Nanomed. J., 2(4): 299-304, Autumn 2015
DOI: 10.7508/nmj. 2015.04.008

Combined effects of PEGylation and particle size on uptake of PLGA particles by macrophage cells

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Received; 16 August 2015 Accepted; 7 September 2015

ABSTRACT:

Objective*:* At the present study, relationship between phagocytosis of PLGA particles and combined effects of particle size and surface PEGylation was investigated.

Materials and Methods*:* Microspheres and nanospheres (3500 nm and 700 nm) were prepared from three types of PLGA polymers (non-PEGylated and PEGylation percents of 9% and 15%). These particles were prepared by solvent evaporation method. All particles were labeled with FITC-Albumin. Interaction of particles with J744.A.1 mouse macrophage cells, was evaluated in the absence or presence of 7% of the serum by flowcytometry method.

***Results*:** The study revealed more phagocytosis of nanospheres. In the presence of the serum, PEGylated particles were phagocytosed less than non-PEGylated particles. For nanospheres, this difference was significant (P<0/05) and their uptake was affected by PEGylation degree. In the case of microsphere formulation, PEGylation did not affect the cell uptake. In the serum-free medium, the bigger particles had more cell uptake rate than smaller ones but the cell uptake rate was not influenced by PEGylation.

Conclusion: The results indicated that in nanosized particles both size and PEgylation degree could affect the phagocytosis, but in micron sized particles just size, and not the PEGylation degree, could affect this.

Key words: *PLGA particles, PEGylation, Phagocytosis, Size*

INTRODUCTION

Nanocarrier drug delivery systems (nDDS) such as polymeric nanoparticles, solid lipid nanoparticles and nanoliposomes could be used as a novel mean to treat some of the major diseases (1-4). Delivery via nanocarrier-based formulations is being pursued because of enhancing therapeutic effects or alleviating the safety problems. A wide range of hydrophilic and macromolecular drugs, such as peptides, proteins and genes have been encapsulated within polymeric nanoparticles (5-7).

Poly (D, L-lactic-co-glycolic acid) (PLGA) polymers possess unique features such as being biodegradable, biocompatible, and FDA-approved. Many drugs have been encapsulated with biodegradable PLGA nanospheres. PLGA is also frequently used for mucosal delivery of drugs and antigens (8-11).

Despite of these advantages, a few nanoparticles were successful in clinical trials. It is mainly due to mononuclear phagocytic system (MPS) which is also known as the reticuloendothelial system (RES). RES uptakes the nDDSs and removes them from systemic circulation. These processes are generally called opsonisation, which is one of the most important biological barriers against nDDSs (12-13).

After IV administration, macrophages of the MPS are able to immediately remove the non-stealth nDDSs from the bloodstream, and led to ineffective treatment. Subsequently, preventing from RES uptake is a challenge for nDDSs (14).

It is well-established that biological fate of the particles is influenced by various parameters such as their particle size, surface charge and surface hydrophilicity (15-17).

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Note. This manuscript was submitted on August 16, 2015; approved on September 7, 2015

It has been proved that PEGylation could be an anti-opsonisation strategy and is the most effective approach to prevent from phagocytosis (18-20). PEG chains create a barrier layer and subsequently prevent particles from adhesion of macrophage cells. This can make the particles camouflaged or invisible to phagocytic cells. Changing the physicochemical properties of particles is another approach to increase their half-life. It has been shown that phagocytosis of particles was drastically influenced by their mean size and size distribution and controlling the particle size is essential to reduce the phagocytosis (21).

The aim of the present study was to evaluate the simultaneous effects of particle size and surface PEGylation on phagocytosis of PLGA particles.

MATERIALS AND METHODS

Materials

PLGA 50:50 (Resomer® RG 502H, intrinsic viscosity 0.18 dl/g), PLGA 50:50-PEG Resomers® (9% PEG, iv 0.72 dl/g and 15% PEG, iv 0.5 dl/g) were obtained from Boehringer Ingelheim (Germany). Polyvinyl alcohol (PVA, MW 30–70 KDa) and Bovine serum albumin (Fraction V) were purchased from Sigma (St. Louis, MO). Fluorescein-4-isothiocyanate (FITC-1) was purchased from Fluka (Switzerland). Cell culture media and materials were products of Gibco BRL (Grand Island, NY). J744.A.1 macrophage cells were obtained from the Pasteur Institute (Tehran, Iran).

