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Supporting Information

Oral Biologics Carrier from Modified Halloysite Nanotubes

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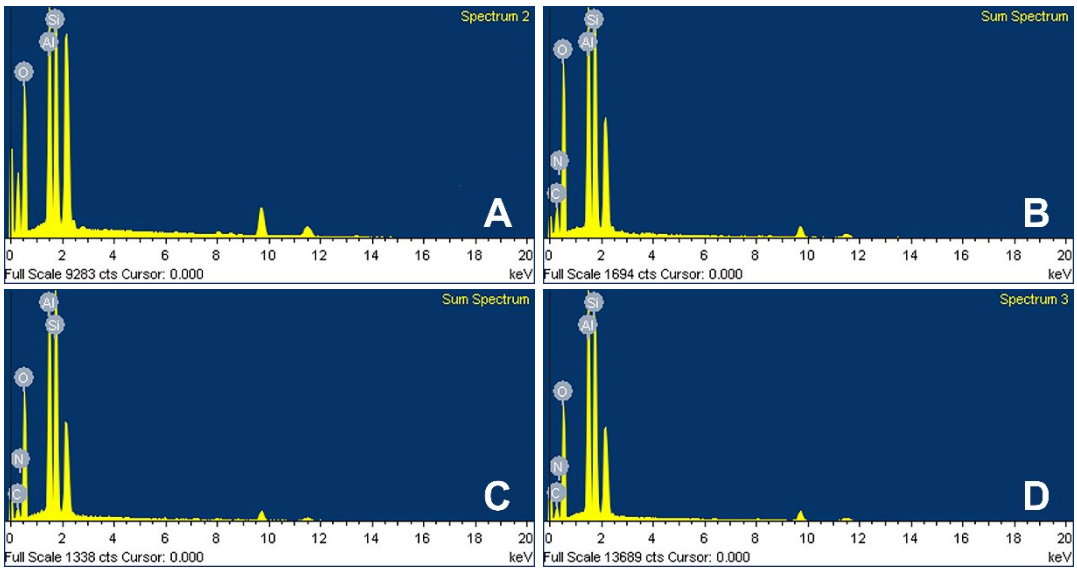
Supplementary Table 1. Percentage of elemental compositions in H, HA, HAC, and HC.

Samples	Weight (%)					Atomic (%)				
	O	Al	Si	C	N	O	Al	Si	C	N
H	54.74	14.37	30.89	-	-	67.70	10.54	21.76	-	-
HA	48.88	8.12	16.65	23.26	3.10	50.03	4.93	9.71	31.71	3.62
HAC	46.53	10.42	21.99	16.17	4.89	50.38	6.69	13.56	23.32	6.05
HC	45.59	11.19	21.40	18.58	3.24	49.09	7.15	13.13	26.64	3.99

H, halloysite nanotube, or HNT. HA: HNT-APTES. HAC, HNT-APTES-chitosan. HC, HNT-chitosan.



Supplementary Figure1. EDX spectra of H (a), HA (b), HAC (c), and HC (d). H, halloysite nanotube, or HNT. HA: HNT-APTES. HAC, HNT-APTES-chitosan. HC, HNT-chitosan.

Supplementary Table 2. TGA data of pure chemical and modified halloysites.

Sample name	$M_{L\ 150}$ (wt%)	$M_{R\ 800}$ (wt%)	$M_{D\ 800}$ (wt%)
APTES	88.71	5.08	6.21
Chitosan	11.75	2.09	86.16
H	1.77	84.57	13.66
HA	2.39	80.07	17.54
HA-Glutaraldehyde	1.40	79.56	19.04
HAC	2.18	77.59	20.23
HC	1.69	82.50	15.81

H, halloysite nanotube, or HNT. HA: HNT-APTES. HAC, HNT-APTES-chitosan. HC, HNT-chitosan.

The degraded masses of sample at 800°C ($M_{D\ 800}$) was calculated as

$$M_{D\ 800} = 100 - (M_{R\ 800} + M_{L\ 150})$$

where $M_{L\ 150}$ = mass losses at 30-150°C

$M_{R\ 800}$ = residue masses at 800°C

For example, chitosan loading on halloysite was determined based on the rule of mixture. The degraded masses at 800°C of HC ($M_{D\ 800\text{-}HC}$) was demonstrated as

