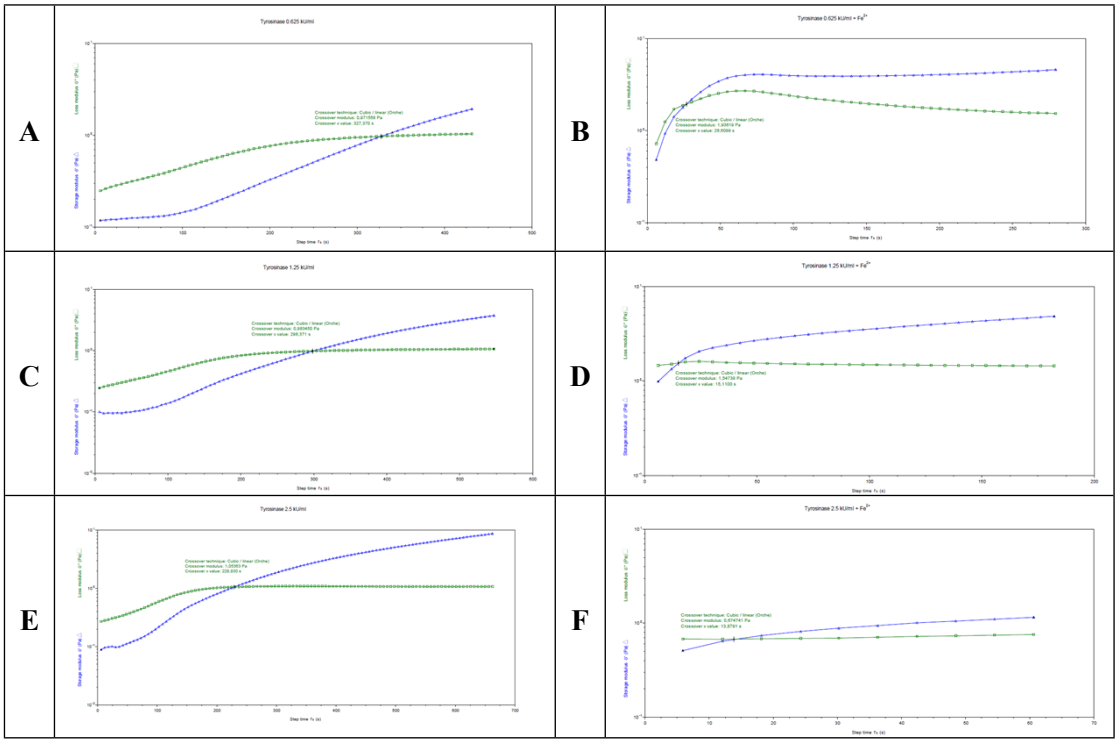


Fig. S1. Rheological determination of kinetics of gelation. The influence of 0.9 mM iron(II) on the varying concentrations of tyrosinase . (A) 0.625 kU/mL (B) 0.625 kU/mL + Fe 2+ (C) 1.25 kU/mL (D) 1.25 kU/mL + Fe2+ (E) 2.5 kU/mL (F) 2.5 kU/mL + Fe2+

Fig. S2. The influence of 0.9 mM iron(II) on the varying concentrations of tyrosinase.

B. The hydrogel network structure analysis

The mesh size of HATA hydrogels was calculated using the following equation (1) obtained from (Peppas et al., 2000).

$$\xi = l v_{2,s}^{-\frac{1}{3}} \left(\frac{2C_n M_c}{M_r}\right)^{\frac{1}{2}} \quad (1)$$

where l is the bond length along the polymer backbone (0.52 nm) [2]

□$_{2,□}$ is the polymer volume fraction at the equilibrium swollen state;

□$_{□}$ is the Flory characteristic ratio of HA (27) [2];

□$_{□}$ is the molecular weight of the repeat (HA disaccharide) unit (400 g/mol);

□$_{□}$ is the molecular weight between two adjacent crosslinks.

