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Leveraging Engineering of Cells for Drug Delivery

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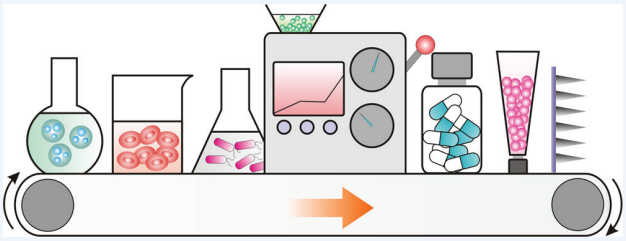
CONSPECTUS: Cell therapy has become a momentum-gathering treatment strategy for a variety of diseases, including cancer, diabetes, hemophilia, and cardiomyopathy. However, clinical applications of conventional cell therapies have often been compromised by rapid decline in viability and function of the transplanted cells due to host recognition and subsequent foreign body rejection. Along this line, cell engineering technologies such as cell encapsulation within microcapsules and immobilization in porous scaffolds have been implemented to address the immunosuppression concerns. As a recent emerging research topic, drawing inspiration from the ways that natural cells interact with the body has opened new avenues for cell engineering, such as direct modification of whole cells with synthetic materials and "top-down" integration of biological membranes with micro/nanomaterials, which aim to alleviate immune response while harnessing the complex biological functions of cells.



In this Account, we summarize our recent contribution to the field of cell engineering methodologies, with which we have demonstrated their promising applications for cancer immunotherapy, targeted drug delivery, and blood glucose regulation. For example, inspired by the inherent ability of platelets to accumulate at wound sites and interact with circulating tumor cells, we exploited a targeted checkpoint antibody delivery strategy for treatment of postsurgical cancer recurrence and metastatic spread by covalent binding of platelets' cell surfaces with a monoclonal antibody against programmed-death ligand 1 (aPDL1). Without interfering with the platelets' surgical-site homing property, the conjugated aPDL1 could be triggered to release in the form of microparticles after in situ activation. As an extension, we then engineered the platelet membrane to cloak nanoparticles for anticancer drug delivery, mimicking the targeting capability of the source cells while possessing prolonged circulation lifetime and insignificant immunogenicity. At the same time, we also found that the subcellular compartment membrane-derived particulates exhibited high specificity toward homotypic cells, by which enhanced intracellular drug delivery was achieved. Moreover, by taking advantage of the reversible interaction between glucose-derivative-modified insulin and the red blood cell membrane, we constructed a glucose-responsive smart insulin delivery system for long-term maintenance of blood glucose levels within a normal range. Recently, by virtue of painless microneedle patches as convenient cell engineering platforms, a minimally invasive intradermal antitumor vaccine was invented by integrating whole-tumor lysis into near-infrared light-illuminated microneedle patches. The microneedle patches also showed promise in combining with conventional cell encapsulation techniques, by which an externally positioned β-cell engineering strategy was proposed for diabetes treatment. The results presented in this Account demonstrate distinct approaches to the development and application of cell engineering strategies for drug delivery.

1. INTRODUCTION

Cell therapy holds tremendous promise for treating various diseases. For instance, genetically engineering isolated T cells from patients themselves with chimeric antigen receptors (CARs) has redirected the T cell specificity and opened a new era for cancer therapy.[1] Exciting news is that CD19-specific CAR T cells have recently been approved by the U.S. Food and Drug Administration for treating children and young adults with B-cell acute lymphoblast leukemia.[1] Moreover, clinical studies on cell transplantation are under investigation for treating cell function deficiencies.[2,3] In addition, novel strategies are currently being developed in various stages to leverage stem cells for tissue engineering.[4] However, these approaches are often limited by the difficulty in supplying clinical-scale demand of donor cells as well as the lifelong need for immunosuppressive or other adjuvant drugs that are used to boost the cell efficiency but have adverse effects.[5] To this end, cell engineering methods aiming to address the concerns of rejection by host immune systems by immunoisolation of the cells with microcapsules or three-dimensional scaffolds have been proposed.[6−8] Although promising, challenges arise with the foreign-body response induced by the implant materials, which are manifested by the recruitment of immune cells, formation of fibrous deposits, hindered passage of nutrients, and eventual failure of the cells.[8] Besides, additional work is

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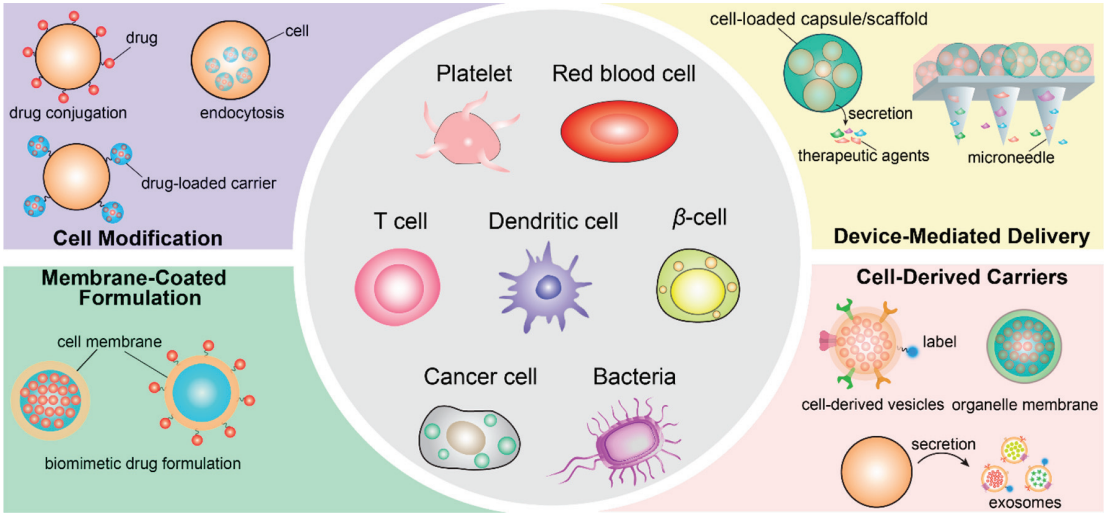

Figure 1. Schematic illustration of the recent advances in cell engineering technologies from different perspectives for drug delivery applications.

