

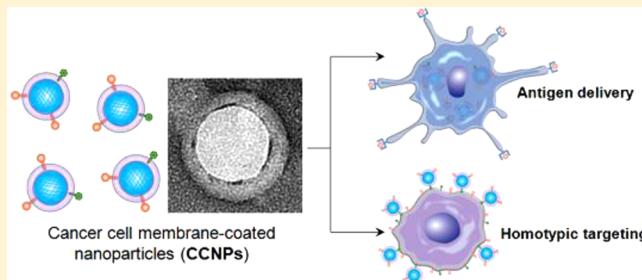
Cancer Cell Membrane-Coated Nanoparticles for Anticancer Vaccination and Drug Delivery

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S Supporting Information

ABSTRACT: Cell-derived nanoparticles have been garnering increased attention due to their ability to mimic many of the natural properties displayed by their source cells. This top-down engineering approach can be applied toward the development of novel therapeutic strategies owing to the unique interactions enabled through the retention of complex antigenic information. Herein, we report on the biological functionalization of polymeric nanoparticles with a layer of membrane coating derived from cancer cells. The resulting core–shell nanostructures, which carry the full array of cancer cell membrane antigens, offer a robust platform with applicability toward multiple modes of anticancer therapy. We demonstrate that by coupling the particles with an immunological adjuvant, the resulting formulation can be used to promote a tumor-specific immune response for use in vaccine applications. Moreover, we show that by taking advantage of the inherent homotypic binding phenomenon frequently observed among tumor cells the membrane functionalization allows for a unique cancer targeting strategy that can be utilized for drug delivery applications.



KEYWORDS: Nanomedicine, biomimetic nanoparticle, cellular membrane, cancer immunotherapy, targeted drug delivery, homotypic targeting

Nanoparticle technology has enabled a wide array of improvements in the treatment of cancer, ranging from improved efficacy in cancer drug delivery^{1,2} to enhanced immunogenicity of cancer vaccines.³ More recently, there has been interest in leveraging the increased understanding of biological systems to make nanoparticles with new and enhanced functionalities.^{4–6} The inspiration behind this pursuit lies in the fact that natural components have evolved very specific functions over time, and these are difficult to fully recreate with synthetic materials. The unique properties displayed by different cell types can, in large part, be attributed to the complex antigenic profile present on their membranes. Identification of individual membrane factors has enabled researchers to enhance synthetic platforms with biomimetic features for specific applications such as advanced drug delivery.^{7,8} Membrane-bound tumor antigens have also been used to train the immune system to recognize and fight cancers,⁹ and cancer-mimicking particulate vectors decorated with these surface antigens have been prepared to improve vaccine potency.^{10,11} These examples demonstrate the vast potential of biomolecule functionalized nanoparticles, the design and development of which continue to benefit from increased understanding of cell surface markers.

Among the different bioinspired strategies, utilization of cellular membrane material for nanoparticle preparation

presents a unique top-down approach that offers the advantage of being able to completely replicate the surface antigenic diversity of source cells.^{12,13} This approach, which involves fashioning cellular membranes directly into nanoparticle form, circumvents the labor-intensive proteomics¹⁴ and the engineering hurdles behind multivalent nanoparticle functionalization.¹⁵ Using this emerging approach, researchers have successfully created nanoparticles possessing many desirable features. Examples include RBC membrane-cloaked nanoparticles with long-circulating properties,¹² stem cell-derived “nanoghosts” with cancer targeting capabilities,¹⁶ and leukocyte membrane-coated silica microparticles with the ability to traverse endothelium.¹⁷ This strategy has also given rise to novel applications that transcend traditional therapeutic motifs, such as in the case of toxin nanosponges that exploit particle-stabilized RBC membranes to neutralize pore-forming virulence factors.^{18,19} The work done thus far in this field has provided a glimpse of the new possibilities enabled by cell membrane-derived nanoformulations.

In this study, we functionalize biodegradable polymeric nanoparticles with cancer cell membrane and demonstrate that

