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Nanoparticles camouflaged in platelet membrane coating as an antibody decoy for the treatment of immune thrombocytopenia

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Abstract

Immune thrombocytopenia purpura (ITP) is characterized by the production of pathological autoantibodies that cause reduction in platelet counts. The disease can have serious medical consequences, leading to uncontrolled bleeding that can be fatal. Current widely used therapies for the treatment of ITP are non-specific and can, at times, result in complications that are more burdensome than the disease itself. In the present study, the use of platelet membrane-coated nanoparticles (PNPs) as a platform for the specific clearance of anti-platelet antibodies is explored. The nanoparticles, whose outer layer displays the full complement of native platelet surface proteins, act as decoys that strongly bind pathological anti-platelet antibodies in order to minimize disease burden. Here, we study the antibody binding properties of PNPs and assess the ability of the nanoparticles to neutralize antibody activity both *in vitro* and *in vivo*. Ultimately, we leverage the neutralization capacity of PNPs to therapeutically treat a murine model of antibody-induced thrombocytopenia and demonstrate considerable efficacy as shown in a bleeding time assay. PNPs represent a promising platform for the specific treatment of antibody-mediated immune thrombocytopenia by acting as an alternative target for anti-platelet antibodies, thus preserving circulating platelets with the potential of leaving broader immune function intact.

Graphical abstract

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Keywords

autoimmune disease; platelet membrane-coated nanoparticle; biomimetic nanoparticle; nanosponge; antibody decoy

1. Introduction

Platelets, also known as thrombocytes, are a blood component that is essential for maintaining hemostasis. One of their main functions is to stop bleeding via initiation and propagation of the coagulation cascade [1, 2]. Platelet count is universally regarded as the key indicator of bleeding risk, and the normal range in healthy people sits between 150,000 to 450,000 platelets per microliter of blood. A count under the normal range, termed thrombocytopenia, can be due to either decreased platelet production or increased platelet destruction. Clinically, the disease can manifest itself as purpura, a delay in the normal process of clotting, and spontaneous or excessive bleeding. When platelet counts drop substantially lower than normal values, internal hemorrhaging can occur, a severe condition that can potentially be fatal [3].

Immune thrombocytopenia purpura (ITP), which is oftentimes also referred to as idiopathic thrombocytopenic purpura, is an immune-mediated hematological disorder characterized by low level of platelets and easy or excessive bleeding due to the presence of anti-platelet autoantibodies [4, 5]. These pathological antibodies bind to specific antigens on the platelet surface, leading to sequestration and destruction by the reticuloendothelial system. The age-adjusted prevalence of ITP is estimated to be 9.5 per 100,000 persons in the United States [6]. While the condition may appear secondary to a known autoimmune condition or infection, oftentimes the underlying etiology is unclear [7-9]. Given this fact, chronic ITP is classically treated using nonspecific therapies such as corticosteroids. While capable of eliciting a rebound in platelet levels in many patients, such treatments are susceptible to relapse and can cause unwanted side effects [5, 10]. For those that fail to respond to frontline treatments, invasive and irreversible splenectomy is a common intervention, but has the chance of postoperative complications such as infection, bleeding, and hospitalization [11, 12]. Other second- and third-line treatments include intravenous immunoglobulin (IVIg) [13], intravenous Rho immunoglobulin (RhIg) [14], rituximab (anti-CD20) [15], and

thrombopoietin receptor agonists [16]. Most carry significant iatrogenic risk given their generally non-specific modes of action. With the probability of high side effects, treatment can ultimately be more burdensome than the original disease. With these considerations in mind, the development of a treatment modality that can specifically target the pathological moieties responsible for ITP is highly desirable.