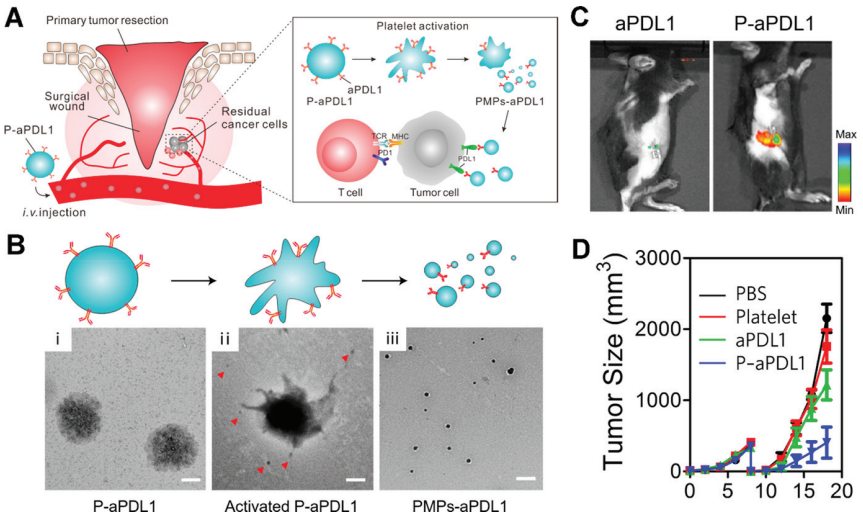

Figure 2. (A) Schematic illustration of delivery of an aPDL1-conjugated platelet to the surgical wound site. (B) Transmission electron microscopy (TEM) images of P-aPDL1 (i) before and (ii) after activation and (iii) release of PMPs (scale bars: 0.5 μm). (C) In vivo fluorescence imaging of mice 2 h after intravenous injection of free aPDL1-Cy5.5 (left) and P-aPDL1-Cy5.5-conjugated platelets (right). (D) Sizes of recurrent tumors with different postsurgical treatments. Error bars represent the standard error of the mean. Reprinted with permission from ref 26. Copyright 2017 Nature Publishing Group.

required in order to assess the accuracy of transplantation and the extent of the engrafted cells for optimizing the therapeutic outcome.[9] Therefore, further transformative technologies for cell engineering are in high demand for biomedical applications.

In the body system, the ability of cells to communicate with other cells and interact with the environment allows them to perform complex tasks and adapt sophisticated biological entities. As one of the classic examples, the various proteins presented on the membrane of red blood cells (RBCs) facilitate information exchange with phagocytes, which relieves the attack by the complement system and thus leads to prolonged circulation time.[10] Moreover, the recruitment of specific cells in response to chemokines has been demonstrated to contribute to immune reactions, disease development, and tissue construction.[11] Taking lessons from these processes, attempts have been prompted to transfer therapeutic constructs into natural cells to devise the next generation of delivery platforms.[11−13] As a further evolution, "top-down" procedures in which synthetic nanomaterials are camouflaged with cellular membranes have been proposed to recreate the key functions of the original cells for enhancing the utility of nanomaterials.[14−16] These cell engineering approaches not only alleviate the host immune response but also retain the complexity and, most importantly, the biological functions of the natural cells, endowing augmented therapeutic efficacy. This Account will focus on recent studies from our laboratory directed toward newly developed cell engineering technologies for applications in cancer immunotherapy, targeted drug delivery, and diabetes treatment (Figure 1).

2. CELL ENGINEERING FOR CANCER IMMUNOTHERAPY

Cancer immunotherapy, which boosts the immune system response to tumor cells, has emerged as a new pillar in the war against cancers.[17] Among the various treatment strategies, engineering of immune cells by chemical methods and/or with

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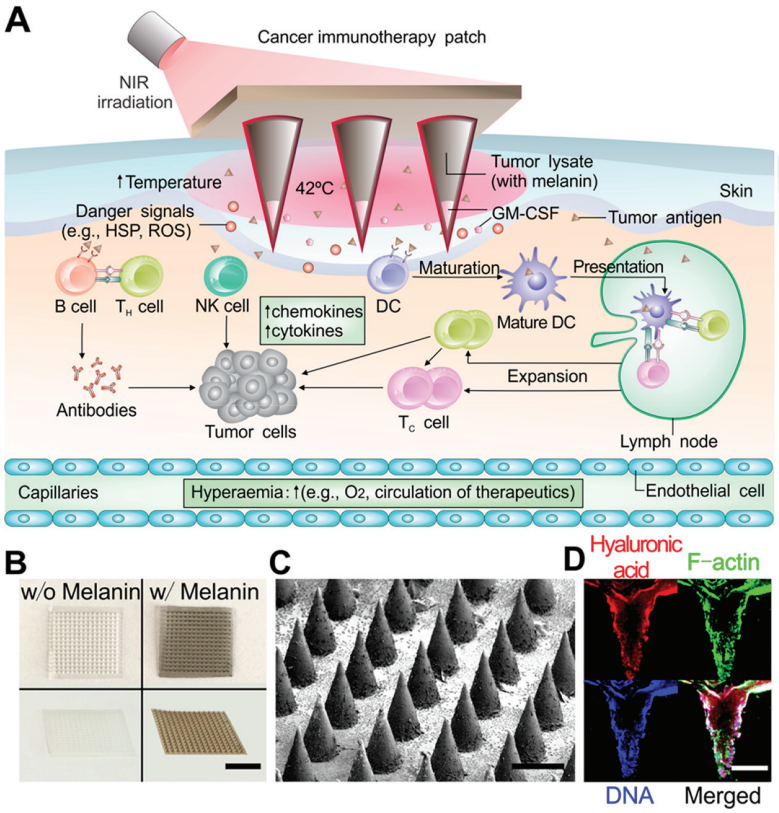