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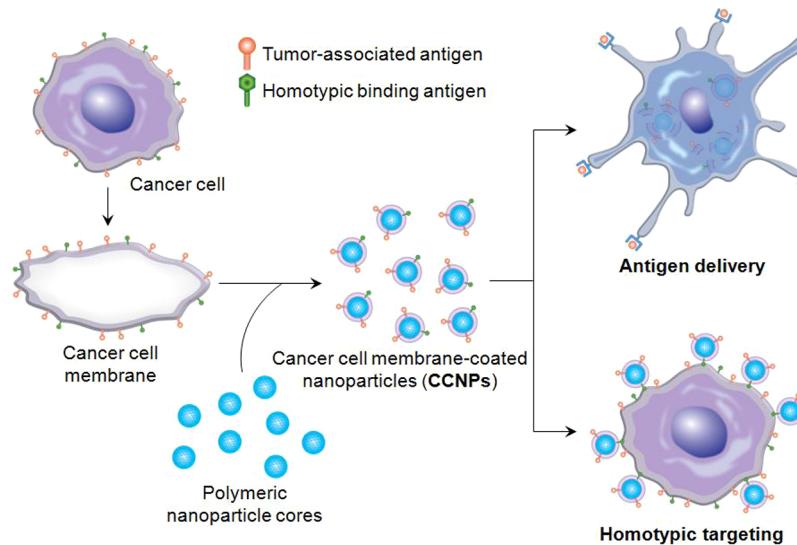


Figure 1. The cancer cell membrane-coated nanoparticle (CCNP). Schematic representation of CCNP fabrication and two potential applications. Cancer cell membrane along with its associated antigens is collected from source cancer cells and coated onto polymeric nanoparticle cores made of poly(lactic-*co*-glycolic acid) (PLGA) polymer. The resulting CCNPs can then be used to deliver tumor-associated antigens to antigen presenting cells or to homotypically target the source cancer cells.

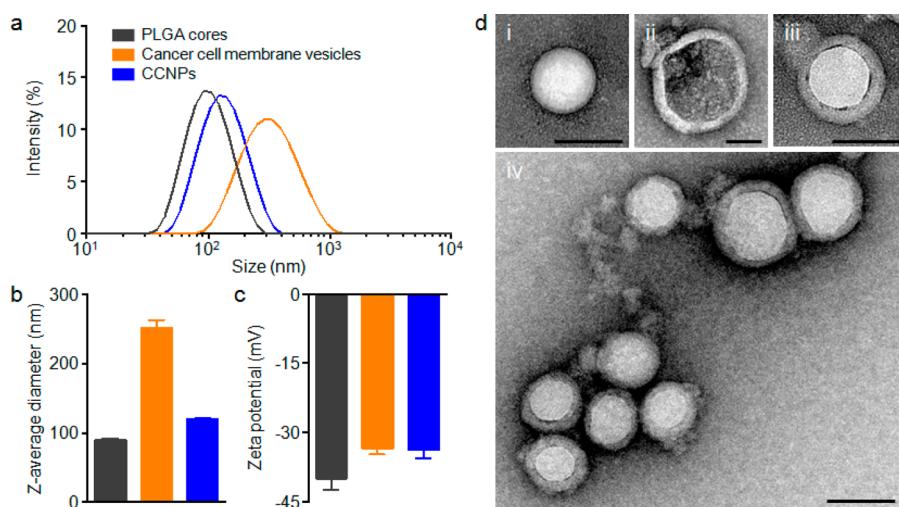


Figure 2. CCNP physicochemical characterization. (a) Size intensity curves of PLGA cores, cancer cell membrane vesicles, and CCNPs measured by dynamic light scattering (DLS). (b) Hydrodynamic size of PLGA cores, cancer cell membrane vesicles, and CCNPs. Bars represent means \pm SD ($n = 3$). (c) Surface zeta potential of PLGA cores, cancer cell membrane vesicles, and CCNPs. Bars represent means \pm SD ($n = 3$). (d) Transmission electron micrographs of (i) a PLGA core, (ii) a cancer cell membrane vesicle, (iii) a CCNP, and (iv) multiple CCNPs. Samples were negatively stained with uranyl acetate. All scale bars = 100 nm.

the resulting particles possess an antigenic exterior closely resembling that of the source cancer cells. These cancer cell membrane-coated nanoparticles (CCNPs) provide a platform that can be used toward the development of two distinct anticancer modalities (Figure 1). We demonstrate that these CCNPs allow membrane-bound tumor-associated antigens, together with immunological adjuvants, to be efficiently delivered to professional antigen presenting cells to promote anticancer immune responses. In addition, as the CCNPs possess the same cell adhesion molecules as their source cells, they are shown to exhibit source cell-specific targeting that reflects the homotypic binding mechanism frequently observed in cancers.^{20,21} Given the polymeric core's capacity to encapsulate therapeutic payloads,²² the platform presents an innately targeted nanocarrier for cancer drug delivery.

Ultimately, the studies presented demonstrate the broad applicability of the cell membrane coating approach for nanoparticle functionalization, which bridges the properties of natural membrane components with those of synthetic nanomaterials.

