Functionalization of porous CoCrMo alloy with dexamethasone-PLGA using compressed carbon dioxide

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Porous CoCrMo alloy specimens were functionalized with dexamethasone embedded in biodegradable polylactic-co-glycolic acid (PLGA) polymer for sustained release of the anti-inflammatory drug. Dexamethasone loaded PLGA microspheres and thin films were impregnated onto the alloy surface using carbon dioxide bonding and dip-coating methods, respectively. In the carbon dioxide bonding method, dexamethasone loaded PLGA microspheres were prepared using the oil-in-water emulsion/solvent evaporation method, dispersed into the porous specimens and exposed to carbon dioxide (CO_2) for plasticization and bonding to the porous alloy surface. Exposure to compressed CO_2 at 40°C and 10 bar was effective to bond the microspheres to the pore surface and to maintain its spherical morphology, while at 50 and 100 bar, significant microsphere agglomeration was observed. In a more direct approach, alloy specimens were dip-coated into dexamethasone-PLGA solutions, air-dried, and exposed to CO_2 to produce dexamethasone loaded PLGA thin films. The release of dexamethasone in PBS at 37°C and under sink conditions from both the microspheres and the thin film were investigated. Complete release of dexamethasone from microspheres bonded to glass plates exposed to CO_2 at 10 bar was attained in less than 50 days, while at 50 and 100 bar, significant microsphere agglomeration resulted in long delay phase of dexamethasone release profile. No effect of CO_2 pressure on the release from thin layer PLGA as dexamethasone was completely released in 3 days. Exposure to CO_2 at 40°C and 10 bar was sufficient to reduce the amount of residual dichloromethane in thin films and bonded microspheres to less than 50 mg/kg.

INTRODUCTION

Cobalt chromium-based alloys are commonly used in orthopedic surgery due to their excellent mechanical strength and hardness as well as corrosion characteristics. However, as metal objects are implanted into the body, release of corrosion and wear products may induce adverse effects including inflammatory response by the local tissue and even implant failure [1-[2]. Adverse reactions to CoCrMo, an alloy commonly used in implants for total joint replacement, have been reported in several papers [3-[4]. Functionalization of porous CoCrMo alloy with dexamethasone as a widely used anti-inflammatory drug encapsulated within polylactic-co-glycolic acid (PLGA) as a safe biodegradable polymer for controlled release, could help solve the inflammation problems associated with the CoCrMo implants. Functionalization of the porous alloy can be extended to a combined anti-inflammatory drug and a growth hormone system [5] and to other controlled release systems as well. Dexamethasone encapsulated in PLGA have been investigated for many applications, including release from alginate hydrogels [6], PVA hydrogels [7], metallic stents [8], nanospheres for ocular delivery [9], copolymers [10], porous scaffolds [11], gelatin coated PLGA [12], and biosensor [13-[14].

Functionalization of the porous CoCrMo alloy can be achieved by bonding dexamethasone loaded PLGA micro/nanospheres onto the pore surface using organic solvent or heating above the glass transition temperature (T_g) of polymer substrates. Use of compressed CO_2 is desirable since it

is non-toxic, inexpensive, and leaves no residual residue upon depressurization step. Additionally, supercritical CO_2 extracts residual organic solvents from PLGA such as dichloromethane [15] and chloroform [16] to acceptable levels. As carbon dioxide pressure is raised, T_g of PLGA decreases nearly linearly with the increasing solubility of the gas in the polymer, making interfacial fusion of PLGA microstructures at low temperatures possible [17-[18]. At elevated pressures, rapid depressurization step following CO_2 sorption resulted in PLGA foam with a closed pore structure [19-[20].