Figure 3. (A) Schematic illustration of engineering tumor lysate with microneedle patches for transdermal vaccination. Th cell: T helper cell. Tc cell: cytotoxic T cell. (B) Photograph of microneedle patches without (w/o) and with (w/) melanin (scale bar: 4 mm). (C) Scanning electron microscopy image of the microneedle patch. (D) Cross-sectional fluorescence images to show the lysate inside the microneedle patches: actin filaments, DNA fragments, and the hyaluronic acid matrix were visualized by Alexa Fluor 488−phalloidin (green), Hoechst (blue), and rhodamine (red), respectively (scale bar: 200 μm). Reprinted with permission from ref 28. Copyright 2017 American Association for the Advancement of Science.

synthetic nanomaterials without altering the key cellular functions has received considerable attention for enhancing cancer immunotherapy.[18] For instance, to overcome the rapid loss of T cell effector function in adoptive cell therapy (APC), direct surface conjugation of T cells with nanoparticles encapsulating cytokines or immunomodulators not only enabled the sustainable stimulation of the T cells after transplantation but also avoided the side effects induced by high doses of adjuvants given systematically.[5,19,20] Such engineering strategies offer a new paradigm in modulating signaling events at T cell−APC interfaces. In the development of therapeutic cancer vaccines, engineering of dendritic cells (DCs) by in vitro endocytosis of antigen-conjugated nanoparticles with bioimaging functionalities has been validated to enhance antigen-specific T cell response to inhibit tumor growth while simultaneously allowing for tracking the fate of DCs during the processes.[21,22]

In addition, checkpoint blockade immunotherapy, which works by interfering with the inhibitory pathways in T cells, has elicited durable clinical responses and long-term remissions in treating different types of cancer; however, insufficient lymphocyte infiltration and off-target binding of intravenously administered checkpoint inhibitors remain the bottlenecks in further improving the effectiveness.[23] Inspired by the inherent ability of platelets to home in on vascular injury at wound sites, interact with circulating tumor cells (CTCs), and modulate immune responses (discussed below),[24,25] our group exploited a checkpoint inhibitor delivery platform for postsurgical cancer immunotherapy by surface conjugation with aPDL1 (P-aPDL1) (Figure 2).[26] Once activated in vitro, aPDL1 could be released in the form of platelet plasma membrane-derived microparticles (PMPs) (Figure 2B), accompanied by the release of several pro-inflammatory cytokines. After intravenous injection, P-aPDL1 was enriched at the surgical wounds around the residual tumors (Figure 2C), highlighting the merits of the injury-homing nature of platelets in immune checkpoint delivery. In vivo therapeutic performance substantiated that the release of aPDL1-tagged PMPs and various inflammatory factors synergistically boosted the activation of T cells for destroying tumor cells with high efficiency (Figure 2D). Moreover, the potential of P-aPDL1 for treating tumor metastasis was demonstrated by subsequent intravenous injection of B16F10 cells and antibodies. The versatility of this engineering technique was proved by further inhibiting the recurrence of triple-negative breast cancer models. This strategy of engineering platelets with immune checkpoint inhibitors provided an effective and generalizable strategy for targeted delivery of antibodies to augment their immunotherapeutic potential in preventing postsurgical cancer recurrence while limiting side effects.

The rich reactive chemical groups on cell surfaces, including thiols, amines, and carboxylates, provide sufficient sites for covalent conjugation of synthetic materials. Also, nano−bio interface interactions such as cellular uptake of nanomaterials

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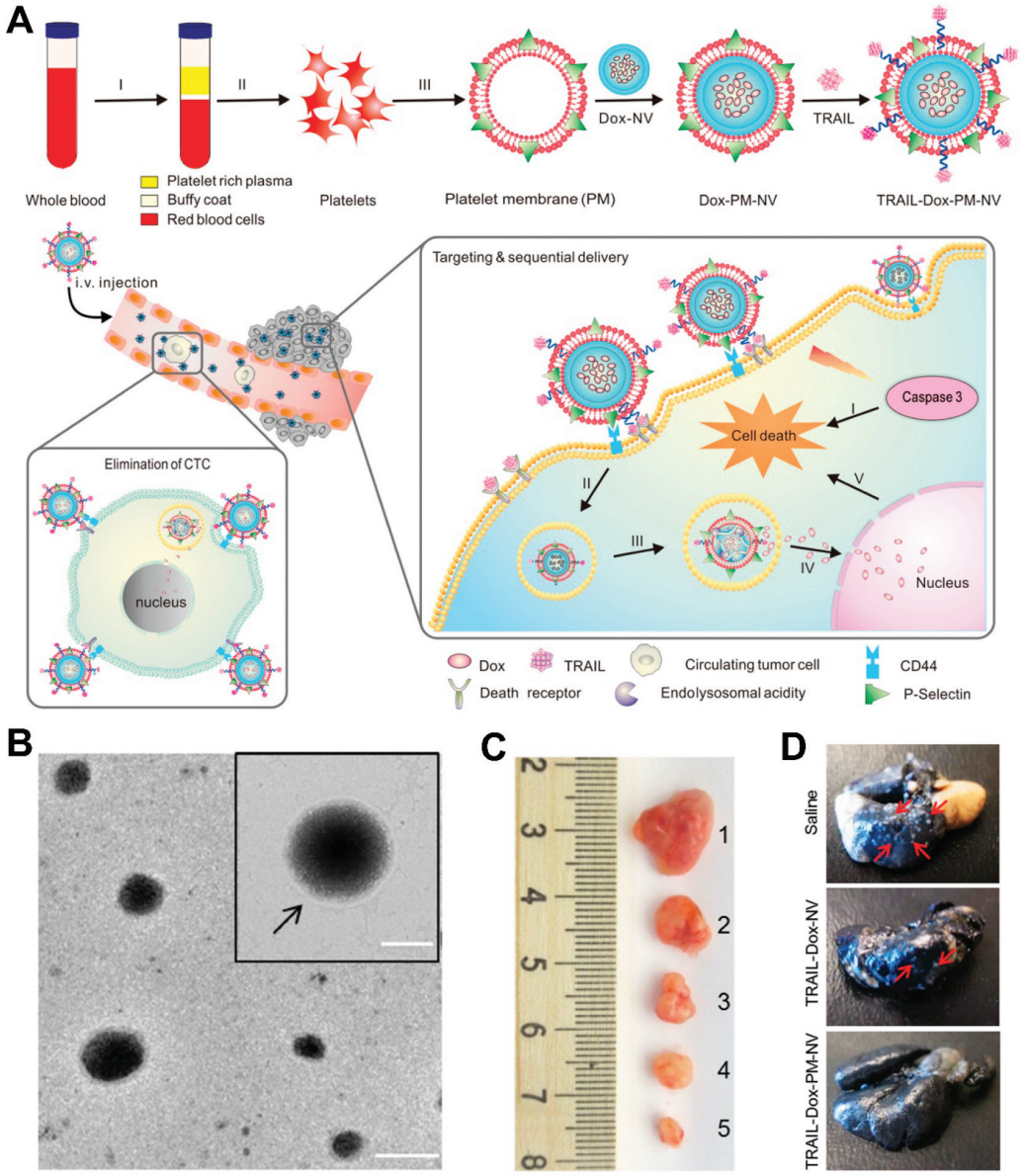

