Supporting Information

# Fabrication of multifunctional hydrogel with robust interface bioinspired by the structure of dentogingival junction

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## Experimental section

**Materials** Acrylamide (AM), N, N'-Methylenebisacrylamide (MBA), Irgacure 2959 (I2969), silver nitrate ($AgNO_3$), calcium nitrate tetrahydrate ($Ca(NO_3)_2 \cdot 4H_2O$), diammonium hydrogen phosphate ($(NH_4)_2HPO_4$), and ammonia were purchased from J&K Scientific Ltd. Agar (gel strength > 600 g/cm$^2$) and vinylphosphonic acid (VPA) were obtained from Sigma-Aldrich (Shanghai, China). All the reagents were used without further purification. Quantum dots composed of citric acid and m-phenylenediamine was synthesized by hydrothermal method (180 ℃ for 8 h).

**Preparation of single hydrogel** The native hydrogel ($AP_0$) was prepared according to the method of reported by Chen *et al*. [1]. Briefly, agar (100 mg)， AM （900 mg）, I2959 (28.4 mg) and MBA solution (59 μL， 10 mg/mL) were mixed with water (5 mL) in the dark. The suspension was heated under 95 ℃ and magnetic stirring until the solution is colorless and transparent. Then the hot solution was poured into the model for cooling. Finally, the cold forming hydrogel was exposed to the ultraviolet light (365 nm wavelength, 8W) for 1 hour. Hydrogels with single function, i.e. $AP_{VPA}$, and $AP_{Ag}$, were made through adding VPA solution (200 μL， 136.7 mg/mL) and $AgNO_3$ (50 mg) to the suspension, respectively. Silver nanoparticles was reduced by the UV light [2]. $AP_{HA}$ was formed on the basis of Hu *et al*. work

[3]. After heating, the pH of solution was adjusted to 10-10.5 by ammonia. Then the $Ca(NO_3)_2 \cdot 4H_2O$ and $(NH_4)_2HPO_4$ (Ca/P molar ratio = 1.67, organic/inorganic ratio = 7:3) were added to the solution under gentle agitation for 30 min. Gelation process of $AP_{HA,}$ $AP_{Ag}$ and $AP_{VPA}$ were same as that of $AP_0$.

**Preparation of multilayer hydrogel** Chemical method: Putting two pieces of cooled hydrogel together, then the multilayer hydrogel was UV -cured to form attached interface (365 nm, 8W, 1 hour). Physical method: Pouring the hot solution (95 ℃) on the cooled UV-cured lower hydrogel, the upper hot solution was cooled into hydrogel and then the lower hydrogel was covered by the silver paper and the UV light path was parallel to the interface during the photo-curing of upper hydrogel. Physical-chemical method: Pouring the hot solution (95 ℃) on the cooling hydrogel, then the multilayer hydrogel was cooled and UV-cured in sequence (365 nm, 8W, 1 hour).

**Mechanical properties** The stress-strain curves of the tensile test with typical dimensions of 8 × 15 mm (width× length) and a thickness of 3.0 ± 0.2 mm were characterized using commercial test machine equipped with a 10 N load sensor (HZ-1004B, Dongguan Lixian Science and Technology Ltd.) in force control mode at a force ramp of 100 mm/min until the sample fractured. The samples were fixed between two clamps with an 8 mm interval. The specimens of compressive test were cylinder with typical dimensions of 10 × 8 mm (diameter× height). For compression measurement, hydrogel specimens were first compressed to a maximum stress 100 KPa and then unloaded. The interface of multilayered hydrogel was perpendicular to the direction of force and in the middle of specimens.

**Swelling ratio of hydrogel** The hydrogel was firstly free-dried to get the dry weight ($m_0$), then the dried hydrogel was putted into the water for 48 h to reach the equilibrium swelling state and the swollen weight (m) was recorded. According to the equation, the swelling ratio is calculated as:

$$swelling\ ratio\ (\%) = \frac{m - m_0}{m_0} \times 100\%$$

**Morphology of hydrogel** The morphology and element mapping of dry hydrogels were recorded by scanning electron microscope (SEM, Quanta 250, FEI, US, 20 kV) and Energy Dispersive X-ray Detector (EDX, Quanta 250, FEI, US)

**Antibacterial behavior** Previously reported method[4] was used to evaluate the antibacterial behavior by using Escherichia coli (*E. coli*). The *E. coli* suspension of $10^8$ CFU/mL was flat coated on the nutrient agar medium, then the $AP_{Ag}$-$AP_{HA}$ hydrogel was put on the plate. After 24 hours of incubation at 37 ℃, the plate was used to observe inhibition zone. The hydrogels co-cultured with *E. coli* suspension of $10^5$ CFU/mL at 37 ℃ for 24 h was stained using LIVE/DEAD™ BacLight™ Bacterial Viability Kit (ThermoFisher Scientific, USA) in the dark for 15 min. The fluorescent staining images was photoed by fluorescence microscope (IX-71, Olympus, Japan).

