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| Original Article |

Nanocomposite Microspheres of PLGA/HA with Antibiotics for Injectable Bone-Graft Materials

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Abstract : This study investigated the possibility of an injectable bone substitute consisting of methylcellulose aqueous solutions and poly lactide-co-glycolide acid/hydroxyapatite (PLGA/HA) composite microspheres containing sustained releasing antibiotics. HA nanoparticles were synthesized by coprecipitation. PLGA/HA composite microspheres were prepared by oil-in-water emulsion/solvent evaporation with tetracycline. They were mixed with a 2% (w/v) aqueous solution of methylcellulose. The particle size of the composite microspheres increased linearly with increasing PLGA concentration from 17.8±4.5 to 185.4±29.5 μm. As the size of PLGA/HA composite microspheres increased, the content of tetracycline increased from 0.8% to 70.3%. The release rate of tetracycline decreased with increasing size of the composite microspheres. Tetracycline exhibited a burst release with nearly 100% escape within 24 hrs when the PLGA concentration was less than 20%. Sustained continuous release until 2 weeks when PLGA content was 20%. The gelation temperature of methylcellulose can be adjusted to normal body temperature when NaCl content exceeds 5%. PLGA/HA composite microspheres mixed with a methylcellulose aqueous solution was easily and completely ejected through an 18G needle when the mixing ratio was below 100%.

Key words: *HA, PLGA, injectable, antibiotic, tetracycline*

1. Introduction

There is an increasing demand for bone substitutes in the field of orthopedic surgery due to the increasing use of advanced procedures in reconstructive surgery after various traumatic pathologies and iatrogenic bone losses secondary to bone resections for tumors, infections or pseudoarthroses. In addition, the increasing number of elderly patients or individuals with various systemic pathologies and biological shortcomings related to the bone healing processes often necessitates the use of bone substitutes as an adjuvant therapy to prosthetic implants in order to improve the level of biological fixation and osseointegration processes.[1,2]

Recently, injectable gels have been developed in orthopedics. These gels can fill defects of any shape, can incorporate various therapeutic agents, and do not contain residual solvents. Hence, they can reduce surgery time, minimize damaging effects, reduce the size of scars, decrease post-operative pain, and allow patients to achieve a rapid recovery. Several injectable biomaterials have been developed including collagen,[3] polyethylene oxide,[4] calcium alginate,[5] and fibrin glue.[6,7]

Hydroxyapatite (HA) is a well-known biomaterial used as a bone substitute to fill bone defects and as a coating agent on biomedical implants. Methylcellulose (MC) and hydroxypropylmethylcellulose (HPMC) have the unique property of reversible thermogelation. Upon cooling, the gelation process is completely reversed and the gel formed reverts to the sol state. The temperature at which the gelation process begins as well as the strength of the gel formed depend on the type and degree of substitution of the gum, molecular weight, concentration, and the presence of electrolytes.[8]

Many studies have shown benefits of *in vitro* and *in vivo* associations of therapeutic agents using drug delivery systems (DDS).[9] Controlled DDS can help optimize the therapeutic efficiency and reduce serious side effects.[10,11]

Poly[DL-lactide-co-glycolide](PLGA)-based microparticles can help to accurately control the drug release kinetics over periods of days to months, and can be administered easily using standard syringes or needles with complete biodegradability

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and good biocompatibility. The size of the microsphere, which is a key factor in the release rate, might have a significant effect on product performance and safety. The particle size can also influence the injectability of the product.

The aim of this study was to assess the potential of a MC aqueous solution containing PLGA/HA composite microspheres and antibiotics as a sustained drug release-bone substitute.

2. Materials and Methods

Needle-shaped HA nanoparticles, 20 nm in width and 100 nm in length, were synthesized by coprecipitation. A 0.3 M aqueous solution of $(NH_4)_2 \cdot HPO_4$ was added drop wise to a 0.5 M aqueous solution of $CaCl_2$ with vigorous stirring at 10,000 rpm. The pH of the reacted solution was adjusted to pH 10 using a NH_4OH solution and the temperature was maintained at 60°C. The coprecipitated HA was filtered, washed with NH_4Cl_2 and freeze-dried.

