Efficient non-viral CAR-T cell generation via silicon-nanotube-mediated transfection

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The advances in engineered nano–bio cellular interfaces—driven by vertically configured nanostructure arrays—have fostered unprecedented progress in modulating and regulating cellular processes at the nanoscale. In particular, nanoinjection has been developed to realize delivery of diverse bioactive payloads into a wide range of cells and tissues both in vitro and in vivo. Nevertheless, progress has been slow in relation to nanoinjecting primary T lymphocytes, which are central players during adaptive immune responses and hold great clinical potential in fighting diseases including cancer. In particular, chimeric antigen receptor (CAR)-T therapy holds great promise in treating cancer and other diseases; but the current viral-based method raises a significant cost and safety hurdle. In this study, we show for the first time successful CAR transfection into primary T cells via vertically aligned silicon nanotube (SiNT) arrays. By co-culturing with target lymphoma Raji cells, we prove that transfected CAR-T cells can suppress Raji cell growth, indicated by significant increase in effector:target (E:T) ratio (by up to 30.7-fold). SiNT-generated CAR-T cells using non-activated (N_SiNT) T cells perform comparable or even higher lymphoma suppression compared with pre-activated (A_SiNT) T cells, indicating that SiNT-mediated transfection can bypass the requirement of antigen stimulation and preserve higher immune potency. N_SiNT CAR-T cells produce significantly higher amounts of cytotoxic agents, demonstrating stronger inhibition on Raji cells' luciferase expression, compared to their A_SiNT counterparts or those generated by electroporation, particularly with a larger E:T ratio (4:1) and a longer co-culture period (72 h). The results demonstrate the capacity of SiNT-mediated transfection of generating effective anti-lymphoma CAR-T cells. Considering the growing potential of cell-based therapies, we expect that a non-viral nanoinjection platform such as ours will facilitate the full realization of their therapeutic promise.