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Research Article

HIGH INITIAL BURST RELEASE OF GENTAMICIN FORMULATED AS PLGA MICROSPHERES IMPLANT FOR TREATING ORTHOPAEDIC INFECTION

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ABSTRACT

Antibiotic treatment of orthopaedic infection is complicated by systemic toxicity and the need of effective therapeutic concentration necessary to ensure optimum killing of bacteria. To overcome the problem of systemic toxicity and to achieve a high initial release followed by sustained release of antibiotics, a new method of delivering gentamicin is attempted by encapsulating gentamicin into PLGA using multiple emulsion, solvent-evaporation method. Gentamicin was first extracted from the microspheres and quantified using ninhydrin assay before the concentration was measured using UV spectrophotometer. Gentamicin efficacy after encapsulation was preserved when CTAB (83.51 ± 1.42%) and low molecular weight (LMW) PLGA (82.38 ± 9.08%) were used as indicated by drug loading efficiency of more than 80% in the disc-diffusion assay. LMW PLGA enabled high burst release (~90%) of gentamicin within the first 10 hours corresponding to zone inhibition of 13.78 ± 0.86 mm, only 30% smaller than the positive control (10 mg/ml gentamicin). The effects of T_g and molecular weight rather than surfactant types influence the initial burst release. The in vitro release profile suggests that by having a mixture of various PLGA microspheres in one dosage implant system, the high burst release can be sustained within therapeutic concentration for a prolonged period (> 1 months). This biodegradable delivery system does not entail another surgery to remove the implant hence reducing the high treatment cost usually associated with the non-biodegradable proprietary gentamicin-polymethyl-methacrylate (PMMA) beads currently in use.

Keywords: Micro particles, Gentamicin; Surfactant, PLGA, Controlled-release

INTRODUCTION

Current treatment for acute and chronic orthopaedic infection using non-biodegradable, antibiotic implant is frequently hampered by high cost and disabling problem that severely affects patient'squality of life. A long-standing infection in an orthopaedics case often causes long-term complications, requiring numerous surgeries and prolonged antibiotic treatments. Implant removal inflates the medical expense further. It is estimated that implant removal, 6 weeks of parenteral antibiotics, andre-implantation can cost approximately USD50, 000per patient.

A common, debilitating infection in orthopaedics setting is osteomyelitis, mainly caused by *Staphylococcal aureus*[1].It is an inflammatory bone disease with deep bone involvement infiltrating the medullary cavity, cortex and periosteum[2].Osteomyelitis normally arises as a nosocomial infection due to post-operative orthopaedic surgeries, introduced during implantation of a prosthesis or carried to the biomaterial surface by a temporary bacteraemia where they adhere and grow to form a biofilm[3]. Osteomyelitis is especially complicated if patients are immune-compromised[4].

One of the conventional treatments for osteomyelitis is by local administration of antibioticeither by spray or by injection to the infected site followed by oral administration of the antibiotic to optimize its therapeutic effect[5]. Parenteral injection can provide adequate bioavailability of gentamicin, however the long-term indwelling catheter coupled with repeated doses of antibiotics are major disadvantages of this treatment regime[4]. The risk of otoxicity and nephrotoxicity of gentamicin following uncontrolled prolonged use, especially in orthopaedic infection, is the main concern in patient administered with gentamicin[6]. This risk however can be mitigated by using an implant system for localized effect in which the risk of systemic toxicity is minimized[6, 7]. For example, polymethyl-methacrylate (PMMA) containing gentamicin bead that is locally implanted at infection site, has been used as standard treatment option for orthopaedic infection[4]. Unfortunately, a major disadvantage associated with PMMA beads is its non-biodegradability which requires a second surgery to remove the beads resulting in substantially high overall treatment cost.

Gentamicin-loaded PLGA microsphere prepared by using solvent evaporation method[8] has been proposed to overcome the shortcoming of PMMA beads. However, none that has been approved to be used clinically despite several encouraging reports. PLGA remains the best candidate for this delivery system[9]; nevertheless other polymers had also been tested such as namely poly(L-lactic-co-hydroxymethyl glycolic acid)[6] and poly(3-hydroxybutyrate)[10] which exhibited varied critical formulation parameters that influence encapsulation efficiency and the initial release of the micro-carrier system.

It is well-known that there are myriad of factors contribute synergistically and antagonistically depending on the nature of the microspheres including polymer concentration[6, 10], types of polymers[7] and molecular weight of the polymer[11]. For example, synergistic effect affecting positively the encapsulation efficiency by the addition of chitosan as co-polymer to stabilize the microencapsulation process has been demonstrated in earlier studies[12, 13]. In view of the lack of data that establish suitable release profile of the gentamicin-PLGA microspheres intended as antibiotic implant for orthopaedic infection, this study tested various formulation variables with the objective of obtaining a desirable release profile with preserved antimicrobial efficacy post-encapsulation. This study also demonstrated feasible way to quantify gentamicin extracted from PLGA microspheres using ninhydrin assay.

