Supporting Information

3D printed multilayer biomimetic scaffold with gradient-oriented structure for articular cartilage repair

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1. XRD and FTIR analysis of the superficial layer hydrogel bioink 7Gt-3Alg

XRD analysis of the superficial layer 7Gt-3Alg hydrogel bioink was performed to evaluate the effect of physical blending on the crystal structure of the polymer. As shown in Figure S1-(a), the XRD pattern of 7Gt-3Alg has four diffraction peaks at different positions, presenting as weak broad peaks near 2θ=13° and 22° and strong sharp peaks at 2θ=45.35° and 56.40°, indicating the semi-crystalline property of 7Gt-3Alg polymer [1]. Compared with pure Alg and pure Gt, the peak positions and peak intensities of 7Gt-3Alg show significant changes, which is obviously not a simple superposition of their diffraction patterns after the blending of the components. It indicates that the existence of interactions such as hydrogen bonding or electrostatic gravitational forces between Gt and Alg [2], thus destroying the original crystal structure of Gt and Alg. It is proved that the good compatibility between the blended components.

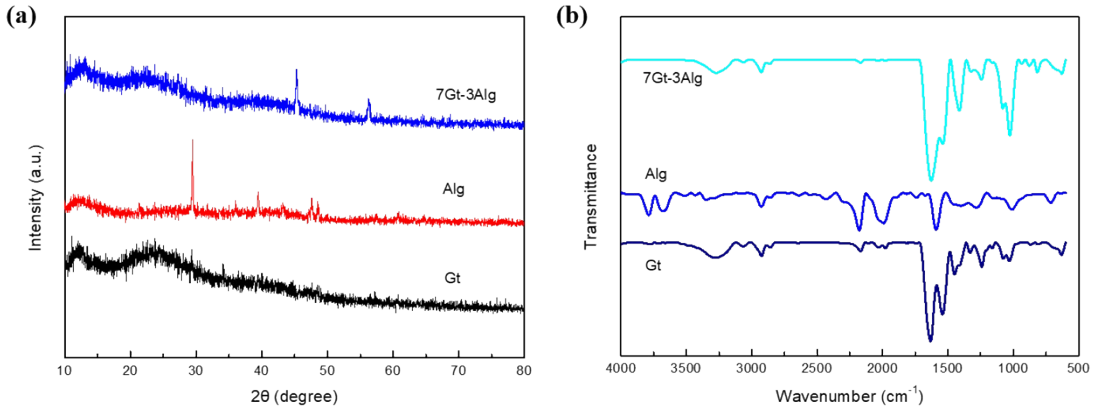

Figure S1. (a) XRD and (b) FTIR spectrum of superficial hydrogel ink 7Gt-3Alg

The characterization of Fourier transform infrared spectra (FTIR) helps to confirm the formation of new chemical groups in the polymer. The FTIR spectra of 7Gt-3Alg bioink and each component are shown in Figure S1-(b). From the spectrum of Alg, it can be seen that the absorption

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peaks located at 3640cm^{-1}~3610cm^{-1} are the characteristic peaks of free -OH, the absorption peak around 3445cm^{-1} is the stretching vibration peak of -OH, and the absorption peak around 1627cm^{-1} is the antisymmetric stretching vibration peak of COO^-. In the spectrum of Gt, the broad absorption peak at around 3328cm^{-1} is caused by the superposition of the peaks of the stretching vibration of -O-H and -N-H. The absorption peaks at around 1651cm^{-1} and 1571cm^{-1} are attributed to the absorption peaks of the amideIband (C=O antisymmetric stretching vibration) and the amide II band (-NH bending vibration), respectively. Compared with the spectrum of Alg, the characteristic absorption peak of free -OH located at 3640cm^{-1}~3610cm^{-1} in the spectrum of Gt-Alg disappeares, and the vibration peak of -OH around 3445cm^{-1} moves to the lower wave number and the peak shape becomes wider. This may be due to strong hydrogen bonding between the hydroxyl group (-OH) in the Alg molecule and the amino group (-NH_2) or carboxyl group (-COOH) in the Gt molecule. Compared with the spectrum of Gt, the absorption peak of the amide I band at 1651cm^{-1} was stronger, and that of the amide II band at 1571cm^{-1} was weaker, indicating that the peptide bonds formed by -NH_2 and -COOH between Gt and Gt molecules were reduced, which also proved that the intermolecular interactions between Gt and Alg did occur, consistent with the above conclusions.

