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# Supporting Information

# Development of biomimetic trilayer fibrous membranes for guided bone regeneration

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Data shown in the supporting information were about the basic properties of synthetic polyurethane (PU) and the composite of polyurethane and nano hydroxyapatite (PUHA).

## Characterize

1. Gel permeation chromatography

Gel permeation chromatography (GPC, Waters-1515, USA) measurements were used to detect the number-average molecular weight ($M_n$), weight-average molecular weight ($M_w$), and molecular weight distribution at 40 °C with a Waters system. N, N-Dimethylformamide (DMF) was chosen as the mobile phase and the concentration of polyurethane solution was 2 mg/mL. The standard curve of molecular weight was calibrated by Monodispersed polymethyl methacrylate.

2. ATR-FTIR spectrometry

To investigate the chemical structure of PU and PUHA compounds, the infrared spectra of the samples were collected using attenuated total reflectance Fourier transform infrared spectrometry (ATR-FTIR, Nicolet 6700, Thermo Fisher Scientific, USA). Every spectrum was acquired in transmission mode with a resolution of 4 $cm^{-1}$ and a spectral range of 4000–650 $cm^{-1}$.

3. X-ray diffraction (XRD)

To study the crystal structure of PU and PUHA, XRD patterns was performed using a diffractometer (DX-2500X, Dandong Fangyuan Instrument, China) and Cu Kα radiation with a wavelength of 1.5406 Å. Data were collected for 2θ values of 5-80°, with a step width of 0.02° and a counting time of 1 s per step.

4. Thermogravimetric analysis (TGA)

TGA was used to observe the thermal stability of the PUHA fibers and calculated the weight content of n-HA in PUHA. The measurement was carried out with a Mettler-toledo TGA/DSC 1 thermogravimetric analyzer under a nitrogen atmosphere (nitrogen flow rate 20 mL/min) and a heating rate of 10 °C/min. The scan range was from 30 to 600 °C.

5. Tear testing

To study the adhesion behavior between layers, bilayer membranes fabricated by outer layer (PCL layer) and inter layer (co-PUPCL layer) have been prepared in different electrospinning processes for comparison. PU solution was dyed with alizarin red for clear observation. Other

electrospinning conditions were as the same as the preparation of trilayer membranes. Then, the experimental samples have been fabricated via the step-by-step method, of which the process was successively performed with first PCL solution (outer layer) and then dying PU and PCL solutions (inter layer) by using conjugate electrospinning. The control samples have been fabricated via a layer-by-layer method, of which the electrospinning process is not continuous even if the same equipment has been used. After an outer layer (PCL fibers) was prepared and set completely, the interlayer (co-PUPCL fibers) was collected on the set outer layer. Finally, tape was pasted on two sides of the bilayer membranes and teared with the grips of Universal Mechanical Testing Machine (AG-IC 50KN, SHIMADZU, Japan). The testing was done at the speed of 25 mm $min^{-1}$ at room temperature with a load cell capacity of 250 N.

**Results**

Table S1 The molecule weight of synthetic polyurethane

| $M_w$ (g/mol) | $M_n$ (g/mol) | $M_w/M_n$ |
|---|---|---|
| 45,856 | 4,744 | 9.67 |

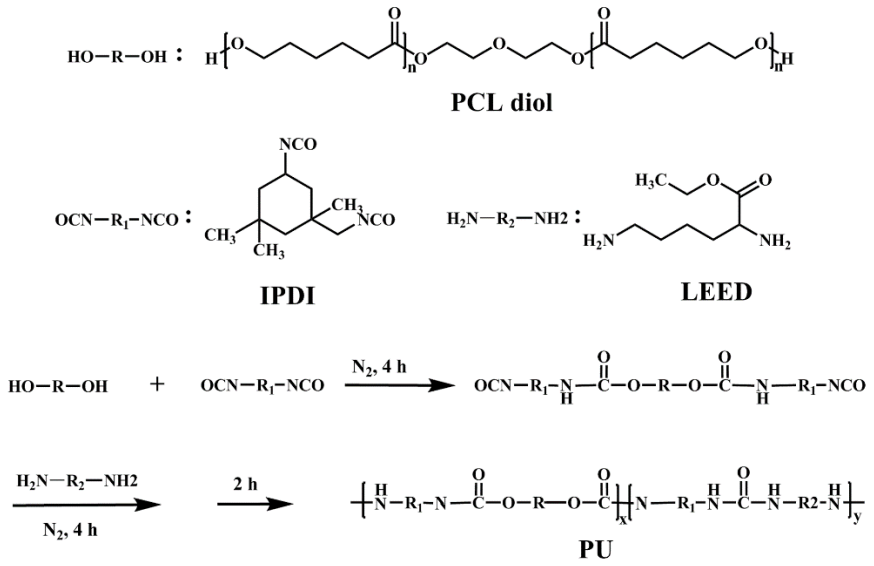


Figure S1. The chemical structure of components and schematic diagrams of the polymerized procedure of PU