The aim of our study was, therefore, to evaluate methods suitable for functionalization of porous CoCrMo alloy with dexamethasone/PLGA that can be used for continuous delivery of an anti-inflammatory drug at an implant site. In this study, dexamethasone loaded PLGA microspheres were prepared using the oil-in-water emulsion/solvent evaporation method, dispersed into the porous alloy, heated and exposed to CO_2 for plasticization and bonding to the porous alloy surface. Dip-coating, as a more direct approach for bonding was also investigated. This method consisted of dipping the porous alloy into the dexamethasone/PLGA solution, drying with air, and exposing the resulting dexamethasone loaded PLGA thin films to CO_2. The amount of dexamethasone released in PBS at 37°C under sink conditions from both the microspheres and the thin film were investigated, whereas standard microspheres and microspheres heated without CO_2 exposure were used as control. In addition, effectiveness of compressed carbon dioxide as an extractant for residual solvent in PLGA was also investigated.

I - MATERIALS AND METHODS

Materials

Poly(D,L-lactide-*co*-glycolide) 50:50 (PLGA, average MW 40-75 kDa) and dexamethasone (98% HPLC-grade) were obtained from Sigma. Poly(vinyl alcohol) 99% hydrolyzed (PVA, average MW 30–50 kDa) and high performance liquid chromatography (HPLC) grade solvents (dichloromethane, methanol, isopropanol and acetonitrile) were obtained from Sigma-Aldrich. Deionized water used for the preparation of all solutions was obtained from a Millipore Milli-Q water filtration system. Porous CoCrMo alloy specimens having 20 pore per inch porosity was provided by FT Innovations B.V., Nijmegen, The Netherlands.

Dexamethasone loaded PLGA microsphere preparation

PLGA microspheres loaded with dexamethasone were prepared using the oil-in-water (o/w) emulsification and solvent evaporation method based on previous report [14]. The oil phase, consisted of 10 mg of dexamethasone and 50 mg of PLGA dissolved in 5 ml of 9:1 (v/v) dichloromethane and methanol mixture, was added into 100 ml of 0.2% (w/v) aqueous PVA solution and the resulting stirred at 1,250 rpm for 30 min to obtain the desired o/w emulsion. The resulting emulsion was stirred on a magnetic stirrer plate at 600 rpm for 16 h to allow for complete evaporation of the organic solvents, centrifuged at 10,000 rpm for 5 min (Allegra X-22, Beckman Coulter) and decanted. The obtained microspheres were resuspended in deionized water, centrifuged, vacuum-filtrated using 0.2 μm nylon membrane filters (Nylaflo, Pall), and washed with deionized water to remove the remaining PVA. The microspheres were washed several times with isopropanol and deionized water to remove the non-encapsulated dexamethasone, dried using a water ejector, and stored in an amber glass jar at 4°C to extend its shelf life [21].

Microsphere and film morphology using optical microscopy and SEM

The microspheres were observed for presence of any non-encapsulated dexamethasone crystals using a Nikon optical microscope Optiphot 200 and their morphologies examined using a Jeol JSM 5400 scanning electron microscopy (SEM).

Microspheres bonding and thin film coating

Approximately 20 mg of microspheres were dispersed into the porous CoCrMo alloy by gently shaking the specimens (1x1x1 cm). Each specimen was weighed before and after the

dispersion, put inside a partially covered petri dish to protect the microspheres from being blown away by CO_2. The petri dish was put inside a high pressure vessel maintained at 40°C, the vessel was flushed with CO_2 and the samples exposed to CO_2 at the desired pressures (10, 50, and 100 bar) for 30 minutes. Pressurization was carried out slowly to make sure that CO_2 is always at supercritical condition as contact with liquid CO_2 could significantly change the morphology of the microspheres [19]. At 10 bar, CO_2 exposure was carried out in a batch mode while at higher pressures, a constant flow of 100 g/min CO_2 was used. The total exposure time was less than 40 min, including 5-10 min of depressurization time. In addition to using the porous alloy, the same procedure was applied to approximately 20 mg of microspheres dispersed into glass plates.

Dexamethasone loaded PLGA films were obtained by dipping the specimens into a mixture of 9:1 (v/v) dichloromethane to methanol containing dexamethasone and PLGA, repeating this step three times, and drying in vacuum for 16 hours. Several formulations were used to obtain dexamethasone loadings similar to those of the standard microspheres. All of the dip-coated specimens were washed, dried, and exposed to CO_2 following the same procedures applied to the bonded microspheres.

