Bioprosthetic heart valve cross-linked by non-glutaraldehyde reagent with improved biocompatibility, endothelialization, anti-coagulation and anti-calciafication property

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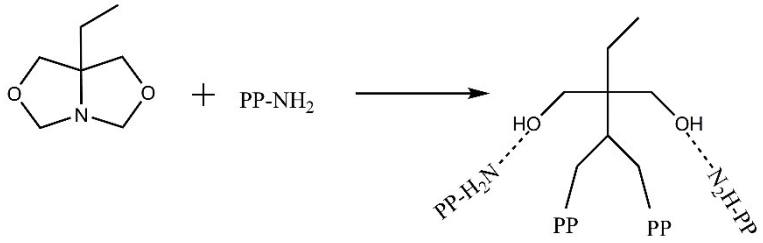

Fig. S1 The chemical reaction between OX-Et and PP

Methods

Decellularization

The method of decellularization was followed by the relevant literatures[29, 30]. After removing the connective tissue and fat on the surface, PP was cut into suitable size and rinsed by PBS. Then, PP was incubated with 0.5% sodium dodecyl sulfonate, 0.5% sodium deoxycholate, and 2% penicillin-streptomycin solution for 24 h at room temperature to obtain decellularized PP (D-PP).

Crosslinking of PP

OX-Et treated PP (OX-Et-PP): The decellularized PP was stretched and reacted in a solution containing 6% NaCl and 1% OX-Et (pH=5) for 24 h. Then, the pH was raised to 7 by using

saturated sodium bicarbonate solution, and the decellularized PP was reacted for another 24 h. After being washed by PBS, the resulting PP was incubated with 5% aminoglycerin for 24 h to obtain OX-Et-PP.

Glutaraldehyde-treated PP (Glut-PP): The decellularized PP was stretched and reacted with a solution containing 6% NaCl and 1% glutaraldehyde (pH=5) for 24 h. Then, the pH was raised to 7 by using saturated sodium bicarbonate solution, and the decellularized PP was reacted for another 24 h to obtain Glut-PP.

Crosslinking degree

D-PP and OX-Et-PP cross-linked by OX-Et in different concentrations were cut into suitable size (1 x 1 cm^2) and weighed respectively. Each sample was immersed in 2 mL ninhydrin solution (1% w/v ninhydrin, pH=5) and reacted in water bath at 95 ^oC for 40 min. After the reaction and cooling down to the room temperature, the supernatant fluid was taken out from the tube and measured the absorbance at 567 nm by using microplate reader. The results were calculated according to the following formula:

$$\text{Crosslinking degree} = 1 - \frac{OD_{sample}/W_{sample}}{OD_{D\text{-}PP}/W_{D-PP}}$$

Thermal shrinkage temperature

The thermal shrinkage temperature was measured by using differential scanning calorimetry (Mettler Toledo, Switzerland). D-PP, OX-Et-PP, and Glut-PP were freeze-dried, cut into suitable size (1 x 1 cm^2), and tested by using DSC 8000 under N_2 atmosphere with heating rate at 10 ^oC/min.

Collagenase and elastase degradation

D-PP, OX-Et-PP and Glut-PP were freeze-dried, cut into suitable size (1 x 1 cm^2) and weighed respectively. Each sample was placed into a tube and incubated in 5 mL collagenase Ⅱ solution (1

mg/mL) or 5 mL elastase (1 mg/mL) at 37 °C for 24 h. The enzyme-treated PPs were rinsed by PBS, then freeze-dried and weighed respectively. The results were calculated according to the following formula:

$$\text{Weight Loss Rate} = \frac{W_0 - W_1}{W_0} \times 100\%$$

The average weight of D-PP was recorded as W_0, and the weight of samples was recorded as W_1.

Uniaxial tensile test

The mechanical property was characterized by uniaxial tensile testing (Instron 4310, USA). D-PP, OX-Et-PP, and Glut-PP were cut into suitable size (3 x 1 cm^2, n = 6). All samples were hydrated and tested by a universal testing machine with constant velocity of 10 mm/min until failure.

