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Fabrication of covered porous PLGA microspheres using hydrogen peroxide for controlled drug delivery and regenerative medicine

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ABSTRACT

Poly(lactic-co-glycolic acid) (PLGA) microsphere has been a useful tool in delivering therapeutic drugs and biologically active proteins. In this study, a covered porous PLGA microsphere was manufactured using $W_1/O/W_2$ double emulsion solvent evaporation method, utilizing hydrogen peroxide as a novel porogen. An enzymatic reaction between hydrogen peroxide and catalase produced oxygen bubbles and thus many internal pores within microsphere were naturally developed. When different molar ratios between lactide and glycolide in PLGA were examined, the ratio, 50:50 showed the most organized porous microstructure. Higher molecular weight of PLGA seemed to be favorable in creating a porous structure. By testing various concentrations of hydrogen peroxide, it was found that rather concentrated one was more efficient in developing a porous network in the microspheres. The source of the skin layer that covers the whole surface of the microsphere was found to be PLGA, not polyvinyl alcohol (PVA). The residual amount of hydrogen peroxide was negligible after a thorough evaporation of PLGA microsphere. When release profiles of dexamethasone (Dex) with morphologically different microspheres such as, nonporous, covered porous, and porous, were investigated for up to 28 days *in vitro*, their release patterns were found to be significantly different on a temporal basis. The present work demonstrated that the covered porous PLGA microspheres could be successfully fabricated using hydrogen peroxide and that the covered skin layer on the PLGA microsphere played an important role in determining the characteristic release profiles of Dex.

1. Introduction

A controlled release of therapeutic agents that target specific diseases or injured sites is very important in the area of drug development and regenerative medicine. To achieve the goal of controlled release, a proper delivery system is of particular interest in terms of both efficiency of drug loading and effectiveness of treatment at target sites. Specifically focused on the viewpoint of tissue regeneration, the major role of carriers is to deliver bioactive growth factors that are essential for the induction of cell differentiation into a specific lineage, especially for stem cells [1–3]. Among a variety of candidates, polymeric microspheres have been widely utilized as a favorable tool in delivering various cytokines and proteins, due mainly to their biodegradability in human body and plasticity in fabrication. Silva et al. developed starch-based microparticles to incorporate and to release platelet-derived growth factor (PDGF) [4]. They reported a constant release for up to 8 weeks and the maintenance of biological activity after the incorporation. Meanwhile, transforming growth factor-beta (TGF-β) and insulin-like growth factor-1 (IGF-1) were incorporated together in PLGA microspheres and then photoencapsulated with bovine articular chondrocytes in polyethylene oxide (PEO)-based hydrogels for *in vitro* culture [5].

While biodegradable polymeric microspheres are manufactured using various techniques, water-in-oil-in-water (W/O/W) double emulsion method is considered to be the most common one. The whole procedure of microsphere preparation seems to be simple but many parameters that affect the morphology of microsphere have been addressed. Crotts et al. investigated the effect of inner water volume to observe the internal and external structure of PLGA microsphere [6]. While the hollow microsphere with nonporous surface layer was prepared with an initial aqueous volume fraction of 5.6%, the hollow one with porous surface was obtained with that of 22.7%. On the other hand, volume change in oil phase or varying polymer concentrations would result in different patterns of solvent exchange and precipitation mechanism that caused morphological difference and altered loading efficiency [7]. Higher stirring speed was also a contributing factor, reducing the microsphere size and decreasing the yield of microsphere [7]. Even the preparation temperature influenced a final morphology of PLGA microsphere as well as the release profile of encapsulated protein [8]. In fact, both

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external and internal morphologies have a great influence in determining the release profile of incorporated components within microspheres. When recombinant human growth factor was encapsulated in either porous or pore-closed surface of microsphere, two totally different release profiles were obtained, where extremely high initial burst occurred with the porous surface, while sustained release was obtained with the pore-closed surface [9]. Meanwhile, microspheres themselves are often united in a three-dimensional (3D) scaffold as a subunit. For site-specific delivery of basic fibroblast growth factor (bFGF) to a grafted skin, bFGF-loaded chitosan–gelatin microspheres were fabricated and incorporated into a porous chitosan–gelatin scaffold [10]. Biodegradable PLGA microspheres containing bovine serum albumin (BSA) were even fused together to form a 3D scaffold in which the pore size was controllable, depending on the size of microspheres [11].

In this study, a novel method to fabricate covered porous PLGA microspheres was introduced. The key factor was hydrogen peroxide, a gas-forming agent that is decomposed into water and oxygen in the presence of catalysts. During the preparation of microspheres using double emulsion solvent evaporation method, a variety of contributing factors were evaluated. Release profiles of dexamethasone (Dex) were also investigated using Dex-incorporated, hydrogen peroxide-derived PLGA microspheres with different morphological features. We hypothesize that the use of hydrogen peroxide is simple and particularly effective in making porous PLGA microspheres having a thin layer cover over the surface.

2. Materials and methods

2.1. Materials

PLGA polymers were purchased from Boehringer Ingelheim (Ingelheim, Germany). Polyvinyl alcohol (PVA, 87–89% hydrolyzed, Mw 13,000–23,000) and Dex were obtained from Sigma (St. Louis, MO). Hydrogen peroxide (H_2O_2, 50 wt.%) was obtained from Sigma-Aldrich (St. Louis, MO) and catalase (Trilon Net-100) was purchased from Kookje Industrial Co. (Gyeonggi, Korea). Other chemicals and solvents were of analytical reagent grade.

