Wireless magnetic microrobots are envisioned to revolutionize minimally invasive medicine. While many promising medical magnetic microrobots are proposed, the ones using hard magnetic materials are not mostly biocompatible, and the ones using biocompatible soft magnetic nanoparticles are magnetically very weak and, therefore, difficult to actuate. Thus, biocompatible hard magnetic micro/nanomaterials are essential toward easy-to-actuate and clinically viable 3D medical microrobots. To fill such a crucial gap, this study proposed ferromagnetic and biocompatible iron platinum (FePt) nanoparticle-based 3D microprinting of microrobots using the two-photon polymerization technique. A modified one-pot synthesis method is presented for producing FePt nanoparticles in large volumes and 3D printing of helical microswimmers made from biocompatible trimethylolpropane ethoxylate triacrylate (PETA) polymer with embedded FePt nanoparticles. The 30 μm long helical magnetic microswimmers are able to swim at speeds of over five body lengths per second at 200 Hz, making them the fastest helical swimmer in the tens of micrometer length scale at the corresponding low-magnitude actuation fields of 5–10 mT. It is also experimentally in vitro verified that the synthesized FePt nanoparticles are biocompatible. Thus, such 3D-printed microrobots are biocompatible and easy to actuate toward creating clinically viable future medical microrobots.
the printed structure. After pyrolization, the organic material is burned off, and only a metallic structure remains, though while retaining the original shape, the structure shrinks by a factor of 5.\footnote{11}

For biocompatible magnetic microrobots toward medical use, fully metallic structures may not be needed if the net magnetization is sufficient for the desired function. Also, the polymer matrix can be used to incorporate therapeutic, medical imaging, and other functional compounds.\cite{12-15} Since the early 2000s, iron platinum (FePt) alloys have been of interest to researchers of magnetic storage media due to their high remanent magnetization and coercivity.\cite{16-18} These films have been deposited through sputter deposition, though the best results have been obtained through the annealing of self-assembled monolayers. To obtain these monolayers, highly controlled experimental conditions are required, and a small volume is produced. Thus, these methods have been inaccessible to researchers outside of the magnetic material synthesis field. Nanoparticles that have weaker magnetic properties have been used as magnetic resonance imaging (MRI) contrast materials and for potential biocompatible hyperthermia techniques.\cite{19,20}

Recently, Kadiri et al. have used physical deposition to deposit a 350 nm section of highly ordered FePt to enable the actuation of biocompatible helical microswimmers.\cite{21} While this form of FePt possesses greater intrinsic properties than the method presented here, such process cannot produce 3D-printable complex microrobots, which are essential for different specific applications. Therefore, we present the synthesis and characterization of FePt nanoparticles for two-photon lithography-based 3D microprinting of biocompatible and ferromagnetic microrobots.

We 3D print biocompatible helical microrobot structures made of trimethylolpropane ethoxylole triacrylate (PETA; 912 Da molecular weight) with embedded FePt nanoparticles. Such FePt/PETA helical microswimmers have swimming speeds 500% higher than equivalently produced microrobots with embedded SPIONs. We also show that the in vitro cytotoxicity of untreated FePt nanoparticles is negligible after 24 h, though cell division is inhibited at some level.

The FePt nanoparticle synthesis process is shown in Figure 1A. A single-round bottom flask is used with the mouth of the flask connected to the condenser column top to ensure Argon, heavier than air, can purge the system of residual atmosphere. A vacuum pump is used in aiding the purging, and a pressure sensor is used to monitor the system. Synthesis details are provided in the Experimental Section. Magnetic characterization is conducted by a vibrating sample magnetometer (VSM) (Microsense EZ7). The particles were weighed and affixed to a thin wax layer, which, in turn, is mounted to the magnetometer’s sample holder. The hysteresis loop on Figure 1B shows that at the maximum field of 1.8 T, an intrinsic magnetization maximum of 60.3 emu g$^{-1}$ is achieved. The remanent magnetization is approximately half the saturation value, 32 emu g$^{-1}$. The coercive field is slightly above 300 mT, placing the coercive field well above the actuating fields of electromagnetic coil systems, apart from MRI scanners, but an order of magnitude greater than strong elemental permanent magnetic materials, such as cobalt and nickel.