Dexamethasone loading

Dexamethasone loading was determined using a Varian Prostar high performance liquid chromatograph equipped with a mobile phase delivery system and a ChromSpher C18 reversed-phase column. Approximately 20 mg of standard microspheres were dissolved in 2.5 ml of acetonitrile using an ultra sonic bath and analysis was performed using a UV detector at 246 nm. The mobile phase used was a mixture of 60:40 (v/v) acetonitrile and 2 mM acetate buffer solution (pH 4.8) flowing at 1 ml/min. Acetate buffer and dexamethasone standard solutions were prepared weekly. The same procedure was also applied in the determination of dexamethasone loading in bonded microspheres and thin films.

Residual solvent

Residual solvents were analyzed on a Chrompack CP 9001 Gas Chromatography System equipped with an auto sampler and a TCD detector. Samples equivalent to 20 mg of standard microspheres were dissolved in THF using an ultrasonic bath and diluted with deionized water. Liquid samples were obtained using a syringe attached with 0.2 μm nylon membrane filters (Nylaflo, Pall) to obtain a clear solution. Analysis was performed using a Varian Cp-Sil-5Cb column at 50°C.

In vitro release of dexamethasone

Dexamethasone release profiles from standard microspheres, bonded microspheres and thin films were determined under sink conditions where dexamethasone concentrations in phosphate buffered saline (PBS) were kept at less than 10% of its saturation solubility. Samples equivalent to 30-40 mg of microspheres were placed in amber glass jars containing 50 ml of PBS (pH of 7.4) and the jars were shaken at 50 rpm in an incubator maintained at 37°C. At set time intervals, 2 ml samples were collected by syringe through a 0.2 μm nylon membrane filter and the microspheres retained in the filter were returned into the jars by backwashing using 2 ml of fresh PBS.

II RESULTS AND DISCUSSION

Dexamethasone loaded PLGA microspheres and thin films

The standard microspheres, obtained using the emulsion evaporation method, were spherical with varying diameter of less than 50 μm. The average loading of dexamethasone was 0.16±0.01 %, close to the reported value of 0.17% loading in PLGA (50:50) nanoparticles [9] and the average dexamethasone encapsulation efficiency was ~1%. PLGA microspheres placed on top of SEM holder were bonded around their contact points after exposure to CO_2 at 10 bar, as shown in **Figure 1(a)**. The microspheres maintained their original morphology, indicating fusion of the PLGA surface due to CO_2 plasticization [17[18]. On the other hand, bonding at 40°C and at a CO_2

pressure of 50 bar resulted in significant PLGA foaming and microsphere agglomeration as can be seen from the SEM picture given in **Figure 1(b)**. The observed CO_2 effect on PLGA (50:50) morphology at this condition is consistent with the reported data of carbon dioxide sorption over 10% by weight and PLGA swelling by 13% [22]. A similar effect of CO_2 on PLGA was also observed in the case of the microspheres dispersed in the porous CoCrMo alloy. **Figure 2(a)** shows fused and agglomerated microspheres bonded to the surface of the alloy after exposure to CO_2 at 100 bar.

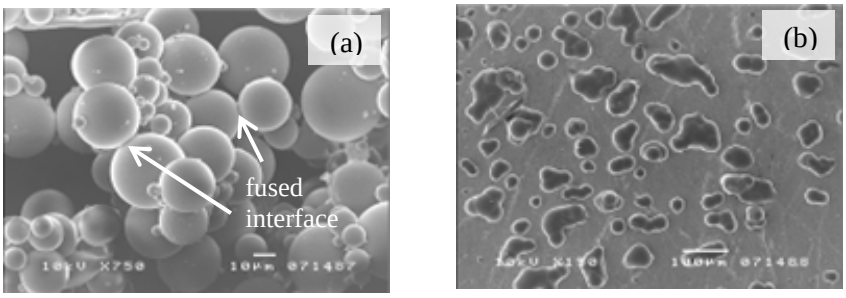

Figure 1. SEM images of dexamethasone loaded PLGA microspheres bonded to SEM plates after exposed to CO_2 at 10 bar (a) and at 50 bar (b).