The effective cross-linking density (□$_{□}$) was determined with equation (2) [3,4]:

$$(2) \quad e = \frac{\rho_p}{M_c}$$

The molecular weight between two adjacent crosslinks (Mc) was calculated from rubber elasticity theory using equation (3) summarized by [1]:

$$\tau = \frac{\rho_p RT}{M_c}\left(1 - \frac{2M_c}{M_n}\right)\left(\alpha - \frac{1}{\alpha^2}\right)\left(\frac{v_{2s}}{v_{2r}}\right)^{\frac{1}{3}} \quad (3)$$

where □ is the shear stress applied to the polymer sample;

□ is the universal gas constant;
□ is the absolute experimental temperature;

□ is the extension ratio;

□$_{□}$ is density of the dry polymer (1.229 g/cm^3)[5]

M_n is the average molecular weight of the monomers (average molecular weight of HATA

derivative 340 · 10^3 g/mol);

$\square_{2,\square}$ is the polymer volume fraction in the relaxed state (after crosslinking but before swelling).

The polymer volume fraction at the equilibrium swollen state $\square_{2,\square}$ and relaxed state $\square_{2,\square}$ was calculated based on [6] equation (4a and 4b):

$$v_{2,s} = \left(1 + \frac{\rho_p}{\rho_s}.\ (Q_{M,s} - 1)\right)^{-1} \quad (4a)$$

$$v_{2,r} = \left(1 + \frac{\rho_p}{\rho_s}.\ (Q_{M,r} - 1)\right)^{-1} \quad (4b)$$

where $\square_{\square}$ is density of the dry polymer (1.229 g/cm^3 [7]).

$\square_{\square}$ is density of the solvent (NS 1.009 g/cm^3);

$\square_{\square,\square}$ is the equilibrium mass swelling ratio;

$\square_{\square,\square}$ is the relaxed-state mass swelling ratio;

$\square_{\square,\square}$ a $\square_{\square,\square}$ were calculated as follows (equation 5):

$$\square_{\square,\square} = \frac{\square_{\square}}{\square_{\square}} \qquad \square_{\square,\square} = \frac{\square_{\square}}{\square_{\square}} \quad (5)$$

where $\square_{\square}$ is a mass of the hydrogel at the equilibrium swollen state;

$\square_{\square}$ is a mass of the hydrogel at the relaxed state;

$\square_{\square}$ is a mass of the dry hydrogel.

The relationship between the shear stress ($\square$) and the shear modulus ($\square$), is summarized in the following equation (6) adopted from [8]:

$$\tau = G\left(\alpha - \frac{1}{\alpha^2}\right) \quad (6)$$

Substitution □ by □ in equation (3) yields following equation (7) allowing to determine Mc:

$$M_c = \frac{\rho_p RT\left(\frac{v_{2,s}}{v_{2,r}}\right)^{\frac{1}{3}}}{G + \frac{2\rho_p RT}{M_n}\left(\frac{v_{2,s}}{v_{2,r}}\right)^{\frac{1}{3}}} \quad (7)$$

The shear modulus shown in this equation is the modulus of hydrogels at the equilibrium swollen state. The shear modulus obtained in this study was measured for matured hydrogels at the relaxed state before swelling. The equilibrium-swollen modulus thus was calculated by equation (8) obtained from [9]

$$\frac{G_S}{G_r} = \frac{v_e RT\ (v_{2,s})^{\frac{1}{3}}}{v_e RT\ (v_{2,r})^{\frac{1}{3}}} = \frac{v_e RT\ (v_{2,s})^{\frac{1}{3}}}{v_e RT\ (v_{2,r})^{\frac{1}{3}}} \quad (8)$$

where □□ is the equilibrium swollen modulus;

□□ is the relaxed-state modulus;

□□ is the hydrogel cross-linking density.

Table S1: Mesh size □, crosslinking density □□ and average molecular weight Mc between crosslinks highlighted in yellow

	1% HATA_0.625 kU/mL tyrosinase	1% HATA_0.625kU/mL tyrosinase _0.9mM Fe^{2+}
Mw (Da)	1 000 000	1 000 000
PI	1.5	1.5
Mn (Da)	666 667	666 667
$□_{2,□}$	0.008576	0.00816
$□_{2,□}$	0.007716	0.007716
Mc (Da)	320 856	331 213
□ (nm)	528	555
□□ ($g.m^{-3}$)	4.04	3.92
Gs (Pa)	368	426

