Framework nucleic acids as programmable carrier for transdermal drug delivery

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DNA nanostructures are promising drug carriers with their intrinsic biocompatibility, uniformity and versatility. However, rapid serum disintegration leads to low bioavailability at targeted sites following systemic administration, hindering their biomedical applications. Here we demonstrate transdermal delivery of framework nucleic acids (FNAs) through topical applications. By designing FNAs with distinct shapes and sizes, we interrogate their penetration on mice and human skin explant. Skin histology reveals size-dependent penetration, with FNAs ≤75 nm effectively reaching dermis layer. 17 nm-tetrahedral FNAs show greatest penetration to 350 µm from skin periphery. Importantly, structural integrity is maintained during the skin penetration. Employing a mouse melanoma model, topical application of doxorubicin-loaded FNAs accommodates ≥2-fold improvement in drug accumulation and tumor inhibition relative to topically-applied free doxorubicin, or doxorubicin loaded in liposomes and polymeric nanoparticles. Programmable penetration with minimal systemic biodistribution underlines FNA potential as localized transdermal drug delivery carriers.
DNA origami is an emerging technique that exploits programmable folding of single-stranded DNA molecules to generate complex framework nucleic acids (FNAs) ranging from periodic arrays to three-dimensional (3D) architectures. DNA can self-assemble into arbitrary shapes with full adddressability, allowing the fabrication of nanostructures with well-defined form and precisely arranged heteroelements. Given their biocompatibility, versatility, and controllable size and shape, FNAs hold great promise for drug delivery. However, DNA origami based in vivo application has predominantly been explored through invasive needle injection. In such systemic delivery, DNA structures are subjected to rapid disintegration and digestion besides immune- and renal-clearance, leading to low bioavailability at targeted sites. In mouse model, their half-life time in circulation is only of minutes or up to a few hours after chemical modification of DNA backbones3,6,7, forming a major barrier for widespread application of DNA origami-based nanomedicine.

Transdermal drug delivery (TDD) is an attractive mean of drug administration, given its non/minimally invasive nature, high-patient compliance, and direct route of entry bypassing gastrointestinal or liver metabolism. Consequently, intended treatment can be achieved with minimal side effects. Studies have revealed that nanoparticles (NPs) of appropriate size, shape, and surface properties can reach viable epidermis or even dermis layer for potent therapy in vivo. One-specific case is the skin penetration of spherical nucleic acids (SNAs), gold NPs coated with tightly organized oligonucleotides. Without assistance from external devices, these SNAs can penetrate through the stratum corneum and epidermis barrier, to reach the dermis region. Although the mechanism remains mostly unclear, the similarity of size and surface chemistry between FNAs and SNAs leads us to hypothesize that FNAs would penetrate the skin as well. If successful, this attempt would provide an alternative carrier for TDD with precisely controlled size, shape and surface chemistry, while concurrently open new application route to circumvent the susceptibilities of FNAs in systemic drug delivery. Skin diseases like melanoma, abnormal scarring, and atopic dermatitis would benefit from the resulted formulations such as FNAs containing RNA interference moieties and/or antisense oligonucleotides.

This study explores the skin penetration ability of FNA structures between 20 and 200 nm in size. We observe a size-dependent skin penetration phenomenon, with 20 nm tetrahedron FNA (TH) reaching into the dermis region. FNA integrity was well maintained during the skin penetration. Taking the optimized system, we loaded 20 nm TH structures with doxorubicin (DOX) through intercalation for the topical treatment of melanoma tumor in mice. Compared with other topical carriers (i.e. liposomes and polymeric NPs), we observe twofold enhancements in DOX delivery and tumor inhibition, corroborating FNA efficacy in achieving TDD.

Results

In vitro characterization of FNAs. To examine the topical penetration and drug carrier capability of well-defined FNAs, we have designed 8 types of FNAs including 3 tetrahedrons, 2 cylindrical rods, 2 rectangles, and 1 triangle (Fig. 2a), ranging from 81.9 to 4711.9 kDa in size (Supporting Table 1). FNAs were synthesized through self-assembly via base pairing hybridization, assisted by the presence of divalent cations (e.g. Mg2+) to minimize electrostatic repulsion from the negatively charged nucleotides.

FNAs were examined through atomic force microscopy (AFM) or gel electrophoresis. AFM confirmed the desired assembly of large FNAs, including tetrahedron with 337 base pairs (bps) (TH337), 6-helical rods with 14,498 bps (6H14498), rectangular plane with 13,730 bps (R13730), rectangular box with 14,498 bps (B14498) and triangular plane with 14,498 bps (T14498) (Supporting Fig. 1A). Tetrahedron with 21 bps (TH21), tetrahedron with 37 bps (TH37) and 6-helical rods with 714 bps (6H714) were too small for AFM imaging and were confirmed using gel electrophoresis. As shown in Supporting Fig. 1B, TH21 migrated the furthest in the gel, matching the 220 bps band from the DNA ladder, followed by 6H714 and TH37 which migrated less distance from the 300 bps band. Interestingly, 6H714 migrated slightly further than TH37 despite its larger molecular weight, which might be due to its rod-like shape. Finally, dynamic light scattering examination revealed their hydrodynamic diameters (Dh) (Supporting Fig. 1C & D). The sizes of all tetrahedron structures have gaussian distribution with the average Dh of 17 nm for TH21, 44 nm for TH37 and 187 nm for TH337. Both 6-helix (6H714 and 14498) structures have two peaks, likely corresponding to the width (~30 nm) and length of the rods (~66/220 nm). Meanwhile, the remaining large rectangular or triangular nanostructures (R13730, B14498, and T14498) have a similar average Dh between 140 and 170 nm. Notwithstanding slight alteration due to shape differences, average Dh increased in correlation with the molecular weight of FNAs (Supporting Fig. 1E).

