Colloids and Surfaces B: Biointerfaces 121 (2014) 395–399

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Uniform polydimethylsiloxane beads coated with polydopamine and their potential biomedical applications

Dae-Ryong Jun [1], Seung-Kwan Moon [1], Sung-Wook Choi *

Department of Biotechnology, The Catholic University of Korea, 43 Jibong-ro, Wonmi-gu, Bucheon, Gyeonggi-do 420-743, Republic of Korea

ARTICLE INFO

Article history:
Received 9 February 2014
Received in revised form 9 June 2014
Accepted 10 June 2014
Available online 17 June 2014

Keywords:
Poly(dimethylsiloxane)
Uniform beads
Polydopamine
Cell culture
Filler materials

ABSTRACT

Based on oil-in-water emulsion, uniform poly(dimethylsiloxane) (PDMS) beads were prepared using a simple fluidic device and then modified with polydopamine (PDA) to improve cell attachment. The size of the PDMS beads could be easily tuned by changing the flow rates of the discontinuous and continuous phases, and PDMS concentration in oil phase. The PDA-coated PDMS beads exhibited a dark and rough surface, whereas the pristine PDMS beads had a clear and smooth surface. The PDA layer at the surface of the PDMS beads was found to provide a favorable environment for cell culture due to its hydrophilic property. The PDA-coated PDMS beads can potentially be employed as filler materials for tissue engineering.

1. Introduction

Injectable microspheres have been of great interest as implant materials in a wide variety of biomedical applications such as plastic surgery, bone reconstruction, and among others [1–6]. Basically, filler materials can be classified into two types: hydrogel and solid powders/beads. There are a number of generic requirements for ideal implantable constructs: (1) the ingredients should be biocompatible, bioinert or biodegradable; (2) the constructs should have suitable size, surface morphology, and mechanical strength for specific applications; (3) the constructs should provide a favorable surface for host tissue/cells [7]. So far, many investigations have been conducted for developing implantable constructs capable of filling the defect or injured site, eventually for restoring the function of the target tissue or organ [3–6]. Among many issues on filler materials, migration of implanted powders/beads from injection site to other tissue or organ is one of the most challenging tasks [8]. As pointed out by many researchers, characteristics of filler materials, including their composition, size, shape, and surface morphology play an important role in the migration in the body [9–11]. The migration of the implanted powders/beads was more critical in the case of muscle, folding skin, and trachea [12].

Generally, hydrogels have intrinsic shortcomings, including the low mechanical strength and durability in the human body. Recently, many research groups have fabricated biocompatible powders or beads from inorganic (e.g., hydroxyapatite [13,14], tricalcium phosphate [15], silica [16]) and organic (e.g., polymethyl methacrylate (PMMA) [17–19], bisphenol-a-glycidyl methacrylate (Bis-GMA)-based resin [20], dextran [21]) materials using various techniques mainly based on emulsification.

To address these problems, we fabricated uniform poly(dimethylsiloxane) (PDMS) beads using a simple fluidic device based on oil-in-water emulsion and modified with polydopamine (PDA) at their surface. In addition, the size of the PDMS beads could be finely tuned by changing the flow rates and PDMS concentration. The rationale for the selection of PDMS is its excellent biocompatibility and non-biodegradability for duration in the human body [22,23]. Generally, tissues and cells are hard to attach on the PDMS surface due to its inherent hydrophobic property. Therefore, PDA was employed for surface modification due to its cell-adhesion property [24–28], leading to improve the adhesion interaction between beads and host cell/tissue. We believe that the uniform PDMS beads modified with PDA can be clinically used as filler materials in tissue engineering and regenerative medicine.

