Effect of Sizes on the Lung-Targeting and Biocompatibility of Intravenously Administered Biodegradable PLGA Microspheres

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Research

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Abstract

Background: In the treatment of lung diseases, drug showed low bioavailability and efficacy by conventional administration methods. Passive lung-targeting microspheres provide a method to deliver drugs from the vascular side, there is still a paucity of systematic studies on the biocompatibility of biodegradable-polymer microspheres.

Results: We used poly (lactic acid-glycolic acid) (PLGA) microspheres with different particle sizes (3, 10, 25, and 40 μm) as a model. The optimal lung-targeting particle size of the PLGA microspheres is 10 μm and the corresponding range of maximum tolerated dose is 125-150 mg/kg. We hypothesized that the decrease of blood oxygen saturation under treatment of microspheres was caused by pulmonary embolism. We found varying degrees of blood circulation loss of lungs by micro computed tomography test, which indicated severe pulmonary embolism subsequent to intravenous injection of microspheres with a particle size $>25 \mu\text{m}$. Furthermore, we found lung injury and microspheres leaked from the blood vessels into the alveoli by H&E staining. This process was likely induced by increased secretion of matrix metalloproteinases (MMPs), which destroyed the alveolar-capillary barrier and trigger an inflammatory cascade. Finally, we found that optimal particle size and dose conditions did not affect normal physiological activities after the microspheres degraded. The upregulation of vascular endothelial growth factor (VEGF) and platelet endothelial cell adhesion molecule-1 (PECAM-1 or CD31) induced vessel recanalization and reestablishment, which promoted the progress of lung repair.

Conclusions: Collectively, our results highlight the importance of accurately designing the optimal size and dose of microspheres for passive lung-targeting delivery.

Introduction

Currently, the recommended treatment for bacterial infections in the lungs includes oral or intravenous administration of amoxicillin, tetracyclines, macrolides, and fluoroquinolones [1]. However, such administration routes lead to systemic distribution and low distribution of the drug in lung. Inhalation administration is widely considered as an appealing noninvasive alternative to conventional invasive techniques. However, for patients with compromised lung function or respiratory obstruction, it is difficult to deliver drugs deep into the lungs by inhalation administration [2, 3, 4]. Administration to the lungs through the capillaries for innovative formulation is a promising method [2]. Passive lung-targeting microspheres based on microembolism can directly reach the lungs, allowing localized precision treatment of the respiratory system disease, and greatly improving drug bioavailability [5, 6, 7]. Studies have proven that biodegradable microspheres have achieved effective pulmonary delivery of drug and treatment of pulmonary diseases in animal models [8, 9, 10]. However, these lung-targeted microspheres may have safety risks of generating pulmonary embolisms that can damage the pulmonary capillaries and alveoli, severely limiting clinical applications.

The biodistribution and toxicity of microspheres are believed to be related to particle size [2, 11, 12, 13], dose [14], rigidity [11, 15, 16], and shape [13, 17, 18]. Studies have shown that regardless of the type of microspheres (non-biodegradable or biodegradable), a particle size of 7–12 μm is the best for passive lung-targeting [2, 19]. Small microspheres (MPs, < 7 μm) are captured by the reticuloendothelial system (RES), leading to that the majority are retained by the liver (80–90%), spleen (5–8%), bone marrow (1–2%) and other organs [20, 21, 22]. Large microspheres (> 20 μm) can easily block arteries and be used for embolization therapy under controlled conditions, which are dangerous *in vivo* for drug delivery [23, 24]. Previous results inferred that microspheres with even larger particle sizes (> 40 μm) can block upstream blood vessels and caused pulmonary embolism and lung injury [25]. The risk of pulmonary embolism and lung injury can be minimized by controlling the particle size of microspheres and administering the appropriate injection dose, which made microspheres trapped in the capillaries of the lungs. At present, it remains unclear that systematic studies on optimal particle sizes/doses for the safety of lung targeting using biodegradable microspheres. Therefore, designing optimal particle sizes/doses of lung-targeted microspheres are key factors for the clinical safety and application.

In this study, we designed four monosized microspheres to systematically evaluate the lung-targeting and biocompatibility under treatment with different sizes/doses in the passive lung-targeting process (Fig. 1). The optimal particle size of monosized PLGA microspheres were obtained by the micro computed tomography (micro CT) and small animal *in vivo* imaging system images. Histopathological analysis and pulse oximeter analyses revealed that the presence of pulmonary embolism was caused by the microspheres. Then we observed severe lung injury, including destroyed alveolar-capillary barrier and triggered an inflammatory cascade, caused by microembolism. We found that the levels of lungs injury were decreased by using optimal particle size and doses, it was further alleviated by the degradation of microspheres. Lastly, the increased number of capillaries promotes the recovery of lungs within 14 days.