Synthesis and Characterization of CCNPs. In order to fabricate CCNPs, purified cancer cell membrane was first collected. Using B16-F10 mouse melanoma cells as a model cancer cell line, membrane derivation was achieved by emptying harvested cells of their intracellular contents using a combination of hypotonic lysing, mechanical membrane disruption, and differential centrifugation. With the collected membrane, cancer cell membrane vesicles were then formed by physical extrusion through a 400 nm porous polycarbonate membrane. Concurrently, poly(lactic-*co*-glycolic acid) (PLGA),

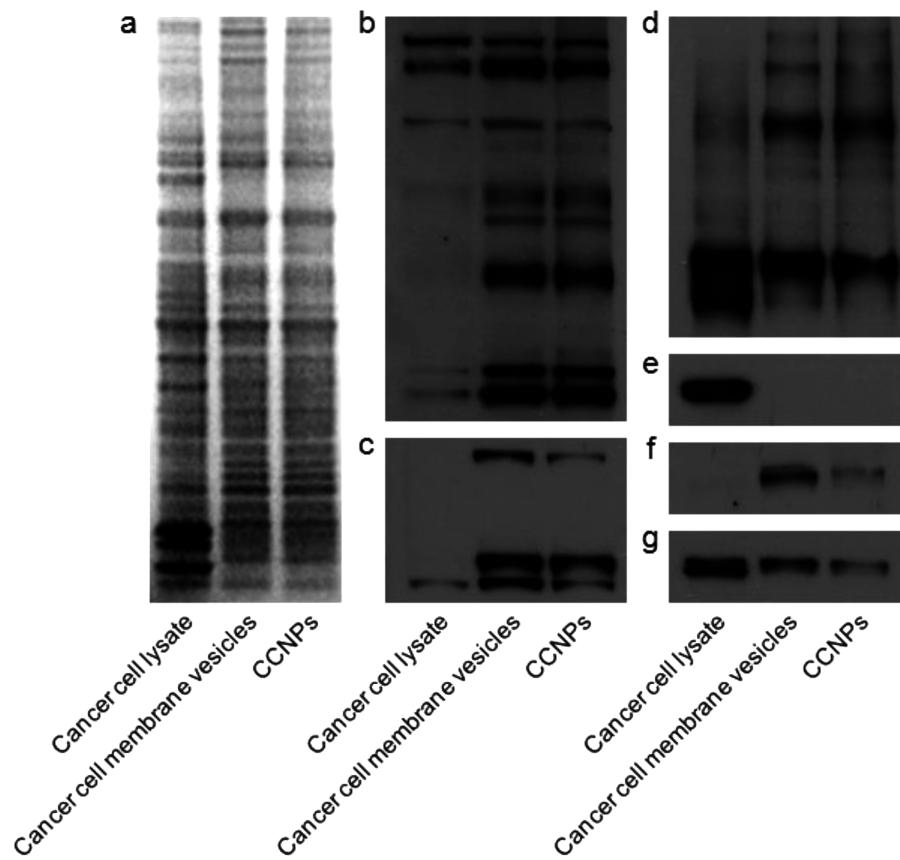


Figure 3. CCNP membrane antigen characterization. (a) SDS-PAGE protein analysis of cancer cell lysate, cancer cell membrane vesicles, and CCNPs. Samples were run at equal protein concentration and stained with Coomassie Blue. (b–g) Western blotting analysis for membrane-specific and intracellular protein markers. Samples were run at equal protein concentration and immunostained against membrane markers including (b) pan-cadherin, (c) Na⁺/K⁺-ATPase, and (d) gp100, and intracellular markers including (e) histone H3 (a nuclear marker), (f) cytochrome c oxidase (a mitochondrial marker), and (g) glyceraldehyde 3-phosphate dehydrogenase (a cytosolic marker).

an FDA-approved polymer, was used to prepare cores through a well-studied nanoprecipitation process.²³ Briefly, PLGA dissolved in acetone was added into water as an antisolvent, resulting in the spontaneous formation of polymeric nanoparticle cores. The acetone was then allowed to evaporate. In order to coat the PLGA cores with cancer cell membrane, the two components were coextruded through a 200 nm porous polycarbonate membrane. As measured by dynamic light scattering, the membrane vesicles were under 300 nm in hydrodynamic diameter. Upon fusion of the membrane vesicles with the polymeric cores, the final CCNPs were approximately 110 nm in size (Figure 2a,b). Zeta potential measurements suggested successful coating, as the surface charge of the PLGA cores increased to approximately the level of the membrane vesicles after being coated (Figure 2c). Membrane coating around the polymeric core was visualized using transmission electron microscopy (TEM), and the final CCNPs were spherical in shape and exhibited a core–shell structure upon negative staining with uranyl acetate (Figure 2d). From the TEM micrographs, the membrane coating appeared to be consistent as there were few uncoated PLGA cores. Upon the basis of our previous studies on RBC membrane-coated nanoparticles,¹³ the coating on the CCNPs is expected to occur as the faithful translocation of the membrane bilayer structure onto the nanoparticle surface, resulting in a right-side-out conformation that allows the membrane to retain its ability to interact with the environment while providing stability.

