Synthesis of hydrogel microspheres with tunable pore size and their application in alkaline protease immobilization

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1 Immobilization conditions of alkaline protease

The enzymatic loading of Alk-P(H-A)HMs was studied when the mass ratios of alkaline protease to carrier were 1:8, 1:6, 1:4, and 1:2. As shown in Fig. S1a, when the mass ratio of alkaline protease/P(H-A)HMs increased from 1:8 to 1:4, the enzymatic loading of Alk-P(H-A)HMs increased dramatically. The mass ratios of alkaline protease/P(H-A)HMs at 1:4 were the optimal enzyme loading ratios for Alk-P(H-A)HM1, Alk-P(H-A)HM2 and Alk-P(H-A)HM3, which were 10.39 mg g^{-1}, 20.20 mg g^{-1} and 13.44 mg g^{-1}, respectively. With the increase of the mass ratios of alkaline protease/P(H-A)HMs (from 1:4 to 1:2), the enzymatic loading of Alk-P(H-A)HM1, Alk-P(H-A)HM2 and Alk-P(H-A)HM3 decreased to 5.11 mg g^{-1}, 11.31 mg g^{-1} and 8.96 mg g^{-1}, respectively. The reason for the above phenomenon might be ascribed to that the more alkaline protease piled up in Alk-P(H-A)HMs with the increase of alkaline protease content, which inhibited the substrate from entering deeper catalytic sites. Therefore, the mass ratio of 1:4 for alkaline protease /P(H-A)HMs was chosen as the best enzymatic loading ratio.

The immobilization temperature had a critical influence on the immobilized enzyme. Too low temperature would affect the immobilization rate of the enzyme, and the high temperature could induce conformational the changes of enzymes. Based on this phenomenon we chose mild temperatures ranging from 20 to 50 ℃. As shown in Fig. S1b, the enzymatic loading of Alk-P(H-A)HMs increased with the increase of the temperature. When the temperature rose to 40 ℃, the enzymatic loading of Alk-P(H-A)HM1, Alk-P(H-A)HM2 and Alk-P(H-A)HM3 reached the maximum of 11.98mg g^{-1}, 20.75 mg g^{-1} and 13.39 mg g^{-1}, respectively. The temperature increased from 40 to 50 ℃, the enzymatic loading of Alk-P(H-A)HMs decreased, which might be because of the temperature sensitivity of alkaline protease. The hydrogen bonding between the carrier and alkaline proteases became unstable at high temperature, and the structure of alkaline protease would be destroyed and even became inactive. Therefore, 40 °C was the optimum immobilization temperature.

The incubation time of alkaline protease on the carrier plays a significant role in

improving the loading of alkaline protease. The immobilization times were separately controlled at 3, 6, 9, and 12 h to investigate the effect of immobilization time on loading of alkaline protease. As shown in Fig. S1c, the enzymatic loading of Alk-P(H-A)HM1, Alk-P(H-A)HM2 and Alk-P(H-A)HM3 displayed a tendency to grow from 5.61 mg g^{-1}, 15.30 mg g^{-1} and 7.41 mg g^{-1} to 10.52 mg g^{-1}, 20.47 mg g^{-1} and 13.49 mg g^{-1} over time (from 3 to 9 h), respectively, indicating more alkaline protease molecules were immobilized on P(H-A)HMs. Further prolonging the reaction time to 12 h did not cause a significant increase in the enzymatic loading. The long-time incubation could disrupt the enzymatic activity. Therefore, in thefollowing experiments, 9 h was selected as the optimum immobilization time for further studies.

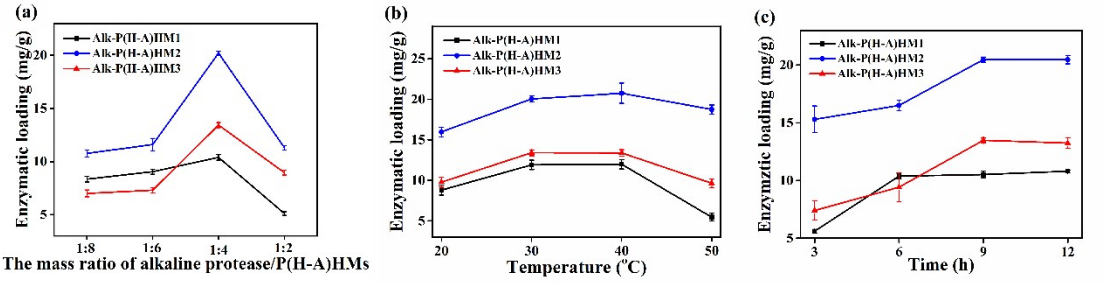

Fig. S1 The enzymatic loading of different mass ratio of alkaline protease/P(H-A)HMs (a), immobilization temperature (b) and immobilization time (c)