Results And Discussion

Effect of microspheres sizes on the lung-targeting

We obtained PLGA microspheres of different particle sizes (3 mm: PLGA-3; 10 mm: PLGA-10; 25 mm: PLGA-25; 40 mm: PLGA-40,) through improving a method described by Qu *et al.* (Fig. S1) [5]. To obtain the optimal particle size for lung-targeting, we performed imaging experiments for lung blood vessels using micro CT (Fig. 2A). It has been reported that the occlusion of blood vessels by microspheres made the downstream blood vessels unable to be imaged through micro CT [26]. We found that the area of blood vessels in PLGA-3 and PLGA-10 groups were comparable (Fig. 2B). The density of microvessels was reduced compared with the PLGA-10 group in the lungs. This is because the PLGA-3 group have the characteristics of aggregation, causing microcirculation embolism. Interestingly, we found the number of imaged blood vessels in the lung of the PLGA-25 and PLGA-40 groups were significantly reduced, indicted that the microspheres were trapped in arterioles rather than capillaries (Fig. 2A, B). These results proved that PLGA microspheres with a particle size greater than 25 mm can cause severe pulmonary embolism in a short time.

To demonstrate the lung-targeting of microspheres with different particle sizes, the biodistribution of fluorescent label microspheres (Rhodamine B, Solarbio, Beijing, China) in mice was performed using the small animal *in vivo* imaging system. The biodistribution of microspheres with different particle sizes and materials were different in various animal models. Sinko *et al.* revealed that PS-3 were quickly entrapped in the rats of liver and spleen [2]. Schroeder *et al.* reported that the PS-10 can achieve lung-targeting in rats or dogs [27, 28, 29]. However, it has reported that 3 mm silica microspheres were distributed in the lung, due to its adhesion and aggregation in the lung capillaries [13]. The PLGA-3 quickly distributed in pulmonary capillaries due to its adhesion and aggregation nature (Fig. 2D). We also found that the PLGA-10 were quickly entrapped in the lung (Fig. 2D), similarly to previous reports for PLGA or PS microspheres [2, 19]. However, we did not observe that the distribution of microspheres in main organs from PLGA-25 group. It most likely due to their entrapment in other blood vessels, and unable to be detected by system. To evaluate the inflammation in mice treated with PLGA-3 and PLGA-10 microspheres, the lung tissues were collected to measure the inflammation factors (IL-1 β , TNF- α and IL-8) by ELISA. We found that the levels of inflammation factors in the PLGA-3 group were higher than that in the PLGA-10 group and PLGA-3 were easy to cause microcirculation embolism, indicated that the PLGA-3 caused more serious injuries to the lung tissue (Fig. 2E, S2). Collectively, the 10 mm of PLGA microspheres is the optimal particle size for lung-targeting in mice.

Effect of microspheres sizes on the lung biocompatibility

Cell viability and hemolytic activity tests proved that PLGA microspheres have good biocompatibility *in vitro* (Fig. S3 and S4). To obtain the maximum injection dose (the appearance of death in mice within 7 days or syringe blockage), PLGA and PS microspheres of different particle sizes (3 mm: PLGA-3, PS-3; 10 mm: PLGA-10, PS-10; 25 mm: PLGA-25, PS-25; 40 mm: PLGA-40, PS-40) were injected through the tail-vein of mice. We found that the maximum injection dose of microspheres was decreased in a particle size-dependent manner (Fig. 3A). To verify that the biocompatibility of PLGA and PS microspheres, the lung tissues were collected to calculate the lung coefficient. All the microsphere groups showed a significant increase in lung coefficient from the PBS control group (Fig. 3B), suggesting that all microsphere groups had lesions or injuries in the lungs. Furthermore, we found that the lung coefficient of the PS-10 and PS-25 was 1.4 times greater than that of the PLGA microspheres groups, proved that the degree of injury was more serious compared with the PLGA microspheres groups with the same sizes. The lung coefficient and survival rate of PS-3 and PLGA-3 were similar, but PS-3 have a major disadvantage that PS microspheres (< 6 mm) cannot achieve lung targeting [2]. Moreover, the survival rate of PLGA-10 treated mice was increased by 72.6% compared with PS-10 under the same dose (Fig. 3C, D), and confirm that PLGA microspheres are safer. These results showed that the biocompatibility PLGA microspheres were suitable for lung targeted drug delivery.

To observe the morphological features of lungs caused by the microspheres with maximum injection dose, mice were perfused with normal saline, then fixed with 10% (v/v) neutral buffered formalin. We observed that the color of the lung tissue treated with PLGA microspheres was darker than that of with the PBS group (Fig. 3E). Combined with the black spots on the surface of the lung tissue, suggesting that

the microspheres may cause lung injury. To further demonstrate the level of lung injury, histopathological analysis of the lung tissues was performed. We observed serious pathologic lesions in microsphere groups, including the generation of hyaline membrane in lung interstitium and alveoli in 3 mm and 10 mm microsphere groups, and the destruction of the alveolar-capillary structure in 25 mm and 40 mm microsphere groups (Fig. 3F). Microspheres are rapidly covered by plasma proteins after contact with blood [30]. Such protein adsorption can mediate the adhesion of the microspheres to endothelial cells [31]. This characteristic of microspheres was triggered when the particle size is less than 5 mm [13]. Therefore, we observed that PLGA-3 adhered and aggregated in capillaries (Fig. 3F). We also observed that microspheres were deformed in PLGA-25 and PLGA-40 groups (Fig. 3F), suggested that the vasoconstriction was happened [32]. Due to the aggregated and deformed characterization of PLGA microspheres, the particle size of all PLGA microsphere groups tend to same-size *in vivo* (Fig. 3F), but microspheres with a particle size greater than 25 mm still pose a major threat to the lungs, such as pulmonary thrombosis (Fig. S5). Overall, these results have demonstrated that the biocompatibility of passive lung-targeting using biodegradable microspheres could be improved by optimization of particle size.

