Supporting Information

Multi-Functionalized Micro Helical Capsule Robot with Superior Loading and Releasing Capabilities

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1. Supplementary Video Description

Video S1 Helical microfiber in alginate solution

Video S2 Helical microfiber in chitosan solution

Video S3 Suface coating of magnetic nanoparticles on helical microfiber

Video S4 Locomotion of helical magnetic microrobot in microcapillary at 7 Hz frequency

Video S5 Locomotion control of helical microrobot in chip

Video S6 The encapsulant release by helical microcapsule

Video S7 The encapsulant release by pure helical microfiber

2. Supplementary Figures Description



Characterization of helical alginate hydrogel microfiber

Figure S1 (a) Schematic illustration of egg-box structure unit of Ca-alginate hydrogel microfiber. The helical Ca-alginate microfibers generated via ion cross-linking reactions between alginate and Ca²⁺. (b) Optical image of one segment of helical Ca-alginate microfiber after dicing. (c) SEM image of the helical Ca-alginate microfiber after freeze-drying and (d) is enlarged SEM image in the red zone in (c).

Based on the liquid rope coiling effect, helical Ca-alginate microfiber with the desired pitch can be stably generated in the microcapillary device by adjusting the flow rate of the inner phase ranging from 0.15 to 0.35 mL/h while keeping the outer phase CaCl₂ at 15 mL/h. Therefore, the desired helical Ca-alginate hydrogel microfiber can be scalably generated and obtained by the ionic cross-linking gelation in the co-axial capillary

microfluidic system. Moreover, the formation of the egg-box structures is schematically displayed in Figure S1 (a). A segment helical microfiber is dicing into a short piece, as shown in Figure S1 (b). The average wire diameter, amplitude, and length of the segment of Ca-alginate microfiber are 69.4 μ m, 554.9 μ m, and 1948.2 μ m, respectively. Figure S1 (c-d) displays the SEM images of the helical Ca-alginate microfiber after dehydration. The surface of helical Ca-alginate microfiber was not smooth compared to the original hydrogel microfiber contains water as shown in Figure S1 (a). Moreover, the dimensions of the helical microfiber decrease by a third after dehydration.

Characterization of the magnetic functionalized helical microrobot

In order to observe electrostatic adsorption of magnetic nanoparticles on the surface of Ca-alginate microfiber, we used the same concentrations of Fe_3O_4 -NH₃⁺ solutions but with a reduced reaction time of 5 mins. Figure S2 (a) illustrates the mechanism of the surface coating process. The positively charged amino groups (NH₃⁺) of magnetic nanoparticles can electrostatically interact with the negatively charged carboxyl groups (COO⁻) of alginate and the Fe₃O₄-NH₃⁺ nanoparticles can absorb to the surface of microfiber and form a thin magnetic layer. As shown in Figure S3, the 5 mins coating resulted in the undesirable partial nonuniform coating on some parts of the helical microfiber. Once the helical microfiber segment was placed in the magnetic nanoparticle solution, the dispersed nanoparticles are absorbed on the surface of the helical Ca-alginate microfiber emphasize as the red circles in Figure S2 (b).



Figure S2 (a) Schematic illustration of magnetic nanoparticles functionalized helical microfiber by electrostatic interaction. (b) Bright filed images of the helical Ca-alginate microfiber during magnetic coating at different times. The red circles display that more and more magnetic nanoparticles are absorbed on the surface. Scale bars are $500 \mu m$.



Figure S3 SEM image (a-d) of a magnetic helical microfiber with different magnification times after freezedrying. The red rectangle zones represent the enlarged area. Inset (a) is the bright field image of the magnetic helical microfiber.

Furthermore, the magnetic coated helical microfiber was observed by SEM microscopy, as shown in Figure S3. The $Fe_3O_4-NH_3^+$ nanoparticles that are by electrostatic attraction are not completely uniformly coated on the surface of microfibers. The may cause by the non-uniform surface charge distribution of the Ca-alginate network. The number of COO- groups will not entirely the same in the whole microfiber, some regions may have higher charge density and the activity sites for the magnetic modification. The distribution of magnetic nanoparticles immobilized on the surface of Ca-alginate helical microfiber can be observed in Figure S3 (a-c) with different magnification times. Figure

S5 (d) clearly displays the magnetic nanoparticles with sphere shapes with the diameters around 300~400 nm.



Characterization of the magnetic helical microcapsule

Figure S4 SEM image (a-d) of ACA membrane coated magnetic helical microfiber with different magnification times after freeze-drying. The red zone rectangles represent the enlarged area. Inset (a) is the bright field image of ACA membrane coated.