C. Measurement of dopa standard determined by UV-visible spectroscopy

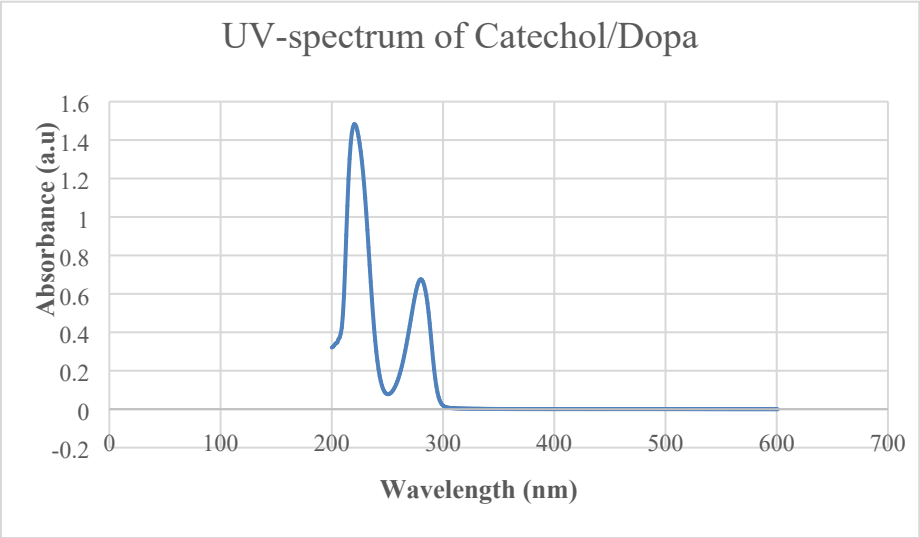
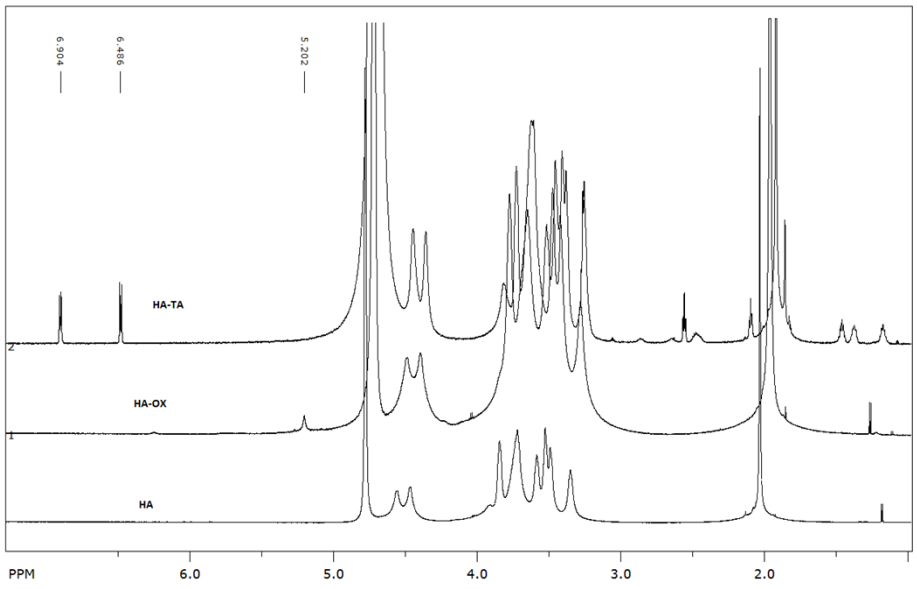


