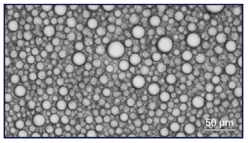
Ethiodized Oil Emulsion for Sustained Release of Anti–PD-L1 Antibodies



Hiroki Satomura, MD, Yasushi Kimura, MD, PhD, Norifumi Kennoki, MD, PhD, Kosuke Tomotake, MD, Hiroki Yano, MD, Yuji Koretsune, MD, Daisuke Katayama, MD, PhD, Kaishu Tanaka, MD, PhD, Yusuke Ono, MD, PhD, Hiroki Higashihara, MD, PhD, and Noriyuki Tomiyama, MD, PhD

ABSTRACT

Purpose: To develop and assess an ethiodized oil (Lipiodol) Pickering emulsion containing anti–programmed cell death ligand 1 (PD-L1) antibodies through in vitro experiments.

Materials and Methods: The emulsion was created by combining ethiodized oil with poly (lactic-co-glycolic acid) (PLGA) nanoparticles and anti–PD-L1 antibodies. Confocal laser microscopy was used to evaluate the encapsulation of the antibodies within the Pickering emulsion. To assess the stability, the emulsion was visually examined, and droplet sizes were measured under a light microscope. For the sustained release evaluation, the emulsion was introduced into saline and incubated in a shaking bath, after which the supernatant was collected over time. The concentration of anti–PD-L1 antibodies in the supernatant was determined using a bicinchoninic acid assay. Western blotting and flow cytometry were employed to confirm the functionality of the released antibodies. A conventional ethiodized oil emulsion was used as a control for comparison.

Results: The anti–PD-L1 antibodies were encapsulated within the layer of PLGA nanoparticles, positioned at the interface between the water and oil phases, as confirmed by confocal laser microscopy. The ethiodized oil Pickering emulsion demonstrated long-term stability with significantly smaller droplet sizes ($P < .001$). Moreover, the emulsion facilitated a gradual and sustained release of the anti–PD-L1 antibodies over an 8-week period ($P < .001$). The antibodies released from the emulsion specifically targeted PD-L1.

Conclusions: This study demonstrated that ethiodized oil Pickering emulsions effectively encapsulate anti–PD-L1 antibodies and enable their sustained release, highlighting their potential as a therapeutic agent for primary and secondary liver cancers.

ABBREVIATIONS

FDA = U.S. Food and Drug Administration, HCC = hepatocellular carcinoma, IgG = immunoglobulin G, IRB = institutional review board, PLGA = poly (lactic-co-glycolic acid)

The development of immune checkpoint inhibitors has revolutionized cancer treatment. Anti–PD-L1 antibodies are widely used as a first-line therapy in the treatment of various cancers. In particular, the combination of an anti–PD-L1 antibodies and an anti–vascular endothelial growth factor antibodies has become the first-line therapy for advanced hepatocellular carcinoma (HCC) (1–3). Despite achieving potent antitumor effects, this treatment only benefits a small number of patients (1,4). Recently, an observational meta-analysis (5) suggested that soluble PD-L1 in serum may impede the distribution of anti–PD-L1 antibodies in tumors, potentially leading to treatment resistance, although this hypothesis remains primarily theoretical. In addition, immune-related adverse events remain a considerable challenge (6). Therefore, strategies to reduce the injected dose of anti–PD-L1 antibodies while sustaining prolonged local delivery and avoiding contact with soluble PD-L1 are needed.

Ethiodized oil (Lipiodol)–based emulsions are currently used for the local delivery of chemotherapy, such as transarterial chemoembolization, to unresectable HCC (7). The widely recognized and used procedure is favored

Figures E1–E3 can be found by accessing the online version of this article on *www.jvir.org* and selecting the Supplemental Material tab.

J Vasc Interv Radiol 2025; 36:861–868
https://doi.org/10.1016/j.jvir.2025.01.040

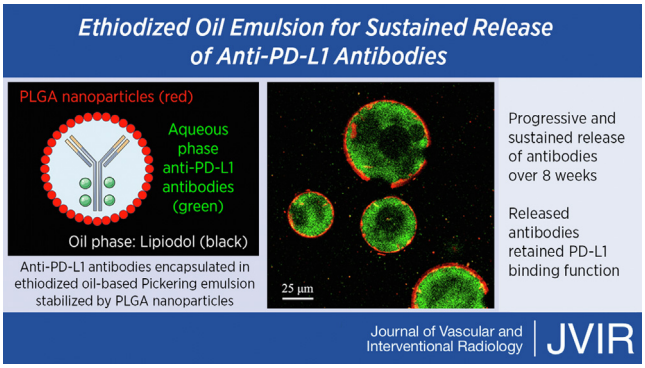
RESEARCH HIGHLIGHTS

- Anti–PD-L1 antibodies were successfully encapsulated within an ethiodized oil–based Pickering emulsion stabilized by poly (lactic-co-glycolic acid) nanoparticles, achieving high stability and small droplet sizes.
- The ethiodized oil–based Pickering emulsion exhibited a progressive and sustained release of anti–PD-L1 antibodies over 8 weeks, significantly surpassing the performance of conventional emulsions.
- The released anti–PD-L1 antibodies retained their ability to bind to PD-L1, highlighting the potential effectiveness of this emulsion as a targeted drug delivery system for the treatment of primary and secondary liver cancers.

STUDY DETAILS

Study type: Laboratory investigation

VISUAL ABSTRACT



owing to its selectivity for tumor tissue over prolonged periods. The benefits of using ethiodized oil emulsions for HCC treatment include enhanced embolic effects of water-in-oil emulsions (8) and the slow release of anticancer drugs from the emulsion (9). Additionally, its radiopacity allows easy monitoring under X-ray fluoroscopy.

Despite these advantages, the inherently low stability of these emulsions remains a challenge. This instability manifests as rapid phase separation, potentially leading to unintended systemic exposure to the chemotherapeutic agent (10). Various strategies, including adjusting the ratio of the water phase to the oil phase (11,12), incorporating different emulsifiers (13–15), and using glass membrane–pumping emulsification devices (16,17), have been developed to enhance emulsion properties. However, a universally acceptable solution for the enhancement of emulsion properties remains elusive.