Preparation, labeling and characterization of PLGA particles

In preliminary studies, three different methods were used for preparation of the PLGA particles, emulsion solvent diffusion (ESD), oil-in-water emulsion-solvent evaporation and water-in-oil-in water emulsion-solvent evaporation. Based on the early results the single oil-in-water emulsification – solvent evaporation method was used for the main study.

In this method, 100 mg of PLGA was dissolved in 300 µl of DCM (oil phase). To prepare various sizes of particles, different concentrations of PVA solution (1-5% w/v) in deionized water were used as aqueous phase. The oil phase was added dropwise to the aqueous phase. The preparation parameters were listed in table 1.

The o/w emulsion was added to bigger volumes of PVA solution, and the solvent was evaporated under magnetic stirring. Particles were separated by centrifugation at 10000 g for 10 min and washed twice with deionized water. and then resuspended in 1 ml water. Before freeze-drying step, to prevent from aggregation, 5% (w/v) mannitol was dissolved in final suspension (10).

For labeling of particles, fluorescein isothiocyanate (FITC) labeled albumin was used. 200-300 µl of FITC-albumin (20% w/v) was added to the aqueous phase before emulsification (22). The details of preparation methods and types of polymers were listed in table 1.

The physicochemical properties of particles such as particle size, size distribution and zeta potential were afterward measured. To show the florescent properties of particles, fluorescent microscope images were recorded.

Cell uptake studies

The fabricated particles were incubated with J744 A.1 mouse macrophage cells, both in the absence and presence of serum. Briefly, the cell line was cultured in DMEM F12 medium supplemented with 10% fetal calf serum (FCS). For uptake study, 6-well plates were used to seed the cells (5 x $l0^3$ cells per well) and the cells were allowed to attach for 2 h.

The medium in each well was replaced with 1 ml of freshly prepared particle suspension in PBS (100 or 200 µg/well) and the plates were incubated for 1 h. The cells were then washed twice with PBS to remove the non-internalized particles and harvested using trypsin-EDTA treatment followed by centrifugation and resuspending in PBS. Flow cytometry analysis was performed within 1 h.

In this method, 10000 cells were counted and analyzed by measuring signal from FITC channel of the flowcytometer (Partec AG, Switzerland).

Statistical analysis

All tests were performed at least in triplicate. One-way analysis of variance (ANOVA) was performed by Graphpad Instat for testing of differences between groups. $P < 0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

Characterization of particles

Among preparation methods tested in preliminary studies (data not shown), single emulsion - solvent evaporation method showed the best results (Table 2). The fluorescence of particles was showed by fluorescent microscope (Fig 1).

Table 1. The details of preparation methods and types of polymers used for each kind of particles

	Polymer	% PVA	Type of preparation
Microspheres F-1	PLGA	1%	Homogenization, 8000 rpm, 1 min
Microspheres F-2	PLGA-PEG 15%	1%	Homogenization, 20500 rpm, 90s
Nanospheres F-1	PLGA	5%	Probe sonication, 80 S
Nanospheres F-2	PLGA-PEG 15%	5%	Probe sonicaton, 120 s
Nanospheres F-3	PLGA-PEG 9%	5%	Probe sonication, 80S

Table 2. Size, polydispersity index (PDI) and zeta potential of particles

Formulations	Z-average Mean (nm)	PDI	Zeta potential (mv)	
			- serum	+ serum
Microspheres F-1	3433	0.349	-2.61	-3.43
Microspheres F-2	3676	0.098	-3.21	-2.61
Nanospheres F-1	742.8	0.174	-2.68	-2.42
Nanospheres F-2	1995	1	-2.32	-1.72
Nanospheres F-3	1167	0.543	-3.48	-3.56

To optimize the formulations, various parameters such as PVA concentration, type of surfactant and the effect of mannitol were tested. Based on the DLS results, for non-pegylated particles, lower concentrations of PVA (1% w/v) could decrease the particle size. However, for PEGylated particles, higher PVA concentration was able to reduce the size of particles.