Optimized dose of microspheres improves the lung biocompatibility

To obtain the maximum tolerated dose (MTD) of microspheres, we evaluated the changes of SpO₂ under treatment of PLGA microspheres with five possible doses (less than the maximum injected dose) for 10 days (Fig. 4A). The MTD of PLGA microspheres with different particle size (3, 10, 25, and 40 mm) were 125, 150, 75, and 62.5 mg/kg, respectively (Fig. 4B and S6). We found that the SpO₂ was decreased in a dose-dependent manner when doses above the MTD, suggesting that the capacity of pulmonary gas exchange was declined (Fig. 4B, S7 and S8). Next, the time-dependent level in SpO₂ of mice treated with PLGA-10 were evaluated. However, we found that mice were injected with PLGA microspheres in a larger dose than MTD, their SpO₂ had recovered to a standard level at 8 days (Fig. 4C). The degradation of PLGA microspheres *in vivo* or recruitment and distention of the capillary bed contributed to the recovery of SpO₂ [14]. Furthermore, we found that the SpO₂ remains standard level after the injection of doses below the MTD within 10 days (Fig. 4C), suggested that we could improve the biocompatibility of pulmonary delivery by optimizing the dose (Fig. S9). Consistent with the results of SpO₂, histopathology showed that no lung injury was induced by the treatment of PLGA microspheres with a lower dose than MTD (Fig. S10).

To assess the capacity of the lungs to retain microspheres, we tested the cumulative amount of fluorescence in lung tissues using the small animal *in vivo* imaging system. The images showed that when the dose is lower than the MTD (150 mg/kg), the fluorescence accumulation remained stable (Fig. 4D). However, we found that the fluorescence accumulation quickly reduced to the stable state (similar to 125 mg/kg) after injected a larger dose of microspheres (exceed the MTD) (Fig. 4D), it is possible that the microspheres in the lung were shunted [33]. These results proved that the lung can retain a certain dose (the optimal particle size of PLGA microspheres is 10 mm and the corresponding range of MTD is 125-

150 mg/kg) of microspheres. Overall, these results have demonstrated that the biocompatibility of passive lung-targeting PLGA microspheres could be improved by optimization of dose.

Pulmonary embolism was eliminated by self-repair of lungs

Given the biocompatibility of pulmonary delivery of microspheres under the optimal particle size and dose, we next sought to explore the repaired mechanism of lung tissue. Previous reports have demonstrated that due to the phagocytosis of endothelial cells within blood vessels, which contributed to the extravasation of emboli from capillaries and crossed into the alveolar space [34]. Carson *et al.* showed that complex cellular and molecular events, including the degradation of tight-junction proteins, adherens junctions, and extracellular matrix, participated in the process of envelopment and extravasation of emboli [35, 36]. Matrix metalloproteinases (MMPs) were able to disrupt these structures and involved in guidance, lumen formation, and barrier function in the development of blood vessels. [36, 37]. However, the degradation of the extracellular matrix indicated that the angiophagy was detrimental during the early stages of the extravasation of emboli [35]. To confirm whether the PLGA microspheres extravasated and crossed into the alveoli, we examined the expression of MMP-9 after treated with PLGA microspheres by immunofluorescence and western blot, and tracked the position of PLGA microspheres in lung tissue using H&E staining. We observed that the expression of MMP-9 significantly increased compared with the PBS group, indicated that the extravascular matrix was disrupted (Fig. 5A, B). Furthermore, previous studies have reported that MMP-9 could promote the expression of inflammatory factors (TNF- α) [36, 38], consistent with above results that PLGA microspheres promoted the secretion of inflammatory factors (Fig. 3E). Furthermore, we found that the PLGA microspheres appeared in the alveoli (Fig. 5C, S11), proved the occurrence of angiophagy and extravasation of the PLGA microspheres. These data explained that the PLGA microspheres caused lung injury at the early stages of engulfment. However, the pulmonary embolism was relieved by the extravasation of PLGA microspheres to alveoli. It ensured that the PLGA microspheres can retain in the lung for a long time to sustained release.

VEGF has been shown to have pro-angiogenic and pro-permeability features [39]. The images of immunofluorescence and western blot showed that the expression of VEGF was significant increased (Fig. 5A, B). The results suggested that it plays a positive role in the extravasation of PLGA microspheres and the vessel recanalization and reestablishment. The study showed that the VEGF plays a key role in the vessel of recanalization and reestablishment [39]. Similarly, the CD31 could rebuilt the capillaries by recruited more epithelial cells to promoted lung repair [40]. Consistently, the level of CD31 and microvessel density (MVD) were significantly increased under the injection of PLGA microspheres with a dose lower than 150 mg/kg (Fig. 5D, E). These results indicated that recruitment and distention of the capillary were used to relieve pulmonary embolism caused by PLGA microspheres [41, 42, 43]. Lastly, we found that the CD31 and MVD returned to a normal level (Fig. 5D, E), no pathological injury was observed by histopathological analysis at 14 day and no significant difference in growth rate from PBS group within 14 days (Fig. S12, S13). Collectively, our findings indicated that the optimal size (10 μ m) and MTD (125-150mg/kg) provided the evidence for safe delivery to the lungs in mice.