**Cell-adhesion behavior** We use mouse osteoblast-like cells (MC3T3-E1) to evaluate the cell-adhesion behavior [5] MC3T3-E1 were cultured in alpha-minimum essential medium (α-MEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin solution. The cells (passage 3) with a density of $3\times10^5$/mL were used in the following assays, and were cultured at 37 ℃ in a humidified 5% $CO_2$ atmosphere. The MC3T3-E1 cells were seeded on the surface of $AP_0$-$AP_{VPA}$ hydrogel placed in 24-wells plates and cultivated in the culture medium for 24 h. Then the sample was rinsed in the PBS buffer for further fluorescent staining. The staining liquid was the mixture solution of Fluorescein Diacetate (FDA, 1000 μg/mL) and Propidium iodide (PI, 20 μg/mL). The hydrogel was immersed in staining liquid for 15-20 min and observed in the fluorescence microscope (IX-71, Olympus, Japan) after twice resining of PBS buffer.

## References

[1] Q. Chen, L. Zhu, C. Zhao, Q. Wang, J. Zheng, *Adv. Mater.,* 2013, **25**, 4171-4176.

[2] M. Darroudi, M. B. Ahmad, A. K. Zak, R. Zamiri, M. Hakimi, *Int. J. Mol. Sci.,* 2011, **12**, 6346-6356.

[3] J. Hu, Y. Zhu, H. Tong, X. Shen, L. Chen, J. Ran, *Int. J. Biol. Macromol.,* 2016, **82**, 134-143.

[4] H. Xu, D. Zhang, J. Li, *J. Bioresources and Bioproducts* 2019, **4**, 177-182.

[5] X. Xu, D. Zhang, S. Gao, T. Shiba, Q. Yuan, K. Cheng, H. Tan, J. Li, *Biomacromolecules* 2018, **19**, 1979-1989.

## Additional figures

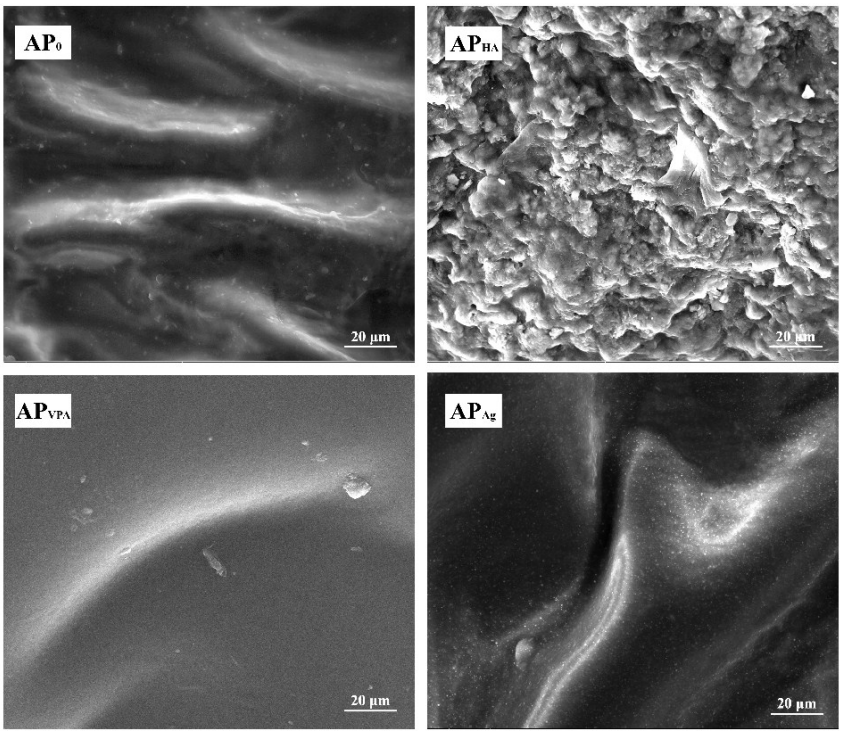


*Figure S1.* SEM images of diverse hydrogels with single function.

