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5 Therapeutic

Facial Surgery

Preliminary Report

Short-Term Effects of Poly-L-Lactic Acid-b-Polyethylene Glycol Microsphere Injection on Different Adipose Tissue Types in Rats

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Abstract

Background: Wrinkles and sagging, characteristics of aging, are associated with reductions in collagen and fat. Poly-L-lactic acid (PLLA) is widely used clinically as a tissue filler owing to its good biocompatibility and ability to improve wrinkles and signs of aging. Despite extensive studies of the mechanism of action of PLLA when used as a dermal filler, few studies have examined its effects on adipose tissue.

Objectives: The short-term effects of PLLA-b-polyethylene glycol (PEG) microspheres implanted in subcutaneous back adipose tissue (BAT) and visceral epididymal adipose tissue (EAT) of rats were examined.

Methods: The authors divided 15 male Sprague–Dawley rats into 5 groups based on implantation time, and PLLA-b-PEG microspheres were implanted into the BAT (3 groups were sampled at 6, 8, and 12 weeks) and EAT (2 groups were sampled at 6 and 12 weeks) of rats. Tissue samples were collected at different time points postimplantation and subjected to histological analyses using hematoxylin and eosin, Masson's trichrome, and immunofluorescence staining.

Results: Implantation of PLLA-b-PEG microspheres into different adipose tissues resulted in a mild and persistent inflammatory reaction, increased fibrous connective tissue, and noticeable collagen regeneration. Immunofluorescence showed the upregulation of uncoupling protein (UCP) 1 and UCP2 in the visceral adipose tissue surrounding the implant.

Conclusions: PLLA-b-PEG microspheres exhibited good tissue compatibility and induced an increase in fibrous connective tissue postimplantation, potentially mitigating oxidative damage and improving adipose tissue quality.

Level of Evidence: 5 (Therapeutic)

Adipose tissue is a crucial factor influencing facial aging, which is primarily manifested by the loss of fat volume and changes in fat layer positioning.[1] In the face, fascial layers are divided into superficial and deep fat layers. Within each layer, adipose tissue is further compartmentalized into numerous lobules by fibrous connective tissue anchored to the fascial layer.[2] There is a strong linkage between the facial muscles (especially those involved in facial expressions) and the collagenous meshwork surrounding the adipose tissue and the skin. The adipose tissue interposed between the collagen fibers and muscle network is wrapped and fixed by the fibrous meshwork to prevent displacement.[3] Volume filling of adipose tissue and the anchoring role of fibrous connective tissue are essential for maintaining the fullness and firmness of the facial skin. With aging and external stimuli, such as ultraviolet (UV) radiation, oxidative stress can accelerate adipocyte apoptosis,[4] gradually reducing the facial fat volume

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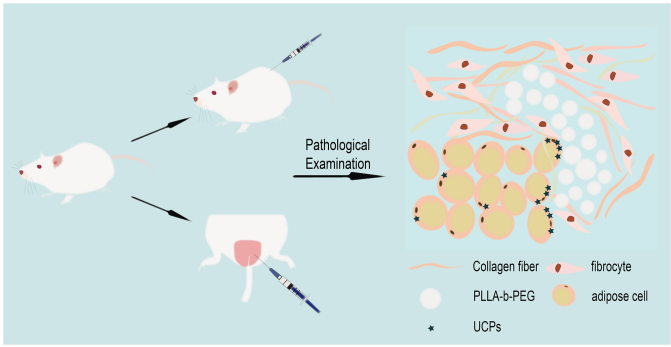

Figure 1. Experimental design diagram. UCP, uncoupling protein; PLLA, poly-L-lactic acid; PEG, polyethylene glycol.