Cell membrane-coated nanoparticles represent an increasingly popular platform for a variety of applications, including drug delivery [17], vaccination [18, 19], and detoxification [20, 21]. A significant factor behind their appeal is the ability to replicate the surface properties of different cell types faithfully on nanoparticle surfaces. Employing biological materials through a top-down coating approach bestows synthetic nanoparticles with native cell functionalities. For example, it has been shown that coating with red blood cell membrane actively modulates residence time in the bloodstream via the display of self-markers that are recognized by the immune system [22]. Functionalization with platelet membrane enables biomimetic targeting by taking advantage of the natural interactions between platelet surface markers and different targets, including damaged vasculature and pathogens [23, 24]. Given the wide range of biological interactions that natural cell membranes participate in, the potential of cell membrane-coated nanoparticles extends far beyond traditional nanodelivery applications. One such area is biodetoxification where the membrane coating serves as an ideal substrate for interaction with biological toxins, enabling their neutralization and subsequent clearance. For example, red blood cell membrane-coated nanoparticles have previously been shown to bind and clear both bacterial toxins [20] as well as small molecule poisons [21].

Here, we demonstrated the use of platelet-derived membrane as a natural biomaterial for the design of nanoparticulate decoys that can effectively bind and clear the pathological antibodies responsible for ITP (Fig. 1). The binding capacity and specificity of platelet membrane-coated nanoparticles (PNPs) were evaluated before studying the neutralization capacity of PNPs against anti-platelet antibodies both *in vitro* and *in vivo*. Finally, an antibody-induced murine model of ITP was employed in order to assess treatment efficacy. As a possible new treatment for ITP, PNP administration holds distinct advantages compared to current therapies. By using the natural substrate of the pathological agent, the treatment is highly specific, which may prevent the immune compromising side effects commonly seen with other treatments. Further, by diverting anti-platelet antibodies away from healthy platelets, PNPs directly act to preserve normal hemostatic function. Ultimately, employing this biomimetic nanoparticle system for the specific treatment of ITP may serve to improve patient outcomes in the clinic.

2. Materials and methods

2.1 Animals

Male CD-1 mice (6-week old; 20–24 g body weight) were purchased from Harlan Laboratories. All animal experiments were performed in accordance with NIH guidelines and approved by the Institutional Animal Care and Use Committee (IACUC) of the University of California, San Diego.

2.2 Platelet isolation and membrane derivation

Whole blood was collected from male adult CD-1 mice (Harlan Laboratories) via puncture of the submandibular vein with ethylenediaminetetraacetic acid (EDTA; USB Corporation) as the anticoagulant. To isolate platelets, the blood was first centrifuged at $300 \times g$ for 5 minutes at room temperature. The supernatant then was collected and spun at the same speed for another 5 minutes. The resulting supernatant, representing a platelet rich plasma, was then centrifuged at $2000 \times g$ for 4 minutes in order to pellet down the platelets, which were resuspended in water, aliquoted, and stored at -80°C for further use. Platelet membrane was derived by a repeated freeze-thaw process. A frozen aliquot of purified platelets was allowed to thaw at room temperature, centrifuged at $21,000 \times g$ for 7 minutes, and the pellet was resuspended in water. The platelet suspension was refrozen, and the process was repeated three times. The pellet was finally resuspended in water, and the membrane protein concentration was quantified using a commercial BCA assay (Pierce).

2.3 Preparation and characterization of platelet membrane-coated nanoparticles (PNPs)

PNPs were prepared using a previously reported sonication method.[23] Polymeric nanoparticle cores were prepared using carboxyl acid-terminated 0.67 dL/g 50:50 poly(DL-lactic-co-glycolic acid) (PLGA; LACTEL Absorbable Polymers) in a nanoprecipitation process. A volume of 1 mL of a 10 mg/mL PLGA solution in acetone was added rapidly to 4 mL of water. The acetone was then allowed to evaporate under vacuum for 3 hours. PNPs were prepared by fusing platelet membrane onto PLGA cores via sonication using a Fisher Scientific FS30D bath sonicator at a frequency of 42 kHz and a power of 100W for 2 minutes. The size and zeta-potential of PNPs were measured by dynamic light scattering (DLS) using a Malvern ZEN 3600 Zetasizer. To study the morphology of PNPs by transmission electronic microscopy (TEM), samples were deposited onto a 400-mesh carbon-coated copper grid (Electron Microscopy Sciences) and negatively stained with vanadium (Abcam).

