Neutrophil membrane-coated nanoparticles for enhanced nanosecond pulsed electric field treatment of pancreatic cancer

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ABSTRACT
Objective: Pancreatic cancer is one of the leading causes of cancer-related deaths worldwide. Poor prognosis and low survival rates have driven the development of novel therapeutic strategies. Nanosecond pulsed electric field has emerged as a novel, minimal invasive and non-thermal treatment for solid tumors. It is of great significance to study the combination therapy of nsPEF and other treatment strategies for pancreatic cancer.

Methods: We developed neutrophil membrane-wrapped liposomal nanoparticles loaded with gemcitabine (NE/Lip-GEM) and investigated their use as a complementary agent for nsPEF treatment.

Results: Our results showed that neutrophil-mediated delivery of liposomal-gemcitabine (NE/Lip-GEM) efficiently inhibited the growth of pancreatic tumors in mice whose has been treated with incomplete nsPEF ablation.

Conclusions: The combination of nsPEF and NE/Lip-GEM may be a promising synergistic strategy for pancreatic cancer therapy.

1. Introduction
Pancreatic cancer is one of the leading causes of cancer-related deaths worldwide. Most patients lost the chance of surgery because of insidious onset, rapid progression and difficult early diagnosis [1]. For patients with unresectable pancreatic cancer and postoperative recurrence, current guidelines recommend systemic chemotherapy or combined radiation therapy. However, the prognosis is still poor, with an average survival of only 6–12 months [2–3]. Therefore, new therapeutic strategies are necessary to improve patient survival.

Nanosecond pulsed electric field (NsPEF) is a novel and effective strategy for tumor ablation [4–7]. Pulses with high electric field strength (KV/cm) are released in extremely short time. Nanosecond pulses penetrate the cell membrane to induce the formation of microbubbles. Especially, nsPEF could create nanopores in intracellular organelles. Subsequently, intracellular events such as calcium release, caspase activation, and the release of cytochrome C into the cytoplasm are induced [8]. NsPEF selectively ablates tumor cells without damaging the surrounding important structures which has shown great advantages in the treatment of pancreatic cancer [9–11]. However, nsPEF has its limitations: the effects are correlated to the electric field in the tissue (related variables include voltage intensity, distance between electrodes, and depth of electrode insertion). If the above requirements are not met, insufficient ablative energy leads to residual tumors. It has been proven that nsPEF combined with chemotherapy can enhance the anti-tumor effect [12–14]. Thus, it is of great significance to study the combination therapy of nsPEF and other treatment strategies for pancreatic cancer.

Previous studies have proposed cell membrane as a potential drug delivery system [15–17]. Cell membrane coating enables the camouflage of nanoparticles for evading reticuloendothelial system elimination and immunological surveillance [18–19]. Coating nanoparticles with cell membrane extends their half-life in systemic circulation and improves their tumor targeting ability [15,20–22]. Neutrophils are the most abundant immune cells in the circulatory system, and they play a critical role in the immune system [23].
They can be effectively recruited to inflammation sites and can be recognized as applicable drug delivery platforms for tumor targeting [24–26].

Our study revealed that nsPEF triggered immunological responses with the release of the inflammatory factors, such as tumor necrosis factor-α (TNF-α). After nsPEF treatment, pathological examinations revealed necrosis and large amounts of neutrophils in the ablated tumors [27]. We hypothesized that this amplification of inflammatory signals supports an enhanced tumor targeting. In this study, we developed neutrophil membrane-wrapped liposomal nanoparticles loaded with gemcitabine (NE/Lip-GEM) and investigated their use as a complementary agent for nsPEF treatment (Figure 1). It was expected that the NE/Lip-GEM accumulate in the ablated tumors and can significantly enhance the efficacy of nsPEF therapy. We believe that this scheme will offer new opportunities for pancreatic cancer therapy.

