Loading of Gentamicin Sulfate into Poly (Lactic-Co-Glycolic Acid) Biodegradable Microspheres

[1]Hanieh Nojehdehian [2]Malihe Ekrami [*3]Zahra Jaberi Ansari

[1]Assistant Professor, Dept. of Dental Materials, School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
[2]Postgraduate student, Dept. of Operative Dentistry, School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
[*3]Professor, Dept. of restorative Dentistry, School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran. E-mail: zahrajaberiansari@yahoo.com

Abstract

Objective: In dental treatments, use of carriers for targeted antibiotic delivery would be optimal to efficiently decrease microbial count. In this study, gentamicin was loaded into polylactic co-glycolic acid (PLGA) microspheres and its release pattern was evaluated for 20 days.
Methods: In this experimental study, PLGA microspheres loaded with gentamycin were produced by the W/O/W method. The correct morphology of loaded microspheres was ensured using scanning electron microscopy (SEM). The rate of drug release from polymeric microspheres into the phosphate buffered saline (PBS) solution was measured during a 20-day period using spectroscopy. Data were analyzed using one-way ANOVA.
Results: SEM micrographs showed that the produced microspheres had smooth and nonporous surfaces and 30-micron diameter. Assessment of the pattern of drug release from the PLGA microspheres loaded with gentamycin revealed a burst release on day six followed by a stable pattern of release until day 20.
Conclusion: Considering the biocompatibility of PLGA and optimal pattern of drug release, PLGA microspheres loaded with gentamicin can be successfully used for infection control and reduction of microbial count in dental treatments.
Key words: Antibiotic, Controlled release, Drug release system, Gentamicin, Polylactic co-glycolic acid, Polymer microspheres.
Please cite this article as:
Nojehdehian H, Ekrami M, Jaberi Ansari Z. Loading of Gentamicin Sulfate into Poly (Lactic-Co-Glycolic Acid) Biodegradable Microspheres. J Dent Sch 2015; 33(2): 145-151.
Received: 09.07.2014 Final Revision: 22.11.2014 Accepted: 06.01.2015

Introduction:

Antibiotics are a group of drugs widely used for treatment of infections. Antibiotics have a short half-life and variable side effects. Designing drug release systems that enable effective and controlled drug delivery over a long period of time is a necessity for medical applications. Antibiotics are commonly administered systemically and locally in medicine and dentistry.

Direct pulp capping with the use of pulp capping agents is a common treatment modality for traumatic or mechanical pulp exposures. In this treatment, the exposed pulp is covered with a specific pulp capping agent that induces the formation of reparative dentin at the exposure site. The pulp tissue then recesses and the tooth remain vital. In the past decade, materials such as calcium silicate cements like mineral trioxide aggregate (MTA) and calcium-enriched mixture were introduced as ceramic materials capable of repair and induction of regeneration of pulp tissue.

Poly alpha hydroxy ester is a synthetic polymer and has three subgroups of aliphatic polyesters, polyanhydrides and poly ortho esters. Poly alpha hydroxy esters have different types such as polyglycolic acid and polylactic acid. Glycolic acid and lactic acid are by-products of various

metabolic pathways in the human body and are eliminated after production. Their main difference is in their degradability since polyglycolic acid is broken down faster than polylactic acid. Behavior and degradability of PLGA copolymer is controllable and this characteristic is the main advantage of this polymer and the reason for its application in medicine and dentistry (1-6). Use of biodegradable polymers in the form of microspheres/microparticles containing drugs in novel drug delivery systems enables controlled release of drug and is a new approach and an alternative to complex medical and dental treatments. Microspheres are mainly made of PLGA and are used for in vitro cell proliferation. They are injected at the injured site for tissue repair (7-10).

Many studies in the fields of pharmaceutics and orthopedics have been conducted on the production of PLGA microspheres and assessed the amount of released antibiotics from loaded microspheres. In restorative dental treatments and use of pulp capping agents, the main objective of treatment is to eliminate microorganisms from the pulp chamber and induce the formation of dentinal bridge. Antibiotic carriers would be ideal for decreasing the microbial count as much as possible.