Figure 4. (a) Schematic design of the platelet-mimicking drug delivery system. (b) TEM characterization of PM-NV. The black arrow indicates the presence of the platelet membrane. Scale bars: 100 nm and (inset) 50 nm. (c) In vivo antitumor efficacy of TRAIL-Dox-PM-NV. Shown are tumors obtained upon treatment with (1) saline, (2) TRAIL-Dox-NV, (3) TRAIL-PM-NV, (4) Dox-PM-NV, and (5) TRAIL-Dox-PM-NV. (d) In vivo antitumor metastasis efficacy. Red arrows indicate the visible metastatic foci. Reprinted with permission from ref 31. Copyright 2015 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

can be explored to equip cells with different functions. Notably, state-of-the-art bioorthogonal chemistry, which has shown great viability in selective modification of biological species,[27] may offer new avenues to this field. These methods are convenient and robust to impart cells with new functions while maintaining the cell's own biological competence, which can be considered as powerful complements to traditional genetic engineering technologies in enriching living cell functions.

Besides engineering living cells, we recently engineered B16F10 whole-tumor lysate (containing melanin) for cancer immunotherapy by integrating it with granulocyte-macrophage colony-stimulating factor (GM-CSF) into near-infrared light (NIR)-irradiated microneedle patches (Figure 3).[28] The sustained transdermal delivery of GM-CSF could effectively recruit and activate DCs, which consequently internalized the released lysate and presented the tumor antigens. Meanwhile, the NIR irradiation stimulated the melanin to generate a high local temperature, which further facilitated migration of DCs and other immune cells to the vaccination sites by elevating local microcirculatory blood/lymphatic perfusion and releasing danger signals, including reactive oxygen species and inflammatory cytokines. In this way, increased filtration of polarized T cells and topical release of cytokines were elicited, and B16F10-specific immune response was finally initiated. The feasibility of the vaccine design was demonstrated by the high survival level of mice after tumor challenge as well as the antitumor effects toward primary and distant tumors.

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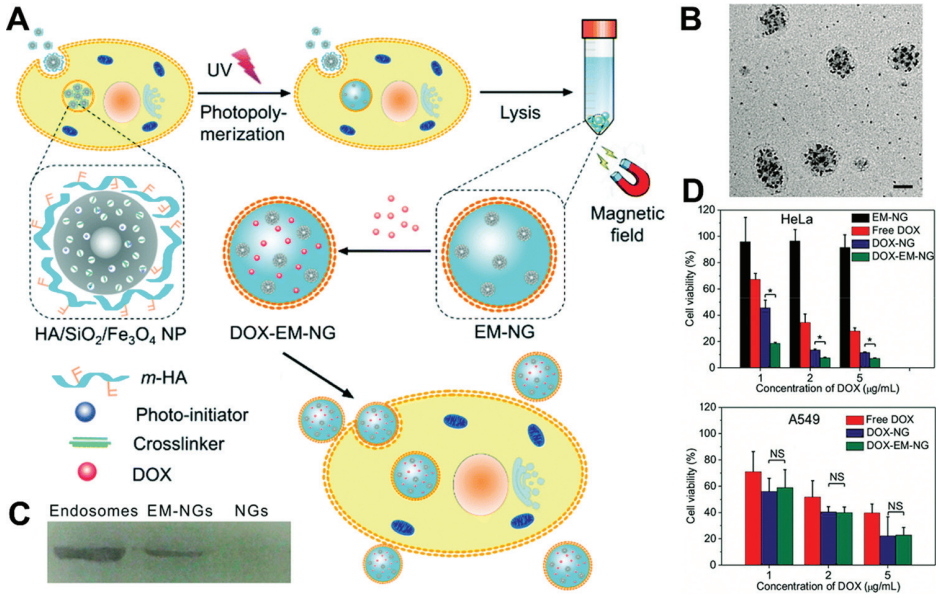

Figure 5. (A) Schematic illustration of the process of engineering nanoparticles with endosome membranes for homotypic cell-targeted drug delivery. (B) TEM image of EM-NGs (scale bar: 200 nm). (C) Western blotting analysis of endosomes, EM-NGs, and bare NGs against early endosome antigen 1. (D) In vitro cytotoxicities of DOX-EM-NG toward (top) HeLa and (bottom) A549 cells. *, $p < 0.05$; NS, not significant. Reprinted with permission from ref 38. Copyright 2016 Royal Society of Chemistry.