Pickering emulsions use solid particles to stabilize the interface between the 2 immiscible liquids, namely the aqueous and oil phases (18). The use of poly (lactic-co-glycolic acid) (PLGA) improves emulsion stability and sustained drug release. These results are based on ethiodized oil Pickering emulsions containing oxaliplatin (19,20), doxorubicin (21), and anti–CTLA-4 antibodies (22). However, evaluation of ethiodized oil Pickering emulsions that incorporate anti–PD-L1 antibodies remain limited. Therefore, this study aimed to develop and characterize a ethiodized oil Pickering emulsion incorporating anti–PD-L1 antibodies for the treatment of primary and secondary liver cancers.

MATERIALS AND METHODS

Formulation of Ethiodized Oil Pickering Emulsions

Ethiodized oil (Lipiodol; Guerbet Japan, Tokyo, Japan) was used for the oil phase, and anti–PD-L1 antibodies (Genentech, South San Francisco, California) were used for the water phase. To formulate a water-in-oil emulsion, the oil and water phases were mixed in a 2:1 ratio using 2 2.5-mL syringes connected by a 3-way stopcock (**Fig E1**, available online on the article's Supplemental Material page at *www.jvir.org*). The mixture was pumped back and forth 20 times to ensure proper emulsification, as previously described (11). For the experimental group (ethiodized oil Pickering emulsion), the water phase was prepared by mixing 180 μL of anti–PD-L1 antibody stock solution (16.7 mg/mL), 670 μL of purified water, and 150 μL of PLGA nanoparticles (10% weight/volume; Lilac Pharma, Sapporo, Japan). This water phase was then combined with 2 mL of ethiodized oil and emulsified through the pumping process. For the control group (conventional ethiodized oil emulsion), the water phase was prepared by mixing 180 μL of anti–PD-L1 antibody stock solution and 820 μL of purified water. This water phase was then combined with 2 mL of ethiodized oil and emulsified in the same manner. Both emulsions maintained a consistent anti–PD-L1 antibody concentration of 1 mg/mL, ensuring comparability between groups. The Pickering emulsion incorporated a PLGA concentration of 0.5% weight/volume.

Confirmation of Anti–PD-L1 Antibodies Encapsulation

To confirm the encapsulation of anti–PD-L1 antibodies within the emulsion, the PLGA nanoparticles were stained with Rhodamine B (Nacalai Tesque, Kyoto, Japan), and the anti–PD-L1 antibodies were labeled with Alexa Fluor 488. Using these components, an ethiodized oil Pickering emulsion was prepared and observed using confocal laser microscopy (LSM710; Carl Zeiss, Tokyo, Japan). The excitation and emission wavelengths were 488 nm and 499–547 nm for Alexa Fluor 488 and 561 nm and 566–703 nm

for Rhodamine B, respectively. The laser intensity was set to 2.0% for both 488-nm and 561-nm lasers. Imaging was performed using a Plan-Apochromat 10×/0.45 objective lens (Carl Zeiss, Oberkochen, Germany). The pinhole sizes were set to 91 μm for Alexa Fluor 488 and 98 μm for Rhodamine B. The imaging parameters ensured optimal resolution and clear visualization of the emulsion components.

Emulsion Stability

To evaluate the stability of the emulsions, macroscopic observations were performed at room temperature at the following time points: immediately after preparation and 1 hour, 3 hours, 6 hours, 24 hours, 3 days, 5 days, 1 week, 2 weeks, and 4 weeks after preparation. For macroscopic observations, the emulsions were visually inspected at each time point to assess the presence and degree of phase separation between the aqueous and oil phases. Additionally, droplet size measurements were conducted at the time of preparation, 1 hour later, and 24 hours later using a light microscope (BZ-X800L; Keyence, Osaka, Japan). For droplet size measurements, the diameters of 250 emulsion droplets in each group were measured, and the mean diameter was calculated.

Sustained Release of Anti–PD-L1 Antibodies

To evaluate the sustained release from the emulsion, 0.5 mL of the emulsion was dropped into 10 mL of normal saline and incubated in a shaking water bath at 37 °C and 150 rpm as previously reported (22). These conditions were chosen to mimic physiological conditions as closely as possible, including temperature (37 °C) and an isotonic saline environment. At 1 hour, 5 hours, 24 hours, 1 week, 2 weeks, 4 weeks, 6 weeks, and 8 weeks after emulsion preparation, 1 mL of the supernatant was collected. The concentration of anti–PD-L1 antibodies in the supernatant was then measured using the bicinchoninic acid assay, which specifically quantifies the amount of protein released from the emulsion but does not directly assess the functionality of the antibodies. After collecting the supernatant at each time point, 1 mL of normal saline was added to maintain a constant total volume of the liquid (**Fig E2**, available online at *www.jvir.org*). The amount of anti–PD-L1 antibodies released at each time point was calculated based on these measurements.

Western Blotting

Western blotting was performed to confirm the functionality of the preserved antibodies. Recombinant murine PD-L1 (catalog number: 1019-B7; R&D Systems, Minneapolis, Minnesota) was resolved using SDS-PAGE under reducing conditions. In brief, the PD-L1 protein was mixed with Laemmli reducing sample buffer (Bio-Rad, Hercules, California) and incubated at 95 °C for 5 minutes. Sample solutions (10, 20, 50, 100, 200, and 500 ng/lane) were separated by electrophoresis and transferred onto polyvinylidene fluoride membranes. After transfer, the membrane was blocked with a blocking buffer and incubated with primary antibodies. Both native anti–PD-L1 and anti–PD-L1 antibodies isolated from the 2-week supernatant were used as primary antibodies. The reduced PD-L1 protein was probed with primary antibodies (1:100 for antibodies from the supernatant and the same concentration for the native antibodies) in Tris-buffered saline containing Tween 20 for 1 hour. Subsequently, the membrane was incubated with horseradish peroxidase–conjugated anti-mouse immunoglobulin G (IgG) secondary antibodies (diluted 1:2,000 in Tris-buffered saline containing Tween 20) for 1 hour. The horseradish peroxidase signal was detected using an enhanced chemiluminescence reagent. The blots were imaged using a western blot imaging system (Chemidoc Touch MP; Bio-Rad). Western blot bands were quantified using ImageJ software (National Institutes of Health, Bethesda, Maryland). To evaluate the effectiveness of the primary antibody in western blotting, the R^2 value of the calibration curve was calculated using known concentrations of PD-L1 protein (100 ng, 200 ng, and 500 ng) with Prism 9 (GraphPad Software, Boston, Massachusetts).

