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**Supplementary information:**

# Synthesis of Poly(L-lactide)/*β*-cyclodextrin/citrate Networks Modified Hydroxyapatite and its Biomedical Properties

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## 1. Materials and methods

### 1.1 Chemicals

Calcium nitrate tetrahydrate ($Ca(NO_3)_2 \cdot 4H_2O$), ammonium phosphate ($(NH_4)_3PO_4$), ammonium hydroxide ($NH_3 \cdot H_2O$) (28 wt% aq.), *β*-CD and Zinc lactate. All these reagents were in analytical purity and purchased from commercial companies, and they were used as received without further purification. L-lactide was synthesized according to the method in literature[1]. PLLA (commercial poly(L-lactide)) was bought from Natural work (4032D; *ca.* $1.67 \times 10^5$ $g \cdot mol^{-1}$). Acetone, dichloromethane and dimethylbenzene were in analytical purity and dried by 4A molecular sieves.

### 1.2 Synthesis of the materials

**Synthesis of HA.** A aqueous solution of $Ca(NO_3)_2$ (250 mL 1.0 M) was adjusted to pH 10.0 by a 28 wt% ammonia aqueous solution, and then it was heated to 60 $^oC$. An ammonia aqueous solution (3.4 mL 28%) and 3.0 g urea was added to an aqueous solution of $(NH_4)_3PO_4$ (150 mL 0.6 M) under stirring to form a clear solution, and this solution was drop added to the above $Ca(NO_3)_2$ aqueous solution under stirring. After 24 h of stirring at 60 ℃, the mixture was filtered. The filtrate was repeatedly washed with deionized water, and then dried overnight at 100 ℃. The thus-obtained filtrate was denoted as HA, which was employed in the subsequent synthesis step.

**Synthesis of CD-HA**. A 6.0 g HA sample was first dispersed into 30 mL deionized

water, and then a certain amount of citric acid was slowly added to the above suspension under stirring. After that, 3.2 g *β*-CD was added into the above mixture under stirring. After 24 h of stirring, the suspension was filtered, and then, the filtrate was directly heated at 140 $^{o}$C for 24 h to form citrate/*β*-CD network *via* the crosslink reaction of *β*-CD and citric acid. The thus-obtained filtrate was milled in an agate mortar for 10 min and washed with deionized water. Then the procedure was repeated another two times to remove the unreacted *β*-CD and citric acid, and then it was dried overnight at 100 ℃. The thus-obtained powder was denoted as CD-HA, which was employed in the subsequent synthesis step.

**Synthesis of PLA-CD-HA-x**. The CD-HA (0.2 g), L-lactide (1.0 g), zinc lactate (0.01 g) and xylene (10 mL) were first added together in a Schlenk tube, and then, the tube was purged five times by $N_2$. After that, the temperature was raised to 130 $^{o}$C. The reaction at that temperature was allowed to run for a preset period of time, enabling the generation of poly(L-lactide) (PLA) on the surface of CD-HA *via* the ring opening polymerization (ROP) of L-lactide. The mixture was centrifugated, and the solid obtained was washed sequentially by dichloromethane and acetone for three times, followed by drying at 100 $^{o}$C. These obtained powders were named as PLA-CD-HA-x (x = 24, 36, 48, 72) with x representing the reaction time for the ROP of L-lactide. For example, PLA-CD-HA-24 referred to the samples synthesized with a lactide ROP reaction time of 24 h.

As a comparison, a sample was also synthesized *via* a 72 h of lactide ROP reaction directly on HA without modification by CD, according to the same procedure for PLA-CD-HA-x, and this sample was named as LT-HA.

**1.3 Characterization**

Fourier transform infrared (FT-IR) spectroscopy was conducted over a Varian 3100 instrument. The spectrum was recorded as an accumulative result of 32 scans from 500 $cm^{-1}$ to 4000 $cm^{-1}$ at a resolution of 2 $cm^{-1}$.