Fig. S3 UV-visible spectroscopy of DOPA standard

D. Synthesis of the tyramine derivative of HATA

The tyramine derivative of hyaluronic acid (HA) was synthesized through a two-step process (Fig. S5). SEC-MALLS and ^{1}H NMR (Fig. S4) were used to determine the molecular weight and degree of substitution of either aldehyde groups or tyramine moieties respectively. In the first step, a polyaldehyde derivative of hyaluronic acid was obtained by regioselective oxidation at position six of the N-acetylglucosamine unit. Acetamido-2,2,6,6-tetramethylpiperidine-1-oxyl (Acetamido-TEMPO), NaClO, and NaBr were used for the oxidation reaction. Signals between 3.4 and 3.9 ppm correspond to the protons of the secondary alcohols in the sugar rings. The signals at 4.4 and 4.5 ppm represent the anomeric protons of the D-glucuronic acid and N-acetylglucosamine units. It is worth mentioning that in aqueous medium, approximately 95% of the aldehyde moieties exist as geminal diols. Therefore, the signal at 5.2, which was absent in native hyaluronic acid (HA) but present in its oxidized form (HA-OX), was assigned to the aldehyde moieties introduced at position six of the N-acetylglucosamine unit.[10] Tyramine was then conjugated to hyaluronan polyaldehyde by the reductive amination reaction using complex picoline borane (Pic-Bor). The signals at 6.5 and 7.0 represent the four aromatic protons of the tyramine phenolic ring. [11]

Ai



Aii

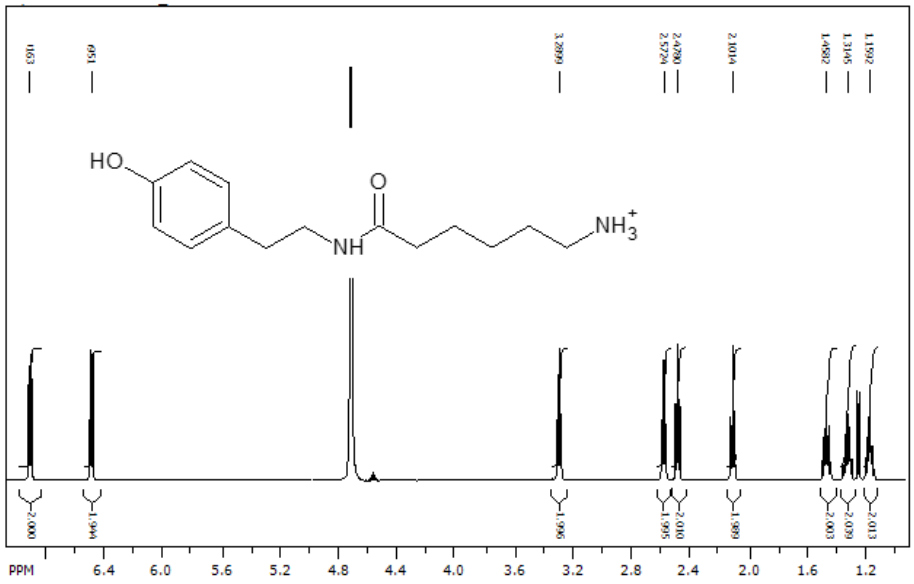


Fig. S4 (Ai) ^{1}H NMR spectra of unmodified hyaluronic acid (HA), oxidized hyaluronic acid (HA-OX), and tyramine-modified hyaluronic acid (HA-TA). (Aii) ^{1}H NMR spectrum of 6-amino-N-[2-(4-hydroxyphenyl)ethyl]hexanamide, the derivative of tyramine used for the synthesis of the tyramine derivative of HA.

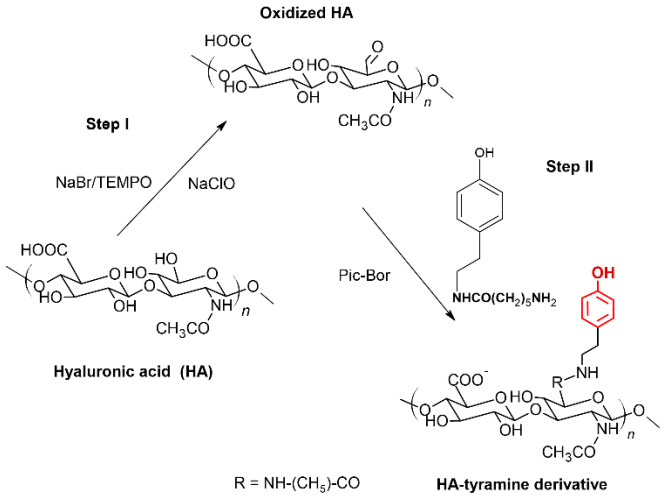


Fig. S5. Reaction scheme for the modification of hyaluronic acid (HA) to the oxidized form (HA-OX) and tyramine derivative (HA-TA).

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