The PLGA/HA composite microspheres were prepared by oil-in-water (O/W) emulsion/solvent evaporation. Various PLGA concentrations ranging from 2.5 to 20% were dissolved in dichloromethane (DCM), as shown in Table 1. The resulting solution was mixed with HA and tetracycline (TC) at a fixed ratio of 10:1:1 (PLGA:HA:TC). The resulting mixture was poured into an aqueous solution of 2% polyvinyl alcohol (PVA) and homogenized at 3,000 rpm. After rotary evaporation under vacuum, the PLGA/HA composite microspheres were washed and freeze-dried. The morphology and the average size of the composite microspheres were determined using an optical microscope (Olympus CK2, Olympus optical, Japan).

The release rate of TC from the composite microspheres was determined using a UV/Visible Spectrophotometer (UVD-3200, LaboMed, USA). 200 mg of the composite microspheres were immersed into 20 mL of saline and shaken continuously at 37°C. At set time intervals, the intensity of the absorption spectra at 275 nm of the saline was measured using a UV/Visible Spectrophotometer.

The PLGA/HA composite microspheres containing TC were mixed with a 2% (w/v) aqueous solution of methylcellulose (MC; viscosity of 400 cP). The mixing ratio of the microspheres/solution was varied from 40/60 to 60/40 (w/v). Before mixing, the gelation temperature of the 2% MC aqueous solution was adjusted to normal body temperature by adding NaCl. The gelation temperature according to the amount of NaCl added (up to 6%) was determined by examining the rheological behavior at temperatures up to 60°C using a fluid rheometer (RVDV-III, Brookfield, USA).

In order to verify the optimum injection condition through a needle, the injectability of the MC aqueous solution containing the dispersed PLGA/HA composite microspheres was measured using a slight modification of the method reported by Bohner and Baroud.[12] A syringe with an 18G needle was filled with 1 mL of the aqueous solutions of MC containing the PLGA/HA composite microspheres. The needle was then placed in a cylinder-shaped holster, which was attached to a universal test machine (AG-5000G, Shimadzu, Japan). A stainless steel rod, 4 mm in diameter, was pressed vertically on the gasket at a crosshead speed of 10 mm/min, and the load-displacement curves were obtained.

Table 1. Particle size and content of tetracycline in PLGA/HA microspheres

Sample code	PLGA concentration (% w/v)	Particle size (mm)	Content of TC (%)
PHM1	2.5	17.8±4.5	0.8
PHM2	5	48.7±9.4	10.6
PHM3	10	96.3±17.7	21.8
PHM4	20	185.4±29.5	70.3

3. Results

Fig 1 shows a representative optical micrograph of the PLGA/HA composite microspheres. All the particles had a spherical shape. Fig 2 shows the particle size according at PLGA concentrations up to 20%. The particle size of the composite microspheres increased linearly with increasing PLGA concentration from 17.8±4.5 to 185.4±29.5 μm. It is believed that at a fixed stirring shear force, the higher concentration of PLGA increase the viscosity of the oil phase.

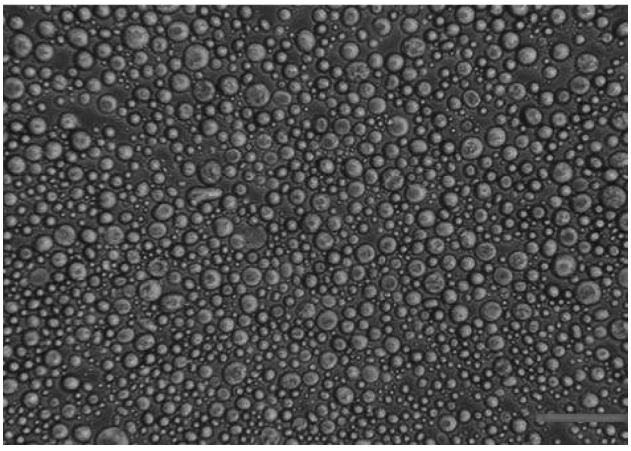

Figure 1. Representative image of PLGA/HA microspheres (scale bar = 100 μm).