2. Materials and methods

2.1. NsPEF treatment

All animal experiments were approved by the Ethics Committee for Zhejiang Center of laboratory Animals. The application electrodes, pulse generator, voltage, and pulsing pattern of the nsPEF were used as described previously [28]. To establish the incomplete ablation model, the C57BL/6 mice with Panc02 tumors were treated with nsPEF of 24 kV/cm amplitude, 300 ns duration for 100 pulses in a single treatment (Figure 2(A)).

2.2. Assessment of cytokines concentration using enzyme linked immune sorbent assay (ELISA)

The levels of TNF-α, IL-1β and CXCL1 in the ablated tumor samples were analyzed after treatment using TNF-α ELISA kit (70-EK282HS-96; MULTI SCIENCES, China), IL-1β ELISA kit (EK201B/3-96; MULTI SCIENCES, China), and CXCL1 ELISA kit (70-EK296/2-96; MULTI SCIENCES, China) according to the manufacturer’s instructions [29].

2.3. Single-cell RNA-sequencing (scRNA-seq) and sequencing data analysis

NsPEF ablated tumor and non-treated tumor were minced into small pieces and digested in gentle MACS C Tube using tissue dissociation kit (130-096-730, Miltenyi Biotec, Germany) according to the protocol. Library preparation was conducted fusing the 10X Chromium single-cell kit processed by OE Biotech. Co., Ltd. (Shanghai, China). The libraries were sequenced on a HiSeq 2500 platform with paired-end sequencing (Illumina, USA). The Cell Ranger software pipeline (version 3.0.0) was applied to quantify and filter the 10X genomics raw data. Next, t-distributed stochastic neighbor embedding (t-SNE), clustering, and comparison analyses were performed using the Seurat and Monocle packages. KEGG analysis of cell type-specific marker genes were performed with online tools (cloud.oebiotech.cn).

2.4. Isolation of neutrophils and neutrophil membrane

Neutrophils were isolated from murine blood of 20–30g ICR mice following protocols using Neutrophil Isolation Kit (P9201, Solarbio, China). Specifically, before blood collection, lipopolysaccharide (LPS) from Escherichia coli (L2880, Sigma-Aldrich, USA) was injected intraperitoneally into the mice to activate neutrophils and blood was collected by eyeball extirpating [26]. Fresh anticoagulant whole blood was taken. When the blood volume was less than 5 mL, 4 mL reagent A was first added into the centrifuge tube, and then 2 mL reagent C was carefully folded on top of reagent A, forming a gradient interface. Then add the blood above the liquid level of the separation solution. Centrifugation was performed at room temperature at 1000 g for 20–30min. After centrifugation, there will be two circular milky cell layers in the centrifuge tube, and the upper layer is thin, the cells are mononuclear cells, and the lower cells are neutrophils. Carefully siphon the

![Figure 1](image-url)
neutrophils in regents A and between reagents C and A. The collected neutrophils were placed into a 15 ml centrifuge tube and washed with 10 ml PBS. Neutrophils were purified by washing with 1:C2 PBS three times, suspended, and stored at -80°C for subsequent membrane derivation. Mouse neutrophils were co-stained with FITC anti-mouse CD11b (101205, Biolegend, USA) and PE anti-mouse Ly-6G/Ly-6C (Gr-1) (108407, Biolegend, USA) antibody to identify neutrophil cells. Washed mouse neutrophils were resuspended in PBS and analyzed using FACS Canto-II flow cytometer. The resulting data were processed using FlowJo (version 10.0.7) software. Membrane of neutrophils was harvested following a previously published protocol [30]. The cells are broken moderately by ultrasound. The cell nucleus and a few unbroken cells were removed by low-speed centrifugation, and then the supernatant was taken to obtain the cell membrane precipitate and the supernatant containing cytoplasmic protein by high-speed centrifugation. Then the membrane protein was extracted from the precipitate by optimized membrane protein extraction reagent (P0033, Membrane and Cytosol Protein Extraction Kit, Beyotime, China). Membrane content was quantified using a BCA kit (23225, Pierce, USA) in reference to a bovine serum albumin standard.