Flow Cytometry

Flow cytometry was used to perform an in vitro competitive binding assay. The murine colorectal carcinoma cell line (CT26) was used in this study. CT26 cells were kindly provided by Dr. Keisuke Tamari, Department of Radiation Oncology, Osaka University Graduate School of Medicine. The cells were cultured in Dulbecco's Modified Eagle Medium (Fujifilm-Wako Pure Chemicals, Osaka, Japan), supplemented with 10% fetal bovine serum (Nichirei Bioscience, Tokyo, Japan) and antibiotics (Antibiotic-Antimycotic; Cytiva, Tokyo, Japan), at 37 °C and 5% CO_2. Before the experiment, CT26 cells were stimulated with interferon gamma overnight, which induced PD-L1 expression on the cell membrane (23). After stimulation, the cells were incubated either with native anti–PD-L1 antibodies, released anti–PD-L1 antibodies collected at the 2-week time point, or with isotype IgG (Mouse IgG2a Isotype Control; Proteintech Japan, Tokyo, Japan) for 1 hour at 37 °C. To detect unblocked PD-L1 on the cell surface, native anti–PD-L1 antibodies were tagged with fluorescein (Fluorescein Antibody Labeling Kit; Dojindo Laboratories, Tokyo, Japan). Subsequently, the cells were harvested and incubated with fluorescein-conjugated anti–PD-L1 antibodies. After incubation for 1 hour at 4 °C, fluorescence of fluorescein was measured using flow cytometry (FACS Canto II; BD Biosciences, Franklin Lakes, New Jersey). The mean fluorescence intensity was measured using FlowJo version 10 (FlowJo LLC, Becton Dickinson, Ashland, Oregon).

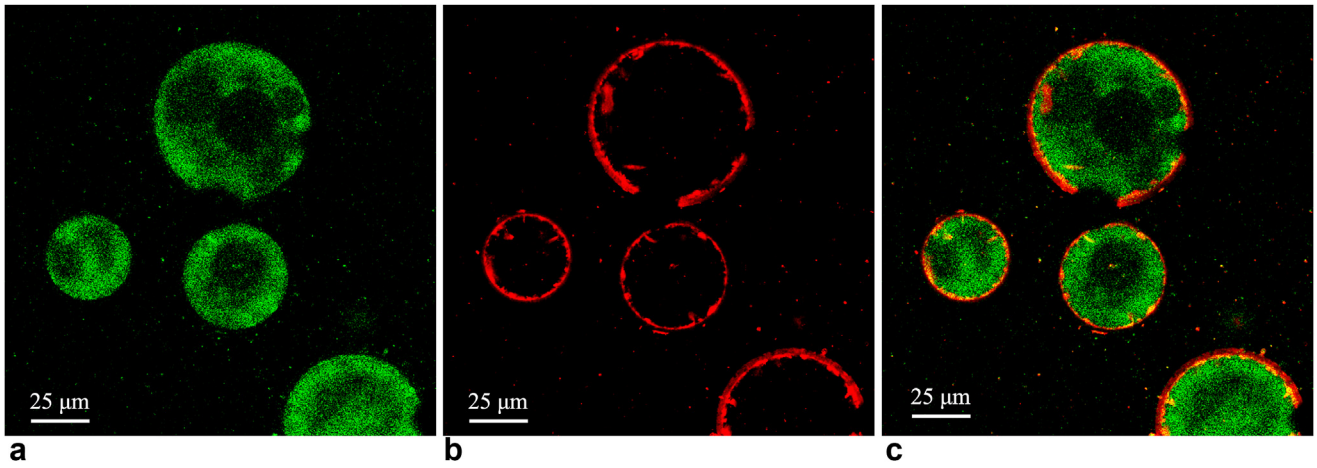


Figure 1. Confocal laser microscopy images. **(a)** Anti–PD-L1 antibodies labeled with Alexa Fluor 488 (green). **(b)** Poly (lactic-co-glycolic acid) (PLGA) nanoparticles stained with Rhodamine B (red). **(c)** Fusion image. The anti–PD-L1 antibodies were present in the water phase, whereas ethiodized oil, which constitutes the oil phase, is depicted in black. The PLGA nanoparticles were located at the interface between the oil and aqueous phases, with anti–PD-L1 antibodies encapsulated within the PLGA nanoparticles.

Statistical Analysis

Continuous variables were expressed as means, and comparisons were made using Student *t*-tests. All tests were 2-tailed, and statistical significance was set at $P < .05$. Statistical analyses were conducted using R software version 4.2.0 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Encapsulation of Anti–PD-L1 Antibodies

Confocal laser microscopy showed that the anti–PD-L1 antibodies were encapsulated in the emulsion (Fig 1a–c). The PLGA nanoparticles were located at the interface between the water and oil phases, forming a Pickering emulsion.

Emulsion Stability

No significant differences were observed macroscopically between the 2 emulsions during the 4-week observation period (Fig 2). Microscopic images captured on the first day after preparation revealed that the droplet size in the Pickering emulsion was more uniform, whereas the conventional emulsion contained many larger droplets (Fig 3a–d). The mean droplet size in the Pickering emulsion (31.2 μm) was significantly smaller than that in the conventional emulsion (54.1 μm; $P < .001$).

Sustained Release of Anti–PD-L1 Antibodies

The release profile of anti–PD-L1 antibodies over time, as measured by the bicinchoninic acid assay, revealed that the Pickering emulsion consistently demonstrated a significantly greater cumulative drug release than the conventional emulsion

Figure 2. Macroscopic appearance of emulsions. The left side contains ethiodized oil Pickering emulsions, and the right side contains conventional ethiodized oil emulsions. Each 1 mL syringe contains 0.8 mL of emulsion. During the 4-week observation period, no differences were detected between the 2 emulsions.

(Fig 4). The amounts of anti–PD-L1 antibodies released from Pickering emulsions were as follows: 209 μg at 1 hour, 314 μg at 1 day, 471 μg at 1 week, 717 μg at 2 weeks, 1,604 μg at 4 weeks, 2,186 μg at 6 weeks, and 2,610 μg at 8 weeks. After 2 weeks, drug release in the Pickering emulsion was approximately 4.8 times higher than that in the conventional emulsion ($P < .001$), and after 4 weeks, drug release in the Pickering emulsion was approximately 3.8 times higher than that in the conventional emulsion ($P < .001$).

