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FROM DYNAMIC STRUCTURES TO FUNCTIONAL MATERIALS

D4 peptide nanofibrils as efficient transduction enhancers for ex vivo gene transfer in CAR-T and NK cell generation

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Chimeric antigen receptor (CAR)-T and natural killer (NK) cell therapies are promising treatments for hematologic malignancies and are increasingly explored for solid tumors and autoimmune diseases[1, 2]. However, their manufacturing remains labor-intensive and costly. A key limitation is the inefficient viral transduction due to electrostatic repulsion between negatively charged viral and cellular membranes. Transduction enhancers, such as peptide nanofibrils (PNFs), can overcome this limitation, thereby reducing manufacturing costs and improving cell yields. However, established enhancers like Vectofusin-1 show variable performance depending on the viral vector system[3].

We identified a novel PNF, D4, through in silico screening followed by experimental optimization. Its transduction efficiency was evaluated across different viral vectors and cell types and compared to Vectofusin-1. Mechanistic studies were done including imaging of virus–PNF–cell interactions as well as a simulation of a CAR-T cell production process to assess stability and degradation.
D4 PNFs demonstrated comparable or superior transduction efficiency relative to Vectofusin-1. Notably, D4 enhanced transduction independently of the viral vector type (lentiviral (LV) or retroviral (RV)) and glycoprotein used for pseudotyping, including VSV-G–pseudotyped LV, which are poorly enhanced by Vectofusin-1. In NK cells, D4 increased transduction efficiency without the need for spinfection, improving infection rates from 0.4% to ~23% for RD114-LV and from 15% to 65% for baboon endogenous virus envelope pseudotypes (BaEV-LV)[3].

Mechanistically, D4 PNFs exhibited a high density of viral binding sites, facilitating the formation of virus–PNF complexes that are actively captured by filopodia and transported to the cell body. This promotes efficient membrane fusion or endocytic uptake. Following internalization, PNFs are degraded in lysosomes. Approximately 98% of D4 aggregates were degraded within three days in a CAR-T production simulation. Importantly, D4 did not negatively affect cell viability or proliferation. In cell-free conditions, D4 aggregates gradually disassembled, suggesting a favorable safety profile due to reduced persistence and lower risk of immune activation or vascular occlusion[4].

These findings highlight the potential benefits of using the transduction enhancer D4 in ex vivo gene transfer processes, such as CAR-T cell production.[1]

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