MATERIALS AND METHODS

Materials

All chemicals were of analytical grade. Dichloromethane (DCM) was obtained from Fisher Scientific (United Kingdom), Poly vinyl alcohol (PVA) with MW 115 kDa was purchased from BDH Laboratory Supplies (England).Different intrinsic viscosities of PLGA (50:50) (0.2 dl/g, 0.4 dl/g and 1.0 dl/g) were purchased from PURAC (Holand). Tween 20, Tween80, Span 80, Span 85, Triton X100 and Sodium dodecyl sulphate (SDS) were supplied by MERCK (Germany). Cetyltrimethylammonium bromide (CTAB) was purchased from SIGMA® (Denmark). Gentamicin Sulphate was obtained from CALBIOCHEM® (Germany). Ninhydrin was supplied by Fisher Scientific (United Kingdom).

Microencapsulation of gentamicin

Modified multiple-emulsion solvent evaporation method was adopted from Mohamed and van der Walle[14]. Briefly, primary emulsion was prepared by dissolving 100 mg of PLGA in 2 ml DCM

(oil phase). This was homogenized (14, 500 rpm, 1 min)with 200 µl gentamicin solution consisting of 100 mg of the drug (dissolved in PBS) and 22 µl of respective surfactants, producing 1% w/v of primary emulsion.

The primary emulsion was injected directly into secondary continuous phase (1% w/v aqueous PVA)of 10 times the volume of primary emulsion and homogenized (14, 500 rpm, 3 min) to produce the secondary emulsion.

Subsequently, this secondary emulsion was transferred into a continuously stirred hardening tank containing 100 ml of 1% w/v PVA (2 hour). The microspheres were collected by centrifugation (4000 rpm), washed and then lyophilized overnight. Dried microspheres were kept in air-tight container prior to further evaluation.

Characterization of Microspheres

Particle size analysis and morphology

Prior to lyophilization, the microspheres suspension were subjected to particle size analysis using Laser Particle Size Analyzer BT-9300H (Better size Instrument Ltd., China). The particle size distribution was expressed as the volume weight diameter. For every sample, the measurement was done in triplicate.

Scanning electron microscope (SEM), Carl Zeiss Evo® 50 (Germany) was used to capture images for evaluation of shape, size and external morphology of the microspheres. Briefly, small amount of lyophilized microspheres were mounted on aluminum stubs, pre-pasted with double-sided copper tapes. The samples were sputter-coated with a thin layer of gold[15] and placed inside the specimen chamber at an accelerating voltage of 5-10 kV at 20 °C and 10^{-5}Torr.

Quantification of gentamicin and encapsulation efficiency (EE)

Colorimetric assay developed and validated by Frutos' research group was employed to quantify gentamicin extracted from microspheres[16]. Briefly, 5 – 8 mg of lyophilized microsphere was suspended in 1 ml PBS to which 1 ml of DCM was added to solubilize PLGA. The tube was rotated end-to-end for 1 h prior to centrifugation (6000 rpm, 5 min). 800 µl of supernatant was then transferred to a fresh tube to which 240 µl of freshly prepared 2 mg/ml ninhydrin previously dissolved in PBS was added. The mixture was vortexed, heated at 95°C (15 min) and then cooled in ice bath (10 min). The mixture was transferred to CELLSTAR® 96 well plate flat bottom (Greiner bio-one) and subjected to UV absorbance measurement at 400 nm. The absorbance values were substituted into a standard curve of linear regression of known gentamicin concentration to obtain the actual concentration of extracted gentamicin. Encapsulation efficiency was calculated based on the ratio of actual gentamicin concentration to theoretical loading, expressed as percentage.

Glass transition temperature

Method employed by Mohamed & van der Walle was adopted[14]. A Differential Scanning Calorimeter (DSC1, Mettler Toledo) with sensor accuracy of 0.1 °C was used to measure the glass transition temperature (T_g) under nitrogen atmosphere as the purge gas. Approximately 2 mg of microspheres were accurately weighed on a microbalance and evenly spread onto 40 µl aluminium crucibles making sure the edge of the crucible was spill-free from any microspheres before it was hermetically sealed with a pinhole in the lid. The reference against which the sample was measured consisted of an empty pin-holed aluminium crucible of the same geometry and mass as the microsphere crucibles. Both crucibles were first allowed to equilibrate at 0 °C (5 min) to ensure isothermal starting condition. The crucibles were then heated at a rate of 10 °C/min from zero to 85 °C, quench cooled to -20 °C and heated again to 85 °C at the same heating rate. The bisector method of T_g determination was employed on the scanning curve and calculated using Mettler Toledo STARe® software version 9.10.

Antimicrobial study

Antimicrobial study was conducted to evaluate the efficacy and stability of the encapsulated gentamicin. An established method known as "Disk Diffusion Method" was adopted from Reller's research group[17]. Each paper discs (Oxoid, Hampshire, England) were impregnated with 100 µl of gentamicin samples previously extracted from individual PLGA microspheres and were placed on freshly prepared nutrient agar lawned with *S. aureus*. The zone inhibitions were measured following 24 hour incubation at 37 °C. Data of triplicate samples were recorded.

