Layer-by-layer Coated Microneedles for Cancer Immunotherapy

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Therapeutic cancer vaccines are a type of immunotherapy that boosts the immune system to recognize, target, and eliminate cancer cells. Over the past few decades, research has achieved remarkable advances in both pre-clinical and clinical studies identifying new pathways and developing novel delivery vehicles. Compared to preventive vaccination, therapeutic vaccination against established diseases, in this case cancer, has been proven to be more challenging since it needs to combat an immune system that has already been restrained to sustain the disease, such as an immunosuppressive tumor microenvironment. One major breakthrough has been the advent of the checkpoint inhibitors which effectively upregulate a defense against cancer. Yet, only a portion of cancer patients respond to checkpoint inhibitors. A major factor in the treatment resistance is the lack of tumor T cell infiltration and activation. Hence, strategies to boost antigen presentation and T cell priming in order to increase the fraction of patients responding to immunotherapy remains an urgent need. This thesis is dedicated to designing vaccines with effective T cell priming over a sustained period.

We engineered microneedle (MN) skin patch as our delivery platform, as research has shown that transdermal delivery of vaccines may result in more effective antigen presentation than deeper injections with larger doses, due to the activation of the potent epidermal Langerhans cells and dermal dendritic cells. The drug was incorporated as a releasable multilayer coating on the microneedle surface constructed with alternating absorption of oppositely charged species including protein or nucleic acid drugs and biocompatible polymer carriers. This layer-by-layer (LbL) coating approach offered excellent control over the drug release profile, but also makes the entire film ‘sticky’ as each layer is electrostatically attracted to its adjacent neighbors. Past LbL MN strategies have all retained this ‘sticky’ nature and therefore require a long epidermal application time (15-90 mins) for drug implantation. To resolve this problem, we devised a pH-induced charge-invertible polymer as a lift-off layer that significantly shortens the application time to 1 min. Our approach has inspired other work involving rapid film lift-off with charge-invertible species.

On the drug side, we explored several bioinspired strategies in developing potent vaccine components with ribonucleoprotein complexes. We eventually focused on the stimulator of interferon genes (STING) pathway, which plays an important part in the recognition of tumor cells and type I IFN-dependent antitumor immune response. Current existing strategies mostly focus on developing synthetic liposomes or

polymersomes to deliver the STING agonist, cyclic GMP-AMP (cGAMP) into the cells. However, this assumes the presence of fully functional STING protein in the cell to bind cGAMP. STING signaling has not only been shown to be frequently impaired in cancer cells due to epigenetic silencing of the protein; it is also under debate whether the overall population is responsive to delivery of just the agonist, since 19% of humans carry a mutated STING variant called HAQ STING that has been suggested to exhibit impaired function. In cancer cells, STING signaling is frequently impaired by epigenetic silencing of STING or the cGAMP synthase (cGAS). To address these challenges, we engineered a recombinant STING protein without the transmembrane domain (STINGΔTM) that could be used as a functional carrier for cGAMP delivery and elicit type I IFN expression in cell lines deficient of STING or with defective endogenous STING. *In vivo*, our cGAMP-STINGΔTM signaling complex elicited enhanced antigen specific B and T cell responses as well as robust anti-tumoral immunity in a B16 melanoma and a MC38 colon cancer mouse model.

To sum up, we have developed a new LbL MN administration platform based on a charge-invertible polymer and a ribonucleoprotein vaccine component based on the STING pathway. Together, these work laid strong foundation for continued development of a potent cancer vaccine microneedle skin patch.

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