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Technological challenges of biomembrane-coated top-down cancer nanotherapy

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Cancer nanotherapy suffers from low-yield delivery that is imposed by tumour pathophysiological barriers. Top-down drug delivery strategies, including exosomes and cell membrane-coated particles, can improve safety and efficacy owing to the innate biointerfacial properties of these platforms. Here, we discuss the technological challenges that need to be overcome for their clinical implementation.

Nanodelivery enables the modulation of drug biodistribution and improves target site accumulation, thereby reducing toxicity and off-target effects[1]. However, a striking imbalance exists between the number of preclinical studies that use synthetic complex nanosystems and their poor clinical trial performance. One reason for such discrepancy is the formation of a protein corona, which masks surface ligands and triggers immunological responses. This protein coating is difficult to detect as its components vary between different animal models. Furthermore, the functionalizing ligands in synthetic nanosystems have heterogeneous orientation, pattern of distribution and concentration, thereby reducing targeting efficiency. Even polyethylene glycol (PEG) biointerfacing, which improves circulation half-life by reducing renal- and macrophage-mediated hepatic clearance, and has proved its efficacy in the mRNA vaccines against SARS-CoV-2, can lead to immunogenic events at higher doses and accelerate clearance and off-target release[2]. Nevertheless, simple systems (for example Doxil, Abraxane and Myocet) remain the gold-standard in oncology.

Current bottom-up conjugation and delivery strategies against cancer suffer from low specificity and efficacy owing to tumour heterogeneity and its associated pathophysiological barriers. Top-down systems, including biological exosomes and natural membrane-coated nanosystems, can help overcome these issues by preserving and expressing structural and functional self-recognition markers.

Top-down biointerfacial nanosystems

Having a specific size, shape, charge or functional moiety alone is no longer sufficient to ensure efficient cargo delivery to tumours. Unlike synthetic techniques, membrane-camouflaged abiotic nanosytems and exosomes benefit from the innate biointerfacial properties of natural membranes, including fluidity and antigenic profile. These top-down strategies have improved delivery efficiency owing to lower nonspecific uptake and improved homotypic targeting, which increase circulation half-life and promote immune modulation. Cell membrane-coated nanosystems first employed red blood cell (RBC) membranes, but they rapidly expanded to platelets, leukocytes, stem cells, cancer cells and bacteria. Exosomes have complex biomolecule-decorated bilayers that are enriched with tetraspanins, adhesion, cell-targeting and immune-escaping molecules, and a smaller size (typically 30–130 nm) than membrane-coated systems. Exosome-based systems differ from cell membrane-coated nanosystems in their manufacturing process, which can be either by isolation of natural exosomes or disintegration of large structures into smaller units. Furthermore, the methods of cargo loading in exosomes can be either exogenous, such as membrane-coating, or endogenous, which uses genetic and metabolic manipulation (Fig. 1a). The latter prevents the protein and nucleic-acid-based payload degradation that is normally observed in exogenously fabricated systems[3].

Pre-clinical efficacy

The choice of a proper parent cell is crucial to ensuring targeting efficiency, because exosomal and membrane-coated systems retain most of the original membrane properties. For example, tumour cell membrane-coated nanosystems with surface markers, such as E-cadherin, CD44 and CD326, display homotypic targeting, and inhibit primary and metastatic tumour growth of breast cancer in rodents[4,5]. Similarly, intratumoural administration of platelet-coated polylactic acid nanoparticles carrying the toll-like receptor 7 (TLR7) agonist resiquimod results in tumour regression and protection against tumour challenges in a murine model of colorectal and breast cancer[6].

Exosome-coated systems also possess homotypic targeting. Preclinical results have shown promising results with improved tumoural delivery in primary and metastatic targeted chemo-, gene- and immunotherapy. For example, intrapleural delivery of a single dose of autologous exosomes loading methotrexate resulted in tumour and $CD163^{+}$ macrophage reduction in lung and colon rodent models with malignant pleural effusion[3]. Human and non-human derived drug-loaded exosome-based nanosystems are undergoing clinical trials (NCT01159288, NCT01294072, NCT02507583, NCT01550523, NCT01294072, NCT03608631 and NCT02657460).

