Development of a Cell-Loading Microrobot with Simultaneously Improved Degradability and Mechanical Strength for Performing In Vivo Delivery Tasks

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Microrobots with simultaneously improved degradability and mechanical strength are highly demanded in performing in vivo delivery tasks in clinical applications. The properties of degradability and mechanical strength are contradictory for many materials used to make microrobots. This article proposes a new design that can result in 3D cell culture microrobots with improved degradability and mechanical strength from the following perspectives. First, the mechanical strength of a microrobot is improved using triangle patterns to replace hexagon pattern in the microrobot structure, which can provide more supporting grids to obtain increased mechanical strength. Second, the relationship between structural design and material composition in relation to the mechanical strength of microrobots is investigated. The study reveals that triangle-patterned microrobots have increased mechanical strength compared with hexagon-patterned microrobots, thereby allowing high composition of degradable material that leads to the fast degradation of the microrobot. It is also shown that the triangle-patterned microrobots can maintain the same structural integrity and cell capacity as hexagon-patterned microrobots. Finally, the demonstration shows that the triangle-patterned microrobot can be precisely navigated in microfluidic channels. This article successfully demonstrates that the degradability and mechanical strength can be improved simultaneously through the microrobot structural design.

1. Introduction

Microrobots have been increasingly used for the delivery of biological cells in precise cell therapy. A 3D cell-loading microrobot with advantages of degradability and mechanical strength is essential to many clinical applications.[1,2] Traditional magnetic microrobots are fabricated on the basis of the nondegradable substrate and magnetic materials, such as photoresists, Au nanowire, silica, and nickel.[3–7] These foreign materials stay for a long time in the body and bring side effects of immune activation and thrombin formation to organisms.[8,9] Therefore, degradable materials should be used to make microrobots.[10,11] Given the soft nature of degradable materials, microrobots made using these materials generally have low mechanical strength and poor magnet-driven capability.[12,13] Thereby hindering the functionality and movability of microrobots for practical applications.[14,15] Many in vivo environments, such as defects in bone tissues, request microrobots to have good mechanical strength as cell carriers.[16–18] In addition, the ability of 3D cell culture is also important for microrobots loaded with cells, because it will be beneficial to cell adhesion and proliferation, promote local tissue repair, and generate appropriate nutrient supply.[3,4] Therefore, pursuing a 3D cell culture microrobot with good degradability and mechanical strength is highly demanded.[19,20]

Considerable literature has reported hydrogel-based degradable microrobots.[21,22] For example, helical microswimmers are fabricated using methacryloyl hydrogel and are enzymatically degraded, allowing accurate navigation and precise delivery in vivo.[23] In this article, a microrobot with improved degradability and mechanical strength is designed and fabricated using a new material system. The mechanical strength is enhanced using triangle patterns to replace hexagon patterns in the microrobot structure, which can provide more supporting grids to obtain increased mechanical strength. The relationship between structural design and material composition in relation to the mechanical strength of microrobots is investigated. The study reveals that triangle-patterned microrobots have increased mechanical strength compared with hexagon-patterned microrobots, thereby allowing high composition of degradable material that leads to the fast degradation of the microrobot. It is also shown that the triangle-patterned microrobots can maintain the same structural integrity and cell capacity as hexagon-patterned microrobots. Finally, the demonstration shows that the triangle-patterned microrobot can be precisely navigated in microfluidic channels. This article successfully demonstrates that the degradability and mechanical strength can be improved simultaneously through the microrobot structural design.

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degradable.[23,24] Naturally extracted microalgae are used as template for degradable microrobots in a physiological environment.[25,26] Other microrobots fabricated using silk fibroin, chitosan, and proteins are also reported to be degradable.[27–29] Most of these hydrogel microrobots show good degradability with a degradation time ranging from days to weeks in vitro but suffer from low mechanical strength.[30,31] Degradability and mechanical strength are contradictory properties for materials used to fabricate microrobots, and microrobots with strong mechanical strength usually have weak degradability.[32–34] Some efforts to solve this problem have been reported in the literature, such as magnetically actuable microriggers, which rely on degradable polymers with good mechanical strength to capture single cells or clumps for biopsy.[35–37] However, the cells loaded on the above-mentioned microdevices are based on 2D cell culture, which may cause loss of function and morphology of the cells.[38] Simultaneously improving the degradability and mechanical strength of microrobots while maintaining 3D cell culture ability is challenging.