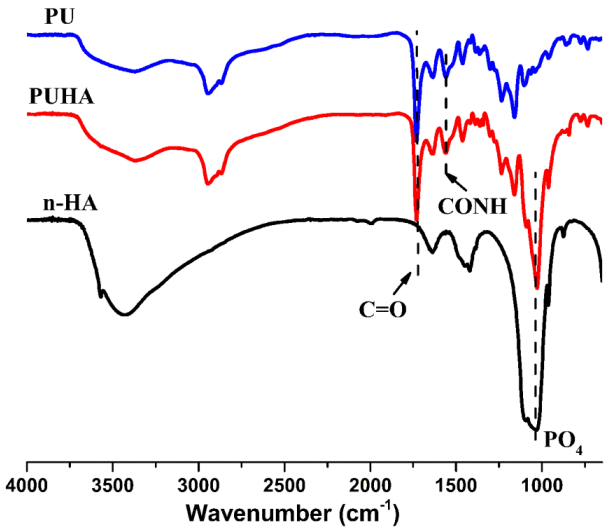


Figure S2. FTIR spectra of PU, PUHA and n-HA

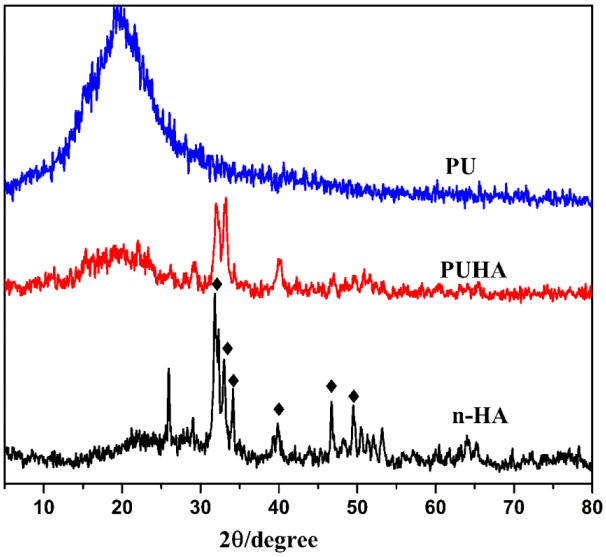


Figure S3. XRD patterns of PU, PUHA and HA

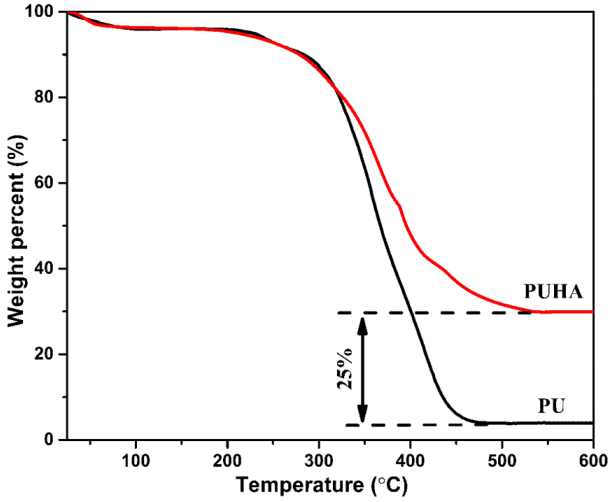


Figure S4. TG curves of PU and PUHA.

XRD, ATR-FTIR and TG were used to characterize the basic properties of PU and PUHA fibers. Figure S2 showed the ATR-FTIR spectra of polyurethane fibers with or without n-HA addition. The FTIR peak at 2,260 $cm^{-1}$ for the NCO groups was not observed in PU and PUHA compounds (Figure S2), meaning -NCO groups of IPDI completely reacted during polymerization. Strong peaks at 1730 $cm^{-1}$ and 1558 $cm^{-1}$ were observed for PU and PUHA fibers, which could be attributed to the carbonyl (C=O) stretching of urethane (CONH) groups of the PU matrix [S1]. Few peaks of polyurethane affected by the