2.4 Platelet membrane to nanoparticle core ratio optimization

To optimize the platelet membrane to PLGA core ratio, PNPs were synthesized at membrane-to-core weight ratios ranging from 0.125 to 2 at a final polymer concentration of 1 mg/mL. PLGA cores without membrane coating were included as a control. The sizes of each set of particles were first measured by DLS immediately after synthesis. Afterwards, the particle solutions were adjusted to $1 \times \text{PBS}$ by adding an equal volume of $2 \times \text{PBS}$ and the particle sizes were measured again. An increase in size upon introduction of PBS was used as an indicator of particle instability.

2.5 In vitro binding capacity and specificity studies

To evaluate the *in vitro* binding capacity of PNPs, 10 μg of the nanoparticles was mixed with different amounts (2, 4, 8, 16, 32, 64, and 128 μg) of fluorescein isothiocyanate (FITC)-labeled polyclonal anti-mouse thrombocyte antibodies (Lifespan Biosciences). The precise antigen specificity of the antibodies was unknown. After mixing the PNPs with antibodies, the fluorescence intensity of the fluorescently labeled antibody was measured using a Tecan Infinite M200 plate reader. The mixtures were incubated for 10 minutes at 37°C , then

centrifuged at $21,000 \times g$ for 8 minutes to pellet the PNP/anti-platelet complexes. The fluorescence intensity of the supernatant was measured and used to calculate the amount of antibody that had bound to the PNPs. To evaluate binding specificity, either 10 μg of PNPs or 10 μg of polyethylene glycol-functionalized nanoparticles (PEG-NPs) [25] were mixed with 32 μg of FITC-labeled antibody. To test the binding capacity in serum, 10 μg of PNPs were incubated with 128 μg of FITC-labeled antibody in either PBS or 50 vol% mouse serum.

2.6 In vitro neutralization

For the pre-incubation study, 20 μg of FITC-labeled anti-platelet antibody was incubated with varying amounts of PNP (5, 10, 20, 50, and 100 μg) or PBS at 37 °C for 15 minutes. The mixture was then added to a solution containing the number of platelets equivalent to 40 μg worth of membrane material and incubated at 37 °C for 15 minutes. For competitive co-incubation study, the same amounts of PNPs and antibody were concurrently added to the platelets. All samples were then washed by centrifugation at $2,000 \times g$ three times with PBS. The amount of antibody binding to platelets was measured by flow cytometry on a Becton Dickinson FACSCanto II flow cytometer and analyzed using Treestar Flowjo.

2.7 In vivo binding stability

To establish a mouse model of thrombocytopenia, 6-week old CD-1 mice were injected intraperitoneally with PBS or 50 μg of anti-thrombocyte antibody. The mice were bled before injection as well as 4 hours and 24 hours after injection for platelet enumeration. Male 6-week old CD-1 mice were injected intraperitoneally with either 50 μg of anti-mouse thrombocyte antibody (Lifespan Biosciences) pre-incubated with 100 μg of PNPs, 50 μg of antibody alone, or PBS. Blood was sampled by submandibular vein puncture both before and 24 hours after injection using EDTA as the anticoagulant. To enumerate the platelets, a 1 μL volume of blood was diluted 1,000 times in 1% bovine serum albumin (Sigma Aldrich) in PBS. The diluted solution was then stained with FITC-labeled anti-mouse CD41 (Biolegend) for labeling of platelets, and flow cytometry was used to count the number of FITC⁺ events per given volume.

2.8 In vivo treatment

Male 6-week old CD-1 mice were injected intraperitoneally with 50 μg of anti-thrombocyte antibody to induce thrombocytopenia. After 15 minutes, mice received either 400 μg of PNPs, 400 μg of PEG-NPs, or PBS via tail vein injection. Blood was sampled both before and 24 hours after administration of antibody. To assess the effect of treatment on bleeding time, mice were first anesthetized 24 hours after antibody administration with a cocktail of 150 mg/kg ketamine (Zoetis) and 10 mg/kg xylazine (Lloyd Laboratories). For the bleeding time assay, a tail segment 5 mm from the distal end was excised by a sterile blade, and the cut end of the tail was immediately placed into 37 °C PBS in a 50 mL tube. The time from amputation to complete cessation of bleeding was recorded for each mouse. Those mice bleeding longer than a pre-determined time limit of 20 minutes were euthanized immediately.