Validation of Cancer Cell Membrane Antigen Functionalization. Analysis of the protein content on the CCNPs was carried out to confirm successful functionalization of the nanoparticles with cancer cell membrane antigens. Gel electrophoresis followed by protein staining showed modulation of the protein profile when comparing the purified membrane material to raw cell lysate (Figure 3a). CCNPs were purified by centrifugation in order to separate out the coated particles from free vesicles, and their protein profile matched closely with that of the purified membrane. To confirm the presence of specific antigens on the CCNPs, Western blotting analysis was conducted on a series of membrane and intracellular protein markers (Figure 3b–g). There was a significant enrichment of cadherins and Na⁺/K⁺-ATPase, both plasma membrane-specific markers, in the final CCNP formulation. Glycoprotein 100 (gp100), a widely reported transmembrane protein that is a tumor-associated antigen for melanoma,⁹ was also present on the purified CCNPs. Conversely, protein markers for the nucleus, mitochondria, and cytosol were lowly present on the final nanoparticles, demonstrating preferential retention of membrane antigens through the fabrication process.

Optimization of Membrane Coating and CCNP Stability Studies. In order to optimize the membrane coating, CCNPs were synthesized at membrane-to-core weight ratios ranging from 0.125 to 4 mg of membrane protein per 1 mg of PLGA particles (Figure 4a). At lower membrane-to-core ratios,

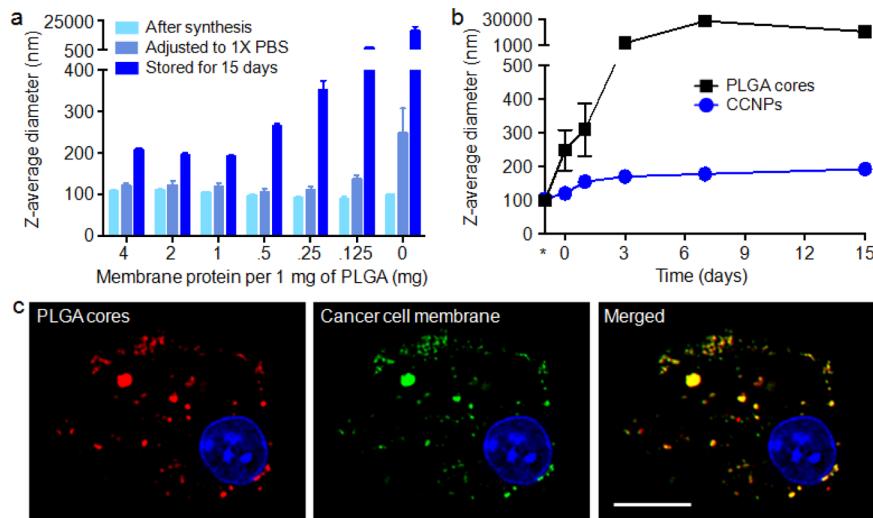


Figure 4. CCNP optimization and stability. (a) Hydrodynamic size as measured by DLS of CCNPs at varying membrane protein to PLGA weight ratios right after synthesis, after adjusting to 1× PBS, and after storage for 15 days in PBS. Bars represent means \pm SD ($n = 3$). (b) Stability of CCNPs made at a membrane-to-core ratio of 1 mg protein per 1 mg PLGA versus bare PLGA cores over time. * = particles before adjusting to 1× PBS. Symbols represent means \pm SD ($n = 3$). (c) Colocalization of PLGA cores and cancer cell membrane upon cellular uptake. CCNPs were fabricated with PLGA cores loaded with DiD (red channel) and membrane labeled with FITC (green channel). The nucleus was stained with DAPI (blue channel). All channels were deconvolved by software to eliminate out of focus fluorescent signal. Yellow color represents colocalization of the core and the membrane signals. Scale bar = 10 μ m.

