



A simple coating method of PDMS microchip with PTFE for synthesis of dexamethasone-encapsulated PLGA nanoparticles

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Abstract

Dexamethasone is a widely used drug in medical and biological applications. Since the systematic and controllable release of this drug is of significant importance, encapsulation of this anti-inflammatory drug in poly(lactic-co-glycolic acid) (PLGA) nanoparticles can minimize uncontrolled issues. As dexamethasone-encapsulated PLGA nanoparticles are synthesized in the presence of organic solvents, poly(dimethylsiloxane) (PDMS)-based microchannels collapse due to the swelling problem. In present study, PTFE nanoparticles were used for the surface modification of the microchannels to prevent absorption and adhesion of solvents into the microchannels' wall. The contact angle analysis of microchips after coating showed that the surface of microchannels bear the superhydrophobicity feature (140.30°) and SEM images revealed that PTFE covered the surface of PDMS, favorably. Then, the prepared microchip was tested for the synthesis of dexamethasone-loaded nanoparticles. SEM and atomic force microscopy (AFM) images of the synthesized nanoparticles represented that there was not any evidence of adhesion or absorption of nanoparticles. Furthermore, the monodispersity of nanoparticles was discernible. As AFM results revealed, the average diameters of 47, 63, and 82 nm were achieved for flow ratios of 0.01, 0.05, and 0.1, respectively. To evaluate the drug efficiency, cumulative release and encapsulation efficiency were analyzed which showed much more efficiency than the synthesized nanoparticles in the bulk mode. In addition, MTT test revealed that nanoparticles could be considered as a non-toxic material. Since the synthesis of drug-loaded nanoparticles is ubiquitous in laboratory experiments, the approach presented in this study can render more versatility in this regard.

Keywords PTFE coating · Dexamethasone · Microfluidics · Nanoparticle synthesis · Numerical simulation

Introduction

Dexamethasone has been a matter of significant interest for researchers, i.e., biologists in medical and biological applications. Dexamethasone, also known as an anti-inflammatory agent, can

be used in treatments of rheumatic-related fevers, allergies, skin diseases, asthma, and brain swelling, clinically [1]. Besides what was already mentioned, dexamethasone is a potent morphogen and utilized in the multipotent mesenchymal stem cells differentiation [2]. To shed more light on this matter, this drug plays a

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significant role in osteogenic differentiation medium and is able to differentiate mesenchymal stem cells to the osteoblasts [3]. Nonetheless, this drug suffers from certain side effects where using uncontrolled, high dosage of dexamethasone in cell culture media may lead to decrease in the osteoblastic cells proliferation [4]. As a result, systematic and controlled release of this drug has to be used in order to minimize consequences and maximize the efficiency of drug delivery. Furthermore, it has to be encapsulated on a carrier to provide better handling with researchers.

Recently, a surge of efforts has been performed to enhance the durability of dexamethasone release of polymeric nanocarriers. For instance, Larrañeta et al. succeeded to incorporate hydroxypropyl- β -cyclodextrins and Tween 85 into the hydrogel system to increase dexamethasone loading and release period [5]. Also, inserting of calcium ions to alginate/dexamethasone sodium phosphate and forming a hybrid hydrogel structure could extend the release period of dexamethasone [6]. Poly(lactic-co-glycolic acid) (PLGA) is one of the most widely used biological materials for drug encapsulation and drug release. It is a synthetic co-polymer composed of lactic acid and glycolic acid. This polymer breaks down into the harmless monomers that routinely exist in the body and is a well-known biocompatible and biodegradable material [7, 8]. These features allow us to use several doses of this polymer without worrying about side effects for patients. Also, the decomposition time of PLGA is well characterized, and it is possible to predict the decomposition time based on the polymer compounds [9].

Dexamethasone-encapsulated PLGA nanoparticles traditionally are prepared in bulk mode where dropwise addition of polymer is a matter of vital importance [10]. However, this approach cannot guarantee such features of nanoparticles as a desired size or polydispersity. Microfluidic approach with a short diffusive length at the microscale level has provided better control over nanoparticle physiochemical characteristics like hydrodynamic diameter and surface charge [11, 12]. Thus, better and controllable synthesis of dexamethasone encapsulated by PLGA in the presence of organic solvents is possible via microfluidic.

Poly(dimethylsiloxane) (PDMS) has emerged as a robust soft material for fabrication of microfluidic devices. Nonetheless, of particular concern is swelling of this material when is exposed to the organic solvents which leads to detrimental effects on the function of microfluidic device [13, 14]. Therefore, this phenomenon impacts negatively upon a wide range of application of microfluidic devices with PDMS substrates for organic-based solvents. To address this issue, factors found to be influencing the swelling of PDMS microchannels have been explored in several studies [15–17].

Recently, some silicon polymers, all of which are fluorinated, have been proposed; however, those are neither widely available nor resistant to all organic solvents. It is now well established from a variety of studies that coating of the PDMS channel with Teflon can efficiently prevent the whole chip to be swelled [18–20]. Teflon, a DuPont Company material which is utilized for its fluoropolymers, the best of which is polytetrafluoroethylene

(PTFE), has been broadly applied in biomedical applications due to its transparency and excellent feature of solvent resistant. PTFE enjoys certain characteristics that enable it to reduce the friction and adhesion of a surface [21, 22]. As a result, it is a preferable candidate for treating the surface to decrease the level of surface energy and ease the flow. In addition, since its melting point is in high temperature, it can be applied in a wide range of applications for microfluidic devices. Coating the surface of PDMS microfluidics chip with PTFE is a promising method for surface modification of PDMS which is already used by different structures of PTFE like powder and films [21, 23].

Recently, Sadrabadi et al. encapsulated dexamethasone in chitosan nanoparticles using a T-junction microchip to enhance the bone remodeling process in human mesenchymal stem cells [24]. Also, Chronopoulou et al. encapsulated dexamethasone in PLGA nanoparticles using a stainless steel capillary microfluidic device. Even though the mentioned article could gain acceptable result in term of adjusting the size of nanoparticles in the range of 35 to 350 nm, the burst dexamethasone release as well as its uncontrolled release behavior was a challenging issue and needs to be addressed properly [25].

As such, the aim of this study was to present a convenient method for synthesis of PLGA nanoparticles using microfluidic devices and investigate encapsulation and release behavior of dexamethasone within the synthesized nanoparticles. PLGA was used as a carrier for dexamethasone to be efficiently encapsulated in the nanoparticles. At first, since organic-based solutions exist for the synthesis of nanoparticles, a convenient and easy to reach method for coating of the PDMS surface with PTFE nanoparticles was presented to prevent adhesion and absorption of solvents. Then, SEM images and contact angle analysis were used to evaluate the surface of the bare and coated PDMS. Also, in order to evaluate the effect of flow ratios (FRs), which describe as the ratio between the core flow to the sheath flows on physiochemical characteristics of synthesized nanoparticles, including their hydrodynamic diameter and also obtain the proper range of FRs for the synthesis procedure, finite element method was used to simulate the fluid behavior. More importantly, for better comprehension of flow condition, a comparison between simulated flows and the experimental ones which is performed by fluorescent microscope was carried out. In addition, to characterize nanoparticles after synthesis, atomic force microscopy (AFM) and SEM images of nanoparticles were captured. In the end, encapsulation efficiency, release efficiency, and cytotoxicity test were performed for all different sizes of synthesized nanoparticles.