3. Results

3.1. Preparation and characterization of PNPs

PNPs were prepared by fusing mouse platelet-derived membrane onto the surface of poly(lactic-co-glycolic acid) (PLGA) nanoparticle cores [23]. In brief, platelets collected from whole blood were subjected to repeated freeze-thaw cycles and centrifugation in order to obtain purified membrane. The membrane was then coated onto the surface of preformed PLGA nanoparticles by a sonication process. After the membrane coating, dynamic light scattering indicated an approximately 20 nm increase in the average hydrodynamic diameter over that of the bare PLGA cores (Fig. 2A). Zeta potential measurements also suggested successful coating, as evidenced by the increase in surface charge of the coated nanoparticles to approximately the same level as a membrane vesicle only sample (Fig. 2B). Moreover, transmission electron microscopy of negatively stained PNPs revealed a characteristic core-shell structure with a layer of membrane coated over the polymer core (Fig. 2C). In order to optimize the membrane coating ratio, PNPs were prepared using different membrane to PLGA core weight ratios ranging from 0.125 to 2 (Fig. 2D). After adjusting to 1× phosphate buffered saline (PBS) solution, which represents physiological salt concentrations, bare PLGA cores with no membrane coating aggregated immediately due to charge screening effects. With increasing amounts of membrane, there was a trend of decreasing aggregation. At a ratio of 1 to 1, no apparent size increase was observed, and this formulation was chosen for further studies. The particles also demonstrated little change in size and distribution when subjected to high shear conditions (Fig. S1).

3.2. Antibody binding capacity and specificity

To investigate the binding capability of PNPs to anti-platelet antibodies, 10 µg (polymer weight) of PNPs were incubated with different amounts of fluorescently labeled polyclonal anti-platelet antibodies ranging from 2 µg to 128 µg (Fig. 3A). Quantification based on the fluorescent signal showed a linear increase in antibody binding at lower concentrations, after which the binding plateaued. From the plotted data, it was interpolated that 50% binding occurred at a polyclonal antibody input of approximately 25 µg. The experiment was also repeated keeping the amount of antibody constant while varying the PNP concentration (Fig. S2). To assess the specificity of the PNP-antibody interaction, binding was compared to a control polyethylene glycol-functionalized lipid-polymer hybrid nanoparticle (PEG-NP) [25] (Fig. 3B). Using an equivalent amount of either PNPs or PEG-NPs, it was demonstrated that, comparatively, the PEGylated nanoparticles exhibited the near absence of antibody binding. The different results observed between the two types of nanoparticles indicate that the platelet membrane bestows specific binding properties. Additionally, an isotype antibody not specific to platelet membrane also showed no binding to the PNPs (Fig. S3). Furthermore, to evaluate the effect of the presence of other proteins, the binding of antibody to PNPs was tested in the presence of serum (Fig. 3C). Compared with binding in PBS, there was little difference observed for the sample tested in serum, indicating the potential of the nanoparticles retain their function within the complex biological environment found *in vivo*.

3.3. In vitro dose-dependent neutralization of anti-platelet antibodies by PNPs

To characterize the ability of PNPs to neutralize anti-platelet antibodies *in vitro*, different amounts of PNPs ranging from 0 to 100 μg were pre-incubated with a constant amount of fluorescently labeled anti-platelet antibodies. This was followed by the addition of fresh platelets to the mixture and analysis of antibody binding to the platelets by flow cytometry (Fig. 4A, B). It was shown that fluorescent signal sharply decreased with increasing amount of PNPs. Using 20 μg of polyclonal anti-platelet antibodies, it was observed that approximately 5 to 10 μg of PNPs were able to reduce mean fluorescence intensity by 50%, a ratio that was in line with what was observed from the antibody binding study. To evaluate the neutralization capacity in a competitive setting, both PNPs and fresh platelets were simultaneously incubated with the antibodies (Fig. 4C, D). In this scenario, mean fluorescence intensity was halved at approximately 20 μg of PNPs using 20 μg of antibodies. The antibody signal on the platelets decreased slower with increasing PNP concentration compared with the pre-incubation scenario, reflecting the increased challenge in neutralizing antibodies when both nanoparticle and fresh platelets compete for binding at the same time. Despite this fact, a great deal of neutralization capacity was still observed, indicating strong potential for therapeutic use.