We then examined interaction of these FNAs with skin cells in vitro. First, FNA internalization kinetic was evaluated using smallest Cy5.5-tagged TH21 on skin fibroblast cells (Supporting Fig. 2A & B). There were ~18 and 50-fold increases in cell fluorescence after 6 and 24-h incubation respectively. Intracellular localization of FNAs was revealed through confocal imaging. As shown in Supporting Fig. 2C, TH21 signal (Cy5.5-tagged; red) localized in the cytoplasm (i.e. outside of Hoechst33342-stained nuclei; blue) and partially overlapped with Lysotracker signal.
(staining endo-lysosomes; green), confirming that FNAs entered the cells through the endolysosomal pathway. Comparing internalization of all FNAs after 24-h incubation, TH21 showed greatest intracellular accumulation, followed by TH37 and 6H714 (Supporting Fig. 3 and Supporting Fig. 4A). The remaining structures had negligible cellular uptake post signal normalization. This aligns well with previous reports in which there was better cellular internalization of compact, low-aspect ratio FNAs in the size of 50–80 nm. Scavenger receptors, facilitating cellular uptake of DNA moieties, obviously prefer to bind and transport FNAs in this size range. All FNAs showed minimal influence on the viability of the skin fibroblasts and keratinocytes, even at the high concentration of 1 µg/mL (Supporting Fig. 4B). In fact, most of them promoted cell proliferation, presumably by providing useful bioproducts following its catabolism.

**Fig. 2** Topical delivery and penetration of FNAs through mice and human skin: a Schematic illustration of 8 kinds of FNAs (not drawn to scale). b Representative fluorescence images of mouse skin histology post various FNAs penetration (24 h; red: Cy5.5-tagged FNAs, blue: Hoechst33342-stained skin); c profile of Cy5.5 fluorescence signal from FNAs along skin depth in b. d Representative fluorescence images of human skin explant histology post TH21 FNA penetration (24 h; red: Cy5.5-tagged FNAs, blue: Hoechst33342-stained skin); e profile of fluorescence signal from Cy5.5-TH21 FNA along human skin depth in d. f Hematoxylin & eosin staining showing TH21 treatment minimally affects human skin morphology (left: untreated skin, right: TH21-treated skin). Source data are provided as a Source Data file. Scale bar: 200 µm. Error bars represent standard deviation.
Topical delivery of FNAs in mouse and human skin explant. Eight kinds of FNAs were mixed with the moisturizer (i.e. Aquaphor®) under the same mass concentration (100 µg/ml). To adjust for molecular weight differences, one fluorophore was tagged on each TH21, TH37, and 6H714, 3 fluorophores on T14498 and 4 on each TH337, 6H14498, R13730, and B14498 (Supporting Table 1). In ensuring successful tagging and maintenance of purity, excess fluorophore strands were added during assembly of large FNAs, which were subsequently removed through repeated chromatography or centrifuge filtrations (Supporting Table 2–Supporting Table 9). Overall, comparable fluorescence level (within an order) was observed from the FNAs at the same mass concentration (Supporting Fig. 5). The formulations were topically applied on the back of mice and covered with Tegaderm™ dressing for 24 h (Supporting Fig. 6A). After formulations were topically applied on the back of mice and covered with Tegaderm™ dressing for 24 h (Supporting Fig. 6A). After wipping off excess cream, the mice were imaged with in vivo imaging system (IVIS). Finally, mice were killed, and treated skin regions were taken for histological analysis.

IVIS showed skin treated with TH21, TH37, and 6H714 had greater fluorescence signal than skin samples treated with the other 5 FNA structures (Supporting Fig. 6B). Skin histology revealed significant penetration by TH21, TH37, and 6H714 among others (Fig. 2b, c). Fluorescence signal of 6H714 remained strong until ~100 µm deep, signifying noteworthy penetration through the epidermis and up to early dermis region. Meanwhile, even more remarkable entry was noted for TH37 and TH21, reaching ~350 µm beneath the skin surface. Larger structures (TH337, 6H14498, R13730, B14498, and T14498) were mostly retained only at the epidermis region (~50–75 µm from the skin surface).

Upon closer examination through keratin14 (Krt14) counterstaining, we noted that FNAs localize in deep dermis region predominantly around the hair follicles and sweat glands (Supporting Fig. 7A, white arrow). This suggests that trans-appendageal route played a crucial role for extensive FNA skin penetration, in agreement with reported transdermal study with liposomes or polymeric NPs.10,21. It is noteworthy that FNA penetration until epidermis-dermis boundary was also observed at area without follicles or glands, suggesting partial role of trans/intercellular route in enabling topical delivery of FNAs (yellow arrow). By scanning and correlating fluorescence signals of FNA and Krt14-stain, we estimate 20–25% internalization of FNAs occurring via non-trans-appendageal route (Supporting Fig. 7B & C).