Materials and methods

Materials

Acid-terminated PLGA (lactide:glycolide 50:50; Mn = 31,500 g mol⁻¹; Mw = 48,000 g mol⁻¹; Mw/Mn =

1.52(GPC); inherent viscosity 0.53 dL g^{-1}), dexamethasone and polyvinyl alcohol (PVA) (99% hydrolyzed, MW 133 kDa), fluorescein isothiocyanate, and Rhodamine B were acquired from Sigma-Aldrich. Methylene chloride (DCM) and dimethyl sulfoxide (DMSO) were purchased from Merck Millipore. Purified water was obtained from a Milli-Q Advantage A10 System (Millipore, France). Dulbecco's modified Eagle's medium (DMEM) was provided by Gibco.

Microchip fabrication and surface coating

For the fabrication of microfluidic chip, at first, the design model was drawn in Solidworks 2016, a capable CAD and CAE program. Then, this design was printed by a high-resolution printer, and a chrome mask was created, and SU-8100 photoresist was centrifuged on a silicon wafer with a height of 3 mm. This SU-8 was baked 2 min to attach on the silicon wafer. After that, the chrome mask was transferred on the SU-8 and exposed to UV lights. Ethyl oxalate was used to clean the regions where affected by UV lights. The mixture of PDMS with its curing agent in a ratio of 9:1 was poured on the created mask, and it was degassed by a vacuum and cured at the temperature of $60\text{--}65^\circ\text{C}$. The designed microchip was easily detached from the SU-8, and it was bounded by oxygen plasma on a glass. For coating of the microchip, the 60% weighted solution of PTFE nanoparticles with average diameter of 100–200 nm was used and injected by a syringe pump (KDS-210, KD Scientific Inc.) in the microchip and after that removed by vacuum. In order to have a film layer of PTFE on the microchannels' wall, the coated microchip was cured at 70°C for 10 min and at 150°C for 25 min in an oven. The schematic procedure of microchip fabrication as well as its coating by PTFE is illustrated in Fig. 1.

Characterization of coating in microchannels

SEM of microchannels

In order to better grasp the function of PTFE nanoparticles coated on the microchannels in molecular dimensions, the surface of the microchip has to be analyzed. Thus, the characterization of bare PDMS microchannels, coated PDMS ones before curing, and coated PDMS ones after curing was evaluated using SEM apparatus (HITACHI S-4160 Tokyo, Japan). The surface morphologies and structure of the microchannel were gained through this method.

Contact angle measurement

The water contact angle of the microchannel was measured before coating of PDMS, after coating of PDMS and before its curing, and after coating of PDMS and after its curing. A specific amount of dropped distilled water was poured on each sample while they were firmly fixed by a double-sided adhesive tape surrounded by glass slides. Thereafter, water contact angle was analyzed by a Theta Optical Teniometer (KSV Instruments, Ltd.) on which a digital camera connected to a computer was mounted, and the fitting method of Young-Laplace curves was applied.

Nanoparticle synthesis and encapsulation of dexamethasone

Synthesis of nanoparticles was performed both by bulk and microfluidic methods. In the bulk approach, under the stirred condition, PLGA solution in DCM (10 mg ml^{-1}) was

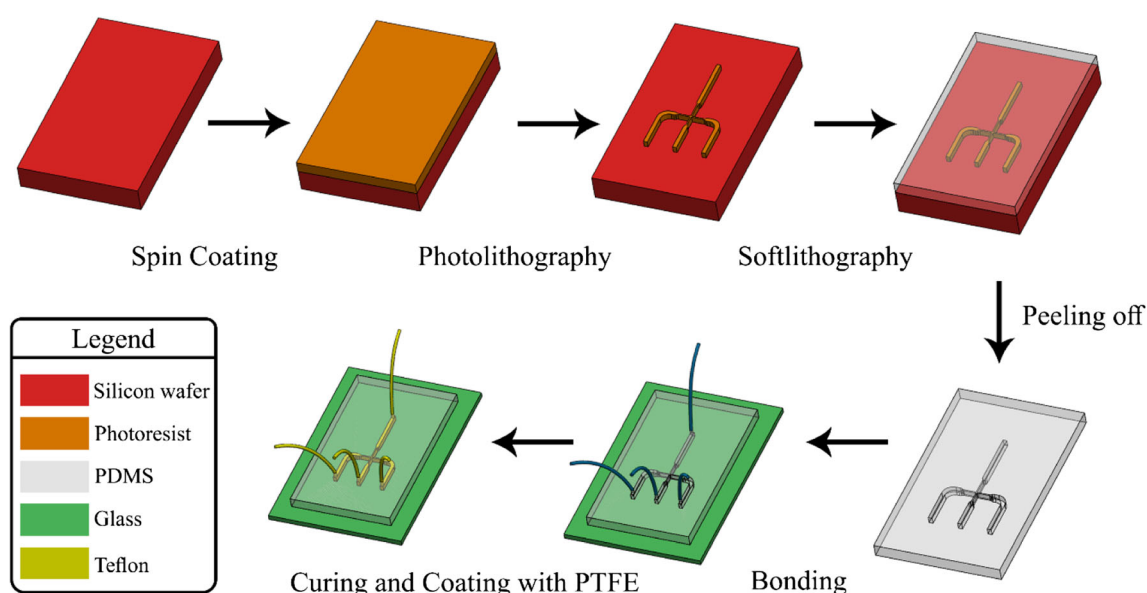


Fig. 1 The procedure of microchip fabrication as well as its surface coating for the synthesis of PLGA nanoparticles

prepared. The formation of droplets happened with the meticulous addition of the prepared solution to 50 ml of the stirred water. In order to synthesize the drug-loaded nanoparticles, at first, dexamethasone (Sigma-Aldrich) was dissolved in DCM, and then, it was mixed with PLGA. After that, this solution was used to be synthesized and formed nanoparticles by the mentioned bulk method. In the method of synthesis via microfluidic approach, first of all, the core solution flow which was PLGA in DCM (10 mg ml^{-1}) was prepared and injected into the microchip. At the same time, water as the sheath flow was injected into the microchip. FRs were adjusted by a syringe pump. To synthesize dexamethasone encapsulated in the nanoparticles, dexamethasone was dissolved in DCM with initial loading of $1 \text{ } \mu\text{g ml}^{-1}$ and then mixed with PLGA. Then, this solution was introduced into the microchip as the core flow, and the rest of the method is same as above.

Characterization of PLGA nanoparticles

DLS analysis

In order to determine the size distribution of the nanoparticles as well as their zeta potential in the solution, dynamic light scattering (DLS) was used. For this aim, the diluted solutions in water were analyzed using a Zetasizer (Zetasizer 3000HS, Malvern Instrument Ltd., Worcestershire, UK) at 173°C in the mode of backscattering. Measurements were repeated three times, and results were reported as mean \pm standard deviation.

SEM analysis

The evaluation of morphological features of PLGA nanoparticles was performed via SEM approach. The interaction of the electrons with the nanoparticles could get the topography of them. The images were captured in different scales to investigate the adhesion and aggregation of the nanoparticles, as well.