2.2. Preparation of covered porous PLGA microspheres

The covered porous PLGA microspheres were prepared using $W_1/O/W_2$ double emulsion evaporation method. Once PLGA was dissolved in 6 ml of methylene chloride (MC) and then 5 ml of hydrogen peroxide was added, a W_1/O emulsion phase was created using homogenizer (Ultra Turrax® T18 basic, IKA, Staufen, Germany) at 5000 rpm for 3 min. The primary emulsion was transferred to a beaker containing 300 ml of PVA solution (0.2%, w/v) and re-emulsified using an overhead propeller for 4 h at 70 rpm. A catalytic enzyme, catalase was then added to the $W_1/O/W_2$ emulsion phase. As the solvent was evaporated, the PLGA microspheres were precipitated and harvested in centrifugation. The covered porous microspheres were then washed three times with distilled water and lyophilized using a freeze dryer.

2.3. Fabrication of different morphology of microspheres

While the covered porous PLGA microspheres were fabricated using hydrogen peroxide, various factors that affect the final morphology of microspheres were selectively tested. First, influence of the final concentrations of PLGA (w/v) was screened using 5, 8, and 11%, respectively. To examine the effect of the molar ratio between lactide and glycolide, the different ratios of PLGA – 50:50, 75:25, 85:15, and 95:5 – were used in manufacturing microspheres, respectively. Dependence of polymer molecular weight (Mw) was evaluated using different Mw of PLGA: 20,000, 50,000, 80,000, and 230,000 Daltons. In addition, different concentrations (v/v) of hydrogen peroxide – 3, 10, 15, 35, and 50% – were individually added in the W_1/O emulsion phase and their effects on the formation of porous PLGA microspheres were evaluated. Meanwhile, the influence of the volume ratio (v/v) between hydrogen peroxide and MC in the primary emulsion phase was also investigated, maintaining the ratios at 1:9, 1:4, 2:3, 1:1, and 3:2, respectively.

2.4. Analysis of covered porous PLGA microspheres

Morphological characteristics of microspheres were observed with a field emission-scanning electron microscope (FE-SEM; S-4100, Hitachi, Tokyo, Japan). For the sample preparation, freeze-dried microspheres were spread on the sticky surface of an aluminum stub and they were coated with platinum/palladium for 80 s using an ion sputter (E-1030, Hitachi, Tokyo, Japan) at 0.1–0.05 Torr and 10 mA of ion current. The microspheres were cut into halves and then subjected to SEM inspection for their internal morphology. Microsphere diameter was analyzed using laser diffraction particle size analyzer (LS230, Beckman Coulter Inc., CA) and pore size was manually calculated from the SEM images. Microspheres were also analyzed using differential scanning calorimeter (DSC; Model 2910, TA instruments, New Castle, DE) in order to investigate glass transition temperature (T_g) and melting temperature (T_m). The DSC measurements were carried out under nitrogen atmosphere in the temperature range from −50 to 250 °C, with a heating rate of 10 °C/min. The Mw of PLGA microspheres was determined by using gel permeation chromatography (GPC; VE2001, Viscotek, Houston, Texas). A calibration curve was obtained using different molecular weights of the polystyrene standards and the weight average Mw of each sample was then determined. For surface chemical analysis, Fourier transform infrared spectrometer (FTIR; JASCO615, Jasco Inc., Easton, MD) was harnessed in the frequency range of 400–4000 cm^{-1}. The surface of microspheres was further analyzed by using proton nuclear magnetic resonance spectrometer (^1H NMR, Gemini 200, Varian Inc., Palo Alto, CA), operating at 200 MHz. Meanwhile, the diameter of the microspheres was analyzed using a laser diffraction particle size analyzer and its pore size was manually determined from the SEM images. In addition, the internal porosity of PLGA microspheres was approximately sought from a manual calculation, based on the pore-occupied area over the entire cross-sectional area of microsphere.

2.5. Determination of residual hydrogen peroxide

The residual amount of hydrogen peroxide in the PLGA microspheres was determined following the early report [12]. In brief, 25 ml of isopropanol was added to the microspheres (10 mg), followed by the addition of 1 ml of saturated potassium iodide (KI) and 1 ml of glacial acetic acid. When the mixed solution was heated to the boiling point and PLGA microspheres were melted, the color of hydrogen peroxide-containing solution turned yellow. Titration was conducted using sodium thiosulfate solution and the endpoint of titration was the disappearance of the yellow color.