Conclusion

In summary, we obtained optimal particle size and MTD of PLGA microspheres to improve the biocompatibility of passive lung-targeting delivery, using a mice model. The optimal lung-targeting particle size of the PLGA microspheres is 10 μm and the corresponding range of MTD is 125–150 mg/kg. PLGA microspheres can migrate from the lumen of the blood vessel to the alveolar under the angiophagy, which contributed to relieve pulmonary embolism caused by PLGA microspheres. However, the process of extravasation of PLGA microspheres may cause overexpression of MMP-9 in the lung tissue, leading to trigger an inflammatory cascade. The mechanism of lung repair indicated that VEGF and CD31 promoted vessel recanalization and reestablishment within 14 day. The optimization of particle size and dose was essential for improving the biocompatibility of a passive lung-targeting drug delivery system.

Materials And Methods

Materials

PLGA (Mw, 20 kDa; lactic acid: glycolic acid, 25:75) with a terminal carboxyl group was purchased from Daigang (Jinan, China). Fluorescent, internally labeled, polystyrene microspheres (PS microspheres) of various sizes (3, 10, 25 and 40 μm) were purchased from Unibead Scientific Co., Ltd (Tianjin, China). Rhodamine B and RPMI 1640, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Solarbio Science & Technology Co., Ltd. (Beijing, China). Microfil was purchased from Flow Tech, Inc. (Massachusetts, USA). All other chemicals and reagents were obtained from commercial sources with the analytical reagent available.

Animals

Male Kunming mice 4 weeks old, 20-22 g were purchased from Pengyue (Shandong, China), and were housed under specific-pathogen-free conditions, with a 12-h light/12-h dark cycle. All experimental animal protocols were approved by the Animal Protection and Research Ethics Committee of Qingdao Agricultural University.

Acute toxicity evaluation

Mice were randomly assigned to nine groups ($n = 6$ per group), including one control group with PBS injection and eight experimental groups intravenously administered PLGA (3 mm: PLGA-3, 10 mm: PLGA-10, 25 mm: PLGA-25, or 40 mm: PLGA-40) or PS microspheres (3 mm: PS-3, 10 mm: PS-10, 25 mm: PS-25, or 40 mm: PS-40). For the microspheres groups, 0.5 mL of the microspheres in phosphate-buffered saline (PBS) was injected, while 0.5 mL of PBS was administered to the control group. After administration, we observe the mice daily for abnormal behaviors. Dead mice were immediately weighed and dissected. The lungs were harvested, photographed, and weighed to calculate the lung coefficient, which is defined as the percentage of organ weight to body weight. Lung samples were fixed in 10% (v/v) neutral buffered formalin at 24-26°C. The fixed samples were embedded in paraffin, cut into 5 mm-thick

sections, and stained with a hematoxylin and eosin (H&E) staining kit (Jiancheng Biotech, Jiangsu, China) using a standard histological method.

Micro CT imaging

Lung perfusion and casting were performed according to a previous study [26]. Briefly, mice were divided into five groups (PBS, PLGA-3, PLGA-10, PLGA-25, PLGA-40). Mice have injected with PLGA microspheres (250 mg/kg) and the mice were anesthetized with 5% chloral hydrate after injected for 1 hour. After removing the anterior chest wall, a syringe was inserted through the right ventricle, and the lungs were perfused with normal saline to remove blood. The Microfil was then mixed (MV-122: MV diluent: MV curing agent = 4:5:0.5) and perfused through the lung. After solidifying at 4°C overnight, the lungs dehydrated with an ethanol gradient, and finally immersed in methyl salicylate to render the lung tissue transparent. The lungs were scanned on a micro CT scanner (Quantum GX2, PerkinElmer, USA) and established a 3-dimension (3D) model of the pulmonary blood vessels. The scans were acquired using the following settings: field of view (36 mm), energy or intensity (90 kV, 88 mA), and integration time (14 min).

Fluorescence imaging

Fluorescence imaging of mice was performed using a small animal *in vivo* imaging system (IVIS Spectrum, PerkinElmer, USA). DsRed excitation (λ_{ex} = 500–550 nm) and emission (λ_{em} = 575–650 nm) filters were used. Mice were injected with microspheres (3, 10, and 25 mm) in PBS through the tail vein. 30 min after the injection of the microspheres, the mice were anesthetized by isoflurane and imaged using the *in vivo* imaging system. The heart, lungs, liver, spleen, and kidneys were then removed intact and imaged intact *ex vivo*. Additionally, different pro-inflammatory mediators, namely tumor necrosis factor- α (TNF- α), and interleukins 1 β (IL-1 β), and interleukins 8 (IL-8) were measured in the Lung tissue homogenate (PBS, pH 7.4, 1:9) using enzyme-linked immunosorbent assay (ELISA) kits (Meimian, Jiangsu, China).