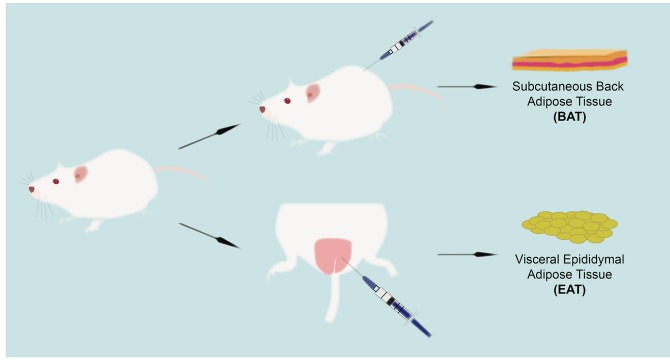

Figure 2. Schematic illustration of poly-L-lactic acid-b-polyethylene glycol microsphere injection and sample collection in rats. BAT, back adipose tissue; EAT, epididymal adipose tissue.

and causing skin laxity. Additionally, decreased collagen fibers in the superficial fat layer and shear forces caused by facial expressions lead to a downward displacement of the fat layer, resulting in sagging skin and wrinkles.[5]

Autologous fat transplantation in the early stages is an effective method to address fat volume loss; however, the adipose cell survival rate is low and the filling effect is not maintained for long periods. With advances in biotechnology, attention has shifted toward synthetic fillers, such as hyaluronic acid (HA), poly-L-lactic acid (PLLA), polycaprolactone, and calcium hydroxyapatite (CaHA).[5] PLLA has garnered significant attention as a biodegradable filler owing to its excellent biocompatibility, degradability, and suitable mechanical properties.[6] It has been approved for correcting signs of facial fat loss since the early 21st century.

Technological advances have enabled the development of amphiphilic PLLA-b-polyethylene glycol (PEG) microspheres by grafting PEG onto PLLA chains.[7] Clinically, PLLA-b-PEG microspheres are commonly injected into the subcutaneous fat layer for facial filling and demonstrate effective and long-lasting clinical volumizing effects.[7] PLLA materials not only physically and biologically stimulate the filling effect but also had an impact on the adipose tissue. Although in vitro studies indicate that PLLA materials directly regulate adipocytes, enhance viability after UVB irradiation, and upregulate collagen Types IV and VI,[8] in vivo studies of the effects of PLLA on adipocyte growth and other components of adipose tissue are limited.

In this study, the short-term effects of PLLA-b-PEG microspheres implanted in rat subcutaneous back adipose tissue (BAT) and visceral epididymal adipose tissue (EAT) were evaluated (Figure 1). EAT is more prone to the dysregulation of inflammation, oxidative stress,[9] and fibrosis[10] than BAT. The results of this study provide a foundation for future research on the application of PLLA-b-PEG microspheres.

METHODS

Materials

Sterile PLLA-b-PEG microspheres were prepared by Beijing Engineering Lab of Neo-Biodegradable Materials (Beijing, China). The microspheres were suspended in sodium hyaluronate solution at a concentration of 180 mg/mL. The configuration was prepared in a sterile environment.

Animals

Fifteen male Sprague–Dawley rats, 8 weeks old, were purchased from SiPeiFu (Beijing) Biotechnology Co., Ltd (Beijing, China) and housed in controlled conditions with a temperature of 23 ± 3 °C, relative humidity of 40% to 70%, and a 12 h light/12 h dark cycle. They were provided food and purified water. After a 1-week acclimatization period, the rats were randomly assigned to 5 groups based on the tissue type (BAT or EAT) and observation period (6, 8, or 12 weeks). Animal experiments were conducted in compliance with Ethics Committee guidelines (IACUA no. YXKT2023L011) from Beijing YongXin Kangtai Technology Development Co., Ltd. The study dates ranged from December 2023 to May 2024.

Experimental Process

Anesthesia was induced using isoflurane (Qingdao Orbiepharm Co., Ltd, Qingdao, Shandong, China) in a sterile surgical room. The rats were shaved, and the skin was disinfected with alcohol and iodophor. BAT injections (0.5 mL per injection site) were administered to 3 groups (6, 8, and 12 weeks) on one side of the spinal column (at the exact location on the back) near the scapula using a 27 G needle. In the remaining 2 groups (6 and 12 weeks), a 1 cm skin incision was made in the lower abdominal area to expose the unilateral testis and surrounding EAT. The injection (0.5 mL per injection site) was administered to the adipose tissue, followed by closure of the skin by suturing (Figure 2).