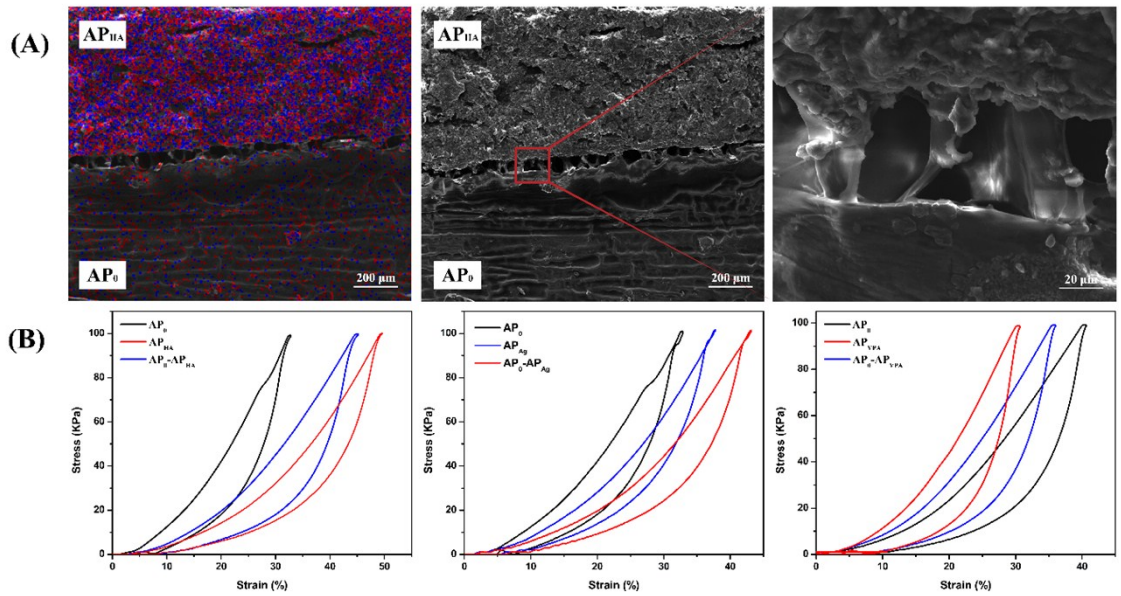


*Figure S2.* (A) Interface morphology of $AP_0$-$AP_{HA}$ hydrogel. The left is element mapping tested by EDX analysis. Ca: Red dots, P： Blue dots. The middle one is SEM image and the right one is the enlarged image of red box in the middle one. (B) Compressed stress-strain curve of single and multilayered functional hydrogels.

