The hydrogel template method for fabrication of homogeneous nano/microparticles

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ARTICLE INFO

ABSTRACT

Nano/microparticles have been used widely in drug delivery applications. The majority of the particles are prepared by the conventional emulsion methods, which tend to result in particles with heterogeneous size distribution with sub-optimal drug loading and release properties. Recently, microfabrication methods have been used to make nano/microparticles with a monodisperse size distribution. The existing methods utilize solid templates for making particles, and the collection of individual particles after preparation has not been easy. The new hydrogel template approach was developed to make the particle preparation process simple and fast.

The hydrogel template approach is based on the unique properties of physical gels that can undergo sol–gel phase transition upon changes in environmental conditions. The phase reversible hydrogels, however, are in general mechanically too weak to be treated as a solid material. It was unexpectedly found that gelatin hydrogels could be made to possess various properties necessary for microfabrication of nano/microparticles in large quantities. The size of the particles can be adjusted from 200 nm to >50 μm, providing flexibility in controlling the size in drug delivery formulations. The simplicity in processing makes the hydrogel template method useful for scale-up manufacturing of particles. The drug loading capacity is 50% or higher, and yet the initial burst release is minimal. The hydrogel template approach presents a new strategy of preparing nano/microparticles of predefined size and shape with homogeneous size distribution for drug delivery applications.

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1. Introduction

Nano/microparticles play an important role in drug delivery. They have been usually prepared by well known emulsion methods [1,2]. Nano/microparticles prepared by the conventional emulsion methods have polydisperse size distributions. The shape of the particles is limited to sphere, and thus, it has been difficult to examine the effect of size and shape on biological responses [3]. These particles also possess sub-optimal drug loading and release properties. Their drug loading capacity is usually less than 10% with an average of about 5%, while the drug loading efficiency is less than 50% [4]. It is important to develop nano/microparticulate formulations with higher drug loading efficiency and capacity.

Microfabrication methods, because of their ability to control microarchitecture and feature size, have been used successfully to develop novel nano/microparticles for applications in drug delivery. Optically encoded microparticles have been prepared by in situ photopolymer-ization in microfluidic channels [5]. Mesoporous silicon particles have been used as multistage drug delivery systems [6]. Several soft lithography methods, such as microcontact hot printing [7], particle replication in nonwetting templates [8], and step and flash imprint lithography [9], have been developed using polymer templates to prepare homogeneous particles. These methods, despite their success in producing homogeneous microstructures, are not readily applicable for fabrication of particles designed for drug delivery or for scale-up. The presence of any impurities resulting from in situ polymerization steps will prevent clinical applications, and the difficulty in mass production resulting from multi-step particle recovery procedures could make commercial applications difficult. Successful translation of microfabrication technique to development of clinically useful drug delivery formulations requires development of easier and faster processing steps that do not affect the desirable drug loading and release properties.

2. The hydrogel template approach

Since the drug-containing nano/microparticles are prepared for ultimate use in clinical applications, it is necessary to produce particles
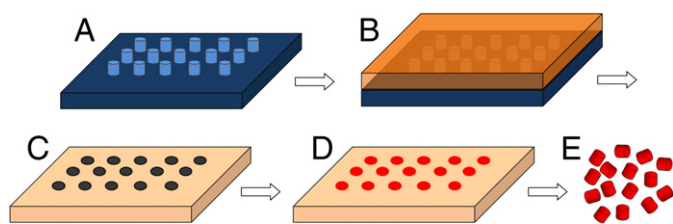
Fig. 1. Schematic description of the hydrogel template approach for fabrication of homogeneous microstructures. See text for descriptions of steps A–E.

in large quantities in a reproducible manner by simple microfabrication processing steps. Thus, it is desirable to develop a method that can collect formed particles by simply dissolving the templates in aqueous solutions. It would be even more desirable if the drug-containing particles can be delivered while they are still in the templates. For this reason, a hydrogel system that can be fully dissolved in aqueous solution was contemplated. It was not clear, however, whether this approach would be feasible, because it is well known that hydrogels, especially sol–gel phase reversible hydrogels, do not have a mechanical strength high enough to be used as templates, and the condition for sol–gel phase transition can be too harsh for loaded drugs. It was unexpectedly found, however, that gelatin could be manipulated to possess a mechanical strength sufficiently high enough for use as templates with a gel-to-sol transition temperature low enough not to adversely affect the loaded drugs.