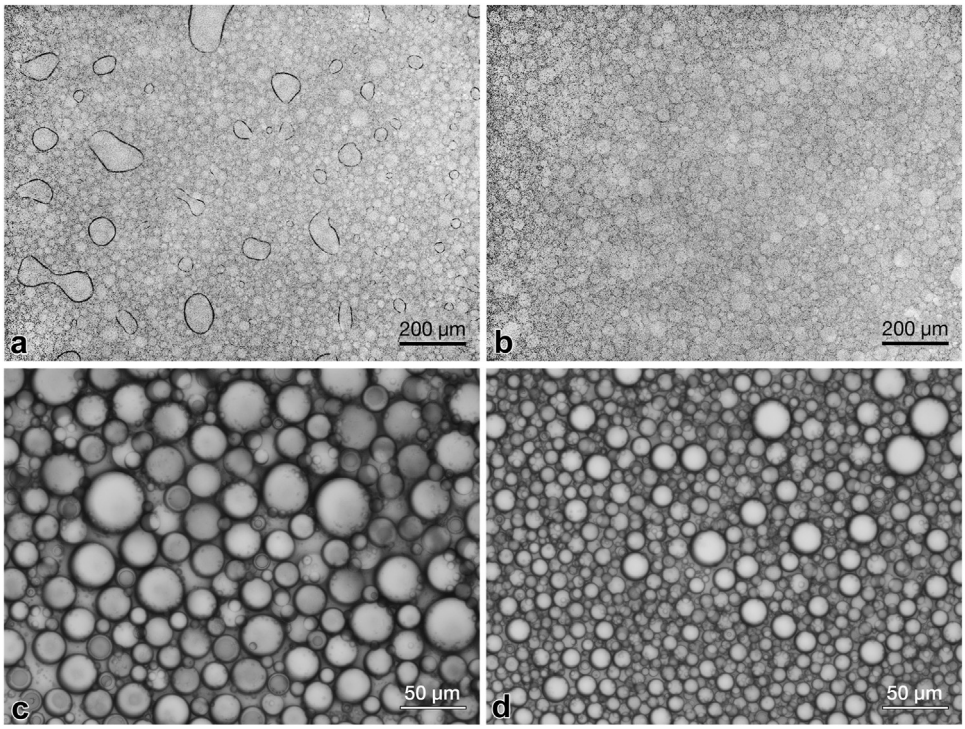


Figure 3. Light microscope images. **(a)** Conventional ethiodized oil emulsions and **(b)** ethiodized oil Pickering emulsions at 24 hours after preparation (×10). Numerous coalesced droplets were observed in **Figure 3a**, whereas the size of the droplets was relatively uniform in Figure3b. Higher magnification images of **(c)** conventional ethiodized oil emulsions and **(d)** ethiodized oil Pickering emulsions at 24 hours after preparation (×40). The droplets tended to be larger in conventional ethiodized oil emulsions, whereas smaller droplets were observed in ethiodized oil Pickering emulsions.

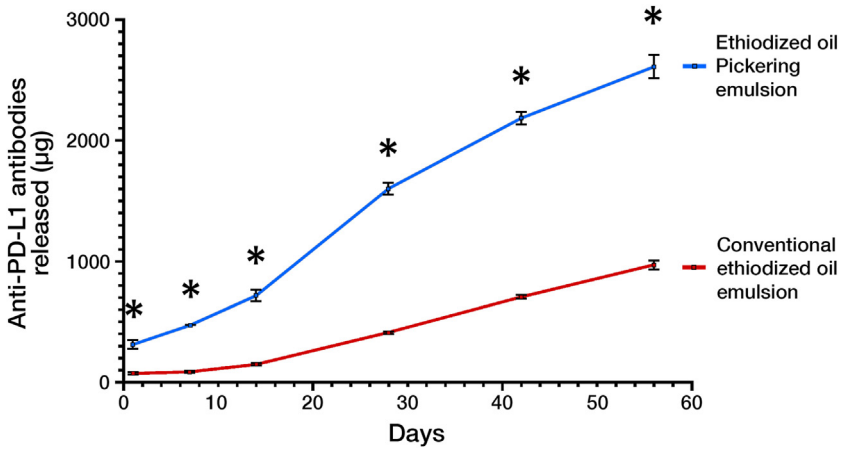


Figure 4. Sustained release of anti–PD-L1 antibodies. This shows the changes in antibodies concentration in the supernatant measured using the bicinchoninic acid assay. At all the time points, the Pickering emulsion exhibited a significantly higher release of antibodies. *$P < .001$.

Western Blotting

The bands of the released anti–PD-L1 antibodies corresponded with the molecular weight of reduced PD-L1 protein (79 kDa), similar to those of the native antibodies (**Fig 5a, b**). Furthermore, the calibration curves were similar for native and anti–PD-L1 antibodies. R^2 value of the calibration standard curve was 0.91 and 0.95, respectively.

Flow Cytometry

The competitive binding assay confirmed that both native anti–PD-L1 antibodies and those released from the ethiodized oil Pickering emulsion bound to PD-L1 expressed on the CT26 cell membrane (**Fig 6a, b**). The fluorescein-labeled anti–PD-L1 antibodies successfully bound to PD-L1 expressed on CT26 cells when compared with the