X-ray diffraction (XRD) spectroscopy was performed with a Bruker D8 Advance X-Ray diffractometer, under the following condition: Cu target Kα radiation (λ = 1.54187 Å), scanning voltage 40 kV, scanning current 40 mA, scanning speed 6$^{o}$ min$^{-}$

$^{1}$, scanning step 0.02°.

Thermal gravimetric analysis (TGA) was performed with a TA SDT Q500 equipment. The measurement was carried out by heating the sample from room temperature to 800 ℃ at a rate of 10 ℃·min$^{-1}$, with air as carrier gas.

Transmission electron microscopy (TEM) were performed with a JEM-2100F transmission electron microscope operated at an acceleration voltage of 200 kV. Before determination, the specimen was dispersed into ethanol by ultrasonic treatment and the resultant suspension was dropped onto a carbon-coated copper grid.

The atomic force microscopy (AFM) was performed with a Bruker Bioscope Catalyst (Bruker, Germany). The samples were dispersed in ethanol via ultrasonic to form a suspension (10 μg·mL$^{-1}$). Then suspensions were droped on the mica muscovite substrates.

The Zeta-potential and average particle size were measured with Zetasizer Nano ZS (Malvin, UK). The samples were dispersed in deionized water.

The water contact angles of all samples were measured with a contact angle analyzer (DSA100) at room temperature.

### 1.4 Bioactivity test

#### 1.4.1 The cultivation of the cells

The mesenchymal stem cells (MSCs) of Wistar rat (Stem Cell Bank, Chinese Academy of Sciences) contained in a cell culture medium (DMEM/F12 = 1:1; purchased from Hyclone), with a culture density of $2.0 \times 10^4$ cells·cm$^{-3}$, were cultured in a cell culture flask, which was mounted in a humidified incubator exposed to an environment of 5% $CO_2$ at 37 °C. The culture medium was changed every 3 days. The monolayer cells were removed from the cell culture flasks by trypsin (2.5 mg·mL$^{-1}$) and EDTA (0.2 mg·mL$^{-1}$) (1:1 v/v) treatment after 80-90% cell covered the wall of cell culture flask, and the cells were rinsed three times with 0.1 mol·L$^{-1}$ phosphate buffer solution (PBS) by centrifugation at 1000 rpm for 5 min. The obtained MSCs of Wistar rat were suspended in the medium and the cell density was adjusted to $1.0 \times 10^5$

cells·mL$^{-1}$.

### 1.4.2 Cell proliferation

The proliferation of MSCs of Wistar rat on various samples was determined using a Cell Counting Kit-8 (CCK-8, Dojindo) assay. Briefly, 200μL each sample (1 μg·mL$^{-1}$) was seeded by 1 × 10$^4$ MSCs of Wistar rat. After culture for 24, 48 and 72 h, 200μl of mixed solution (including 180μl culture medium and 20μl CCK-8 solution) replaced old culture medium of each well and incubated at 37$^o$C for 3 h. Then, 100μl of mixed solution was extracted to a new 96-well plate for the measure of absorbance at 450 nm using a microplate reader (Bio-Rad, USA).

### 1.4.3 The cultivation of cells on the prepared specimen

The specimen was prepared as follow: The samples (10 μg·mL$^{-1}$ of HA, CD-HA and PLA-CD-HA-72) were first dispersed in ethanol by ultrasonic treatment to form a suspension, and then dropped the suspension (200 μL) onto a clean coverslip (24 × 24 mm), followed by drying at room temperature in a lamiar flow clean bench. The thus-obtained specimen was sterilized by UV light for 30 min for the subsequent biocompatibility test.

The MSCs of Wistar rat were seeded on the coverslips coated with HA, CD-HA, and CD-HA-72, respectively, in 6-well cell culture plates (Costar) at a density of 1.0 × 10$^5$ cells·well$^{-1}$. Then 3 mL of the medium was added to each well and mixed with the cells. The cells were cultured for 24 h, respectively. After a certain time, the coverslips were washed with PBS for three times, and fixed with 3% glutaraldehyde in PBS for 8 min. After carefully washing, the cells were dyed with crystal violet (0.1mL, 9%). The cell attachment and the cell morphology were observed under a reverse microscope (TE2000U, NIKON) and 9 pictures of different places of the coverslip were taken by using a Digital Camera DXM1200F (Nikon). Number of the well attached MSCs were accounted for each picture.