This article proposes a new method to develop a magnet-driven 3D cell culture microrobot with improved degradability and mechanical strength. First, the mechanical strength of a microrobot is improved from a structural design perspective. In our previous research,[3,39] the hexagon-patterned structure is used to design the microrobot. In the current study, the hexagon-patterned structure is replaced with a quadrilateral- or triangle-patterned structure, which can provide more supporting grids to enhance structural integrity. Second, the relationship between a pattern-based structural design and material composition is investigated. The study reveals that the use of triangle-patterned structure can provide the microrobot stronger mechanical strength than that of other patterns, such as hexagon-based patterns. The triangle-patterned structure subsequently allows for the use of a higher composition of degradable material to make the microrobots, resulting in fast degradation. Experimental results show that compared with hexagon-patterned microrobots that use a low composition of degradable material to make the microrobots, triangle-patterned microrobots that use a high composition of degradable material can maintain the same structural integrity and capacity for loading cells while having much improved degradability. This research reveals, for the first time, that the degradability and mechanical strength of a magnet-driven 3D cell culture microrobot can be simultaneously improved through an optimized design of patterned structure.

2. Results

The synthesis scheme and morphology control of the proposed 3D cell culture microrobot have been introduced in our previous work.[3,39] According to synthesis scheme,[19] an optimized composition of materials containing 74 vol% poly(ethylene glycol) diacylate (PEGDA), 24 vol% pentaerythritol triacylate (PETA), and 2 vol% magnetic Fe₃O₄ nanoparticles can balance the microrobot degradability, mechanical strength, and magnetic actuation capability. According to morphology control,[19] the microrobot uses a 3D porous spherical structure, where each pentagon is surrounded by hexagons in its body, and extruded burrs are used to load cells (Figure 1a). With this microrobot structure, each microrobot burr is supported by three microrobot grids.

The mechanical strength of microrobots is significantly affected by two factors. The first one is composite materials used to fabricate microrobots, such as the material that contributes to the mechanical strength (e.g., PETA) and the degree of material polymerization. The other factor is the structural design of the microrobot. A high composition of mechanical material (e.g., PETA) increases the mechanical strength but decreases the degradability of the microrobot. By contrast, the high composition of degradable materials (e.g., PEGDA) increases degradability but decreases mechanical strength. Our strategy is to increase the composition of the degradable material (i.e., PEGDA) as high as possible to ensure degradation performance and compensate for the loss of mechanical strength caused by the reduced amount of mechanical strength material (i.e., PETA) through the optimization of the structural design of the microrobot. Different microrobot structures are investigated in this study to reveal the influence of the structural design of the microrobot on the mechanical strength. Given that hexagon, quadrilateral, and triangle are commonly stable patterns in natural structures,[40] these patterns are used as the base of the designed microrobot structures. Figure 1a shows the microrobot structure filled with hexagon patterns, and Figure 1b,c shows the microrobots with quadrilateral and triangle patterns, respectively. In Figure 1b, three supporting grids are added into each hexagon. Thus, each pentagon is surrounded by quadrilaterals. With this design, each burr is supported by four grids. In Figure 1c, six and five supporting grids are added to hexagon and pentagon patterns, respectively. Thus, each microrobot burr is supported by six grids. Figure 1 and Figure S1, Supporting Information, also illustrate the force analysis for each structural design, indicating that the additional supporting grids in quadrilateral and triangle structures can help share the force applied from the microrobot burr and reduce the force exerted on each grid of quadrilateral and triangle microrobots.