addition of n-HA particles, except the phosphate groups between 900-1100 $cm^{-1}$ shown characteristic hydroxyapatite FTIR bands in PUHA. As shown in Figure S3, the XRD patterns confirmed the presence of a typical HA crystalline phase and a poorly crystallized PU phase. Two major 2θ reflection peaks at around 26° were the (002) diffraction of HA crystals, and around 32° being the overlapped diffractions of (211), (300), and (202) were observed in the n-HA filler, The reflection peaks at 26° and 32° shown in PUHA fibers indicate the introduction of crystal HA into the amorphous matrix of PU [S2]. The TG cures of PU and PUHA testified that the content of n-HA in PUHA fiber was about 25 wt% (Figure S4).

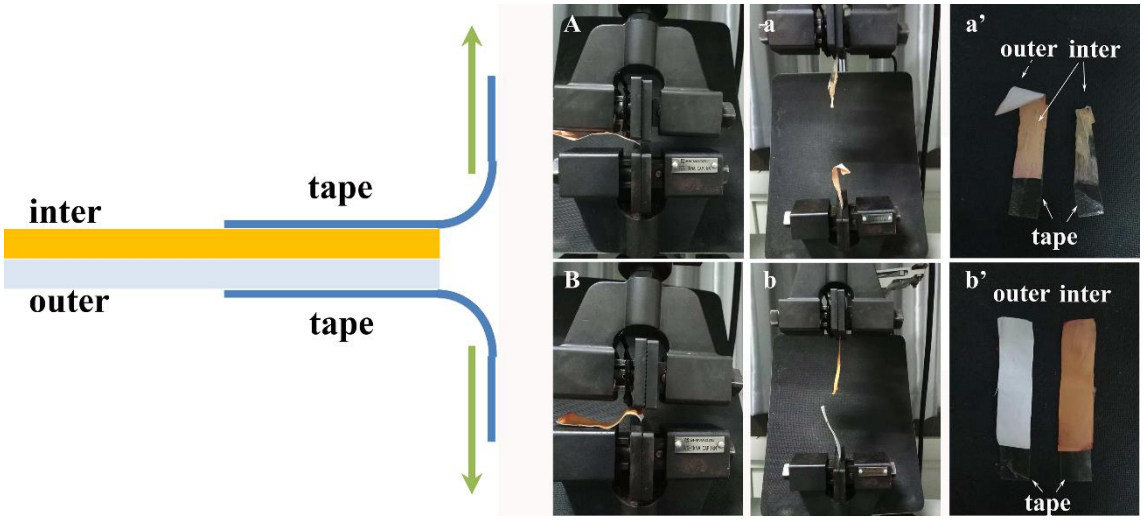


Figure S5. The tear testing on adhesion behavior of the experimental sample ((A), (a) and (a')) fabricated by step-by-step method, and the control sample ((B), (b) and (b')) fabricated by layer-by-layer method for comparison. The experimental membrane (Figure S5 (a')) was broken fragmentarily and the orange color could be seen on both two fragment parts of the bilayer membrane. In contrast, the control membrane (Figure S5 (b')) was completely stripped separated distinctly as different layers with white color for PCL fibers (out layer) and orange color for inter layer (co-PUPCL fibers). The step-by-step method of conjugated electrospinning could provide better adhesion between different layers than the layer-by-layer method.

Representative photos of the tear testing of the bilayer membranes were observed from Figure S5. When the bilayer membrane was teared by force, the fracture of the experimental membrane (Figure S5 (a')) was fragmentary. No complete layer has been reached under force and the orange color could be seen on both two fragment parts of the bilayer membrane. On the contrary, the fracture of the control membrane (Figure S5 (b')) was broken in continuity and separated distinctly as different layers with white color for PCL fibers (out layer) and orange color for inter layer (co-PUPCL fibers). The fractured phenomena described that the experimental membrane had a successive interphase structure between two layers (outer layer for PCL fibers and inter layer for dying PCL/PU fibers). Therefore, the step-by-step method of conjugated electrospinning could provide good adhesion between different layers and fabricate a unique fibrous structure for multi-layer membranes.

**References**


S1. J. Han, R. W. Cao, B. Chen, L. Ye, A. Y. Zhang, J. Zhang and Z. G. Feng, *Journal of biomedical materials research. Part A*, 2011, **96**, 705-714.

S2. L. Li, Y. Zuo, Q. Zou, B. Yang, L. Lin, J. Li and Y. Li, *ACS applied materials & interfaces*, 2015, **7**, 22618-22629.