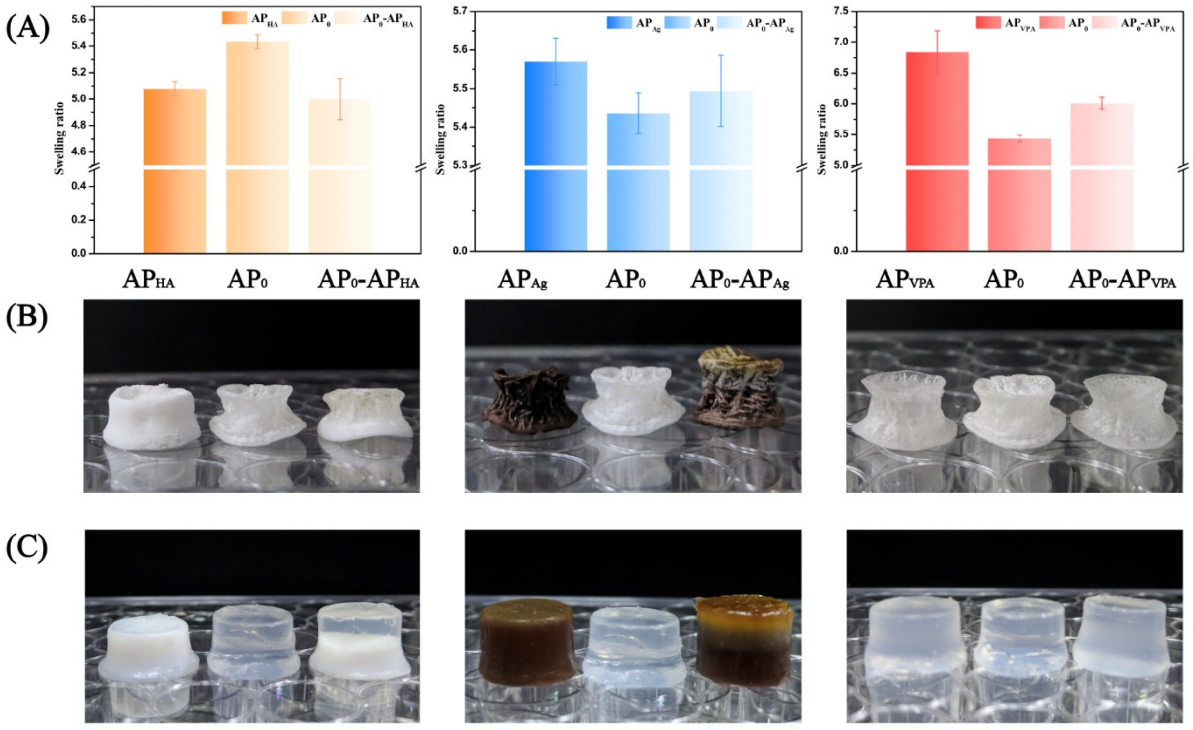


*Figure S3.* (A) Swelling ratio of functional multilayered hydrogels. Optical images of (B) freeze-dried hydrogels and (C) swollen hydrogels.

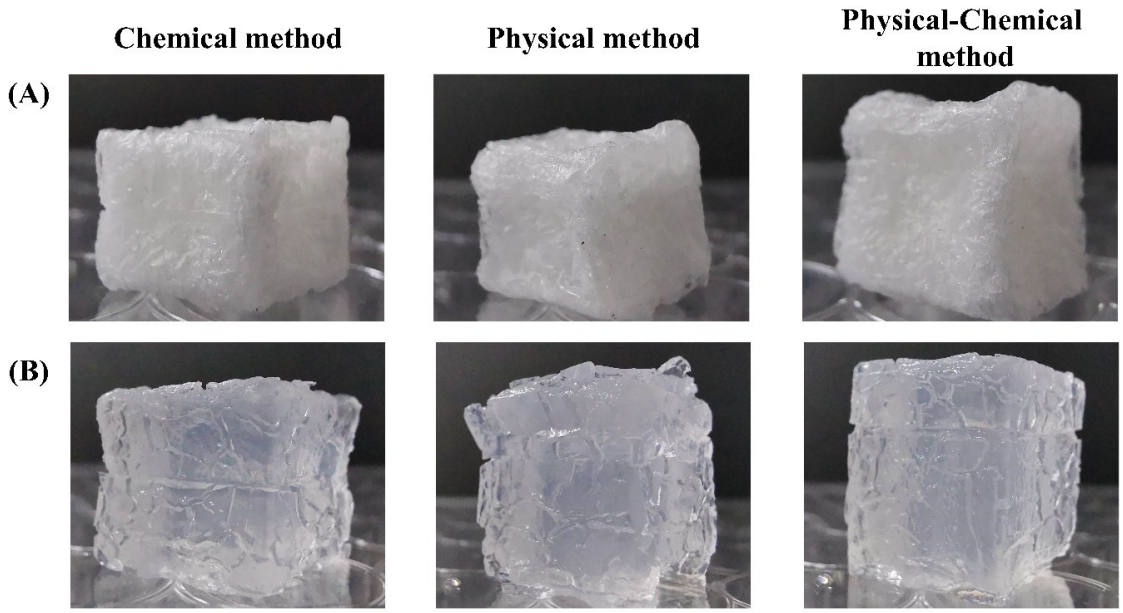


*Figure S4*. Optical images of (A) freeze-dried and (B) swollen hydrogels made by different methods.