AFM

To gain three-dimensional shape or topography of the nanoparticles, AFM analysis was performed. This kind of measurement was used in the mode of contact on a Bruker Multimode 8 equipped with a scanner type “E.” Cantilevers of MSNL silicon nitride with a spring contact of 0.1 N m^{-1} and Si tips with a radius of 2 nm (MSNL-10, Bruker, Coventry, UK) were used.

Encapsulation and release efficiencies

To evaluate encapsulation and release efficiencies, the loaded dexamethasone PLGA nanoparticles (1 mg) were dissolved in

acetonitrile. Then, dexamethasone (Dex) concentrations were measured by UV–Vis spectrophotometer (UV-2450, Shimadzu, Kyoto, Japan) at a wavelength of 242 nm . The encapsulation efficiency was calculated by Eq. (1).

Dex Encapsulation Efficiency

$$= \frac{\text{Amount of Dex in nanoparticles}}{\text{Initial amount of Dex}} \times 100 \quad (1)$$

The in vitro profile of drug release was calculated by dispersing lyophilized dexamethasone-loaded nanoparticles (1 mg) in phosphate-buffered saline (PBS) (1 ml , and $\text{pH } 7.4$). After that, the prepared solution was poured into a dialysis cartridge (3500 Da , Thermo Scientific, IL, USA) and immersed in 1 l of PBS, and at the same time, it was slowly shaken in a water bath of 37°C . At a certain specific time, 1 ml of the buffer solution was collected for the analysis purpose while the equivalent amount of fresh PBS was added to the water bath. In addition, the concentrations of dexamethasone were measured by a UV spectrophotometer at 242 nm wavelength.

MTT

MTT colorimetric assay was used to investigate nanoparticles cytotoxicity. Human mesenchymal stem cells (hMSCs) were gained from Bon Yakhteh Institute and utilized as received. These hMSCs were seeded in 96-well tissue culture plates plated at a density of $10,000$ per well in triplicate (10% fetal bovine serum, 50 units/ml penicillin, 50 g/ml streptomycin in low glucose Dulbecco’s modified Eagle’s Media (DMEM, Sigma-Aldrich)) at 37°C in a $5\% \text{ CO}_2$ incubator. After that the unloaded PLGA nanoparticles were exposed to cells with a variety of concentrations, cell viability was measured. The critical point in utilizing different values of concentration is that to comprehend whether there is any dependency between the toxicity and concentration and to find out the safest amount of concentration of nanoparticles. The cytotoxicity of the nanoparticle was obtained after 72 h of incubation.

Simulation settings

To better comprehend the fluid flow in the microchip, three-dimensional continuum and Navier–Stokes equations were solved numerically by finite element method via Comsol 5.3a, a commercial computational fluid dynamic (CFD) solver [26]. In addition, the diffusion process was also modeled, and the convection–diffusion model was coupled with mentioned equations. The continuity, Navier–Stokes, and convection–diffusion equations are expressed by Eqs. (2), (3), and (4) [27].

$$\nabla \cdot \mathbf{V} = 0 \quad (2)$$

$$\frac{\partial \mathbf{V}}{\partial t} + \rho(\mathbf{V} \cdot \nabla) \mathbf{V} = -\nabla P + \mu \nabla^2 \mathbf{V} \quad (3)$$

$$\frac{\partial c}{\partial t} + (\mathbf{V} \cdot \nabla) c = \frac{1}{Re \cdot Sc} \nabla^2 c \quad (4)$$

where P is the fluid pressure, \mathbf{V} is the velocity vector, c is the concentration of two fluids, and Sc is the Schmidt number which indicates the ratio of momentum and mass diffusivity and can be obtained by Eq. (5).

$$Sc = \frac{\nu}{D} = \frac{\mu}{\rho D} \quad (5)$$

where ν is the kinematic viscosity. The input velocity of the fluids was adjusted by Reynolds (Re) number which is given by Eq. (6) [28].

$$Re = \frac{\rho V D_h}{\mu} \quad (6)$$

where ρ is the density, μ is the dynamic viscosity, and D_h is the hydraulic diameter which can be calculated by Eq. (7).

$$D_h = \frac{4A}{P_w} \quad (7)$$

where A is the cross-section area of the fluids at the inlet and P_w is the wetted perimeter of the cross section. The fluids were assumed to be steady state, Newtonian, and incompressible during this simulation. The velocity was applied at the inlets while the outlet was set to be pressure outlet and zero pressure outlet with suppress backflow was specified. In addition, consistent stabilization technique was considered for solving Navier–Stokes equation in order to make it closer to the exact solution of the equations.

Results and discussions

Coating with Teflon PTFE

PDMS gradually become the first selected material for the fabrication of microfluidic devices [29, 30]. This material has certain unique features, including flexibility, low Young's modulus, low surface energy, and gas permeability. However, it has the problem of swelling for organic solvents. This phenomenon leads to disruption of flow rates in the device due to the variation of cross-sectional area (swelling caused this problem) [31]. Furthermore, it makes it difficult to control over the size of nanoparticles in the microchannel. The absorbance of solvent into the walls of PDMS microchannel leads to disruption in monodispersity of the nanoparticles and batch to batch different size [13]. As a result, it is hardly possible to carry out the synthesis of organic-based materials in the microchannels made by PDMS [32]. To

meet this demand, the microchip was covered with a solution of PTFE nanoparticles to overcome the mentioned issue. To obtain a flat and continuous layer of PTFE, the PDMS chip was cured at 70 °C for 10 min and 150 °C for 25 min. PTFE nanoparticles stick to the surface of the device immediately after that they were in touch with the surface, and after heating, they formed a continuous hydrophobic layer on the surface. Since the preparation of the PDMS chip is easy and fast, this easy-to-reach and trouble-free coating procedure provides a coated layer on the surface of the PDMS. Since using PTFE before fabrication of microchip may cause severe problems on the SU-8 mold [18], the methodology presented in this study can overcome this problem, as well. The procedure of microchip fabrication as well as its surface coating is illustrated in Fig. 1.

To confirm surface coating of PDMS by PTFE nanoparticles, the bare PDMS as well as coated PDMS were tested by contact angle method and SEM. The contact angle test was used to compare the contact angle of the bare PDMS with the coated PDMS before and after curing at 150 °C to better understand the function of heating on the coated PDMS, the results of which are illustrated in Fig. 2.

As is clear from Fig. 2, the contact angle of the coated PDMS after curing was more than before curing, and these were more than the bare PDMS. The contact angle of bare PDMS was 59.37°. This amount reached to 108.01° when it was coated by PTFE nanoparticles, and it went to the highest level when it was heated at 150 °C for 25 min, and the contact angle was 140.3° which could be considered as a superhydrophobic surface. In general, superhydrophobicity is made up the micro- and nanoscale roughness as well as the utilized material which has lower surface energy [33]. Rual et al. proved that PTFE coating played a vital role in the hydrophobicity of PDMS surface and concluded that the more the amount of PTFE, the higher the hydrophobicity. In order to consider a surface as hydrophobic, its contact angle has to be more than 90° [23]. Since the bare PDMS is inherently hydrophobic, by coating, its hydrophobicity increases dramatically. Figure 3 illustrates the result of SEM analysis of microchips. The credibility of the results was approved by the SEM images of the bare PDMS, the coated PDMS before heating, and the coated PDMS after heating which are illustrated with different scales. As presented by Fig. 3, the PDMS surface after applying the PTFE solution is completely covered with PTFE nanoparticles and after heating at two different temperatures for a specific duration, the PTFE nanoparticles start to melt and form a continuous layer on the PDMS surface. Thus, the PTFE nanoparticles covered more surfaces of the PDMS, resulting in higher water contact angle.