ACA multilayered shell coated Ca-alginate helical microfiber by sequential adsorption of alginate and chitosan was also functionalized with magnetic nanoparticles. magnetic nanoparticles (Fe_3O_4 - NH_3^+) are surface-modified on the structure for magnetic-response ability. One segment of ACA membrane functionalized helical microfiber with surface-modified magnetic nanoparticles is shown inset Figure S4 (a). The surface morphology of the magnetic ACA helical microfiber was analyzed by SEM microscope, as shown in Figure S4, which revealed the multifunctional morphology of microstructure. As shown in Figure S4 (b), magnetic nanoparticles are immobilized on the ACA membrane coated microfiber. The surface property of multifunction structure exhibiting both the

surface folding and wrinkle compared with the smooth surface of Ca-alginate hydrogel microfiber and magnetic nanoparticle immobilized at the same time in Figure S4 (b-c) Figure S4 (d) clearly displays the magnetic nanoparticles with sphere shape with the diameter around 300~400 nm.



Figure S5 SEM image (a-d) of magnetic helical microcapsule with different magnification times after freezedrying. The red rectangle zones represent the enlarged area. Inset (a) is the bright field image of the magnetic helical microcapsule.

Another segment of ACA membrane functionalized helical microfiber with surfacemodified magnetic nanoparticles are immersed in chelating agent sodium citrate solution to liquefy the solid inner core to form a liquified helical microcapsule. The immobilization of the magnetic nanoparticles at the surface of the multilayered shell is evidenced in the bright field images of Figure S5 (a). The presence of nanoparticles at the surface of the ACA multilayered membrane of microcapsules indicates that they did not get a part, even after the liquefaction process. This behavior is due to the electrostatic attraction between the positively charged magnetic nanoparticles, and the negative charged Na-alginate solution in the multilayered ACA shell. The electrostatic attraction between carboxyl functional groups (COO⁻) on alginate chain and magnetic nanoparticles (Fe₃O₄-NH₃⁺). Although the helical microcapsule is swollen during liquefication and the structure undergoes expansion and flattens, the nanoparticles can be kept stably on the surface of the membrane. The surface morphology of the magnetic microcapsules was also analyzed by SEM microscope, as shown in Figure S5 (b-d), which revealed the surface structure and immobilization of magnetic nanoparticles. The morphology of the magnetic microcapsule exhibits hollow membrane properties compared with the solid ACA membrane coated magnetic helical microfiber due to the liquefied lumen. Figure S5 (d) demonstrates that magnetic nanoparticles are immobilized on the microcapsules.

The locomotion control of magnetic helical microrobot

The magnetic torques exerted on the microrobots are proportional to the magnetic field strength and act to bring their internal magnetization into alignment with the field. The magnetic forces exerted on the microrobot are proportional to the spatial gradient of the magnetic field, and act to move them to a local maximum. The magnetic torques and forces are determined using the general relations, integrating over the volume of the magnet c:

$${}^{\mathbf{L}}_{m} = \int_{V} \stackrel{\mathbf{L}}{M} \times \stackrel{\mathbf{L}}{B} (x, y, z) \mathrm{d}V$$
⁽¹⁾

$$F_{m} = \int_{V} \left(M \bullet \nabla \right) \bullet B(x, y, z) \mathrm{d}V$$
(2)

where \overline{T}_m is the torque the magnetic microrobot experiences, \overline{M} is the magnetization of the microrobot, assumed to be uninformed, and \overline{F}_m is the force the single magnetic microrobot experiences. Far from the field source, \overline{B} becomes relatively constant over the volume and these equations can be simplified to the single dipole approximation.



Figure S6 (a) The helical magnetic microrobot rotates following the direction change of magnetic induction line under the magnetic torque, which generates a translational propulsion force to make the microrobot move forward. (b) The principle of magnetic helical microfiber rotating under magnetic field. Microrobots with arbitrary posture in the (i) XYZ coordinate system without the magnetic field. The magnetization orientation M of microfiber is radial distribution and parallels to the applied magnetic field B on the YZ plane (ii). When the magnetic field B rotates and has a different angle with the magnetization orientation M on XZ plane (iii), the magnetic torque will generate and applied to helical microfiber until the magnetization orientation M is

consistent with the magnetic field B.(c)The magnetization direction of helical microrobot is along the radial direction and rotate around the central axial axis the rotating magnetic field.