2.6. Incorporation of Dex and in vitro release test

To incorporate Dex into the PLGA microspheres, 50 mg of Dex was added in the W_1/O emulsion phase. After the recovery of the fabricated Dex-loaded microspheres, they were dissolved in 10 ml of acetonitrile and vigorously mixed, and the solution was then filtered. Concentration of the encapsulated Dex was quantified using an UV spectrophotometer (J-560, JASCO, Tokyo, Japan) at 235 nm. The loading efficiency (%) was determined based on the ratio of the amount of encapsulated Dex to the initial amount. Meanwhile, a release pattern of Dex from the PLGA microspheres was monitored *in vitro*. Four groups of microspheres with different morphologies – one nonporous, two covered porous, and one porous – were prepared,

controlling the volume of hydrogen peroxide. For the nonporous microsphere, hydrogen peroxide was not used, while the other three groups (two covered porous microsphere and one porous microsphere) were fabricated using 10, 35, and 50% of hydrogen peroxide, respectively. The samples (n=3, each group) were weighed in 10 mg and immersed in a 5 ml vial containing 1 ml of phosphate-buffered saline (PBS, pH 7.4) at 37 °C. While each sample stayed for up to 28 days under static condition, the PBS solution was collected at the predetermined time interval and replaced with a fresh one. The amount of the released Dex was determined by the UV spectrophotometer at 242 nm.

2.7. Statistical analysis

All the data were expressed as means ± standard deviation (SD). Statistically significant difference was sought using Student's t test. The difference was considered significant when p value is lower than 0.05.

3. Results and discussion

3.1. Effect of hydrogen peroxide

The covered porous PLGA microsphere was successfully manufactured using $W_1/O/W_2$ double emulsion solvent evaporation method in which hydrogen peroxide was utilized, instead of using common effervescent salts. While microspheres have been made from a variety of methods [13–16], to our best knowledge, no studies have taken advantage of hydrogen peroxide as a key player of creating a porous structure of microsphere. Without hydrogen peroxide in the W_1 phase, PLGA microsphere was very poor in developing the internal pores. While there were no pores formed inside the microspheres from the PLGA/MC phase, some pores of irregular size and shape were noticed in the microspheres from the PLGA/MC phase to which H_2O was added (Fig. 1a and b). The effect of hydrogen peroxide was clearly visible with the nicely distributed and interconnected pores in which the pore diameters were ranged from 5 to 20 μm (Fig. 1c). It is reasoned that when the hydrogen peroxide-containing W_1/O emulsion phase was dropped into the W_2 phase of both PVA solution and catalase, an enzymatic reaction could occur between hydrogen peroxide and catalase. It seemed that the catalyst, catalase passively diffused into the primary emulsion droplets and reacted with hydrogen peroxide in the W_1 phase. This phenomenon generated oxygen bubbles and as a result, many internal pores were naturally developed during the solvent evaporation and the following polymer solidification. Although a full understanding of such reactions, e.g., intensity and duration, is unobvious at this time, the present condition is supposed to be feasible to make the covered porous PLGA microspheres. In the early report, Kim et al. used ammonium bicarbonate as an effervescent salt in the primary W_1 droplets [17]. This system produced gas bubbles during the solvent evaporation process and thus small and large pores were created inside PLGA microspheres as well as on the surface. As compared to the hydrogen peroxide-derived ones, some notable differences were the diameter of microsphere and skin layer. While the early study showed the diameter ranged from 100 to 400 μm without a skin layer, the current work displayed a diameter less than 150 μm with the skin layer. Details about the skin layer are discussed later.

3.2. Effect of various factors on morphology

During the fabrication of the covered porous PLGA microsphere, many contributing factors are involved in determining a final morphology of the microsphere. When various final concentrations of PLGA solution (5, 8, and 11%, w/v) were tested, the covered porous PLGA microspheres were successfully fabricated in all cases (data not shown). Upon the inspection of them, the microspheres from 8% PLGA solution were chosen for further study. Taken into consideration the molar ratio of PLGA copolymer, the effect of changing molar ratios between lactide and glycolide – 50:50, 75:25, 85:15, and 95:5 – was investigated. While the ratio of 50:50 showed a well-organized, covered porous microstructure, the remainders presented a poor morphology with enlarged internal pores (Fig. 2a). Interestingly, with the increment of lactide ratio, the internal pore size significantly increased but the total number of pores decreased. This result can be explained by the unique property of the copolymer: as the lactide ratio increases, the polymer becomes mechanically stiff. In fact, the formation of interconnected pore structure with poly (L-lactic) acid (PLLA) was nearly impossible using hydrogen peroxide (data not shown). It just produced a few randomly scattered pores. The mechanism is mostly likely that hydrogen peroxide-containing W_1 droplet might be unable to be dispersed uniformly in the W_1/O emulsion phase of higher molar ratios that was physically stiff and inflexible, as compared to PLGA with the ratio of 50:50. Such an environment could enforce a coalescence of droplets themselves, leading to some inflated and localized W_1 droplets. Therefore, when the primary emulsion phase was contacted with the catalase during