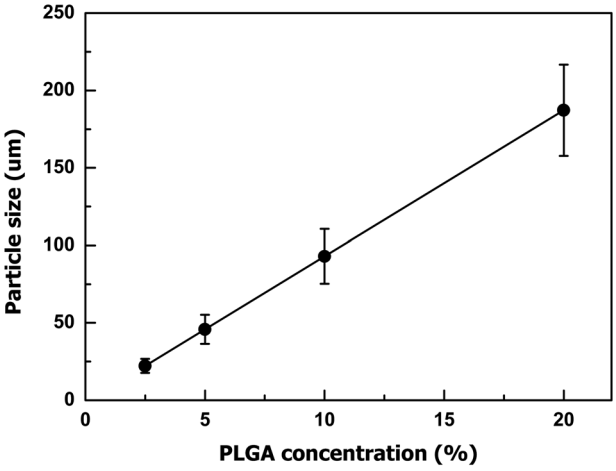

Figure 2. Effect of PLGA concentration on the particle size of PLGA/HA microspheres.

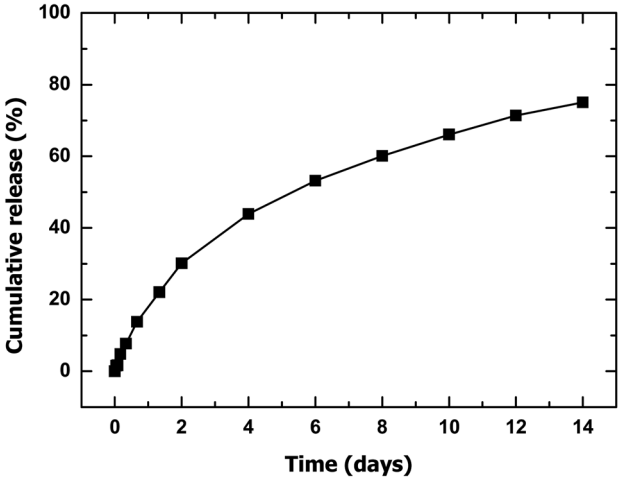

Figure 4. Cumulative release of tetracycline from PLGA/HA composite microspheres with 20% of PLGA up to 2 weeks in saline at 37°C.

This makes it difficult for small droplets to form because it is well known that the size of the emulsion droplets depends on the balance between the stirring shear force and the level of droplet cohesion. This trend is in agreement with the findings reported by Klose *et al.* in that DCM diffuses from the solution into the water carrying some PLGA molecules with it.[13] The amount of DCM increases with increasing PLGA concentration with the other conditions fixed, which increases particle size.[14] The TC content increased from 0.8% to 70.3% with increasing particle size of the PLGA/HA composite microspheres, as shown in (Table 1).

Fig 3 shows the effect of the size of the PLGA/HA composite microspheres on the release rate of TC in saline. A high initial burst phenomenon was observed with the exception of PHM4.

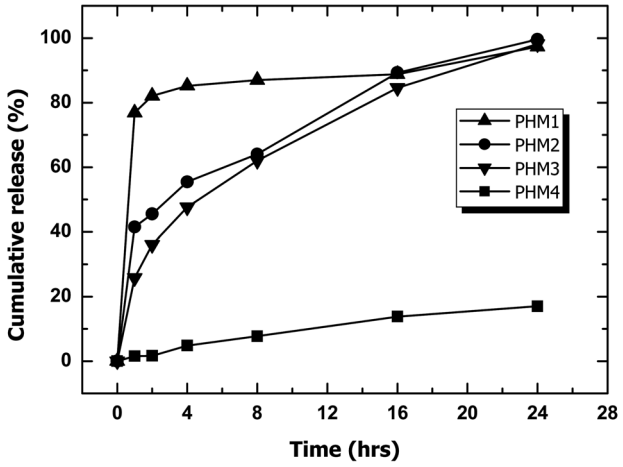

Figure 3. Cumulative release of tetracycline from PLGA/HA composite microspheres with various amount of PLGA in saline at 37°C.

