Electronic Supplementary Material (ESI)

Multi-functional SiO₃²⁻-releasing hydrogel with bioinspired mechanical properties and biodegradability for vascularized skeletal muscle regeneration—**Supporting Information**

1 Supplementary methods

1.1 Fourier Transform Infrared (FTIR) analysis

The chemical composition of PVA, PVA-Si, PVA/starch and SRHs were analyzed by Fourier transform infrared spectrometer (FTIR, Nicolet 6700, Thermo Fisher, USA) in transmission mode in the wave number range of 4000–700 cm⁻¹. Samples were prepared by dry pressing the mixture of KBr powder and freezing-dried hydrogel powder (mass ratio 50:1) into a disc (1 cm in diameter and 0.5 mm in thickness).

1.2 X-ray diffraction (XRD) analysis

The phase composition of hydrogels was characterized by X-ray diffractometry (XRD, Empyrean, panalytical, Holland) with CuKα radiation. Diffraction patterns were recorded at 2θ between 10° and 50° using a step scanning (0.025° per step and 2°/min).

1.3 Mechanical property of the Hydrogel

First, we performed compression tests on the PVA, PVA-Si, H2W2, H3W1, H4W0 and CRH, which were molded into a cylinder with a diameter of 18 mm and a think of 10 mm. The samples were placed on a universal testing machine (HY-0580, Shanghai Hengyi Co., Ltd., China) and compressed at a rate of 10 mm min⁻¹. The compression modulus was calculated from the linear
slope of the first 10% of the stress-strain curve. Next, tensile tests were performed on the samples using a constant strain rate mode at 20 mm/min on a universal testing machine. Similarly, the tensile modulus was calculated from the linear slope of the first 10% of the stress-strain curve. The tensile strength and elongation at break of samples were also obtained.

1.4 Cytotoxicity of silicate ions to C2C12 myoblast

To evaluate the effect of silicate ions on cell viability, the half maximal inhibitory concentration (IC50) of silicate ion was determined. Briefly, we prepared DMEM with ascending (0.1, 0.5, 1, 2, 3 or 4 mM) silicate ion levels using Na$_2$SiO$_3$. Normal DMEM served as control. C2C12 myoblasts were seeded in 96-well plates at a density of 3000 cells per well. After 24h post seeding, the culture medium was replaced with the aforementioned high silicate medium. After incubation for 24 h, the cell viability was measured according to the method described above.

1.5 Electrical conductivity

The PVA/starch, CRH and H3W1 hydrogels were cut into strips (20× 5 × 4 mm in length, width, and thickness) and connected with two platinum plates to form a circuit. A constant voltage of 3 V was applied to the circuit through a constant voltage power supply (IT6833, ITECH, China). The current in the circuit was measured by a multimeter (17B, Fluke, USA) to calculate the conductivity of hydrogels.

\[
\sigma = \frac{L \times I}{U \times S}
\]

where \(\sigma\) is electrical conductivity, \(L\) is the distance between two platinum wires, \(S\) is the cross-sectional area of the hydrogel, \(I\) is current, and \(U\) is voltage.

1.6 Bacteria-inhibition test
The test was performed according to the method reported in the literature\(^1\). PVA/starch and H3W1 hydrogels with a thickness of 2 mm were tiled in a 24-well plate, then added with 100 μL of \(S.\) aureus suspension at \(1 \times 10^6\) CFU mL\(^{-1}\). The bacteria were cultured at 37 °C in a humidified atmosphere of 5% CO\(_2\). After incubation for 24 h, the hydrogels were rinsed with phosphate buffered saline (PBS) for 3 times to remove the non-adhered bacteria. Then the hydrogels were incubated with 10 μM SYTO® green fluorescent nucleic acid stain (Cat. no. S34854) for half an hour. Bacterial growth on hydrogel surface were observed under inverted fluorescence microscope (Carl Zeiss, Germany) and the number of \(S.\) aureus per unit area (mm\(^2\)) were quantified by ImageJ software.

2 Supplementary text

2.1 FTIR spectra of pure PVA, PVA-Si, PVA/starch and SRH hydrogels

Fig. S3 showed the FTIR spectra of pure PVA, PVA-Si, PVA/starch and SRHs. The peak at 1024 cm\(^{-1}\) and 1081 cm\(^{-1}\) appeared in PVA/starch and all SRH groups corresponds to the stretching vibration peak of C-O in C-O-C and C-O in C-O-H on the starch molecule chain respectively\(^2\). The deformation vibration of -CH\(_2\) in -CH\(_2\)OH of PVA molecule at 1422 cm\(^{-1}\) were found in the spectra of all hydrogels\(^3\). Compared to PVA, the absorption wavenumber of hydroxyl groups in PVA/ starch shift from 3299 cm\(^{-1}\) to 3313 cm\(^{-1}\), due to the higher wavenumber of hydroxyl groups of starch (3384 cm\(^{-1}\)). With the addition of Na\(_2\)SiO\(_3\), the absorption wavenumber of hydroxyl groups in each SRH hydrogel decreased compared with that of PVA/starch, indicating that the interaction between inorganic salts and PVA/starch...
molecules weakens the hydrogen bonds within the starch and PVA molecules\textsuperscript{4} and enhance the hydrogen bonding interactions between starch and PVA\textsuperscript{5, 6}, benefiting the formation of a biocompatible structure. FTIR analysis also showed that there was no chemical reaction between PVA, starch and Na$_2$SiO$_3$, and that the three components interacted physically.