Taking TH21 as a proof-of-concept, the FNAs were additionally tested on human skin explant for their penetration ability and biocompatibility. As shown in Supporting Figure 8 & Fig. 2d (for ×40 and ×200 imaging magnification, respectively) and quantified in Fig. 2e, Cy5-tagged TH21 formulation (red) penetrated significantly through the epidermis, reaching into the dermis region (Hoechst33342 signal: blue). Lack of red fluorescence from control group (PBS+ cream) confirmed minimal fluorescence signal from the cream itself. TH21 signal reached ~160–170 µm beneath the skin surface, around the stratum basale and into the dermis region on intact human skin. Noting the more robust, denser barrier of the human skin, FNA penetration could be enhanced with assistive technology (e.g. microneedles, iontophoresis) to enable deeper delivery into the dermis region. Nonetheless, FNAs remain a promising tunable TDD nanocarrier, with certain extent of penetration and minimal alteration/damage-induced towards skin morphology during its delivery (Fig. 2f).

Beyond the extent of skin penetration, integrity of FNAs during this process is also crucial for TDD application. As proof-of-concept, we examine this issue using symmetrical TH21. To see whether the stability of one TH21 edge can represent the stability of all edges, we ran gel electrophoresis study on all permutations of TH21 chain assembly (Fig. 3a). As shown in Fig. 3b, similar distances were traveled for all permutations containing same number of ssDNA chains (e.g. missing 2 chains: 2A-2F, missing 1 chain: 3A-3D). This, supported with equivalent A/T and C/G ratio of each chain, substantiates our hypothesis regarding uniformly-stable TH21. We then designed a dual (Cy3/Cy5)-tagged TH21 structures without or with BHQ3 quencher (TH or TH’, respectively; Fig. 3c and Supporting Table 10). Due to the proximity of BHQ3 with Cy5 fluorophore in TH’, the structure had significantly lesser Cy5 fluorescence compared to unquenched TH, while retaining similar Cy3 fluorescence. In vitro, Cy5/Cy3 signal of TH’ was 35% of that of TH. Meanwhile, incomplete TH’ (i-TH’, lacking one side to mimic degraded TH’) exhibited recovered Cy5 fluorescence (Cy5/Cy3 ratio of 98% of that of TH; Fig. 3d). In this manner, Cy5/Cy3 fluorescence ratio following topical penetration of TH’ can be compared to that of unquenched TH, to evaluate the extent of disintegration of the TH’ structure. Following the topical application on mice, TH’ showed lower Cy5/Cy3 signal than TH from both IVIS (Fig. 3e) and fluorescence imaging of skin histology (Fig. 3f, g). Under IVIS imaging, the Cy5/Cy3 ratio of TH’ was 44% of that of TH (Fig. 3e). The slight disparity from the in vitro result might be due to variability in mouse skin condition. This is supported by histological result, which showed an average of 48% for Cy5/Cy3 ratio from TH’ group to TH group, across its penetration depth (Fig. 3f, g). Significant Cy5 fluorescence of the quenched TH’ group at the skin surface is likely due to signal saturation from the high FNA concentration applied. Overall, these results suggest that integrity of the tetrahedron structure was well-retained during the skin penetration process.

Transdermal drug delivery with topical FNAs. We finally explored the role of FNAs as drug carrier in transdermal delivery. As a proof-of-concept, we loaded DOX into TH21 (TH-DOX). Slower migration rate of TH-DOX on gel electrophoresis as compared to Cy5.5-tagged TH or TH only indicated successful DOX loading (Supporting Figure 9A). Moreover, fluorescence measurement of TH-DOX solution against solutions of known DOX concentrations allowed the precise quantification of DOX amount, ~0.57 µg DOX for 100 µg of TH21 or 49.72 nmol DOX per 1 nmol of TH21. When these results were normalized with the number of nucleotide (nt) in the TH21 (252 nt, i.e. ~0.2 DOX/nt), this loading efficiency is comparable to previously-reported 1–9 µM DOX loading in 1 nmol of ~9000 nt DNA nanotube (i.e. ~0.1–1 DOX/nt). Performing gel electrophoresis study on unloaded (TH21) or loaded TH-DOX post distinct incubation period in 10% serum condition, the presence of intercalated DOX significantly extended TH lifespan from enzymatic degradation (half-life of > 48 h than 26 h on unloaded TH, Supporting Figure 10). Thus, while TH21 can be expected to remain effective as carrier within 1–2 days, TH-DOX formulation can remain efficacious for even longer.

The prepared TH-DOX had a 72-h inhibitory concentration (IC50) of 153.45 nM against B16F10 mice melanoma cells, more efficient than free DOX (IC50: 335.81 nM; Fig. 4b). This observation is consistent to previous report on breast cancer cells (MDA-MB231, MDA-MB468, and MCF7), where lower DOX IC50 value was reported following DNA nanostructures intercalation and intracellular delivery. Performing possible explanations for this are related to disruption of endo-lysosomal pathway (through prevention of acidification) and sustained release of DOX from FNAs, leading to greater cellular distribution and overall availability.