2 Standard curve

The standard curve of protein content was determined by Bradford protein assay. Fig. S2 showed that the linear equation of the protein standard curve was Y = 0.0087X + 0.0111, where R^2 = 0.99959, Y was the absorbance at 595nm, and X was the protein content (μg). The results indicated that the standard curve was well linear when the protein content ranged from 0 to 90 μg.

Each result was obtained by averaging three individual experiments.

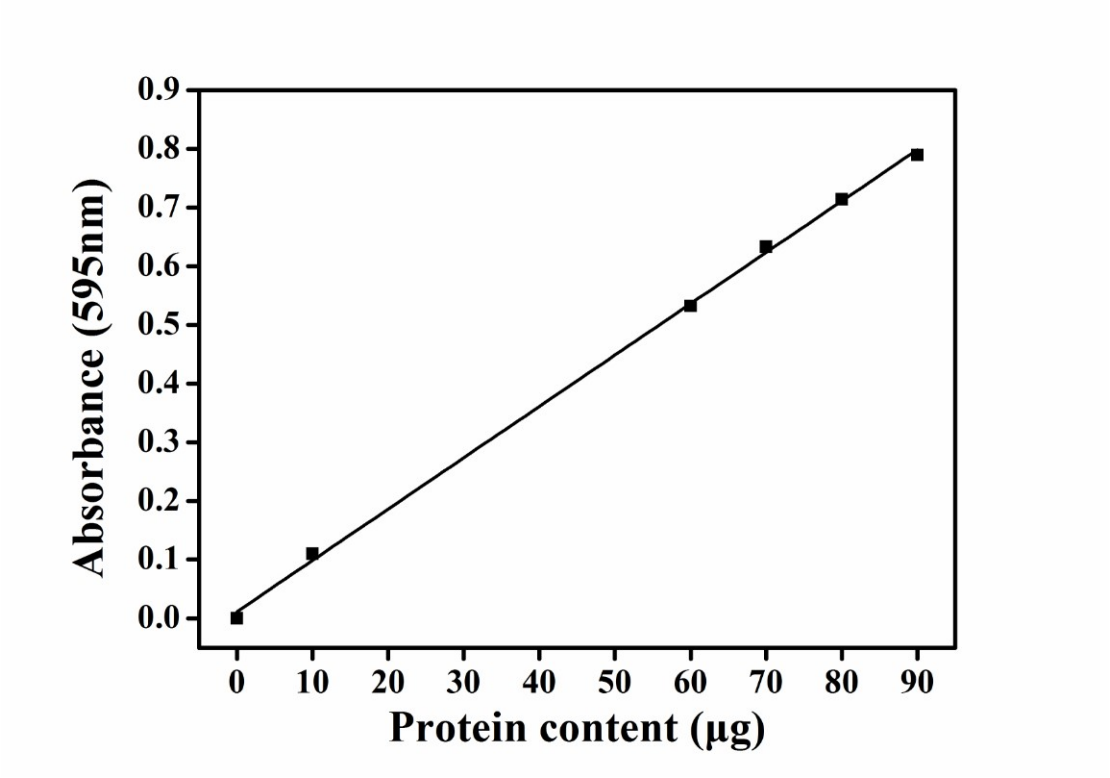

Fig. S2 The protein standard curve of BSA

3 The SEM and EDS of free alkaline protease

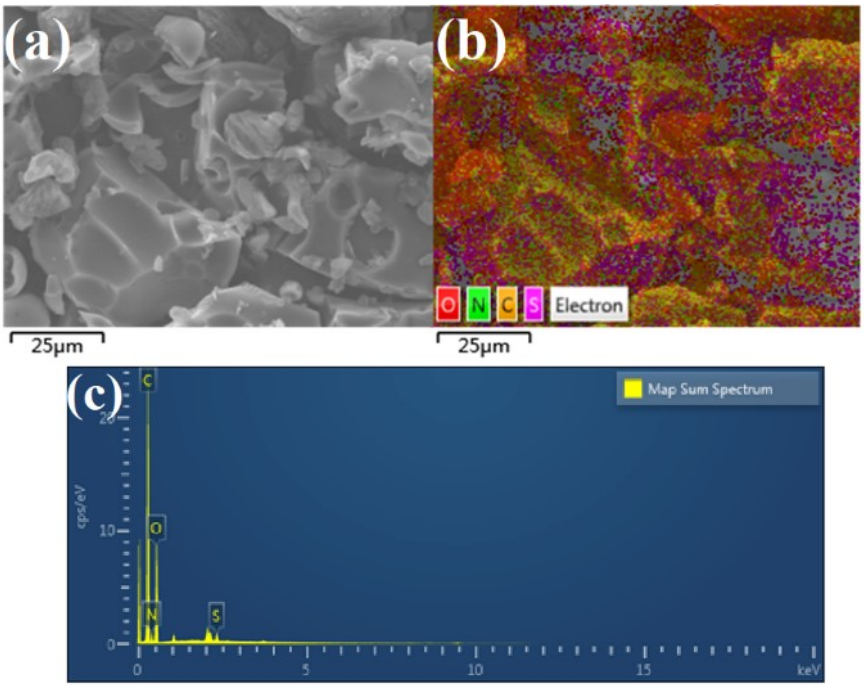

Fig. S3 The SEM image (a) and EDS map (b-c) of free alkaline protease

4 The BET analysis of P(H-A)HMs

Table S1 The specific surface area and pore size of the P(H-A)HMs

Samples	Surface area (m^2/g)	Average pore diameter (nm)