In Vitro Release Profile

Real-time release of individual microspheres

Only microspheres samples that gave 20% or higher encapsulation efficiency were further evaluated for their in vitro release performance. Approximately 10 mg of microspheres were placed into Eppendorf tubes and 1 ml of PBS was added. The tubes were inverted 5 times before they were left undisturbed at 37°C. At pre-determined time points (1, 2, 3, 5 and 10 hour followed by 1, 2, 3, 4, 5, 6 and 7 days, followed by 2, 3 and 4 weeks), 0.8 ml of samples were withdrawn following centrifugation (6000 rpm, 1 min). The samples were subjected to ninhydrin assay to quantify the gentamicin being released. 0.8 ml of fresh PBS was added into original Eppendorf tube containing microspheres to replenish the amount being withdrawn.

Theoretical prediction of release from microspheres blends

Based on the in vitro release performances of all formulated microspheres here, the release is expected to show desirable results if all of the microspheres were to be blended into one dosage unit, yielding one unique release profile. In order to test our hypothesis, a simple way of deriving the in vitro release profile of the blends was proposed. Since each formulation had different encapsulation efficiency, common encapsulation efficiency must be calculated retrospectively. This was done by blending together 4 samples of microsphere with equivalent amount in such a way that each would maintain the same (10 mg) employed in real-time in vitro release study. Therefore, total weight for all four tested formulations was calculated to be 40 mg.

For microsphere fabricated using CTAB, Triton X-100, 6% PLGA and PLGA0.2, the encapsulation efficiency were 83.51%, 24.66%, 22.83% and 82.38% respectively. From 10 mg of each formulation, the corresponding theoretical amounts of gentamicin were to be 8.35 mg, 2.46 mg, 2.28 mg and 8.23 mg respectively. Hence, total theoretical amount of gentamicin were to be 21.33 mg from 40 mg of which the encapsulation efficiency of the blended were to be53.35%. Calculation of cumulative release of one dosage unit was based on this retrospective encapsulation efficiency and used to plot release profile graph.

RESULT AND DISCUSSION

Characteristics of microspheres as a function of multiple variables

Particle size and surface morphology

Fig. 1 shows the physical characteristics of gentamicin-loaded microspheres fabricated according to the variables being studied i.e. types of surfactant. Microspheres fabricated by employing 1% PVA as surfactant in the primary emulsion was regarded asa control due to the presence of PVA which is commonly used in microencapsulation protocol[14].

The study found that size distribution of microspheres fabricated with Tween 20 and CTAB were significantly ($P<0.05$) smaller than microspheres fabricated with PVA. While CTAB seemed to produce the highest encapsulation efficiency attributed to its ability to produce more stable emulsion, the result also suggests that particles size of microspheres employing similar microencapsulation technique with similar variables can yield significantly different particle size depending on agent being encapsulated. When macromolecules (DNA; peptides) were encapsulated, the particles seemed to be as 10 times larger than when small molecules drug, such as gentamicin, was encapsulated[14, 18]. This can be a useful guideline to predict range of particle size employing similar techniques for small molecules and macromolecules.

Contrary to previous findings on the effect of sorbitan-based surfactants towards surface morphology of the microspheres[14, 18], it is demonstrated in this study that gentamicin microspheres fabricated with Span 80 (HLB 4.3) and Span 85 (HLB 1.8) appeared with some dimples on the surfaces (Fig. 1). The dimple geometry that was formed was probably due to the presence of small molecular weight materials (hydrophilic gentamicin) in the nascent microspheres that could not resist the concavity action of Spans surfactants at the interfaces of the multinary systems. In contrast, macromolecules like DNA and peptides have sufficient molecular energy to resist and compromise the concavity effect as manifested by smooth surface DNA-loaded PLGA microspheres[14]. The results also suggest that surface templating activity causing dimples is not necessary applicable due to absence of strong hydrophilic-hydrophobic contrast within the molecular chain of these sorbitan-based surfactants, unlike Pluronic surfactants.