3. ENGINEERING NANOMATERIALS WITH BIOMEMBRANES

The recent "top-down" engineering of cellular membranes to camouflage nanomaterials is yielding insights that are aiding in the design of biomimetic platforms to recapitulate natural cellular biological functions, such as targeting and immune evasion.[29] To date, many cell types, including leukocytes, cancer cells, stem cells, bacteria, and platelets, have been utilized as membrane source cells, each with a set of its own specific properties.[30] This section will focus on our recent studies on engineering synthetic nanocarriers with biomembranes to impart local therapeutic effects.

3.1. Platelet Membrane

As one of the most important bioparticulates in the body, platelets actively participate in various biological processes, including hemostasis, wound healing, and immune response.[24,25] For instance, platelets can augment immunity by targeting the injury site and releasing pro-inflammatory factors to recruit leukocytes.[25] Also, recent studies have revealed that platelets can facilitate tumor metastasis by aggregating on the CTCs to camouflage them from immune attack and migrate to new tissue for tumor development, in which biomolecular binding between P-selectin and the CD44 receptor plays the key role.[24]

As a starting point, we leveraged advantage of the interactions between platelets and cancer cells to develop a platelet-mimicking drug delivery system (Figure 4A).[31] We utilized the "top-down" method to wrap the platelet membranes onto the surface of doxorubicin (Dox)-loaded nanogels, where the translocation of abundant surface-associated proteins enabled replication of the specific interactions between platelets and cancer cells. Afterward, the platelet membranes were decorated with tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). Mediated by the specific interaction between P-selectin on the platelets and CD44 receptors on the cancer cells, the platelet-membrane-coated nanovehicle (PM-NV) (Figure 4B) could efficiently target the surface of cancer cells to facilitate the therapeutic efficacy of TRAIL. Upon internalization, the nanogel inside the PM-NV composite containing acid-responsive cross-linkers could dissociate inside the acidic endosomes, releasing the loaded drugs. This sequential drug delivery fashion finally led to synergistic cytotoxicity. Moreover, the preservation of an abundant "self-recognition" protein like CD47 on the membrane minimized the uptake by microphage cells and prolonged the in vivo circulation time.[32] Using the MDA-MB-231 tumor model, we demonstrated the superior anticancer potency of PM-NV, which was attributed to the selective cancer homing capability of PM-NV and synergistic anticancer effect based on the site-specific delivery of TRAIL and Dox (Figure 4C). Moreover, the versatility of PM-NV was further confirmed by a reduction in the number of CTCs after intravenous administration, as evidenced by decreased lung metastasis (Figure 4D).

Following this study, we reported a relay drug delivery strategy to amplify the tumor targeting signals by inducing a local inflammation of tumor blood vessels.[33] On the basis of the inherent property of platelet homing to the injured blood vessel and specific interactions between platelets and cancer cells, a two-module delivery method was designed to maximize the anticancer treatment effect. The signaling transmission nanocarrier (NC_A) was made from an Arg-Gly-Asp peptide-functionalized polymeric nanoparticle loaded with TNFα, an inflammation inducer. The execution nanocarrier (NC_B) was composed of platelet-membrane-coated nanoparticles encapsulated with paclitaxel to receive the broadcasting inflammation signal and accumulate at the tumor site. NC_A was demonstrated to induce a strong inflammation response on human umbilical vein endothelial cells in vitro, as proved by upregulated cell adhesion molecules and increased release of cytokines. After in vivo administration, NC_A could selectively accumulate at the tumor site and trigger local inflammation of blood vessels.

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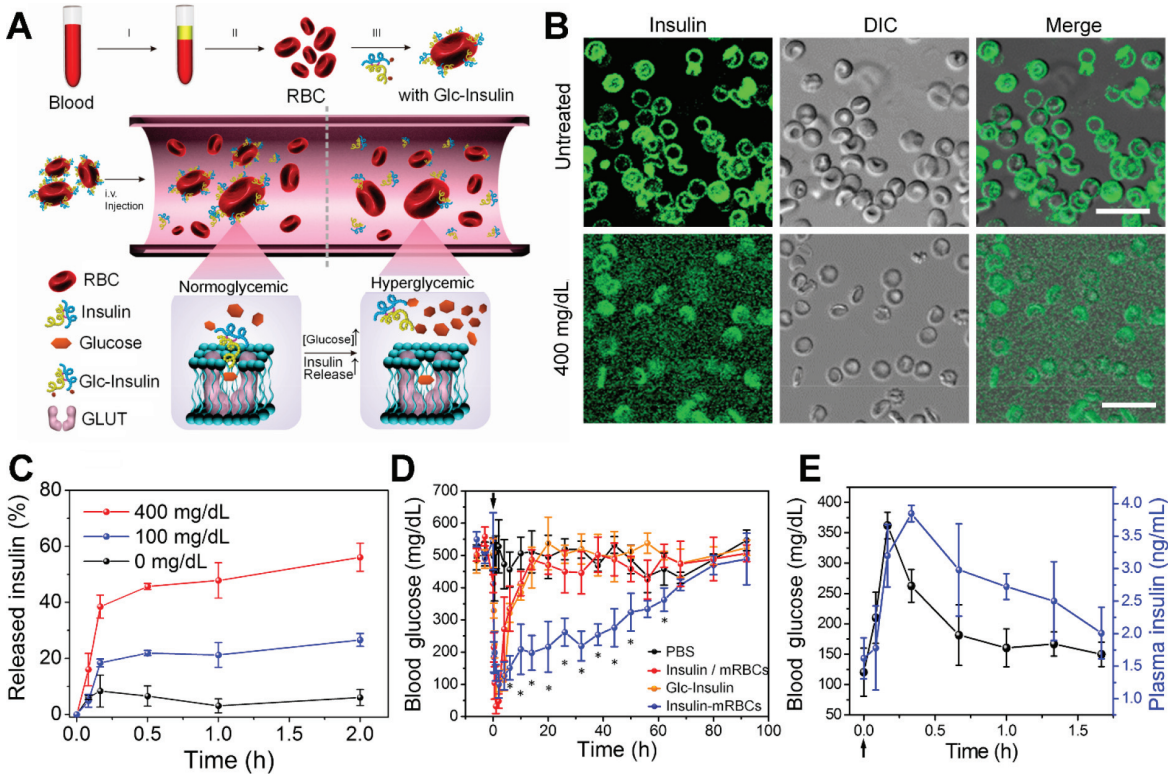