3.4. In vivo binding stability of PNPs to anti-platelet antibodies

After confirming that PNPs could neutralize anti-platelet antibodies *in vitro*, their binding stability *in vivo* was assessed (Fig. 5). To conduct the experiment, a previously established murine model of immune thrombocytopenia was employed [26]. When anti-platelet antibodies alone were administered intraperitoneally, their diffusion across the peritoneal membrane induced very obvious thrombocytopenia. Platelet counts dropped dramatically even 4 hours post-injection and the challenged mice exhibited a more than 90% reduction in platelet counts 24 hours post-injection (Fig. S4). When the antibodies were pre-incubated with PNPs, followed by injection of the mixture, platelet counts were preserved to levels not statistically different from those of mice administered with only blank solution. The results suggest a strong binding interaction of the anti-platelet antibodies with the PNPs, which prevents the release of the pathological antibodies and thereby preventing their ability to cause the clearance of healthy platelets. The ability of the PNPs to maintain antibody neutralization within the complex *in vivo* biological environment was encouraging and motivated further study on the ability of the nanoparticles to perform this function *in situ* in a therapeutic setting.

3.5. Treatment of a murine model of immune thrombocytopenia

Finally, the ability of PNPs to be used as a means for the therapeutic treatment of immune thrombocytopenia *in vivo* was assessed. Mice were first intraperitoneally administered a bolus dose of anti-platelet antibodies capable of causing a marked reduction of platelet counts. This was followed by intravenous administration of either blank solution, PEG-NPs, or PNPs. Blood was sampled both before and 24 hours after challenge with anti-platelet antibodies, and platelet count was determined (Fig. 6A). Without any treatment, platelet counts dropped dramatically after 24 hours and were approximately 10% of their original value. A similar drop was seen when mice were treated with PEG-NPs, which could not

bind the antibodies and were unable to rescue platelet counts. In contrast, those mice treated with PNPs exhibited a marked increase in preservation of platelet number with final values at approximately 70% of pre-challenge values.

In order to demonstrate the importance of this platelet preservation on maintenance of hemostatic capacity, a bleeding time test, which is a commonly used *in vivo* assay for evaluating platelet function, was carried out (Fig. 6B). After tail tip excision and immediate immersion into a warmed saline solution, the amount of time to bleeding cessation was recorded. For unchallenged mice, the bleeding stopped on average between 1 and 2 minutes after excision, whereas those of anti-platelet antibody-challenged mice with no treatment exhibited increased bleeding times of at least 5 minutes; more than half of the untreated mice bled longer than a predetermined threshold of 20 minutes. The same level of bleeding was seen for those mice treated with PEG-NPs, whereas those treated with PNPs had bleeding times that were consistent with the unchallenged group. The results of the bleeding time assay correlate with the post-challenge platelet counts for each group and demonstrate that the amount of platelets retained in PNP-treated mice is sufficient to retain full hemostatic capabilities. This is in line with previous research that platelet counts need to decrease below a certain threshold in order to translate to increased bleeding times [27]. The treatment efficacy results here confirm the ability of PNPs to bind and neutralize pathological antibodies in circulation, thus preserving the function of healthy platelets.

4. Discussion

ITP is a hematological disorder characterized by a decreased number of circulating platelets, which generally manifests as an increased tendency to bleed as well as susceptibility to bruising. While this can generally affect quality of life, severe cases can have serious consequences, such as the induction of intracranial hemorrhaging that carries with it a high mortality rate [28, 29]. The thrombocytopenic condition is very often acute and, in most cases, patients spontaneously recover platelet levels within a short period time without any specific treatment [30]. However, a small proportion may develop chronic ITP, which usually occurs in adults and is characterized by persistence of significantly low platelet counts for longer than 6 to 12 months. Unfortunately for patients suffering from chronic ITP, the disease commonly occurs in response to an unknown stimulus [31], making effective treatment a difficult task for clinicians. Drugs that either modulate or distract the immune system are generally employed, including the use of corticosteroids or IVIg as frontline therapies [32]. Splenectomy is the approach often taken after failure of initial treatment and can lead to the restoration of the platelet counts to a normal level by removing the organ responsible for both clearance of opsonized platelets and pathological antibody production [33]. The procedure, however, can be associated with infection, bleeding, hospitalization, and vascular complications [11, 12]. Moreover, a splenectomy is irreversible and can likely lead to long-term impairment of hematologic and immunological functions [12, 34]. Other secondary or tertiary treatment options exist, including use of cytotoxic drugs, platelet transfusions, and thrombopoietin receptor agonists among others. To our knowledge, none of the current available therapies directly address the pathological moieties that contribute directly to the clearance of healthy platelets from circulation.