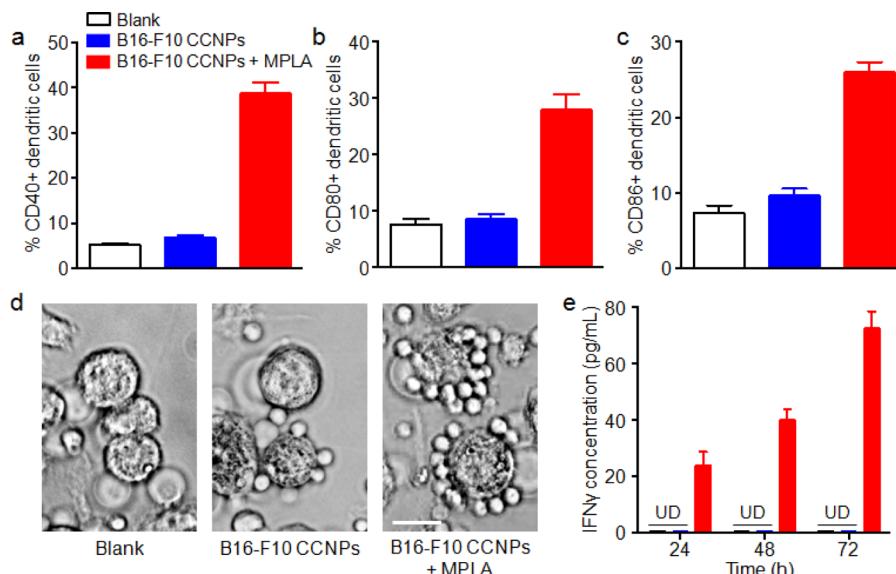


Figure 5. CCNPs for the delivery of tumor-associated antigens. (a–c) Maturation of dendritic cells. Dendritic cells were pulsed with blank solution, CCNPs derived from B16–F10 cells, or CCNPs with MPLA as an adjuvant for 48 h. Afterward the cells were immunostained with antibodies against CD11c as a dendritic cell marker and (a) CD40, (b) CD80, or (c) CD86 as a maturation marker and analyzed by flow cytometry. For the analysis, CD11c+ cells were gated first before gating on the maturation markers. (d,e) Antigen-specific T-lymphocyte stimulation. Dendritic cells pulsed with blank solution, CCNPs, or CCNPs with MPLA for 24 h were then cocultured with splenocytes derived from pmel-1 transgenic mice. (d) Phase contrast microscopy image of cells 72 h after coculture. T-lymphocytes can be seen clustering around dendritic cells. Scale bar = 25 μ m. (e) Specific response against the presentation of a melanoma-associated gp100 antigen was assayed using an ELISA for IFNy at 24, 48, and 72 h after coculturing. UD = undetectable by the ELISA (bars were made visible to distinguish samples). Bars represent means \pm SD ($n = 3$).

a significant increase in the hydrodynamic diameter was observed when the particles were transferred to 1× PBS. This suggested incomplete coverage, which exposes the surfaces of the cores to charge screening,²⁴ resulting in low stability in ionic buffers. This effect was even more pronounced after 15 days of storage, as samples with membrane coverage lower than 0.25 mg of protein per 1 mg of PLGA aggregated significantly. The lowest membrane-to-core ratio at which the particles maintained size stability over time was around 1 mg of protein

per 1 mg of PLGA. At this ratio, there was minimal size increase throughout the 15 days of observation (Figure 4b). To further test for the long-term storage capacity of the CCNPs, the particles were lyophilized in 5 wt % sucrose solution (Supporting Information Figure S1). Upon reconstitution in water, the particles exhibited a hydrodynamic size consistent with that prior to freeze-drying.

Integrity of the membrane coating around the polymeric cores was studied by observing the CCNPs upon cellular