Tissue Morphological Observation

Adipose tissues were biopsied under pentobarbital sodium anesthesia at 6, 8, and 12 weeks postinjection. Following collection, tissues were fixed in 10% neutral-buffered formalin for paraffin embedding. Subsequently, vertical sections (4 μm thick) were prepared from the samples by longitudinal axis cutting using a microtome. The sections were stained with hematoxylin and eosin (H&E) and Masson's trichrome and visualized under a light microscope (Olympus Corporation, Tokyo, Japan). Immunofluorescence staining was performed to characterize the expression of uncoupling protein (UCP) 1 and UCP2, and the fluorescence intensity was analyzed using a fluorescence microscope (Leica, Wetzlar, Germany).

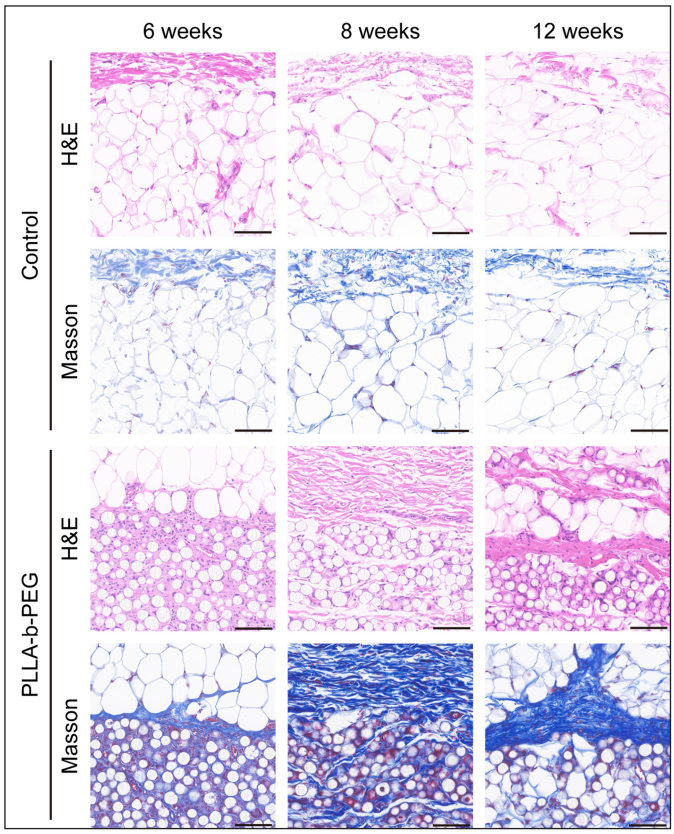

Figure 3. Histological response to back adipose tissue after injection based on hematoxylin and eosin and Masson staining. The adipose structure of the control group exhibited no pathological changes. After poly-L-lactic acid-b-polyethylene glycol microsphere injection, the fibrous envelope, a number of microvessels, and a small number of foreign body macrophages were observed in the implantation area (scale bar: 100 μm). PLLA, Poly-L-lactic acid; PEG, polyethylene glycol.

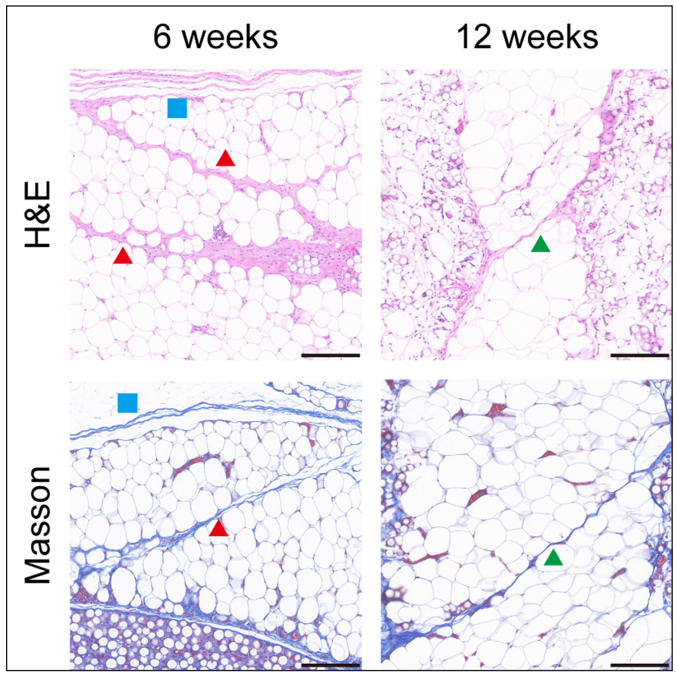

Figure 4. Histological response to adipose tissue after injection based on hematoxylin and eosin and Masson's trichrome staining. Newly formed collagen fibers (triangle in left) connected with the superficial fascia (square) at 6 weeks after poly-L-lactic acid-b-polyethylene glycol (PLLA-b-PEG) microsphere injection (left; back adipose tissue; scale bar: 200 μm). Bridging fibrous tissue (triangle in right) between the adjacent implantation sites was clearly observed at 12 weeks after PLLA-b-PEG microsphere injection (right; epididymal adipose tissue; scale bar: 200 μm).

