Pharmaceutical Research, Vol. 25, No. 5, May 2008 (© 2008)
DOI: 10.1007/s11095-008-9563-x

Short Communication

A Comparison Between Polymeric Microsphere and Bacterial Vectors for Macrophage P388D1 Gene Delivery

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Received January 7, 2008; accepted February 26, 2008; published online March 15, 2008

Purpose. The purpose of this study was to compare bacterial and polymeric gene delivery devices for the ability to deliver plasmid DNA to a murine macrophage P388D1 cell line.
Methods. An 85:15 ratio of poly(lactic-co-glycolic acid) (PLGA) and poly(β-amino ester) polymers were formulated into microspheres that physically entrapped plasmid DNA encoding for the firefly luciferase reporter gene; whereas, the same plasmid was biologically transformed into a strain of *Escherichia coli* engineered to produce recombinant listeriolysin O. The two delivery devices were then tested for gene delivery and dosage effects using a macrophage cell line with both assays taking advantage of a 96-well high throughput format to quantify and compare each vector type.
Results. Gene delivery was comparable for both vectors at higher vector dosages while lower dosages showed an improved delivery for the microsphere vectors. Delivery efficiency (defined as luciferase measurement/mg cellular protein/ng DNA delivered) was 881 luminescence mg^{-1} ng^{-1} for polymeric microspheres compared to 171 luminescence mg^{-1} ng^{-1} for the bacterial vectors.
Conclusion. A first head-to-head comparison between polymeric and bacterial gene delivery vectors shows a delivery advantage for polymeric microspheres that must also be evaluated in light of vector production, storage, and future potential.

KEY WORDS: bacterial vectors; *E. coli*; gene delivery; high throughput; polymeric microspheres.

INTRODUCTION

Immune therapy takes advantage of a patient's own immunological properties to combat a certain disease or illness. Vaccination is one example where an antigen is used to trigger, activate, and prepare a native immune system for disease challenge. In doing so, the antigen is generally recognized by antigen presenting cells that operate on the border of the innate and adaptive immune systems. Antigen presenting cells such as macrophages and dendritic cells have an ability to recognize and process a foreign antigen and present this information to the adaptive immune system so as to optimally address the current or future illness.

Macrophage and dendritic cells will recognize and phagocytize foreign objects, and this is the first step towards the presentation process that will trigger a final immune response. One early recognition feature is the size of a non-native particle or organism within the body. Particles or foreign biological cells ranging between 0.5 and ~10 μm are naturally engulfed by macrophage and dendritic cells (1,2). Antigen presenting cells also posses a range of receptors (termed Toll-like receptors) that recognize motifs such as bacterial cell wall, membrane, and DNA components (3,4). As might be expected, a major function of antigen presenting cells is to combat bacterial cell invasion, first by recognizing and internalizing foreign bacterial cells and then digesting and processing bacterial macromolecules which serve as subsequent antigens. Such a processing pathway typically leads to antibody formation as a means to combat future bacterial invasion. However, viral infections, which generally involve nucleic acid delivery by the viral pathogen, can trigger a second type of immune response. Here, foreign protein units derived from foreign viral DNA expression within the infected cell lead to a cellular response on the part of the immune system in which cytotoxic T cells recognize and kill similarly infected cells. The steps of foreign object recognition, uptake, and processing by antigen presenting cells provides a framework for designing delivery systems with the purpose of protein or gene delivery and eventual immune therapy application.

In this regard, polymeric microspheres and bacterial vectors have been explored as delivery devices. Though intended for the same purpose of immune therapy, each vector is fundamentally different. First and foremost is the nature of the delivery device: polymeric microspheres are composed of biodegradable and biocompatible polymers physically formulated into microscopic spheres that (1) match the optimal size range to target the phagocytic antigen presenting cells and (2) physically entrap either protein or genetic antigens for delivery to the antigen presenting cells; whereas, bacteria innately match the size and recognition

