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Enhanced Cell Adhesion to the Dimpled Surfaces of Golf-Ball-Shaped Microparticles

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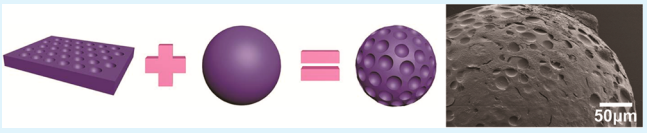
Supporting Information

ABSTRACT: Engineering surface morphology as in honeycomb-structured planar films is of great importance for providing new potential application and improved performance in biomedical fields. We demonstrate potential new applications for the uniform biocompatible golf-ball-shaped microparticles that resembles 3D feature of honeycomb-structured film. Dimple size controllable golf-ball-shaped microparticles were fabricated by microfluidic device. Surface dimples not only can act as picoliter beaker but also enhance cell adhesion without any chemical modification of the surface.



KEYWORDS: *microparticle, biodegradable polymer, cell adhesion, drug release, golf-ball-shape, surface morphology*

Microporous honeycomb-structured films with hexagonally arranged pores on their surfaces have attracted considerable interest, because of their potential applications in electronics, photonics, and biomedical science.[1,2] Fabrication variables and how to utilize the structural advantages derived from surface dimples have been studied intensively, and such efforts have led to the ability to successfully control the surface morphology of 2D honeycomb-shaped planar films.[1−3] Inspired by the potentials of 2D honeycomb-shaped structures to be used in various applications, attempts to produce nonplanar honeycomb-shaped features have recently been reported in an effort to expand the advantages of the honeycomb shaped to more complex structures.[4,5] However, few works have attempted to elucidate the possible applications of dimpled 3D structures and the effects of dimples remain unexplored. Microparticles, which are representative forms of 3D structures, provide platforms for various technological applications. Generally, microparticles are fabricated in aqueous media and conventional microparticles are smooth and round, an entropically favorable morphology. Inspired by various shapes and surface morphologies of naturally occurring microparticles, versatile types, such as anisotropic, Janus, patch, and surface patterned particles are believed to be capable of exceeding the limitations of conventional shapes and providing microparticles with new functionalities.[6] Because they mimic naturally occurring microparticles, of the various fields in which such unconventional microparticles could be used, biomedical applications, such as controlled drug release, tissue engineering, and sensors, are being paid the most attention. Of the various microparticle surface microstructures, surfaces with dimpled structures, golf-ball-shaped microparticles,[7−9] are appealing because they resemble the 3D honeycomb structures of flat films (Figure 1a). We recently succeeded in developing a facile fabrication method for producing golf-ball-shaped microparticles and observed that dual-imaging-agent encapsulated microparticles with dimpled surfaces showed remarkable cell

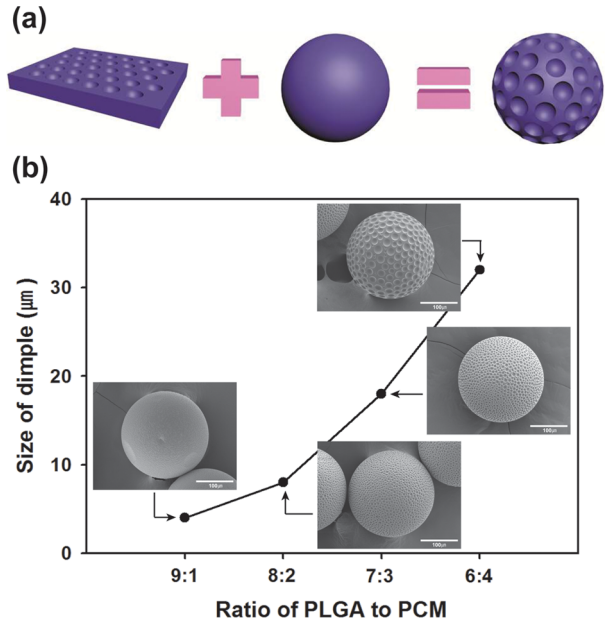


Figure 1. (a) Schematic showing the resemblance of honeycomb-shaped 2D surfaces to golf-ball-shaped microparticles. (b) Average surface dimple diameters of PLGA (lactic acid: glycolic acid = 65:35) microparticles as a function of the ratio of the phase change material (PCM) to the organic phase. Size of the microparticles were same (235 μm ± 5 μm) regardless of PLGA/PCM ratio selected.