Figure 6. (A) Schematic illustration of engineering RBCs with Glc-Insulin for diabetes treatment. (B) Confocal microscopy images showing the release of fluorescence-labeled insulin from the cell surfaces under hyperglycemic conditions (scale bars: 50 μm). (C) Profiles of in vitro Glc-Insulin release from the mRBCs at several glucose concentrations. (D) BGL variations in diabetic mice after different treatments. *, $p < 0.05$. (E) Changes in plasma insulin levels and glucose levels after intraperitoneal glucose tolerance tests. Reprinted with permission from ref 46. Copyright 2017 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

Moreover, it was shown that NC_B could be efficiently internalized by MDA-MB-231 tumor cells, leading to enhanced cytotoxicity. Furthermore, the sequential administration of NC_A and NC_B significantly increased the drug availability and retention time at the tumor site compared with the NC_B alone, which led to enhanced inhibition of tumor growth. More importantly, this relay delivery induced negligible systemic inflammation response and did not cause any pathological abnormalities of normal tissues.

As a further extension, we designed platelet-membrane-coated nanoparticles for combined treatment of multiple myeloma and thrombus.[34] Complications such as thrombus formation impose great challenges to the successful treatment of multiple myeloma and increase the mortality. A nanoplatelet delivery system was developed by coating bortezomib-loaded nanoparticles with platelet membranes. After that, tissue plasminogen activator (tPA) and alendronate (Ald) were covalently conjugated to the surface of the platelet membranes for clot lysing and bone targeting, respectively. Both in vitro and in vivo results confirmed that these nanoplatelets could increase the drug accumulation at the myeloma site by first targeting bone via the calcium chelating ability of Ald and then binding myeloma cells through cellular interactions, such as the affinity between P-selectin on the platelet membrane and CD44 receptors on the tumor cells, leading to enhanced therapy efficacy. Moreover, the nanoplatelets could prevent the thrombus complication by readily dissolving the clot.

The development of biomimetic nanoparticles mimicking structural and functional aspects of biological assemblies is being actively pursued in these years for therapeutic purposes.[11] Given the advantages of the intrinsically biocompatible and nonimmunogenic nature of natural cells, functionalizing synthetic nanoparticles with cell membrane will continue to contribute to bridging the gap between natural cells and synthetic formulations and encourage researchers to develop more effective drug delivery systems that are inspired by nature.

3.2. Subcellular Membranes

Generally, upon cargo internalization, vesicles are formulated by invagination of the plasma membrane. These vesicles then converge upon early endosomal systems, followed by sorting of the internalized substances.[35] The majority of the endocytosed membrane components, including lipids and proteins, are recycled back to the plasma membrane via early and recycling endosomes,[35] where the membrane-associated proteins and receptors are randomly distributed over the endosome membrane.[36] Recently, the multiple molecules on the plasma membrane have been demonstrated to mediate the aggregation of homotypic cells.[37]

In this circumstance, our group proposed an alternative cell engineering method to fabricate endosome-membrane-encapsulated nanogels (EM-NG) (Figure 5A) for homotypic cancer drug delivery.[38] Mesoporous-silica-coated magnetic nanoparticles with cross-linkers and photoinitiators loaded inside the channels and methacrylated hyaluronic acid coated on the surface were utilized as the model nanomaterials. Cross-linking of the endocytosed nanoparticles by UV irradiation led to the formation of nanogels, which appeared as round or oval in shape with multiple silica/magnetic nanoparticles clustered

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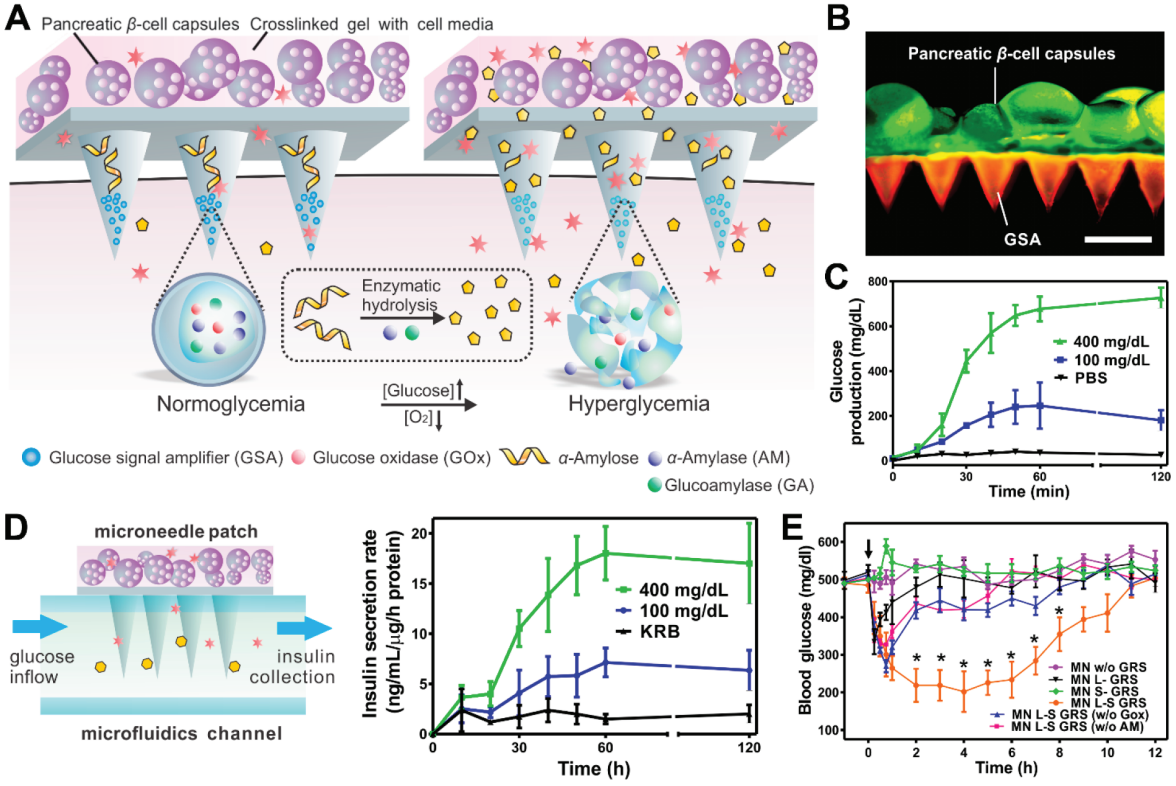