In general, direct pulp capping has a poor prognosis due to the high risk of internal resorption, pulp calcification, necrosis and damage to the tooth surrounding bone (11-15). Nonetheless, direct pulp capping of primary teeth is performed by clinicians as a conservative pulp treatment (16). However, an ideal direct pulp capping agent for primary teeth has not yet been introduced (17). PLGA microspheres loaded with antibiotics may be used for direct pulp capping to increase the success of treatment. However, this topic needs to be further evaluated.

In this study, gentamicin was loaded into PLGA microspheres and its release pattern was evaluated over a 20-day period.

Methods:

In this in-vitro study, the following materials were used for the fabrication of PLGA microspheres loaded with gentamicin:

PLGA (RG504H, Sigma Aldrich, MO, USA), chloroform (Merck, Germany), gentamicin sulfate (SinaDarou, Tehran, Iran), polyvinyl alcohol (hydrolyzed 98%-99%; 50000-31000 MW, Sigma Aldrich, MO, USA) and phosphate buffered saline (PBS).

Preparing gentamicin solution:

To prepare gentamicin solution, deionized water was used in order to prevent the effect of ions on the surface of PLGA microspheres. The initial water phase included 1cc of polyvinyl alcohol (PVA) solution (1%w/v) containing 0.1 g gentamicin. The solution was vortexed in order for the gentamicin to dissolve completely.

Preparing PLGA microspheres containing gentamicin:

Microspheres were prepared using double emulsion method (W/O/W). In this method, the oil phase included 0.2 g PLGA dissolved in 650 µL chloroform. To produce microspheres, the initial water phase (gentamicin solution in PVA) was added to the oil phase. The mixture was homogenized at 13,000 rpm for one minute using a homogenizer (Heidolph, Schwabach, Germany) to obtain water-oil (W1/O) emulsion. The obtained emulsion was added to 30cc of the secondary water phase (0.01% w/v PVA solution).

The W1/O/W2 double emulsion was mixed by a magnetic mixer (Heidolph, Schwabach, Germany) for 2 hours at room temperature in order for the solvent to evaporate. To collect microspheres, the obtained emulsion was centrifuged at 11,000 rpm for 10 minutes. To wash microspheres, this procedure was repeated three times (18). The specimens were then frozen at -20°C and dried using freeze dryer

(Christ, Shropshire, UK) for 48 hours. Next, microspheres were thoroughly collected and stored at 0°C.

Particle size and surface morphology:

SEM (VEGA, TESCAN, LMU, USA) was used for assessment of surface morphology. To obtain SEM micrographs of PLGA microspheres, 5mg of specimens was weighed and placed on a piece of aluminum foil. Specimens were gold coated and evaluated under SEM in terms of size, shape and surface morphology of microspheres.

Release profile:

To determine the concentration of drug released from the microspheres into the PBS solution, a spectrophotometer (Unico 2100uv, New Jersey, USA) was used. Based on the results of scanning gentamicin in buffered solution, the drug was found to have a 330nm wavelength.

By preparing the stock solution and diluting it, different concentrations were obtained and for each concentration, drug absorbance at 330nm was measured. Standard curve of gentamicin concentration based on UV absorbance at 330nm was drawn by the spectrophotometer.

Drug release curve:

In three small containers, 3mg of PLGA microspheres containing gentamicin were added to 3mL of PBS solution and placed in incubator shaker at 37°C. PBS in each container was refreshed at specific time points. To assess the amount of released drug, UV spectroscopy was performed using a spectrophotometer at 330nm wavelength. The drug release measurement was performed every other day for 20 days.

Gentamicin loading:

To determine the encapsulation efficiency of gentamicin, 15 mg of drug-containing microspheres was dispersed in 10mL of 5%w/v sodium dodecyl sulfate (SDS) in NaOH solution and the specimens were then mixed for 12 hours by a magnetic mixer for complete degradation of particles and release of drug. Twelve hours after the degradation, to separate any possible remaining particle, the samples were centrifuged at 8000 rpm for 5 minutes and the supernatant was collected. The amount of drug extracted from the microparticles present in the solution was measured using a spectrophotometer and compared with the baseline value of drug prior to loading to assess the amount of loaded drug (encapsulation efficiency).

Encapsulation efficiency (EF)%=

$$\frac{\text{Mass of drug in microspheres}}{\text{Mass of initial drug}} \times 100$$

Statistical analysis:

Data were analyzed using SPSS version 18. The mean, standard deviation, minimum and maximum values of released drug at different time points were calculated and changes in the pattern of drug release at different time points were assessed using one way repeated measure ANOVA. Type one error was considered as 0.05 and $p \leq 0.05$ was considered statistically significant.