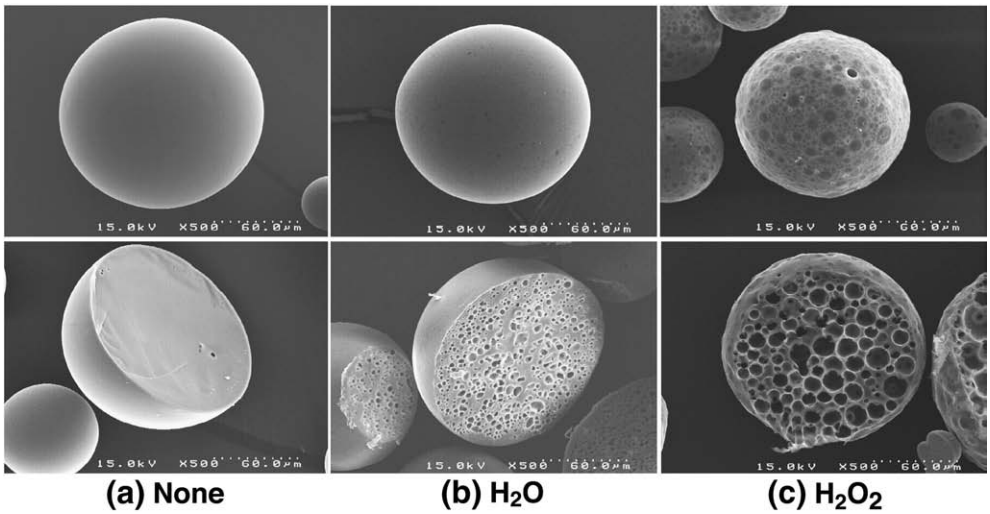

Fig. 1. Effect of hydrogen peroxide as a porogen. Different morphologies of PLGA microspheres were prepared using: no porogen (a), water (b), and H_2O_2 (c). The efficacy of hydrogen peroxide was notable in making porous structure inside PLGA microsphere.

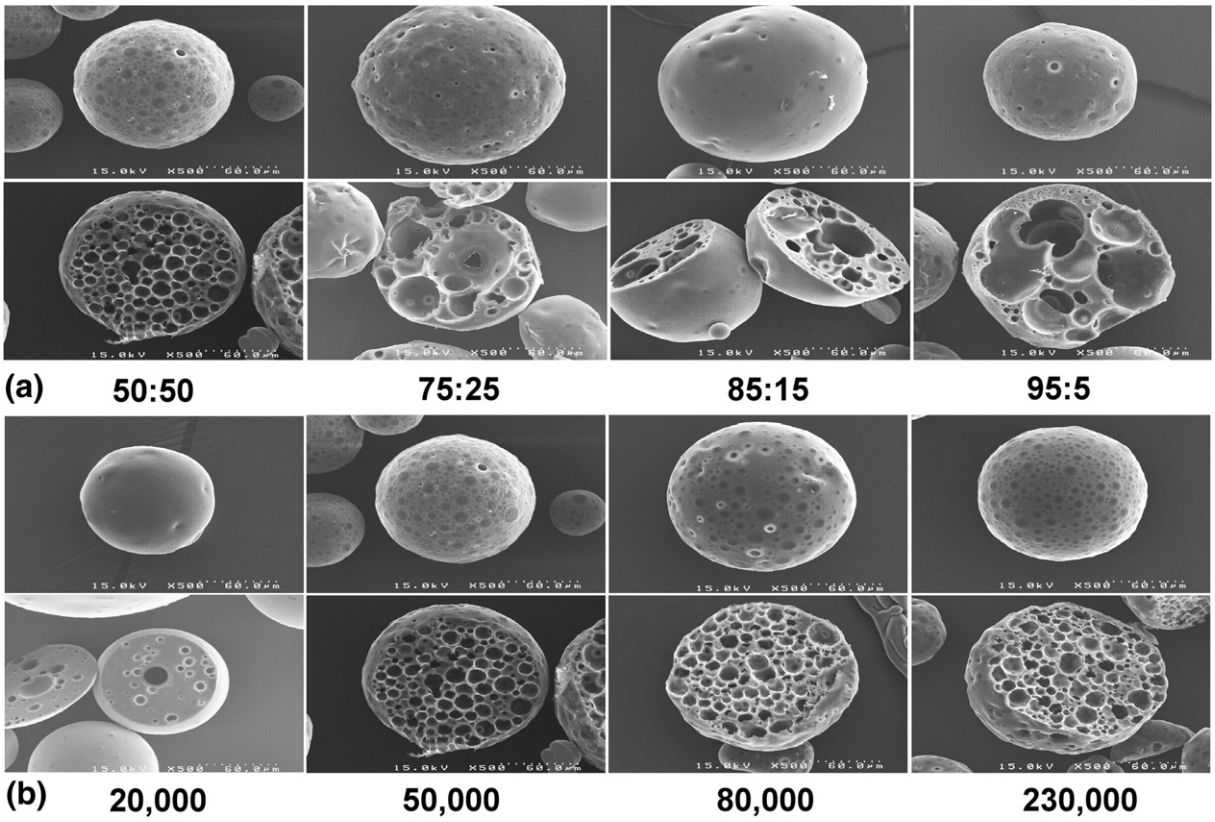

Fig. 2. Effect of either PLGA molar ratio (a) or molecular weight (b) on the formation of covered porous microspheres. The molar ratio of 50:50 showed a well-organized porous microstructure, whereas other ratios presented a poor morphology. The formation of porous network was unsuccessful with lower Mw of 20,000 but for PLGA polymers with higher Mw, they developed a large number of homogeneous pores that were evenly distributed and interconnected.

the re-emulsification, this condition would develop a small number of large pores.