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features antigen presenting cells have evolved to identify and through recombinant DNA technology can be designed to hold specific protein or genetic antigens. As might be expected, options for both vector types have been pursued (5–7). Early reports for polymeric microspheres used the well-characterized, biocompatible, and biodegradable polymer poly(lactic-co-glycolic acid) (PLGA) (8). This study served as a basis for additional microsphere delivery efforts with one example formulating microspheres containing mixtures of PLGA and poly(β-amino esters), with the latter polymer added due to tailor-able pH responsive properties that positively influenced gene delivery (9–11). Early efforts with bacterial vectors focused on organisms (*Salmonella*, *Shigella*, *Listeria*) that inherently possessed a pathogenic nature (12–14). This was partially because such vectors would naturally trigger antigen presenting cell recognition and uptake but also because such pathogens had their own active mechanisms for cellular entry and survival upon uptake (15,16). Attenuated versions of these strains have since been applied to both gene and protein delivery applications. In addition, recombinant DNA technology tools have been used to transfer the innate delivery features from these organisms to those less pathogenic but also less adept at gaining entrance to and surviving within the antigen presenting cell. As an example and as a way to preface the study here, the listeriolysin O (LLO) protein, which naturally destabilizes intracellular phagosomal vesicles and allows *Listeria monocytogenes* access to the mammalian cytoplasm, has been recombinantly expressed in strains of *E. coli* to aid the delivery of proteins and genes (17,18). Hence, for both polymeric and bacterial vectors, different innate features and tools (used to provide acquirable delivery features) are available to influence the final delivery and, hence, immune therapy outcome.

In this particular study, gene delivery to a murine macrophage P388D1 cell line was compared between similarly modified polymeric microsphere and bacterial vectors. An 85:15 mixture of PLGA and a poly(β-amino ester), formulated into 3–11 μm microspheres, was compared to a recombinant *E. coli* strain inducibly producing the delivery-facilitating listeriolysin O protein. Both systems were thus similarly engineered to improve gene delivery and evaluated using the same reporter gene within the context of a high-throughput delivery assay to allow a direct and measurable comparison. Such a study is one of the first to directly compare polymeric microsphere and bacterial delivery vectors and will follow with an analysis of the relative advantages and disadvantages, based on the results obtained, provided by each.

MATERIALS AND METHODS

Cell Lines and Strains

The murine P388D1 macrophage cell line was used for gene delivery assays and was maintained in media prepared as follows: 50 ml fetal bovine serum (heat inactivated), 5 ml 1 M HEPES buffer, 5 ml 100 mM MEM sodium pyruvate, 5 ml penicillin/streptomycin solution, and 1.25 g D(+)-glucose were added to 500 ml 1640 RPMI media and then filter sterilized. Cells were housed in T75 flasks and cultured at 37°C in a humidified incubator at a 5% CO_2 level. The strain of *E. coli* containing the listeriolysin O gene (*hly*) has been described more completely elsewhere (Parsa and Pfeifer, in press). Briefly, the strain is a derivative of the BL21(DE3) *E. coli* cell line (Novagen) with the *hly* gene placed under inducible *lac* expression with a T7 promoter; the *hly* gene was integrated into the *E. coli* chromosome using the λ Red recombination protocol at the *clp* position. The strain was stored frozen at −80°C in 8% glycerol. For the assays described below, a plasmid containing a firefly luciferase reporter gene under the control of a cytomegalovirus (CMV) promoter was introduced to produce the final strain YWT7-*hly*/pCMV-Luc using standard molecular biology protocols for competent cell preparation and electro-transformation (19).