Fig. 1 shows the main idea of the hydrogel template approach. The first step is to form a pattern of vertical posts on a silicon wafer master template (Fig. 1A). If an intermediary silicone rubber template is to be used, then the vertical posts on the master template will become vertical cavities. On top of the master template is poured a warm aqueous gelatin solution, and then the temperature is lowered to form a gelatin hydrogel imprint (Fig. 1B). Once the gelatin layer is solidified, the gelatin mold is peeled off and the gelatin mold is placed on the flat surface to expose the cavities (Fig. 1C). The cavities in the gelatin mold are filled with a solution or a paste of drug/polymer mixture (Fig. 1D). In this study, poly(lactic-co-glycolic acid) (PLGA) was used as a model biodegradable polymer, and Nile Red, a fluorescent probe, was used for easy visualization unless specified otherwise. The organic solvent present inside the cavities is removed by drying, and the formed particles are collected by simply dissolving the hydrogel mold, followed by centrifugation or filtration (Fig. 1E).

3. Materials and methods

3.1. Materials

Gelatin (from porcine skin, Type A, 300 bloom), 1,6-diphenyl-1,3,5-hexatriene (DPH), 6-propionyl-2-dimethylaminonaphthalene (Prodan), and Nile Red were purchased from Sigma (St. Louis, MO, USA), and poly(lactic-co-glycolic acid) (PLGA) of different molecular weights (MW 36,000, IV 0.7 dL/g; MW 65,000, IV 0.82 dL/g; MW 112,000, IV 1.3 dL/g) were purchased from Lactel (Pelham, AL).

3.2. Fabrication of a silicon wafer master template by photolithography

A silicon wafer was spin coated with SU8 2010 photoresist (Microchem, MA) at 3500 rpm for 30 s followed by baking at 95 °C for 3 min. The photoresist coated silicon wafer was exposed to UV radiation through a mask containing a 10 μm diameter circular pattern for 12 s. Please note that the diameter can be changed to any other value of interest, and any specific sizes used in this article are just for a demonstration purpose. After exposure, the silicon wafer was post baked at 95 °C for 3 min followed by development in SU-

8 developer for 2 min. The silicon wafer was rinsed with isopropanol and dried with nitrogen gas. The wafer thus fabricated contained 10 μm diameter wells.

3.3. Fabrication of silicon master templates by electron beam (e-beam) lithography

Making surface patterns of submicron sizes requires e-beam lithography. Circular patterns of 500 nm squares were designed using the Auto CAD 2007 program. A 3-in. diameter silicon wafer covered with 1 μm thick SiO_2 layer (University Wafer, South Boston, MA) was spin coated with poly(methyl methacrylate) (PMMA, Microchem, Newton, MA) photoresist of 300 nm thick layer using a spin coated at 3500 rpm for 30 s (SCS P6708 spin coating system, Indianapolis, IN). The coated PMMA photoresist layer was exposed to e-beam in a preprogrammed pattern using a Leica VB6 High Resolution Ultrawide field e-beam lithography Instrument (Bannockburn, IL) operating at 100 KV, transmission rate 25 MHz current 5 nA. After e-beam lithography, the silicon wafer was developed in 3:1 isopropanol:methyl isobutyl ketone solution to remove exposed regions of the photoresist. A 5 nm thick chromium layer and a 20 nm thick gold layer were successively deposited on to this pattern followed by liftoff of the residual PMMA film in refluxing acetone. The pattern was transferred to the underlying silicon oxide by deep reactive ion etching with SF_6/O_2 plasma. The generated silicon master template was used in the fabrication of hydrogel templates.

3.4. Fabrication of hydrogel templates

A clear gelatin solution (30% w/v in aqueous solution, 10 ml) at 50–55 °C was transferred with a pipette onto a silicon master template (3 in. diameter) containing circular pillars (e.g., of 10 μm diameter and 10 μm height). The gelatin solution was evenly spread to form a thin film completely covering the master template and cooled to 4 °C for 5 min by keeping it in a refrigerator. Cooling resulted in formation of a gelatin template which was subsequently peeled away from the master template. The obtained gelatin template was ~3 in. in diameter, and contained circular wells (e.g., of 10 μm diameter and 10 μm depth). The gelatin template was examined under a bright field reflectance microscope to determine its structural integrity. The preliminary experiment suggested that a solution of 30% gelatin resulted in templates which were elastic and mechanically strong enough for further processing.