On the other hand, with the molar ratio fixed at 50:50, the influence of Mw of PLGA was also found to be significant on the morphology of internal pores: as Mw of PLGA increased, lots of internal pores were nicely created (Fig. 2b). For Mw of 20,000, the formation of porous network was barely possible. However, in case of higher Mw of polymers, they successfully developed a large number of homogeneous pores that were evenly distributed and interconnected to each other. Because the only difference was the Mw of each PLGA, it was reasoned that the intrinsic characteristics of low Mw of PLGA, such as unfavorable physical and mechanical properties were mostly responsible for the failure of making a good porous structure. Effect of hydrogen peroxide itself was also examined, with the Mw of PLGA at 50,000. Since the as-received concentration of hydrogen peroxide was 50%, a serial dilution (v/v) of the stock solution was prepared in order to produce five different concentrations: 3, 10, 15, 35, and 50%, respectively. As expected, since the morphological difference was remarkable for each concentration, it seemed that there was a threshold concentration of hydrogen peroxide to generate uniform pores inside the polymer microspheres (Fig. 3). The less concentrated hydrogen peroxide resulted in a poor porous structure, whereas more

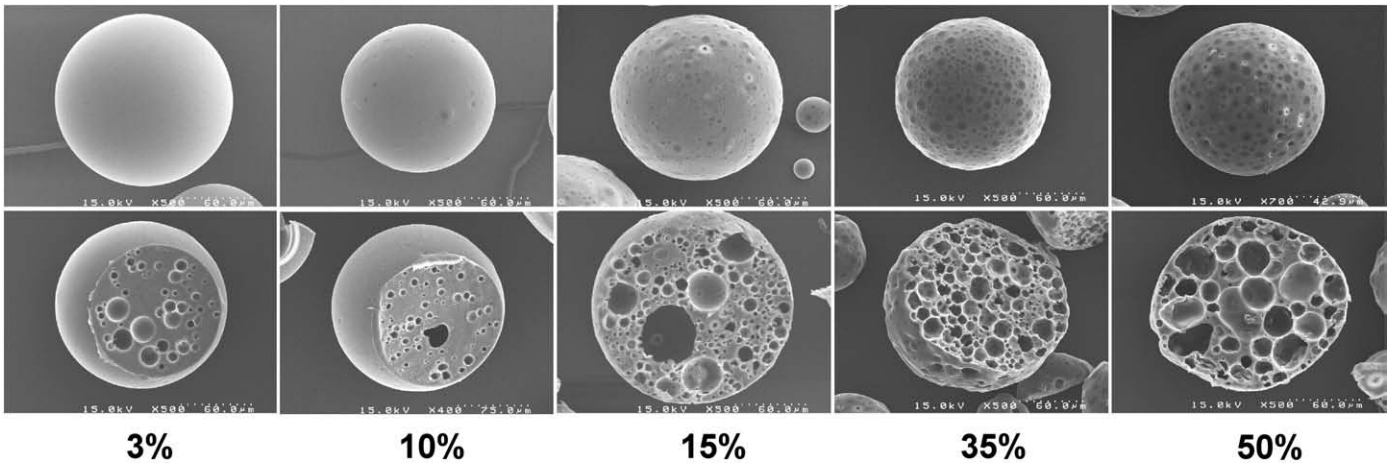

Fig. 3. Influence of hydrogen peroxide concentrations on the formation of covered porous PLGA microspheres. Depending on the contents of hydrogen peroxide, the morphological differences were remarkable.