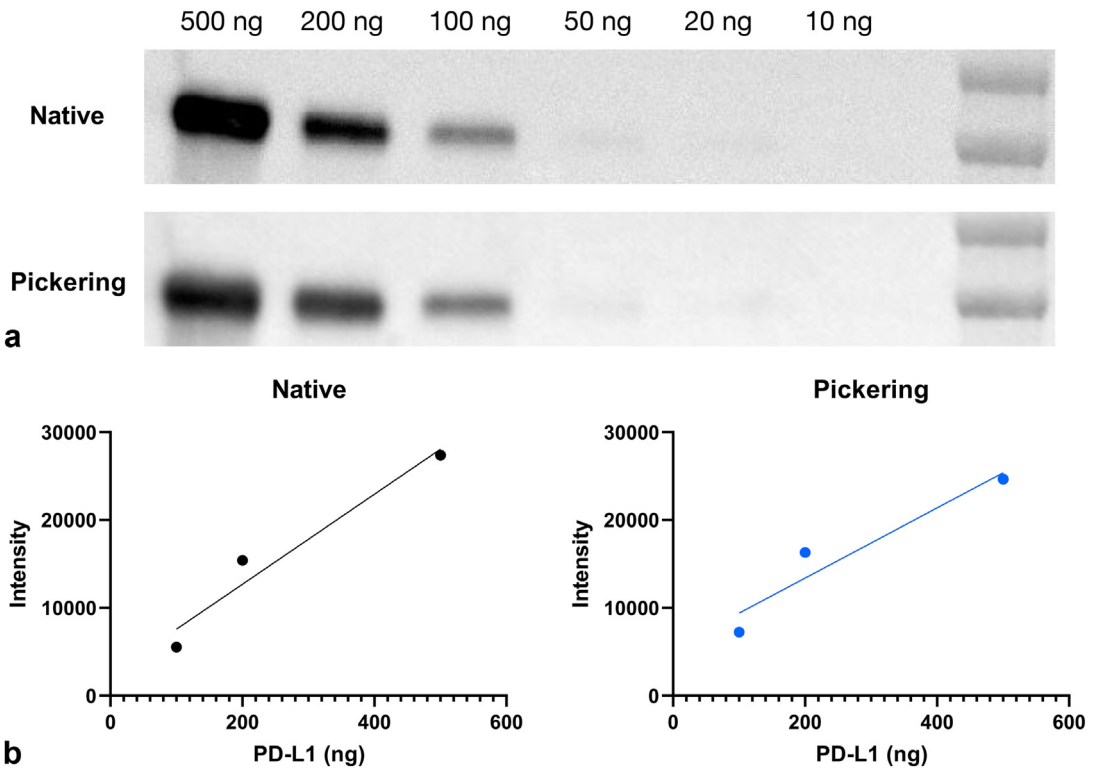


Figure 5. Western blot analysis comparing band detection and densitometry of protein samples using a native anti–PD-L1 antibodies and anti–PD-L1 antibodies from ethiodized oil Pickering emulsion. **(a)** Representative western blot showing PD-L1 protein bands detected using native antibodies and antibodies from ethiodized oil Pickering emulsions. **(b)** Standard curve generated from known concentrations of the target protein by densitometric analysis. R^2 value of the calibration curve of antibodies released from the ethiodized oil Pickering emulsion and native antibodies was 0.91 and 0.95, respectively.

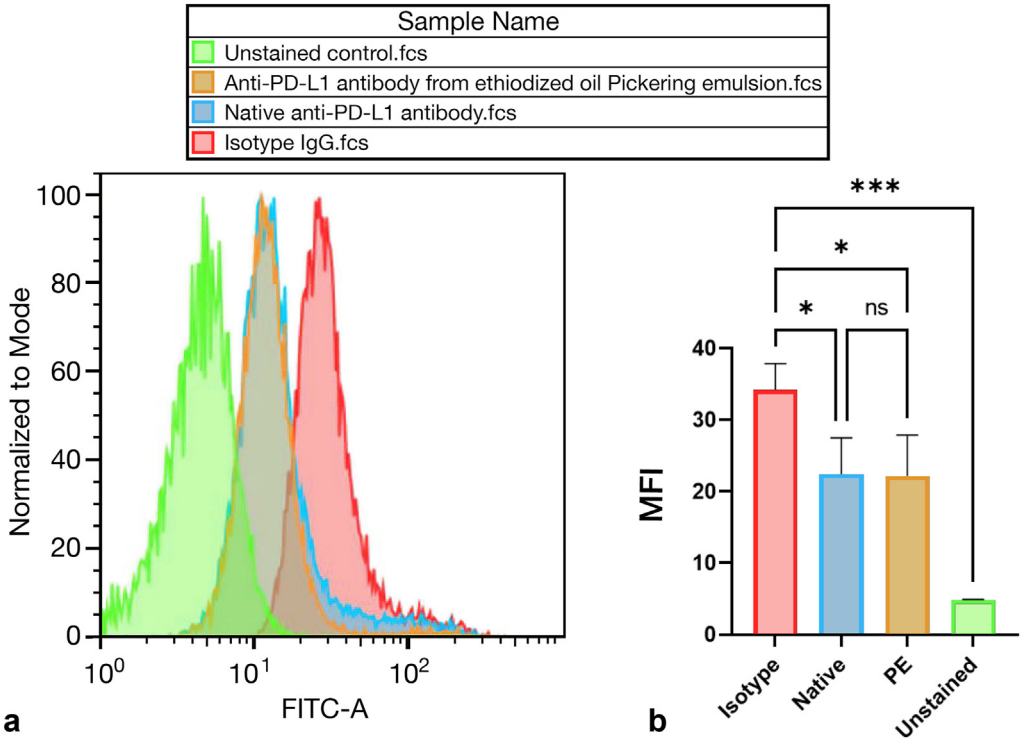


Figure 6. Flow cytometry results for the in vitro competitive binding assay. **(a)** The histogram showed that the antibodies released from the ethiodized oil Pickering emulsion (PE) bind to PD-L1 on the CT26 cell membrane, similar to native antibodies. **(b)** Similarly, there was no significant difference between the mean fluorescence intensity (MFI) of the antibodies released from the ethiodized oil PE and that of the native antibodies. IgG = immunoglobulin G; ns = not significant. *$P < .05$; ***$P < .001$.

isotype control and unstained samples. A decrease in fluorescence was observed for both native anti–PD-L1 antibodies and those released from the Pickering emulsion. There was no significant difference in mean fluorescence intensity between the native anti–PD-L1 antibodies and those released from the Pickering emulsion.

DISCUSSION

This study successfully developed and characterized an ethiodized oil Pickering emulsion incorporating anti–PD-L1 antibodies, demonstrating its potential as a drug delivery system for the treatment of primary and secondary liver cancers. Encapsulation of the anti–PD-L1 antibodies within PLGA nanoparticles resulted in a smaller water-in-oil emulsion. Over an 8-week period, the emulsion provided sustained release of the antibodies while preserving their function and PD-L1 binding capacity, as confirmed by western blotting and flow cytometry.

The ethiodized oil Pickering emulsion was formulated by mixing ethiodized oil and anti–PD-L1 antibodies with PLGA nanoparticles. Ethiodized oil has been used as a drug delivery system since the 1980s, and its safety and effectiveness as an HCC treatment have been well established. PLGA nanoparticles, composed of copolymers of lactic acid and glycolic acid, are highly biodegradable and biocompatible (**Fig E3**, available online at *www.jvir.org*) (24). PLGA has been approved for medical use by the U.S. Food and Drug Administration (FDA) since the 1970s (25). Therefore, an ethiodized oil Pickering emulsion with PLGA nanoparticles can be expected to be safe for the treatment of primary and secondary liver cancers.