To better assess the performance of TH-DOX as TDD carrier, we also synthesized commonly adopted topical drug carriers in
liposomes and degradable polymeric (i.e, poly(lactic-co-glycolic acid)/PLGA) NPs as comparison. The drug loading efficiencies in these larger (~100 nm) NPs were ~2-folds of TH nanostructures (Fig. 4a). Upon longer term storage (i.e. 1 week at 4 °C), however, PLGA-DOX and LIP-DOX showed significant drug leakage (19.3 and 10.8%; Fig. 4c). Moreover, LIP-DOX are limited with tendency to aggregate upon storage (Fig. 4d). We then investigate skin penetration ability of TH-DOX against these carriers, free DOX, free DOX following microneedle (MN) application, and DOX-intercalated 21-bp dsDNA segments (DS-DOX). To note, drug loading was similar between DS21 and TH21 (Fig. 4a). In particular, solid poly(methyl methacrylate) (PMMA) MN (600 μm tall) was finger-pressed for 1 min to disrupt skin membrane integrity (Fig. 4e). As represented and quantified in Fig. 4f, g, TH-DOX facilitated deep penetration in pig skin reaching ~400 μm from periphery, comparable to when free DOX could diffuse following PMMA MN pre-application. LIP-DOX reached ~250–300 μm depth from periphery. DS-DOX (~8 nm in hydrodynamic diameter) showed significantly less penetration than TH-DOX, reaching 150 μm from periphery. Likely, they were retained by the initial few cell layers through interaction with membrane receptors. Coupled with its susceptibility to degradation, they were consequently unable to maintain integrity to penetrate and reach deeper skin layers. PLGA-DOX penetrated minimally to a depth of 100 μm, comparable to that achieved by free DOX.

**Efficacy of topical FNAs for cancer treatment.** TH-DOX formulation was later topically applied on the mouse melanoma model, carrying ~6 mm-sized subcutaneous melanoma tumor in both hind legs (Fig. 5a). Following 24 h treatment and removal of excess cream, IVIS revealed threefold DOX delivery at tumor
region following TH21 application than free DOX application (Fig. 5b). This observation is consistent over six-independent experiments (Fig. 5c). Histology further shows that TH21 brought DOX as deep as 400–450 μm from the skin surface, significantly deeper than the mouse skin covering the tumor area (Fig. 5d, Supporting Figure 11). The mice skin here is notably thinner (100–125 μm) than physiological skin thickness (≥350 μm), presumably from the stretching pressure exerted by underlying tumor. DOX signal decreased along increasing skin/tumor depth (Fig. 5f), matching that of TH21 (Fig. 5e). In comparison, topical delivery of free DOX reached only ~50–75 μm beneath the skin surface, mainly retained at the skin without entering the tumor (Fig. 5f, Supporting Figure 11). Through DOX signal quantification at the subcutaneous tumor region (i.e. signal from 100 μm depth onwards), a 5.67-fold increase in DOX accumulation was achieved via TH-DOX delivery, as compared to free DOX treatment (Fig. 5g). These results suggest the efficacy of TH-DOX to deliver drugs to subcutaneous tumor site.

To corroborate the successful FNA-facilitated DOX delivery to subcutaneous tumor, we further evaluated its effectiveness in inhibiting tumor growth. As depicted in Fig. 6a, we extended the study 2 weeks after the first application of TH-DOX, with three topical treatments a week and bi-daily recording of tumor volume. For comparison purposes, the same amount of DOX was topically delivered by itself (tDOX), within DS-DOX, PLGA NPs (PLGA-DOX), liposomes (LIP-DOX) or through intra-tumoral injection (iDOX) under the same frequency (Fig. 6a). As shown in Fig. 6b and Supporting Figure 12, TH-DOX treatment significantly hindered the tumor growth over other topically applied DOX carrier (DS-DOX, PLGA-DOX, LIP-DOX). At the final time point (day 17 post injection), tumor size on mice treated with TH-DOX was ~33% of the untreated controls (544 mm³ relative to 1653 mm³; Fig. 6c). This result was most comparable to that achieved through PMMA MNs (397 mm³, 24% of the untreated tumors; Supporting Figure 13A). In contrast, DS-DOX, LIP-DOX, PLGA-DOX, and tDOX treatment had less inhibitory effect on tumor growth.
growth, with 68.0, 53.9, 74.5, and 84.5% of the untreated tumors, respectively (Fig. 6d). Notably, TH only group showed negligible inhibition compared to untreated tumors (Fig. 6b, d).

The isolated tumor tissues were further examined with IVIS and histology. IVIS imaging revealed that repeated tDOX and PLGA-DOX treatment resulted in minimal DOX fluorescence over untreated tumor, likely due to insufficient penetration. In comparison, TH-DOX and iDOX, as well as PMMA MN+tDOX significantly enhances DOX content (Fig. 6e and Supporting Figure 13B). By homogenizing these tumors in PBS and plotting it against a standard curve, we found that every gram of TH-DOX and iDOX-treated tumors contained ~7.1 and 8.5 µg of DOX, significantly >1.4 µg found in tDOX-treated tumors, 2 µg in PLGA-DOX, 3.3 µg in DS-DOX and 4.3 µg in LIP-DOX tumors (Fig. 6f and Supporting Figure 13C), TH-DOX facilitated 5.1-fold accumulation of DOX as compared to tDOX, matching results from fluorescence quantification in Fig. 5g. By imaging sliced tumor samples under confocal microscopy, we confirmed the presence of considerable amount of DOX following TH-DOX, PMMA MN+tDOX and iDOX treatment (Fig. 6g and Supporting Figure 14), with concomitant increase in Annexin V expression (indicative of apoptotic tissue; Fig. 6h and Supporting Figure 14). Overall, these results demonstrated efficacious transdermal DOX treatment through FNA delivery, validating its effective penetration.