RESULTS

Histological Evaluation of Rat Adipose Tissue

H&E staining revealed that in BAT, 6 weeks after implantation, the PLLA-b-PEG microspheres were evenly dispersed and fibroblasts and collagen fibers surrounded the implantation site. Low levels of inflammatory cell infiltration were observed within the implant. There was some visible neovascularization, and fibrous connective tissue encapsulated the microspheres. The newly formed fibrous connective tissue extended along the adipose tissue clefts and separated the adipose tissue into different regions, with some fibrous tissue growth directed toward the superficial fascial layer. By the 8th and 12th week postimplantations, the HA used to suspend the microspheres was partially metabolized, and the gap between the microspheres decreased, leading to a temporary reduction in implant volume. The amount of fibrous connective tissue and neovascularization further increased. Masson's trichrome staining results demonstrated that PLLA-b-PEG microspheres induced collagen neogenesis around the implant. Collagen gradually thickened with increasing implantation time; newly formed collagen connected to the muscular fascial layer and gradually replaced the metabolized HA to achieve a filling effect (Figures 3, 4).

Similar responses were observed in the EAT following the implantation of PLLA-b-PEG microspheres. A small quantity of inflammatory cells were detected around the implant, which was surrounded by fibrous sacs, accompanied by the regeneration of collagen fibers. Fibrous tissue bridging was observed between adjacent implants by the 12th week (Figure 4).

Uncoupling Protein (UCP) Expression in Rat Epididymal Adipose Tissue

Immunofluorescence staining revealed that in the adipose tissue not implanted with PLLA-b-PEG microspheres, there was minimal expression of UCP1, indicating that EAT primarily consists of white adipocytes. In contrast, by the 6th week postimplantation, there were significant increases in the expression levels of UCP1 and UCP2 within the adipocytes surrounding the implant. Because the implantation duration increased, the fluorescence signal intensified gradually (Figure 5).

DISCUSSION

Based on the results of this study, the authors indicate that PLLA-b-PEG microspheres result in a mild early inflammatory response after implantation into adipose tissue, with noticeable collagen production, collagen synthesis, and angiogenesis (demonstrating a time-dependent increase), consistent with previous research.[11] In particular, we observed that newly formed fibrous connective tissue extends around the