The mechanical strengths of three microrobot structures fabricated using PEGDA and PETA materials in different ratios of 75:25, 80:20, 85:15, and 90:10 are compared to reveal the relationship between structural design and materials in terms of mechanical strength. The laser exposures for fabricating all microrobots are the same to exclude the influence of the degree of material polymerization on the microrobot mechanical strength. A measurable force is loaded on the microrobot and unloaded in a test range of three micrometers for all tests. Figure 2a shows the mechanical tests of three types of microrobots, namely, hexagon, quadrilateral, and triangle microrobots fabricated using 75 vol% PEGDA:25 vol% PETA and 90 vol% PEGDA:10 vol% PETA. For the same microrobot structure, the mechanical strength decreases as the PEGDA composition in microrobot materials increases, and this finding is consistent with our previous report.[19] Under the same composition of PEGDA and PETA materials, triangle and quadrilateral microrobots show high mechanical strength. This finding indicates that quadrilateral and triangle designs with additional supporting grids can enhance the mechanical strength of the microrobot than the hexagon design. Notably, the hexagon microrobot in 90 vol% PEGDA:10 vol% PETA breaks when compressed to a deformation of 2.9 μm. The triangle microrobot fabricated using
90 vol% PEGDA:10 vol% PETA shows higher mechanical strength than the hexagon microrobot fabricated using 75 vol% PEGDA:25 vol% PETA. This finding implies that the triangle microrobot fabricated using 90 vol% PEGDA:10 vol% PETA can be used to replace the hexagon microrobot fabricated using 75 vol% PEGDA:25 vol% PETA.

The degradability properties of hexagon microrobots fabricated using 75 vol% PEGDA:25 vol% PETA and triangle microrobots fabricated using 90 vol% PEGDA:10 vol% PETA are tested. The microrobot is tested in alkaline environment and phosphate buffer saline (PBS) solution to demonstrate its degradation performance. Figure 3a shows that the microrobot rapidly degrades and disappears after 10 h in an alkaline environment. Figure 3b shows that the microrobot in the PBS environment degrades slowly, and only a few burrs disappear after 24 h, which is similar to our previous report. Since the degradation mechanism of microrobots in alkaline environment is similar to that of PBS, alkaline environments are usually used to shorten the degradation time of microrobots. Figure 3c,d shows that the fluorescence intensity of the triangle microrobot fabricated using 90 vol% PEGDA:10 vol% PETA decays faster than that of the hexagon microrobot fabricated using 75 vol% PEGDA:25 vol% PETA in these two environments. The in vivo demonstration of microrobot degradation is then performed in the subcutaneous tissue of mouse (Balb/c, 6 weeks). During the experiment, tissues are excised and sliced to examine the fluorescence intensity of implanted microrobots. Figure 3e,f shows that in 4 weeks, the fluorescence intensity of hexagon microrobots fabricated using 75 vol% PEGDA:25 vol% PETA decays by 62%, whereas that of triangle microrobots fabricated using 90 vol% PEGDA:10 vol% PETA decays by 80%. These results show that the use of triangle-patterned structure in microrobot with 90 vol% PEGDA:10 vol% PETA can significantly improve the microrobot degradability than the use of hexagon-patterned structure in microrobot with 75 vol% PEGDA:25 vol% PETA and maintain equivalent (or improved) microrobot mechanical strength.

The biocompatibility of the microrobot was verified by co-culturing three different cell types with the degradable materials of microrobot in our previous study. In this study, a proliferation test by co-culturing mesenchymal stem cells (MSCs) with microrobots was also conducted. Figure 3g shows that the cells in both experimental and control groups proliferate in the same growth trend, indicating that the microrobot will not affect the growth and proliferation of the cells.

The cell-loading capability is further tested on the two microrobot prototypes. One prototype is hexagon microrobot fabricated using 75 vol% PEGDA:25 vol% PETA and the other is triangle microrobot fabricated using 90 vol% PEGDA:10 vol% PETA. The results show that the cell-loading capacity of triangle microrobot is higher than that of hexagon microrobot.
using 75 vol% PEGDA:25 vol% PETA, and the other is triangle microrobot fabricated using 90 vol% PEGDA:10 vol% PETA. Figure 4a shows that after loading the cells, two microrobot prototypes are structurally integral, indicating that the newly designed triangle microrobot with 90 vol% PEGDA:10 vol% PETA maintains the necessary mechanical strength for loading.