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internalization compared to smooth microparticles, indicating that they have potential for use in biomedical applications.[10,11] Herein, we report on dimple size control, potential uses for surface dimples, and improved cell adhesion to golf-ball-shaped microparticles.

The microfluidic approach has been recognized as a powerful method for the fabrication of uniform microparticles and a combination of several methods can be used to produce microparticles with morphologies that are not round and smooth.[12] However, the use of biodegradable and biocompatible polymers still poses a challenge to the application of new fabrication methods. Incorporating low molecular mass materials that alter smooth surfaces to give specific morphologies is a characteristic approach to obtaining microstructured microparticles that avoids the use of photoreactions in microfluidic fabrication approach.[13] Golf-ball-shaped microparticles were fabricated in a fluidic apparatus as previously reported.[10] The incorporation of an inert organic phase change material (PCM) is a key step to achieving microparticles with surface dimples. Briefly, an oil phase droplets, which is PLGA and PCM (2-methyl pentane) dissolved in dichloromethane, were discontinuously introduced into 1 wt % PVA aqueous solution that flows continuously throughout the tube-type fluidic apparatus. As the oil phase droplet travels with continuous flow, droplet solidifies with the evaporation of DCM and simultaneously phase separation occurs between polymer and PCM. Because there is no remaining of PCM after microparticle fabrication, PCM drops spread on the surface of oil phase droplet result in surface dimple formation where PCM drops inside the oil phase droplet forms closed porous structure.[10] The average dimple size of golf-ball-shaped microparticles produced in this manner can be controlled by varying the PLGA: PCM ratio of the organic phase (Figure 1b) using a tube-type microfluidic device. Increasing PCM content results in increased dimple diameters. Also number of dimples on the one side of microparticles is counted from the SEM image shown in Figure 1b and approximately 3058, 1590, 970, and 213 dimples existed on the same size microparticles for PLGA/PCM ratio of 9:1, 8:2, 7:3, and 6:4, respectively. Because the PCM, used to prepare the golf-ball-shaped microparticles, has a bp of 62 °C, the effect of microparticle fabrication temperature on dimple formation was observed by placing tubing in a fixed temperature water bath. PLGA with two different composition, lactic acid: glycolic acid = 65:35 and 50:50, were used to prepare microparticles. Golf-ball-shaped microparticles were fabricated at various temperatures from 16 to 48 °C, using increments of 2 °C. Separate fabrications were conducted at each temperature examined. Starting temperature, 16 °C, was selected because the temperature could be continuously maintained throughout the fabrication device at room temperature condition. However, golf-ball-shaped microparticles can be obtained at 8 and 10 °C, which imply that surface dimples can be also observed below 16 °C. Surface dimple structures were obtained up to 46 and 48 °C for PLGA 65:35 and PLGA 50:50, respectively. These results demonstrate that PCM, which is responsible for dimple formation, should be in the liquid state during fabrication. Because the glass transition temperature of the two polymers used differed by 10 °C and there was a minute difference between their fabrication temperature ranges, glass transition temperature also has a little effect on the available fabrication temperatures (see Figures S1−S3 in the Supporting Information). Microparticles fabricated at temperatures greater than those mentioned above showed only porous internal structure; no dimples were observed on their surfaces (see Figure S4 in the Supporting Information).