The relative release rates of TC increased up to 100% within 24 hrs except for PHM4. This means that the relative release rate decreased with increasing particle size of the composite microspheres. Fig 4 shows that PHM4 has a sustained release rate of 80% for 2 weeks.

Fig 5 shows the rheological behavior of MC at various NaCl concentrations. The viscosity increased with increasing temperature. The temperature at which the viscosity of the solution increased suddenly is regarded as the gelation temperature. The gelation temperature of MC decreased from 54°C to 32.5°C with increasing amount of NaCl added, concentrations of 0, 2, 4, 5, 6% NaCl where tested. The gelation temperature was lower than body temperature when the amount

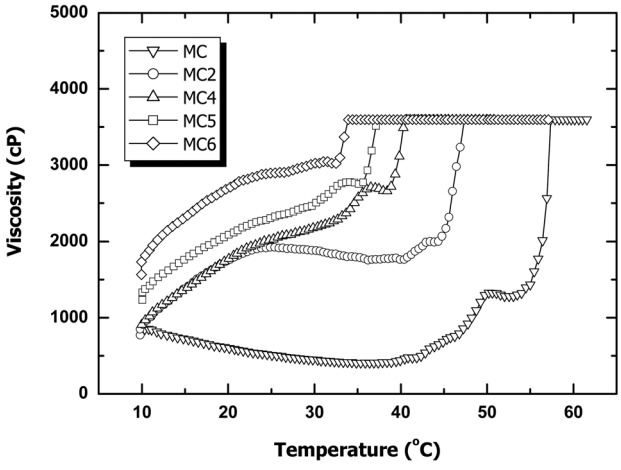

Figure 5. Rheological curves of 2% methylcellulose aqueous solution blended with various amount of NaCl up to 6%.

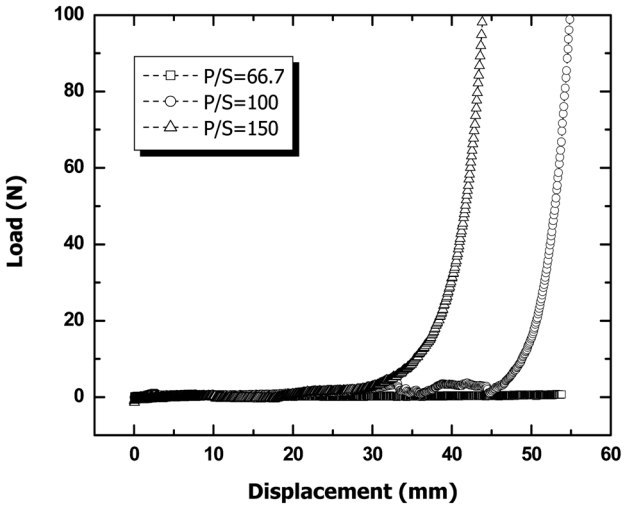

Figure 6. Load-displacement curves of methylcellulose aqueous solution dispersed with various amount of PLGA/HA composite microspheres through a syringe.

Table 2. Gelation temperature and fluidity of methylcellulose aqueous solution according to the amount of NaCl

Sample code	Concentration of NaCl (%)	Gelation temperature (°C)	Fluidity
MC	0	54.0	O
MC2	2	44.4	O
MC4	4	38.4	O
MC5	5	35.7	X
MC6	6	32.5	X

of NaCl in MC exceeded 5%.

Fig 6 shows the load-displacement curves of the MC solutions containing the PLGA/HA composite microspheres through a needle from a syringe. The ratio between the PLGA/HA composite microspheres and the MC solutions was varied from 66.7% to 150.0% (w/v) with the PLGA concentration fixed at 5%. When the ratio was 66.7%, the MC solutions containing the PLGA/HA composite microspheres were easily and completely ejected through the 18G needle using a small load. The load needed to eject the mixtures increased with increasing particle/solution ratio with uninjected amounts remaining inside the syringe at the higher ratios.