Certain previous studies have been focused on the enhancement of hydrophobicity of PDMS with PTFE nanoparticles [18, 22, 34]. Lue et al. studied the function of PTFE on hydrophobicity, and they could increase the water contact angle

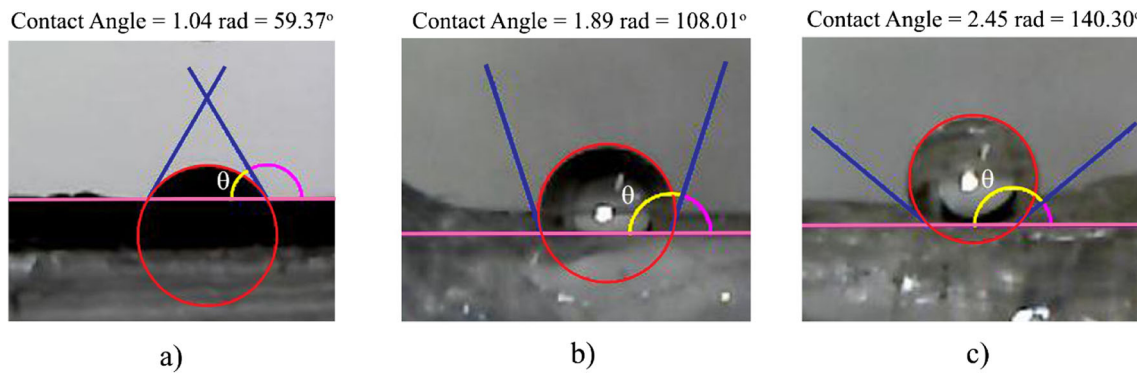


Fig. 2 Contact angles of **a** the bare PDMS, **b** the coated PDMS before heating, and **c** the coated PDMS after heating. Surface coating of the PDMS leads to having a superhydrophobic surface which prevents adhesion and absorption of the solvent

to 126° [35]. Also, the resistance of wear with polyphenylene sulfide (PPS)/PTFE increased significantly compared to the mere coating with PPS. Also, it was reported before that using

a solution containing PTFE nanoparticles with diameter of 200 to 350 nm in comparison with the PTFE solution with diameter of 2 to 3 μm had better hydrophobicity [36].

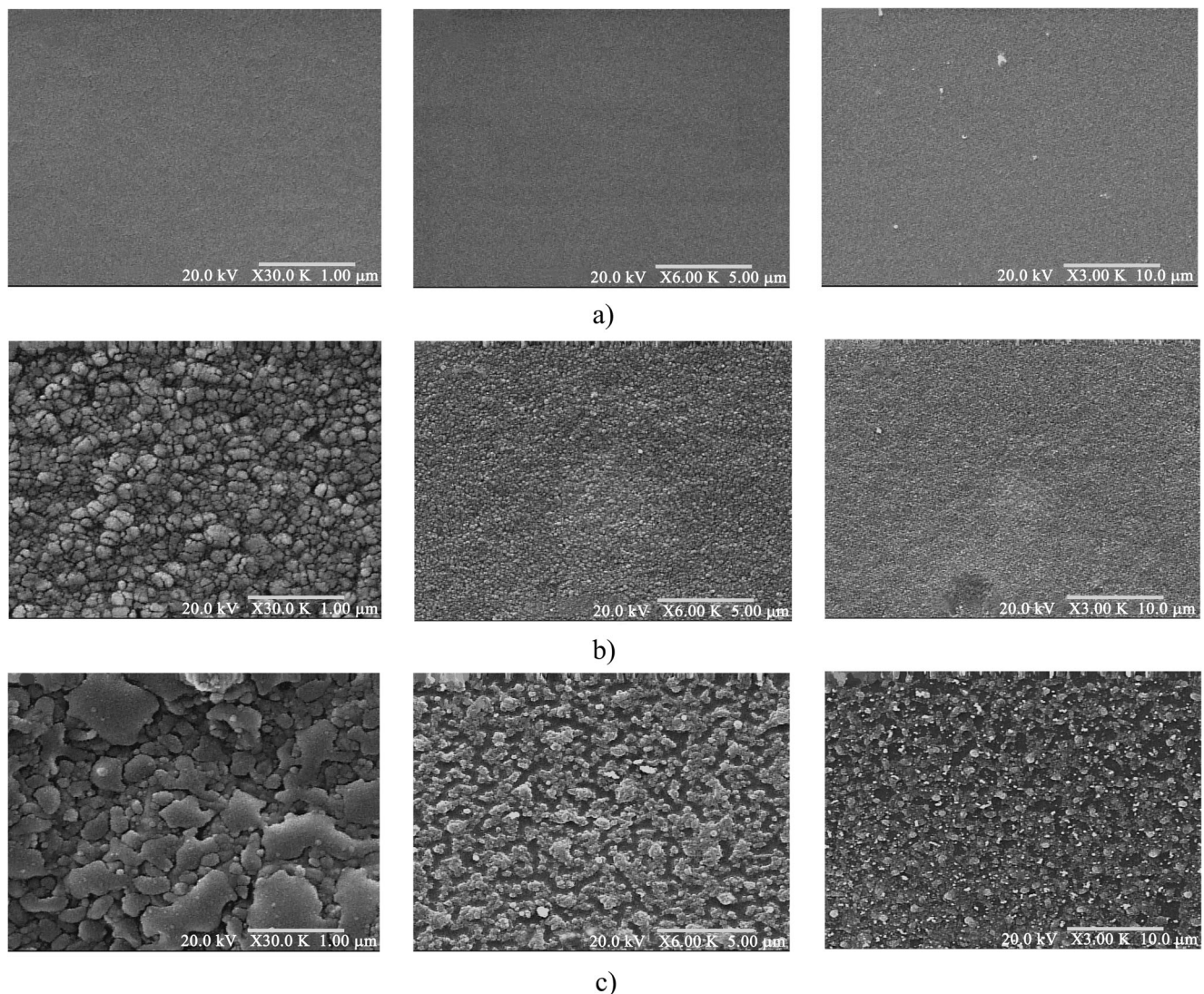


Fig. 3 SEM images of the microchip for **a** bare PDMS, **b** coated PDMS before heating, and **c** coated PDMS after heating

Coated microchip performance and simulation results

To evaluate the performance of the coated PDMS by PTFE nanoparticles, a dichloromethane solvent labeled with the fluorescein was used and flowed through the microchip. As was expected, the coated PDMS with PTFE showed excellent resistance against the organic solvent. Figure 4 illustrates the coated microchip filled with the dichloromethane. The simulation results for different flow rates for core and sheath flows are also depicted in Fig. 4.