Safety of FNAs for localized transdermal drug delivery. Finally, we examined the accumulation of DOX in the major tissues/ organs following different delivery formulations. As shown in Fig. 6b, intra-tumoral DOX injection (iDOX) led to greatest accumulation of DOX as compared to tDOX, matching results demonstrated that TH-DOX presented low-serum DOX group contained >2.4-fold DOX as compared to that from other DOX carriers. Of note, TH-DOX presented low-serum DOX concentration and the least organ DOX signal when normalized against tumor signal (Fig. 7b). Such localized delivery can be attributed to the penetration ability of TH-DOX to reach subcutaneous tumor region. In the case of other carriers (i.e. DS-DOX, PLGA-DOX, and LIP-DOX) and iDOX which did not show significant penetration, DOX likely had accumulated over time within the skin layers instead. Consequently, greater redistribution of free DOX is expected through the skin lymphatic or blood vessels. Hematoxylin & eosin staining corroborates the safety of FNA carrier, with minimal differences seen between organs from TH-DOX-treated mice and those from the healthy mice (Supporting Figure 15). Another striking observation is related to the utilization of PMMA MN to disrupt the integrity of skin overlying the tumor. During early timepoints (i.e. day 5/7 post tumor injection), MN application results in reversible mark which faded after 5 min. Towards the later timepoint however (e.g. day 12), thumb-pressed MN resulted in significant bleeding, presumably due to thinner skin tissue from the stretching pressure exerted (Supporting Figure 16). To this end, MN application can be considered to have induced consequential damage.

Discussion

Transdermal penetration ability of drug carriers is greatly dependent on its physical parameters, including size, morphology, charge, and material composition. Skin penetration has so far
been observed on small liposomes, dendrimers, lipid and polymeric NPs and nanoemulsions, with or without adjunctive treatments (e.g. ultrasound). Predominant-positive charge promotes transdermal permeation of polymeric NPs, though it may also limit permeation by promoting significant adsorption on top few cell layers. In this study, we find that highly ordered FNAs can penetrate the skin barrier (Fig. 2). Three structures with <75 nm in size (TH21, TH37, 6H714) showed considerable skin penetration, in which small tetrahedron TH37 (44 nm) and TH21 (17 nm) reached deeply to ~350–400 µm beneath skin surface in mice. This result aligns well with reported size-dependency on carrier skin penetrative ability. However, the three distinct-shaped structures with similar size (~180 nm; TH337, R13730, and T14498) all showed minimal skin penetration (~50–75 µm from the skin surface). This suggests minimal shape dependency on FNA skin penetration, minor as compared to size dependency.

One challenge in evaluating skin penetration of wide variety of nanostructures is the choice of dosage. Herein, there is a big discrepancy in molecular weight of the 8 structures, ranging between 81.9 kDa and 4711.9 kDa. In this case, fixing same FNA mole concentration would result in 58-fold greater amount of NA to be internalized between largest structure (T/B14498) and smallest structure (T21). Considering this, same mass concentration (i.e. equivalent NA content) was chosen for the skin penetration tests (100 µg/mL). We realize that different molar concentration gradients may result in distinct diffusion rates. Thus, 24 h were given in all experiments to ensure sufficient diffusion of all 8 FNAs. Multiple fluorophores were also conjugated on larger FNAs while small structures (TH21, TH37, and 6H714) only had a single fluorophore (Supporting Table 1). An interesting observation is that the ratio of fluorophores didn’t match the ratio of mass. The addition of 2 or 3 fluorophores were sufficient to enhance the fluorescence of the larger FNAs to fluorophore solution. However, this 3D structure of FNA and the location of fluorophore tagging influence the relation between fluorophore concentration and fluorescence yield, which is distinct from a fluorophore solution.

In ensuring successful drug delivery, sufficient integrity/stability of the drug carrier is required throughout the administration and distribution. By examining the fluorescence change of a dual tagged, symmetrical tetrahedron structure (TH) during its skin penetration process, we demonstrated the retention of integrity of FNA structures during topical application and skin penetration steps (Fig. 3). This is supported with ~26-h half-life of FNA under 10% serum condition, suggesting an effective carrier period of...
1–2 days (Supporting Figure 10). The stability of FNAs and their capability in skin penetration allowed us to explore their potential applications in skin disease treatment. Relative to conventional TDD carrier in PLGA NP and liposomes, FNAs confer physical stability with regards to aggregation and drug leakage, for intercalated DOX tested here and expectedly for chemically conjugated drugs (Fig. 4)31,32. DOX-intercalated FNA exhibited a half-life of >48 h, significantly longer than prior to DOX loading (26 h). Such stability complements its precisely tunable size and penetration ability to achieve consistent, effective TDD. Nuclease-resistant backbone modification (e.g. 2-O'-methylated), salt-modulated concentration of cations (e.g. Mg2+), and inclusion of additional coating (e.g. oligosyline) can further stabilize FNA and extend its lifespan for even less frequent application. This will open broader range of TDD treatments, including those requiring extended treatment (e.g. chronic wound), although its influence over FNA interactions with skin cells and thus penetration requires further clarification33–35.