### 2.5. Preparation and characterization of the NE/Lip-GEM

The Lip-GEM was provided by SunLipo NanoTech (Shanghai, China). Lip-GEM was prepared by a pH gradient method which uses a transmembrane pH gradient to induce an influx of drug and locks the drug in the core as a sulfate complex [31–33]. Then, the neutrophil membrane-enveloped Lip-GEM nanoparticles were prepared according the well-studied technique [34–36]. For membrane coating, neutrophil membrane was mixed with Gem-Lip at a Lip-GEM-to-membrane protein weight ratio of 2:1. The mixture was then sonicated with a sonicator for 2 min (Fisher Scientific FS30D, Thermo Scientific, USA) and incubated on ice for 30 min. Subsequently extruded serially through 400-nm and then 200-nm polycarbonate porous membranes using a liposome extruder. For fluorescence imaging, Dil and neutrophil membrane (or red blood cell membrane) were quickly mixed, followed by sonication for 2 min on ice, and incubated at 37°C for 30 min. The suspension was centrifuged at 1000 g to remove free Dil.

The size and zeta potential were measured with a Zetasizer Nano S (Malvern Instruments, Worcestershire, UK). Nanoparticle morphology was examined under a transmission electron microscope (Hitachi High-Tech, Tokyo, Japan). The protein samples were separated by 4–12% SDS-PAGE (M00653, GenScript, China). To examine the protein profile, the obtained gels were subsequently stained with Coomassie Blue and then washed overnight to visualize protein bands (G4540, Solarbio, China). The obtained gels probed with antibodies specific for integrin β2 (10554-1-AP, PTG, USA) and β actin (4970L CST, US). HRP-conjugated secondary antibodies were used for secondary staining for 1 h at room
temperature. The immunoblots were detected with an imaging system (Bio-Rad, USA) using enhanced chemiluminescence detection kit (P1050, Applygen Technologies Inc., China). The release profiles of gemcitabine from NE/Lip-GEM were assessed in PBS at pH 7.4 by dialysis method. Briefly, 1 ml of NE/Lip-GEM suspension was loaded into dialysis bag (MWCO 3500 Da) and then put it into 20 mL of PBS with continuous shaking at 120 rpm. At programmed time intervals, replace 20 mL of the incubated solution with fresh release buffer. The quantitation of released was detected by high performance liquid chromatography (HPLC). In HPLC experiment, the mobile phase (methanol: acetonitrile = 9:1) was used at a flow rate of 1.0 mL/min. The release experiments were performed in three counterparts.

2.6. Cell culture
Murine pancreatic cancer cell line Panc02 were purchased from BeNa Culture Collection (Beijing, China). Panc02 cells were cultured in Dulbecco’s Modified Eagle Medium (DMEM, Gibco, USA) containing 10% (v/v) fetal bovine serum (FBS, Gibco, USA) and incubated in 5% CO₂, 37°C incubator (Thermo Scientific, USA) with a humidified atmosphere.

2.7. Cellular uptake assay
Panc02 cells were seeded into confocal dishes (1 × 10⁴ cells/well) and incubated for 24 h. Then NE/Lip-GEM was labeled using ExoSparkler Exosome Membrane Labeling Kit-Green (EX01, DOJINDO, Japan). After 2 or 4 h incubation, the cells were washed with PBS and fixed by immunostaining fix solution for 20 min. After washing with cold PBS, the cells were stained with lyso-tracker red (L8010, Solarbio, China) for 60 min according to the protocol. Then the cells were stained with DAPI (C0060, Solarbio, China) for 20 min in the dark. The cells were then washed with PBS three times and visualized using a Zeiss LSM 710 laser scanning confocal microscope (LSCM, Carl Zeiss Inc., USA).

2.8. Animals and tumor model
C57BL/6 mice (4-6 weeks) were purchased from Shanghai Experimental Animal Center of Chinese Academic of Sciences (Shanghai, China). 2 × 10⁶ cells resuspended in 100 μL DMEM were subcutaneously injected to the left flank of the mice which were randomly divided into several groups. Tumor sizes were measured every 2 to 4 days. The tumor weights were recorded and the volumes were estimated with the formula: 1/2 × length × width × depth. The overall health of the mice was monitored and euthanized upon reaching ethical endpoint of the experiment (tumor volume ≥2000 mm³).