This finding was in contrast with previously published data. It is expected that in the higher concentrations of PVA, as a surfactant, smaller size and PDI could be attained (23). This discrepancy could be attributed to the lower intrinsic viscosity of the PLGA polymer (0.18 dl/g) compared with PEG-PLGA polymers (0.5 and 0.72 dl/g respectively for 15% and 9% PEG-PLGA). It is supposed that in preparation of PLGA particles, low viscosity of polymers allows for preparation of small sized particles in presence of lower concentration of surfactant. But in the case of PEG-PLGA, because of higher viscosity of polymers, higher concentration of PVA is required for decreasing the particle size.

The effect of mannitol in the freeze drying step - was also evaluated. The results indicated that in the absence of mannitol, the particle size slightly increased (Data not shown). The data indicated that mannitol, as a cryoprotective agent, could prevent from aggregation of particles in freeze-drying step.

Cell uptake studies

The results of cell uptake studies were illustrated in table 3.

Fig.1. Fluorescent microscopic image of particles coated with FITC-BSA, prepared by single emulsion method

In the presence of serum, there were no significant differences between cell uptake of nanospheres and microspheres ($P>0.05$). Upon PEGylation of particles, the extent of cell uptake was decreased. The lower uptake of 9% PEGylated nanospheres over 15% PEGylated nanospheres was observed in flowcytometry results (Table 2) ($P<0.05$). The results relating to the microspheres showed that PEGylation did not influence the rate of cell uptake and there were no significant differences between PEGylated and non-PEGylated forms ($P >0.05$). The least uptake was observed with 9% PEGylated nanospheres.

In the absence of the serum, the bigger particles had more cell uptake than smaller ones.

Table 3. Particle uptake by J744 macrophage cells. The data shows mean ± SEM of cell fluorescence intensities as a function of nanoparticle uptake by the cells (n=3)

	Negative control	Microspheres F-1	Microspheres F-2	Nanospheres F-1	Nanospheres F-2	Nanospheres F-3
+ serum	308 ± 33	581 ± 146	459 ± 10	525 ± 34	446 ± 32	345 ± 4
- serum	317 ± 20	699 ± 52	521 ± 33	515 ± 7	432 ± 14	347 ± 13

The PEGylation effects were similar to the results in the presence of serum.

The dependency of phagocytosis on particle size was previously described (21, 24). These observations were consistent with present study. In both the presence and absence of the serum, the phagocytosis of nanospheres was lower than microspheres. It was shown that particles with approximately 3 μm were phagocytosed more readily than smaller ones (21). Due to these observations and the particle size of microsphere in present study, enhancing the uptake of microsphere with respect to the nanospheres could be explained.

Surface modification of the particles is another approach to reduce the phagocyctosis level. PEGylation is a promising strategy (25). As seen in table 2, upon PEGylation of particles, the phagocyctosis level was decreased. These results indicated the influence of PEGylation on the prevention of phagocytosis. It was shown that preparation of stealth nanoparticles is appropriate strategy to enhance the circulation time (26). The presence and absence of serum is another factor in evaluation of the cell uptake results. Phagocytosis was reduced in the presence of serum. The results of present study were consistent with Leroux et al (27).

Their results also indicated that PEGylated PLA nanospheres showed reduced uptake level in presence of the serum, as compared with the absence of it. Therefore it can be deduced that other parameters beyond physicochemical properties or modification of particles are involved in macrophage-particle interactionOne possible reason is the role of serum albumin in opsonisation of particles. Serum albumin does not have the opsonic ability but is able to alter the surface hydrophobicity of particles. It should be also mentioned that among the serum proteins contained in FCS, albumin is at the most ýabundant. Therefore, particles placed in medium with serum will be coated predominately with ýalbumin. Suppression of phagocytosis of particles by the addition of serum may also be explained in terms of adsorption of albumin on to the surface, followed by lowering of surface hydrophobicity. It is well-known that a more hydrophobic surface is more readily adsorbed with serum albumin which suppresses phagocytosis (28).