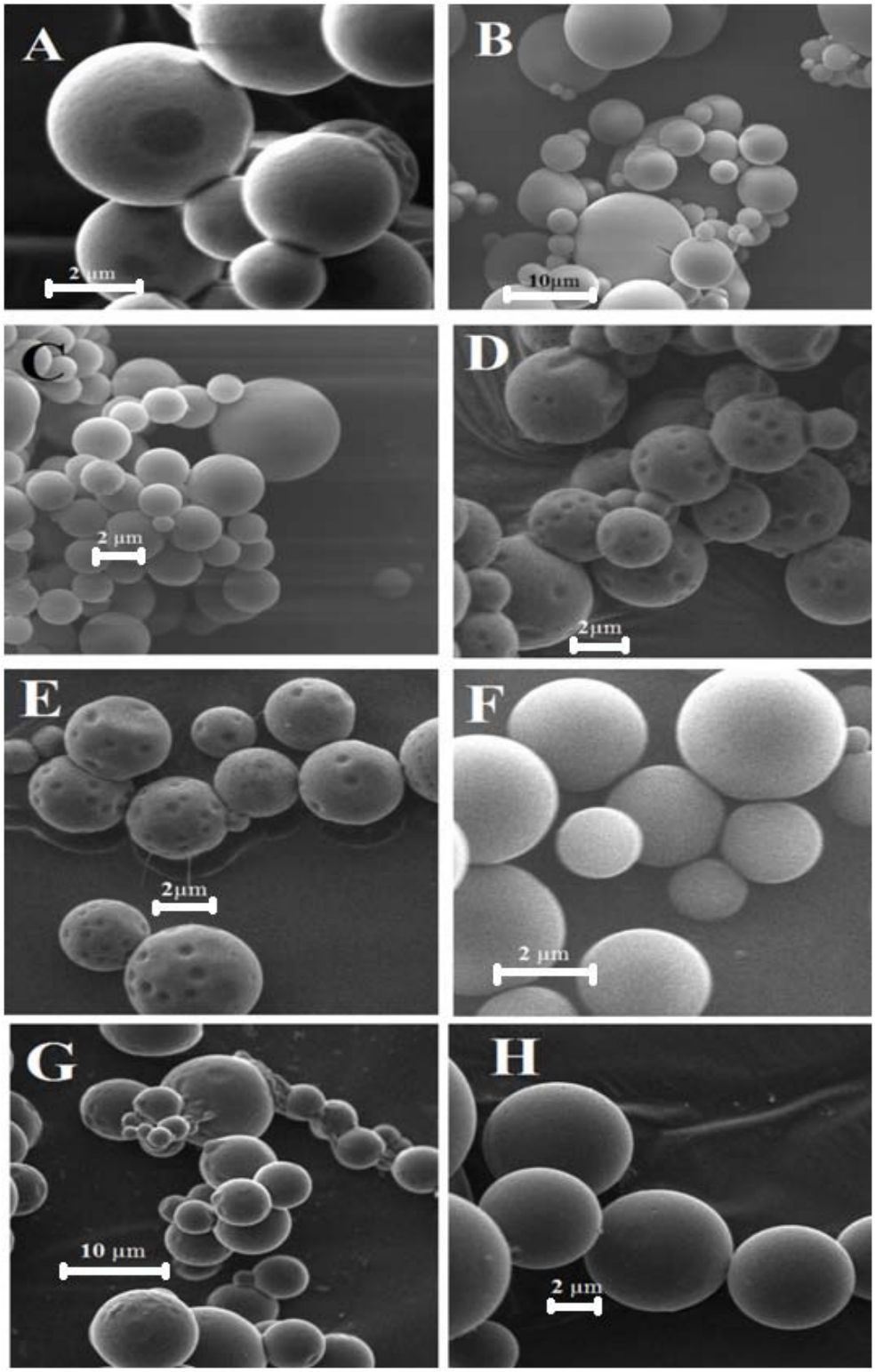

Fig. 1: Surfaces morphologies of the microspheres fabricated using 1% w/v PVA in the secondary emulsion and the following surfactants in the primary emulsion: (A) PVA; (B) Tween 20; (C) Tween 80; (D) Span 80; (E) Span 85; (F); CTAB (G) SDS and (H) Triton X-100.

Drug encapsulation efficiency

Based on surfactants as variables, CTAB gave the highest encapsulation efficiency, as high as 83.51% ±1.42, in comparison to other surfactants (Fig. 2). In fact, other surfactants produced not more than 25% encapsulation efficiency as could be observed for Triton X-100 (24.66% ±3.84). The gentamicin is a positively charged molecules[19]and this observation is in contrast to pure manner of electrostatic interaction. One possible reason is that this gentamicin molecule may act as a specific adsorb ion on CTAB/PLGA surfaces and during the microspheres synthesis, this condition caused charge reversal to gentamicin sulphate 'catalysed' by the presence of sulphate (from dissociation of gentamicin sulphate) and phosphate ions (from PBS). This could also explain the low encapsulation efficiency obtained when SDS was employed. The phenomena have been observed whenever the particles' zeta potential went beyond its potential of zero charge (PZC) causing potential-determining ions (PDI) to predominate. The latter interaction is commonly observed in corrosion study[20] and none microspheres-related references had also mentioned this. However, the use of buffer system in the internal and external aqueous phases corroborated understanding that present of salts that control osmotic pressure play an important role in promoting incorporation of water soluble drugs into PLGA systems[6, 21].

As expected, employment of Tween 20 produced lowest encapsulation efficiency due to its preferential stabilizing effect for O/W emulsion. In contrast, Triton X-100, which is also a hydrophilic surfactant, seemed to favour encapsulating gentamicin into PLGA/DCM front. It follows that the Span groups of surfactants did not favour encapsulation of gentamicin molecules despite its prevalence use in stability of W/O emulsion. This unusual observation is perhaps due to relatively small micellar molecular weights of sorbitan-based surfactants[22] as compared to Triton X-100, the former having insufficient micellar molecular energy to resist dilution collapsing its critical micelles concentration.