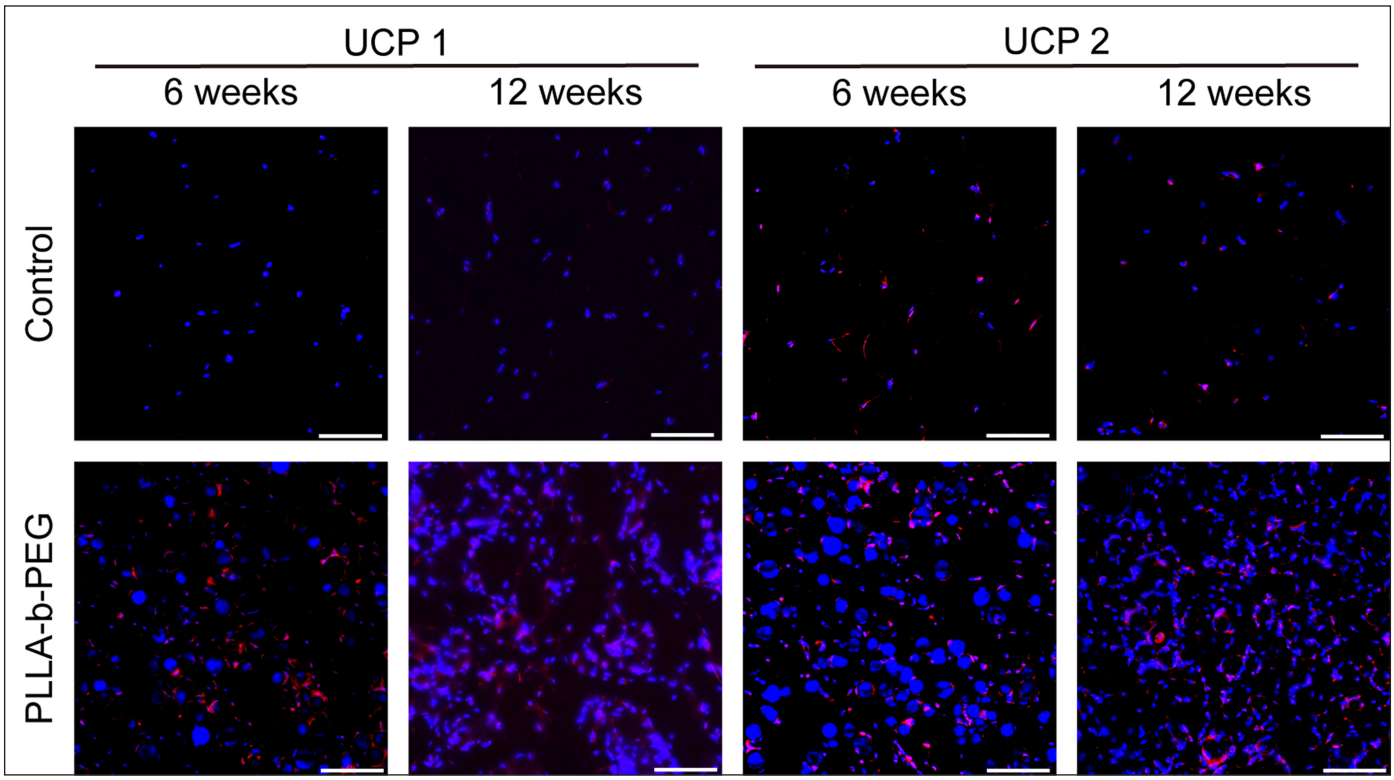

Figure 5. Immunofluorescence staining of epididymal adipose tissue at 6 and 12 weeks after PLLA-b-PEG microsphere injection. UCP1 (red), UCP2 (red), nucleus (DAPI, blue; scale bar: 100 μm). UCP, uncoupling protein. PLLA, poly-L-lactic acid; PEG, polyethylene glycol.

adipose tissue interstices, especially toward the superficial fascial layer, with fibrous tissue bridging observed between adjacent implants, as is often found in studies with threads.[12] Increases in UCP1 and UCP2 expression were evident in adipocytes within the implantation area.

One of the primary manifestations of skin aging is the loss of fat volume, which is attributed not only to intrinsic factors but also to extrinsic factors, such as UV exposure, diseases, and malnutrition. Common clinical interventions include autologous fat transplantation, HA, CaHA, and Polymenthyl Methacrylat (PMMA) fillers.[5] In the early 21st century, PLLA was approved by the FDA for the restoration and correction of facial fat loss (lipoatrophy) based on its excellent safety profile and filling effect, making it popular in clinical practice.

In this study, we implanted bimodal PLLA-b-PEG microspheres into the subcutaneous BAT and visceral EAT of rats. Although both are white adipose tissues, EAT is more prone to inflammatory reactions and fibrosis compared with BAT.[10] Histopathological examination revealed no acute inflammation or abnormal fibrosis in adipose tissue after PLLA-b-PEG microsphere implantation. Throughout the implantation period, a mild and persistent tissue response was observed, confirming its good biocompatibility and low risk of adverse reactions, such as granuloma formation postinjection.

In clinical practice, volumetric filling should not be the sole aim for facial rejuvenation. Excessive filler use and improper injection techniques can lead to overfilling syndrome, which is characterized by an unnatural facial appearance and impaired facial movements.[13] To mitigate this syndrome, a strategy involving multiple injections at low doses is often employed, utilizing facial fascial tissues for the segmentation and fixation of fillers.[14] Moreover, the biomechanical effects of facial structures, particularly dense superficial subcutaneous fat, exert directional pressure on fillers, which may lead to uncontrollable displacement with an age-related reduction in fibrous connective tissue.[15] In this study, PLLA-b-PEG microspheres induced substantial collagen synthesis and neovascularization upon implantation. We observed fibrous bridging between adjacent implantation sites, a phenomenon commonly observed in thread implantation studies.[16] These collagen fibers extend outward in a spiderweb-like pattern, reinforcing soft tissues by connecting with the existing fibrous connective tissue, thereby reducing facial skin sagging and enhancing filler stability. This phenomenon supports reduced filler displacement and frequency of use, thereby mitigating the overfilling syndrome.