Taking the xenograft melanoma tumor model as an example, capability of TH21 to deliver DOX into subcutaneous tumor site was compared to other DOX formulations (i.e. free, PLGA NPs, Liposomes, and MNs). Reaching as deep as 400–450 μm from the skin periphery (Fig. 5), TH-DOX effectively reduced the tumor growth (~33% of untreated ones), slightly less than those treated with intra-tumoral injection of DOX or with topical DOX application following PMMA MN disruption (Fig. 6b). In comparison, DS-DOX did not penetrate deep from skin periphery (likely due to receptor interaction and premature degradation), resulting in poor tumor growth inhibition. Similarly, inferior penetration of both PLGA-DOX and LIP-DOX as compared to TH-DOX was reflected as well in unsatisfactory tumor inhibition. More excitingly, this performance was accompanied with minimal non-targeted delivery on other organs. This contrasts with the great accumulation of DOX in other organs following intratumoral injection (Fig. 7a, b), in which locally injected DOX easily migrated through the tumor vasculature and lymph system36. Overall, although transdermal delivery of TH-DOX was relatively slow compared to when skin barrier was disrupted by MN application or when DOX was injected directly, better isolation of transdermal TH-DOX from the circulation enabled similar inhibition effect as the latter two. Additionally, there is minimal risk of pain and injury, unlike needle injection or microneedle application under special conditions (e.g. skin thinning)37.

Another key advantage of FNAs as TDD carrier is its fully-biodegradable safety with natural degradation products. This contrasts with polymeric NPs (e.g. PLGA), inert NPs (e.g. Ag, Au) or metal-oxide NPs (e.g. ZnO, TiO2) NPs adopted in various cosmeceutical skin products, which may degrade into acids and affect physiological pH, accumulate over extended usage or release inflammatory-inducing ions38,39. Minimal skin irritation and better patient compliance can be expected from frequent, repeated application of FNA-based products. Finally, with its predictable hybridization and folding, FNAs of various shapes and sizes can be conveniently fabricated1,40. Such versatility allows careful tuning of structures to match intended delivery site/depth. As shown herein, larger FNAs such as 6H14498 and R13730 could be employed as...
sustained-release drug carrier when minimal transdermal penetration is desired. Meanwhile, smaller FNAs such as TH21 can be adapted to target dermis layer (e.g. to modulate collagen production by fibroblast cells)\(^1\).

To our knowledge, this is the first study to explore DNA origami nanostructures for TDD application. Preliminary study reveals that both cellular and trans-appendageal route contribute to DNA nanostructure skin penetration, with the latter mechanism responsible for ~75% delivery into deep dermis region. Future study will elaborate on this penetration mechanism responsible for ~75% delivery into deep dermis.

**Methods**

**Materials.** Chemicals and reagents were obtained from Sigma-Aldrich unless stated otherwise. Dulbecco’s modified eagle medium (DMEM) containing t-glutamine was purchased from Lonza. Trypsin–EDTA (0.05%), fetal bovine serum (FBS) and streptomycin (PS; 10kU/mL) were obtained from Gibco. NucBlue Live Ready Probes and LysoTracker® were obtained from ThermoFisher Scientific. Aquaphor Healing Ointment (Eucerin) was obtained from pharmacy. Animal studies were performed in compliance with guidelines set by Nanyang Technological University’s Institutional Animal Care and Use Committee (IACUCs: NTU #BN16098).

**Fabrication and characterization.** FNAs were assembled in accordance to previous studies\(^2–4\). Detailed assembly of each FNA structure can be found in supporting information. Briefly, small FNAs were assembled using equimolar ratio of ssDNA component strands. Meanwhile, large FNAs were assembled by mixing together long ssDNA scaffold with short staple oligonucleotides (designed by fold different parts of scaffold DNA) at 1:10 ratio (excess of short strands) in 1X Tris-Acetate-EDTA (TAE) buffer containing MgCl\(_2\) and other constituents as listed in Supporting Table 1. Sequences for FNA structures are as listed in Supporting Table 2. Super-resolution imaging was performed using AFM nanoscale IIIa AFM (Veeco/Digital Instruments), coupled with a silicon nitride cantilever with sharp pyramidal tip (OMCL-TR400PSA, Olympus).

**Histology.** For histological examination, mice and human skin explants after injection of free DOX were performed to deliver ~2.4 μg DOX each treatment. This matches previous reported topical dosage of 120 μg/kg DOX\(^49\). Following 24-h treatment period, similar VIS procedure was done to measure total DOX and TH-DOX concentration in target region (DOX: 490/595 nm, cy5.5: 675/695 nm). For efficacy and evaluation, the study was extended for another 2 weeks, with treatments repeated thrice a week. As control, several tumors were treated with unloaded TH or thumb-pressed for 1 min with PMMA MN (3 M) was thumb-pressed on skin for 1 min prior to free DOX application. Excess cream was wiped for further processing.

**Topical application of FNAs on mice and ex vivo porcine skin.** Fresh porcine ear skin was obtained from Jurong Abattoir (Singapore) and cut into 1 cm by 1 cm pieces. Ten microliter of free DOX, TH-DOX, DS-DOX, PLGA-DOX, and LIP-DOX solutions (adjusted to contain 5 μg of DOX) was mixed with Aquaphor \((1:1 \text{ weight ratio})\) and topicaly applied for 24 h. Control, 600 μm-tall PMMA MN (3 M) was thumb-pressed on skin for 1 min prior to free DOX application. Excess cream was wiped for further processing.