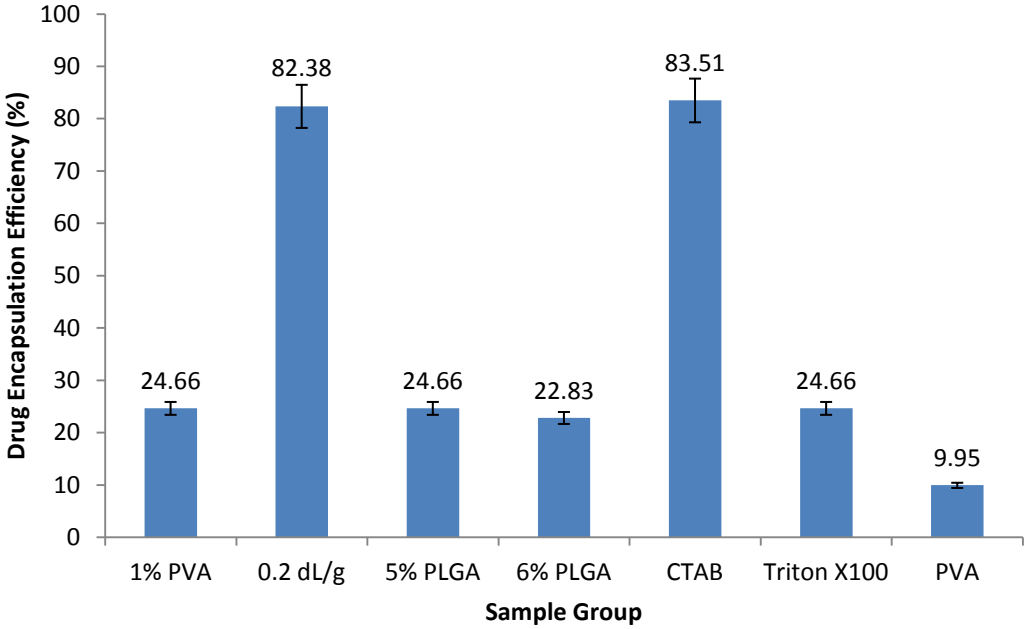

Fig. 2: The 6 batches of gentamicin microspheres with more than 20% drug encapsulation efficiency and a control group (PVA). The values were the average taken from triplicate measurements (n=3).

Glass transition temperature

The thermal analysis of the microspheres was done to investigate effects of different variables towards the onset of the glass transition (T_g) temperature as this would influence the activity of the microspheres from the perspective of drug release and storage. As water is known to hydrolyze PLGA, storing the microspheres in a place with high humidity will cause prominent physical effects on the microspheres that can unknowingly alter the drug release profile[23].

Table 1: The onset of glass transition for the gentamicin-loaded microspheres

Sample Group	T_g Onset (°C)
PVA	46.3
Tween 80	44.5
Tween 20	44.4
Span 85	45.9
Span 80	45.6
Triton X-100	44.6
SDS	47.0
CTAB	46.4
14 kDa(0.2 dL/g)PLGA	32.9
34 kDa(0.4 dL/g)PLGA	40.0
100 kDa(1.0 dL/g)PLGA	43.9

Based on the data obtained (Table 1), lower MW PLGA exhibited lower T_g compared to higher MW PLGA. However, for the same MW of PLGA, effects of surfactants can be seen and the T_g values seem to suggest that all surfactants had anti-plasticizing effects on the gentamicin-loaded PLGA microspheres. This is especially so for PVA whose anti-plasticizing effect was more prominent compared to if the therapeutic agent being encapsulated were macromolecules[14]. This implies that the presence of bigger molecules in the PLGA microspheres had in some way restricted the molecular re-arrangement of the polymer when being heated at high temperature. It also suggests that, PLGA microspheres encapsulating small molecules will need adequate amount of moisture content to prevent excessive brittleness of the microspheres to ensure sufficiently long shelf-life.

PLGA microspheres fabricated using 14 kDa (0.2 dL/g) PLGA, had the lowest T_g (32.9 °C) when compared to other microspheres especially those fabricated using 100 kDa (1.0 dL/g) PLGA which was 43.9 °C. This T_g was well below the body temperature which indicates that some anti-plasticizer will be required as adjuvant to sustain the stability of the microspheres prior to its administration into body. It also explains the reason behind high initial burst release (>80%) demonstrated by this microspheres. Despite having comparably high encapsulation efficiency, only the low MW PLGA

exhibited more than 80% release within 10 hour. The other CTAB-PLGA microspheres were all fabricated with higher MW PLGA. It appears that T_g value and low MW weight PLGA, and not surfactants, were the dominant factors that could influence the burst release of PLGA microspheres as depicted in Fig.4.

Antimicrobial study

Apart from high encapsulation efficiency, preservation of the antimicrobial efficacy of gentamicin reflecting the stability of encapsulated gentamicin is also crucial. Antimicrobial study was conducted to evaluate stability of gentamicin by observing its effect on bacterial growth. *Staphylococcus aureus* was selected to be tested against encapsulated gentamicin that achieved more than 20% encapsulation efficiency. This bacterial strain was selected due to its prevalence in causing infection related to bone[3, 4, 24].

Table 2: Data of zone of inhibition expressed as the mean ± SD of triplicate samples.