2. Materials and methods

2.1. Materials

Sylgard 184 (Sewang Hitech, Korea) and poly(vinyl alcohol) (PVA) (Sigma–Aldrich, USA) were used as materials for the discontinuous and continuous phases, respectively. Sylgard mixture was prepared just before use according to the manufacturer's

* Corresponding author. Tel.: +82 2 2164 4449; fax: +82 2 2164 4865.
E-mail address: choisw@catholic.ac.kr (S.-W. Choi).
[1] These authors equally contributed to this work.

http://dx.doi.org/10.1016/j.colsurfb.2014.06.027
0927-7765/

instructions. Dichloromethane (Junsei, Japan) was used as a solvent for Sylgard 184. A syringe needle (BD Medical, USA), a glass capillary (Ace Glass, USA) and Tygon® tube (Saint-Gobain Corp., USA) were used for the fabrication of a simple fluidic device. Dopamine hydrochloride (Sigma–Aldrich, USA) was used to modify the surface of PDMS beads.

2.2. Fabrication of uniform PDMS beads and surface modification with PDA

A simple fluidic device consisted of a 30 G needle, a glass capillary (0.5 mm i.d. × 0.9 mm o.d.), and Tygon® tube (1/32 in. i.d. × 3/32 in. o.d.), as previously reported [29–31]. An aqueous PVA solution (3 wt%) was introduced into the fluidic device as the continuous phase and an organic solution containing the Sylgard mixture served as the discontinuous phase. Each of the phases was introduced into the fluidic device by syringe pumps (NE-1000, New Era Pump Systems Inc., USA) at predetermined flow rates. The resultant PDMS droplets were collected in a tall beaker (1 L) containing the aqueous PVA solution (collection phase). The collection phase was heated at 40 °C for 3 h to evaporate solvent and then at 80 °C for another 3 h to crosslink PDMS, obtaining uniform PDMS beads. The PDMS droplets and beads prepared at the different flow rates were carefully collected on a concave glass and analyzed by optical microscopy (B-350, Optika, Italy). The average sizes and standard deviations were calculated from the optical microscopy images using ImageJ (National Institutes of Health, USA) (n = 300). The PDMS beads were coated with PDA by modifying a previous method [32]. The PDMS beads (0.5 g) were stirred in a solution (10 mL) of dopamine hydrochloride (0.5 wt%) and Tris–HCl (1 M, pH 8.5) for 3 days using an orbital shaker. Scanning electron microscopy (SEM) (S-4800, Hitachi, Tokyo, Japan) was used to observe the morphology of the PDMS beads. The hydrophobicity of pristine PDMS and PDA-coated (0.1–1.0%) PDMS were measured by contact angle analyzer (Phoenix300, S.E.O, Korea). The pristine PDMS and PDA-coated (1.0 wt%) PDMS beads were analyzed by FT-IR spectrophotometer (Spectrum 2000, PerkinElmer, USA).

2.3. Cell culture on the PDMS and PDMS beads

For cell proliferation assay, NIH-3T3 fibroblasts (Korean Cell Line Bank, Korea) were cultured on PDMS and PDA-coated PDMS surface in two- and three-dimensional fashions. For two-dimensional culture, the bottom of each well of the 24-well plates were modified with PDMS by pouring the Sylgard mixture (1 g) and then crosslinking, followed by the modification with PDA as described in above section. Prior to cell seeding, the culture plates were sterilized by immersion in 70 wt% ethanol overnight, and subsequently washed with PBS five times. Cells (2×10^4/mL) were seeded into the culture plates. For three-dimensional culture on the PDMS beads, the cells (1×10^5/mL) in culture media were incubated with the PDMS and PDA-coated PDMS beads in a conical tube (50 mL) under a gentle stirring at 80 rpm for 6 h. Afterwards, the PDMS beads with cells were gently washed with PBS three times to remove the unattached cells, and then transferred into a 96-well culture plate (approximately 20 beads per well). The culture medium consisted of DMEM (Invitrogen Corp. Grand Island, NY) supplemented with 10% FBS (Invitrogen Corp. Grand Island, NY) and 1% penicillin and streptomycin (Invitrogen Corp. Grand Island, NY). The cultures were maintained in an incubator at 37 °C in a humidified atmosphere containing 5% CO_2, and the media were changed every other day. In two-dimensional culture, the cells were fixed with 4% formaldehyde, consecutively treated with DAPI (Sigma–Aldrich) and rhodamine–phalloidin (Invitrogen), and observed with fluorescence microscope (Axio Imager D2, Carl Zeiss, Germany) at 1, 4 and 24 h. In three-dimensional culture, the cells on the beads were stained with DAPI at 7 days of culture and observed with a confocal microscope (LSM710, Carl Zeiss, Germany).