The surface pores of honeycomb films are known as picoliter beakers and they are of importance in analytical chemistry.[14] Similarly, the possibility of using the surfaces of golf-ball-shaped microparticles as tiny vials was investigated. Cubic NaCl crystals can reside in the dimples of these microparticles (Figure 2a, b) (see the Supporting Information for the detailed

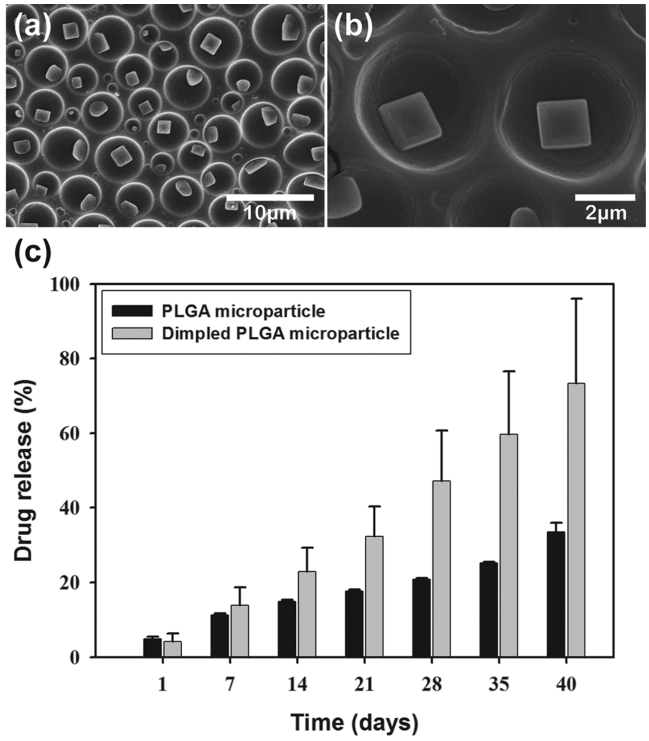

Figure 2. (a-b) SEM images of NaCl crystal formations in dimples. They indicate that the surface dimples act like tiny beakers. (c) Cumulative release profile of smooth, round, and golf-ball-shaped microparticles incubated in a phosphate buffer saline (PBS) at 37 °C. Nifedipine was used as a model drug.

procedure). Dimples held aqueous NaCl solutions and evaporation resulted in NaCl crystallization.[15] This means that these microparticles could be used in cases where faster release of one active agent, from surface dimples, and a slower release of another, from the interiors of microparticles, is desired. The drug release behaviors of golf-ball-shaped and smooth, round microparticles, fabricated with nifedipine (used as a model drug) using microfluidic devices, were compared. Preparation of the nifedipine loaded microparticles were same as procedures used to fabricate drug free particles except for additional dissolving nifedipine in oil phase (1 wt %). Monodispersed microspheres are much more appealing, in terms of behavior reproducibility, for biomedical applications.

It has been reported that microfluidic microparticle based controlled release systems exhibit slower drug release behaviors and significantly lower initial bursts than polydispersed particle systems.[16] As shown in Figure 2c, both types of microparticles showed low initial bursts. However, the golf-ball-shaped microparticles showed higher drug release percentages. Because golf-ball-shaped-microparticles have internal pore structures and larger surface areas than smooth, round microparticles,