When it comes to the helical structure at the microscale under the circumstance of the liquid environment, spiral propulsion force powered via magnetic torque is more efficient than gradient pulling force. Thus, the electromagnetic coils control system will be developed to provide external rotating magnetic fields to control this kind of functionalized helical microrobot motion. When this functionalized helical microrobot is controlled by external rotating magnetic fields, thus applying a magnetic torque, the robot is rotated about its helical axis as depicts in Figure S6. We define several parameters to model the robot motion, the velocity of forwarding and angular motion is defined as v and ω , the applied torque and applied force occur on the robot under the circumstance of without fluid are defined as τ and f so that the matrix of propulsion is calculated as:

$$\begin{bmatrix} f \\ \tau \end{bmatrix} = \begin{bmatrix} a & b \\ b & c \end{bmatrix} \begin{bmatrix} \upsilon \\ \omega \end{bmatrix}$$
(3)

where a, b and c can be represented as:

$$a = 2\pi n\sigma \left(\frac{\xi_{\rm P}\cos^2\theta + \xi_{\perp}\sin^2\theta}{\sin\theta}\right)$$
(4)

$$b = 2\pi n\sigma^2 \left(\xi_{\rm P} - \xi_{\perp}\right) \cos\theta \tag{5}$$

$$c = 2\pi n\sigma^{3} \left(\frac{\xi_{\perp} \cos^{2} \theta + \xi_{P} \sin^{2} \theta}{\sin \theta} \right)$$
(6)

in which n represents the helix number, $\xi \perp$ and $\xi \parallel$ are represented as the viscous drag coefficients along the helical robot's cylinder axis and along the length of the robot's axis, which can be represented as:

$$\xi_{\perp} = \frac{4\pi\eta}{\ln\left(\frac{0.36\pi\sigma}{r\sin\theta}\right) + 0.5} \tag{7}$$

$$\xi_{\rm P} = \frac{2\pi\eta}{\ln\left(\frac{0.36\pi\sigma}{r\sin\theta}\right)} \tag{8}$$

Benefiting from the corkscrew motion, the functionalized helical microrobot can be actuated forward under the circumstance of low Reynolds number so that we can control the robot motion speed via changing the switching frequency of magnetic field and electromagnetic coils' current. Meanwhile, the robot motion direction can be controlled by adjusting the six electromagnetic coils' current.



The encapsulant stability and control release of the helical microcapsule

Figure S7 Histogram of length, amplitude, and wire diameter variations of the helical microstructure during different steps.

During the liquefication process, the volume expansion and swelling of the helical microcapsule are shown in Figure S7. The dimensions variation of the helical microstructure during different steps were independently repeated at 5 individual samples. The statistical analysis data were measured based on the pixels in time-lapse images acquired from the LbL assembly experiment videos and curved as a mean \pm standard deviation. After different functionalized steps, the length, amplitude, and wire diameter of the ACA helix microcapsule increase from $52.77 \pm 3.57 \,\mu\text{m}$ to $95.05 \pm 7.54 \,\mu\text{m}$, $352.16 \pm 6.57 \,\mu\text{m}$ to $487.24 \pm 10.53 \,\mu\text{m}$, and $1710.21 \pm 12.73 \,\mu\text{m}$ to $2251.48 \pm 24.43 \,\mu\text{m}$, respectively. The above dimension change phenomenon during different steps can be attributed to the presence of the polyelectrolyte complexation of ACA membrane on the surface and ion exchange induced liquefaction process.



Figure S8 Release of encapsulants from helical microfiber through the chelating agent. Bright field of (a) resulted dissolve helical microfiber and (b) fluorescence microbeads release from the microfiber.



Figure S9 Stability of ACA helical capsule microrobot tests with FITC-BSA. (a-e) are histograms of the fluorescent spectrum intensity of collected buffers samples after 1 day, 2 day, 3 day, 4 day and 5 day in different buffers 1 ml MES (pH=4), TRIS (pH=7 and 9), and PBS (pH=1.2 and 7.4) overtimes, respectively. (f) is the histogram of the fluorescent spectrum intensity of collected samples at 37 °C after 1 h, 6 h, 12 h. Including the comparison of ACA helical microcapsule and pure Ca-alginate helical microfiber.

It is noted that the stability of helical capsule microrobot is highly influenced by different pH environments. These results indicated the ACA helical microcapsules are stable in acidic conditions while being sensitive to alkaline environments. Comparing intensity in different pH and temperature environments reveal that the stability of ACA helical microcapsules is relatively higher than that of pure Ca-alginate microfiber counterparts. The above results in Figure S9 could be explained by the following reasons: (a) all helical microstructures have relatively lower swelling degrees at acidic conditions; (b) the alkaline pH condition would cause a volume change of the polyelectrolyte ACA membrane-based structure capsule, which may result in possible rupturing of the structures and fast release of the encapsulants; (c) Ca-alginate hydrogel 3D matrix was not stable and would erode by chelating agents in the buffer solutions such as PBS buffers.