X-ray diffraction (XRD) is used to ensure that the synthesized FePt nanoparticles have the desired crystallography and can help

![Figure 1](https://www.advancedsciencenews.com/wiley-vch-gmbh/)

**Figure 1.** Synthesis and characterization of the iron platinum (FePt) nanoparticles for 3D-printable and biocompatible magnetic microrobots. A) The synthesis of FePt nanoparticles can be accomplished through a single-pot chemical reaction. The core components, illustrated here from left to right, are the argon source, vacuum pump, pressure sensor, round bottom flask, condenser column, and water bubbler. B) The hysteresis loop characterization of the synthesized FePt nanoparticles using VSM. The key magnetic properties are a 60 emu g$^{-1}$ saturation magnetization, a 32 emu g$^{-1}$ remanent magnetization, and a coercive field of 320 mT. C) XRD plot indicating the presence of crystalline FePt nanoparticles. D) TEM image of the FePt nanoparticles image, showing the presence of polypdispersity in FePt nanoparticles. E) Magnified image of TEM showing the presence of highly crystallized FePt nanoparticle. Scale bar is 2 nm. F) EELS image of FePt nanoparticles. G) EELS color-coded image of the particles with Fe in green and Pt in red. Scale bars are 5 nm.
give an approximation of the elemental composition of the particles. This is useful for engineering particles to have higher coercive fields, which occur when the particles are comprised of a ratio of 55:45 iron to platinum along with an annealing temperature of 600 °C. The peaks are fitted using a Bruker XRD software (PANalytical HighScore Rel.: 3.0), and the spectrum shows characteristic peaks at (001), (110), (111), (200), (210), (112), and (200). These compare with the characteristic peaks of the FePt nanoparticles demonstrated in the literature. In addition, the sharpness of the peaks increases with the particle size when the particle diameter is on the single-nanometer scale. A particle crystal structure is observed by transmission electron microscopy (TEM). Figure 1D–G shows the TEM images of the representative nanoparticles. Such images show the presence of nanoparticles in the range of 2–50 nm. The particles are polydispersed due to the nature of the colloidal synthesis process. Figure 1E shows the magnified image of a single nanoparticle, where the particle is highly crystalline in nature, and its crystal facets can be observed. The presence of crystalline structures increases the magnetic properties of the FePt nanoparticles and makes them more viable for microrobotic applications. Electron energy loss spectroscopy (EELS) provides elemental information and the distribution of Fe and Pt composition within a nanoparticle. Figure 1F,G shows the EELS images of FePt nanoparticles and their colored image, with Fe in green and Pt in red. From Figure 1G, it is shown that the Fe forms in the particle outer shell, and the Pt is mostly present inside the particles. The FePt alloy is rather homogeneous within the particle, whereas the Fe part is segregated toward the outer shell. This is possibly a function of the annealing process length, phase separation, and subsequent oxidation of Fe. The presence of Fe on the outer shell has the potential to make the particles more biocompatible. Further energy-dispersive X-ray spectroscopy (EDS) measurements inside TEM are obtained to validate the presence of only Fe and Pt within the nanoparticle and also their elemental composition. EDS images in Figure S1, Supporting Information, show that the elemental composition is 58% Fe and 42% Pt, indicating that the synthesized FePt nanoparticles are similar to those present in the literature. A dynamic light scattering (DLS) analysis of the nanoparticles is also shown in Figure S2, Supporting Information. They show the hydrodynamic radius of the nanoparticles being around 72 nm. The hydrodynamic radius is always larger than the actual radius of the particles, indicating that all the particles are smaller and there is no or negligible aggregation.

Pre-polymer solution is prepared for two-photon lithography by adding 500 mg Irgacure 369, to 15 mL of PETA and mixing well. Magnetic helices are fabricated by dispersing the particles in polymer solution to achieve the desired concentration. Approximately 1 mL of particle–polymer solution is generated using a 5 mg mL\(^{-1}\) density of FePt nanoparticles. The microswimmers were tested in a rotating magnetic field of 5 mT magnitude. A maximum speed increase from 10 to 50 μm s\(^{-1}\) for the FePt nanoparticle-embedded helical microswimmers is observed, which is faster by a factor of 5. A subsequent batch of microswimmers using 10 mg mL\(^{-1}\) density of FePt nanoparticles is tested, and the results are shown in Figure 3B. This sample is tested at 5 and 10 mT rotating field magnitudes. As SPIONs were not able to be loaded at 10 mg mL\(^{-1}\) due to 3D-printing limitations, another common magnetic material for microrobots, nickel, is chosen and sputter-coated onto microswimmers at 100 nm thickness, the maximum amount of nickel typically demonstrated in the literature. While nickel resists oxidation, its toxicity typically requires a barrier nanofilm layer, such as gold, to prevent undesired destruction of biological tissues.