Figure 7. (A) Schematic illustration of integrating β-cells and GSAs with microneedle patches. (B) Fluorescence microscopy image of the designed structure (scale bar: 500 μm). β-Cells and GSAs were labeled with calcium AM and rhodamine, respectively. (C) Glucose production induced by the GSAs in different glucose solutions. (D) Accumulated insulin secretion (right) simulated by the inflow of different glucose solutions through a microfluidics device (left). (E) In vivo therapeutic efficiency treated with the design (MN L-S GRS) and the control designs. *, $p < 0.05$. Reprinted with permission from ref 47. Copyright 2016 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

inside (Figure 5B). Simultaneously, endosome membrane components were well-preserved on the shell of the EM-NG (Figure 5C). The much higher in vitro cytotoxicity induced by DOX-loaded EM-NGs (DOX-EM-NGs) compared with DOX-loaded nanogels (DOX-NGs) and free DOX indicated the targeting effects of the EM-NGs, whereas the negligible differences in cytotoxicity for human lung adenocarcinoma epithelial cells treated with DOX-EM-NGs and DOX-NGs underlined the homotypic targeting ability of the EM-NGs (Figure 5D). Compared with the process of utilizing plasma membranes to coat nanomaterials by mechanical sonication or extrusion,[15,29] the reconstitution of endosomal membranes onto nanogels relies on the internalization process, which could facilitate maintaining the functions of biomolecules. Although the detailed mechanism underlying the function of endosome-encapsulated nanogels still needs further study, this novel cell engineering method has implications for the development of alternative drug delivery systems, especially personalized therapeutics.

Other subcellular compartments can also be exploited to engineer synthetic materials for many user-specified purposes. Major advances in this area include the extensively studied extracellular vesicles, which are important mediators for intercellular communication.[39] The key roles extracellular vesicles play in regulating both normal pathological processes and disease pathogenesis provide clues to engineer and modify such natural particulates for therapeutic interventions.[39] Additionally, engineering cellular membranes with antigens or quantum dots to derive micro/nanoparticles has shown promising potential in theranostic applications.[40,41] Related studies of engineering cell-derived nanovesicles for cancer immunotherapy are under development in our lab.

4. RED BLOOD CELL ENGINEERING FOR SMART INSULIN DELIVERY

Diabetes mellitus is a complex and progressive disease characterized by high blood glucose levels that currently affects hundreds of millions of people worldwide. Although insulin injection has remained the standard method in treating diabetes, it is always associated with pain and failure in tight blood glucose control.[42] To this end, great efforts have been devoted to the development of alternative treatment strategies such as cell-based therapy and insulin delivery with synthetic materials.[43] In view of the concerns regarding cell transplantation mentioned above, glucose-responsive materials have shown great potential for insulin delivery.[44] Nevertheless, constructing a system that can respond quickly to elevated blood glucose levels is a great challenge in closely mimicking the body's physiological needs for insulin.[45]

To address this challenge, our group engineered RBCs with a glucose derivative (glucosamine)-modified insulin (Glc-Insulin) as a new insulin delivery formulation by leveraging the reversible interaction between glucose and the abundant glucose transporters (GLUTs) on the surface of RBC membranes (Figure 6A).[46] The Glc-Insulin could specifically bind GLUT1 on human RBCs and GLUT4 on mice RBCs with high efficiency without inducing obvious damage of the cells. Driven by competitive binding of free glucose with the GLUTs,

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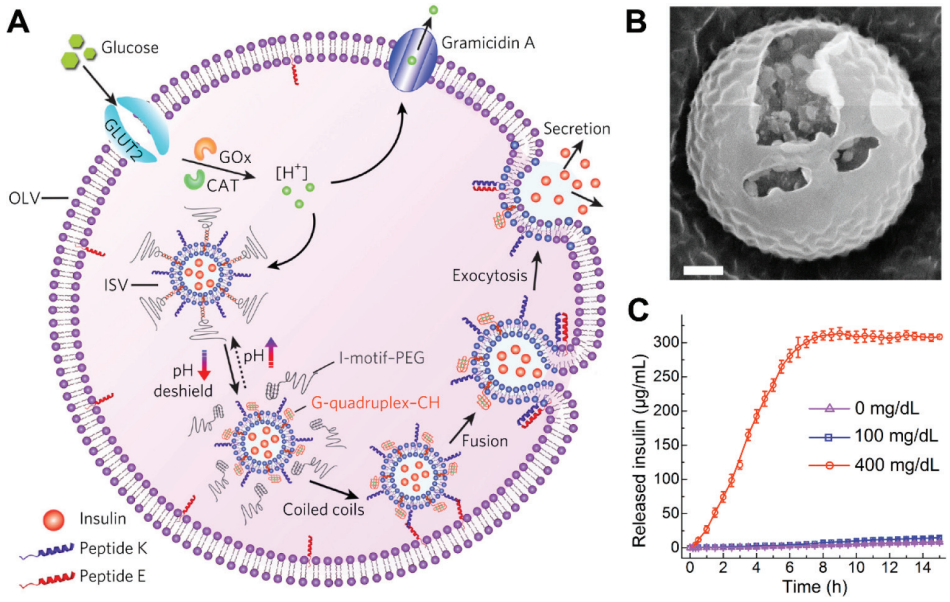