Pharmacokinetics and pharmacodynamics

Pharmacokinetic (PK) and pharmacodynamic (PD) properties of exosomes and cell membranes depend on the cell of origin and administration route. RBC and platelet coatings that express 'don't-eat-me' signals outperform PEGylated nanomedicines in extending circulation half-life, passive tumour accumulation and safety[5]. Both cells can be

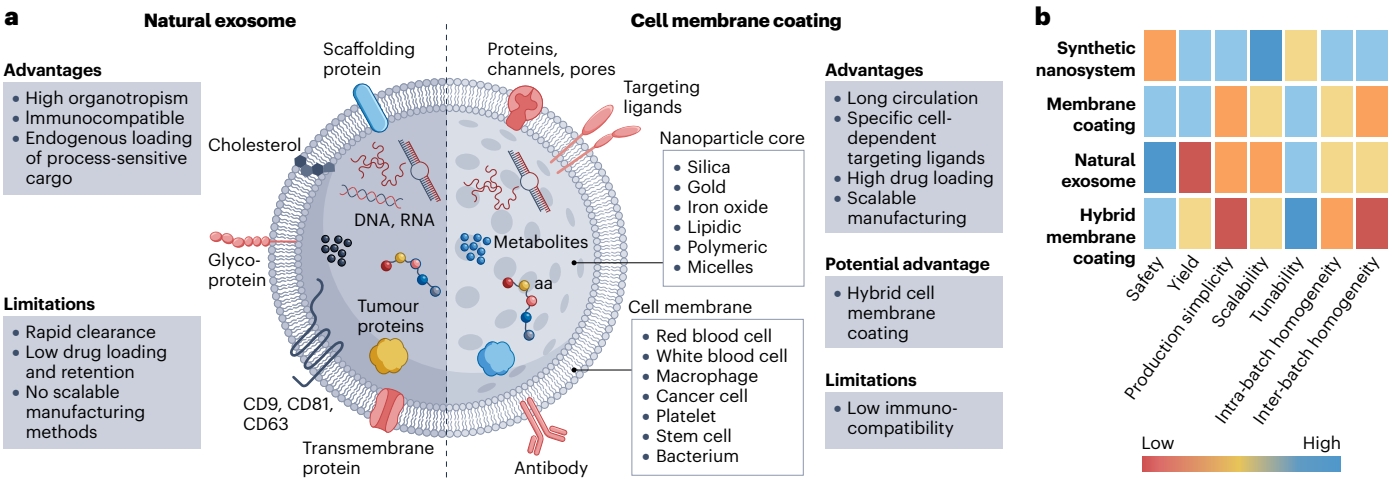

Fig. 1 | Comparison of naturally secreted exosomes and engineered cell membrane-coated nanosystems. a, Main characteristics of natural exosomes and cell membrane-coated nanosystems. **b**, Comparative analysis of end-product and manufacturing properties of natural exosomes and cell membrane-coated nanosystems. aa, amino acids.

conveniently collected autologously or from accessible blood banks. However, current studies on tumour cell membrane coating employ membranes from murine sources, and their clinical efficacy and safety in humans remain to be verified.

The half-life of exosome-based nanosystems is in the minute scale when injected systemically, and direct PK comparison with synthetic nanosystems is scarcely reported. For example, genetically enriched siRNA-packed exosomal nanosystems result in a 10-fold reduction of siRNA therapeutic dose compared with synthetic lipid systems[7]. However, how the immune system, parent cell and biomolecular composition affect PK and PD remain to be addressed.

Manufacturing

Similar exogenous manufacturing processes exist for cell membrane- and exosome-coated nanosystems, including membrane extraction, synthetic core production and coating (fusion process). Nucleus-free cells are substantially easier to isolate and purify, which favours RBC and platelet usage. Extrusion and sonication are common coating methods in laboratory settings with moderate scalability[8]. The former method suffers from high material loss, whereas sonication leads to polydisperse coating, which can translate into unpredictable PK and PD. Tandem microfluidic–electroporation is a versatile alternative that grants homogeneous and biocompatible coatings[9].

Delivery of hydrophilic payloads is more challenging for exosome-based systems compared with membrane-coated particles as the cargo does not diffuse through the vesicle membrane during exogenous incubation. Endogenous genetical and metabolic manipulation can address this issue, but it suffers from low production yield and heterogeneous loading efficiencies. This process is less aggressive than exogenous approaches, which improves safety and biodistribution by ensuring high targeting and cell-specific uptake[3]. However, it suffers from low yields and poor scalability. Furthermore, disintegration of parent cells into smaller units by extrusion or microfluidics improves production yield, compared to natural exosomes, but suffers from low homogeneity and biomimetic profile[10].