**Figure 2.** Microrobot mechanical test. a) Mechanical tests of hexagon, quadrilateral, and triangle microrobots fabricated using 75 vol% PEGDA:25 vol% PETA and 90 vol% PEGDA:10 vol% PETA. b) Mechanical strength of three microrobot structures in relation to material compositions.
cells such as the hexagon microrobot in 75 vol% PEGDA:25 vol% PETA. Figure 4b shows that as the density of loaded cells increases, the number of cells loaded onto the microrobot increases. Under the same density of cells, the triangle microrobot with 90 vol% PEGDA:10 vol% PETA can load a comparable number of cells to the hexagon microrobot with 75 vol% PEGDA:25 vol% PETA. These results indicate that the triangle microrobot can maintain the same structural integrity and cell-loading capacity as the hexagon microrobot.

Finally, an automatic navigation experiment of the magnetic microrobot is performed in a Y-shaped microfluidic channel to confirm that the above-designed microrobot can be precisely controlled. Figure S2, Supporting Information, shows the laboratory-made magnetic system, which provides a gradient magnetic force to drive the microrobot. A proportional integral derivative (PID) controller is used to determine the current input to magnetic coils in the microrobot motion control. Figure 5a and Video, Supporting Information, show that the microrobot can move from the starting position a to the target position d along the planned paths of a and b, b and c, and c and d. The position error analysis in Figure 5b indicates that the magnetic microrobot can be precisely navigated to the targeted position.
3. Conclusion

This article reports a method of developing a magnet-driven 3D cell-cultured microrobot with improved degradability and mechanical strength. The hexagon-patterned microrobot filled with pentagons with surrounding hexagons is previously reported in our work.\(^{[39]}\) In this article, the hexagon pattern is replaced with quadrilateral or triangle pattern to provide supporting grids for improved mechanical strength from the structural perspective, which can subsequently reduce the mechanical composition of materials for improved degradability. Mechanical and degradation tests indicate that the triangle-patterned microrobot fabricated using 90 vol% PEGDA:10 vol% PETA exhibits equivalent mechanical strength but faster degradation than the hexagon-patterned microrobot fabricated using 75 vol% PEGDA:25 vol% PETA as reported in our previous work.\(^{[39]}\) After loading the cells, two microrobot prototypes maintain similar structural integrity and cell capacity.

For the advantages of insensitiveness to biological tissues and noncontact high-positioning capability, the magnetic microrobot shows potential to carry and deliver cells for cell-based therapy. For example, MSC-equipped microrobots were used to regenerate knee articular cartilage\(^{[16]}\); magnetic microrobots delivered human-induced pluripotent stem cells-driven MSCs to the liver tumor site to inhibit the tumor growth\(^{[39]}\) and soft sphere microrobots delivered MSCs to the bile duct through natural orifices to heal ulcers\(^{[42]}\). Magnetic microrobots have been extensively reported previously, but most microrobots suffer from the disadvantage of either low mechanical strength or poor degradability. In most of the literature works, the microrobot degradability is only determined by materials used to fabricate microrobots, and few reports have revealed how to improve microrobot degradability and maintain necessary mechanical strength based on these available materials.

This article addresses this important issue from the structural optimization perspective. The relationship between the pattern-based structural design and material composition in relation to microrobot mechanical strength is revealed. The original hexagon-patterned microrobot\(^{[39]}\) is structurally enhanced by providing increased supporting grids for strong quadrilateral or triangle patterns. The triangle-patterned microrobot exhibits higher mechanical strength than the hexagon-patterned microrobot to reduce the mechanical composition of materials and increase the degradable composition of materials in making

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**Figure 4.** Cell loading on microrobots. MSCs with different densities are loaded on two microrobot prototypes. a) SEM images of cells-cultured hexagon microrobot fabricated using 75 vol% PEGDA:25 vol% PETA and triangle microrobot fabricated using 90 vol% PEGDA:10 vol% PETA. b) Statistical results of loading cells on two microrobot prototypes (n = 3).
microrobots, thus benefitting microrobot degradability. The experimental study shows that the triangle microrobot can maintain similar structural integrity and cell-loading capacity while having higher degradability. This research reveals, for the first time, that degradability and mechanical strength can be improved simultaneously from a structural perspective.