2.9. Fluorescence imaging and biodistribution analysis
The mice were randomly divided into two groups (n = 4) and treated with PBS or nsPEF respectively. The injected gemcitabine dosage was 10 mg/kg of total mouse body weight. Images were taken at 4 h and 24 h after injection using the CRI Maestro in vivo Imaging System to obtain the Dil fluorescence signals (549 nm excitation and 565 nm filter). The C57BL/6 mice were sacrificed at 24 h after injection and the organs including heart, liver, spleen, lung, kidney and tumor were collected for imaging and semi quantitative biodistribution analysis.

2.10. In vivo therapy
When the diameters of the tumor reached approximately 8-10 mm, the Panc02 tumor-bearing mice were randomly assigned into six groups and injected intravenously on days 3, 6, 9, 12 with the following formulation: (1) nsPEF, (2) GEM, (3) NE/Lip-GEM, (4) nsPEF + GEM, (5) nsPEF + Lip-GEM, (6) nsPEF + NE/Lip-GEM. The gemcitabine dosage was 10 mg/kg of total mouse body weight. Each group consisted of six mice. For the incomplete nsPEF ablation, the tumors were treated with 24 kv, 300 ns, 100 pulses. The tumor volumes and weights of mice were recorded. The overall health of mice was monitored daily and euthanized if the tumor burden was too large or the animal became moribund.

2.11. Histopathology and immunohistochemical analysis
To assess postoperative inflammation, mice were sacrificed and tumors were excised and fixed in paraformaldehyde solution before and after nsPEF treatment. Then the specimens were embedded in paraffin, sliced into 5-mm sections and stained with hematoxylin and eosin (H&E) to evaluate the morphological features of tumor. To evaluate the effectiveness of in vivo therapeutic effect, some mice were sacrificed and tumor were excised and fixed in paraformaldehyde solution on day 21. Then the specimens were stained with H&E to evaluate the morphological features of each organ and tumor. For Ki-67 staining, the slides of tumors and other organs were observed by an optical microscope under bright field.

2.12. Safety evaluation
After treatment with different formulations, the blood samples of the mice were harvested. The quantities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and creatinine (CREA) in the serum were determined using the corresponding assay kits (NINGBO PUREBIO BIOTECHNOLOGY CO., LTD., China). Histological analyses on the normal organs were performed using H and E staining including the heart, liver, spleen, lung and kidney.

2.13. Statistical analysis
Statistical analysis was performed with GraphPad Prism 5 (GraphPad Software). Data are expressed as the mean ± SD. One-way ANOVA was used to analyze the differences among multiple groups. Significant differences between or among the groups are implied by *p < 0.05, **p < 0.01, ***p < 0.001.
3. Results

3.1. NsPEF induced changes on the neutrophils in pancreatic cancer mouse models

Using immunohistochemistry staining, deeply cytoplasmic-stained cells (the nuclei are lobulated, and uniform neutrophilic particles are found within the cytoplasm) were infiltrated after nsPEF. We found that the number of neutrophils was significantly increased after nsPEF treatment (Figure 2(B)).