To create a platform capable of specific autoantibody depletion for the treatment of ITP, platelet membrane was directly employed in order to fabricate nanoparticles that mimic the surface properties of the original cell [17, 22]. One major advantage of this approach is that the nanoparticle surface serves as a natural substrate for autoantibodies against endogenous cellular targets [35]. Further, the nanoparticles present the relevant epitopic targets recognized by the antibodies without the need to identify antigen specificity, which can vary among patients [36]. Cell membrane-coated nanoparticles have previously demonstrated favorable safety profiles *in vivo* as well as lack of both acute and chronic anti-nanoparticle immune responses [19, 37]. Another advantage of the nanoparticulate platform is that storage after lyophilization is common practice [23], significantly extending shelf-life compared with whole platelets, which need to be carefully processed and expire within a week after collection [38].

In the present study, *in vitro* binding of PNPs to anti-platelet antibodies was demonstrated to be both stable and specific. According to the information obtained from Fig. 3A, the apparent weight binding ratio between PNPs and the anti-thrombocyte antibody was approximately 1 to 5. However, the antibody preparation that we employed was polyclonal, and Fig. S2 suggests that only about 15 percent of the antibody was actually specific to platelet membrane. Based on the information from these two pieces of data, we calculated that one PNP could sequester around 280 platelet membrane specific anti-thrombocyte antibodies. Pre-incubation and binding of antibodies to the nanoparticles appeared to preclude further interaction with platelets, a result that was confirmed *in vivo*. Impressively, the PNPs performed well in a competitive setting. As shown in Fig. 4C and D, 20 μ g of PNPs, which contain 20 μ g of platelet membrane material, was able to reduce binding of antibodies to platelets by half, despite the fact that they were in the presence of 40 μ g worth of membrane material from the native platelets. This suggests that nanoparticulate membrane may have an inherent advantage in binding that can be exploited for other biodetoxification applications. Administration of PNPs in a therapeutic scenario demonstrated considerable efficacy, and a bleeding time assay was used to highlight the importance of preserving platelet counts. Compared with a 93% drop in platelet count for the PEG-NP treatment group, PNPs preserved 70% of the platelets, presumably due to their ability to bind the pathological antibodies and remove them from circulation. The results are not likely due to any thrombotic effect from the PNPs, as it has been demonstrated that the particles are absent most intracellular activating factors. Given the relative scarcity of platelets in the blood, it should be feasible to administer enough nanoparticles to significantly outnumber healthy circulating platelets. While the long-term consequences of administering cell membrane-derived antigenic material in a nanoparticulate format have yet to be fully explored, it should be noted that platelet transfusion is a well-established clinical procedure; it can be reasonably expected that administration of membrane material only should be less burdensome given the lack of the biologically active components present in the intracellular compartment of intact platelets.

5. Conclusion

In conclusion, we have successfully demonstrated the application of PNPs towards the treatment of ITP. The nanoparticles showed the ability to specifically bind anti-platelet

autoantibodies, which are directly responsible for reducing platelet counts. Upon binding, it was demonstrated that the interaction between the PNPs and the antibodies was strong, effectively neutralizing biological activity *in vivo*. In an antibody-induced thrombocytopenia animal model, mice treated with PNPs after challenge with antibodies were able to retain their platelet counts. Further, in a bleeding time assay, mice treated with PNPs exhibited normal hemostasis via effective clot formation, and average values were nearly identical to unchallenged controls. On the other hand, untreated mice or those administered with control nanoparticles bled excessively due to lowered platelets counts and impaired hemostasis capacity. The ability to specifically neutralize anti-platelet antibodies in ITP presents a new option in the current landscape of treatment for the disease. Currently, most therapies are non-specific and can significantly impair broad immune function. By targeting the pathological antibodies directly, it may be possible to treat the disease while leaving the immunity intact, giving patients an increased opportunity for natural recovery of platelet counts without damaging and irreversible interventions. Alternatively, PNPs may also be used as an adjuvant therapy to either synergize with current treatments or enable a decrease in drug dosages to help limit unwanted side effects. Ultimately, PNPs represent a promising platform for the treatment of ITP and further study towards translation is warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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Fig. 1.