CONCLUSION

The results indicated that in nanosized particles both size and PEgylation degree could affect the phagocytosis, but in micron sized particles just size, and not the PEGylation degree, could affect this.

CONFLICT OF INTEREST

All authors declare that there is no conflict of interest in this study.

ACKNOWLEDGMENT

This study was supported by a grant from Vice Chancellor for Research of Mashhad University of Medical Sciences, Mashhad, Iran. The results reported in this paper were part of a Pharm.D. thesis.

REFERENCES

1. Poovi G, Lekshmi UMD, Narayanan N, Reddy N. Preparation and characterization of repaglinide loaded chitosan polymeric nanoparticles. Res J Nanosci Nanotechnol. 2011; 1(1): 12-24.
2. Khameneh B, Iranshahy M, Ghandadi M, Ghoochi Atashbeyk D, Fazly Bazzaz BS, Iranshahi M. Investigation of the antibacterial activity and efflux pump inhibitory effect of co-loaded piperine and gentamicin nanoliposomes in methicillin-resistant Staphylococcus aureus. Drug Dev Ind Pharm. 2014 May 20.
3. Atashbeyk DG, Khameneh B, Tafaghodi M, Fazly Bazzaz BS. Eradication of methicillin-resistant Staphylococcus aureus infection by nanoliposomes loaded with gentamicin and oleic acid. Pharm Biol. 2014 Nov; 52(11): 1423-8.
4. Mohajer M, Khameneh B, Tafaghodi M. Preparation and characterization of PLGA nanospheres loaded with

inactivated influenza virus, CpG-ODN and Quillaja saponin. Iran J Basic Med Sci. 2014 Sep; 17(9): 722-6.