Dose assessment of blood oxygen saturation and lung injury

The four monosized PLGA and PS microspheres were dispersed in PBS (pH 7.4). The microspheres were vortexed immediately before mice (n = 3 per group) tail vein injection. The mice were anesthetized by 5% chloral hydrate after injection. Arterial hemoglobin oxygen saturation (SpO₂) of the mice was monitored by a pulse oximeter (YK-820 miniA, USA) for 10 days. After 10 days, the lung tissues of the mice were collected for histopathological examinations and stained with H&E.

To further verify the maximum injection dose tolerated in mice, we used a small animal *in vivo* imaging system to quantitatively measure the fluorescence accumulation in the mouse lung tissue. Microspheres with the optimum particle size were used as injection drugs, and the lung tissues of the mice were imaged and fluorescence quantitative analysis at 15, 30, and 60 min after tail vein injection with different doses (125, 250, 375, 500 mg/kg).

Lung injury and post-injury repair

The mice were injected with high-dose (250 mg/kg) PLGA microspheres (3, 10 mm, n = 3 per group) through the tail vein. Lung samples were collected after seven days. One portion was used for western blot analysis, and the other portion was fixed in neutral formalin. The fixed samples were embedded in paraffin, stained with H&E, and subjected to immunofluorescence.

The mice were injected intravenously with PLGA microspheres (10 mm) of different doses (75, 150, 225 mg/kg, n = 3 per group). The lung tissues of the mice were formalin-fixed and paraffin-embedded on day 7 and day 14, stained with H&E, and subjected to immunofluorescence. Image J was used to calculate the microvessel density.

Western blot

The expression of TNF- α (Abcam, UK, 1:200), IL-1 β (Abcam, Cambridge, UK, 1:200), p38 (Abcam, Cambridge, UK, 1:200), vascular endothelial growth factor-A (VEGF-A, Abcam, Cambridge, UK, 1:200), and matrix metalloproteinase-9 (MMP-9, Abcam, Cambridge, UK, 1:200) was detected by western blot analysis. The lung tissues were collected and the protein was isolated by RIPA lysis buffer, quantified by BCA protein assay. After the standard procedure of western blot, protein bands were visualized by enhanced chemiluminescence (ECL) detection reagents and captured by Tanon-4200 Gel Imaging System (Tanon Science, China). The quantitative analysis of western blot was carried out by Image J software.

Immunofluorescence staining

Immunofluorescence analysis was performed to detect the expression of MMP-9, VEGF, and platelet endothelial cell adhesion molecule-1 (PECAM-1 or CD31, Bioss, Beijing, China, 1:200). The paraffin sections of the collected samples were deparaffinized with xylene and ethanol, rehydrated, subjected to antigen retrieval, blocked with bovine serum albumin (BSA, 5%, 30 min), and incubated overnight at 4°C with specific primary antibodies. After rinsing with PBS, the samples were incubated with appropriate corresponding secondary antibodies and then counterstained with 4',6-diamidino-2-phenylindole (DAPI, Solarbio, Beijing, China). All images were captured with a fluorescence microscope (CKX53, Olympus, Japan) and analyzed using the NIS Elements Advanced Research software (Nikon, Japan).

Statistical analysis

All data are expressed as the means $\pm$ standard deviation (SD). Statistical analysis was performed using GraphPad Prism 8.0. Tests for significant differences were analyzed by one-way ANOVA. $P < 0.05$ was considered statistically significant.

Declarations

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Authors' contributions

Zhihui Hao have full access to all data in the study and are responsible for the integrity of the data and the accuracy of data analysis. Fengting Lang contributed to the research design, conducted experiments, assisted the analysis of samples, and participated in the interpretation of the results and the writing of the manuscript. Shaoqi Qu contributed to the experimental design. Shaoqi Qu, Kang Li, Muqi Zhong and Cuncai Wang participated in the discussion of the results. The authors read and approved the final manuscript.

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Availability of data and materials

All the data and materials are available. These are all openly available.

Ethics approval and consent to participate

All animal procedures described in this study were conducted in accordance with the guidelines for the care and use of laboratory animals approved by Qingdao Agricultural University.

Consent for publication

No personal information is included in this study.

Declaration of Competing Interest.

The authors declare no competing financial interest.

References

1. Woodhead M, Blasi F, Ewig S, Huchon G, Ieven M, Ortqvist A, et al. Guidelines for the management of adult lower respiratory tract infections. Eur Respir J. 2005;26 6:1138-80; doi: 10.1183/09031936.05.00055705.
2. Kutscher HL, Chao P, Deshmukh M, Singh Y, Hu P, Joseph LB, et al. Threshold size for optimal passive pulmonary targeting and retention of rigid microparticles in rats. J Control Release. 2010;143 1:31-7; doi: 10.1016/j.jconrel.2009.12.019. <https://www.ncbi.nlm.nih.gov/pubmed/20043961>.
3. Hirst PH, Pitcairn GR, Weers JG, Tarara TE, Clark AR, Dellamary LA, et al. In vivo lung deposition of hollow porous particles from a pressurized metered dose inhaler. Pharm Res. 2002;19 3:258-64; doi:

10.1023/a:1014482615914.