Sample Group	Zone of Inhibition (mm) ± S.D
CTAB	14.11 ± 0.13
Triton X-100	12.04 ± 0.19
1% PVA	12.57 ± 0.32
5% PLGA	12.04 ± 0.41
6% PLGA	11.36 ± 0.16
PLGA 0.2 dl/g	13.78 ± 0.86
Free gentamicin (positive control) 10mg/ml	20.00 ± 0.29

Based on the zone inhibition data (Table 2), all 6 batches of gentamicin microspheres showed that the antibiotic loaded in the microspheres retained its stability post-fabrication implying that technique employed is suitable to the molecular stability of gentamicin. Diameters of zone of inhibition for all batches were directly related to their respective encapsulation efficiency. For example, CTAB that produced highest encapsulation efficiency managed to release the drug within 24 hour of measurement, with highest amount reflected by largest zone inhibition, followed by microspheres fabricated from 14 kDa (0.2 dL/g) PLGA, which were second highest in encapsulation efficiency. Both formulation produced results that were statistically significant difference ($P < 0.05$) to other groups. Interestingly, the zone inhibition by CTAB, was only 30% smaller than free gentamicin (10 mg/ml).

In vitro release profile

This study was conducted only for microspheres having encapsulation efficiency more than 20%. Based on Fig.3, total release of gentamicin was observed by day 6 from gentamicin microspheres fabricated using the lowest PLGA MW. The rate of release tends to accelerate due to its T_g that was lower than the temperature of dissolution media. As the surrounding temperature was higher than the T_g, the rubbery states of the microspheres predominated, increasing the molecular chain mobility of the polymer promoting intake of water and rate of hydrolysis[25]. The rate of release seems to fasten with the high encapsulation efficiency achieved by this PLGA as suggested by other study[26]. Apart from that, hydrophilic surfactant, Triton X-100, employed for this microsphere, also contributed to the high initial burst release seen within 10 hour since it can enhance wetting effect of the PLGA microspheres promoting release of gentamicin located at the microsphere surface. This significantly high encapsulation efficiency coupled with high initial burst release compared to other microspheres were attributed to a combined effect of appropriate surfactant with low MW PLGA, the release of which is desirable since it can provide high antibiotic concentration available at the site of implant.

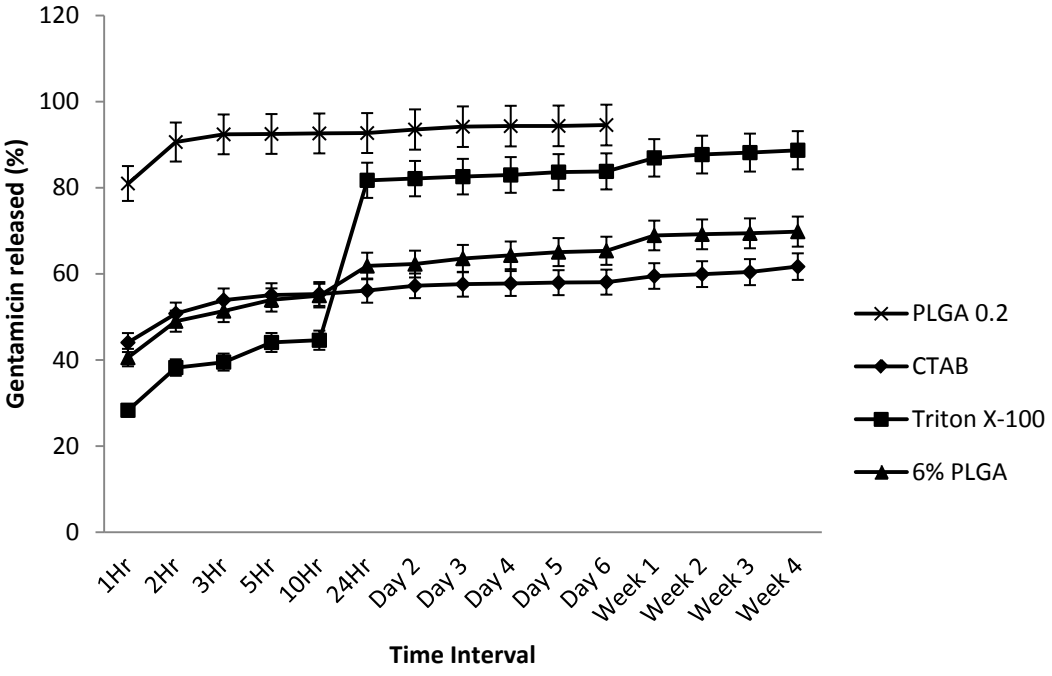

Fig. 3: The cumulative release expressed as percentage of gentamicin from four different formulation of microspheres: PLGA 0.2 employing 5% 0.2 dl/g PLGA with Triton X-100; CTAB employing 5% IV 1.0 dl/g PLGA; Triton X-100 employing 5% IV 1.0 dl/g PLGA and 6% IV 1.0 dl/g PLGA employing Triton X-100. Plotted data are the mean values with error bar of triplicate samples.

On the other hand, although comparable encapsulation efficiency was achieved in CTAB-PLGA microspheres, but it did not display a high burst effect as that of low MW Triton X100-PLGA microspheres. The reason perhaps is dueto the higher MW of the former formulation and the type of surfactant employed. Approximately 50% of gentamicin was released within 10 hour and release continued to be extremely minimal for the next hours with total release less than 60% for a period of one month. CTAB may had higher binding strength than water to gentamicin and hence reduce the release rate of gentamicin from microspheres, unlike Triton

X100-PLGA microspheres whose release was initially low but drastically increase after 24 hour to a plateau despite similar MW PLGA. Interestingly, microspheres fabricated with 1% higher concentration than the others showed almost similar rate of release to that CTAB, despite using Triton X100 as surfactant.