The cell proliferation was evaluated by cell proliferation and cytotoxicity assay. After harvesting the cells on the beads at 1, 3, 5 and 7 days, the medium was removed, 200 μL fresh medium and 20 μL Cell Counting Kit-8 (Dojindo Laboratories, Tokyo, Japan) were added to each well, and incubated at 37 °C, 5% CO_2 for 2 h. The absorbance at a wavelength of 450 nm was measured using a microplate reader (EON, BioTek Instruments Inc., USA) after transferring from the incubated 100 μL medium to the 96-well plate. The absorbance was normalized against the total weight of the beads.

3. Results and discussion

Uniform PDMS beads were fabricated using a simple fluidic device as a previously reported method [29,30]. In brief, a mixture of PDMS base and curing agent in the ratio of 10:1 was added into an organic solvent (DCM). The organic phase containing the PDMS mixture served as the discontinuous phase in the fluidic device, where an aqueous PVA solution was used as the continuous phase. Based on the oil-in-water emulsion, uniform oil droplets of the PDMS mixture were collected in the aqueous PVA solution and stirred to allow solvent evaporation and PDMS curing, finally resulting in PDMS beads. For the surface modification with PDA, the PDMS beads were soaked in a dopamine solution for 3 days and then washed with water for further experiments.

The size of the PDMS beads should be tuned to suit a specific application. Therefore, the effects of the flow rates of the discontinuous and continuous phases on the size variation of the oil droplets were evaluated. Fig. 1A shows optical microscopy images and a plot of size variation of the oil droplets with respect to the flow rate of the continuous phase, where the flow rate of the discontinuous phase was kept as 0.05 mL/min. The average size of the oil droplets decreased with an increase in the flow rate of the continuous phase due to a high shear stress [29,30]. Note that the PDMS droplets had a highly monodisperse size distribution with coefficient of variance (CV) less than 3% even at 5 mL/min of flow rate of the continuous phase. We also evaluated the effect of the flow rate of the discontinuous phase on the size variation, while the flow rate of the continuous phase was kept as 3 mL/min (Fig. 1B). The size of the PDMS droplets increased from 189.27 ± 3.93 to 283.40 ± 5.78 μm as the flow rate of the discontinuous phase increased from 0.05 to 0.25 mL/min.

Fig. 2 shows the size variations of the PDMS droplets and cured PDMS beads after solvent evaporation with respect to the PDMS concentrations. There was no significant difference in the size of the PDMS droplets even at the different PDMS concentrations. The PDMS beads with a smaller size can be obtained at a lower PDMS concentration. The PDMS beads with 104.50 ± 9.61 μm in size were achieved at 2.5 wt% of the PDMS concentration. Much smaller PDMS beads could be likely obtained at a lower PDMS concentration and a higher flow rate of the continuous phase. However, the polydispersity and amount of obtained PDMS beads will become major problems.

The cell attachment on the PDMS surface is known to be difficult to achieve due to its extreme hydrophobic property [33]. Therefore, the resulting PDMS beads were modified with PDA to improve cell attachment [18]. The dopamine concentration was varied at 0.1, 0.5, and 1.0 wt% to control the amount of the PDA at the surface of the PDMS beads. Fig. 3 shows optical microscopy and SEM images of the pristine and PDA-coated PDMS beads. The PDMS beads coated with a higher dopamine concentration had a darker surface, whereas the pristine PDMS beads were transparent. The inset images show the surface morphologies of the PDMS beads coated with different dopamine concentrations. Small PDA clusters were sparsely