Electronic Supplementary Material (ESI) for Materials Horizons.

Supporting Information

Magnetically Driven Piezoelectric Soft Microswimmers for Neuron-like Cell Delivery and Neuranol Differentiation

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1. Experimental Section

Materials: P(VDF-TrFE) 70/30 mol% was purchased from Piezotech. PLLA was provided by Sulzer tech. Copper wires were purchased from Goodfellow. Other reagents are purchased from Sigma-Aldrich. All of the chemicals are used as received without further purification.

Fabrication of the microswimmers: 1.5 g P(VDF-TrFE) 70/30 mol% was dissolved in 8.5 g diethylketone with overnight magnetic stirring to form a clear solution. The magnetic stirring bar was then taken out, and 0.45 g of $CoFe_2O_4$ nanoparticles were added to the solution. The vial containing the mixture was put on a vortex mixer (VWR) for 10 mins and then sonicated with a tip-sonicator (Bandelin HD2070) for 10 mins. A cool water bath was used to keep the sample vial from overheating during sonication. This mixing process was repeated three times. The copper wire was washed in acetone, isopropanol, and a deionized water ultrasonic bath before dip coating. The dip coating of the copper wire was performed on a micromanipulator (Sutter MP-285) with a drawing speed of 4 mm/s. After being dried in air, the coated wire was put on a hotplate and annealed at 130 °C for half an hour. A similar process was adopted for dip-coating copper wires with PLLA-based composites. To prepare the PLLA solution, 1.5 g PLLA was dissolved in 17.25 g chloroform, and 0.45g of magnetic nanoparticles were mixed with the solution. The dip coating of the copper wires was performed at a drawing speed of 4 mm/s. The wire was mounted on a custom-made wire holder (Figure S1) and slightly stretched between two concentric chucks to keep the wires straight. The wire holder was able to rotate the wire around its long axis, which is a key feature for creating a helical pattern. The pattern was made using a Lasermill (New Wave Research). The laser intensity was set to penetrate and ablate the polymer. The laser was moved with a constant speed along the rotating wire while the wire was rotating, resulting in a helical pattern. The laser ablation process for an individual helical swimmer usually requires a few minutes. The working space of our custom-made wire holder is around 2 cm. Therefore, 5 to 20 microhelices can be manufactured in series (depending on the length of each microdevice) in approximately 30

min. Finally, the copper wires were etched by acidic ferric nitrate solution to obtain several microrobotic structures. The fabrication process is reproducible with a yield of approximately 90%.

Characterization of Microswimmers: SEM images were taken with an FEI Nova NanoSEM 450 scanning electron microscope. XRD patterns were acquired on a Bruker D8 Advance X-ray diffractometer equipped with a Cu target with a wavelength of 1.542Å. Magnetic measurements were obtained using a vibrating sample magnetometer (VSM EZ9, Microsense).

Manipulation of the microswimmer: The microswimmers were manipulated using an eight-coil Magnetic Field Generator (Octomag) in both the DI water and the silicon oil (AK 350). An optical microscope was used to record the video. Microswimmers with PC12 cells swimming in the capillary tube (inner diameter 400 μm) were conducted using a magnetic control system, which included an eight-coil Magnetic Field Generator (MFG) commercialized by MagnetbotiX AG (www.magnebotix.com). An inverted fluorescent optical microscope (Olympus IX-81) equipped with a UV light source was used for video recording.

Cell Culture: The neuron-like PC12 cells were maintained in Dulbecco's minimum essential medium (DMEM) (Gibco, catalog no. 11965-092) supplemented with 10% fetal bovine serum (FBS; Gibco, catalog no. 10270-106), penicillin (100 U/ml), and streptomycin (100 U/ml; Invitrogen, catalog no. 15240-062) at 37°C in a humidified atmosphere of 5% CO_2. The PC12 cells were subsequently trypsinized and resuspended at a concentration of 2×10^5 cells/ml for cell seeding. The microswimmers were sterilized in 75% alcohol for 1h and then coated with poly-l-lysine (1 mg/ml; PLL) (Sigma Chemical Company, catalog no. P-7890). The PC12 cells were then seeded into one culture dish containing the microswimmers. The dish conatining the cells and the microswimmers was stored overnight in an incubator at 37°C

under a humidified atmosphere of 5% CO_2. After PC12 cells were adhered to the microrobots, PC12 cells were fixed with polyformaldehyde, and then dyed with Hoechst 33342 (Thermo Fisher, catalog no. 62249) for 30 min.