In addition, the droplet size of the Pickering emulsion was smaller and more uniform than that of the conventional emulsion, which displayed many coalesced droplets, resulting in size irregularities. In Pickering emulsions, solid particles adsorb at the oil–water interface, forming a dense particle layer that prevents droplet coalescence. Consequently, droplet growth and coalescence are suppressed, resulting in more uniform and stabilized droplets (26,27). The observations conducted in this study were made within 24 hours; therefore, stability over longer time frames should be evaluated in future experiments. Considering transarterial administration, the smaller droplet size enables closer contact with and penetration into tumors. On the contrary, it is essential to note that droplets that are excessively small might pass through the tumor vasculature without being retained, potentially migrating to the lungs and causing undesired side effects. Given that the blood vessels near tumors typically range between 40 and 60 μm in diameter, the optimal emulsion droplet size is 20–40 μm (21,28). In this study, the mean size of the Pickering emulsion droplets was 31 μm, indicating that it fits within the optimal range for efficient transarterial delivery and potential tumor targeting.

In this study, the ethiodized oil Pickering emulsion was shown to allow for the loading and release of anti–PD-L1 antibodies. Delivery of anti–PD-L1 antibodies to the tumor reactivates cytotoxic T cells by inhibiting the interaction between PD-1 and PD-L1, leading to the elimination of tumor cells (29). On the contrary, intravenous administration of anti–PD-L1 antibodies results in a significant distribution in normal tissues, necessitating higher doses to achieve therapeutic effects (30). Additionally, soluble PD-L1 in the serum has been proposed as a factor that may reduce the effective distribution of anti–PD-L1 antibodies to tumors, although this hypothesis is based on observational meta-analysis and remains theoretical (5). Consequently, the local administration and release of anti–PD-L1 antibodies is considered reasonable.

Several factors contribute to the superior sustained release of anti–PD-L1 antibodies from the ethiodized oil Pickering emulsion compared with conventional emulsions. First, the stability of Pickering emulsions against droplet coalescence helps maintain the emulsion structure over an extended period (31). Furthermore, the PLGA nanoparticles formed a physical barrier around the droplets, slowing the diffusion of antibodies and enabling more controlled, sustained release, as opposed to the faster, less regulated release seen with conventional emulsions (31,32). In addition, previous studies have shown that antibody aggregation occurs in conventional emulsions (22), which may reduce the amount of intact antibodies in the aqueous phase. Furthermore, aggregated antibodies can hinder the diffusion of antibodies from the aqueous phase, resulting in slower release rates and reduced overall release.

Additionally, it was demonstrated that the released antibodies maintained their original function, retaining the ability to bind specifically to PD-L1. Several studies have investigated localized PD-L1 administration (33,34). In these studies, multiple intratumoral injections were required due to the lack of a controlled-release mechanism. In contrast, the anti–PD-L1 antibody-releasing Pickering emulsion developed in this study demonstrated sustained drug release over several weeks, serving as an alternative approach for sustained intratumoral delivery of anti–PD-L1 antibodies. Although repeated intra-arterial infusion of anti–PD-L1 antibodies is a viable option, the ethiodized oil Pickering emulsion offers the potential advantage of improved intratumoral retention of antibodies, reduced systemic exposures, and a lower risk of acute infusion reactions, which is caused by systemic immune response (35). Future in vivo experiments are necessary to determine the effectiveness of the sustained-release ethiodized oil Pickering emulsion encapsulating anti–PD-L1 antibodies.

This study has several limitations. First, the evaluations were conducted solely in vitro; therefore, the antitumor effects in vivo remain unknown. Second, the emulsion stability was evaluated only at room temperature, although this may not fully replicate the physiological conditions within the human body. Third, both western blotting and flow cytometry were performed using supernatant samples collected at a single time point (2 weeks after emulsion preparation), and the functionality of antibodies released at later time points was not evaluated. This limitation is particularly important, as antibodies released at later time

points, such as 8 weeks, may exhibit differences in activity. Fourth, the stability of the emulsion and the elution behavior under varying pH conditions were not assessed in this study. Future research should investigate the influence of pH and other physiological factors, such as electrolyte concentrations, on emulsion behavior.

In conclusion, this study confirmed that the ethiodized oil Pickering emulsion successfully encapsulated anti–PD-L1 antibodies and enabled sustained drug release. This study highlights ethiodized oil Pickering emulsions as a promising therapeutic agent for the treatment of primary and secondary liver cancers.

ACKNOWLEDGMENTS

This work was supported by JSPS KAKENHI, Grant-in-Aid for Early-Career Scientists (Grant Number 23K14867) and JST SPRING (Grant Number JPMJSP2138). The confocal laser microscopy observations were conducted with support from Dr. Oiki and Center for Medical Research and Education, Graduate School of Medicine, Osaka University. The measurement of antibody concentration using the bicinchoninic acid assay and the western blotting procedures were carried out with the assistance of Dr. Keisuke Tamari and Dr. Shohei Katsuki from Department of Radiation Oncology, Osaka University Graduate School of Medicine.

AUTHOR INFORMATION

From the Department of Diagnostic and Interventional Radiology (H.S., K.To., H.Y, Y.Ko., D.K., K.Ta., Y.O., N.T.) and Department of High Precision Image-guided Percutaneous Intervention (Y.Ki., N.K., H.H.), Osaka University Graduate School of Medicine, Suita, Osaka, Japan. Received October 24, 2024; final revision received January 10, 2025; accepted January 15, 2025. Address correspondence to Y.Ki., Department of High Precision Image-guided Percutaneous Intervention, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan; E-mail: y-kimura@radiol.med.osaka-u.ac.jp; Twitter handle: @j_irist

N.T. reports payments for lectures (December 14, 2021, to October 20, 2023) from Guerbet Japan. None of the other authors have identified a conflict of interest.

From the 2024 SIR Annual Scientific Meeting (Abstract No. 237, "An Innovative Lipiodol Pickering Emulsion for Sustained Release of Anti-PD-L1 Antibodies").