The simulated concentration distribution in the microchips with different FRs is compared with the experimental ones.

The comparison reveals that the simulation can well predict the flow behavior of the solutions. In this microchip, the main interfering force in the synthesis of nanoparticles is FR where by adjusting FRs, the control over the size of nanoparticles is feasible. As shown in Fig. 4a, the core flow was completely squeezed by two side flows. As a result, the mixing occurs in a narrower area and mixing takes place faster than the time scale for aggregation of the particles, leading to synthesis of smaller nanoparticles. In the same way, compared to other FRs, Fig. 4d had the lowest sheath flow velocity. Hence, the area which mixing of precursor is take place is wider, and mixing happens slower in comparison to the lower FRs and larger

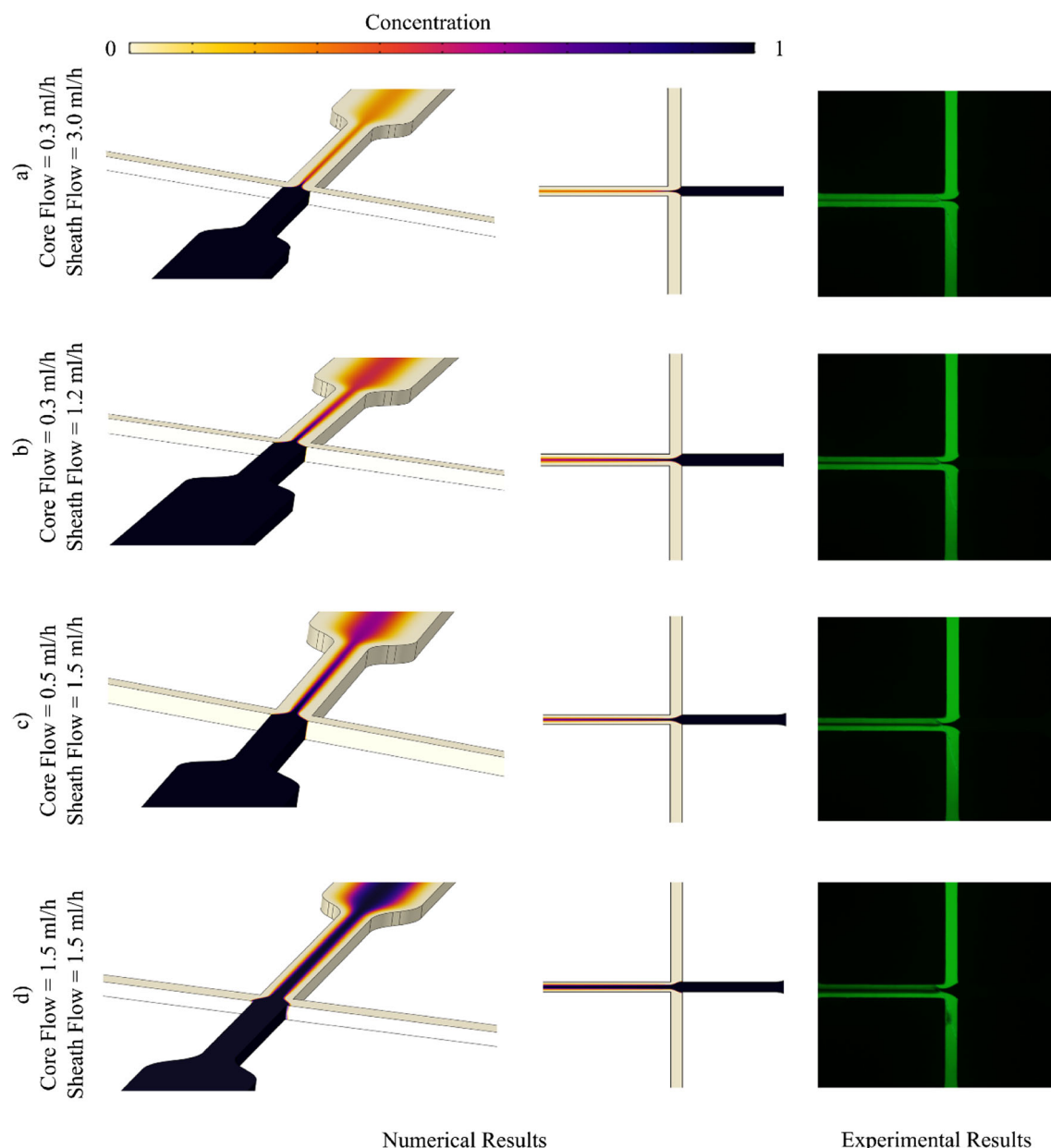


Fig. 4 The coated microchips filled with the dichloromethane with different flow rate ratios. For better sympathy, three-dimensional view of the microchips is depicted to show the interface of two fluids. As it

is obvious, numerical results are consistent with experimental ones and the flow behavior can be extracted from the numerical simulation in a great detail

nanoparticles are generated. Numerical simulation of flow behavior has been already studied in two- and three-dimensional microfluidic chips, the results of which supported our results. Recently, Amrani et al. investigated the effect of changing FRs on diameter of synthesized nanoliposomes in a two-dimensional flow focusing microfluidic platform [37].

After 10 h, there was no swelling in the channel so that the microchip prepared with this method can be used in long-term applications such as cell culture. In this study, by the simple proposed fabrication process, a smoothly coated microchip was fabricated which was resistant to the solvents and had the feature of transparency. This microchip could provide more versatility compared with the bare PDMS for nanoparticle synthesis as well as cell culture. Thus, the use of PDMS-based microchips can be broadly enhanced.

It is good to mention that for surface modification of PDMS, a material has to be used that is commercially available or easy to make. In this regard, PTFE, a perfluorinated polymer, is a proper choice. This material can be used in various environments from anti-corrosion to superclean for variety of chemical-involved processes [22]. In addition, their inert feature to almost all solvents, either organic or not, and chemicals make them a promising candidate. In addition, PTFE by decreasing the surface friction and adhesion is an appropriate choice for surface modification of materials, i.e., PDMS, to reduce the level of surface energy and enhance tribological features for industrial facilities [35].

Using PTFE for coating, the PDMS surface is a good strategy which already used to overcome the swelling problem in PDMS microfluidics chips. Recently, Ren et al. managed to make a Teflon-made microfluidic chip using Teflon films. In that study, a proper strategy was developed to fabricate a microchip that had considerable resistance to the organic solvents such as dichloromethane and chloroform and also prevented absorption of biomolecules to the PDMS context [21]. However, the complex fabrication procedure impedes its usage. The Teflon coating in this study can be performed easily and in a short time while there is no need to have any clean room or advanced facilities. Thus, it can be a proper choice even for mass production. The surface of microchannel changes to be superhydrophobic by PTFE nanoparticles coating which leads to assist the anti-fouling effects of the surface while clogging does not occur in the microchannel. Thus, these microchips can be handled for long-term usage. The function of the coated microchip is also tested by different solvents, and there is not any evidence of leakage or collapse in the microchannels. The result of our study is completely in line with previous studies which focused on PDMS surface modification to overcome the swelling problem for the synthesis of micro- and nanoparticles [15–17, 38–41].