After nsPEF, the expression levels of inflammatory cytokines were quantified in the tumors after treatment. The level of TNF-α, IL-1β and CXCL1 in tumors showed a significantly elevated expression after nsPEF treatment (Figure 2(C–E)). In our previous study, we observed the inflammatory cytokines changes in the serum [27]. Furthermore, scRNA-seq results showed that nsPEF induced the remodeling of tumor-infiltrating neutrophils three days after treatment (Figure 2(F,G)). The percentage of the neutrophils present in tumors significantly increased from 2.98% to 26.71% (Figure 2(H)). In addition, scRNA-seq identified three distinct subsets of neutrophils. The top 10 markers of each cluster based on fold change are shown in the Figure S1. Il1a was highly expressed in cluster 1. Esd and Hmox1 were highly expressed in cluster 2. Ccl6 and Sell were highly expressed in cluster 3. The proportions of these subclusters significantly increased after nsPEF treatment (Figure 2(G)). KEGG enrichment analysis revealed that neutrophils were involved with genes and pathways associated with ribosome and leukocyte transendothelial migration, IL-17 signaling and chemokine signaling pathways (Figure 2(I,J)). ScRNA-seq analysis suggested that nsPEF treatment could induce recruitment of neutrophils within the tumors. In summary, these results confirmed that an inflammatory stress reaction occurred after nsPEF, and increased amounts of the relevant cytokines were retained in the tumor, which can facilitate attracting the neutrophils as well as NE/Lip-GEM to tumor sites.

3.2. Ne/Lip-GEM preparation and characterization

Three steps were involved in the preparation of NE/Lip-GEM. First, the membranes were obtained from activated neutrophils. We extracted activated neutrophils from mouse blood using neutrophil isolation kit (Figure 3(A)). Lipo-GEM nanoparticles were prepared according to a previous protocol [37], and the nanoparticles were characterized as shown in Figure S2. Finally, the neutrophil membrane and the nanoparticles were mixed to prepare NE/Lip-GEM via sonication and repeated extrusion with a liposome extruder. The NE/Lip-GEM were visualized using transmission electron microscopy (TEM) after uranyl acetate staining. They exhibited a spherical core–shell structure with a membrane coating the liposome core (Figure 3(B)). Owing to the membrane cloaking, compared with Lipo-GEM, NE/Lip-GEM possessed a slight elevation of 10–20 nm in size (Figure 3(C)). The zeta potential of NE/Lip-GEM was closer to the zeta potential value of neutrophil membrane vesicles and confirmed the formation of a biomimetic surface charge (Figure 3(D)). To ensure that NE/Lip-GEM retain membrane-associated proteins of neutrophils, proteomic analysis of membrane-associated proteins was performed. Protein separation via SDS-PAGE indicating

Figure 3. Preparation and characterization of NE/Lip-GEM. (A) Flow cytometric analysis of the purity of NEs doubly stained with FITC-CD11b and PE-Gr1. The average population of the cells shown in the corner of each quadrant(n = 3); (B) Images of NE/Lip-GEM observed by TEM; (C) Histogram of particle-size distribution of NE/Lip-GEM, Lip-GEM, Neutrophil membrane vesicles(n = 4); (D) Zeta potential of NE/Lip-GEM, Lip-GEM, Neutrophil membrane vesicles(n = 4); (E) SDS-PAGE analysis of Lip-GEM, NEs and NE/Lip-GEM(n = 3); (F) Western blot of actin, intergrin β2 on cytosol, NEs and NE/Lip-GEM(n = 3); (G) The release of gemcitabine from NE/Lip-GEM(n = 3).
that there was no significant damage to the extracellular proteins of membrane during the process of the synthesis of membrane-coating (Figure 3(E)). Moreover, as judged by western blotting analyses, the isolated membranes and NE/Lip-GEM expressed integrin β2, while the cytosol only express β actin (Figure 3(F)). These indicates that the separation of the plasma membrane was successful and further confirming the presence of specific homologous molecules on NE/Lip-gem. As shown in Figure 3(G), 43.66% of gemcitabine was released from NE/Lip-GEM after 4 h and the cumulative release reached up to 87.1% after 24 h. Taken together, these results demonstrated the successful generation of NE/Lip-GEM.

3.3. Cellular uptake of NE/Lip-GEM

To investigate the interaction between NE/Lip-GEM and cancer cells, we incubated NE/Lip-GEM with Panc02 cells. As the results of CLSM displayed in Figure 4, the green fluorescence intensity of NE/Lip-GEM in panc02 cell cytoplasm heightened with the extension of incubation time, indicating that an increasing number of NE/Lip-GEM were ingested by tumor cells through the endocytosis pathway and were endocytosed and co-located with lysosomes where they degrade and rupture.