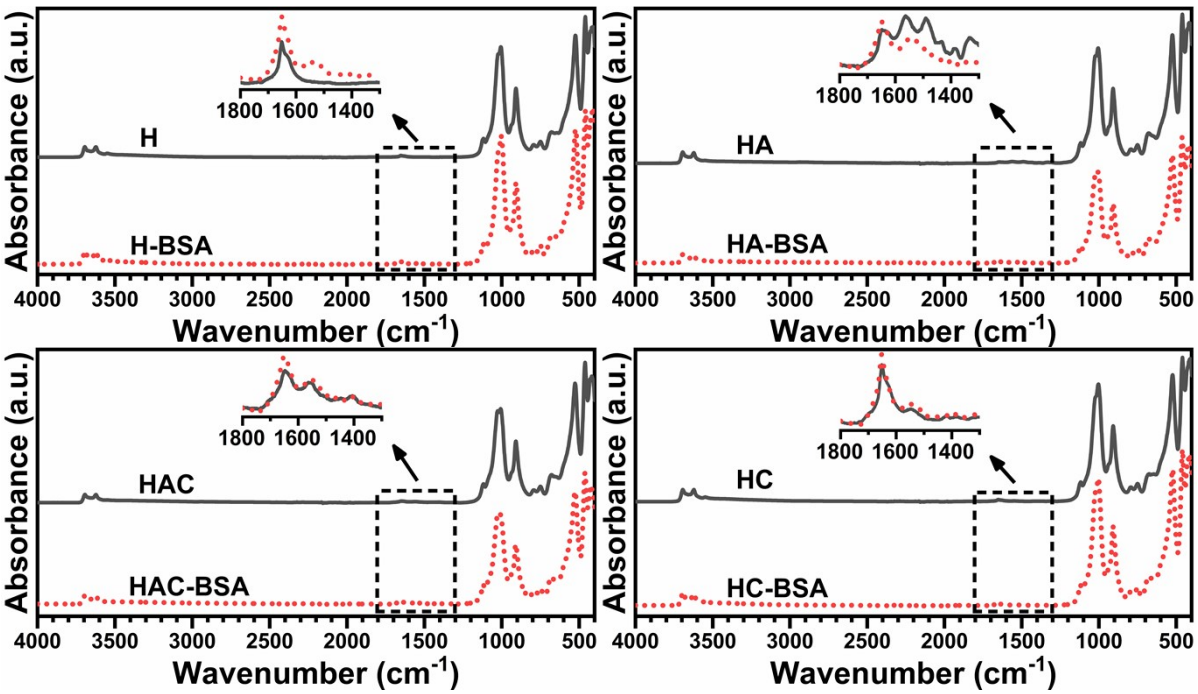
$$M_{D\ 800-HC} = (X_H \cdot M_{D\ 800-H} + X_{CS} \cdot M_{D800-CS})$$