To summarise, the results suggest that, in vitro release of gentamicin-loaded PLGA microspheres was different from predicted results based on other findings. Nevertheless, it is postulated based on the data of in vitro release profile that combination of this release profiles together can result in a better, almost first-order release kinetic. As such, we had constructed a theoretical in vitro release profile and a plot of graph was constructed as shown in Fig.4. The combined release profile of microspheres blends depicted 20% lower initial burst release to that of low MW PLGA (individual release) while maintaining a high concentration over 1 month. The manner of release was most probably a result of multiple microspheres formulation combined in one dosage unit. The second peak at 10 hour time point was most likely attributed to the peak given by individual release from Triton X100-PLGA microspheres. This theoretical release profile is envisaged to give an initial predictor to obtain an optimized release of a final dosage unit. It also suggests that a desirable release profile is feasible to be obtained by appropriate combination of these 4 formulations of gentamicin-loaded PLGA microspheres into one dosage unit. However, this release profile shall be further validated with real experimental data to support the theory.

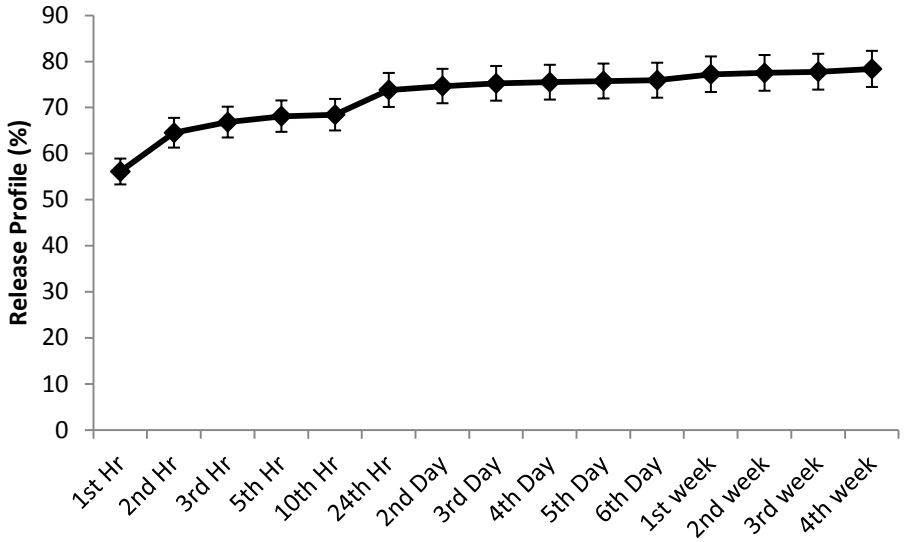

Fig. 4: The theoretical cumulative release expressed as percentage for the combined gentamicin microspheres of 4 different formulation of gentamicin-PLGA microspheres plotted as mean values with error bar of triplicate. The graph was plotted based on retrospective calculation of data having encapsulation efficiency of 53.35% with total amount of microspheres of 40 mg.

CONCLUSION

High initial burst release of gentamicin-PLGA microspheres with preserved antimicrobial effect can be achieved by employing appropriate formulation variables. In contrast to established data on PLGA release profile that always demonstrates triphasic pulsatile release, it is hereby proposed that a high initial burst release coupled with a sustained release at therapeutic concentration over a prolonged period of time (> 1 month) can be achieved by combining different release profiles from different formulation into one dosage unit. It is noteworthy that with appropriate combination of low and high PLGA MW blended with appropriate surfactants can results in desirable release profile that is suitable as implant for orthopaedic infection.