Dexamethasone loaded PLGA thin films were obtained after dip-coating the alloy specimens into dexamethasone/PLGA solutions in a mixture of 9:1 (v/v) dichloromethane and methanol. Several formulations of starting solutions were prepared to obtain similar loading of dexamethasone in PLGA film. The average dexamethasone loading was 0.17 ± 0.01 % although the average dexamethasone encapsulation efficiency was very high ~80%. The solid-state solubility of dexamethasone in PLGA (50:50) (67 and 32 mg/g for the 12 kDa and 143 kDa PLGA, respectively) have been determined using differential scanning calorimetry [23]. It was found that the dexamethasone/PLGA solution did not adhere well to the alloy surface, resulting in patches of thin films in spite of repeated dipping step as shown in **Figure 2(b).**

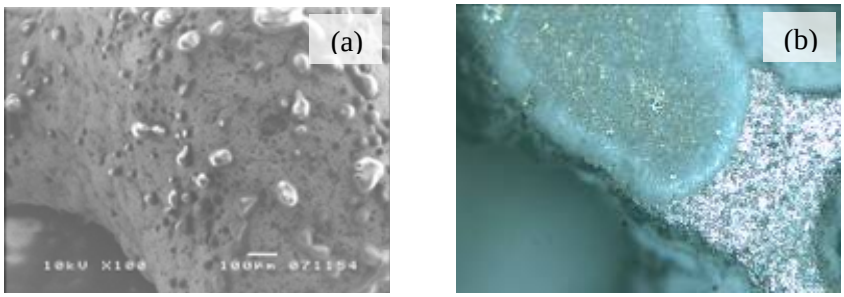

Figure 2. SEM image of dexamethasone loaded PLGA microspheres bonded to the surface of porous CoCrMo alloy after exposed to CO_2 at 100 bar (a) and optical microphotograph of dexamethasone loaded PLGA thin films (b).

Dexamethasone release profile

The release profile of dexamethasone obtained under sink conditions from dexamethasone loaded PLGA as microspheres bonded to glass plates, microspheres bonded to porous CoCrMo alloy, and films coated onto porous Corm alloy are shown in **Figure 3 (a)-(c)**, respectively. The effect of CO_2 pressure on the release rate was more pronounced in the case of release from the glass plates because agglomeration of microspheres was more extensive at higher pressure as shown in **Figure 1(b)**. Formation of highly porous PLGA with a non porous skin [19-[20] could lead to larger diffusion resistances and slower release of dexamethasone as seen in **Figure 3(a)**. The dexamethasone release from microspheres bonded to the porous alloy did not show a strong dependent on the CO_2 pressure as shown in **Figure 3(b)**. It is more likely that since the same

amount of microspheres were dispersed on a much larger surface area of the porous alloy compared to the area of the glass plates, fusion of PLGA was less significant resulting in similar release profiles. It is noted that the data is limited to only 20 days and the release profile will be more obvious pending additional data becomes available. All of the bonded microspheres in PBS solution show a short initial burst release phase up to 5 days, a common occurrence in dexamethasone/PLGA systems prepared using emulsification and evaporation method [5,[7-[9,[13-[14,[23]. No significant effect of CO_2 pressure on the release from thin layer PLGA can be observed as dexamethasone was completely released in 3 days.

Effect of CO_2 on residual solvent

The amount of residual dichloromethane in the standard microspheres as measured by GC was 367 mg/kg. After exposed to CO_2 in a static mode at 10 bar for 30 min, the amount of residual dichloromethane was reduced to less than the detection limit of the GC analysis (50 mg/kg), below the 600 mg/kg limit for dichloromethane in oral medicines [24]. This value is close to the amount residual dichloromethane (107 mg/kg) present in spray-dried PLGA (50:50) after extraction of by CO_2 [15].