3.5. Polymeric microstructures

200 μl of 40% PLGA solution (MW 112,000, IV 1.3 dL/g) w/v in CH_2Cl_2 doped with Nile Red was transferred with a pipette onto a gelatin template containing circular trenches of, e.g., 10 μm diameter and depth. The PLGA solution was evenly spread. The PLGA-filled gelatin template was left to dry at room temperature (~25 °C) for 5–10 min. The gelatin template filled with PLGA solution was characterized by bright field and fluorescence microscopy.

3.6. Collection of free microstructures

Gelatin templates filled with PLGA solution were left at room temperature for 10 min to remove most of CH_2Cl_2 solvent from the templates. For complete removal of the solvent, extended drying at elevated temperatures or freeze drying can be used. A batch of 10 gelatin templates were dissolved in a 100 ml beaker containing 50 ml of Nanopure water at 40 °C and gently shaken for 2 min to completely dissolve the templates. This step resulted in complete release of the free and isolated microstructures into the solution. The dispersion was transferred into conical tubes (15 ml) and centrifuged for 5 min (Eppendorf Centrifuge 5804, Rotor A-4-44, at 5000 rpm, 19.1 RCF,

(relative centrifugal force). The pellet obtained upon centrifugation was freeze dried and stored in a refrigerator. This pellet upon resuspension in 1 ml of Nanopure water formed free and isolated particle dispersion.

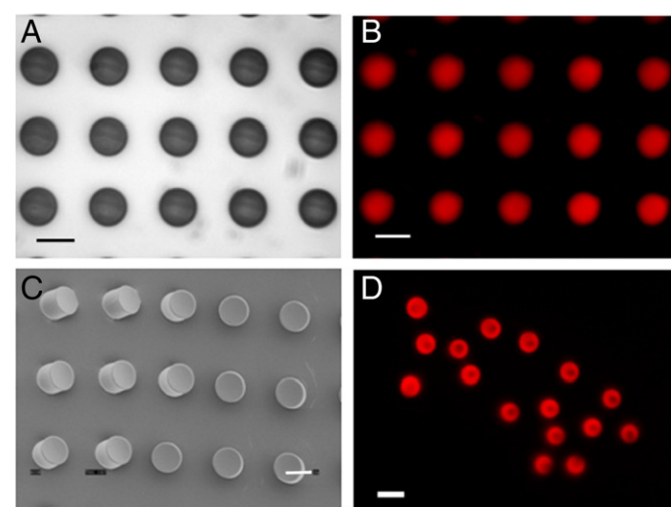### 3.7. Characterization of polymer microstructures

The polymer microstructures were visualized by bright field, confocal fluorescence imaging and scanning electron microscopy (SEM). Bright field and confocal fluorescence imaging was performed on an Olympus Spinning Disc Confocal Imaging Microscope BX61-DSU (Center Valley, PA) which is equipped with Intelligent Imaging Innovations Slide Book 4.0 software for automated Z-stack and 3-D image analysis. Scanning electron microscopy was performed on FEI NOVA nano SEM (Hillsboro, OR) and Hitachi 4800 SEM (Pleasanton, CA).