The FePt swimmer is able to reach the speed of \(175 \mu m s^{-1}\), over five body lengths per second, at 200 Hz in a 10 mT rotating magnetic field. This is around 25% increase in speed and 35% increase in step-out frequency over the microswimmer with 100 nm thick sputtered nickel. Video S1 and S2, Supporting Information, provide examples of a helical microswimmer swimming out-of-plane, away from the surface, and in a square path in-plane; Figure 3D provides the snapshots of the square path-following video. The speed of the microswimmer in the linear regime, from 0 Hz to the frequency which yields the peak speed, is dependent on the spiral's geometry. The fact that the samples in Figure 3B and the samples in Figure 3C are collinear shows that the magnetic materials are not affecting the geometry of the spiral. Principally, if the particles were in large aggregates, then surface roughness could be altered and change the linear velocity–frequency response. This reaffirms the EDX observation.
in Figure 2. Table 1 shows the properties of the common magnetic materials as particles or films used in the fabrication of magnetic microrobots.

As these magnetic microrobots are envisioned to be utilized inside the human body\cite{1a,b,2} for potential medical applications, it is advantageous that the FePt microswimmers are biocompatible, though previous studies\cite{8b} have shown that SPIONs-embedded PETA structures are completely biocompatible. This is due to the embedding of the nanoparticles within the matrix of the PETA, thus effectively shielding them from any form of interactions with the cells. The PETA microswimmer with embedded FePt can be removed from the targeted region after they deliver their cargo. While previous studies have shown that some synthesized FePt nanoparticles are biocompatible depending on the synthesis conditions\cite{19,20b,21}, in vitro biocompatibility of the synthesized FePt nanoparticles with the given specific synthesis process details in this study is tested at various concentrations up to 500 μg mL\(^{-1}\) on Murine macrophage cells (J774A). The cytotoxicity of the particles is measured by incubating the particles along with the cells for two different time periods: 1 and 24 h. Live/dead staining of the cells is performed to measure their viability and their viability at various concentrations of FePt nanoparticles at two time points. The fluorescent images of the viable cells in green are shown in Figure 4B, and the percentage viability is shown in Figure 4A. Though the particles are viable, a reduction in the number of cells after 24 h is observed, indicating that the particles affect the cell growth. The particles can be made more viable using a biocompatible surface coating, which can also be functionalized, as a future work.

In this article, we have shown a 3D-microprinting process to manufacture biocompatible 3D magnetic microrobots using two-photon lithography and embedded strong ferromagnetic FePt nanoparticles. Helical microswimmers prototyped with this process have shown to be five times faster than those with SPIONs. Thus, this is a key milestone step toward real-word medical applications of biocompatible magnetic microrobots by producing faster and easier-to-actuate microrobots in the tens of micrometer length regime, which is important for ensuring control in biological environments\cite{3d}. In addition, both the structural and nanoparticle components of these 3D-printed microrobots are biocompatible. This is confirmed by incubating the particles with murine macrophage cells. As a future work, various 3D-printed biocompatible medical microrobots with various medical functions will be fabricated toward specific in vivo clinical applications.

J774A cells were allowed to reach 80% confluence, observed by optical microscopy. The cellular removal procedure is done by rinsing with dulbecco’s phosphate-buffered saline (DPBS) without Ca\(^+\) and Mg\(^+\) for 5 min. After aspiration of DPBS, a fresh addition of Dulbecco’s Essential Medium (DMEM) is added. The cells were then removed from the flask by cell scraper and counted by hemocytometer. A cell suspension of 5X10\(^3\) cells per mL is created. The samples were placed within 96-well cell
culture plates. After mixing, the cell suspension is added to the wells of the plate containing samples at 5K per well. After a 1 or 24 h exposure time, the plates were removed and observed by microscopy. A serial dilution of the FePt into concentrations up to 500 μg L⁻¹ is added by 100 μL/C₀ into the wells containing cells. A subsequent 24 h is allowed to pass, and the samples were treated with WST-8, and then, after 2 h, the measurement of WST-8 is performed by spectrophotometer at 450 nm. For the different concentrations, a live/dead analysis is performed. This is performed by the addition of live/dead cell imaging kit (Thermo Fisher). The cells are incubated for 30 min, and then observed by fluorescent microscopy (inverted microscope from Nikon Instruments Inc). The fluorescent images of live and dead are collected at the emission and excitation wavelengths of 498/515 and 570/602 nm.

Experimental Section

The synthesis procedure is derived from the literature and modified for a conventional fume hood setup.[16a,b,21] Figure 1A shows an illustration of the experimental setup, which is placed in a conventional fume hood. An argon source and vacuum pump are connected through valves to the

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**Table 1.** Properties of the common magnetic materials as particles or films used in the fabrication of magnetic microrobots. 1 emu is equivalent to 10⁻¹⁵ A m².