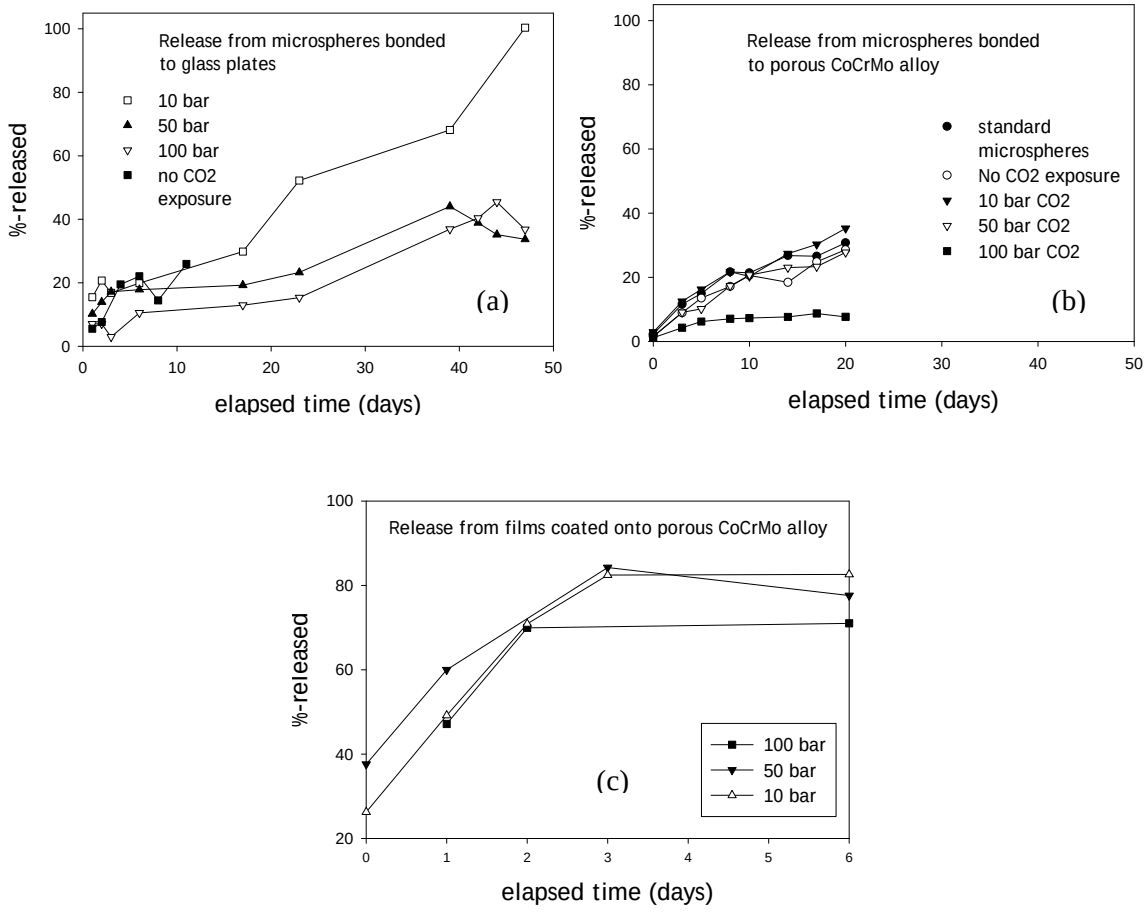

Figure 3. Release profile of dexamethasone from microspheres bonded to glass plates (a), microspheres bonded to porous alloy (b), and thin films coated onto porous alloy (c).

CONCLUSIONS

Porous alloy such as CoCrMo can be functionalized with dexamethasone loaded PLGA either as dispersed microspheres or thin films. Static exposure with carbon dioxide at 10 bar was sufficient for microsphere bonding without agglomeration, to reduce the residual dichloromethane to less than 50 mg/kg, and to achieve complete dexamethasone release from glass plates in less

than 50 days. Complete release from the thin film produced by dip-coating method was achieved only in 3 days while release profiles from the microspheres bonded to the porous alloy will be evaluated pending availability of more extensive data.

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