where $M_{D\ 800\text{-}H}$ = degraded masses at 800°C of halloysite

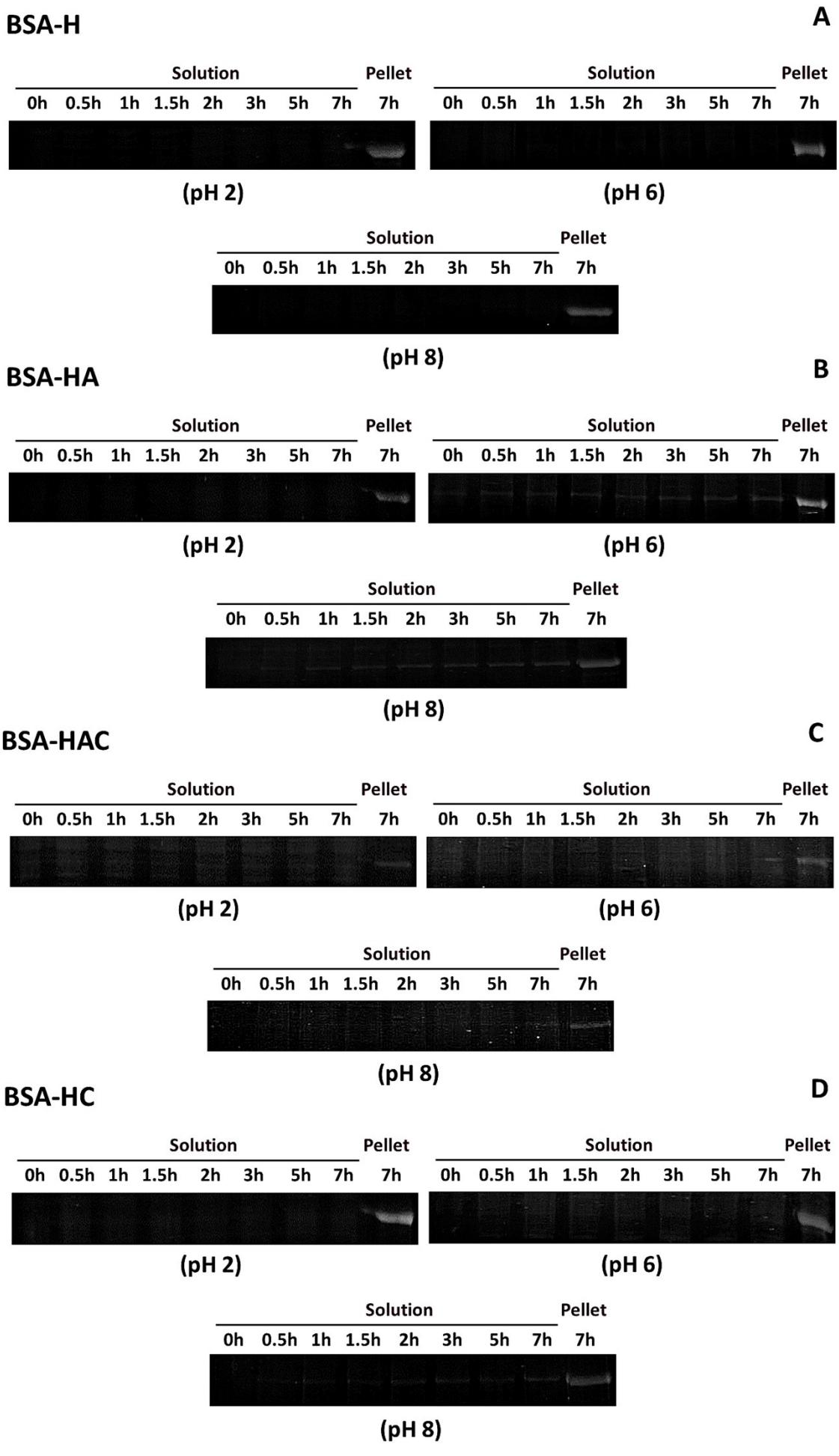
$M_{D\ 800\text{-}CS}$ = degraded masses at 800°C of chitosan