### 1.4.4 Extracellular matrix mineralization

The extracellular matrix (ECM) mineralization of MSCs of Wistar rat co-cultured with the specimen (HA, CD-HA or PLA-CD-HA-72 coated coverslip) was tested by

Alizarin Red staining. The MSCs of Wistar rat of $1 \times 10^4$ cells·mL$^{-1}$ were seeded in the 6-well plate with the blank coverslip and the three kind of specimen. The medium was removed after 80% of the coverslip was covered by MSCs of Wistar rat. The cells were cultured for another 14 days with the differentiation medium (hexadecadrol 100 nmol·mL$^{-1}$, sodium glycerophosphate 10 mmol·mL$^{-1}$, L-anti-thrombotic factor 50 μg·mL$^{-1}$) and the medium was changed for every 2 days. After that, the cells were washed with PBS, and then fixed in 4% paraformaldehyde for 10 min. These fixed cells were stained with 1% Alizarin Red for 5 min. Afterwards, the cells were washed with distilled water for 10 times until the color was stable and images were taken.

**1.5 Statistical analysis**

All sample were cultured and assayed at least in triplicate. All the statistics presented here as a mean ± standard deviation. A two-tailed, unpaired t-test was performed to determine the statistical significance, defined as a P-value less than 0.05 using SPSS (Statistical Package for Social Sciences). The results of the t-test were also marked in the new figures.

**2.1 Characterization and bioactivity test of filler**

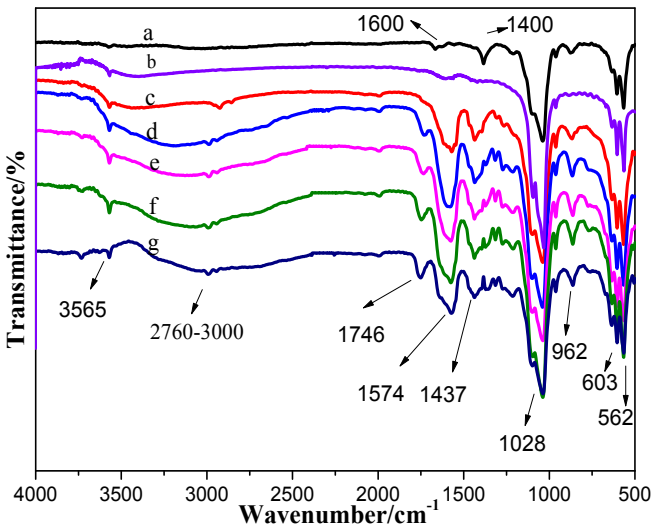


Fig. S1 FT-IR spectra of HA, CD-HA and PLA-CD-HA samples. **(a)** HA; **(b)** LT-HA; **(c)** CD-HA; **(d)** PLA-CD-HA-24; **(e)** PLA-CD-HA-36; **(f)** PLA-CD-HA-48; **(g)** PLA-CD-HA-72

The FT-IR spectra of the synthesized HA, LT-HA, CD-HA and PLA-CD-HA-x (x

= 24, 36, 48, 72) samples are presented in Fig. 1. For the spectra of all samples, The absorption peaks at 562 and 603 $cm^{-1}$ were corresponding to the deformation vibrations of phosphate group, and the absorption peaks at 962 and 1028 $cm^{-1}$ were corresponding to the stretching vibrations of phosphate group[2]. These absorption peaks maintain unchanged in modified samples, which indicates that the modification of HA does not change the structure of HA. The adsorption peaks at 1400 and 1600 $cm^{-1}$ and the wide adsorption peak at 3565 $cm^{-1}$ are assigned to bending vibration and stretching vibration of $H_2O$ adsorbed in the HA, respectively[2].