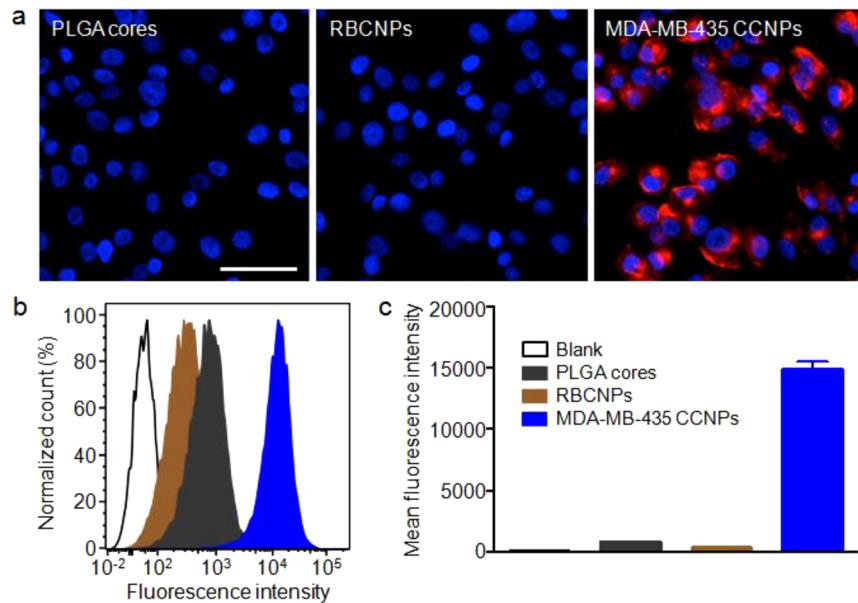


Figure 6. CCNPs as a homotypically targeted delivery vehicle. (a) Fluorescent imaging of MDA-MB-435 cells incubated with PLGA cores, RBC membrane-coated PLGA nanoparticles (RBCNPs), or CCNPs coated with membrane derived from MDA-MB-435 cells. All samples were loaded with DiD (red channel). After 30 min incubation, particles were washed away and the cells were incubated for another 1 h in fresh media before imaging. Nuclei were stained with DAPI (blue channel). All images were taken with the same exposure time for all channels and subjected to the same post acquisition normalization. Scale bar = 50 μ m. (b) Flow cytometric analysis of MDA-MB-435 cells incubated with blank solution, PLGA cores, RBCNPs or CCNPs. All particles were loaded with DiD fluorescent dye and subject to the same incubation conditions as in (a). At the conclusion, cells were detached with trypsin-EDTA for analysis. Histograms are representative of experiments done in triplicate. (c) Quantification of the mean fluorescence intensities of the histograms in (b). Bars represent means \pm SD ($n = 3$).

uptake. PLGA cores were loaded with a far-red fluorescent dye while the membrane proteins were tagged with a green fluorescent dye. In vitro imaging studies demonstrated that the CCNPs were efficiently taken up by bone marrow-derived mouse dendritic cells, allowing for the intracellular delivery of membrane protein antigens (Supporting Information Figure S2). This suggests a stabilization of the relatively fusogenic membrane material upon coating onto the nanoparticle substrate,¹⁸ which facilitates uptake through endocytic pathways.^{25,26} Upon uptake, the fluorescent signals from the cores and the membrane exhibited a high degree of colocalization (Figure 4c). The overlapping fluorescent signals suggest that the core–shell structure of the CCNPs was stable and remained intact upon cellular endocytosis.

Delivery of Tumor-Associated Antigens for Cancer Immunotherapy. The ability of the nanoparticles to deliver tumor antigens and induce dendritic cell maturation was tested by incubating CCNPs made using B16–F10 membrane with dendritic cells derived from C57BL/6 mice. Monophosphoryl lipid A (MPLA), an FDA-approved lipopolysaccharide derivative that binds to toll-like receptor 4 (TLR-4)²⁷ was incorporated with the CCNPs as an adjuvant to boost the immune response against the lowly immunogenic antigens found on the cancer membrane and had little effect on the final physicochemical characteristics of the particles (Supporting Information Figure S3). When incubated with dendritic cells, nanoparticle uptake was visualized by the darkening of the cells as observed under phase contrast microscopy (Supporting Information Figure S4). The CCNPs by themselves, which were derived from a cell line that originates from the exact same mouse strain as the dendritic cells, did not induce additional maturation compared with blank controls (Figure 5a–c). This lack of response indicates limited immunogenicity of the

syngeneic cancer cell membrane material despite being formulated into nanoparticle form, which is consistent with the fact that the B16–F10 cell line has been reported to be lowly immunogenic in C57BL/6 mice.²⁸ Only upon incorporation of MPLA with the CCNPs was a significant response observed with upregulation of the maturation markers CD40, CD80, and CD86 in the dendritic cells.

To confirm that the observed maturation was also coupled with the presentation of tumor antigen-specific epitopes, pulsed dendritic cells were cocultured with splenocytes derived from transgenic pmel-1 mice. T-cell receptors in pmel-1 mice have been genetically engineered to be specific toward a gp100 epitope,²⁹ and consequently their cytotoxic T-lymphocytes can only be stimulated by dendritic cells that properly present the tumor-associated antigen. Phase contrast microscopy showed significant crowding of T-lymphocytes around dendritic cells pulsed with CCNPs incorporated with MPLA (Figure 5d), which reflects the correct presentation of gp100 antigen fragments on the surface of the antigen presenting cells.³⁰ Quantification of the cytokine interferon-gamma (IFN γ), an indicator of cytotoxic T-lymphocyte stimulation, further demonstrated that CCNPs with MPLA were able to successfully elicit an antigen-specific response (Figure 5e). Taken together, the data indicates that the adjuvanted CCNPs were able to correctly deliver tumor-associated antigens to dendritic cells for immune processing, which allowed for the subsequent stimulation of tumor antigen-specific T-cells.

