CHARACTERIZATION OF PLGA COMPOSITE MICROSPHERES CONTAINING HA NANOPARTICLES

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Introduction

The need for bone substitutes is rapidly increasing in the field of orthopaedic surgery, since advanced procedures are now being performed in reconstructive surgery after traumatic pathologies and iatrogenic bone losses secondary to bone resections for tumours, infections or pseudoarthroses. Moreover, the increasing number of elderly patients or individuals with various systemic pathologies and biological drawbacks related to bone healing processes, often requires the use of bone substitutes as an adjuvant therapy to be associated with prosthetic implants in order to improve biological fixation and osteointegration processes. [1]

Recently, injectable gels have been developed in orthopedics. They can fill any shape of defect, may incorporate various therapeutic agents, and need not contain residual solvents. Therefore they should shorten the surgical operation time, minimize the damaging effects, reduce the size of the scars and lessen post-operative pain, and allowing patients to achieve rapid recovery. Several injectable biomaterials have been developed, such as collagen, polyethylene oxide, calcium alginate, and fibrin glue. [2]

Hydroxyapatite(HA) is a well-known biomaterial used as a bone substitute for filling bone defects as a coating agent on biomedical implants. Methylcellulose (MC) and hydroxypropylmethylcellulose (HPMC) possess the unique property of reversible thermogelation. Upon cooling, the gelation process is completely reversed and the gel formed will revert to the sol state. The temperature at which the gelation process starts and the strength of the gel formed depend on the type and degree of substitution of the gum, molecular weight, concentration, and presence of electrolytes. [3]

Many studies have shown the benefit of *in vitro* and *in vivo* associations of therapeutic agents using drug delivery systems (DDS). The controlled DDS could be helpful to optimize the therapeutic efficiency and to reduce serious side effects. [4]

Poly(DL-lactide-co-glycolide)(PLGA)-based microparticles offer the possibility to control accurately the resulting drug release kinetics over periods of days to months and easy administration using standard syringes and needles as well as complete biodegradability and good biocompatibility. The size of the microsphere may have a significant effect on product performance as well as safety. Particle size can also influence the injectability of the product.

The purpose of this study was to investigate the possibility of MC aqueous solution containing PLGA/HA composite microspheres and antibiotics, as a sustained drug release-bone substitute.

Experimental

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

PLGA/HA composite microspheres were prepared by oil-in-water emulsion/solvent evaporation. Various concentrations ranged 2.5~20.0% of PLGA were dissolved in dichloromethane(DCM) and followed by mixing of HA and tetracycline(TC) at a fixed ratio of 10:1:1 (PLGA:HA:TC). Then were poured into an aqueous solution of 2% polyvinyle alcohol(PVA) and was homogenized at 3,000 rpm. After rotary evaporation under vacuum, PLGA/HA composite microspheres were washed and freeze-dried.

The morphology and the average size of the composite microspheres were estimated by an optical microscope. The releasing rate of TC from the composite microspheres was determined using UV/Visible Spectrophotometer.

PLGA/HA composite microspheres containing TC were mixed with 2% (w/v) aqueous solution of methylcellulose (MC; viscosity of 400 cP). Before mixing them, gelation temperature of the 2% MC aqueous solution was adjusted to the body temperature by adding NaCl. The rheological behavior was determined according to the amount of NaCl in order to measure the gelation temperature.

The injectability of MC aqueous solution dispersed with PLGA/HA microspheres was measured in order to verify the optimum injection condition through a needle.

Results and Discussion

All particles exhibited the spherical shapes. Their size was shown in Fig. 1 according to the concentration of PLGA up to 20%. The particle size of the composite microspheres was increased linearly with increasing PLGA concentration from (17.8±4.5) to (185.4±29.5) μm. It is considered that the higher concentration of PLGA, at a fixed stirring shear force, results in a higher viscosity of the oil phase, which makes it difficult for small droplets to form since it is well known that the size of emulsion droplets depends on the balance between stirring shear force and droplet cohesion. This trend is in agreement with the findings of Klose *et al.* that DCM diffuses from the solution into the water carrying some PLGA molecules with it. [5] As increasing the PLGA concentration, the amount of DCM

increases, therefore the particle size increases at a fixed other conditions. As increasing the size of PLGA/HA composite microspheres, TC content was increased from 0.8% to 70.3%.