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REFERENCES

1. Balmayor, E.R., Baran, E. T., Azevedo, H. S., Reis, R. L.Injectable biodegradable starch/chitosan delivery system for the sustained release of gentamicin to treat bone infections. Carbohydrate Polymers. 2011; In Press, Accepted Manuscript.
2. Chung, Y.Y.H.T.W. Microencapsulation of gentamicin in biodegradable PLA and/or PLA/PEG copolymer. Journal of Microencapsulation. 2001;**18**(4): 457-465.
3. Neut, D., van de Belt, H., Stokroos, L., van Horn, J. R., van der Mei, H. C., Busscher, H. J. Biomaterial-associated infection of gentamicin-loaded PMMA beads in orthopaedic revision surgery. Journal of Antimicrobial Chemotherapy. 2001;**48**: 885–891.
4. Brin, Y., Golenser, J., Mizrahi, B., Maoz, G., Domb, A., Peddada, S., Tuvia, S., Nyska, A., Nyska, M. Treatment of osteomyelitis in rats by injection of degradable polymer releasing gentamicin. Journal of Controlled Release. 2008;**131**(2): 121-127.
5. Xue, J. and M. Shi. PLGA/mesoporous silica hybrid structure for controlled drug release. Journal of Controlled Release. 2004;**98**(2): 209-217.
6. Chaisri, W., Ghassemi, Amir H., Hennink, Wim E., Okonogi, Siriporn. Enhanced gentamicin loading and release of PLGA and PLHMGA microspheres by varying the formulation parameters. Colloids and Surfaces B: Biointerfaces. 2011;**84**(2): 508-514.
7. Chang, H.I., Y. Perrie, and A.G.A. Delivery of the antibiotic gentamicin sulphate from precipitation cast matrices of polycaprolactone. Journal of Controlled Release. 2006;**110**(2): 414-421.
8. Behera, A.L., S.V. Patil, and S.K. Sahoo. Formulation and Characteristics of 5 flurouracil Microspheres By Solvent Evaporation Method. Int J Pharm Pharm Sci. 2011;**3**(1): 32-35.
9. Rajeev A, J. The manufacturing techniques of various drug loaded biodegradable poly(lactide-co-glycolide) (PLGA) devices. Biomaterials. 2000;**21**(23): 2475-2490.
10. Francis, L., Meng, D., Knowles, J., Keshavarz, T., Boccaccini, A., Roy, I. Controlled Delivery of Gentamicin Using Poly(3-hydroxybutyrate) Microspheres. International Journal of Molecular Sciences. 2011;**12**(7): 4294-4314.
11. Friess, W. and M. Schlapp. Release mechanisms from gentamicin loaded poly (lactic-co-glycolic acid)(PLGA) microparticles. Journal of Pharmaceutical Sciences. 2002;**91**(3): 845-855.

12. Ji, J., Hao, S., Wu, D., Huang, R., Xu, Y. Preparation, characterization and in vitro release of chitosan nanoparticles loaded with gentamicin and salicylic acid. Carbohydrate Polymers. 2011.
13. Sivakumar, M., I. Manjubala, and K. Panduranga Rao. Preparation, characterization and in-vitro release of gentamicin from coralline hydroxyapatite-chitosan composite micropsheres. Carbohydrate Polymers. 2002;**49**: 281-288.
14. Mohamed, F. and C.F. van der Walle. PLGA microcapsules with novel dimpled surfaces for pulmonary delivery of DNA. International Journal of Pharmaceutics. 2006;**311**(1-2): 97-107.
15. Subha, M.C.S., C. Lakshmi Narayana Reddy, YerriSwamy, B., Venkata Prasad, C., Aswini, C., Mamatha, P., ChowdojiRao, R. Synthesis and Characterization of Poly (Nipamcocaprolactam) Thermoresponsive Microspheres for Controlled Release of Acebutolol Hydrochloride. Int J Pharm Pharm Sci. 2011;**3**(1): 215-221.
16. Frutos, P., Torrado, S., Perez-Lorenzo, M. E., Frutos, G. A validated quantitative colorimetric assay for gentamicin. J. Pharm. Biomed. Anal. 2000;**21**: 1149–1159.
17. Reller, L.B., Weinstein, M., Jorgensen, J. H., Ferraro, M. J., Antimicrobial Susceptibility Testing: A Review of General Principles and Contemporary Practices. Clinical Infectious Diseases. 2009;**49**(11): 1749-1755.
18. Bouissou, C., Potter, U., Altroff, H., Mardon, H., van der Walle, C. Controlled release of the fibronectin central cell binding domain from polymeric microspheres. Journal of Controlled Release. 2004;**95**(3): 557-566.
19. Singh, M.P., Stefko, J., Lumpkin, J. A., Rosenblatt, J. The Effect of Electrostatic Charge Interactions on Release Rates of Gentamicin from Collagen Matrices. Pharmaceutical Research. 1995;**12**(8): 1205-1210.
20. Ma, H., Chen, S., Yin, B., Zhao, S., Liu, X., Impedance spectroscopic study of corrosion inhibition of copper by surfactants in the acidic solutions. Corrosion Science. 2003;**45**(5): 867-882.
21. Pistel, K. and T. Kissel. Effects of salt addition on the microencapsulation of proteins using W/O/W double emulsion technique. Journal of Microencapsulation. 2000;**17**(4): 467-483.
22. Torchilin, V.P. Polymeric Micelles as Pharmaceutical Carriers, in Polymers in Drug Delivery. I.F. Uchegbu and A.G. Schatzlein, Editors. 2006; CRC Press: New York. 111-127.
23. Bouissou, C., van der Walle, C. The Influence of Surfactant on PLGA Microsphere Glass Transition and Water Sorption: Remodeling the Surface Morphology to Attenuate the Burst Release. Pharmaceutical Research. 2006;**23**(6): 1295-1305.
24. Krasko, M., Golenser, J., Nyska, A., Nyska, M., Brin, Y., Domb, A. Gentamicin extended release from an injectable polymeric implant. Journal of Controlled Release. 2007;**117**(1): 90-96.
25. Deng, M. and K.E. Uhrich. Effects of in vitro degradation on properties of poly(DL-lactide-co-glycolide) pertinent to its biological performance. Journal of Materials Science: Materials in Medicine. 2002;**13**(11): 1091-1096.
26. Makadia, H.K. and S.J. Siegel, Poly Lactic-co-Glycolic Acid (PLGA) as Biodegradable Controlled Drug Delivery Carrier. Polymers. 2011;**3**(3): 1377-1397.