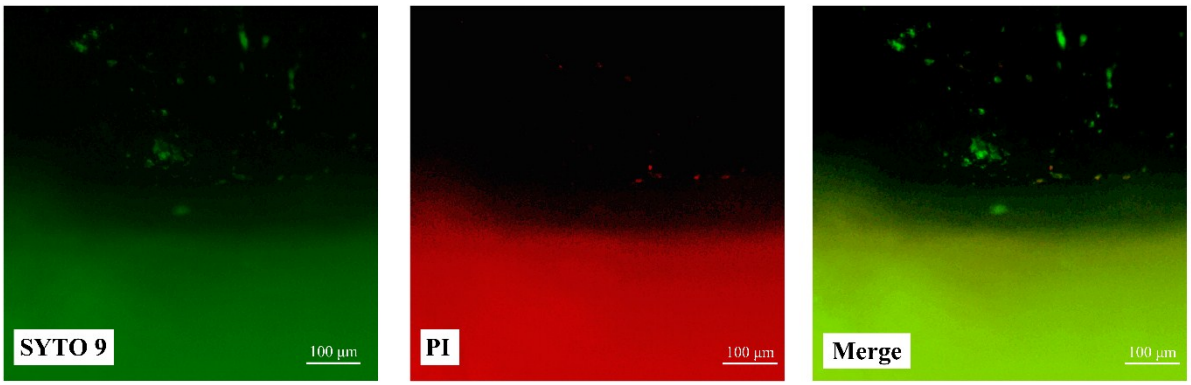


*Figure S5*. Fluorescent staining image of $AP_{Ag}$-$AP_{HA}$ hydrogel with *Escherichia. coli*., the upper hydrogel is $AP_{Ag}$, while the bottom hydrogel is $AP_{HA}$. Live and dead cells are stained by SYTO 9 (green) and PI (red), respectively.

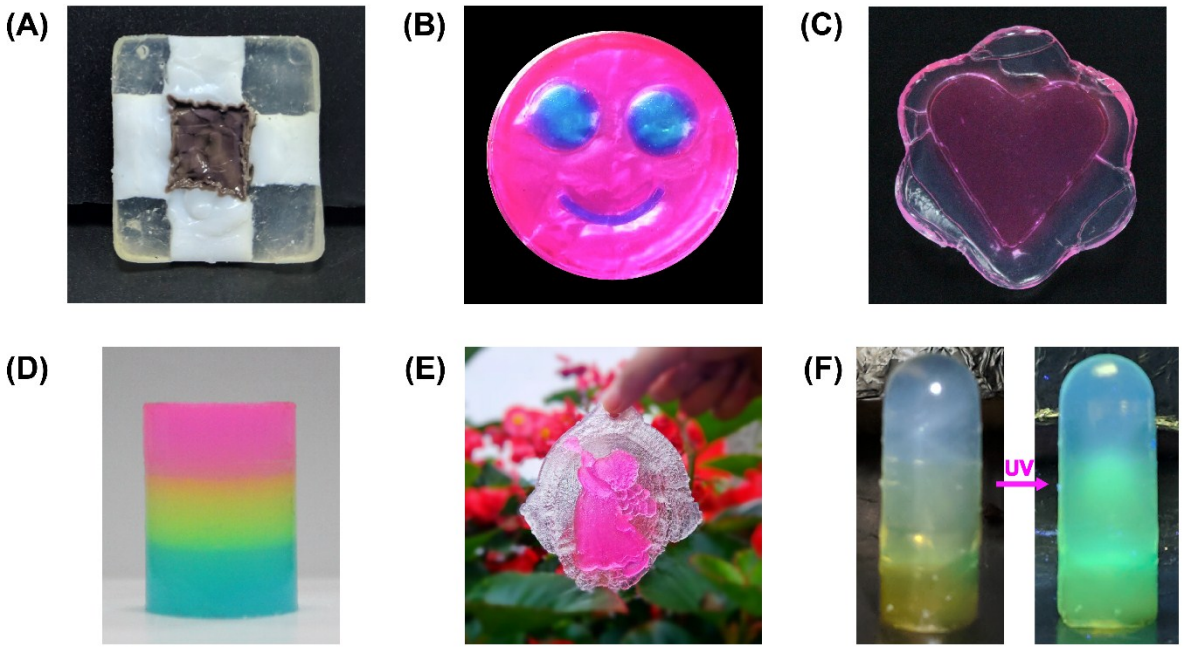


*Figure S6.* (A) Checkerboard-like combination of functional hydrogels, $AP_0$: colorless and transparent, $AP_{HA}$: white and opaque, $AP_{Ag}$: sepia and opaque. (B), (C), (D) and (E) Optical images of combined hydrogels with different shape and structure. Water-soluble pigment was added to distinguish the different parts of hydrogel. (F) Multilayered hydrogel with quantum dots.

**Videos in the Supporting Information**

*Video S1.* The dynamic tensile process of hydrogel made by chemical method.

*Video S2.* The dynamic tensile process of hydrogel made by physical method.

*Video S3.* The dynamic tensile process of hydrogel made by physical-chemical method.

*Video S4.* The dynamic tensile process of $AP_0$-$AP_{Ag}$ hydrogel ($AP_{Ag}$ at the bottom).

*Video S5.* The dynamic tensile process of $AP_{HA}$-$AP_0$ hydrogel ($AP_0$ at the bottom).