4. Leach CL, Davidson PJ, Hasselquist BE, Boudreau RJ. Lung deposition of hydrofluoroalkane-134a beclomethasone is greater than that of chlorofluorocarbon fluticasone and chlorofluorocarbon beclomethasone : a cross-over study in healthy volunteers. *Chest*. 2002;122 2:510-6; doi: 10.1378/chest.122.2.510.
5. Qu S, Dai C, Zhu J, Zhao L, Li Y, Hao Z. Cefquinome-loaded microsphere formulations against *Klebsiella pneumonia* infection during experimental infections. *Drug Deliv*. 2018;25 1:909-15; doi: 10.1080/10717544.2018.1461958.
6. Yang Y, Yuan L, Li J, Muhammad I, Cheng P, Xiao T, et al. Preparation and evaluation of tilmicosin microspheres and lung-targeting studies in rabbits. *Vet J*. 2019;246:27-34; doi: 10.1016/j.tvjl.2019.01.007.
7. Hwang SM, Kim DD, Chung SJ, Shim CK. Delivery of ofloxacin to the lung and alveolar macrophages via hyaluronan microspheres for the treatment of tuberculosis. *J Control Release*. 2008;129 2:100-6; doi: 10.1016/j.jconrel.2008.04.009.
8. Wang W, Cai Y, Zhang G, Liu Y, Sui H, Park K, et al. Sophoridine-loaded PLGA microspheres for lung targeting: preparation, in vitro, and in vivo evaluation. *Drug Deliv*. 2016;23 9:3674-80; doi: 10.1080/10717544.2016.1223210.
9. Harsha S, R C, Rani S. Ofloxacin targeting to lungs by way of microspheres. *Int J Pharm*. 2009;380 1-2:127-32; doi: 10.1016/j.ijpharm.2009.07.020.
10. Garcia-Contreras L, Sethuraman V, Kazantseva M, Godfrey V, Hickey AJ. Evaluation of dosing regimen of respirable rifampicin biodegradable microspheres in the treatment of tuberculosis in the guinea pig. *J Antimicrob Chemother*. 2006;58 5:980-6; doi: 10.1093/jac/dkl369.
11. Xiong S, George S, Yu H, Damoiseaux R, France B, Ng KW, et al. Size influences the cytotoxicity of poly (lactic-co-glycolic acid) (PLGA) and titanium dioxide (TiO₂) nanoparticles. *Arch Toxicol*. 2013;87 6:1075-86; doi: 10.1007/s00204-012-0938-8. <https://www.ncbi.nlm.nih.gov/pubmed/22983807>.
12. Inoue K, Takano H, Yanagisawa R, Koike E, Shimada A. Size effects of latex nanomaterials on lung inflammation in mice. *Toxicol Appl Pharmacol*. 2009;234 1:68-76; doi: 10.1016/j.taap.2008.09.012.
13. Decuzzi P, Godin B, Tanaka T, Lee SY, Chiappini C, Liu X, et al. Size and shape effects in the biodistribution of intravascularly injected particles. *J Control Release*. 2010;141 3:320-7; doi: 10.1016/j.jconrel.2009.10.014. <https://www.ncbi.nlm.nih.gov/pubmed/19874859>.
14. Kutscher HL, Gao D, Li S, Massa CB, Cervelli J, Deshmukh M, et al. Toxicodynamics of rigid polystyrene microparticles on pulmonary gas exchange in mice: implications for microemboli-based drug delivery systems. *Toxicol Appl Pharmacol*. 2013;266 2:214-23; doi: 10.1016/j.taap.2012.10.021. <https://www.ncbi.nlm.nih.gov/pubmed/23142466>.
15. Fish MB, Fromen CA, Lopez-Cazares G, Golinski AW, Scott TF, Adili R, et al. Exploring deformable particles in vascular-targeted drug delivery: Softer is only sometimes better. *Biomaterials*.