REFERENCES

1. Finn RS, Qin S, Ikeda M, et al. Atezolizumab plus bevacizumab in unresectable hepatocellular carcinoma. N Engl J Med 2020; 382: 1894–1905.
2. Llovet JM, Castet F, Heikenwalder M, et al. Immunotherapies for hepatocellular carcinoma. Nat Rev Clin Oncol 2022; 19:151–172.
3. Benson AB, D'Angelica MI, Abbott DE, et al. Hepatobiliary cancers, version 2.2021. J Natl Compr Canc Netw 2021; 19:541–565.
4. Kim JM, Chen DS. Immune escape to PD-L1/PD-1 blockade: seven steps to success (or failure). Ann Oncol 2016; 27:1492–1504.
5. Scirocchi F, Strigari L, Di Filippo A, et al. Soluble PD-L1 as a prognostic factor for immunotherapy treatment in solid tumors: systematic review and meta-analysis. Int J Mol Sci 2022; 23:14496.
6. Wang Y, Kong D, Wang C, et al. A systematic review and meta-analysis of immune-related adverse events of anti-PD-1 drugs in randomized controlled trials. Technol Cancer Res Treat 2020; 19:153303382096745.
7. Wen N, Cai Y, Li F, et al. The clinical management of hepatocellular carcinoma worldwide: a concise review and comparison of current guidelines: 2022 update. Biosci Trends 2022; 16:20–30.
8. Demachi H, Matsui O, Abo H, Tatsu H. Simulation model based on non-Newtonian fluid mechanics applied to the evaluation of the embolic effect of emulsions of iodized oil and anticancer drug. Cardiovasc Intervent Radiol 2000; 23:285–290.
9. Takayasu K, Arii S, Matsuo N, et al. Comparison of CT findings with resected specimens after chemoembolization with iodized oil for hepatocellular carcinoma. AJR Am J Roentgenol 2000; 175:699–704.
10. Gaba RC, Baumgarten S, Omene BO, et al. Ethiodized oil uptake does not predict doxorubicin drug delivery after chemoembolization in VX2 liver tumors. J Vasc Interv Radiol 2012; 23:265–273.
11. Hino T, Kawashima Y, Shimabayashi S. Basic study for stabilization of w/o/w emulsion and its application to transcatheter arterial embolization therapy. Adv Drug Deliv Rev 2000; 45:27–45.
12. Masada T, Tanaka T, Nishiofuku H, et al. Techniques to form a suitable Lipiodol-epirubicin emulsion by using 3-way stopcock methods in transarterial chemoembolization for liver tumor. J Vasc Interv Radiol 2017; 28:1461–1466.
13. Yi SW, Kim YH, Kwon IC, et al. Stable lipiodolized emulsions for hepatoma targeting and treatment by transcatheter arterial chemoembolization. J Control Release 1998; 50:135–143.
14. Ménard N, Tsapis N, Poirier C, et al. Novel surfactants with diglutamic acid polar head group: drug solubilization and toxicity studies. Pharm Res 2012; 29:1882–1896.
15. Deschamps F, Farouil G, Gonzalez W, et al. Stabilization improves theranostic properties of Lipiodol®-based emulsion during liver transarterial chemo-embolization in a VX2 rabbit model. Cardiovasc Intervent Radiol 2017; 40:907–913.
16. Tanaka T, Masada T, Nishiofuku H, et al. Development of pumping emulsification device with glass membrane to form ideal Lipiodol emulsion in transarterial chemoembolization. Eur Radiol 2018; 28:2203–2207.
17. Masada T, Tanaka T, Nishiofuku H, et al. Use of a glass membrane pumping emulsification device improves systemic and tumor pharmacokinetics in rabbit VX2 liver tumor in transarterial chemoembolization. J Vasc Interv Radiol 2020; 31:347–351.
18. Ramsden W. Separation of solids in the surface-layers of solutions and 'suspensions' (observations on surface-membranes, bubbles, emulsions, and mechanical coagulation)—preliminary account. Proc R Soc Lond 1903; 72:156–164.
19. Deschamps F, Harris KR, Moine L, et al. Pickering-emulsion for liver trans-arterial chemo-embolization with oxaliplatin. Cardiovasc Intervent Radiol 2018; 41:781–788.
20. Deschamps F, Tselikas L, Tasaki M, et al. Sustained-hepatic arterial infusion of oxaliplatin: pharmacokinetic advantages over hepatic arterial infusion using a preclinical animal tumour model. Drug Deliv Transl Res 2021; 11:2144–2150.
21. Deschamps F, Isoardo T, Denis S, et al. Biodegradable Pickering emulsions of Lipiodol for liver trans-arterial chemo-embolization. Acta Biomater 2019; 87:177–186.
22. Tselikas L, De Baere T, Isoardo T, et al. Pickering emulsions with ethiodized oil and nanoparticles for slow release of intratumoral anti-CTLA4 immune checkpoint antibodies. J Immunother Cancer 2020; 8:e000579.
23. Yuan W, Deng D, Li H, et al. IFNγ/PD-L1 signaling improves the responsiveness of anti-PD-1 therapy in colorectal cancer: an in vitro study. Onco Targets Ther 2021; 14:3051–3062.
24. Makadia HK, Siegel SJ. Poly lactic-co-glycolic acid (PLGA) as biodegradable controlled drug delivery carrier. Polymers 2011; 3:1377–1397.
25. Danhier F, Ansorena E, Silva JM, Coco R, Le Breton A, Préat V. PLGA-based nanoparticles: an overview of biomedical applications. J Control Release 2012; 161:505–522.
26. Binks BP. Particles as surfactants—similarities and differences. Curr Opin Colloid Interface Sci 2002; 7:21–41.
27. Chevalier Y, Bolzinger M-A. Emulsions stabilized with solid nanoparticles: Pickering emulsions. Colloids Surf A Physicochem Eng Asp 2013; 439:23–34.
28. de Baere T, Dufaux J, Roche A, et al. Circulatory alterations induced by intra-arterial injection of iodized oil and emulsions of iodized oil and doxorubicin: experimental study. Radiology 1995; 194:165–170.
29. Freeman GJ, Long AJ, Iwai Y, et al. Engagement of the Pd-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. J Exp Med 2000; 192:1027–1034.
30. Kurino T, Matsuda R, Terui A, et al. Poor outcome with anti-programmed death-ligand 1 (PD-L1) antibody due to poor pharmacokinetic properties in PD-1/PD-L1 blockade-sensitive mouse models. J Immunother Cancer 2020; 8:e000400.
31. Frelichowska J, Bolzinger M-A, Valour J-P, Mouaziz H, Pelletier J, Chevalier Y. Pickering w/o emulsions: drug release and topical delivery. Int J Pharm 2009; 368:7–15.
32. Prestidge CA, Simovic S. Nanoparticle encapsulation of emulsion droplets. Int J Pharm 2006; 324:92–100.
33. Sakuma K, Kii T, Takahashi H, et al. An in vivo study of local administration of low-dose anti-PD-1 antibody using an oral cancer cell line. Anticancer Res 2022; 42:4293–4303.
34. Machida T, Sakuma K, Fuwa N, Tanaka A. Local low-dose anti-PD-L1 antibodies improve antitumor effects in oral squamous cell carcinoma. Anticancer Res 2024; 44:993–1002.
35. Hansel TT, Kropshofer H, Singer T, Mitchell JA, George AJ. The safety and side effects of monoclonal antibodies. Nat Rev Drug Discov 2010; 9: 325–338.