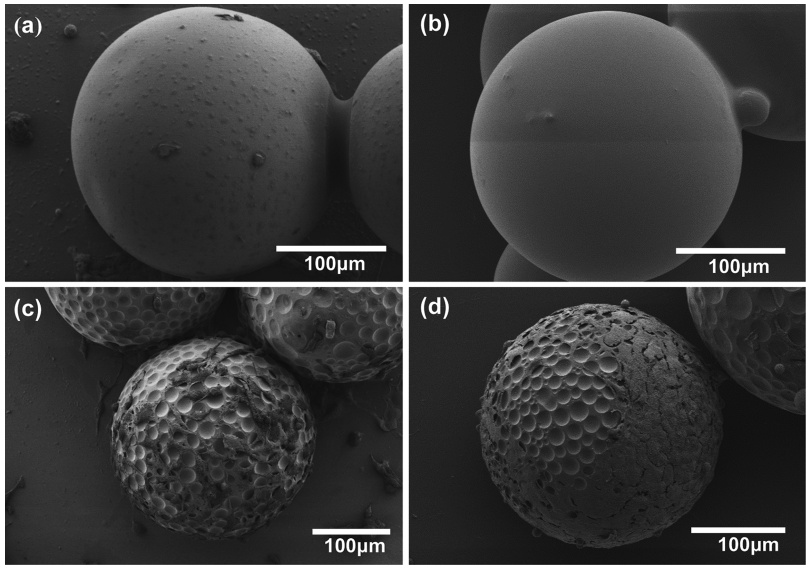

Figure 3. SEM images of postculturing (a, c) MCF-10A and (b, d) HEK293 cell adhesions to the surfaces of conventional smooth, round microparticles (upper) and golf-ball-shaped microparticles (lower).

which do not have internal pore structures, hydrolytic degradation of golf-ball-shaped microparticles is faster (see Figures S5 and S6 in Supporting Information). The expansion of the error bars of the golf-ball-shaped microparticles with increasing incubation time also indicates that release was mainly governed by the degradation after 2 weeks of incubation. Surface patterns are of great importance in tissue engineering, cell biology, optics, and electronics because they can provide new matrix functionalities.[17,18] Considerable efforts have been made to achieve surface micropatterns and new, facile fabrication methods to alter matrix functionalities via physical approaches. It has been reported that the surface structures can be used to improve the performances of honeycomb-shaped film for biomedical applications. It also has been reported that the surface pores of honeycomb-shaped films can be used to achieve improved cell adhesions[19,20] and cell proliferations[21] compared to those of smooth plane surfaces. Thus, honeycomb-shaped films can be used for tissue engineering and cell culture media. Microparticles can be applied to microcarrier cultures, which can be used to mass produce cells because of their large surface areas. Such cultures save space and cost and are more convenient by suspension of culture. Cell carrying microparticles can also be used very effectively in injectable tissue engineering systems because they minimize the needs for invasive surgeries and repair irregular defects.[22,23] When implementing microparticles as cell carriers or scaffolds, cell adhesion is a pivotal step that influence cell growth, proliferation, and biofunction.[24] Synthetic biodegradable polymers, such as poly(L-lactide) (PLLA) and PLGA are suitable for clinical use because of their biocompatibility. However, they exhibit poor cell-matrix interactions owing to their chemical and biological inertias.[25] Previous studies have applied additional treatments, such as prior enzymatic degradation of particles[26] and arginine-glycine-aspartic acid (RGD) peptide coating[22,23] to improve cell adhesion. Otherwise, porous microparticles have been widely considered as cell carriers because their porous structures are similar to those of conventional 2D scaffolds.[27] It is reasonable to assume that honeycomb-shaped films, expanded to have 3D structures, will also have improved cell interactions. Thus, we investigated how the dimples of golf-ball-shaped microparticles influence on cell adhesion. Two different cells, MCF-10A and HEK293, were examined to compare the cell adhesion capabilities of golf-ball-shaped microparticles and conventional smooth, round microparticles.

These results show that cells preferred to stretch out along the micropatterned surfaces of the golf-ball-shaped microparticles, rather than the smooth surfaces of the round microparticles. Dimples provided anchoring points, which gripped the cells, thus spreading them out. The cells covered the surface of the golf-ball-shaped microparticles. However, there was no clear cell adhesion to the smooth, round microparticles. It was explained that macroporous microcarriers enhance cell attachment by the increased surface areas and surface-area-to volume ratio (SAV) has been used to demonstrate effects of microparticles with the geometric features.[28,29] This also applies to golf-ball-shaped microparticles without open porous surface structure. Therefore, Figure 3 clearly shows that increasing surface area enables cell attachment to biocompatible PLGA surfaces. Although it is not clear at this stage, contact splitting may also influence on the adhesion property, as it does with honeycomb-shaped films with surface dimples.[30]