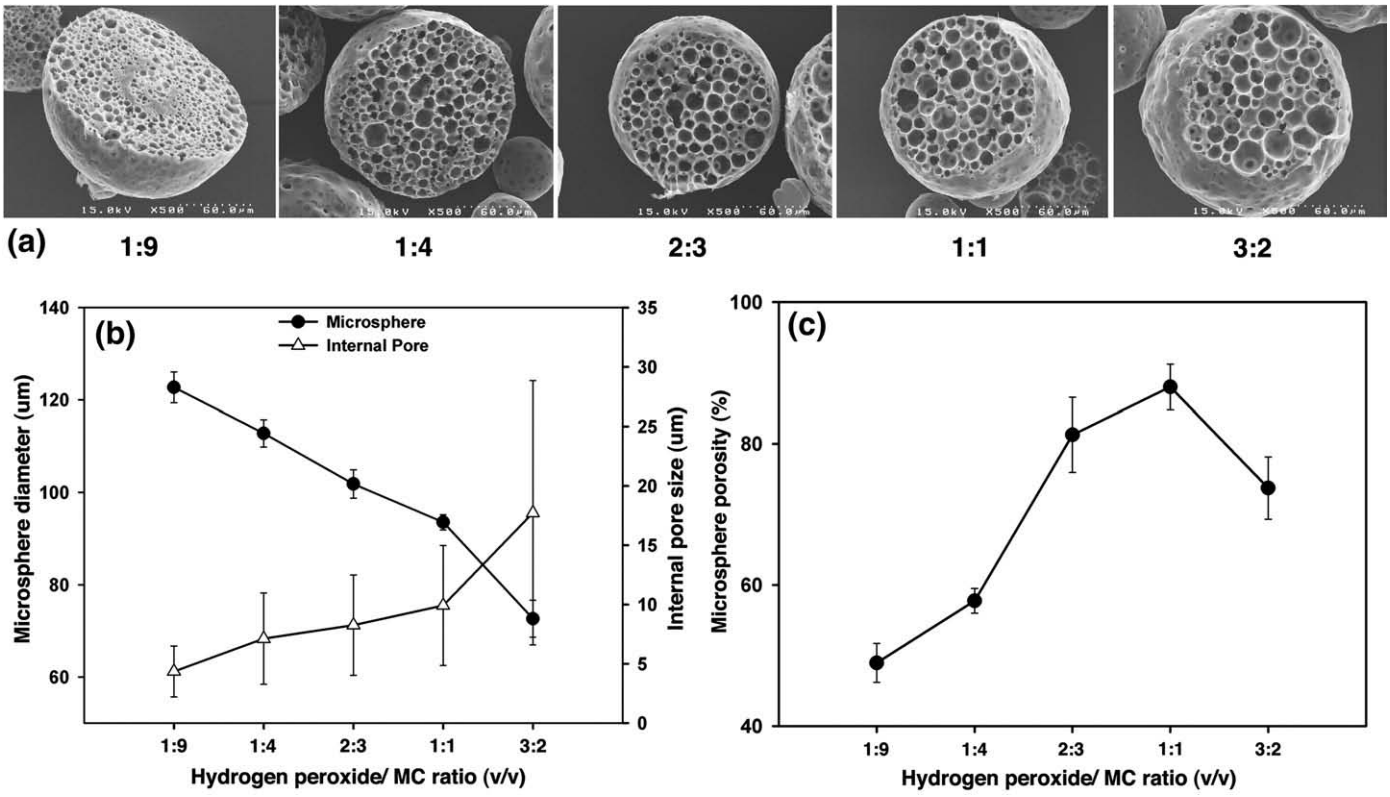

Fig. 4. Effect of the volume ratios of hydrogen peroxide/MC on the construction of porous PLGA microspheres. Overall morphological features appeared insignificant but subtle differences were still present in the tested microspheres (a). The pore size and diameter of microspheres (b) and internal porosity (c) was also measured.

concentrated solution (35%) produced well-distributed pores. Much highly concentrated one (50%), however, created relatively enlarged pores. This result suggests that an excessive amount of hydrogen peroxide can lead to an explosive reaction with the enzyme and as a result, the W_1 droplets may be fused to form largely inflated bubbles during the re-emulsified phase.

The effect of hydrogen peroxide was further examined with different volume ratios (v/v) of hydrogen peroxide and MC in the W_1/O emulsion phase: 1:9, 1:4, 2:3, 1:1, and 3:2, respectively. The overall morphological differences appeared to be insignificant but subtle disparities were still present (Fig. 4a). It was evident that individual pore size increased with the volume ratios, whereas the diameter of microsphere itself decreased. This fact was supported from the direct measurement of both parameters. As compared to the other ratios, the wide variations of internal pore size occurred at the ratio (3:2) that retained relatively higher volume of hydrogen peroxide in the primary emulsion phase (Fig. 4b). Although the results are not dramatic as seen in Fig. 3, they indicate that a fine balance between hydrogen peroxide and MC needs to be coordinately adjusted for W_1/O emulsion phase. Meanwhile, based on the ratio between the void area and the whole cross-sectional area of the microsphere, when the internal porosity was manually determined, it continuously increased from about 50% at 1:9 until a maximum porosity of 85% on average was reached at the volume ratio of 1:1 (Fig. 4c). An excessive volume of hydrogen peroxide (3:2) rather decreased the entire porosity, along with the larger pores. The measurements of porosity demonstrated that the internal porosity of the microsphere was controllable without significantly compromising the porous network.

3.3. Characterization of skin layer

An interesting phenomenon of hydrogen peroxide-derived porous PLGA microspheres was the presence of a thin skin layer that covers the whole surface of porous microsphere. As noticed from the images of microspheres in Fig. 3 (see 3% vs. 35%), the emergence of skin layer was obvious when appropriate conditions were met during the fabrication of porous microspheres. This layer was not seen in the study using ammonium bicarbonate as a porogen during the preparation of open pore PLGA microsphere [17]. The source of skin

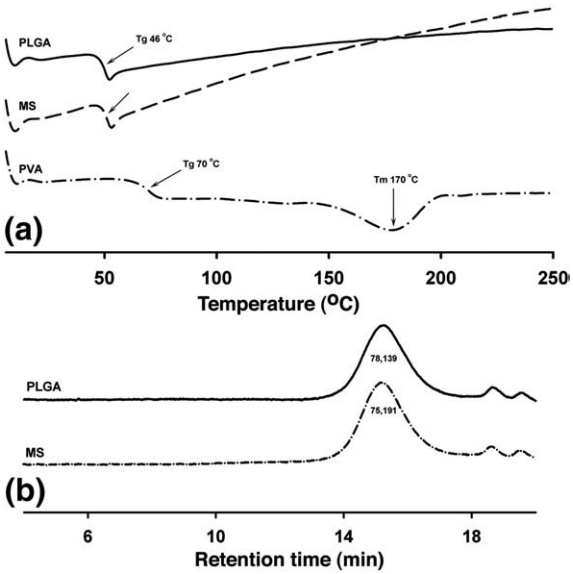

Fig. 5. Analysis of the skin layer of the covered porous PLGA microspheres using DSC (a) and GPC (b), respectively. For PLGA microsphere, the specific peak at 46 °C was exactly overlapped with that of PLGA polymer itself but it failed to match with that of PVA. The results of GPC also showed that the Mw of PLGA coincided with that of PLGA microsphere.