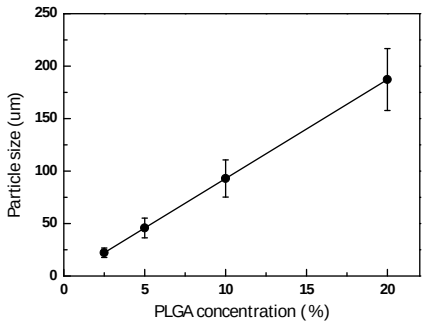

Fig. 1. Particle size of PLGA/HA microspheres according to PLGA concentration.

The effect of the size of the PLGA/HA composite microspheres on the release rate of TC in saline is as follows; High initial bust phenomena were observed except PHM4. The relative release rates of TC were increased up to 100% within 24 hrs except PHM4. In other words, the relative release rate was decreased with increasing the particle size of the composite microspheres. The release rate of PHM4 was shown in Fig. 2. It exhibited the sustained release of 80% until 2 weeks.

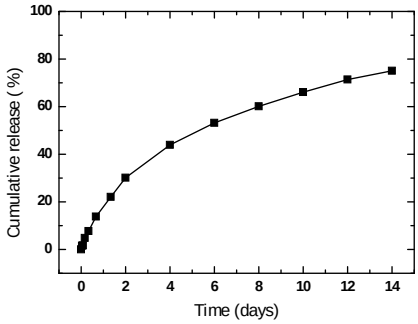

Fig. 2. Controlled release of tetracycline from PLGA/HA composite in saline at 37°C.

The rheological behavior of MC with various concentration of NaCl was shown in Fig. 3. The viscosity was increased with increasing temperature. The temperature at which the viscosity of the solution was suddenly raised is regarded as a gelation temperature. The gelation temperature of MC was decreased by increasing the amount of NaCl from 54 to 32.5°C when the amount of NaCl was increased to 6%. When the amount of NaCl was exceeded 5% in MC, the gelation temperature showed lower than the body temperature.

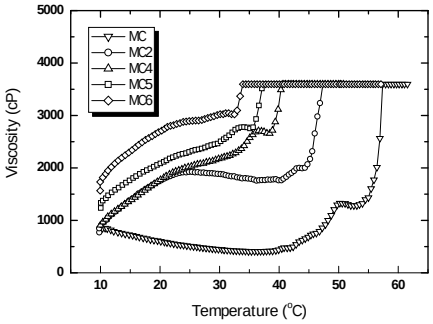

Fig. 3. Rheological curves of 2% methylcellulose aqueous solution blended with various amount of NaCl up to 6%.

Conclusion

The particle size of the composite microspheres was increased linearly with increasing PLGA concentration from (17.8±4.5) to (185.4±29.5) μm. As increasing the size of PLGA/HA composite microspheres, TC content was increased from 0.8% to 70.3%. The release rate of TC was decreased with increasing the size of the composite microspheres. TC was busted out and exhausted nearly up to 100% within 24 hrs under PLGA concentration was less than 20%, while was sustained continuous release until 2 weeks when PLGA content was 20%.

The gelation temperature of MC was adjusted below body temperature when NaCl content was exceeded 5%. PLGA/HA composite microspheres mixed with MC aqueous solution exhibited could be easily ejected through a needle of 18G from syringe with little load under the mixing ratio was below 100%.

Acknowledgements

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References

1.Griffon, D.J., Dunlop, D.G., Howie, C.R., Pratt, J.N., Gilchrist, T.J., Smith, N., An ovine model to evaluate the biological properties of impacted morselized bone graft substitutes. *J. Biomed. Mater. Res*., **56**(2001) 444.

2.Yamada, Y., Boo, J.S., Ozawa, R., Nagasaka, T., Okazaki, Y., Hata, K., Bone regeneration following injection of mesenchymal stem cells and fibrin glue with a biodegradable scaffold. *J. Cranio-Maxillofac. Surg*., **31**(2003)27.

3.Nishinari, K., Hofmann, K.E., Moritaka, H., Kohyama, K., Nishinari, N.. Gel-sol transition of methylcellulose. *Macromol. Chem. Phys*., **198**(1997)1217.

4.Nelson, C.L., The current status of material used for depot delivery of drugs. *Clin. Orthop*., **1**(2004)72.

5.Klose, D., Siepmann, F., Elkharraz, K., Krenzlin, S., Siepmann, J., How porosity and size affect the drug release mechanisms from PLGA-based microparticles. *Int. J. Pharm*., **314**(2006)298.