X_H = mass ratio of halloysite

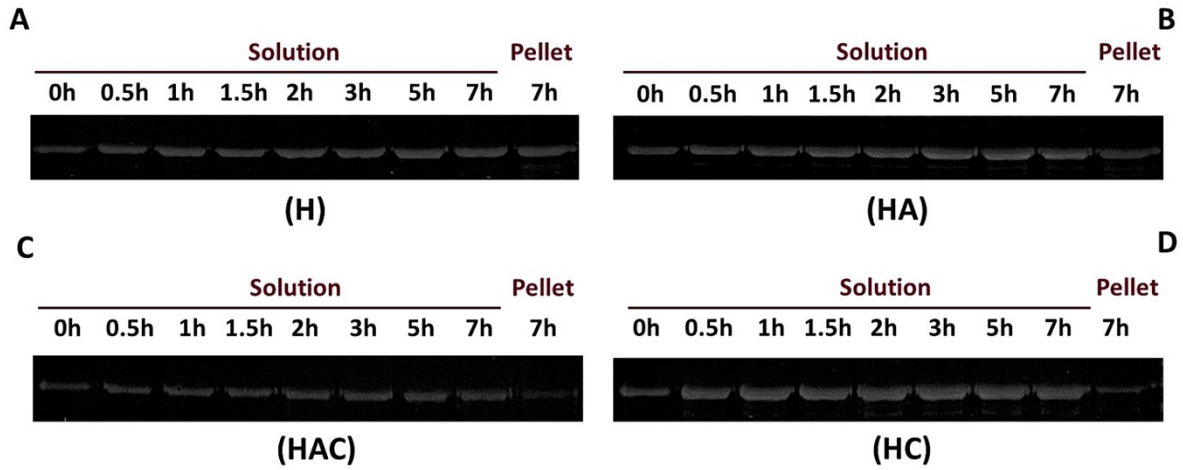
X_{CS} = mass ratio of chitosan



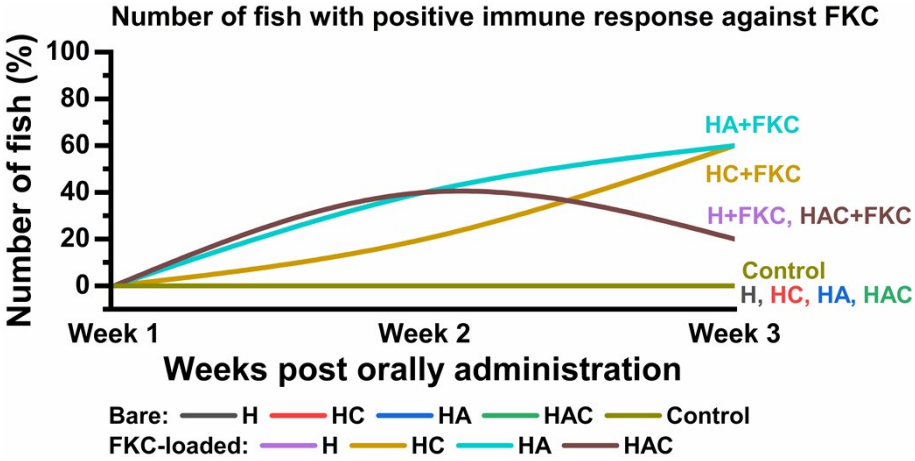
Supplementary Figure 2. FTIR spectra of the unmodified and modified halloysites. H, halloysite nanotube, or HNT. HA: HNT-APTES. HAC, HNT-APTES-chitosan. HC, HNT-chitosan.


BSA-H
A
Solution
Pellet
0h 0.5h 1h 1.5h 2h 3h 5h 7h 7h
(pH 2)
(pH 6)
(pH 8)
BSA-HA
B
BSA-HAC
C
BSA-HC
D

Supplementary Figure 3. Acid tolerance test using SDS-PAGE analysis to reveal BSA band profiles after exposure to 1× PBS solution at pH 2, 6, or 8 for 7 hours. The BSA observed in the solution is the released BSA, whereas the BSA detected in the pellet is the BSA remaining on the HNTs. H, HC, HA, and HAC denote the unmodified HNT, the HNT-chitosan, HNT-APTES, and HNT-APTES-chitosan, respectively.



Supplementary Figure 4. Bile salt tolerance test using SDS-PAGE analysis to reveal BSA bands profile after exposure to 0.3% bile salt for 7 h. The BSA detected in the solution is the released BSA, while the BSA detected in the pellet is the BSA remaining on HNT particles. Notably, samples of 0 h were collected immediately after mixing and resuspending the BSA-HNTs with bile salt solution. H, HC, HA, and HAC stand for the unmodified HNT, the HNT-chitosan, HNT-APTES, and HNT-APTES-chitosan, respectively.



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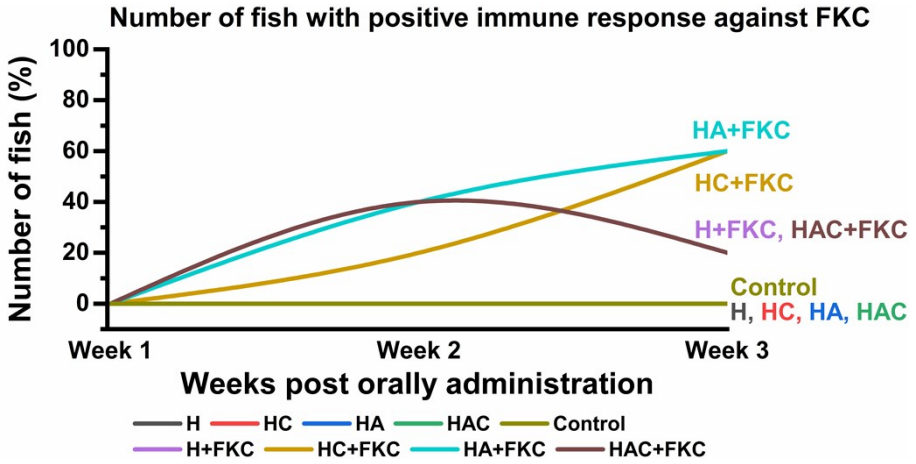

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63 **Supplementary Figure 5.** Antibody titer determination during three weeks HNTs and FKC-
64 loaded HNTs feeding. H, HC, HA, and HAC represent the fish fed with the bare H, HC, HA,
65 and HAC, respectively. Control is the fish fed with original commercial pellet, while
66 H+FKC, HC+FKC, HA+FKC, and HAC+FKC are the fish fed with the FKC-loaded H, HC,
67 HA, and HAC, respectively.

Supplementary Table 3. Number of fish (%) with positive immune response against FKC at week post orally vaccination

Name	Number of fish (%) at Week after post oral vaccination		
	Week 1	Week 2	Week 3
bare			
H	0	0	0
HC	0	0	0
HA	0	0	0
HAC	0	0	0
Mock	0	0	0
FKC-loaded			
H+FKC	0	40	20
HC+FKC	0	20	60
HA+FKC	0	40	60
HAC+FKC	0	40	20

FKC is Formalin killed whole cells vaccine

Mock is control group (no treat)