- 2017;124:169-79; doi: 10.1016/j.biomaterials.2017.02.002.
<https://www.ncbi.nlm.nih.gov/pubmed/28209527>.
16. Aragao-Santiago L, Hillaireau H, Grabowski N, Mura S, Nascimento TL, Dufort S, et al. Compared in vivo toxicity in mice of lung delivered biodegradable and non-biodegradable nanoparticles. *Nanotoxicology*. 2016;10 3:292-302; doi: 10.3109/17435390.2015.1054908.
 17. Thackaberry EA, Farman C, Zhong F, Lorget F, Staflin K, Cercillieux A, et al. Evaluation of the Toxicity of Intravitreally Injected PLGA Microspheres and Rods in Monkeys and Rabbits: Effects of Depot Size on Inflammatory Response. *Invest Ophthalmol Vis Sci*. 2017;58 10:4274-85; doi: 10.1167/iovs.16-21334. <https://www.ncbi.nlm.nih.gov/pubmed/28850638>.
 18. Li L, Liu T, Fu C, Tan L, Meng X, Liu H. Biodistribution, excretion, and toxicity of mesoporous silica nanoparticles after oral administration depend on their shape. *Nanomedicine*. 2015;11 8:1915-24; doi: 10.1016/j.nano.2015.07.004. <https://www.ncbi.nlm.nih.gov/pubmed/26238077>.
 19. Agnoletti M, Rodriguez-Rodriguez C, Klodzinska SN, Esposito TVF, Saatchi K, Morck Nielsen H, et al. Monosized Polymeric Microspheres Designed for Passive Lung Targeting: Biodistribution and Pharmacokinetics after Intravenous Administration. *ACS Nano*. 2020;14 6:6693-706; doi: 10.1021/acsnano.9b09773. <https://www.ncbi.nlm.nih.gov/pubmed/32392034>.
 20. Dobson EL, Jones HB. The behavior of intravenously injected particulate material; its rate of disappearance from the blood stream as a measure of liver blood flow. *Acta Med Scand Suppl*. 1952;273:1-71.
 21. Scheffel U, Rhodes BA, Natarajan TK, Wagner HN, Jr. Albumin microspheres for study of the reticuloendothelial system. *J Nucl Med*. 1972;13 7:498-503.
 22. Yapel AF, Jr. Albumin microspheres: heat and chemical stabilization. *Methods Enzymol*. 1985;112:1-18; doi: 10.1016/s0076-6879(85)12003-3.
 23. Vilgrain V, Pereira H, Assenat E, Guiu B, Ilonca AD, Pageaux GP, et al. Efficacy and safety of selective internal radiotherapy with yttrium-90 resin microspheres compared with sorafenib in locally advanced and inoperable hepatocellular carcinoma (SARAH): an open-label randomised controlled phase 3 trial. *Lancet Oncol*. 2017;18 12:1624-36; doi: 10.1016/s1470-2045(17)30683-6.
 24. Salem R, Hunter RD. Yttrium-90 microspheres for the treatment of hepatocellular carcinoma: a review. *Int J Radiat Oncol Biol Phys*. 2006;66 2 Suppl:S83-8; doi: 10.1016/j.ijrobp.2006.02.061.
 25. Gesler RM, Garvin PJ, Klammer B, Robinson RU, Thompson CR, Gibson WR, et al. The biologic effects of polystyrene latex particles administered intravenously to rats—a collaborative study. *Bull Parenter Drug Assoc*. 1973;27 3:101-13.
 26. Phillips MR, Moore SM, Shah M, Lee C, Lee YZ, Faber JE, et al. A method for evaluating the murine pulmonary vasculature using micro-computed tomography. *J Surg Res*. 2017;207:115-22; doi: 10.1016/j.jss.2016.08.074.
 27. Schroeder HG, Simmons GH, DeLuca PP. Distribution of radiolabeled subvisible microspheres after intravenous administration to beagle dogs. *J Pharm Sci*. 1978;67 4:504-7; doi: 10.1002/jps.2600670417.

28. Kanke M, Simmons GH, Weiss DL, Bivins BA, DeLuca PP. Clearance of ¹⁴¹C3-labeled microspheres from blood and distribution in specific organs following intravenous and intraarterial administration in beagle dogs. *J Pharm Sci.* 1980;69 7:755-62; doi: 10.1002/jps.2600690703.
29. Yokel RA, Sabo JP, Simmons GH, DeLuca PP. Acute toxicity of latex microspheres. *Toxicol Lett.* 1981;9 2:165-70; doi: 10.1016/0378-4274(81)90035-7.
30. Moghimi SM, Szebeni J. Stealth liposomes and long circulating nanoparticles: critical issues in pharmacokinetics, opsonization and protein-binding properties. *Prog Lipid Res.* 2003;42 6:463-78; doi: 10.1016/s0163-7827(03)00033-x.
31. Serda RE, Gu J, Bhavane RC, Liu X, Chiappini C, Decuzzi P, et al. The association of silicon microparticles with endothelial cells in drug delivery to the vasculature. *Biomaterials.* 2009;30 13:2440-8; doi: 10.1016/j.biomaterials.2009.01.019.
32. Lumb AB, Slinger P. Hypoxic pulmonary vasoconstriction: physiology and anesthetic implications. *Anesthesiology.* 2015;122 4:932-46; doi: 10.1097/aln.0000000000000569.
33. Niden AH, Aviado DM, Jr. Effects of pulmonary embolism on the pulmonary circulation with special reference to arteriovenous shunts in the lung. *Circ Res.* 1956;4 1:67-73; doi: 10.1161/01.res.4.1.67.
34. Grutzendler J, Murikinati S, Hiner B, Ji L, Lam CK, Yoo T, et al. Angiophagy prevents early embolus washout but recanalizes microvessels through embolus extravasation. *Sci Transl Med.* 2014;6 226:226ra31; doi: 10.1126/scitranslmed.3006585.
35. Lam CK, Yoo T, Hiner B, Liu Z, Grutzendler J. Embolus extravasation is an alternative mechanism for cerebral microvascular recanalization. *Nature.* 2010;465 7297:478-82; doi: 10.1038/nature09001. <https://www.ncbi.nlm.nih.gov/pubmed/20505729>.
36. Kessenbrock K, Plaks V, Werb Z. Matrix metalloproteinases: regulators of the tumor microenvironment. *Cell.* 2010;141 1:52-67; doi: 10.1016/j.cell.2010.03.015. <https://www.ncbi.nlm.nih.gov/pubmed/20371345>.
37. Stratman AN, Saunders WB, Sacharidou A, Koh W, Fisher KE, Zawieja DC, et al. Endothelial cell lumen and vascular guidance tunnel formation requires MT1-MMP-dependent proteolysis in 3-dimensional collagen matrices. *Blood.* 2009;114 2:237-47; doi: 10.1182/blood-2008-12-196451.
38. Jiang PR, Cao Z, Qiu ZL, Pan JW, Zhang N, Wu YF. Plasma levels of TNF- α and MMP-9 in patients with silicosis. *Eur Rev Med Pharmacol Sci.* 2015;19 9:1716-20.
39. Apte RS, Chen DS, Ferrara N. VEGF in Signaling and Disease: Beyond Discovery and Development. *Cell.* 2019;176 6:1248-64; doi: 10.1016/j.cell.2019.01.021. <https://www.ncbi.nlm.nih.gov/pubmed/30849371>.
40. Woodfin A, Voisin MB, Nourshargh S. PECAM-1: a multi-functional molecule in inflammation and vascular biology. *Arterioscler Thromb Vasc Biol.* 2007;27 12:2514-23; doi: 10.1161/ATVBAHA.107.151456. <https://www.ncbi.nlm.nih.gov/pubmed/17872453>.
41. Lertkiatmongkol P, Liao D, Mei H, Hu Y, Newman PJ. Endothelial functions of platelet/endothelial cell adhesion molecule-1 (CD31). *Curr Opin Hematol.* 2016;23 3:253-9; doi: 10.1097/moh.0000000000000239.