| Material                    | Saturation magnetization | Remanent magnetization | Coercive field [mT] |
|-----------------------------|--------------------------|------------------------|---------------------|
| FePt nanoparticles (this article) | 60 emu g⁻¹               | 32 emu g⁻¹             | 320                 |
| FePt nanoparticles (Kadiri et al.)[21] | 0.7 emu mm⁻³            | 0.65 emu mm⁻³          | 1500                |
| FePt nanoparticles (Shima et al.)[16c] | 1 emu mm⁻³              | 1 emu mm⁻³             | 3600                |
| NdFeB microparticles[35]    | 1.1 emu mm⁻³            | 0.73 emu mm⁻³          | 700                 |
| Cobalt film[37]             | 0.76 emu mm⁻³            | 0.2 emu mm⁻³           | 22                  |
| Nickel film                 | 55.1 emu g⁻¹ [28]        | 0.28–0.50 emu mm⁻³ [32][29] | 0.2–29[24,29]         |
| SPIONs[30]                  | 45 emu g⁻¹               | 0                      | 0                   |
input of a 100 mL round bottom flask. A pressure sensor (Panasonic DP-111A-E-P) is attached to monitor the absolute pressure of the system. The round bottom flask contained one side inlet and one top outlet. Before the flask is fixed with clamps and placed in a 100 mL round heating mantle (Carl Roth HCK6.1), a thermocouple is placed on the bottom of the mantle, and the flask held the thermocouple in place. Thus, the temperature between the mantle and the flask is used as a human in the loop feedback mechanism. The outlet of the flask is attached to a chilled water condenser, which initially has no water flowing through the condenser. The condenser outlet leads to a valve between the system and a pipette tip, which is inserted into a beaker of water to act as a water bubbler. An important note is that the actual length of tubing between the condenser and bubbler is approximately half a meter to prevent aspiration of water into the condenser column.

All chemicals were obtained from Sigma-Aldrich and used as delivered. The flask is cleaned, dried, and prepared with 20 mL of diocetyl ether, 388 mg. 1.5 mmol, of 1,2-hexadecanediol, 197 mg. 0.5 mmol, of platinum acetylacetonate, and a magnetic stir bar. The stirrer is kept at 800 revolution per minute (rpm) during the entire synthesis. Twice in the synthesis, an argon purge is performed. When this is done, the bubbler valve is closed, and the system is vacuumed to 5 l/min, and the solution is centrifuged for 5 min. To precipitate the particles, 20 mL of ethanol is added to each tube and vortexed for 5 min. The solution is centrifuged for 20 min, and the liquid component is removed. After cleaning, the particles were concentrated into 10 mL of hexane and placed in a ceramic boat and set aside to evaporate the hexane, ~30 min. The boat is then placed into an annealing furnace, which is evacuated and filled with forming gas, 5% H2 and 95% Ar. As previously mentioned, the annealing step changes the crystal structure from the FCC to FCT structure. While in the FCC phase, the material will remain magnetically soft, with small magnetic remanence and low coercivity, and in the ordered FCT phase, the particles exhibit a high magnetic anisotropy constant, leading to high coercive fields and remanent magnetization. The temperature is then increased by 1 °C min⁻¹ to 650 °C, and held at this temperature for 1 hr before being allowed to cool as rapidly as the furnace would allow before withdrawing the furnace and removing the material. The Curie temperature of annealed FePt nanoparticle systems is noted by Rong et al. to be around 600 °C for a 1:1 Fe:Pt ratio and an FCT structure, so the material is guaranteed to be demagnetized after annealing. The material is then physically gathered and stored under argon prior to use. Nickel-coated microswimmers were fabricated using IP-S photoresist (Nanoscribe GmbH). The 3D-printed microswimmers were sputtered with 100 nm Ni and 50 nm Au film using a benchtop sputter coating system (Leica EM ACE600, Leica Microsystems); 5 mg mL⁻¹ polyethylene glycol (PEG)-amine-coated 50 nm iron oxide nanoparticles were dispersed into PETA solution with 3% (w/v) Irgacure 369 (fluidMAG-PEG/Amine, Chemicell GmbH). The pre-polymer solution is sonicated for 2 hr to get complete dispersion.

Murine macrophage cells (J774A) were purchased from ATCC. The cells were characterized by surface markers (CD11b, CD80, CD206), morphological, and ability to phagocyte. The cell culture medium is prepared as DMEM (Gibco) supplemented with 10% heat-inactivated Fetal Bovine Serum (HI-FBS) (Gibco) with 1% Penicillin Streptomycin (Gibco). The cells were thawed from cryopreservation at passage 3. The cell culture is all performed in a biosafety cabinet. All cells were stored under cell culture conditions, 5% CO₂, 80% humidity, and 37 °C. The cellular passages for the experiments are between passage 5 up to passage 25.

**Supporting Information**

Supporting Information is available from the Wiley Online Library or from the author.
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Conflict of Interest

The authors declare no conflict of interest.

Keywords

3D microprinting, biocompatible FePt nanoparticles, magnetic microrobots, two-photon polymerization

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