Polymers and Microsphere Formulation

The polymers used for microsphere formulation were commercially available PLGA (RG502H, Boehringer Ingelheim) and a poly(β-amino ester) (termed PolyC) synthesized as previously described (20). Microspheres were generated using a double emulsion/solvent evaporation procedure (21). Initially, the polymers (150 mg PLGA, 50 mg poly(β-amino ester)) were dissolved in methylene chloride (4 ml). Next, 100 μl of an aqueous 300 mM lactose, 1 mM EDTA solution containing 1 mg plasmid DNA (pCMV-Luc purchased from Elim Biopharmaceuticals, Hayward, CA) was added to the organic polymer solution, and the aqueous and organic phases were emulsified by sonication (Vibra Cell, Sonics & Materials, Inc., 40% amplitude setting for 10 s using a stepped microtip probe). The primary emulsion was then transferred to 50 ml of an aqueous 1% PVA, 0.5 M NaCl solution for a 30 s homogenization (L4RT-A, Silverson equipped with a 3/4 in. tubular frame with a square hole high shear screen, 7500 rpm) to form a secondary emulsion. The secondary emulsion was added to an aqueous 100 ml 0.5% PVA, 0.5 M NaCl solution and stirred to evaporate the organic solvent (using a stirplate at room temperature for 2.5 h and an additional 0.5 h at 4°C [needed to help preserve microsphere morphological integrity]). The microspheres were then washed (by centrifugation [150×*g*, 10 min] and resuspension) three times with DI water, frozen with liquid nitrogen, lyophilized to a dry powder using a VirTis Freezemobile 25EL and standard Labconco freeze dry flasks according to the manufacturer's instructions, and stored at −20°C.

Microsphere Size, Zeta Potential, and DNA Encapsulation Characterization

For sizing and zeta potential measurements, microspheres were resuspended at 0.1 mg/ml in filtered distilled water and sonicated (35 W, 5 s). A Coulter microparticle analyzer (Multisizer 3, Beckman Coulter) was used to generate an average diameter for the microspheres (volume average; sample count: 50,000 microspheres). A ZetaPALS dynamic light scattering detector (Brookhaven Instruments Corporation, 15 mW laser, incident beam=676 nm) was used to measure zeta potentials.

Microsphere DNA encapsulation was determined by first resuspending 2.5 mg spheres in 0.25 ml TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 7.7, Sigma). One

milliliter dichloromethane was added and the two phases were then mixed via rotation for 1.5 h at room temperature. After this time, the mixture was centrifuged and the aqueous phase carefully removed and analyzed at A260 (using a SPECTRAmax PLUS 384 UV–vis 96 well plate reader [Molecular Devices Corp.] and flat bottom, acrylic, UV transparent 96 well plates); samples were then compared to a pCMV-Luc DNA standard curve (0–70 μg/ml in TE buffer). Samples with no DNA were tested to ensure that soluble polymer fragments or residual solvent did not interfere with the UV signal. To obtain % DNA encapsulated, the recovered DNA per mg polymer was compared to the initial DNA per mg polymer added during microsphere formation.

Macrophage 96-Well Seeding

For the following luciferase and BCA assays, the P388D1 macrophage cell line was first seeded in 96-well plates. The P388D1 line was maintained in media and growth conditions as described above. However, for tests involving bacterial vectors, the original media formulation containing penicillin and streptomycin was removed and the cells rinsed twice with media containing no antibiotics prior to harvesting for 96-well plate seeding. The cells were then collected via mechanical detachment using a cell scrapper into media without antibiotics, counted using a hemocytometer, and seeded at 50,000 cells/well in 100 μl media/well. For tests with the microsphere vectors, identical seeding procedures were conducted with complete media containing the penicillin and streptomycin antibiotics. Tissue culture treated, sterile, polystyrene 96 well plates were used for the BCA assays; whereas, the luciferase assays were conducted in tissue culture treated, flat bottom, white, polystyrene 96 well plates. Seeded cells were allowed to adhere for 24 h at 37°C/5% CO_2 in a humidified incubator prior to conducting assays.