There is a critical difference between the percentage of dimpled microparticles with cells attached to them and the percentage of microparticles without dimples with cells attached to them. Since only trace numbers of cells were observed attached to the smooth microparticles and the surfaces of the dimpled microparticles were nearly covered in cells, the surfaces of the dimpled microparticles can be said to offer better cell adhesion support than the smooth ones. This result indicates that extra treatments, such as chemical modifications, are required to achieve good cell adhesion to conventional smooth, round microparticles. Confocal fluorescence images (Figure 4b, c) indicate that cells resided on the surfaces of the golf-ball-shaped microparticles. Compared to previously studied cell growth carriers, hollow and open porous microparticles, golf-ball-shaped microparticles are free from problems with mass transport and cell migration to their interiors. In the case of microparticles with pores that extend

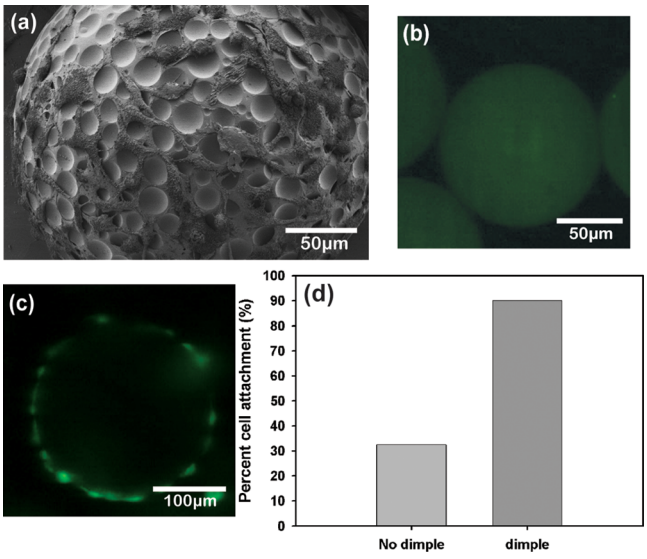

Figure 4. (a) Higher magnified view of Figure 3c, focusing on the surface of a microparticle. (b, c) Fluorescence microscopy photographs of MCF-10A cells on smooth, round and golf-ball-shaped microparticles cultured for 1 day, respectively. (d) Percent of cell attached microparticles from total number of microparticles counted from SEM image. Contrast of image b was enhanced with Photoshop to clearly separate microparticles from the backgrounds.

from the surfaces to the interiors of the structures, the pores should be larger than a single cell to facilitate cell movement toward the inner pores. This may reduce the mechanical strengths of such microparticles. For cell carrier microparticles, injectable systems that often also include controlled-release active agents such as growth factors, it is important to remove the initial burst, which can be a severe problem for microparticles with open-pore surfaces. Thus, microcarriers with closed pore surfaces with micropattern offer an alternative delivery approach for systems with which open-porous microparticles cannot be used. The fabrication of scaffolds for cell carrier and cultures is of paramount importance to the successful development of tissue engineering applications. Biodegradable and biocompatible materials should be used to make the best of injectable cell carrier systems. In addition, a basic requirement of microparticle cell carriers is that they should efficiently accommodate cells. Our approach does not require additional processing of the fabricated microparticles for celluar adhesion. This is beneficial because such processing requires extra effort, and can affect storage stability, and alter the physical and chemical properties of the microparticles. Because pore size has been reported to influence cell adhesion to honeycomb-shaped films,[19,20] further consideration of the effects of dimple size on cell adhesion will be the focus of future study.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Detailed experiment procedures, available dimple formation temperature range, SEM images of microparticles incubated in the PBS buffer solution for biodegradation. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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