5-Aminolevulinic Acid Coated Microneedles for Photodynamic Therapy of Skin Tumors
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Purpose
5-Aminolevulinic acid (5-ALA) based photodynamic therapy (PDT) has been extensively used for the treatment of superficial skin neoplasm. 5-ALA is by itself not a photosensitizer (PS); however it is a biological precursor of protoporphyrin IX (PPIX), which is a potent PS and upon irradiation with visible light produces the cytotoxic oxygen radicals. High polar characteristics of 5-ALA limits its penetrability through stratum corneum. Hence, current clinical practice involves topical application of a high dose of 5-ALA. Excessive curettage of skin lesion to increase dermal penetration of 5-ALA may be needed, which is associated with excessive pain and discomfort to patients. Therefore, the purpose of the present study was to evaluate microneedles for improving dermal delivery of 5-ALA, and assess their potential for PDT.

Methods
A micro-precision dip coating method was used to coat 5-ALA on two dimensional (2D) stainless steel microneedle arrays containing 57 microneedles. Optimization of critical coating parameters was performed to maximize (i) mass of 5-ALA coated on a microneedle patch, and (ii) in vitro delivery efficiency of 5-ALA upon insertion into skin for 5 min. Kinetics of PPIX generation in vivo in skin of Balb/C mice was evaluated after 1, 2, 4, 6, 8, 12, 24 and 48 h of treatment with 5-ALA coated microneedle patch, or conventional formulation (20% w/w 5-ALA cream), or 20% 5-ALA cream on skin area pre-treated with an uncoated microneedle patch. Depth-distribution of PPIX in skin was also evaluated using confocal microscopy, 4h after treatment of mice with aforementioned formulations. Lastly, efficacy of 5-ALA coated microneedles for PDT was assessed in a Balb/c mouse skin tumor model obtained by subcutaneously injecting murine B-cell lymphoma cells.

Results
Optimization of coating parameters revealed that 5 dips into a 25% w/v 5-ALA solution resulted in higher mass of 5-ALA (about 350 µg/patch), which gave a delivery efficiency of 90% in porcine cadaver skin. Microscopic assessment clearly demonstrated uniform coating of 5-ALA on microneedles (Fig 1A). In vivo dermal pharmacokinetic study of 5-ALA conversion to PPIX revealed about 3.2-fold increase in PPIX formation after 4h with coated microneedles as compared to topical cream application of 25 mg on intact skin (Fig 1B). Depth-distribution assessment showed that PPIX was found at deeper locations (~450 µm) with microneedle-based delivery as compared to 5-ALA cream formulation (~150 µm). Finally, 5-ALA coated microneedles suppressed the growth of subcutaneous tumors by ~57% in contrast to conventional 5-ALA cream which did not suppress the tumor volume and revealed tumor growth comparable to untreated control group (Fig. 2).

Conclusion
5-ALA was uniformly coated on microneedles. Compared to 25 mg topical application of 5-ALA, microneedle-based delivery of just 350 µg 5-ALA led to (i) about 3.2 fold higher formation of PPIX in skin, (ii) detection of PPIX at greater depth, and (iii) better tumor regression after PDT. Overall, our findings suggest that coated microneedle may offer a potential approach to deliver photosensitizers into the tumors in a minimally invasive and patient-friendly manner for conducting PDT.