Luciferase Assay

The YWT7-*hly*/pCMV-Luc *E. coli* strain was cultured at 37°C/250 rpm in 2 ml Luria–Bertani medium. To induce *hly* gene expression, 100 μM isopropyl β-D-1-thiogalactopyranoside (IPTG) was added at an OD_{600} of ~0.25 and the culture was allowed to incubate at 30°C/250 rpm for an additional 1 h. Cells were pelleted by centrifugation and resuspended in P388D1 medium without antibiotics. Using an absorbance correlation of $1\ OD_{600}$ unit $= 1 \times 10^8$ cells, the bacteria were then diluted to provide bacterial to macrophage ratios of 1:1, 10:1, and 100:1. After removing the original media, the bacterial vectors were added (50 μl per well) to the macrophage cells. Microspheres were resuspended in media (with antibiotics) and sonicated (35 W, 3 s) before replacing the original media in the 96 well plates with final microsphere concentrations of 1, 10, or 100 μg/ml (added at 100 μl per well). For the bacteria, co-incubation was carried out for 1 h followed by the addition of 50 μl of P388D1 media containing 40 μg/ml gentamicin; the co-incubation period was chosen so as to maximize bacterial and macrophage interaction without negatively impacting the 96-well growth environment which was compromised at longer co-incubation times as determined from a visible color change of the growth media (especially at higher bacterial dosage). Dosages for both bacteria and microspheres were chosen based upon previous studies with these delivery devices and/or this particular assay (11,21–23); in addition, the dosage values cover a wide range (two orders of magnitude) with an upper value approaching the limit for healthy maintenance of the macrophage cells. The assay then continued for 24 h at 37°C/5% CO_2 in a humidified incubator. Following the 24 h incubation, the cells were analyzed for luciferase activity using the Bright Glo system from Promega according to the manufacturer's instructions. Data was recorded with a Perkin Elmer 1420 Multilabel Counter VICTOR^3 luminometer system and luciferase units normalized by cellular protein per well (from separately seeded replicate plates).

BCA Assay

Protein content within the 96 well plates was assayed using a Pierce Micro BCA Protein Assay Kit according to the manufacturer's instructions together with a Molecular Devices VERSAmax microplate reader at 562 nm. Prior to using the kit, cells were washed twice with PBS and lysed in 150 μl of 0.25% deoxycholate.

RESULTS

As opposed to the physical and biological constraints naturally associated with the bacterial vectors, the polymeric microspheres were formulated from their constituent polymers and were, thus, characterized for their size and surface potential. The sizing data recorded was 6.79 μm±3.56 SD and confirms the size range expected for particles readily engulfed by professional phagocytic antigen presenting cells. The zeta potential of the microspheres was measured at 40.9±6.4 SD; both size and zeta potential measurements closely matched previous values recorded for similar formulations (21). The microsphere encapsulation of the pCMV-Luc plasmid DNA was measured at 31.1%.

Figure 1 compares the impact the different vectors have on macrophage cellular protein levels over time. This effect is correlated to general cellular viability and gives a comparison between the negative effect on macrophage cellular health caused by the addition of either microsphere or bacterial vectors. Figure 1 shows the macrophage protein levels as a function of both vector type and dosage level, assayed after the completed total incubation period of 24 h. As expected, cellular macrophage protein levels decreased with increasing vector dosage. Experimentally, this was also observed by noticeable changes to the media color as dosage levels increased, even with efforts to minimize negative effects by, for example, maintaining a relatively short bacterial vector-macrophage co-incubation period of 1 h and total incubation periods of 24 h. Though both vectors share a similar profile for reduced macrophage cellular protein levels with increased vector dosage, the effect is more pronounced for the bacterial vectors.

The two vectors were next compared for their ability to deliver the pCMV-Luc plasmid DNA to the macrophage cells within the context of the 96-well assay used to quantitatively compare gene delivery. It should be noted that other variations of the vectors compared here (i.e., polymeric microspheres with altered combinations of PLGA and poly