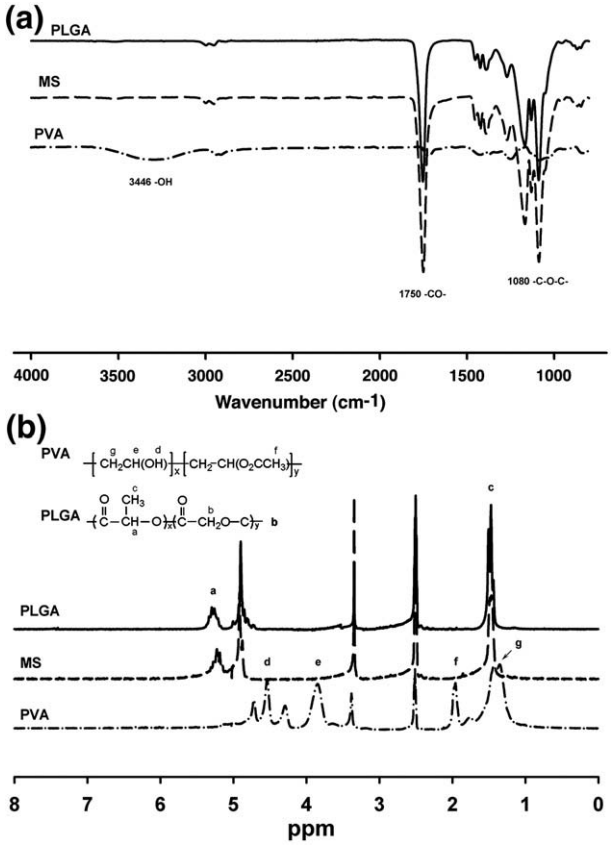

Fig. 6. Analysis of the skin layer of the covered porous PLGA microspheres using FTIR (a) and ^{1}H NMR (b). For PLGA microsphere and PLGA polymer, the multiple peaks were found overlapped in FTIR and ^{1}H NMR spectra.

layer, presumably either PLGA or PVA, was sought using some analytical tools such as, DSC, GPC, FTIR, and ^{1}H NMR. When three candidates, PLGA microsphere, PVA, and PLGA polymer were individually tested, the DSC thermograms represented the characteristic peaks of each at melting (T_m) and glass transition temperature (T_g) (Fig. 5a). For PLGA microsphere, the specific T_g peak at 46 °C was exactly overlapped with that of PLGA but it failed to match with that of PVA. The results of GPC also showed that the Mw of PLGA coincided with that of PLGA microsphere (Fig. 5b). Furthermore, the data from FTIR and ^{1}H NMR supported the same conclusion. For the microsphere and PLGA polymer, multiple peaks were found overlapped in the FTIR (Fig. 6a) and ^{1}H NMR spectra (Fig. 6b). According to these analytical data, the source of skin layer was PLGA itself.

As a matter of fact, skin layer is a unique phenomenon so that only a few studies reported its presence on the microsphere surface. Yang et al. reported that while PLGA microspheres fabricated at high temperature had a uniform internal pore and a very thin dense skin layer, those manufactured at lower temperature carried a thicker but porous skin layer [8]. In the study of Mao et al., PLGA microspheres seemed to have a skin layer on the surface fabricated from $W_1/O/W_2$ double emulsion technique [18]. In addition, prepared by using liquid–liquid phase separation, DNA-loaded polysulfone microsphere retained a thick skin layer on the outer surface and many pores inside [19]. Formation of skin layer on the porous microsphere may be a natural process that is governed by thermodynamics of phase separation and precipitation. During re-emulsification and following solvent removal, as the solvent in the dispersed phase gradually diffuses out into the W_2 phase and continuously evaporates, the polymer concentration in the primary emulsion significantly increases and as a result, a skin layer can be initiated at a specific time point and solidified on the surface.

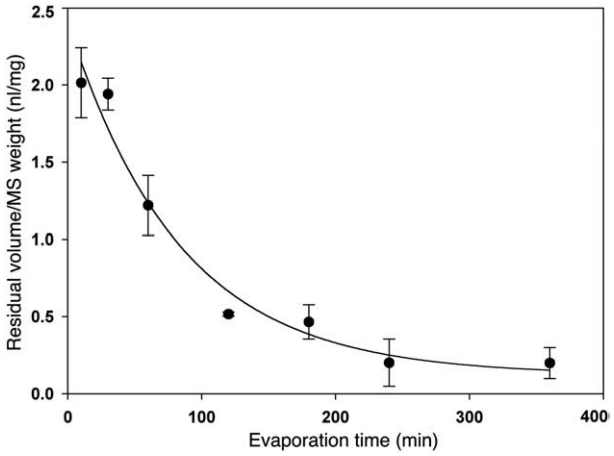

Fig. 7. Determination of the residual concentrations of hydrogen peroxide in the PLGA microspheres after evaporation. The data showed that the residual volume of hydrogen peroxide was significantly declined after 200 min of evaporation and then leveled off in a negligible amount.