2. XRD and FTIR analysis of the intermediate layer hydrogel bioink 2Gt-5Alg-5MMT

Figure S2-(a) shows the XRD diffraction pattern of the intermediate layer hydrogel bioink 2Gt-5Alg-5MMT. As can be seen from the figure, pure Gt shows a broad diffraction peak at around 2θ=21°, which is due to the ordered stacking of the triple helical structure of the Gt molecular chain forming a certain degree of crystallinity [3]. Pure Alg shows a less intense diffraction peak at 2θ = 47.56°, while pure MMT shows stronger diffraction peaks at 2θ of 19.86°, 26.65° and 36.03°. The diffraction pattern of 2Gt-5Alg-5MMT shows that the intensity of the diffraction peaks after homogeneous blending of the three components is considerably lower compared to that of pure Gt, Alg and MMT, indicating a loss of crystallinity during the blending process. This is due to the unique lamellar structure and cation exchange properties of nano-MMT. After mixing with Gt and Alg polymers in aqueous solution, cation exchange between Ca2+ in MMT molecules and Na+ in Alg molecules, Gt and Alg molecular chains or monomers enter between MMT layers, causing MMT to be stripped and dispersed into thinner single crystal sheets, increasing the number of hydroxyl groups (-OH) on the sides of MMT, thus enhancing the crystallinity of cross-linking between MMT and Gt and Alg molecules, while reducing the crystallinity of Gt, Alg and MMT single components.

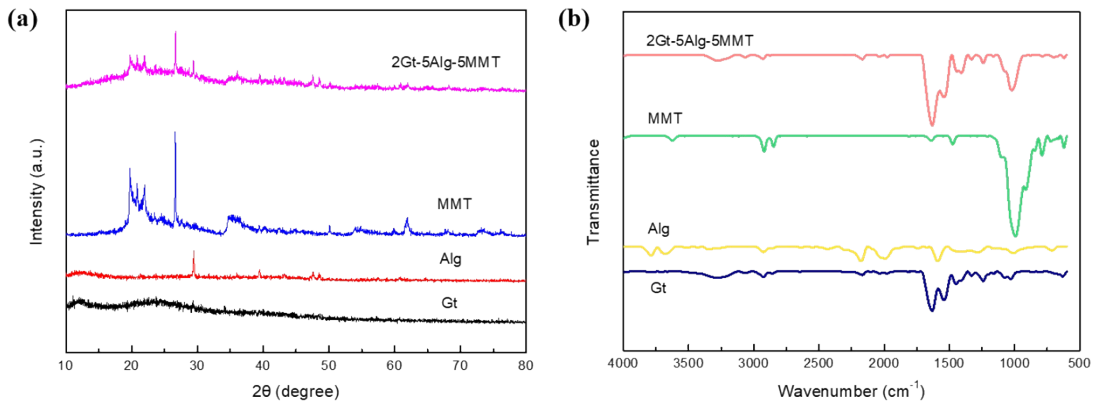

Figure S2. (a) XRD and (b) FTIR spectrum of intermediate layer hydrogel ink Gt-5Alg-5MMT

The FTIR spectra of the intermediate layer hydrogel bioink 2Gt-5Alg-5MMT are shown in Figure S2-(b). In the pure Gt spectrum, the broad peaks corresponding to around 3328 cm^{-1} are the multiple absorption peaks formed by the superposition of the peaks of the stretching vibrations of -O-H and -N-H; the absorption peaks at 1651 cm-1 and 1571 cm-1 correspond to the absorption peaks of the amide I band (C=O antisymmetric stretching vibration) and the amide II band (-N-H bending vibration), respectively. In the pure Alg spectrum, the characteristic absorption peaks of free -OH correspond at around 3640cm^{-1}~3610cm^{-1}; the anti-symmetric stretching vibration of COO^- corresponds at 1627cm^{-1}. In the pure MMT spectrum, the absorption peaks from3640cm^{-1}~3610cm^{-1} are characteristic peaks of free -OH; the absorption peaks between 1088cm^{-1}~913cm^{-1} correspond to the stretching vibration peaks of Si-O [4,5]. The absorption spectrum of 2Gt-5Alg-5MMT shows that the characteristic absorption peaks of free -OH at 3640cm^{-1}~3610cm^{-1} disappear, while the vibrational peaks of associative -OH at 3550cm^{-1}~3200cm^{-1} are enhanced, which indicates that hydrogen bonding interactions between the MMT Sheet layers and Gt and Alg molecules have occurred. In addition, the absorption peaks of the amide I band (C=O antisymmetric stretching vibration) and the amide II band (-N-H bending vibration) at1651cm^{-1} and 1571cm^{-1} are significantly enhanced, so it can be considered that there is also interaction between Gt and Alg macromolecular chains.