**Melanoma mice model.** \(\text{IC}_{50}\) of TH-DOX formulation was tested on B16F10 mice melanoma cells, by performing Alamarblue assay 72 h post treatment. Melanoma disease model was prepared on NCR nude mice or C57B6 mice through subcutaneous injection of \(1 \times 10^6\) B16F10 melanoma cells into its rear flank. Cells were injected in 1:1 PBS:matrigel mixture to ensure retention. Incubation was given to allow tumor to reach ~50 mm\(^3\) in volume prior to any treatment. Once the tumor reached appropriate size, topical application of free DOX, TH-DOX, DS-DOX, PLGA-DOX, and LIP-DOX injection of free DOX was performed to deliver ~2.4 μg DOX each treatment. This matches previous reported topical dosage of 120 μg/kg DOX\(^49\). Following 24-h treatment period, similar VIS procedure was done to measure total DOX and TH-DOX concentration in target region (DOX: 490/595 nm, cy5.5: 675/695 nm). For efficacy and evaluation, the study was extended for another 2 weeks, with treatments repeated thrice a week. As control, several tumors were treated with unloaded TH or thumb-pressed for 1 min with PMMA MN prior to free DOX application. Tumor sizes were recorded every other day, with tumor and organs isolation following mice euthanasia. The tumor volume was calculated by the following formula: \(\text{length} \times \text{width}^2/2\). For serum DOX quantification, mice blood samples were collected at 4000μg for 15 min after 30 min incubation at room temperature.

**Histology.** For histological examination, mice and human skin explants after topical FNA treatment were fixed using 10% neutral-buffered formalin solution for 24 h. Skins were subsequently rinsed thrice with PBS, treated with 30% (w/v) sucrose for 16 h, and immersed into optimal cutting temperature (OCT) solution. Samples were then exposed to liquid nitrogen for cryosectioning. Finally, thin sample slices (15 μm) were stained with Hoechst33342, Annexin V AlexaFluo488 (Invitrogen™ A23204, 50-fold dilution) or anti-Keratin14 antibody (DyLight 488; Novus Biologicals NB22-4475G, 5 μg/ml) according to the manufacturer’s protocol or with standard Hematoxylin & Eosin protocol.

**Fluorescence imaging and signal quantification.** Fluorescence imaging on FNA-labeled cells or cream-treated skin sections was performed on Laser Scanning Confocal Microscope LSM880 at ×100 magnification w/ 10× objective. The respective fluorescence channels were set as follows: (1) Red channel (635 nm excitation/680 nm emission for Cy5.5 and 630 nm excitation/670 nm for Cy3); (2) Green channel (488 nm excitation/525 nm emission for Cy5); (3) Blue channel (405 nm excitation/430 nm emission for Cy3). For quantification, cell viability was determined by AlamarBlue solution at a ratio of 1:100 in culture medium. The change of optical density was monitored at 570/585 nm using a microplate reader (SpectraMax i3; Molecular Devices). Measurement was repeated three times.
Statistical analysis. One-way ANOVA was carried out to calculate the significance $F$-value. A suitable post hoc test (Tukey) was chosen using the IBM SPSS Statistics 22 software. For each experiment, values are reported as mean ± standard deviation at least of three independent samples.

Reporting summary. Further information on experimental design is available in the Nature Research Reporting Summary linked to this article.

Data availability

All relevant data are included in the main manuscript and Supplementary Information. The source data underlying Fig. 2c, e, 3d, e, 4b–d, 5b, e.g. 6b, d, f, 7b, d and Supplementary Fig. 1d–2, 2b, 4a–b, 5b–c, 9b, 13–c and 14b are provided as a Source Data file. Additional data are available from the corresponding author upon reasonable request.