Homotypic Targeting Mediated by Cancer Cell Surface Antigens. To demonstrate the ability of CCNPs to homotypically target cancer cells for drug delivery applications, membrane from the human cell line MDA-MB-435, which has been extensively studied for its homotypic aggregation properties,^{20,21} was collected and coated onto fluorescent

dye-loaded PLGA cores. The membrane and particles were suspended in divalent cation chelator-containing solution throughout the process in order to prevent Ca^{2+} -dependent homotypic binding between particles.^{31,32} Using fluorescence microscopy, it was demonstrated that incubation of these CCNPs with cultured MDA-MB-435 cells *in vitro* resulted in significantly increased uptake as compared to both bare PLGA cores and red blood cell membrane-coated nanoparticles (RBCNPs) (Figure 6a). The decreased uptake of the RBCNPs compared to bare PLGA cores was consistent with previously reported findings using macrophages¹³ and reflects the modulation of cell-to-particle interactions upon membrane functionalization. In order to quantify the difference in uptake, flow cytometric analysis was carried out, and the results indicated that the MDA-MB-435 membrane coating enabled approximately 40-fold and 20-fold increases in uptake compared with RBCNPs and bare PLGA cores, respectively (Figure 6b,c). To further demonstrate that the binding effect was specific to the membrane coating, a heterotypic human foreskin fibroblast cell line was used as a negative control, and it was observed that MDA-MB-435 CCNPs exhibited little increased uptake compared to the bare PLGA cores (Supporting Information Figure S5). These results indicate that coating of nanoparticles with MDA-MB-435 membrane can preferentially increase the affinity of the particles to the source cancer cells, a functionality that can be attributed to the transference of cell adhesion molecules with homotypic binding properties.

The coating of cellular membrane onto nanoparticles as described in this work allows for particle functionalization with an exceptionally high concentration of antigenic material relevant for the replication of specific biological functions. The concomitant removal of nuclear components from the final formulation helps to alleviate the safety concerns regarding genetic material, especially in the case of sourcing membrane from tumorigenic cancer cells. As was observed with previously reported RBCNPs, the nanoparticle core and cancer cell membrane mutually benefit each other when combined into a core–shell structure.^{12,18} The PLGA cores, which can be used to load a wide array of cargoes, are unstable in physiological buffer, but can be stabilized when coated with sufficient membrane. The membrane, which is unstable when unsupported, becomes stabilized upon coating onto a nanoparticle substrate. For CCNPs, the enhanced stability of the two components together can be taken advantage of to promote efficient delivery and internalization of either antigenic or therapeutic material, enabling their use for the design of new anticancer nanotherapeutics.

Therapeutic cancer vaccines represent an emerging anti-cancer regimen that utilizes tumor-associated antigens to promote antitumor immune responses. One example is Provenge, the first FDA-approved cancer vaccine, which is a treatment based on the *ex vivo* pulsing of autologous dendritic cells with prostatic acid phosphatase, an antigen associated with a subset of prostate cancers.³³ Application of the gp100 or MART-1 tumor-associated antigens, combined with immunological adjuvants, has also shown promise in treating melanomas.^{9,34} While encouraging, vaccination approaches based on a single tumor-associated antigen can be inadequate when facing the high heterogeneity and mutation rate of cancer cells.³⁵ Much progress in multiantigen-based vaccination has also been made using cell lysates to prime the immune system against the complete antigenic profile of tumors.^{35,36} In these

formulations, however, treatment efficacy can be compromised by the large presence of intracellular, housekeeping proteins that divert focus away from the relevant antigens, which compose a small percentage of the total protein.³⁷ The present CCNP formulation was shown to be inherently enriched in membrane components, allowing for the delivery of tumor-associated antigens while bypassing the labor-intensive protocols required for individual antigen identification.^{38,39} Also of note is that stabilization of the cancer cell membrane on a nanoparticle substrate facilitated cellular uptake of membrane proteins (Supporting Information Figure S2) and likely contributed to enhanced antigen processing by dendritic cells, an important consideration in vaccine design. Such antigen stabilization by nanoparticles was also previously reported to benefit antigen processing *in vivo*, as the size of the particles can be tuned for enhanced localization to immune organs such as the lymph nodes.⁴⁰