For CD-HA and PLA-CD-HA-x (x=24, 36, 48, 72), The adsorption peak at 2760-3000 $cm^{-1}$ is assigned for the vibration of $-CH_2-$ and $-CH_3$ in the organics (PLA/*β*-CD/citrate). The adsorption peaks at 1437 and 1574 $cm^{-1}$ in the spectra of the CD-HA and PLA-CD-HA-x are attributed to the carboxyl group vibration of calcium citrate. The adsorption peak at 1746 $cm^{-1}$ corresponding to the ester groups is only observed in spectra of PLA-CD-HA-x (x = 24, 36, 48, 72) samples. The vibration of carboxyl group (1437 and 1574 $cm^{-1}$) suggests that calcium citrate is formed on the surface of HA which means the CD was successfully grafted on HA[3]. The vibration of ester group (1746 $cm^{-1}$) suggests the PLA formed on the surface of CD-HA *via* the ROP of L-lactide. All the three peaks (1437, 1574 and 1746 $cm^{-1}$) are absent in the FT-IR spectrum of LT-HA, suggesting that PLA could not directly grafted on the HA under this reaction condition. The *β*-CD/citrate networks play an indispensable role in the modification of HA.

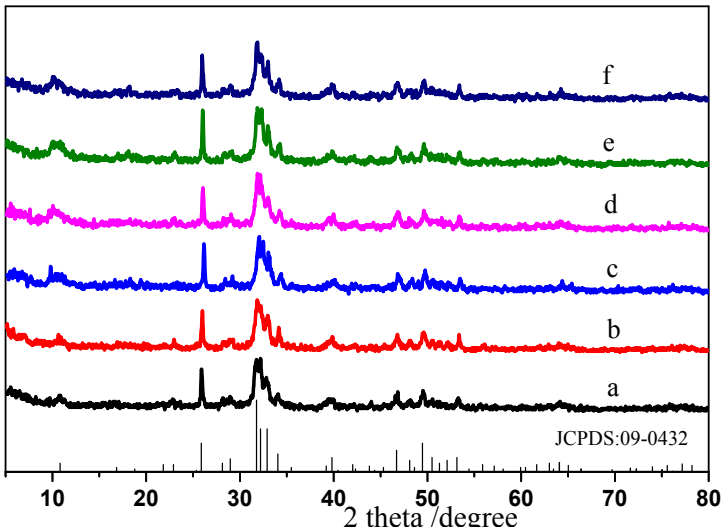


Fig. 2 XRD patterns of HA, CD-HA and PLA-CD-HA samples. **(a)** HA; **(b)** CD-HA; **(c)** PLA-CD-HA-24; **(d)** PLA-CD-HA-36; **(e)** PLA-CD-HA-48; **(f)** PLA-CD-HA-72

The XRD patterns for HA, CD-HA and PLA-CD-HA-x (x = 24, 36, 48, 72) are shown in Fig. 2. All these synthesized samples show similar characteristic diffractions which are consistent with that of hexagonal $Ca_{10}(PO_4)_6(OH)_2$ in P63m space group (JCPDS: 09–0432). The main diffraction peaks, *e.g.* (100), (002), (211), (112), (300), (202), (310), (222), (213) and (004), are almost unchanged before and after the modification of HA. This result indicates that the modification does not destroy the crystal structure of HA and the crosslinking reaction and polymerization only took place at the HA surface.

**Table 1 Grafting amount of HA, CD-HA and PLA-CD-HA-x**

| Entry | Total weight loss[a]/wt% | Grafting amount/wt% | |
|---|---|---|---|
| | | CD-based network[b] | PLA[c] |
| HA | 9.4 | - | - |
| CD-HA | 12.7 | 3.3 | 0.0 |
| PLA-CD-HA-24 | 21.4 | 3.3 | 8.7 |
| PLA-CD-HA-36 | 23.5 | 3.3 | 10.8 |
| PLA-CD-HA-48 | 24.0 | 3.3 | 11.3 |
| PLA-CD-HA-72 | 24.4 | 3.3 | 11.7 |

[a] weight loss calculated by TGA.