Despite risking protein disruption, post-isolation chemical functionalization can unspecifically introduce ligands onto membrane proteins. Lipid tethering is less disturbing, but it still suffers from low coating homogeneity. Alternatively, hybrid cell coatings that combine several parent membranes blend the specific functionality of every parent cell, but substantial batch-to-batch heterogeneity and difficult optimization of the membranes' ratio remain to be addressed.

Outlook

The most valuable aspect of biomembrane-coating technologies in nanotherapeutics is to hijack otherwise inaccessible complex biological functionality. Both natural exosomes and membrane-coated nanosystems can be tailored towards high biocompatibility, low immunogenicity, protection of labile cargo and homotypic targeting. Careful selection of the cell of origin, fabrication process and post-isolation modifications could generate therapeutically superior cancer-tailored medicines (Fig. 1b).

For exosome production, moving to large bioreactors is essential to bypass low isolation yields. To tackle product heterogeneity, chemically defined media need to be implemented. For example, using human fluids and tissues as media could prove beneficial if the overall yield surpasses the problematic purification steps. Moreover, using immortalized cell lines can be considered, but monitoring the encapsulation of exogenous toxic reactants is necessary.

Effective cell membrane manufacturing depends not only on membrane isolation yields, but also on coating efficiency and homogeneity. Aggressive manipulation of the membranes can modify the surface and induce immunogenic responses after adminstration. Large-scale tandem microfluidic-electroporation approaches are a promising alternative to improve yield and end-product homogeneity.

From a regulatory perspective, characterization and quality control requirements are more stringent, including monitoring particle physicochemical properties, storage stability, batch-to-batch variation and surface molecule quantification. Despite the technological challenges that are associated with particle coating reproducibility, early phase clinical trials that use fruit-derived (NCT01294072) and patient-derived (NCT02657460) exosomes coatings have proved to be safe.

Combining natural and engineered structures could provide a more efficient and specific treatment against cancer. However, integrated effort from consortia of academics, clinicians, pharmaceuticals, engineers and regulatory authorities is essential to ensure their manufacture scalability and clinical impact.

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References

1. Mendes, B. B. et al. Nanodelivery of nucleic acids. *Nat. Rev. Methods Primers* **2**, 24 (2022).
2. Shi, J., Kantoff, P. W., Wooster, R. & Farokhzad, O. C. Cancer nanomedicine: progress, challenges and opportunities. *Nat. Rev. Cancer* **17**, 20–37 (2017).
3. Elsharkasy, O. M. et al. Extracellular vesicles as drug delivery systems: why and how? *Adv. Drug Deliv. Rev.* **159**, 332–343 (2020).
4. Qu, Y. et al. Cancer-cell-biomimetic nanoparticles for targeted therapy of multiple myeloma based on bone marrow homing. *Adv. Mater.* https://doi.org/10.1002/adma.202107883 (2021).
5. Fang, R. H., Kroll, A. V., Gao, W. & Zhang, L. Cell membrane coating nanotechnology. *Adv. Mater.* **30**, 1706759 (2018).
6. Bahmani, B. et al. Intratumoral immunotherapy using platelet-cloaked nanoparticles enhances antitumor immunity in solid tumors. *Nat. Commun.* **12**, 1999 (2021).
7. Reshke, R. et al. Reduction of the therapeutic dose of silencing RNA by packaging it in extracellular vesicles via a pre-microRNA backbone. *Nat. Biomed. Eng.* **4**, 52–68 (2020).
8. Lopes, J. et al. Macrophage cell membrane-cloaked nanoplatforms for biomedical applications. *Small Methods* **6**, 2200289 (2022).
9. Rao, L. et al. Microfluidic electroporation-facilitated synthesis of erythrocyte membrane-coated magnetic nanoparticles for enhanced imaging-guided cancer therapy. *ACS Nano* **11**, 3496–3505 (2017).
10. Gimona, M., Pachler, K., Laner-Plamberger, S., Schallmoser, K. & Rohde, E. Manufacturing of human extracellular vesicle-based therapeutics for clinical use. *Int. J. Mol. Sci.* **18**, 1190 (2017).

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Competing interests

J.C. is a co-founder and shareholder of TargTex S.A. The remaining authors declare no competing interests.