The CCNP platform also allows facile coupling of cancer membrane antigens with immunological adjuvants. In the present study, a TLR-4 activator, MPLA, was used as a model adjuvant to raise the immunogenicity of the CCNP formulation. The correct priming of dendritic cells was demonstrated using gp100-specific spleen-derived lymphocytes, as dendritic cells pulsed with adjuvant-incorporated CCNPs showed visibly higher interactions with the lymphocytes and yielded significantly higher secretion of $\text{IFN}\gamma$ (Figure 5d,e), a hallmark of antigen-specific immunity development.⁴¹ The cargo-loading capacity of the PLGA polymeric core can also be applied to carry other adjuvants such as CpG oligodeoxynucleotides and poly(I/C) to further enhance vaccine potency via multivalent TLR activation.⁴² By enabling colocalization and codelivery of multivalent tumor antigens with immunological adjuvant, the CCNP platform can exploit the many unique properties of particulate vaccines to enhance immune responses.⁴³

Regarding drug delivery, CCNPs present a cancer-targeting strategy based on the intrinsic self-adhesive properties of cancer cell membranes. Currently, cancer targeting is achieved primarily via receptor–ligand interactions aimed at overexpressed surface antigens on cancer cells.^{1,44} Numerous targeting ligands have been successfully conjugated to nanoparticles, including antibodies, peptides, aptamers, and small molecules.⁴⁴ These targeted formulations have demonstrated increased tumoral accumulation⁴⁵ and encouraging treatment efficacy has been observed in clinical trials.⁴⁶ In developing novel targeting approaches, the inherent homotypic adhesion property of cancer cells has yet to be considered. It has been demonstrated that many cancer cells express surface antigens with homophilic adhesion domains, which are responsible for multicellular aggregate formation in tumors. For instance, carcinoembryonic antigen and galectin-3 have been identified as homophilic binding proteins frequently overexpressed on cancer cell surfaces for intercellular adhesion.^{47,48} By coating nanocarriers in cancer cell membranes, it is possible to take advantage of this cell-to-cell adhesion for cancer targeting.

In the present study, MDA-MB-435, a tumor cell line with a well-established homotypic binding mechanism that displays homotypic aggregation *in vivo*,^{21,49} was used to demonstrate the cancer targeting potential of CCNPs. Nanoparticles coated in MDA-MB-435 membrane showed significantly increased cellular adhesion to the source cells as compared to bare nanoparticles. It should be noted that structurally analogous RBCNPs showed reduced particle binding to MDA-MB-435

cells, indicating that homotypic cell membrane was responsible for the enhanced particle-to-cell adhesion (Figure 6). Additionally, the MDA-MB-435 CCNPs were shown to have little increase in affinity compared to bare PLGA cores when incubated with human foreskin fibroblasts (Supporting Information Figure S5), which further reflects the cancer cell-specific affinity of the nanoparticles. As CCNPs leverage the adhesive tendencies of cancer cells for targeting, they also have the potential to target distant body sites that are susceptible to cancer metastasis via heterotypic binding mechanisms to subsets of endothelial cells.²¹ It has been found that surface adhesion molecules are an important factor that dictates cancer cell dissemination and determines their metastatic propensities.^{20,21} Thus, by using cancer cell membrane for particle functionalization, it becomes possible to prepare nanocarriers with cancer-mimicking binding properties.⁵⁰ Such a platform can be applied to localize therapeutics directly to cancer cells or to distant sites in the body that are susceptible to metastases for the treatment of aggressive malignancies.

To conclude, coating polymeric nanoparticles with cancer cell membrane presents an effective method for introducing multiple membrane antigens and surface functionalities that are desirable but hard to achieve using traditional synthetic techniques. We have demonstrated successful cancer cell membrane isolation and particle functionalization, and the resulting CCNPs can be used for different modes of anticancer therapy. For cancer immunotherapy, the platform enables colocalization of multiple antigens together with immunological adjuvants in a stabilized particulate form, which facilitates the uptake of membrane-bound tumor antigens for efficient presentation and downstream immune activation. For anti-cancer drug delivery, the membrane coating can be applied to target the source cancer cells via a homotypic binding mechanism. Toward future translation, it is possible to derive cancer cell membranes from primary tumors to develop personalized CCNPs for anticancer treatments. The present study also demonstrates the robustness and versatility of the cell membrane coating approach for nanoparticle functionalization, which provides a feasible method to develop novel, nature-inspired nanotherapeutics with complex antigenic information and surface properties. It can be envisioned that the membrane coating technology presented herein can be further expanded to other cell types for different biomedical applications.

■ ASSOCIATED CONTENT

Supporting Information

Materials and methods and supplementary Figures S1–S5. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Author Contributions

R.F., C-M.H., B.L., and L.Z. conceived and designed the experiments; R.F., C-M.H., B.L., W.G., J.C., Y.T., and D.O. performed all the experiments. R.F., C-M.H., B.L., W.G., J.C., and L.Z. analyzed and discussed the data and wrote the paper. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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