Schematic of platelet membrane-coated nanoparticles (PNPs) for the treatment of immune thrombocytopenia purpura (ITP). (A) To fabricate PNPs, the plasma membrane from fresh platelets is derived and then coated onto poly(lactic-co-glycolic acid) (PLGA) polymeric nanoparticle cores, transferring the surface antigenic material from the original cells onto the outside of the nanoparticles. (B) Without treatment, ITP is characterized by the binding of pathological autoantibodies to healthy platelets, resulting in their clearance by the reticuloendothelial system. (C) When PNPs are administered, they act as decoys that bind to the pathological autoantibodies, neutralizing them from circulation and enabling the survival of healthy platelets.

Fig. 2.

Characterization and optimization of PNPs. (A) Hydrodynamic size of bare PLGA cores, platelet vesicles, and PNPs as measured by dynamic light scattering ($n = 3$; mean $\pm$ SD). (B) Surface zeta potential of bare PLGA cores, platelet vesicles, and PNPs ($n = 3$; mean $\pm$ SD). (C) Transmission electron microscopy images of PNPs negatively stained with vanadium (scale bar = 75 nm). (D) Sizes of PNPs fabricated with varying membrane protein to PLGA weight ratios measured both immediately after synthesis in deionized water and after adjusting to $1\times$ PBS buffer solution ($n = 3$; mean $\pm$ SD).

Fig. 3.

In vitro binding of anti-platelet antibodies to PNPs. (A) Fluorescent quantification of anti-platelet antibody binding to PNPs. A constant amount of PNPs (10 μg) was incubated with varying amounts of fluorescently labeled antibodies ($n = 3$; mean $\pm$ SEM). (B) Relative binding of anti-platelet antibodies to either PNPs or PEGylated nanoparticles (PEG-NPs) ($n = 3$; mean $\pm$ SD). (C) Relative binding of anti-platelet antibodies to PNPs in either PBS or mouse serum ($n = 3$; mean $\pm$ SD).

Fig. 4.

In vitro neutralization of anti-platelet antibodies by PNPs. (A) Representative flow cytometry histograms of platelets labeled with fluorescent anti-platelet antibodies pre-incubated with varying amounts of PNPs (from left to right: 100, 50, 20, 10, 5, and 0 μg). (B) Mean fluorescence intensity of the samples in (A) (n = 3, mean ± SD). Ctrl = no antibody. (C) Representative flow cytometry histograms of platelets labeled with fluorescent anti-platelet antibodies while concurrently incubated with varying amounts of PNPs (from left to right: 100, 50, 20, 10, 5, and 0 μg). (D) Mean fluorescence intensity of the samples in (C) (n = 3, mean ± SD). Ctrl = no antibody.

Fig. 5.

In vivo neutralization of anti-platelet antibody activity by PNPs. Mice were intraperitoneally administered with PBS, anti-platelet antibodies, or the antibodies pre-incubated with PNPs ($n = 8$; mean $\pm$ SEM). Blood was collected both before and 24 hours after administration to quantify platelet counts. *** $P < 0.001$, NS = not significant, Student's t -test.

Fig. 6.

In vivo treatment of antibody-induced thrombocytopenia by PNPs. Mice were intraperitoneally administered with anti-platelet antibodies, followed 15 minutes later by intravenous injections of either blank solution, PNPs, or PEG-NPs via the tail vein. (A) Blood was collected both before and 24 hours after administration to quantify platelet counts ($n = 8$; mean $\pm$ SEM). (B) Bleeding time from the tail vein into PBS. An upper time limit of 20 minutes was established prior to initiation of the study. *** $P < 0.001$, Student's t -test.