Results:

As seen in SEM micrographs, fabricated microspheres had a smooth, non-porous surface and less than 15-micron diameter. Standard concentration curve for gentamicin sulfate based on UV absorbance at 330nm wavelength was drawn by a spectrophotometer and the following equation was obtained:

Log y=9.6661 log x + 1.450

Y= concentration

X= absorbance

The concentration of gentamicin released from the microspheres and the amount of drug loaded into the microspheres were calculated using the above-mentioned formula. The encapsulation efficiency of gentamicin sulfate in the microspheres was calculated to be 45%.

The concentration curve of released drug in µg/mL at different time points is shown in Diagram 1. Assessment of the pattern of release of gentamicin from the microspheres revealed a burst release on day six followed by continuous

stable release until day 20. The results of statistical analyses showed significant differences in terms of the concentration of released drug at different time points (p=0.004) and the release pattern at day six was significantly different from that at different time points (p<0.001). But, no other significant differences were noted in this regard among other time points (p=0.098). On day six, burst release of gentamicin from PLGA microspheres occurred, which is responsible for the significant difference in the pattern of release between day six and other time points.

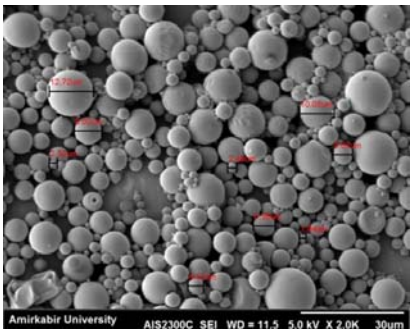

Figure 1- SEM micrograph of PLGA microspheres containing gentamicin

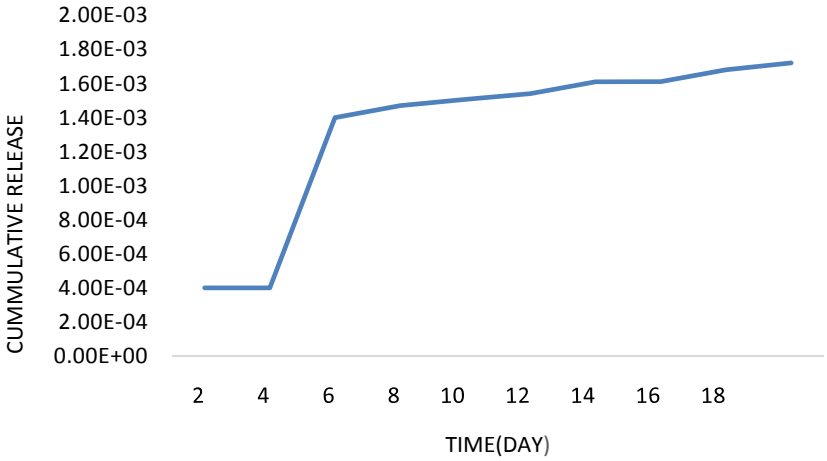

Diagram 1- Gentamicin release pattern (µg/mL) from thePLGA microspheres

Discussion:

Biodegradable microspheres are used for controlled release and targeted delivery of drugs. Considering the need for highly effective antimicrobial agents for root canal infections, in this study we loaded gentamicin into PLGA microspheres.

Gentamicin has the highest efficacy for use in studies on controlled drug release due to its broad-spectrum antimicrobial activity, high solubility and stability at high temperatures (19). Gentamicin has bactericidal effects on aerobic Gram-negative and some Staphylococcus strains. Despite some previous studies on drug release, mechanisms of drug release have yet to be fully recognized and control of release is difficult to achieve.

Considering the specific physical and chemical properties of gentamicin sulfate, several studies have assessed the type of carrier, technique of loading, the amount of water phase and substantivity of drug. In most previous studies, PLGA carriers have been used for loading of this drug. Phosphate salt solution has been mainly used as gentamicin solvent in previous studies, which results in porosities, increased drug release and inadequate drug control. In the

current study, PVA solution was used as the solvent in order to produce nonporous microspheres.