4. Experimental Section

Microrobot Fabrication: PEGDA (Sigma, 437441), PETA (Sigma, 246794), magnetic nanoparticle solution (chemicell GmbH, 260 mg mL\(^{-1}\)), photosensitizer (Parbenate, Curease Chemical, 50 mg mL\(^{-1}\)), and photosensitizer (2-isopropyl-9H-thioxanthen-9-one, Curease Chemical, 10 mg mL\(^{-1}\)) were collected, evenly combined and shaken (MX-S, Dragon Lab). A drop of the mixed material was drop-casted on a clean substrate pretreated with 3-(trimethoxysilyl)propyl methacrylate (J&K Scientific, China) and loaded in the Nanoscribe (GmbH, Germany), which was a two-photon lithography system with a 63× oil immersion objective (numerical aperture = 1.4).

The substrate was developed in toluene (Sigma, 179965) and isopropanol alcohol (Sigma, 67-63-0) and dried in fume hood via airflow.

Microrobot Mechanical Test: The microrobot mechanical test was conducted inside the SEM chamber (field emission scanning electron microscopes (FE-SEM), FEI Nova 450) via an in situ micro/nanomechanical characterization platform with a 100 μm indenter (Hysitron PI85). A measurable force was loaded on microrobots until a 3 μm deformation was reached and unloaded to restore to its original position. For microrobot structures in each composition of PEGDA and PETA materials, the mechanical test was repeated thrice.

Microrobot Degradation Tests: The in vitro degradation tests of microrobots were performed in 1 M sodium hydroxide and PBS solution for the demonstration of microrobot degradation. Microrobot images were captured using a fluorescence microscope (Ts2R, Nikon) at specified degradation time points. The intensity of microrobot fluorescence was analyzed using the ImageJ software.

The in vivo degradation of microrobots was tested in the subcutaneous tissue of mouse (Balb/c, male, 6 weeks). The rhodamine B-poly(ethylene glycol)-thiol (RB-PEG-SH) fluorescence (molecular weight = 1000, Ponsure Biological, China) was added into the prepolymer solution to fabricate microrobots. Microrobots were subcutaneously implanted into the

![Figure 5](image-url). Automatic navigation of the magnetic microrobot in a Y-shaped microfluidic channel. a) Time-lapsed images of magnetic microrobot move from the starting position a to the target position d through positions b and c. b) Actual and planned paths of microrobot, and position error of microrobot during navigation.
left or right flank of mouse through an open incision (>7 mm long). Mice were kept in the animal research laboratory unit of the City University of Hong Kong in accordance with the rules of the animal house. In each week, the mouse was sacrificed to obtain the subcutaneous tissue that embedded microrobots. The excised tissue was fixed in 4% formaldehyde solution (Sigma, 47608) overnight. The tissue was embedded in a cryomatrix frozen medium and cut vertically to a thickness of 50 μm using a cryostat (CryoStar NX70, Thermo Scientific). An image of microrobots embedded into the tissue was captured, and the fluorescence intensity of the microrobot was analyzed using the ImageJ software. Sliced tissues were stained with hematoxylin-eosin staining kit (Abcam, ab245880) in accordance with the protocol of the manufacturer.

**Cell Cultivation and Loading on Microrobots:** The protocols of MSCs were separately maintained in the Dulbecco’s modified Eagle medium (Gibco, 11965-092) and supplemented with 10% fetal bovine serum (Gibco, 10270-106), penicillin (100 U mL⁻¹), and streptomycin (Invitrogen 15240-062, 100 U mL⁻¹). Maintained cells were trypsinized and sus-
pended at a density of 10⁵ cells per milliliter as high density. This tissue sus-
pension was diluted twice and four times as medium- and low-density cell sources, respectively. A drop of cell suspension was dropped onto microrobots and cultured in humidified incubator with 5% CO₂ for half an hour to preattach cells onto microrobots. The full culture medium was used to provide gradient magnetic force to actuate the microrobot, as shown in Figure S2, Supporting Information. Measured images were processed in several steps, including background subtraction, threshold segmentation, and positional correlation. Based on the position of the microrobot image, a PID controller was used to determine the current input to magnetic coils and drive the microrobot for the desired path.

**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.

**Data Availability Statement**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

**Keywords**

cell delivery, degradability, mechanical strength, microrobots

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