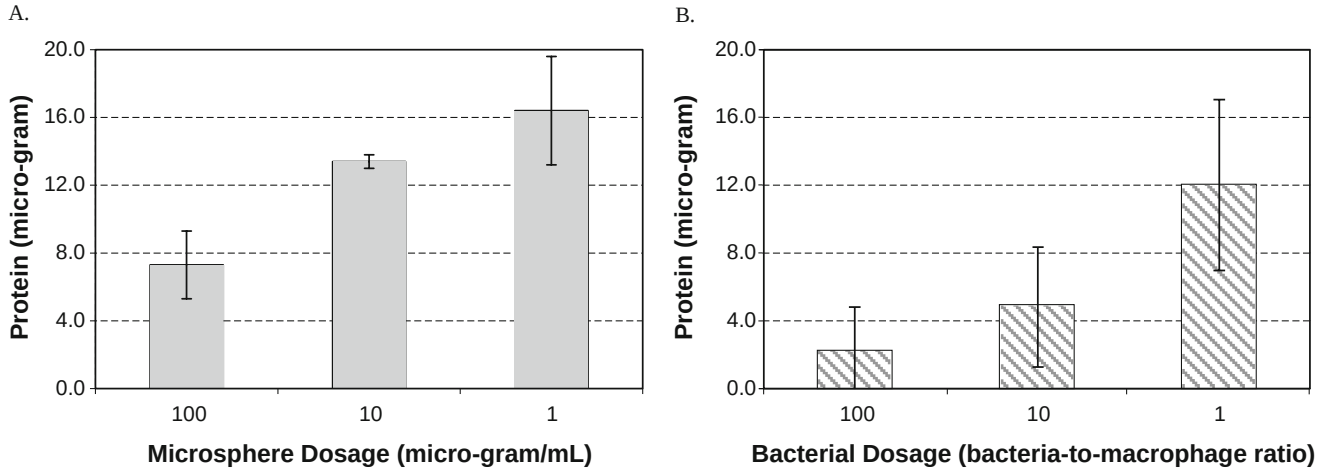

Fig. 1. Macrophage cellular protein as a function of vector type (**A** 15% PolyC-PLGA Microspheres; **B** *E. coli* strain YWT7-*hly*/pCMV-Luc). *Error bars* represent standard deviations from a minimum of three samples. Student *t* test analyses showed statistically significant differences (95% confidence) between vector types for the 100 and 10 dosage levels.

(β-amino ester) and bacterial vectors with different *hly* gene expression parameters) were tested with each analysis confirming the need and benefits of either the poly(β-amino ester) or LLO for gene delivery; final vectors chosen showed the greatest relative gene delivery within their respective vector type. After a total time of 24 h to allow gene delivery and expression, the macrophage cells were tested for luminescence production as a result of successful gene delivery. As shown in Fig. 2, the gene delivery data illustrates a dosage dependent increase in final luminescent signal and a quantitative comparison between the bacterial and polymeric microsphere vectors. As previously observed for this assay, increased dosage for both bacterial and microsphere vectors correlates with improved final gene delivery, indicating the positive effects of escalating dosage (Parsa and Pfeifer, in press) (11), with gene delivery measurements for the two vectors comparable at the highest dosage tested for each. However, at the lower dosages, Fig. 2 shows an ~30 fold improvement in delivery conferred by the polymeric microspheres.

DISCUSSION

This study examined the delivery capabilities of polymeric microsphere and bacterial vectors beginning with a comparison of the physical or biological properties associated with each vectors. Both vectors fall within the particle size range (0.5 to ~10 μm) recognized for uptake by professional phagocytic antigen presenting cells, and previous studies have been conducted to ensure that both vectors are internalized by antigen presenting cells (Parsa and Pfeifer, in press) (11). The microsphere and bacterial vectors possess different geometries with the namesake microsphere geometry contrasted with the cylindrical nature of the bacterial cells. Whether the extent of uptake will vary because of vector geometry is unknown, but this aspect is one difference between the microsphere and bacterial vectors. However, overall, the physical features recorded here bode well for antigen presenting cell uptake, which is one of the first steps to eventual gene delivery. Besides size features that passively target professional phagocytic cells, the two vector types have