42. Lin CK, Lin YH, Huang TC, Shi CS, Yang CT, Yang YL. VEGF mediates fat embolism-induced acute lung injury via VEGF receptor 2 and the MAPK cascade. *Sci Rep*. 2019;9 1:11713; doi: 10.1038/s41598-019-47276-4.
43. Kasprzak A, Surdacka A, Tomczak M, Przybyszewska W, Seraszek-Jaros A, Malkowska-Lanzafame A, et al. Expression of angiogenesis-stimulating factors (VEGF, CD31, CD105) and angiogenetic index in gingivae of patients with chronic periodontitis. *Folia Histochem Cytobiol*. 2012;50 4:554-64; doi: 10.5603/20324.

Figures

Figure 1

Schematic diagram of the passive lung-targeting microspheres based on microembolism for biocompatibility evaluation in mouse model. (A) The microspheres were quickly captured by lung blood

vessels, and it trapped in different levels of blood vessels depend on particle size. (B) Mechanisms to relieve pulmonary embolism is extravasation of microspheres from the lumen of the vascular, which might be accompanied by lung injury. (C) The lung was repaired by recanalization and reconstruction of blood vessels after injection of microspheres.

Figure 2

The optimal particle size of PLGA microspheres for passive lung-targeting. (A) Representative 3D and bright field images of pulmonary vessels after injection of PLGA microspheres with different particle sizes. The pulmonary vessels are shown in yellow using microfil. Red arrows point to the capillary embolism formed by congestion. Scale bar: (first and second row) 5 mm, (third row) 1.5 mm. (B and C) The degree of pulmonary embolism was quantified by the area of the pulmonary vessels (Micro CT) and the number of capillaries per unit area (Bright field). (D) Representative images of the main organ of mice treated with fluorescently labeled PLGA microspheres (3, 10, and 25 μm) were obtained using the small animal in vivo imaging system. (E) Concentrations of TNF- α , IL-1 β , and IL-8 from lung tissues treated with PLGA microspheres. All data were presented as mean $\pm$ SD. Significance was determined by one-way ANOVA. *P < 0.05, **P < 0.01 and ***P < 0.001.

Figure 3

Varying levels of lung injury in mice were caused by the maximum injection dose of different particle-sized PLGA and PS microspheres. (A) The maximum injection dose referred to the injected dose for the appearance of death in mice within 7 days or syringe blockage. (B) The lung coefficient of mice treated with PBS, PLGA microspheres, and PS microspheres was calculated on day 7. (C and D) Survival of mice after injected with PS and PLGA microspheres. (E) Representative pictures of lungs after clearing the lung

blood through lung perfusion. Mice were injected with microspheres under the condition of the maximum injection dose. Black arrow points to the spot formed by congestion. Scale bar: 5 mm. (F) Pathological examination of lung tissues administrated with PLGA and PS microspheres by H&E staining. Red arrows point to the PS and PLGA microspheres; Blue arrows point to the generation of hyaline membrane in lung interstitium and alveoli; Green arrows point to the destruction of the alveolar-capillary structure. Scale bar: 100 μ m. All data were presented as mean $\pm$ SD. Significance was determined by one-way ANOVA. *P < 0.05, **P < 0.01.

Figure 4

The maximum tolerated dose of PLGA microspheres for pulmonary delivery. (A) Timeline of the injection and SpO2 test. (B) Changes in SpO2 of mice under the treatment of PLGA microspheres with different doses. (C) Levels of SpO2 in mice under treatment of 10 μ m PLGA microspheres with different doses in 10 days. (D) Lung accumulation of 10 μ m PLGA microspheres with different doses was determined by

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MMP-9 and VEGF in lung tissues under treatment with PLGA microspheres compared to control PBS. (C) Observe the position of the microspheres by examining the pathological section of the lung tissue. Black arrow points to the PLGA microspheres. Scale bar: 50 μm . (D, E) Expression of CD31 in the lung tissue of mice treated with PLGA-10 at 7 and 14 days, quantified using IF staining. Scale bar: 200 μm . All data were presented as mean $\pm$ SD. Significance was determined by one-way ANOVA. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

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