Results of synthesis of nanoparticles

One significant aspect of this study is to introduce a myriad-minded method for synthesis of dexamethasone-loaded

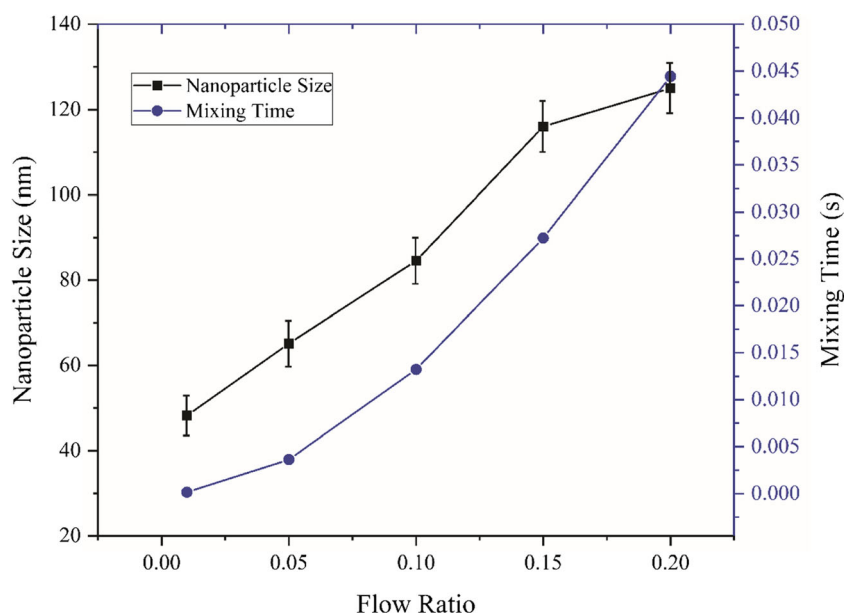
nanoparticles in a coated microchip. Synthesis of nanoparticles in the bulk mode leads to have unstable polydispersed nanoparticles which result in poor control over the encapsulation efficiency and can lead to the loss of the encapsulated material. In this paper, the controlled synthesis of PLGA nanoparticles was carried out using a nanoprecipitation method in a T-junction microfluidic chip. At the intersection of the chip, PLGA solution was cut off by the water phase which results in monodispersed nanoparticles. Dichloromethane is an evaporative solvent which evaporates rapidly when is exposed to air. Microfluidic precipitation is a well-known method in the class of bottom-up technique which works as a continuous production of a variety of materials using mixing, fluid propagation, emulsion, or combination of them. In microfluidic approach, diminutive dimensions lead to high surface to volume ratio [42], and this helps to have careful control over the features of nanoparticles in terms of uniform and rapid mass transfer. The flow condition in this study is laminar. Therefore, reaction region is limited to the interface of two streams and the reaction conditions (e.g., FR, ionic concentration, mole ratio of raw materials) can be well adjusted which helps to have precise control on the parameters in the synthesis [43].

In the current study, the microfluidic method based on the hydrodynamic flow focusing approach was used to obtain high-controlling monodispersed nanoparticles. The device was based on a T-junction microfluidic chip containing two side inlets for aqueous solution, one inlet for PLGA polymer solution with dexamethasone, and one outlet for the extraction of the nanoparticles. By adjusting the FRs, we can control to generate the desired size of the nanoparticles. According to simulation result, FRs were set as 0.01, 0.05, 0.1, 0.15, and 0.2. Mean hydrodynamic diameter for synthesized nanoparticles in every specific FR measured by DLS analysis is presented in Fig. 5. By comparing the results, it is concluded that microfluidic-based synthesized nanoparticles has narrower size distribution and smaller size in comparison to bulk synthesized ones. The average diameter of the nanoparticles synthesized in the mentioned FRs was 47, 63, 82, 115, and 125 nm, respectively. As indicated by Fig. 5, the smallest diameter for synthesized nanoparticles was obtained in lowest FR and, by increasing the FR, the nanoparticles diameter were increased. Also, the mixing time can be calculated by Eq. (8) [44].

$$t_{\text{mixing}} \approx \frac{w^2}{9D} \times \frac{1}{\left(1 + \frac{1}{\text{FR}}\right)^2} \quad (8)$$

where w is the width of the microchannel and D is the diffusion coefficient. Since the width of the microchannel was 120 μm , and FRs changed from 0.03 to 0.2; therefore, mixing time was calculated from 0.044 to 0.157 ms. The result of calculated mixing times is shown in Fig. 5. The findings of present study are consistent with other studies that used microfluidic for synthesis of PLGA nanoparticles [45, 46]. (DLS results are

Fig. 5 The nanoparticle size and mixing time for the synthesis of nanoparticles versus different flow rate ratios. The diameter of nanoparticles and the mixing time is increased with an increase in flow rate ratios



provided in Electronic Supplementary Information (ESI). For example, Karnik et al. could synthesize PLGA nanoparticles in the range of 10 to 50 nm by adjusting parameters like the initial concentration of the polymer, FRs, and the mixing time [11].

According to the results, it has been shown that nanoparticles synthesized by the microfluidic approach, due to the formation in a fast mixing regime, were smaller and more monodispersed than by the bulk mode. DLS results were also illustrated that longer mixing time led to the production of larger nanoparticles. The result of the present work is supported by the other studies which investigated the effect of associated interfering parameter in microfluidics channels like FR and mixing time [45, 47]. By decreasing the mixing time with the microfluidic method, a homogeneous environment for nucleation and growth of nanoparticles was developed. Therefore, mixing was carried out faster, and the nanoparticle precursor self-assembling was done in the direction of the linear flow. As a result, in the microfluidic approach, more homogenous, monodispersed, and smaller particles were achieved which is consistent with the results of previous studies [48]. The ununiform distribution of nanoparticles in bulk synthesis can be attributed to the long mixing time. During the bulk mixing, nanoparticle rearrangement was faster than the microfluidic method which caused heterogeneity in the structure of nanoparticles [47]. Moreover, in the microfluidic method, the ratio between the nanoparticle precursors and the anti-solvent solution could be carefully controlled which is one of the most critical factors that determine the size distribution of the nanoparticles [49]. In the bulk synthesis method, by mixing the nanoparticle precursors into the anti-solvent solution, the ratio of solvent to anti-solvent was increased gradually, while in the microfluidic method, this ratio could be controlled, and the change in this ratio could be prevented.

In the microfluidic approach, it is possible to synthesize more homogenous and controllable nanoparticles [50]. When mixing time is less than the aggregation time, homogenous nanoparticles were achieved. Nanoprecipitation synthesis involved the nucleation of nanoparticles and polymer aggregation into the nanoparticles which resulted in the formation of final size and uniform nanoparticle size distribution. This finding also supports our results which obtained by numerical simulation.

