

MIT Open Access Articles

Nano-Layered Microneedles for Transcutaneous Delivery of Polymer Nanoparticles and Plasmid DNA

The MIT Faculty has made this article openly available. **Please share** how this access benefits you. Your story matters.

Citation: DeMuth, Peter C., Xingfang Su, Raymond E. Samuel, Paula T. Hammond, and Darrell J. Irvine. Nano-Layered Microneedles for Transcutaneous Delivery of Polymer Nanoparticles and Plasmid DNA. *Advanced Materials* 22, no. 43 (November 16, 2010): 4851-4856.

As Published: <http://dx.doi.org/10.1002/adma.201001525>

Publisher: Wiley-Blackwell Publishers

Persistent URL: <http://hdl.handle.net/1721.1/79375>

Version: Author's final manuscript: final author's manuscript post peer review, without publisher's formatting or copy editing

Terms of use: Creative Commons Attribution-Noncommercial-Share Alike 3.0





PubMed
Central

HHMI
HOWARD HUGHES MEDICAL INSTITUTE

AUTHOR MANUSCRIPT

Accepted for publication in a peer-reviewed journal

Published as: *Adv Mater.* 2010 November 16; 22(43): 4851–4856.

Nano-layered Microneedles for Transcutaneous Delivery of Polymer Nanoparticles and Plasmid DNA *

Peter C. DeMuth,

Department of Biological Engineering, Massachusetts Institute of Technology, 77 Massachusetts Ave, Cambridge, MA 02139 (USA)

Xingfang Su,

Department of Materials Science and Engineering, Massachusetts Institute of Technology, 77 Massachusetts Ave, Cambridge, MA 02139 (USA)

Raymond E. Samuel,

Department of Chemical Engineering, Massachusetts Institute of Technology, 77 Massachusetts Ave, Cambridge, MA 02139 (USA)

Prof. Paula T. Hammond, and

Department of Chemical Engineering and Institute for Soldier Nanotechnologies, Massachusetts Institute of Technology, 77 Massachusetts Ave, Cambridge, MA 02139 (USA)

Prof. Darrell J. Irvine

Department of Materials Science and Engineering, Department of Biological Engineering, Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, 77 Massachusetts Ave, Cambridge, MA 02139 (USA); Ragon Institute of MIT, MGH, and Harvard, Boston, MA 02139, Howard Hughes Medical Institute, 4000 Jones Bridge Rd., Chevy Chase, MD

Paula T. Hammond: hammond@mit.edu; Darrell J. Irvine: djirvine@mit.edu

Keywords

Thin Films; Nanoparticles; Polymeric Materials; Drug Delivery

Current vaccine and therapeutic delivery is largely needle-based, [1] but a number of inherent risks and disadvantages to needle-based delivery have been recognized, such as the need for cold storage of liquid formulations, [1, 2] the requirement of trained personnel for administration, and reduced safety due to needle re-use and needle-based injuries. [3] To address these limitations, vaccination and therapeutics administration through the skin represents a promising alternative strategy, [4-6] and technologies promoting efficient transcutaneous delivery of a variety of drugs and vaccines has become a significant focus of recent research (reviewed in [7]). Recent work in this area has demonstrated the utility of microneedle arrays for efficient and pain-free disruption of the stratum corneum (SC), promoting transcutaneous delivery of a variety of bio-active materials. [8, 9] Microneedle delivery is often achieved by coating dried water-soluble drug formulations directly on the surfaces of solid microneedles. Parallel studies in the area of polyelectrolyte multilayer (PEM) engineering have demonstrated the potential for simple and versatile materials encapsulation into conformal thin films, providing robust control over materials release, solid-state stabilization of environmentally-sensitive encapsulated materials, and nanometer-

Correspondence to: Paula T. Hammond, hammond@mit.edu; Darrell J. Irvine, djirvine@mit.edu.

Supporting Information is available online from Wiley InterScience or from the author.

scale control over film structure and composition. [10-16] We recently reported the construction of PEM films loaded with vaccine components prepared on flexible substrates for transcutaneous vaccine delivery. [10] However, these planar multilayer patches require prior SC disruption to permit entry of released cargos from the PEM films into the epidermis. We hypothesized that combining the flexible and highly tunable nature of PEM thin film coatings with microneedle substrates enabling direct entry of films into the viable epidermis could provide a versatile platform for single-step transcutaneous delivery of a broad range of drugs and drug carriers that is effective, generally applicable, inherently safe and pain-free, and potentially cost effective.

In vitro delivery of plasmid DNA from PEMs has been demonstrated using multilayers that deconstruct in aqueous conditions via parallel disassembly and degradation of the constituent polymers. [17, 18] We show here that microneedle arrays coated with DNA-carrying PEMs allows this concept to be translated to *in vivo* transfection in murine skin, an approach of great interest for DNA vaccine delivery. Similarly, we show that biodegradable poly(lactide-co-glycolide) (PLGA) nanoparticles (NPs), ubiquitous in drug delivery, can be embedded within microneedle PEM coatings, and subsequently deposited in the epidermis following a brief application of microneedles to unmanipulated skin. Finally, we show that multilayers combining these two diverse types of therapeutic cargos can be prepared for co-delivery into skin. To our knowledge, this is the first report of functional coating deposition onto microneedle arrays through the use of layer-by-layer (LbL) PEM self-assembly. Furthermore, although transcutaneous plasmid DNA delivery has been demonstrated by topical application to barrier disrupted skin [19, 20] and recently through application of microneedles coated with dried formulations, [21] this is the first reported demonstration of *in situ* DNA delivery from PEM films or PEM-coated microneedles to mediate *in vivo* gene delivery and expression.