4. Results

In a typical experiment, a silicon wafer master template having vertical posts of 10 μm diameter and height was fabricated by photolithography. The prepared gelatin template contained the exact imprint of the features present on the silicon wafer (Fig. 2A). Hydrogel templates could also be prepared with other hydrogel-forming materials, such as alginate, agarose, and carrageenan. These materials form hydrogel templates under different conditions, and they also have different mechanical properties. As seen in Fig. 2B, cavities in the gelatin template were readily filled with a Nile Red-containing PLGA solution without forming a scum layer. The formed microstructures can be obtained by simply dissolving the gelatin template in aqueous solution. In the example in Fig. 2, however, the PLGA-filled gelatin template was pressed on a glass slide to transfer the PLGA microstructures on to it so that they could be imaged by SEM. The SEM image in Fig. 2C revealed that the microstructures were solid cylinders of 10 μm diameter and 10 μm in height, corresponding to the cavity dimension in the gelatin template. The tilted side views of the microparticles are clearly visible in Fig. 2C. Free and homogeneous polymer particles were recovered from gelatin templates by immersing them in warm water (40 °C) for 2 min. Warm water completely dissolved the gelatin template and released the free
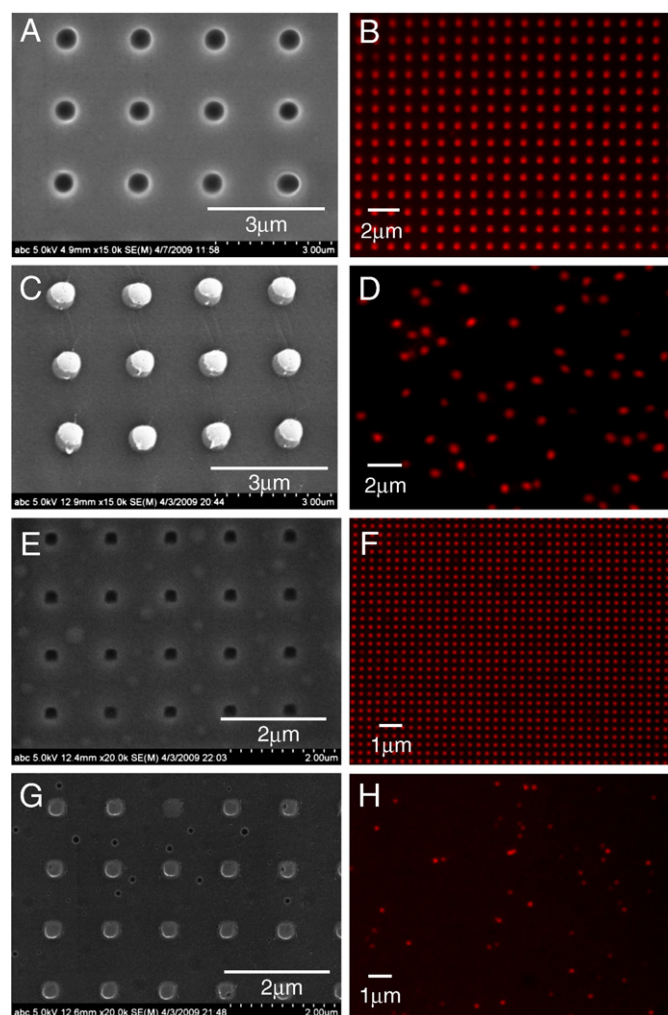
Fig. 3. Fabrication of submicron PLGA particles by the hydrogel template approach. PLGA nanoparticles with diameters of 500 nm (A–D) and 200 nm (E–H). SEM images of gelatin templates (A and E); fluorescence image of a gelatin template filled with PLGA-Nile Red solution (B and F); SEM images of free PLGA particles (C and G); and fluorescence images of free PLGA particles (D and H).
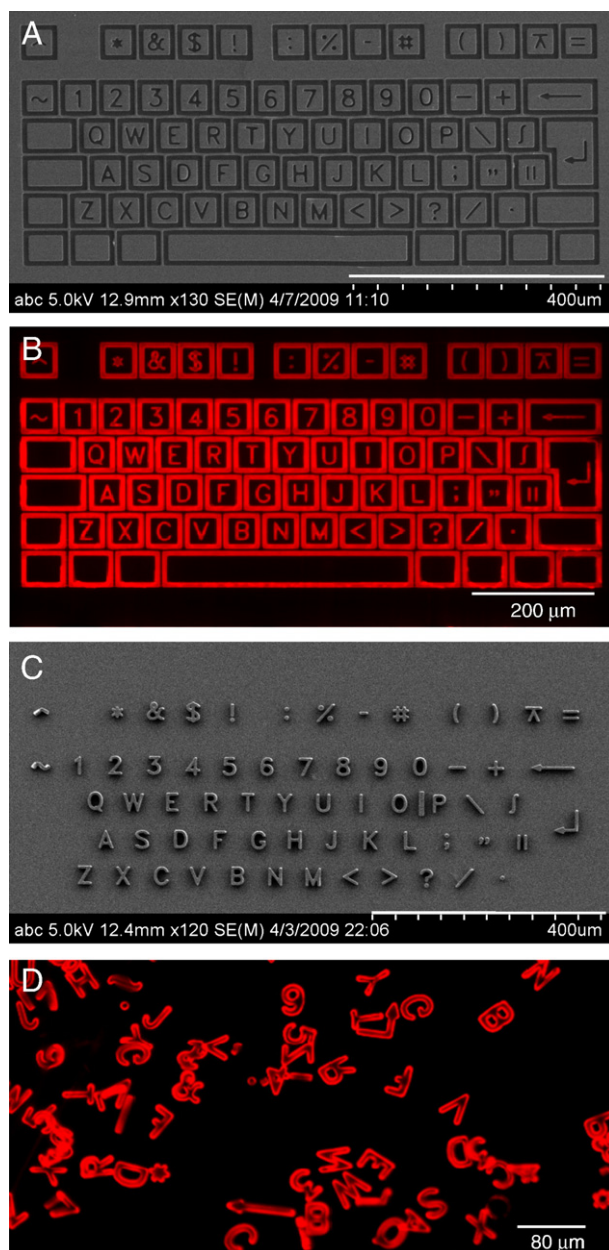
polymer microstructure into the aqueous solution (Fig. 2D). The solution containing PLGA particles was centrifuged and freeze dried to obtain free PLGA particles.