SEM images of nanoparticles

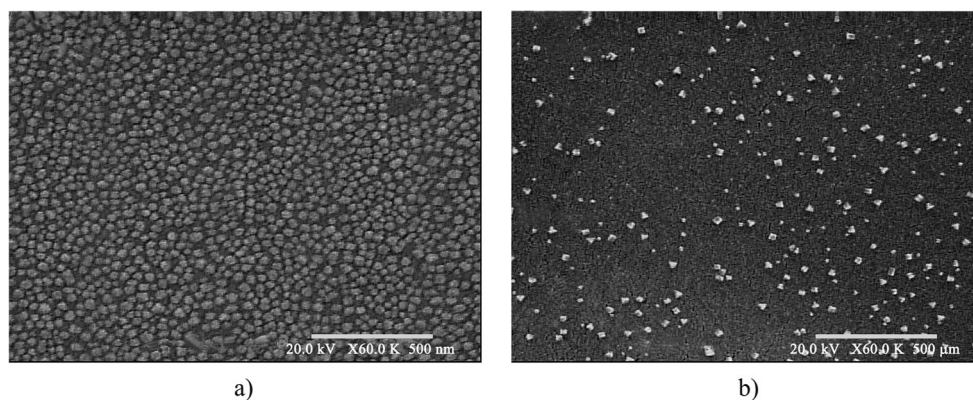
The shape and morphology of the dexamethasone-encapsulated PLGA nanoparticles were analyzed by SEM method and shown in Fig. 6.

As can be concluded from Fig. 6, the generated nanoparticles by microfluidic approach revealed uniform spherical nanoparticles along with smooth surface, and there was not any evidence of crevices. For further discussion about the nanoparticles, AFM images of the nanoparticles have to be analyzed. (Please refer to ESI for further SEM images of synthesized nanoparticles.)

AFM images of nanoparticles

The detailed nanoparticle surfaces as well as their morphology were identified by AFM approach, a method based on atomic level interaction between the tip and the samples. The unique merit of this approach is that it can extract the structure of the nanoparticles with high precision. Indeed, the clear view of the morphology of the surface of nanoparticles can easily be recognized and analyzed. The AFM images of microfluidic-

Fig. 6 SEM results of the synthesized nanoparticles using the developed microfluidic device. **a** Flow rate ratio of 0.05 (nanoparticles size = 65 nm). **b** Flow rate ratio of 0.1 (nanoparticles size = 85 nm)



based nanoparticles synthesized based on different FRs are depicted in Fig. 7.

Based on Fig. 7, the average diameter of PLGA nanoparticles was 47 nm, 63 nm, and 82 nm for FRs of 0.01, 0.05, and 0.1, respectively. The 2D and 3D views of the AFM pictures reveal that there was not any aggregation or even adhesion in the nanoparticles. Furthermore, the AFM images disclose that the nanoparticles approximately have a spherical shape, and the line and also grain analysis reveals that the nanoparticles are in the range of nanometer. In addition, the comparison of SEM and AFM images confirms that the results are consistent with each other.

Nanoparticle encapsulation efficiency and cumulative release

The semi-hydrophilic nature of PLGA polymer allows encapsulation of hydrophobic and hydrophilic compounds.

Simultaneous injection of drug/polymer is an effective strategy for encapsulation of hydrophobic drugs by nanoprecipitation. Many studies have previously used PLGA polymer as a model of biodegradable and biocompatible biomaterial to synthesize nanoparticles by nanoprecipitation for a variety of biomedical applications. As discussed in previous studies, the utilization of nanoprecipitation controlled by microfluidic method increases encapsulation efficiency, significantly, compared to the traditional bulk synthesis methods [11, 45, 51–53]. In this study, dexamethasone drug as a hydrophobic material was encapsulated in PLGA nanoparticles. The calculated encapsulation efficiency via microfluidic method was more than 80% (in 10 wt% of initial dexamethasone content) which was much higher than the bulk mode. Higher encapsulation efficiency leads to an increase in the efficiency of the system. Therefore, the controlled synthesis of nanoparticle in the microfluidic method can enable more drug loading compared to the bulk mode. Figure 8 shows the

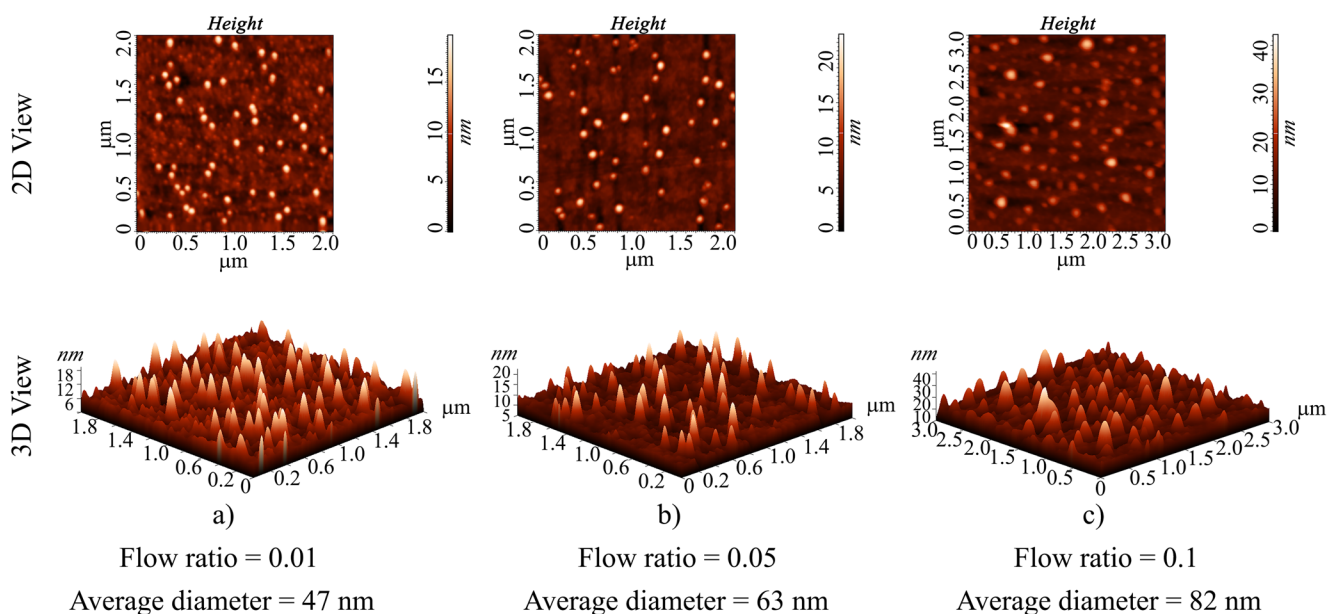


Fig. 7 AFM images of the nanoparticles with two different views of 2D and 3D for better understanding. **a**: flow ratio 0.01, **b**: flow ratio 0.05 and **c**: flow ratio 0.1. The results are presented for three separate FRs

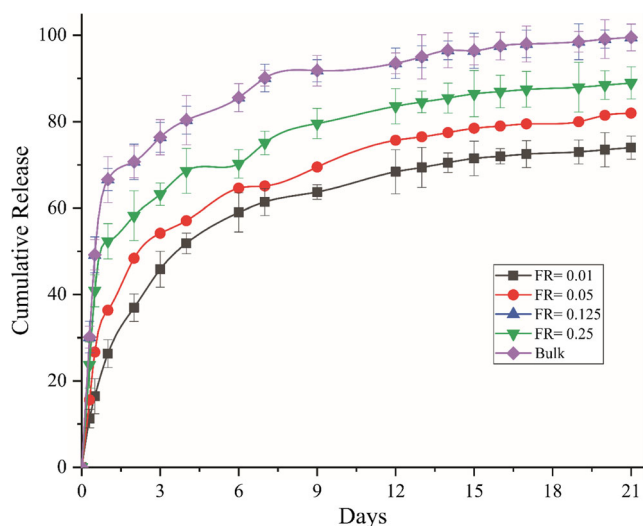


Fig. 8 The cumulative release of the dexamethasone encapsulated in PLGA nanoparticles for 21 days in different FRs

normalized profile of the cumulative release at pH = 7.4 and 37 °C.