Microswimmers stimulate PC12 cells under ultrasound: The PC12 cells were trypsinized and resuspended at a concentration of 1×10^5 cells/ml for cell seeding. The 24-well cell culture plate containing the cells and the microswimmers was stored in an incubator overnight. After the cells adhered to the microswimmers, the culture plate was subjected to ultrasound stimulating for 0, 1, 3, 5 and 7 days. The cells were stimulated by ultrasound in an ultrasonic bath (VWR USC300DF) at 37 °C for 10 mins, three times a day. The media was replaced daily. For positive stimulation experiments, PC12 cells were cultured in a 24 well plate in differentiation media supplemented with 50 ng/mL neuronal growth factors (NGF). PC12 cells were observed at 0, 1, 3, 5 and 7 days and dyed with CFSE (Thermo Fisher, catalog no. C34554).

2. Control of parameters of the helical microswimmer:

The pitch angle α can be calculated as follows:

$$\alpha = \arctan(\upsilon_L/\omega r) = \arctan(180\upsilon_L/\omega_a \pi r) = \arctan(\upsilon_L/2\pi r f)$$

where
υ_L: laser motion speed, μm/s
ω: angular speed of the wire rotation, rad/s
ω_a: angular speed of the wire rotation, °/s
f: turn/s
r: the radius of the wire, μm

The total helical band length:

$$L = 2\pi r(1+(\tan\alpha)^2)^{1/2}t$$

where t represents the number of turns in the helix.

Etched area:

$$A \approx sL$$

where s represents the size of laser spot.

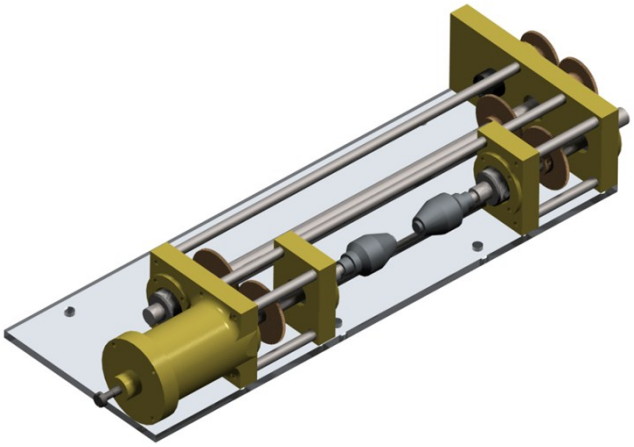

Figure S1. Custom-made wire holder.

3. Frequency-dependent swimming of the helical micro swimmer in DI water.

Table S1. Frequency-dependent swimming velocity and drift angle of an unrestricted helical microswimmer in DI water.

Frequency [Hz]	Drift angle [°]	Lateral velocity [mm/s]	Forward Velocity [mm/s]
4	59.79±6.72	0.5911±0.0869	0.3459±0.0863
7	46.29±1.29	0.6669±0.0232	0.6380±0.0388
11	29.65±0.51	0.3746±0.0613	0.6570±0.0939