While the hydrogel template approach enabled facile fabrication of homogeneous microparticles, it was not apparent whether the same approach could be used to fabricate submicron size particles. The main question was whether the hydrogel template could hold the dimension of cavities at the submicron level, and whether the cavities could be filled with PLGA solution or paste. Fig. 3 shows that the same procedures used to make larger size particles (e.g., 10 μm particles in Fig. 2) could be easily applied to fabricate particles with diameter as small as 200 nm. This demonstrates that the hydrogel template approach can be applied for production of submicron structures of predefined dimensions.

As an example to demonstrate how easily this method works to make diverse structures and sizes, a gelatin template having cavities of a computer keyboard design was fabricated. A computer keyboard containing letters, numerals, and symbols presents rather complicated geometries. As shown in Fig. 4, gelatin templates can be used to imprint not only simple characters (such as I, O, C, etc,) but also complex geometries (such as &, $, #, π, etc.), thus demonstrating its precision imprinting capability. The SEM image of the gelatin

Fig. 2. Fabrication of homogeneous 10 μm PLGA microstructures using the hydrogel template method: (A) bright field image of a gelatin hydrogel template; (B) fluorescence image of a hydrogel template filled with PLGA solution containing Nile Red; (C) SEM image of free standing PLGA microstructures obtained by pressing the gelatin template on a glass slide; (D) fluorescence image of free PLGA microstructures obtained after dissolving gelatin templates. (Scale bars correspond to 10 μm).

Fig. 4. A gelatin template imprinted with a computer keyboard demonstrating the precision imprinting capability. (A) SEM image of a partially dried gelatin template with keyboard imprint as trenches; (B) fluorescence micrograph of a gelatin template filled with PLGA–Nile Red solution; (C) SEM image of a partially dried gelatin template with keyboard imprint as projections; (D) fluorescence micrograph of free letters obtained by dissolving the gelatin template in water.

template (Fig. 4A) clearly shows that the hydrogel template strategy possesses a high precision imprinting capability. The space between each key is clearly visible and even the smallest spaces inside the letters retained their integrity. The line thickness of each character is 5 μm. The filling of the keyboard template with PLGA-Nile Red solution was very clean and no formation of scum layer was observed (Fig. 4B). The gelatin template can also be used to form the letters of the key board in the projection mode (Fig. 4C). The high precision imprinting capability was maintained. Fig. 4D shows the letters obtained by dissolving the gelatin template in Fig. 4B in water. For applications in drug delivery, the shape of delivery systems may need to be more compact for increasing the reservoir space, and thus, particles of different shapes were prepared, such as circles, diamonds, triangles, squares, stars, donuts, packmans, and crosses (Fig. 5).