The cumulative release of drug at any point in time has been normalized to the drug loaded in the nanoparticles. As is clear in Fig. 8, the cumulative release of dexamethasone has a two-phase pattern. The first phase is characterized by a rapid release which may be the result of the solubility of drugs at the surface of the nanoparticles. The second phase is specified by its slow release which can be as a result of degradation of the polymer matrix and the release of the trapped drug. It is concluded from Fig. 8 that synthesized nanoparticles in the bulk mode have a faster release rate than the nanoparticles synthesized via microfluidic approach. The reason behind this fact is related to the compact and robust structure of microfluidic-based nanoparticles. Based on Fig. 8, after 21 days, 74%, 79%, 82%, and 89% of dexamethasone were released from the nanoparticles synthesized with FRs of 0.01, 0.05, 0.0125, and 0.25 while for the bulk synthesis, it reached 99.5%. In addition, the half-release time of drug, known as $t_{50\%}$, was 3 days, 4 days, 3 days, and 1 day for FRs of 0.01, 0.05, 0.0125, and 0.25, whereas for bulk synthesis, it reached the half of an hour. It is good to mention that similar to the corresponding study, release of proteins from nanoparticles was also found in the literature with the same profile [24, 48, 51]. According to previous works, microfluidic synthesized nanoparticles are more compact than bulk synthesized ones, leading to slower diffusion of cargo out of nanoparticle matrix and sustained release of cargo [54]. Sadrabadi et al. produced dexamethasone-loaded chitosan nanoparticles which used for in vitro bone remodeling using mesenchymal stem cells. The synthesized nanoparticles have more encapsulation efficiency and longer period of release in comparison to bulk synthesized ones [24]. Several studies have focused on dexamethasone delivery by micro- and nanocarriers in conventional bulk method. In the bulk synthesis nanoparticles for dexamethasone delivery,

burst release and low encapsulation efficiency are major problems [55–57]. Based on the results obtained by current study, microfluidic-based synthesized nanoparticles have smaller size as well as narrower size distribution compared to nanoparticles synthesized by bulk mode. Also, the microfluidic based nanoparticles have better encapsulation efficiency and efficient controlled release. As previously mentioned, using glucocorticoids in high doses can inhibit the osteogenesis process in vitro and in vivo [58]. For this reason, using constant and controlled doses of this drug is of great importance. In our microfluidics synthesized nanoparticles with smallest size (FR = 0.01), only 16% of drug have exerted out of nanoparticles in first 12 h of release while this amount for bulk synthesized nanoparticles was about 50%. This indicates that reduced burst release of dexamethasone can reduce inappropriate side effect of this drug. Thus, by adjusting the initial dose of dexamethasone, the exact amount of dexamethasone in cell culture media can easily be managed.

MTT results

Although PLGA was already reported as a biocompatible polymer, for assuring of synthesis procedure and removing the organic solution, cytotoxicity of unloaded nanoparticles was evaluated. MTT method was used to investigate the affected cell viability of PLGA nanoparticles in human mesenchymal stem cells (hMSCs). Figure 9 shows the cell viability of hMSCs at 37 °C and after exposure to the PLGA nanoparticles for 72 h.

As is concluded from Fig. 9, the synthesized nanoparticles either by microfluidic approach or by bulk mode showed non-toxic behavior, even though the used concentration was 800 nM. In addition, cell viability was decreased when the nanoparticle size was increased. Furthermore, by increasing the concentration, the cell viability was decreased.

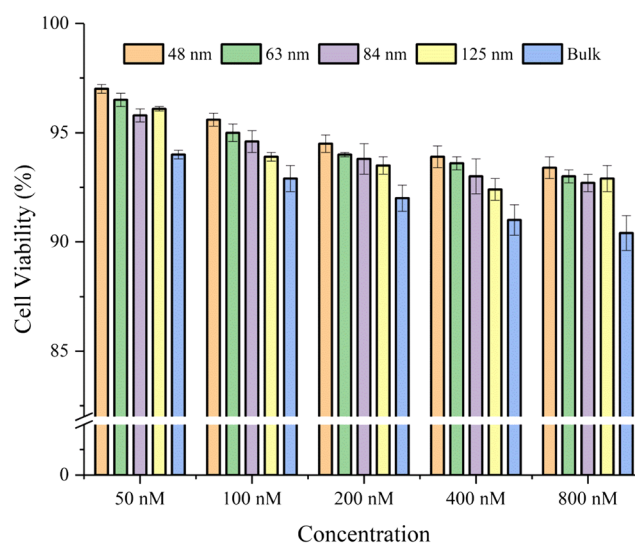


Fig. 9 The percentage of cell viability of the unloaded nanoparticles in different diameter sizes for various concentrations

Dependence of cell viability to size and concentration of nanoparticles are already reported by other studies, and the result of present study was in line them [24, 59]. Generally, PLGA nanoparticles are approved to be low-cytotoxic with perfect biodegradability and biocompatibility.

Conclusion

The main aim of this study was to synthesize the dexamethasone-encapsulated PLGA nanoparticles via microfluidic approach within a PDMS-based microchip. Since in the synthesis process, organic solvents existed, and swelling of the PDMS inhibited the nanoparticle synthesis, hence, a simple and easy-to-reach method was used to coat the PDMS with PTFE nanoparticles in order to prevent adhesion and aggregation of the solvents. The water contact angle illustrated that the contact angle was changed from 59.37 to 140.30° which resulted in superhydrophobicity of the microchannels. Thereafter, the surface of the microchannels was also analyzed by SEM method to check the surface of the coated PDMS. The results elucidated that after heating of the coated microchip, more surfaces of the walls covered with PTFE and thus the hydrophobicity increased. For study of flow behavior inside the microchannels and to optimize the FRs which used in synthesis procedure, finite element method was used for simulation. Afterwards, the dexamethasone-loaded in PLGA nanoparticles were synthesized. SEM and AFM results showed that there was not any evidence of adhesion or aggregation of PLGA nanoparticles and the nanoparticles were monodispersed. In addition, AFM results revealed that average diameters of 47, 63, and 82 nm were achieved in FRs of 0.01, 0.05, and 0.1. The encapsulation efficiency via microfluidic method was also calculated which was more than 80% which enables controlled synthesis compared with the bulk mode. The cumulative release was also reported after 21 days which was 74%, 79%, 82%, and 89% in FRs of 0.01, 0.05, 0.0125, and 0.25 while for the bulk synthesis, it reached 99.5%. At the end, MTT test was revealed that the generated PLGA nanoparticles could be considered as non-toxic ones. Since the synthesis of drug-loaded nanoparticles are mostly performed in the presence of organic solvents, the simple and convenient approach used in this study can be applied in this regard.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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