3.4. Ne/Lip-GEM accumulated in nsPEF-treated tumors

To monitor the accumulation of NE/Lip-GEM in the ablated tumor tissue using in vivo imaging system (IVIS), Dil-labeled NE/Lip-GEM were intravenously injected into untreated or nsPEF-treated Panc02-bearing mice. The fluorescence signal of NE/Lip-GEM was observed at tumor sites after administration for 4 h, and the intensity of fluorescent signal increased as time going on to 24 h (Figure 5). The results showed that more NE/Lip-GEM targeted to nsPEF-treated tumors than to untreated tumors. This was further confirmed by a quantitative ROI analysis of the Dil fluorescence signals at the tumor locations. Quantitative ROI analysis revealed 3.63-, 3.09-fold higher fluorescence signal in the nsPEF-treated tumors than in the control tumors at both 4 and 24 h, respectively. For the biodistribution study, we harvested the tumors and major organs and analyzed their fluorescence signals. Direct ex vivo imaging of the excised tumors and major organs showed that nsPEF resulted in a 1.8-fold increase of the fluorescence signals in tumors; in contrast, there were no significant differences between the organs from nsPEF treated mice and untreated mice. Besides, compared with RBC coated nanomaterials (RBC/Lip-GEM), the tumor sites showed more fluorescence signals of NE/Lip-GEM after administration (Figure S3).

3.5. Therapeutic effect of combination of NE/Lip-GEM and nsPEF in pancreatic cancer model

As nsPEF treatment has been reported to impede tumor growth [5–7,38], we hypothesized that combining it with chemotherapy would enhance the anti-tumor efficacy. The therapeutic potential of NE/Lip-GEM was evaluated in Panc02 tumor-bearing mice after treatment with nsPEF therapy combined with intravenous administration of NE/Lip-GEM at a dosage of equivalent 10 mg/kg GEM according to the administration regimen (Figure 6(A)). Under the nsPEF treatment conditions of 24 KV, 100 pulses, the incomplete ablation tumor models were established. Compared with saline as negative control, the tumor growth was suppressed following injection of various formulations. It was worth noting that nsPEF + NE/Lip-GEM group demonstrated a more significant effect on tumor inhibition than other groups. The tumor volumes in the nsPEF + NE/Lip-GEM treatment group were effectively decreased (Figure 6(B)), while the tumor...
volumes of other groups continued to increase. This was further confirmed by measuring the volumes of the relevant tumors at 21 days post-nsPEF (Figure 6(C)). Histologic images of the tumor sections stained with H&E exhibited massive cancer cell remission after applying nsPEF + NE/Lip-GEM, which further confirmed the efficacy of the combined therapy of nsPEF and NE/Lip-GEM. Furthermore, immunohistochemical analysis of Ki-67 staining, which was utilized to assess the proliferation of tumor, revealed that the expression of Ki-67 was observably reduced in nsPEF + NE/Lip-GEM group compared with that in the other treatment groups (Figure 6(D,E)). The body weights of mice receiving nsPEF and NE/Lip-GEM remained stable, showing no substantial differences with that of other groups (Figure 6(F)). Taken together, it has verified that NE/Lip-GEM can efficiently deliver nanodrug to tumor sites, thereby indicating its potential as an effective combination therapy for treating cancer.

3.6. Safety evaluation of NE/Lip-GEM combined with nsPEF in pancreatic cancer model

H&E staining was carried out to assess the toxicity on the major organs. On the 21th day after nsPEF
treatment, the major organs of treated mice were collected for subsequent histological analysis (Figure 7(A)). No pathological changes were observed from the H&E staining images of the main organs sections of mice receiving. Likewise, AST and ALT levels, which are important indices for hepatic function, were no abnormality in all the groups. Additionally, CREA measurement showed that there was no kidney damage in mice treated with nsPEF and NE/Lip-GEM (Figure 7(B–D)). These results demonstrated that no significant organ damage in the nsPEF + NE/Lip-GEM group was detected, suggesting that there were no noticeable toxic side effects of this treatment.