For particulate systems used in drug delivery, a high drug loading capacity and control of the drug release profile are important. As an example, PLGA particles containing felodipine were prepared using the hydrogel template method. The prepared PLGA–felodipine microparticles were examined for their drug loading efficiency and drug release kinetics *in vitro*. The analysis confirmed that the felodipine-loaded microparticles contained as high as 50% of the drug with an encapsulation efficiency of 80%. The remaining 20% of the drug was recovered and reused and thus, the actual loss is minimal. Despite such a high drug loading the initial burst release of felodipine was about 10% (Fig. 6). Other microparticles containing different drugs showed different release profiles, but all of them exhibited very low initial burst release profiles. More studies will be necessary to maximize the usefulness of this approach, but the initial drug loading study shows that the drug can be loaded at concentrations much higher than possible by conventional emulsion method.

5. Discussion

Phase reversible hydrogels have been used in tissue engineering, drug delivery, diagnostics, and as biosensors [10–13]. Hydrogels, to the best of our knowledge, have never been used as dissolvable template materials in the preparation of nano/microparticles. It was commonly thought that the hydrogel templates would be too weak for the intended applications. Indeed, most synthetic physical hydrogels are mechanically very weak, limiting their usefulness as templates for microfabrication. To demonstrate the proof of principle of the strategy, we examined various natural hydrogels, and selected gelatin as a model system for preparation of hydrogel templates because it was unexpectedly found to have many properties ideal for our applications. Gelatin undergoes a sol–gel phase transition at 40~45 °C. Because gelatin hydrogels turned out to be highly elastic and mechanically robust at the concentration used in our study, it can withstand the physical manipulation during the template preparation and filling of cavities with drug/polymer mixture. The gelatin template maintained a drug–polymer mixture inside the cavities and prevented diffusion of the drug or polymer into the template.

There are several advantages of the hydrogel template approach. It does not require *in situ* thermal or photopolymerization procedures that require purification of the formed nano/micro structures. Hydrogel templates can be readily prepared from a microfabricated silicon wafer master template, or from an intermediate silicone rubber template, by simply covering the master template followed by cooling. The hydrogel template strategy is broadly applicable, mild, and readily scalable for production of homogeneous nano/micro-structures in large quantities. It also offers precise control over structural geometries and lateral dimensions in the range of 200 nm to 50 μm and larger.

Currently available drug delivery systems prepared by emulsion methods have rather low drug loading capacity, usually much less than 10% of the total weight, and often show significant initial burst release of the drug, which can be a half or more of the total loaded drug [14]. Monodisperse particles prepared by microfluidic emulsi-fication also showed such high initial burst release [15]. The drug loading into nano/microparticles prepared by the hydrogel template method was much higher than that achieved by conventional methods, most likely due to the fact that the drug/polymer particles inside the cavities encounter almost no water until they are hardened by drying. In the emulsion methods, the drug/polymer particles are in constant contact with a large amount of water from the emulsion state, probably leading to substantial loss of the drug, as well as accumulation of the drug on the particle surface.

Fabrication of homogeneous particles of non-spherical geometries is important in understanding the influence of shape of the nano/micro
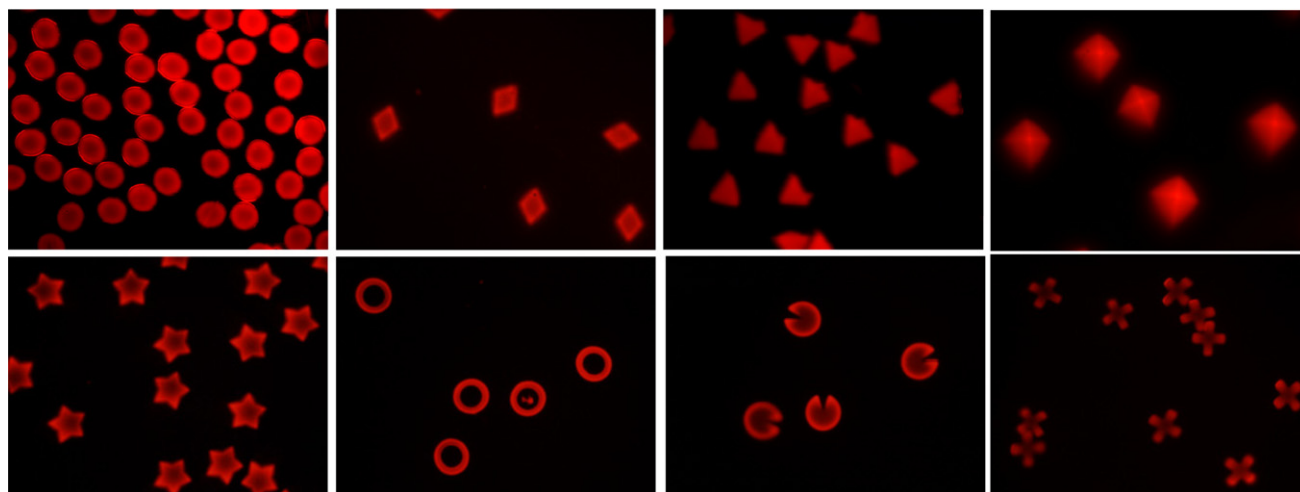
Fig. 5. Fabrication of microparticles of different geometries by the hydrogel template approach. The particle size was 50 μm.