2.2 XRD characterizations of PVA, PVA-Si, and SRH

As shown by Fig. S4, all the PVA, PVA-Si, and SRH samples showed characteristic diffraction peaks in the 2$\theta$ range 19 $\sim$ 21°, corresponding to the 101-crystal plane of PVA. The results also indicated that crystallinity of PVA could be reduced by the addition of Na$_2$SiO$_3$, as reported previously\textsuperscript{2}. The diffraction peak at the 2$\theta$ value around 17° was ascribed to the crystallization of starch\textsuperscript{2}, which was inhibited in the SRH samples probably by the ionic interaction with Na$_2$SiO$_3$ ions\textsuperscript{2, 7}. No characteristic diffraction peaks of Na$_2$SiO$_3$·9H$_2$O appeared in all samples, suggesting that PVA and starch could interact with Na$_2$SiO$_3$ to inhibit the recrystallization of Na$_2$SiO$_3$ during the water loss from network.

2.3 Cell viability of silicate ions to C2C12 myoblast

To investigate cytotoxicity of silicate ions to C2C12 myoblast, we cultured C2C12 myoblast with medium containing varying concentrations of silicate ions to determine the appropriate silicate environment for cell survival. According to the IC$_{50}$ assays, cell viability was enhanced when silicate ion was in the range of 0.1 to 1 mM, but decreased sharply when the concentration exceeded 2 mM. When the concentration reached 3.16 mM, half of the cells were apoptotic (Fig. S6).
### 3 Supplementary Tables

**Table S1.** Compositions of various hydrogels.

| Hydrogel      | PVA (Wt %) | Starch (Wt %) | Na$_2$SiO$_3$$
\cdot$9H$_2$O (Wt %) |
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<td>/</td>
<td>/</td>
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**Table S2.** Primers used for RT-PCR.

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**4 Supplementary Figures**

**Fig. S1.** SEM images of PVA, H1W3 and H2W2 hydrogels.
Fig. S2. Variation of $G'$, $G''$ and $G''/G'$ ratio (loss factor) of the PVA, H1W3 and H2W2 hydrogels with frequency.
Fig. S3. FTIR spectra of the hydrogels.
Fig. S4. XRD patterns of PVA, PVA-Si, SRHs, WS, and HAS.
**Fig. S5.** Compressive and tensile mechanical properties of the hydrogels. (A) The stress-strain curves of the hydrogels under compression. (B) The compression modulus of the hydrogels. (C) The stress-strain curves of the hydrogels under tensile test. (D) The tensile modulus. (E) Tensile strength and (F) elongation at break of the hydrogels. All data are expressed as mean ± S.D (n=3, *p < 0.05, **p < 0.01, ***p < 0.001 and ****p < 0.0001 versus PVA, ^p < 0.05, ^^p < 0.01, +++p < 0.001 and ++++p < 0.0001 versus PVA-Si, ˙p < 0.05, ˙˙p < 0.01 and ˙˙˙˙p < 0.0001 versus PVA).
versus H2W2, *p < 0.05 and ****p < 0.0001 versus H4W0 group). The p-values by one-way ANOVA are indicated.

**Fig. S6.** Viability of C2C12 myoblasts after being cultured in medium supplemented with different concentrations of silicate ions for 24 h (n=3).

**Fig. S7.** (A) Representative fluorescence microscopy images of *S. aureus* adhered to H3W1 and PVA/starch following 24 h incubation. (B) The number of adhered *S. aureus* per square
millimeter on the surface of H3W1 and PVA/starch hydrogels. All data are expressed as mean ± S.D (n=3). The p-values by t-tests are indicated.

**Fig. S8.** pH values of immersion solution after soaked with various silicate-containing hydrogels for different time periods. The immersion solution was refreshed every day.

**Fig. S9.** Electrical conductivity of H3W1 and PVA/starch. All data are expressed as mean ± S.D (n=3). The p-values by t-tests are indicated.
Fig. S10. (A) Rheological behavior, (B) degradation rate, (C) mechanical properties and (D) electrical conductivity of SRH and CRH. All data are expressed as mean ± S.D (n=3). The p-values by t-tests are indicated.
**Fig. S11.** Biocompatibility of CRH and SRH to HUVECs. (A) Optical density values (@450 nm) of CCK-8 assay about HUVECs cultured in the hydrogel extracts or regular cell culture media for 1 and 3 days. (B) Fluorescence micrographs of Live/Dead stained HUVECs after being cultured with the hydrogel extracts or regular cell culture media for 1 and 3 days. Statistical differences were determined with one way ANOVA. All data are expressed as mean ± S.D. NS: no significance.

**5 Supplementary Video**

**Video S1.** Appearance of various SRHs (H0W4, H1W3, H2W2, H3W1 and H4W0).

**References**