5. Ding X, Janjanam J, Tiwari A, Thompson M, Heiden PA. Peptide-directed self-assembly of functionalized polymeric nanoparticles part I: design and self-assembly of peptide-copolymer conjugates into nanoparticle fibers and 3D scaffolds. Macromol Biosci. 2014 Jun; 14(6): 853-71.
6. Yao H, Ng SS, Huo LF, Chow BK, Shen Z, Yang M, et al. Effective melanoma immunotherapy with interleukin-2 delivered by a novel polymeric nanoparticle. Mol Cancer Ther. 2011 Jun;10(6): 1082-92.
7. Sun L, Zhou S, Wang W, Li X, Wang J, Weng J. Preparation and characterization of porous biodegradable microspheres used for controlled protein delivery. Colloids Surf A Physicochem Eng Asp. 2009;345(1–3):173-81.
8. Bian X, Liang S, John J, Hsiao CH, Wei X, Liang D, et al. Development of PLGA-based itraconazole injectable nanospheres for sustained release. Int J Nanomedicine. 2013; 8: 4521-31.
9. Gajendiran M, Divakar S, Raaman N, Balasubramanian S. In vitro drug release behavior, mechanism and antimicrobial activity of rifampicin loaded low molecular weight PLGA-PEG-PLGA triblock copolymeric nanospheres. Curr Drug Deliv. 2013 Dec; 10(6): 722-31.
10. Mohaghegh M, Tafaghodi M. Dextran microspheres could enhance immune responses against PLGA nanospheres encapsulated with tetanus toxoid and Quillaja saponins after nasal immunization in rabbit. Pharm Dev Technol. 2011 Feb; 16(1): 36-43.
11. Jain RA. The manufacturing techniques of various drug loaded biodegradable poly(lactide-co-glycolide) (PLGA) devices. Biomaterials. 2000; 21(23): 2475-90.
12. Misra R, Acharya S, Sahoo SK. Cancer nanotechnology: Application of nanotechnology in cancer therapy. Drug Discov Today. 2010; 15(19-20): 842-50.
13. Nijhara R, Balakrishnan K. Bringing nanomedicines to market: regulatory challenges, opportunities, and uncertainties. Nanomedicine: Nanotechnology, Biology, and Medicine. 2006; 2(2): 127-36.
14. Moghimi SM, Hunter AC, Murray JC. Long-circulating and target-specific nanoparticles: Theory to practice. Pharmacol Rev. 2001; 53(2): 283-318.
15. Hans ML, Lowman AM. Biodegradable nanoparticles for drug delivery and targeting. Curr Opin Solid State Mater Sci. 2002; 6(4): 319-27.
16. Mosqueira VCF, Legrand P, Gref R, Heurtault B, Appel M, Barratt G. Interactions between a macrophage cell line (J774A1) and surface-modified Poly(D,L-lactide) nanocapsules bearing poly(ethylene glycol). J Drug Target. 1999; 7(1): 65-78.
17. Thiele L, Diederichs JE, Reszka R, Merkle HP, Walter E. Competitive adsorption of serum proteins at microparticles affects phagocytosis by dendritic cells. Biomaterials. 2003; 24(8): 1409-18.
18. Kohler N, Sun C, Fichtenholtz A, Gunn J, Fang C, Zhang M. Methotrexate-immobilized poly(ethylene glycol) magnetic nanoparticles for MR imaging and drug delivery. Small. 2006; 2(6): 785-92.
19. Chaudhari KR, Ukawala M, Manjappa AS, Kumar A, Mundada PK, Mishra AK, et al. Opsonization, biodistribution, cellular uptake and apoptosis study of PEGylated PBCA nanoparticle as potential drug delivery carrier. Pharm Res. 2012 Jan;29(1):53-68.
20. Mathaes R, Winter G, Besheer A, Engert J. Influence of particle geometry and PEGylation on phagocytosis of particulate carriers. Int J Pharm. 2014; 465(1-2): 159-64.
21. Champion JA, Walker A, Mitragotri S. Role of particle size in phagocytosis of polymeric microspheres. Pharm Res. 2008 Aug; 25(8): 1815-21.
22. Tafaghodi M, Hadizadeh F, Farahmand F. Determination of encapsulation efficiency of tetanus toxoid in microsphere and liposome drug delivery systems by two different spectroscopic methods. Pharmaceutical Sciences. 2008; 2: 53-9.
23. Sahoo SK, Panyam J, Prabha S, Labhasetwar V. Residual polyvinyl alcohol associated with poly (D,L-lactide-co-glycolide) nanoparticles affects their physical properties and cellular uptake. J Control Release. 2002; 82(1): 105-14.
24. Ahsan F, Rivas IP, Khan MA, Torres Suárez AI. Targeting to macrophages: role of physicochemical properties of particulate carriers—liposomes and microspheres—on the phagocytosis by macrophages. J Control Release. 2002; 79(1): 29-40.
25. Owens Iii DE, Peppas NA. Opsonization, biodistribution, and pharmacokinetics of polymeric nanoparticles. Int J Pharm. 2006 ;307(1): 93-102.
26. Zambaux M-F, Faivre-Fiorina B, Bonneaux F, Marchal S, Merlin J-L, Dellacherie E, et al. Involvement of neutrophilic granulocytes in the uptake of biodegradable non-stealth and stealth nanoparticles in guinea pig. Biomaterials. 2000; 21(10): 975-80.
27. Leroux JC, De Jaeghere F, Anner B, Doelker E, Gurny R. An investigation on the role of plasma and serum opsonins on the internalization of biodegradable poly(D,L-lactic acid) nanoparticles by human monocytes. Life Sciences. 1995; 57(7): 695-703.

28. Tabata Y, Ikada Y. Effect of the size and surface charge of polymer microspheres on their phagocytosis by macrophage. Biomaterials. 1988; 9(4): 356-62.
29. Soppimath KS, Aminabhavi TM, Kulkarni AR, Rudzinski WE. Biodegradable polymeric nanoparticles as drug delivery devices. J Control Release. 2001 Jan 29; 70(1-2): 1-20.
30. Owens DE, 3rd, Peppas NA. Opsonization, biodistribution, and pharmacokinetics of polymeric nanoparticles. Int J Pharm. 2006 Jan 3; 307(1): 93-102.

How to cite this article:
Moayedian T, Mosaffa F, Khameneh-Bagheri B, Tafaghodi M. Combined effects of PEGylation and particle size on uptake of PLGA particles by macrophage cells. Nanomed. J., 2015; 2(4): 299-304.