4. Discussion

This study evaluated the potential of an injectable bone substitute consisting of a MC aqueous solution and the PLGA/HA composite microspheres containing sustained release antibiotics.

PLGA/HA composite microspheres containing TC were used to examine the drug release profiles according to the change in the size of the composite microspheres. It is clear that the release rate of TC decreased with increasing size of the composite microspheres. PHM1, PHM2, and PHM3 showed an initial burst stage and the drug was released completely within 24 hrs, whereas only 17% of TC was released without the initial high burst in the case of PHM4. PHM4 showed sustained release of TC for 2 weeks, which can be explained by properties of both PLGA and TC as follows.

Firstly, the rate of PLGA degradation increases with increasing particle size, irrespective of the presence or absence of the antibiotics. This autocatalytic effect is due to the increase in the diffusion pathways for the acids generated from the release medium with increasing particle size. The decrease in pH becomes more pronounced and the ester bond cleavage is accelerated, followed by an increase in the shorter chain degradation products. Therefore, the average molecular weight of PLGA in the large particles decreases more rapidly than in the small ones.

Another explanation is related to the release mechanism of TC from the PLGA/HA composite microspheres i.e. diffusion.[15] This means that the concentration gradient of TC should decrease with increasing length of the diffusion pathway of larger spheres. The absence of this "increased diffusion pathway length effect" can accelerate the rate of polymer degradation.[16] The resulting release rate of TC is a combination of the above two phenomena.

Encapsulation is a common way of controlling the release of various therapeutic agents,[17] such as antibiotics, anticancer drugs, growth factors[18] or steroid hormones.[19] However, these DDS do not allow drug release for more than a few days. In this study, the PLGA/HA composite microspheres allowed constant release of TC for more than two weeks.

The PLGA/HA microspheres containing TC were dispersed in the MC solutions in order to achieve appropriate injectability, which was the aim of this study. The content of PLGA/HA composite microspheres in the MC solution was found to be a critical factor for injectability. However, it is expected that a larger size and higher particle content in the MC solution can be injected if a larger needle gauge is used.

NaCl is added to the MC solution to adjust the gelation temperature to human body temperature. When the amount of NaCl exceeded 5%, the gelation temperature was less than body temperature. There is a stronger interaction between Cl^- and a water molecule than between water molecules. Therefore, NaCl destroys some of the original hydrogen-bonding network formed by water. This effect is similar to increasing the temperature. The

competition for water molecules from NaCl, and the NaCl-induced destruction of the hydrogen bonds between the MC chains and water molecule decreases the MC solubility in water. As a result, at the same temperature, there are more hydrophobic aggregates of MC in the NaCl-containing MC solution than in the NaCl-free one, which leads to stronger light scattering and a lower transmittance at a given temperature. Therefore, the clouding point of the NaCl-containing samples appears at a lower temperature. The increase in the NaCl content results in fewer free water molecules available around the MC chains and a stronger hydrophobic environment for MC, which causes turbidity of the MC at a given temperature.

5. Conclusion

This study investigated the possibility of an injectable bone substitute consisting of MC aqueous solutions and PLGA/HA composite microspheres for sustained release of antibiotics.

The particle size of the composite microspheres increased linearly with increasing PLGA concentration from 17.8±4.5 to 185.4±29.5 μm. The TC content increased from 0.8% to 70.3% with increasing particle size of the PLGA/HA composite microspheres. The release rate of TC decreased with increasing size of the composite microspheres. At a PLGA concentration < 20%, the TC rapidly diffused out and was almost completely exhausted within 24 hrs, while the rate of diffusion was sustained for 2 weeks when the PLGA content was 20%.

The gelation temperature of MC was lower than body temperature when the NaCl content exceeded 5%. The PLGA/HA composite microspheres mixed with a MC aqueous solution at a mixing ratio < 100% could be ejected through an 18G needle with little load.

It could be concluded that the injectable bone substitute tested were can potentially be used for sustained release of antibiotics and bone tissue regeneration.

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