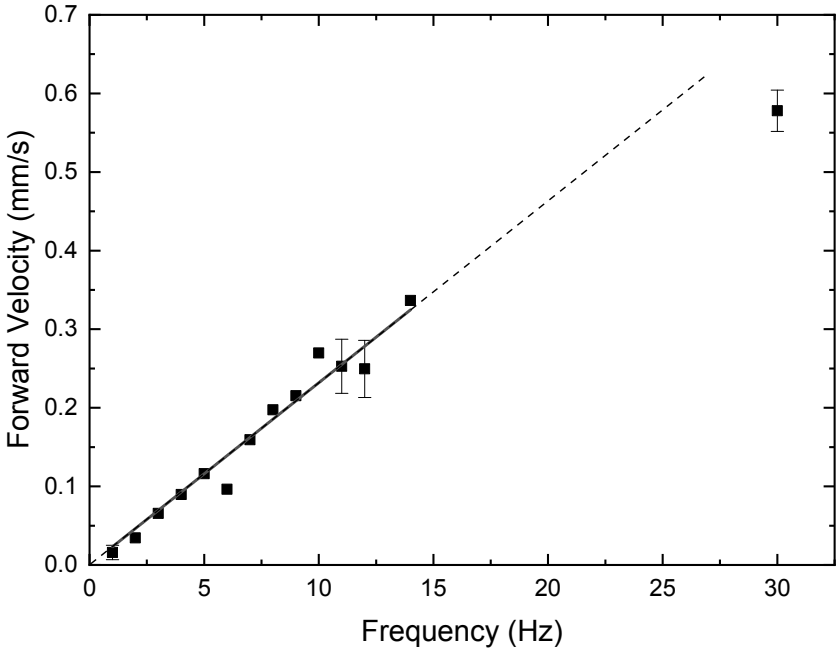

Figure S2. Frequency-dependent swimming velocity of a helical microswimmer in a capillary tube filled with DI water. The solid line was supplied with the data points below 15 Hz. The dashed line is extrapolated from the solid line.

4. Optimization of swimming properties by optimizing the parameter.

4.1 Nomenclature of microswimmers with different parameters.

The proposed micro-robots have been designed by determining their swimming velocity, movement stability, and mechanical strength. We encoded the structure of our micro-robots in the following order: pitch angle, ablated ratio, number of turns, inner diameter and head. The coding method is shown in Table S2. For example, for the micro-robot shown in Figure 1(d), has a pitch angle of 35°, which corresponds to code 2 according to Table S2, so the first digit of its code is 2. The ablated ratio is 60% (code 4), turn number is 4 (code 4), inner diameter is 250 μm (code 2) with no head. Therefore the code of this microswimmer is 2442. Similarly, the code of the micro swimmer in Figure 1(e) is 2442**. We listed these parameters and measured the values of several types of micro-robots in Table S3, in which pitch and head length are design parameters, and thread width and length are measured values.

Table S2. The encoding method of helical micro-robots

Code	Angle [°]	Ratio [%]	Turns	Diameter [μm]	Head
1	25	30	1	125	—
2	35	40	2	250	—
3	45	50	3	500	—
4	55	60	4	—	—
5	65	70	5	—	—
*	—	—	—	—	Cylinder
**	—	—	—	—	Carved cylinder

Table S3. Parameters of several types of micro-robots (all values are in μm)

Code	Pitch [μm]	Thread width [μm]	Head length [μm]	Length [μm]
3331	393	196	—	1200
3332	785	393	—	2400
3332*	785	393	400	2800
2442	550	220	—	2200
2442**	550	220	600	2800

4.2 Swimming characteristics of the helical microswimmers in viscous environment.

The shape and structure of the helix dramatically affects the speed of swimming. To explore which type of micro-robot swims faster, we compared their speeds using different pitch angles, ablated ratios and number of turns in the maximum response frequency of the external

magnetic field in silicone oil. The step-out frequency of most of the structures is lower than 3 Hz. The results are shown in Figure S3. We therefore conclude that type 3332 has the best swimming performance with a speed of ~190 μm/s at 2 Hz, and ~0.5 mm/s at 5 Hz. The step-out frequency for this structure is 5 Hz at 30 mT.