3. XRD and FTIR analysis of the deep layer hydrogel bioink 5Gt-7Alg-3HA

The XRD diffraction patterns of the deep layer hydrogel bioink 5Gt-7Alg-3HA are shown in Figure S3-(a). As can be seen from the figure, pure Gt shows a broad diffraction peak near 21° and pure Alg shows a less intense diffraction peak at 47.56°. For pure HA, characteristic diffraction peaks appear at 2θ of 25.8°, 31.7°, 39.7°, 46.8°, 49.4° and 53.2°, corresponding to (201), (211), (130), (222), (230) and (004) crystal planes of HA, respectively [6]. The diffraction pattern of 5Gt-7Alg-3HA shows that the intensity of the diffraction peaks after homogeneous blending of the three components is significantly lower compared to that of pure Gt, Alg and HA, indicating that the

addition of HA affects the crystallinity of Gt and Alg, while its own crystallinity is also lost. This is mainly because HA contains a large number of hydroxyl (-OH) and calcium ions (Ca^{2+}), Gt contains a large number of amino ($-NH_2$) and carboxyl (-COOH), and Alg contains a large number of sodium ions (Na^{+}). When HA particles are mixed with Gt and Alg polymer solutions, the intermolecular hydrogen bonding force is formed between -OH in HA and $-NH_2$ or -COOH in Gt, and Ca^{2+} in HA has an ion exchange reaction with Na+ in Alg, thus enhancing the cross-linking crystallinity between HA, Gt and Alg, while reducing the crystallinity of single components of Gt, Alg and HA. In conclusion, the presence of the HA phase in the nanocomposite hydrogel ink was demonstrated by XRD diffraction pattern, which will facilitate the subsequent analysis and study of the various properties of the deep layer 5Gt-7Alg-3HA cartilage scaffold.

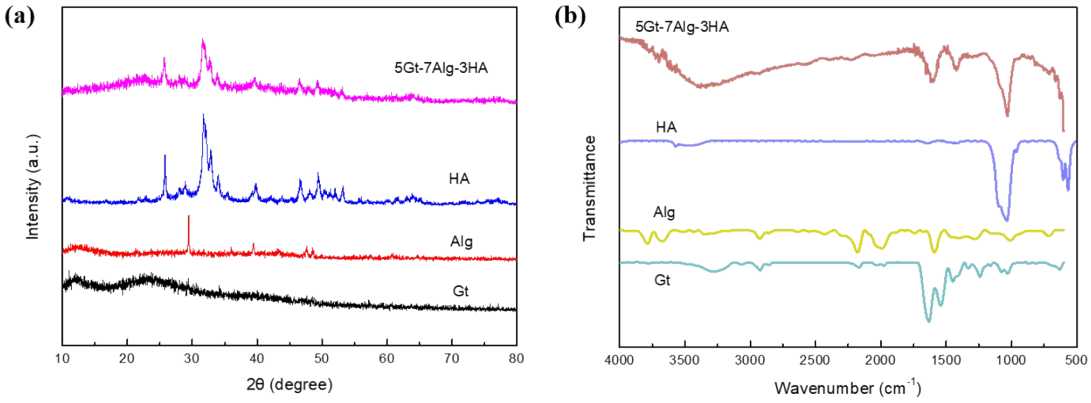

Figure S3. (a) XRD and (b) FTIR spectrum of deeplayer hydrogel ink 5Gt-7Alg-3HA

The FTIR spectra of the deep layer hydrogel bioink 5Gt-7Alg-3HA are shown in Figure S3-(b). As can be seen from the figure, pure Gt at 3328cm^{-1}, 1651cm^{-1} and 1571cm^{-1} respectively corresponds to the superimposed peaks of -O-H and -N-H stretching vibration, the characteristic absorption peaks of amide I band (C=O antisymmetric stretching vibration) and amide II band (-N-H bending vibration). Pure Alg at 3640cm^{-1}~3610cm^{-1} and 1627cm^{-1} corresponds to the characteristic absorption peak of free -OH and the antisymmetric stretching vibration peak of COO^{-}, respectively. In the pure HA spectrum, 3640cm^{-1}~3610cm^{-1} corresponds to the characteristic peak of free -OH, and 1029cm^{-1} corresponds to the characteristic peak of PO_4^{3-} [7]. Comparing the FTIR spectra of 5Gt-7Alg-3HA, it can be seen that the characteristic peak of free -OH at 3640cm^{-1}~3610cm^{-1} disappears while the vibrational peak of association -OH at 3550cm^{-1}~3200cm^{-1} increases, indicating that hydrogen bonds are formed between -OH in HA and Gt and Alg molecules. Moreover, the characteristic peak of $PO4^{3-}$ at 1029cm^{-1} was weakened, which once again proved the interaction between HA and the other two molecules. In addition, the characteristic peak of the amide I band at 1651 cm^{-1} was weakened and the characteristic peak of the amide II band at 1571 cm^{-1} was disappeared, indicating that there was also an interaction between Gt and HA and Alg. In

conclusion, the infrared spectrum analysis confirmed that HA particles formed hydrogen bond interactions with Gt and Alg molecular chains, which provided an important basis for the subsequent analysis of the cross-linking degree and structural stability of the internal network of the deep layer 5Gt-7Alg-3HA cartilage scaffold.

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