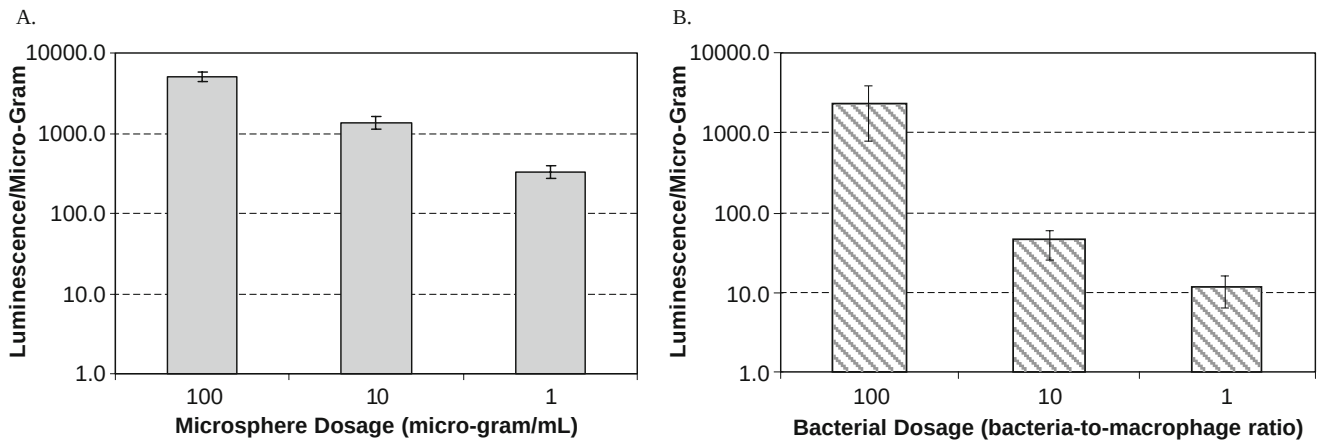

Fig. 2. Gene delivery signal as a function of vector type (**A** 15% PolyC-PLGA Microspheres; **B** *E. coli* strain YWT7-*hly*/pCMV-Luc). *Error bars* represent standard deviations from a minimum of three samples. Student t test analyses showed statistically significant differences (95% confidence) between vector types for the 10 and 1 dosage levels.

also been shown to trigger the recognition features of the sentinel antigen presenting cells that further stimulate immune system activation. It is well-established that bacterial features such as lipopolysaccharide, peptidoglycan, and DNA motifs are readily recognized by antigen presenting cell Toll-like receptors (3). Likewise, the microspheres studied here have been previously characterized for their ability to activate antigen presenting cells with success attributed, at least in part, to the positive charge associated with the microspheres (11,24). This ability to activate or alarm the immune system is considered an advantage as each vector then exhibits an innate adjuvant ability in addition to a design meant to target and optimize gene delivery to antigen presenting cells.

Besides chemical and physical features to aid recognition and uptake by antigen presenting cells, each vector has been designed to improve the intracellular transfer of gene cargo from the delivery vector to the final antigen presenting cell. The microsphere is a composite between a well-established biomaterial (PLGA) and a poly(β-amino ester) shown to solubilize at the pH of the formed phagosomal/lysosomal vesicle (~5.5 pH) (9,11). This feature, in conjunction with a proton sponge-like hypothesis, is attributed to improving gene delivery over using PLGA alone (11,25). Thus, the microsphere formulation used here represents an advanced polymeric microsphere delivery device to be compared to the accompanying bacterial vector also optimized for gene delivery. As opposed to the poly(β-amino ester) used to aid the movement from the phagosome to the macrophage cytosol, the bacterial vector uses a biological mechanism catalyzed by a recombinantly produced lysteriolysin O protein. Prior to co-incubation with the macrophage cells, bacteria are induced for *hly* gene expression and subsequent LLO formation. Upon co-incubation, the macrophage cells are expected to engulf and digest the bacterial cells within phagosomes that mature by fusing with lysosomal vesicles. LLO is active at the 5–6 pH range found within the phagolysosomal vesicles and acts by destabilizing and perforating cellular membranes including the phagosomal membrane (and perhaps remaining bacterial membranes), thus, facilitating movement of the bacterial vector contents to the cytosol and serving a very similar function as the poly(β-amino ester) in the microsphere context (26,27). Given these similarities in engineered activity, the bacterial and polymeric microsphere vectors were compared head-to-head in gene delivery capabilities using a quantitative high throughput assay.