Water content

D-PP, OX-Et-PP, and Glut-PP were cut into suitable size (1 x 1 cm^2, n = 6) and layered between two filter papers. Samples were placed under a 50 g object between the filter paper for 30 s, then took out and weighed to get wet weight. All samples were freeze-dried for 24 h or 48 h and weighed to get the constant dry weight. The results were calculated according to the following formula:

$$\text{Water Content} = \frac{W_{wet} - W_{dry}}{W_{wet}} \times 100\%$$

Water contact angle

D-PP, OX-Et-PP, and Glut-PP were freeze-dried, cut into suitable size (1 x 1 cm^2, n = 6) and fixed on the glass slide. Water contact angle was measured after same volume water was dripped on the sample by AttensionTheta (Biolin, Sweden).

Micro-nano structure observation

D-PP, Glut-PP and OX-Et-PP were firstly prepared to 10 μm thick sections by freezing microtome (Leica Cm 1860, Germany) and fixed on a glass slide by using tissue freezing medium. Then the samples were observed by AFM (Bruker,Germany).

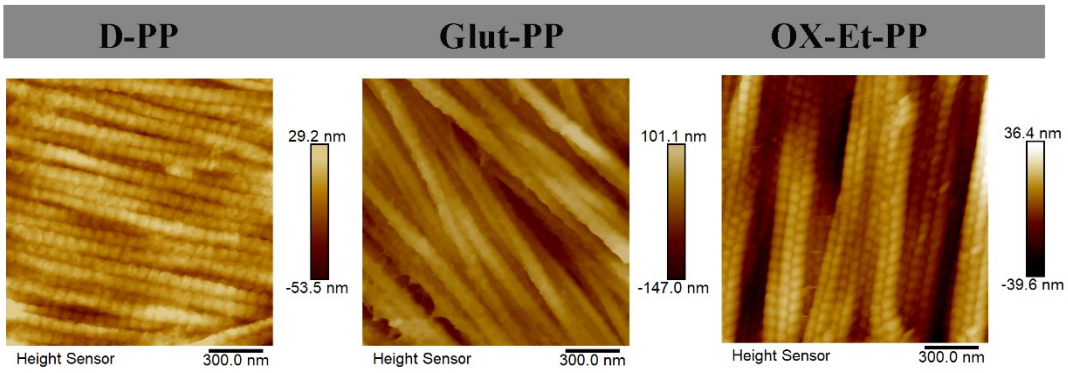

Fig. S2 AFM images of D-PP, Glut-PP and OX-Et-PP.

Young' modulus testing

The Glut-PP and OX-Et-PP were put on a flat glass slide in a wet state. Nanoindentation analysis was carried out by using the displacement controlled Piuma Chiaro Nanoindenter (Optics11, Netherland). A spherical tip with a radius of 45 μm was used to measure the Young's modulus of OX-Et-PP and Glut-PP.

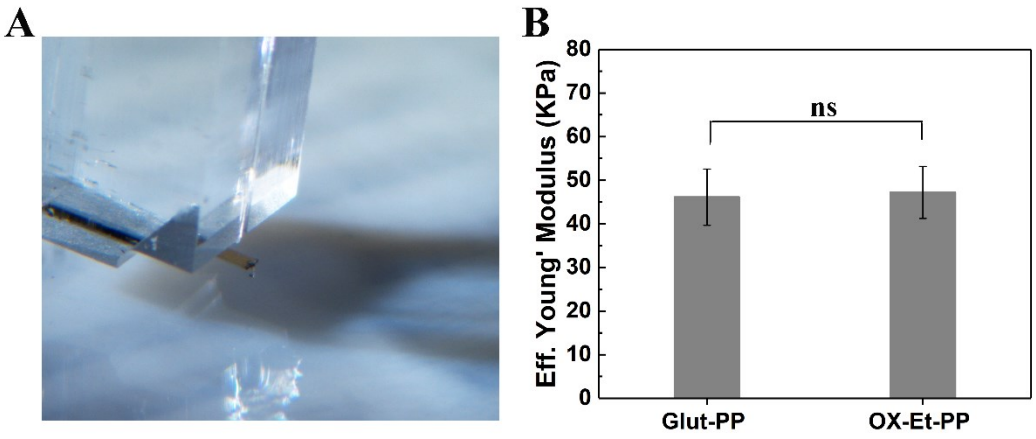

Fig. S3 (A) The testing scenarios of Nanoindenter; (B) The effective Young' modulus of Glut-PP and OX-Et-PP (n = 10).