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References

1. Douglas, S. M. et al. Self-assembly of DNA into nanoscale three-dimensional shapes. Nature 459, 414 (2009).
2. Wang, P., Meyer, T. A., Pan, V., Dutta, P. K. & Ke, Y. The beauty and utility of DNA origami. Chem. Sci. 2, 359–382 (2017).
3. Liu, X. et al. Complex silica composite nanomaterials templated with DNA origami. Nature 559, 393 (2018).
4. Li, J., Green, A. A., Yan, H. & Fan, C. Engineering nucleic acid structures for programmable molecular circuitry and intracellular biocomputation. Nat. Chem. 9, 1056 (2017).
5. Zhang, Q. et al. DNA origami as an in vivo drug delivery vehicle for cancer therapy. ACS Nano 8, 6633–6643 (2014).
6. Lee, H. et al. Molecularly self-assembled nucleic acid nanoparticles for targeted in vivo siRNA delivery. Nat. Nanotechnol. 7, 389 (2012).
7. Perrault, S. D. & Shih, W. M. Virus-inspired membrane encapsulation of DNA nanostructures to achieve in vivo stability. ACS Nano 8, 5132–5140 (2014).
8. Prausnitz, M. R. & Langer, R. Transdermal drug delivery. Nat. Biotechnol. 26, 1261 (2008).
9. Alexander, A. et al. Approaches for breaking the barriers of drug permeation through transdermal drug delivery. J. Control Release 164, 26–40 (2012).
10. Alvarez-Román, R., Naik, A., Kalia, Y., Guy, R. H. & Fessi, H. Skin penetration and distribution of polymeric nanoparticles. J. Control Release 199, 53–62 (2014).
11. Zheng, D. et al. Topical delivery of siRNA-based spherical nucleic acid nanoparticle conjugates for gene regulation. Proc. Natl Acad. Sci. USA 109, 11197–111980 (2012).
12. Yeo, D. C., Wiraja, C., Paller, A. S., Mirkin, C. A. & Xu, C. Abnormal scar barrier and immune dysregulation of at least three-independent samples.
13. Wiraja, C. et al. Near infrared-sensitive fluorescent DNA-origami as a biomaterial. ACS Nano 8, 114 (2014).
14. Jiang, Q. et al. DNA origami as a carrier for circumvention of drug resistance. J. Am. Chem. Soc. 134, 13396–13403 (2012).
15. Zhao, Y.-X. et al. DNA origami delivery system for cancer therapy with tunable release properties. ACS Nano 6, 8684–8691 (2012).
16. Escobar-Chávez, J. J. Nanocarriers for transdermal drug delivery. Skin 19, 22 (2012).
17. Korting, H. C. & Schäfer-Korting, M. In: Drug delivery (ed. Schäfer-Korting, M.) 435–468 (Springer, Berlin Heidelberg, 2010).
18. Wu, X., Landfester, K., Musyanovych, A. & Guy, R. Disposition of charged nanoparticles after their topical application to the skin. Skin. Pharmacol. Physiol. 23, 117–123 (2010).
19. Gupta, R. & Rai, B. Effect of size and surface charge of gold nanoparticles on their skin permeability: a molecular dynamics study. Sci. Rep. 7, 45292 (2017).
20. Troy, C. et al. Size dependent skin penetration of nanoparticles in murine and porcine dermal and subcutaneous tissues. Eur. J. Pharm. Biopharm. 100, 101–108 (2016).
21. Li, J. et al. Self-assembled multivalent DNA nanostructures for noninvasive intracellular delivery of immunostimulatory CpG oligonucleotides. ACS Nano 5, 8783–8789 (2011).
22. Bla, H., Kurisinkal, E. E. & Basting, M. M. C. Engineering a stable future for DNA-origami as a biomaterial. Biomater. Sci. 7, 532–541 (2019).
23. Hahn, J., Wickham, S. F., Shih, W. M. & Perrault, S. D. Addressing the instability of DNA nanoarchitectures in tissue culture. ACS Nano 8, 8765–8773 (2014).
24. Ponnuswamy, N. et al. Oligolysine-based coating protects DNA nanostructures from low-salt denaturation and nucleosome degradation. Nat. Commun. 8, 15654 (2017).
25. Al-Abd, A. M., Hong, K.-Y., Song, S.-C. & Kuh, H.-J. Pharmacokinetics of doxorubicin after intratumoral injection using a thermostable hydrogel in tumor-bearing mice. J. Control Release 142, 101–107 (2010).
26. Shah, A. R. & Kennedy, P. M. The aging face. Med. Clin. North Am. 102, 1041–1054 (2018).
27. Crosora, M. et al. Nanoparticle dermal absorption and toxicity: a review of the literature. Int. Arch. Occup. Environ. Health 82, 1043–1055 (2009).
28. Robertson, T. A., Sanchez, W. Y. & Roberts, M. S. Are commercially available nanoparticles safe when applied to the skin? J. Biomed. Nanotechnol. 6, 452–468 (2010).
29. Xing, S. et al. Constructing higher-order DNA nanoarchitectures with highly purified DNA nanocages. ACS Appl. Mater. Interfaces 7, 13174–13179 (2014).
30. Varani, J. et al. Vitamin A antagonizes decreased cell growth and elevated collagen-degrading matrix metalloproteinases and stimulates collagen accumulation in naturally aged human skin. J. Invest. Dermatol. 114, 480–486 (2000).
31. Rothemund, P. W. Folding DNA to create nanoscale shapes and patterns. Nature 440, 297 (2006).
32. Fu, Y. et al. Single-step rapid assembly of DNA origami nanostructures for addressable nanoscale bioreactors. J. Am. Chem. Soc. 135, 696–702 (2012).
33. Ouyang, X., Chao, J., Su, S. & Fan, C. In: 3D DNA nanostructures (eds Ke, Y. & Wang, P.) 121–132 (Springer, New York, 2017).
34. Cheng, J. et al. Formulation of functionalized PLGA–PEG nanoparticles for in vivo targeted drug delivery. Biomaterials 28, 869–876 (2007).
35. Shroff, K. & Kokkoli, E. PEGylated liposomal doxorubicin targeted to α5β1-integrin expressing MDA-MB-231 breast cancer cells. Langmuir 28, 4729–4736 (2012).
36. Wiraja, C. et al. Near-infrared light-sensitive liposomes for enhanced plasmid DNA transfection. Bioeng. Transl. Med. 1, 357–364 (2016).
37. Tupal, A., Sabzichi, M., Ramezani, F., Khoussani, M. & Hamisheshkar, H. Dermal delivery of doxorubicin-loaded solid lipid nanoparticles for the treatment of skin cancer. J. Microencapsul. 33, 372–380 (2016).
38. Faustino-Rocha, A. et al. Estimation of rat mammary tumor volume using caliper and ultrasonography measurements. Lab Animal 42, 217 (2013).

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Author contributions

C.W. and Y.Z. performed research. M.X., W.F. and Q.L. synthesized and characterized the nanoparticles. S.Y. and W.C. designed and performed the experiments. M.V.S. contributed discussion and analyzed the results, and drafted the manuscript. M.V.S. contributed discussion and analyzed the results, and drafted the manuscript. All authors reviewed and approved the final version of the manuscript.
Additional information

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Competing interests: A patent application has been submitted based on these results. The patent application covers the design criteria and synthetic methods of the Framework Nucleic Acids (FNAs) for transdermal applications, with co-authors C.F., C.J.X., L.W., C.W. and M.X. listed as inventors. Application number: CN201811308594. Status of application: Publication (2019.02.22); Publication number: CN109364261A. The remaining authors declare no competing interests.

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