The direct comparison of the two vectors highlighted the similarities and differences with regards to final macrophage cellular protein levels and final gene delivery. For both vectors, a reduction in macrophage cellular protein levels (correlated to reduction in macrophage cell viability) was observed with increased vector dosage. Such a result is not surprising since dosage levels for even the safest vector systems will eventually cause negative effects at extremely high levels. However, separate studies have more generally characterized the systems studied here for negative impacts on cellular health. PLGA is FDA approved, and the poly(β-amino ester) has been characterized as biodegradable and generally non-toxic to mammalian cells (20). Similarly, the *E. coli* parent strain used for this study was thoroughly assessed for negative *in vivo* effects post administration revealing a generally safe profile (28). Such prior studies bode well for both vector types in future *in vivo* gene delivery experiments, yet regarding the head-to-head cellular protein measurements conducted here, the impact was more pronounced for the bacterial vectors. One explanation is the different measure of dosage used for each vector. Dosage levels were picked based upon established ranges previously used for each vector (11,21–23). However, when compared on a number basis (Table I), significantly more bacteria are being added per macrophage compared to the microspheres. This, coupled with the issues of co-incubating two different biological cells (bacteria and macrophages), may help to explain the greater impact the bacterial vectors had on final macrophage cellular protein levels. Both vectors also showed a similar trend for increased final luminescence signal (i.e., successful gene delivery) with increasing vector dosage. At the highest dosage tested for both vector types, the final gene delivery measured was comparable; however, the lower dosages saw a significant

Table I. Gene Delivery Comparison Between Polymeric Microsphere and Bacterial Vectors for the 10 μg/ml and 10:1 Bacteria-to-Macrophage Dosages

Parameter	PLGA-Poly(β-Amino Ester) Polymeric Microspheres	YWT7-*hly*/pCMV-Luc *E. coli* Strain
Starting DNA	1 mg[a]	~50 ng[b]
DNA recovery	31.1%[c]	100%[d]
Number of microsphere or bacteria added	4,981[e]	500,000
Delivery efficiency (luminescence/mg cellular protein/ng DNA delivered)	881[f]	171[g]

[a] DNA added during the microsphere preparation method described in this work.
[b] Approximate DNA required for standard bacterial transformation (19).
[c] Encapsulation efficiency measured in this work.
[d] Designated 100% due to the consistency between bacterial cells carrying a DNA load dictated by plasmid selection and copy number.
[e] Microsphere number calculated by dividing the total mass of microspheres added per well by an estimated mass of one microsphere. Individual microsphere mass was estimated by using the measured diameter to calculate a microsphere volume and then using a density of 1.22 g/ml (32) to calculate microsphere mass.
[f] Determined by taking the average luminescence/mg gene delivery recorded for the 10 μg/ml microsphere dosage and dividing by the calculated amount of DNA added per sample. The amount of DNA added was calculated by the dosage (10 μg/ml), volume (100 μl), and encapsulation efficiency for the microspheres (31.1%) relative to 100% encapsulation efficiency (1 mg DNA/200 mg polymer).
[g] Determined by taking the average luminescence/mg gene delivery recorded for the 10 bacteria-to-macrophage dosage level and dividing by the amount of DNA added per sample. The amount of DNA was estimated from the number of bacterial added (500,000), the copy number of the plasmid (~100), and the molecular weight of the plasmid (estimated as plasmid bp×650 [Da per base pair]).

improvement in gene expression provided by the polymeric vectors. We should note that there were slight, unavoidable differences between the assay conditions for the bacterial and polymeric vectors. In this particular assay, negative effects caused by macrophage–bacteria co-incubation were minimized (through a 1-h co-incubation period); whereas, the same issues were not a concern with the non-biological microspheres which could be co-incubated throughout the full 24 h length of the assay. Though we believe these differences do not overshadow the ability of the assay to compare these two vectors, they may (1) underestimate the delivery potential of the bacterial vectors and (2) help account for the differences in gene delivery observed. Regardless, the ultimate measure of gene delivery efficacy will come in the way of *in vivo* application. However, before each vector is further evaluated for gene delivery in the context of *in vivo* models, this initial comparison is useful in demonstrating, comparing, and optimizing polymeric and now bacterial vectors for gene delivery to antigen presenting cells; thus, presenting delivery options for *in vivo* applications that would target viral-mediated illnesses and cancer that are particularly susceptible to a cytotoxic T cell response that predominantly originates from genetic antigen delivery (29–31).