SEM analysis showed smooth surface and small size of microspheres, which is in line with the findings of Sivakumar *et al*. in 2002 (20). Also, using homogenizer, small microspheres were produced in our study, which was similar to the ultrasonic method adopted by Virto *et al*, in 2007 (21) with the difference that homogenizer used in our study does not destruct polymer chains (in contrast to ultrasound).

Drug loading depends on the size of microspheres. The larger the microspheres, the greater the amount of loaded drug. This explains the lower loading rate of gentamicin in the produced microspheres in our study.

When microspheres are immersed in an aqueous environment, water penetrates deep into the particles and results in diffusion of drug through the microporosities of the polymer. In macromolecules, diffusion through the porous network is highly limited due to the confined space and occurs gradually as the pores become larger due to the degradation of polymer. Thus, the rate of drug release from the polymeric microspheres is mainly controlled by the amount of loaded drug, size of polymer, degradation of polymer, its porous structure and drug penetration.

Pattern of drug release from the microspheres depends on the type of dug, its hydrophobicity/ hydrophilicity and manufacturing conditions such as type of polymer and method of fabrication. In fact, use of PLGA copolymers with different molecular weights and compositions changes the primary hydration and degradation rate of matrix. However, limitations in type of PLGA copolymer and method of fabrication can be a major obstacle for controlled release of drug. In such conditions, improving the primary morphology (i.e. percentage of primary porosity) or addition of factors affecting drug diffusion through the polymer matrix may be of great help. Gentamicin is a highly hydrophilic antibiotic and thus, its loading into the matrix is very important and is determined based on its desired release rate. Gentamicin-containing microspheres in this study were produced in such way that small microspheres (30-micron diameter) had the lowest encapsulation efficiency. To increase encapsulation efficiency, if required, the share of water phase must be minimized and large microspheres must be produced. In the current study, encapsulation efficiency of gentamicin was 45% and the release period was 20 days, which are similar to the results of Naraharisetti *et al*. in 2005; who reported 50% encapsulation efficiency and release period of 15 days from paclitaxel loaded poly (L-lactic acid (PLLA)microspheres (22). PLGA microspheres undergo mass and homogenous degradation due to the hydrolysis of ester bonds. In 2002, Blanco-Prieto *et al*. stated that by increasing the solubility of gentamicin, loading rate increased, which is in contrast to our findings because in our study, the highest solubility of gentamicin sulfate was considered in the water phase and the microspheres were produced (23).

However, entrapment of gentamicin, its release profile and size of microspheres highly depend on the method of encapsulation, polymer characteristics, biodegradability, position of copolymer and its molecular weight (23-25).

The current study evaluated the release of gentamicin from PLGA microspheres at different days. Moreover, PLGA powders in conjunction with MTA may be suitable for use as an acceptable direct pulp capping agent for placement at the site of pulp exposure. This topic can be the subject of future investigations.

Based on the results of the current study, the concentration of released drug from PLGA microspheres was not significantly different after day six. Burst release of gentamicin from PLGA particles occurred on day six.

Conclusion:

PLGA microspheres loaded with gentamicin sulfate provide controlled and sustained release of drug for over 15 days and thus, they can be used for restorative dental treatments such as direct pulp capping.

Conflict of Interest: "None Declared"

References:

1. Sawicki L, Pameijer CH, Emerich K, Adamowicz-Klepalska B. Histological evaluation of mineral trioxide aggregate and calcium hydroxide in direct pulp capping of human immature permanent teeth. Am J Dent 2008; 21: 262-266.
2. Farsi N, Alamoudi N, Balto K, Al Mushayt A. Clinical assessment of mineral trioxide aggregate (MTA) as direct pulp capping in young permanent teeth. J Clin Pediatr Dent 2006; 31: 72-76.
3. Pace R, Giuliani V, Pagavino G. Mineral trioxide aggregate as repair material for furcal perforation: Case series. J Endod 2008; 34: 1130-1133.
4. Ng FK, Messer LB. Mineral trioxide aggregate as a pulpotomy medicament: an evidence-based assessment. Eur Arch Paediatr Dent 2008; 9: 58-73.
5. Asgary S, Eghbal MJ, Ehsani S. Periradicular regeneration after endodontic surgery with calcium-enriched mixture cement in dogs. J Endod 2010; 36: 837-841.
6. Shi G, Cai Q, Wang C, Lu N, Wang S, Bei J. Fabrication and biocompatibility of cell scaffolds of poly (L-lactic acid) and poly (L-lactic-co-glycolic acid). Polym Adv Technol 2002; 13: 227-232.
7. Hoekstra JW, Ma J, Plachokova AS, Bronkhorst EM, Bohner M, Pan J, *et al*. The in vivo performance of CaP/PLGA composites with varied PLGA microsphere sizes and inorganic compositions. Acta Biomater 2013; 9: 7518-7526.
8. Qutachi O, Vetsch JR, Gill D, Cox H, Scurr DJ, Hofmann S, *et al*. Injectable and porous PLGA microspheres that form highly porous scaffolds at body temperature. Acta Biomater 2014; 10: 5090-5098.
9. Nojehdehian H, Moztarzadeh F, Baharvand H, Nazarian H, Tahriri M. Preparation and surface characterization of poly- L -lysine-coated PLGA microsphere scaffolds containing retinoic acid for nerve tissue engineering: in vitro study. Colloids Surf B Biointerfaces 2009; 73; 23-29.
10. Wen Y, Gallego MR, Nielsen LF, Jorgensen L, Everland H, Møller EH, *et al*. Biodegradable nanocomposite microparticles as drug delivering injectable cell scaffolds. J Control Release 2011; 156: 11-20.
11. Seong KP, Jeon SY, Singh B, Hwang JH, Song SJ. Comparative study of an experimental Portland cement and ProRoot MTA by electrochemical impedance spectroscopy.Ceram Int 2014; 40: 1741-1746.
12. Camilleri J, Sorrentino F, Damidot D. Investigation of the hydration and bioactivity of radiopacified tricalcium silicate cement, Biodentine and MTA Angelus. Dent Mater 2013; 29; 580-593.
13. Tuna D, Olmez A. Clinical long-term evaluation of MTA as a direct pulp capping material in primary teeth. Int Endod J 2008; 27:273-278.
14. Fuks AB. Pulp therapy for the primary and young permanent dentitions. Dent Clin North Am 2000; 44:571-596.
15. Rodd HD, Waterhouse PJ, Fuks AB, Fayle SA, Moffat MA; British Society of Paediatric

Dentistry. Pulp therapy for primary molars. Int J Pediatr Dent 2006; 16:15-23.
16. Asgary S, Kamrani FA. Antibacterial effects of five different root canal sealing materials. J Oral Sci 2008; 50: 469-474.
17. Kopel HM. Considerations for the direct pulp capping procedure in primary teeth: a review of the literature. ASDC J Dent for Child 1992; 59:141-149.
18. Wahlig H, Dingeldein E. Antibiotics and bone cements. Experimental and clinical long-term observations. Acta Orthop Scand 1980; 51: 49-56.
19. Gander B, Johansen P, Nam-Trân H, Merkle HP. Thermodynamic approach to protein microencapsulation into poly (D,L-lactide) by spray drying. Int J Pharm 1996; 129; 51-61.
20. Sivakumar M, Panduranga Rao K. Preparation, characterization and in vitro release of gentamicin from coralline hydroxyapatite–gelatin composite microspheres. Biomaterials 2002; 23: 3175-3181.
21. Virto MR, Elorza B, Torrado S, Elorza Mde L, Frutos G. Improvement of gentamicin poly (D,L-lactic-co-glycolic acid) microspheres for treatment of osteomyelitis induced by orthopedic procedures. Biomaterials 2007; 28: 877-885.
22. Naraharisetti PK, Lew MD, Fu YC, Lee DJ, Wang CH. Gentamicin-loaded discs and microspheres and their modifications: characterization and in vitro release. J Control Release 2005; 102; 345-359.
23. Blanco-Prieto MJ, Lecaroz C, Renedo MJ, Kunkova J, Gamazo C. In vitro evaluation of gentamicin released from microparticles. Int J Pharm 2002; 242: 203-206.
24. Thomasin C, Corradin G, Men Y, Merkle HP, Gander B. Tetanus toxoid and synthetic malaria antigen containing poly (lactide)/ poly (lactide-co-glycolide) microspheres: importance of polymer degradation and antigen release for immune response. J Control Release 1996; 41: 131-145.
25. Pavanetto F, Genta I, Giunchedi P, Conti B. Evaluation of spray drying as a method for polylactide and polylactide-co-glycolide microsphere preparation. J Microencapsul 1993; 10: 487-497.