P(H-A)HM1	1.47	14.52
P(H-A)HM2	1.83	5.41
P(H-A)HM3	2.83	2.00

5 The particle size distribution of P(H-A)HMs before and after swelling

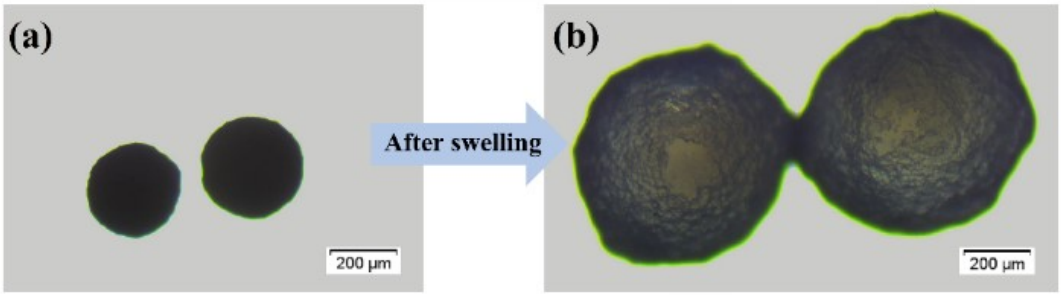

Fig. S4 The optical microscope images of P(H-A)HM2 before (a) and after (b)

6. Mechanical properties of the P(H-A)HMs

Because the particle size of the prepared microspheres was too small to intuitively investigate the strength of the microspheres using a compression instrument, in this paper, the polyacrylamide bulk adhesive was prepared with the same formula as the aqueous phase composition of poly (hydroxyethyl methacrylate acrylamide) microspheres. The mechanical strength of the bulk adhesive was investigated by compression apparatus at room temperature. The specific parameters of the sample block were 10 mm in diameter, 20 mm in thickness and 3 mm/min in compression speed.