The vectors studied here must also be evaluated with regards to gene delivery efficiency (defined as gene delivery signal per DNA delivered) and other desirable properties for overall vector production. Table I compares several metrics for the vectors used in this study. The first two rows present DNA used and recovered during vector formulation. From a process perspective, the polymeric microspheres require polymer synthesis, purified DNA, and a scalable formulation technique. Concerns include reproducible polymerization reactions that yield both PLGA and poly(β-amino ester) chains with consistent chemical, physical, and safety characterization profiles. In addition, as highlighted in Table I, row 1, the formulation process requires a significant amount of DNA. For the formulation procedure in this study, 1 mg of purified DNA was needed for microsphere preparation, with only 31.1% encapsulation efficiency recorded. Though previous efforts have reported improved encapsulation efficiencies, in our experience, it is not uncommon for this number to be in the 10–50% range (11,21). The bacterial vector, on the other hand, requires only ng levels of DNA for bacterial transformation to provide a stable cell line with a consistent DNA loading per cell (19). In addition, production of the bacterial vector would no doubt take advantage of well-established bioreactor schemes for cell production and vector harvesting. Table I next compares the gene delivery signal per DNA delivered for the 10 μg/mL microspheres and 10 bacteria-to-macrophage dosages. These calculations derive from knowledge or estimations of DNA loading per microsphere or bacteria and give an indication of the efficacy of the delivery vector. The comparison shows that the microspheres are more potent delivery vectors by 5 fold. As mentioned, both vectors have been engineered to better influence final gene delivery. Thus, options exist for continued vector engineering to further influence delivery efficiency with significantly different tools available for each vector: polymer chemistry and formulation strategies for the polymeric microspheres and genetic and metabolic engineering for the bacteria. Both tool sets should provide more advanced vectors in the future but it remains to be seen which will provide the greater degree of improvement in vector gene delivery. A final concern and source of comparative evaluation is the need for storage and distribution of the vectors. Here, the microspheres provide a distinct advantage of dry, long-term storage without the need for refrigeration; whereas, it is uncertain how storing the bacterial cells dry or without refrigeration will impact final delivery and overall vector integrity.

In summary, a comparison of polymeric microsphere and bacterial vectors was conducted yielding insight into the relative gene delivery capabilities of each. Though the comparison only holds for these particular microsphere and bacterial vectors within the context of this specific assay, the results obtained represent the first head-to-head comparison between bacterial and polymeric microsphere vectors and help to highlight the strengths, weaknesses, similarities, and differences between these types of vectors. Future studies will no doubt test and compare advanced versions of polymeric microsphere and bacterial vectors both within the context of *in vitro* gene delivery assays, such as those presented here, and in more applied and therapeutically relevant *in vivo* studies.

CONCLUSION

A comparison of gene delivery to a P388D1 macrophage line was made between polymeric microsphere and bacterial vectors. An 85:15 ratio of PLGA:poly(β-amino ester) microspheres were compared to an *E. coli* vector containing a chromosomal copy of the LLO gene; both vectors were designed to improve the delivery of a luciferase reporter gene. These two fundamentally different vectors were then quantitatively compared through the use of a high throughput *in vitro* gene delivery assay. For vector-specific dosage ranges, the two vectors showed comparable gene delivery at higher dosages while the microsphere vectors demonstrated improved gene delivery at lower dosages. When normalizing for amount of DNA delivered, the microspheres showed a relative advantage compared to the bacterial vectors. When placed next to issues of vector manufacture, storage, and the potential for future improvements, direct comparisons of gene delivery provide a seldom reported head-to-head analysis of polymeric microsphere and bacterial vector types.

ACKNOWLEDGEMENTS

We thank Dr. Darren Higgins (Harvard) for providing the plasmid encoding the T7-*hly* construct. B.A.P would like to recognize support from the National Academy of Engineering and S.P. is grateful for support from the Tufts Faculty Research Awards Committee.

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