Furthermore, the decrease and damage of adipocytes contribute to facial aging, possibly through oxidative stress in adipose tissue.[4] UCPs are a class of transport proteins located on the inner mitochondrial membrane that are responsible for regulating the proton gradient generated by respiration, producing heat, and reducing reactive oxygen species (ROS) production. UCP1 is specifically expressed in brown adipocytes, whereas UCP2 is widely expressed in various tissues or cells.[17] In our study, we not only observed the positive effect of PLLA-b-PEG microspheres on collagen production within adipose tissue but also noted their potential regulatory effects on UCP1 and UCP2 in the EAT, which is susceptible to oxidative stress,[9,18] suggesting a potential role in mitochondrial uncoupling. The increases of UCP1 and UCP2 protein expression in surrounding adipocytes indicating a potential role in mitigating oxidative damage to fat cells.[19] We hypothesize that this result

may be related to the lactate produced during microsphere degradation. Lactate enters the tricarboxylic acid cycle and generates ATP and ROS. Because ROS increases, the expression levels of UCP1 and UCP2 in mitochondria also increase.[20] Additionally, lactate accumulation limits local tissue respiration and reduces fatty acid consumption, an important factor for UCP1 activation,[21] leading to increased UCP1 expression. The increased expression of UCP1 and UCP2 is related to the growth of brown adipocytes.[22] In some studies, it has been demonstrated that lactic acid can enhance fatty acid consumption and decrease the volume of adipose tissue by elevating UCP1 expression.[23] In other studies, the intravenous administration of relatively high concentrations of lactate was employed to activate the activity of the G protein-coupled receptors (GPR81) and thereby inhibit fatty acid metabolism.[24] This observation appears to be inconsistent and may be attributed to the multitude of pathways influencing lipid metabolism, with no single pathway exerting a definitive impact on this process. Although our study did not assess changes in adipose tissue volume, existing research indicates that lactic acid plays a role in mediating the browning of white adipocytes,[25] implying that PLLA-b-PEG microspheres administered into adipose tissue could potentially influence adipocyte behavior.

In summary, this study explored the short-term effects of PLLA-b-PEG microspheres on various rat adipose tissues. Implantation in BAT and EAT resulted in mild inflammatory reactions, indicating the safety and biocompatibility of the microspheres. The filling effect of microspheres and collagen generation resulted in long-lasting volumization effects. Additionally, the implantation of microspheres induced spiderweb-like fibrous connective tissue around the implantation site, extending into the fascial layers, thereby stabilizing the adipose tissue. Furthermore, PLLA-b-PEG microspheres upregulated UCPs in surrounding adipocytes, suggesting that they reduce oxidative damage and enhance adipocyte quality.

Our study had some limitations. First, we only explored the short-term effects of PLLA-b-PEG microsphere implantation in adipose tissue; long-term studies are lacking. Second, as an initial exploratory experiment, we only analyzed the impact of PLLA-b-PEG microspheres on UCP protein expression in the adipose tissue; further experiments are needed to validate hypotheses regarding the molecular mechanisms underlying the observed effects. Additionally, this study focused solely on rat adipose tissue, and future research should consider animal models (such as miniature pigs) that closely resemble human skin structures for further validation.

CONCLUSIONS

Our findings demonstrated that the implantation of PLLA-b-PEG microspheres in the subcutaneous BAT and visceral EAT of rats induces mild tissue inflammation with the concurrent regeneration of fibrous tissue and collagen. Furthermore, PLLA-b-PEG microspheres upregulated UCP1 and UCP2 in the surrounding adipocytes, potentially mitigating oxidative damage and could potentially influence adipocytes. These results provide theoretical support for further research on PLLA-b-PEG microspheres.

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Drs Yang and Niu contributed equally to this paper. The data underlying this study will be shared by the corresponding author upon request.

Disclosures

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