[b] $weight\ loss_{CD-HA} - weight\ loss_{HA}$

[c] $weight\ loss_{PLA-CD-HA-X} - weight\ loss_{CD-HA}$

The grafting amount of the HA, CD-HA and PLA-CD-HA-x (x = 24, 36, 48, 72)

are presented in Table 1. The TGA curves of HA, CD-HA and PLA-CD-HA-x (x = 24, 36, 48, 72) are shown in Fig. S1. With the temperature increasing from 25 °C to 800 °C， the mass of HA sample slowly decreases and finally the total weight loss reach 9.4 wt%. For the CD-HA, the total weight loss is 12.7 wt%. The CD-HA show a 3.3 wt% weight loss via the decomposition of *β*-CD/citrate networks. For the PLA-CD-HA-x (x = 24, 36, 48, 72), the total weight loss is 21.4 wt%, 23.5 wt%, 24.0 wt% and 24.4 wt%, respectively. The PLA-CD-HA-x (x = 24, 36, 48, 72) show 8.7 wt%, 10.8 wt%, 11.3 wt% and 11.7 wt% weight loss *via* the decomposition of PLA. It is clear that the PLA grafting amount can be adjusted by the reaction time of the in-situ polymerization. The PLA grafting amount is higher than much of the reported value, which use the stannate as catalyst[4-5]. This is because that the introduced *β*-CD could provide more active sites (hydroxyl groups)to initiate the ROP of lactide[6]. This in-situ polymerization of L-lactide catalyzed by zinc lactate was not found to be applied in the surface modification of HA so far. As is known to all, the non-modified HA would suffer from severe agglomeration because of its strong interaction between HA particles and weak interaction between HA particle with the polymer matrix. The agglomeration of HA would be reduced and cause the dispersibility of HA would be improved *via* grafting organic on the surface of HA[5].

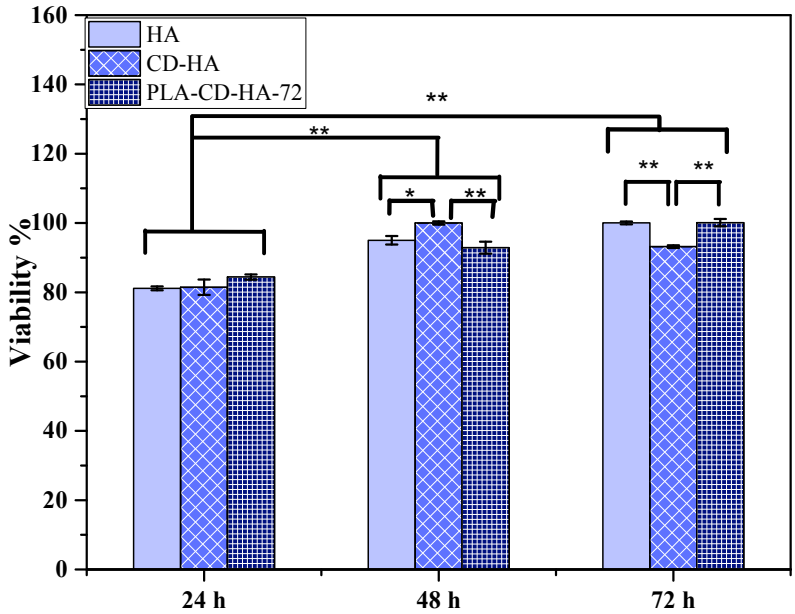


Fig. S3 Viabilities of MSCs in the ethanol solutions (1 μg·mL$^{-1}$) of HA, CD-HA and

PLA-CD-HA-72 with culturing time. There is significant difference between two groups (* $p < 0.05$, ** $p<0.01$)


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