structures on their biological behavior, such as blood circulation times, targeting, and cellular internalization for intravenous administration of submicron sizes [16,17]. But the effect of the shape on biological response has not been fully examined, mainly due to the lack of availability of such particulate systems. Traditional emulsion methods of preparing nano/microparticles result in spherical shape, and only recently, formation of particles in a variety of different geometries became possible as a result of advances in nano/microfabrication techniques. Microstructures with complex geometries influence the anisotropic interactions with biomolecules through steric or spatially segregated surface functionalities. Phagocytosis by macrophages and the renal and hepatic clearance of microstructures are also known to be influenced by the geometry of nano/microstructures [18].

It will be useful to generate a comprehensive library of microstructures with complex geometries to examine their potential benefits of targeted drug delivery. Figs. 2 and 3 show formation of disc shape particles, and Figs. 4 and 5 show the capability of making microstructures with almost any geometry. Furthermore, the hydrogel template approach can also be used to prepare multi(n)-layered microstructures by simply filling the cavities of the template n times. Thus, the same drug can be loaded at different concentrations or different drugs can be loaded into the same particles. Such multi-layered structures are useful in making even more advanced structures, e.g., capsules, for delivery of fragile drugs, such as peptide and protein drugs, and in making multi-functional systems. The ease and flexibility of the hydrogel template approach are expected to find applications in delivery of diverse drugs, ranging from low molecular weight hydrophobic drugs to high molecular weight proteins. The hydrogel template approach provides a new option of fabricating microstructures with various geometries and with multi-functional systems.

6. Conclusions

A hydrogel template approach was developed to prepare homogeneous nano/micro structures of various geometries. This can be readily applicable for the large scale production of nano/microparticles because of its easiness in producing particles. The flexibility in forming different microstructures allows the approach to incorporate diverse drugs with various hydrophilic characters and molecular weights. The study demonstrated production of particles in various geometries and sizes with high precision. Drug loading is also shown to be very high with controllable drug release kinetics. The hydrogel template approach provides a new avenue of preparing nano/microparticles for drug delivery.

Acknowledgment

Fig. 6. Felodipine release profile from PLGA microparticles (20 μm in diameter). The amount of felodipine in the microparticles was 50% (w/w). ($n = 3$).

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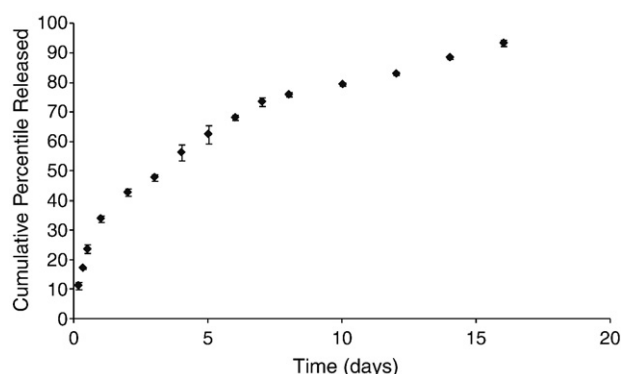
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