3.4. Residual hydrogen peroxide and release profile of Dex

Because hydrogen peroxide is a chemical reagent, potentially unfavorable to cells, the residue that may persist in the microsphere was measured. The data showed that the residual volume of hydrogen peroxide was significantly declined after 200 min of evaporation and then leveled off in a negligible manner (Fig. 7). It seemed that hydrogen peroxide was fully decomposed during the whole procedure and thus the fabricated PLGA microspheres could be safe. Since PLGA microspheres have been applied as a carrier of drugs and other biologically active agents, the present hydrogen peroxide-derived ones were also subjected to a controlled release test of Dex, a critical element for induction of chondrogenesis of mesenchymal stem cells (MSCs). Four different morphologies of microspheres – porous (a), covered porous (b, c), and nonporous (d) – were prepared separately. Two types of covered porous microspheres with a skin layer were fabricated using hydrogen peroxide in varying conditions. Monitored for up to 28 days, the release profiles of the four groups were found to be significantly different on a temporal basis (Fig. 8). The microsphere with the porous surface exhibited an initial burst, losing 60% of Dex by 5 days and the most of Dex was released at the end of experiment (Fig. 8a). On the other hand, the microspheres with

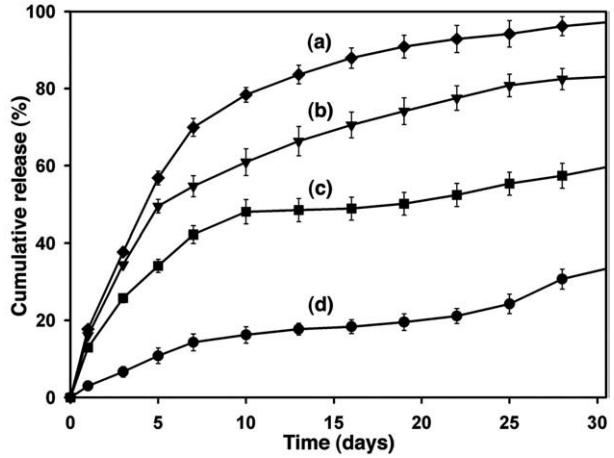

Fig. 8. Comparison of *in vitro* dexamethasone release profiles from different morphologies of PLGA microspheres. Four different groups of microspheres, porous (a), covered porous (b, c), and nonporous (d), were separately prepared and tested ($n=3$, each group) for up to 28 days. Each data point was recorded in the average and standard deviation (SD).

nonporous surface showed a relatively weak initial burst and sustained its release at a lower concentration during the entire time period (Fig. 8d). It even retained more than 60% of Dex after 28 days. Release profiles of hydrogen peroxide-derived covered porous microspheres were of particular interest in that they had neither porous nor nonporous surface morphology. Their release patterns in Fig. 8b and c exhibited a similar initial burst and they maintained a gradual release of Dex. However, the individual difference between (b) and (c) was notable. The curve (c) showed the release kinetics of rather a diphasic profile, in which the initial burst of Dex was followed by a lag phase. The curve (b) also displayed an initial burst within 5 days but a persistent release was observed during the remaining time without a lag phase. In general, the profile (c) appeared to be more favorable than that of (b) simply in terms of a lower rate of initial release and the sustained release of Dex for a longer period. It needs to be addressed at this point that while the rate of release in the early time was quite different, the release kinetics of four groups seemed to be quite similar. Each curve can be divided into two parts, an initial burst and a second phase, and the slopes of the second phases seemed to be highly dependent on the magnitude of the initial burst of each group.

Although the specific role of skin layer in determining the characteristic release profiles are unclear, as compared to the porous and nonporous morphologies (a, d), it is highly probable that the skin layer may serve as a protective barrier in controlling the initial burst of Dex. From the SEM images, it was obvious that the skin layer was gradually eroded and disappeared with time, seemingly past 7 days, during *in vitro* degradation test of PLGA microspheres (data not shown). It is well understood that the external and internal pore structures of biodegradable polymeric microsphere are critical factors in regulating a characteristic release profile of incorporated bioactive agents [9]. On the other hand, applications of Dex-loaded PLGA microspheres as an effective vehicle of drug delivery have been documented in many studies. Hickey et al. evaluated Dex-encapsulated PLGA microsphere and found that the sites with Dex-releasing microsphere had thin deposits of fibrous tissue and reduced the number of immune cells around the implants, indicating a suppression of inflammatory tissue response [20]. Zolnik et al. evaluated *in vivo* and *in vitro* release of Dex from PLGA microspheres [21]. They found that *in vitro* release followed a typical triphasic profile, whereas the *in vivo* release profile was differed in that the lag phase was absent and the release rate was faster than that of the *in vitro*. The reason was speculated as a result of different PLGA degradation mechanism for *in vivo* environment. A combined delivery of Dex and vascular endothelial growth factor (VEGF) in a rat model using PLGA microsphere/PVA hydrogel composite suggested that anti-angiogenic effect of the corticosteroid could be overcome, due to the concurrent elution of VEGF and Dex from the composite [22].

4. Conclusions

In this work, the covered porous PLGA microspheres were effectively fabricated using hydrogen peroxide as a novel effervescent agent. Diverse morphologies of the microspheres were successfully produced in the tests of a variety of contributing parameters. From the *in vitro* release test of Dex, hydrogen peroxide-derived covered PLGA microspheres demonstrated that the release profile could be manipulated in a controlled manner. The hydrogen peroxide-based system thus presents its great potential as a viable option of fabricating the covered porous PLGA microspheres for controlled drug delivery and regenerative medicine.

Acknowledgements

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