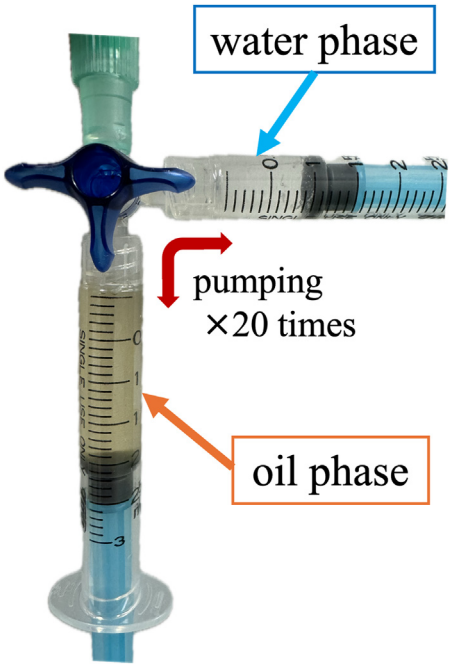


Figure E1. Emulsion formulation. The emulsion was prepared by mixing the oil and aqueous phases in a 2:1 ratio. The mixture was pumped back and forth 20 times using a 3-mL syringe and a 3-way stopcock.

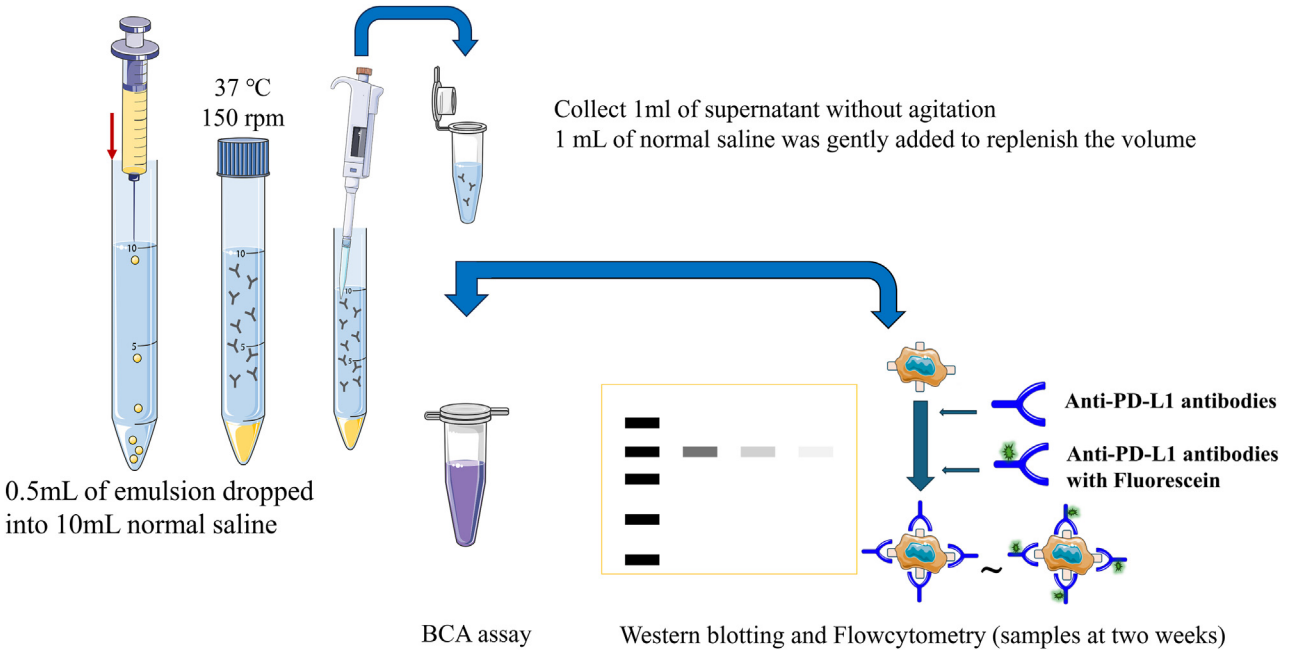


Figure E2. Drug release test: 0.5 mL of the emulsion was added dropwise to 10 mL of normal saline and incubated at 37 °C and 150 rpm. At each time point, 1 mL of the supernatant was carefully collected without agitation, and 1 mL of normal saline was gently added to replenish the volume. The concentration of antibodies was measured using a bicinchoninic acid (BCA) assay. Western blotting and flow cytometry were performed with samples collected at 2 weeks.

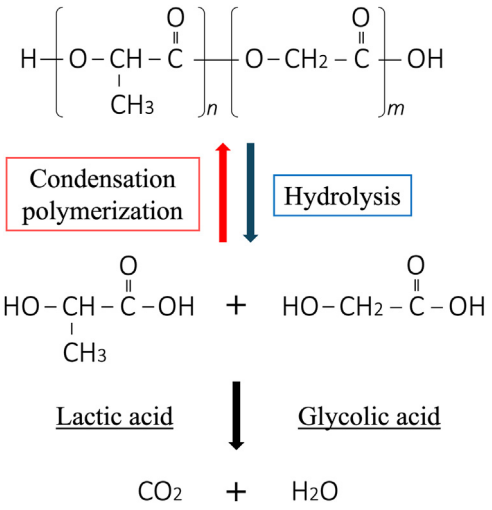


Figure E3. Chemical structure of poly (lactic-co-glycolic acid). Poly (lactic-co-glycolic acid) undergoes hydrolytic cleavage into its monomeric components, lactic acid and glycolic acid.