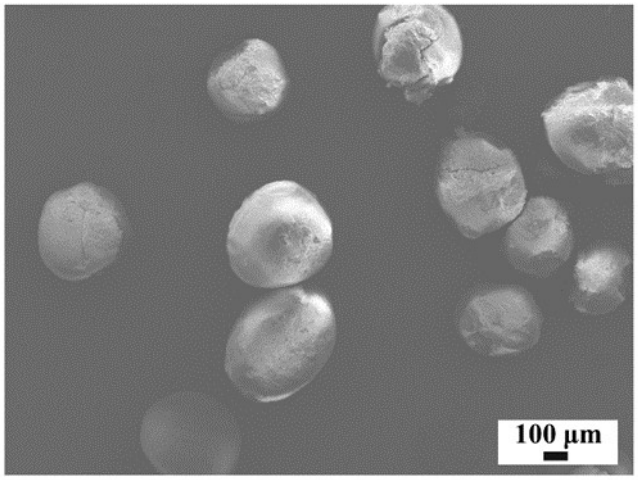

Fig. S5 The SEM images of Alk-P (H-A) HMs

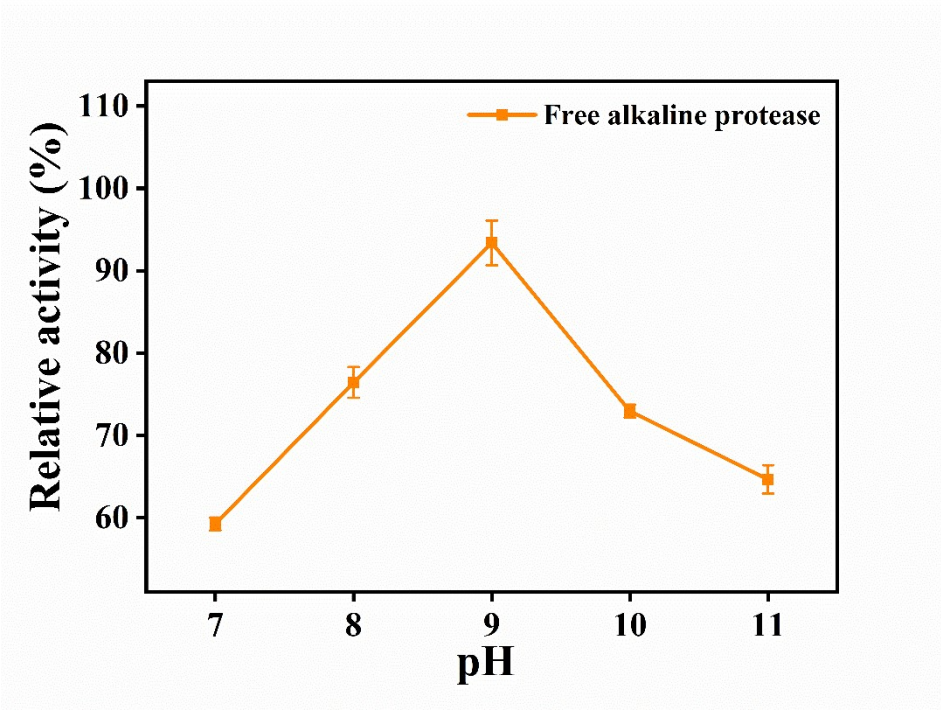

Fig. S6. The curve of free alkaline protease with pH change.

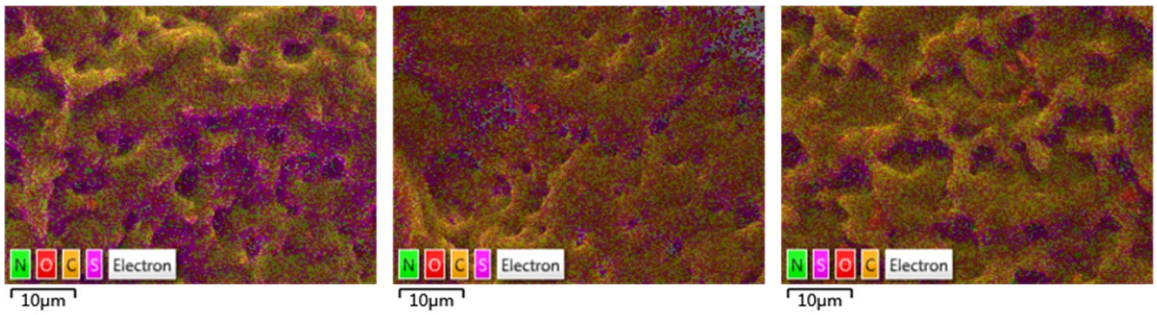

Fig. S7. The EDS results of Alk-P (H-A) HM1, Alk-P (H-A) HM2 and Alk-P (H-A) HM3.