4. Discussion

Tumor ablation using nsPEF is a novel therapeutic modality. Intense nanosecond-duration pulses generate transient nanopores not only in the plasma membrane, but also in intracellular membranous structures including the endoplasmic reticulum and mitochondria [39]. Biological effects are induced including calcium mobilization, cell swelling and activation of phosphocreatine signaling, as well as various response including cytochrome c release from mitochondria and caspase activation associated with apoptosis [40]. NsPEF can uniformly induce apoptosis of tumor cells to achieve the effect of eliminating tumors. The main advantage of nsPEF is
that tissue damage is restricted to the targeted region, important tissues such as blood vessels and nerves can be well protected, which has been confirmed by other studies [41]. However, nsPEF treatment has its limitations: the therapeutic effect depends on the intensity of the electric field applied to the tissues. If the optimal conditions are not provided, insufficient energy is produced, resulting in incomplete ablation. Some studies have demonstrated the feasibility of the combining nsPEF with other treatment methods for a comprehensive treatment strategy that prevents residual tumors [14,42]. Combination therapy has great potential to enhance therapeutic efficacy and ameliorate adverse effects.

Membrane-coated nanoparticles have emerged as promising therapeutic platforms for targeted chemotherapy drug delivery [17,43,44]. Neutrophils are the most abundant white blood cells, accounting for 70% of the white blood cells in peripheral blood circulation. Neutrophils are recruited to sites of inflammation or injury and are involved in various inflammatory responses. Owing to their role in the immune response, neutrophil membrane coating nanoparticles can escape arrest by the immune system and reach target tissues. Thus, activated neutrophils have been designed as targeted drug carriers for the treatment of cancer and inflammatory disease [26,36,45].

In the present study, we found an interesting phenomenon that nsPEF ablation activated the release of inflammatory cytokines at tumor sites which facilitated the migration of neutrophils to the ablation sites. This was confirmed by scRNA sequencing. Based on the above facts and inspired by the targeting of neutrophils to inflammatory tissues, we designed neutrophil-mimicking nanoparticles (NE/Lip-GEM) by cloaking the surface of liposomal with an inflammatory neutrophil-derived membrane. We confirmed that NE/Lip-GEM accumulated in the ablated tumors and significantly enhance the efficacy of nsPEF therapy. In this study, we developed neutrophil membrane-coated liposomal nanoparticles and explored the therapeutic effects of combining NE/Lip-GEM and nsPEF for pancreatic cancer treatment.

To explore the therapeutic efficacy of nsPEF and NE/Lip-GEM, we established a Panc02 tumor-bearing xenograft mice model. Regarding antitumor efficacy in vivo, nsPEF + NE/Lip-GEM treatment significantly inhibited tumor growth compared to other groups, which further demonstrated the improved therapeutic efficacy of this combination strategy in pancreatic cancer. The accumulation of NE/Lip-GEM at the tumor site, following systemic administration was likely mediated by the enhanced permeability and retention effect. Moreover, the nsPEF induced inflammatory response also aided in the accumulation of NE/Lip-GEM at tumor sites. The therapeutic effect of drugs is often limited because of the lack of site-specific distribution. The enhanced tumor-specific distribution of neutrophil-coated nanoparticles after nsPEF in pancreatic cancer models suggests that coating nanoparticles with neutrophil membranes increases their affinity for tumor cells.

5. Conclusions

In summary, we developed a novel cell membrane-based nanoparticle delivery system that can be used in combination with nsPEF treatment for effective pancreatic cancer therapy. The combined treatment strategy significantly inhibited tumor growth, indicating that the release of proinflammatory signal induced by nsPEF contributed to the tumor targeting ability of NE/ Lip-GEM. We believe that this neoadjuvant therapy based on cytopharmaceuticals offers new opportunities for developing antineoplastic protocols, which may have great potentials in clinical application in the future.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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