Figure 8. (A) Schematic illustration of a synthetic β-cell that can distinguish external glucose levels and "secret" insulins via membrane fusion of the inner small vesicles (ISVs) with the outer large vesicle (OLV). GLUT2: glucose transporter 2. GOx: glucose oxidase. CAT: catalase. (B) Fractured cryogenic scanning electron microscopy image of the vesicles-in-vesicle superstructure (scale bar: 200 nm). (C) Accumulated insulin release in solutions with different glucose concentrations. Reprinted with permission from ref 49. Copyright 2018 Nature Publishing Group.

insulin dissociation from the RBCs was quickly detected at 400 mg/dL glucose within 30 min, whereas much slower insulin release was observed under normal glycemic conditions (Figure 6B,C). After intravenous injection of the Glc-Insulin-bound mice RBCs into diabetic mice, the injected cells remained stable even for 72 h, which facilitated the long-term insulin delivery. In vivo diabetes treatment showed that Glc-Insulin-bound mice RBCs could restore the blood glucose level (BGL) for a much longer time than free Glc-Insulin or free insulin plus mice RBCs, which originated from the long-term stability and glucose-responsive property of the designed system (Figure 6D). The dynamics of insulin release detected in intraperitoneal glucose tolerance tests further verified the glucose-dependent responsiveness (Figure 6E). Furthermore, by means of cell membrane engineering technologies, we also built a bioinspired nanoformulation utilizing RBC-membrane-coated nanoparticles for insulin delivery via the same mechanism. Such nanoformulations could be conveniently integrated with microneedle patches for painless intradermal insulin delivery, which is promising for potential clinical applications.

Importantly, the long circulation and biocompatible nature of RBCs effectively avoids the headaches existing in traditional cell therapies. Still, we should note that the inability of RBCs to replenish insulins should inspire new cell engineering methods or engineering of different cell species to achieve more sustainable diabetes treatment methods.

5. β-CELLS INTEGRATED WITH MICRONEEDLE PATCHES

β-Cell-based cellular therapy has always been regarded as the ideal treatment for diabetes because of the inherent ability in tight blood glucose sensing and sustained insulin delivery.[43] To potentially overcome the challenges in conventional direct islet transplantation and immunoisolation techniques, our lab exploited a painless microneedle-patch strategy to externally engineer β-cells for control of internal blood glucose levels (Figure 7A).[47] In this design, alginate microgel encapsulated β-cells and "glucose-signal amplifiers" (GSAs) were respectively positioned on the top and in the matrix of the microneedle patches (Figure 7B). When subjected to hyperglycemic levels, the GSAs could readily generate a local high glucose concentration (~800 mg/dL) via a tandem enzymatic process (Figure 7C). Meanwhile, the amplified glucose could diffuse into the externally located β-cell microcapsules, which in turn stimulated the secretion and delivery of insulin back into the transdermal vascular networks (Figure 7D). An in vivo diabetes study showed that the platform could quickly respond to high BGLs and restore the BGL to the normal range within 2 h; importantly, neither hyperglycemic nor hypoglycemic peaks were observed in the following 6 h (Figure 7E). Engineering β-cells with microneedle patches provides an alternative to conventional cell transplantation for diabetes treatment in a minimally invasive way. This strategy also highlights the potential of engineering other types of cells as living drug depots for treating different diseases involving cell function deficiency. Admittedly, issues regarding the lifetime and inevitable contamination of the externally positioned cells should be addressed.

6. SUMMARY AND OUTLOOK

The studies summarized in this Account demonstrate the strategies of engineering whole cells or parts of cells as chaperones to direct drug delivery, which aims to leverage the unique delivery mechanisms that are employed by natural particulates, including selective targeting, extended circulation time, and evasion of immune surveillance. These techniques could migrate the need for tedious processes or immunosuppressive agents involved in conventional cell transplantation as well as complement traditional genetic engineering to reprogram cell functions. Given the wealth of different functional cells and versatile choices of formulation engineering and chemical conjugation methods, it can be envisioned that

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distinct combinations are worthy of being explored to treat a variety of diseases.

Although considerable advances have been achieved in this field, there are several issues that need to be addressed for acceleration of potential translation. First, for real-world applications, scale-up manufacturing of natural cell-engineered drug delivery formulations must be optimized, especially in cases where cells have low concentrations and are not easy to collect. Second, sorting out a clear and stringent understanding of the delivery mechanisms that are used by the engineered biological particulate systems is necessary to address in vivo safety concerns, since integrity is important for natural cells to communicate with the body. Third, proper standards should be established to resolve problems associated with these cell engineering technologies and to support robust and reproducible modification methodologies, and reliable analytical techniques are also required to support the manufacturing of the biologically derived products. Of note, broad room exists for developing sustainable engineering approaches in a manner of integrating artificial cells. For example, bioinspired approaches through the design of "synthetic cells" fabricated from human-made materials but possessing the "sensing reaction" behaviors of their natural counterparts are a promising alternative to address the combined issues of efficacy, safety, and manufacturing (Figure 8).[48−50]

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Notes

The authors declare no competing financial interest.

Biographies

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