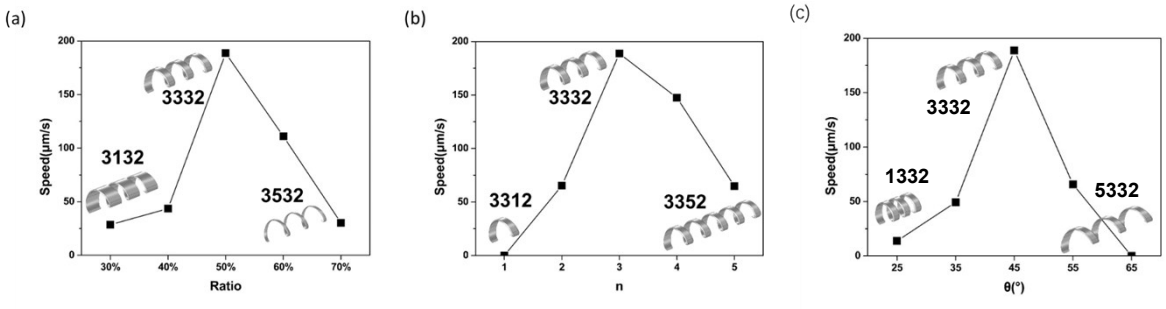

Figure S3. The effect of the ablated ratio (a), the number of turns (b), and the pitch angle (c) on the maximum swimming speed in silicon oil at 2 Hz. The type codes are marked on the diagrams (see section 3.1).

5. The differentiation state of the cells on day 3.

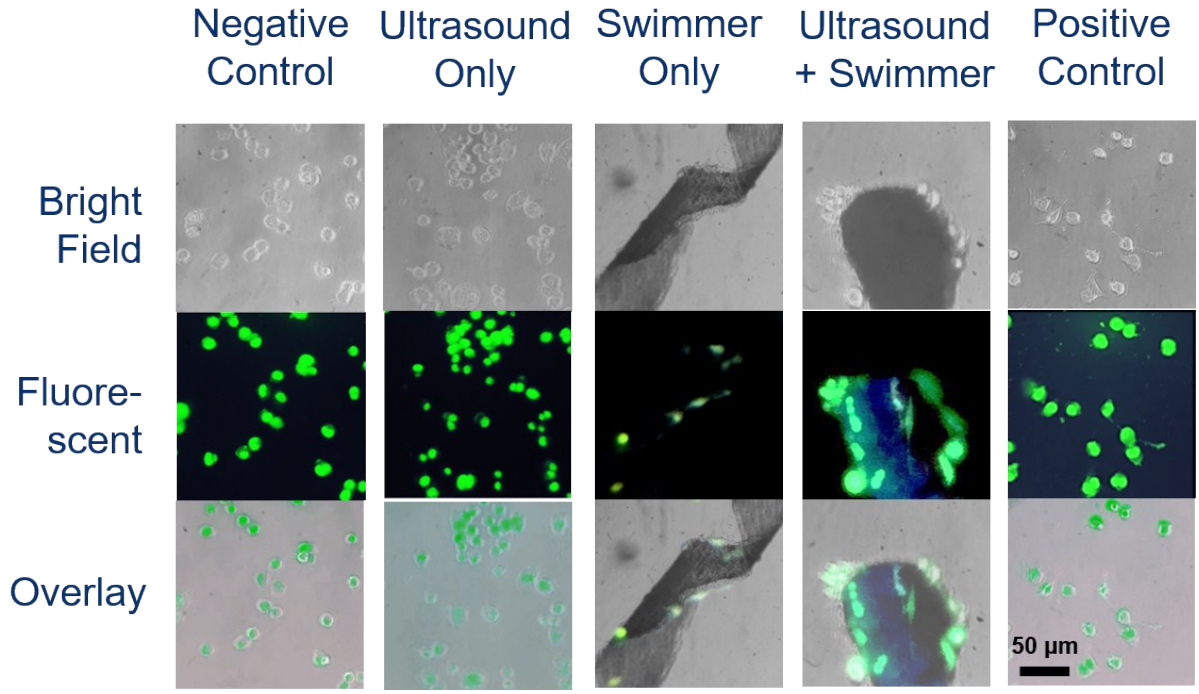

Figure S4. Bright field (BF) and fluorescent images of PC 12 cells cultured under different conditions. The images were taken on day 3. Only the cells on the microswimmer with ultrasonic stimulation show differentiation**.**