We first used laser micromachining to prepare poly(dimethylsiloxane) (PDMS) slabs with arrays of tapered pyramidal or conical microscale cavities across their surface, to serve as molds for polymer microneedle fabrication. Similar to prior reports, [22] PLGA pellets placed over the molds were melted under vacuum, cooled, and separated from the PDMS (Figure S1) to obtain arrays of microneedles each 250 μ m in diameter at their base and 900 μ m in height (Figures 1A, S2). Microneedles of similar dimensions have been shown to produce negligible pain sensations in humans, while maintaining adequate structural integrity to efficiently penetrate the SC. [23] To fabricate a biodegradable PEM coating capable of controlled DNA release *in vivo*, we employed a hydrolytically degradable poly(β -amino ester) (PBAE), designated polymer-1 (poly-1, Figure S3). [24] PBAEs have been previously shown to be biocompatible and degradable, to build multilayers with DNA that transfect cells *in vitro*, and to have adjuvant activity when co-delivered with DNA vaccines. [18, 25, 26] Poly-1 in particular has been used recently by our group to fabricate LbL films with controlled erosion and tunable drug release, [12, 27, 28] and by others to fabricate DNA-releasing PEM films for potential gene delivery applications. [19, 29] To provide a uniform initial surface charge density for PEM film growth on the PLGA microneedles, we first deposited twenty bilayers of poly(4-styrene sulfonate) (SPS), a synthetic polyanion, and protamine sulfate (PS), a mixture of four related biocompatible, highly cationic polypeptides of approximately 30 amino acids (Figure S1). [30, 31] Onto this base film, PEMs were built through the alternating adsorption of poly-1 and plasmid DNA (encoding firefly luciferase, pLUC). Surface profilometry and UV absorbance indicated linear growth of (poly-1/plasmid DNA) multilayers ($\sim 0.5 \pm 0.1 \mu\text{g pDNA}/\text{cm}^2/\text{bilayer}$) when deposited onto the (PS/SPS) base-layer (Figure 1B). Confocal laser scanning microscopy (CLSM) was used to qualitatively examine microneedles coated with Cy3-labeled pDNA PEMs. Microneedle arrays coated in this way showed surface-localized fluorescence conformally coating each

microneedle (Figures 1C and S4A), while control uncoated needles showed no background fluorescence (data not shown).

We next tested whether a similar approach could be used to incorporate biodegradable polymer NPs into microneedle coatings. Lipid-coated PLGA NPs (244 nm in diameter, PDI 0.15) bearing a phospholipid surface layer composed of the zwitterionic lipid DOPC, the anionic lipid DOPG, and containing a lipophilic tracer dye (DiI or DiD) were prepared using an emulsion/solvent evaporation process we recently described. [32] Microneedles were primed with a (PS/SPS) base layer as before, and then alternating layers of poly-1 and PLGA NPs were deposited onto the arrays via spray LbL multilayer self-assembly (Figure S1). [33] CLSM (Figures 1D and S4B) and SEM (Figure 1E) imaging of the nanoparticle PEM-coated arrays revealed conformal coatings on the microneedles, similar to the results seen with (poly-1/DNA) films. Four (poly-1/NP) bilayers produced a coating approximately 2 μm thick as measured by profilometry. In addition, serial deposition of (poly-1/pLUC) followed by (poly-1/NP) bilayers on the same microneedle array permitted the creation of films carrying both functional components (Figures 1F and S4C). Thus, PEM-coated microneedles have the potential to act as multifunctional delivery platforms, carrying cargos with diverse physical properties.

We next analyzed the penetration of microneedle arrays into the dorsal ear skin of C57Bl/6 mice or C57Bl/6-MHC II-GFP mice, transgenic animals expressing green fluorescent protein (GFP) fused to all class II major histocompatibility complex (MHC) molecules. [34] The MHC II-GFP fusion protein provided an *in situ* fluorescence marker for the viable epidermis in skin samples from these mice, as fluorescent epidermal MHC II⁺ Langerhans cells (LCs) are readily detected by CLSM in mouse auricular skin. [12] Prior reports have demonstrated that microneedles prepared from biodegradable polymers with suitable elastic moduli and needle geometries can penetrate human cadaver skin. [23] To confirm that our PLGA arrays could similarly penetrate murine skin, uncoated microneedles were applied to dorsal ear skin. Trypan blue staining revealed efficient and consistent penetration of PLGA microneedle arrays through the SC; [8] light microscopic inspection of arrays before/after application showed some buckling/bending but little breakage of the needle tips (Figures 2A and S5). CLSM imaging of ear skin from MHC II-GFP transgenic mice showed that microneedles readily penetrated into the viable epidermis where LCs were colocalized within the same z-plane (Figure S6). To determine if PEM-coated microneedle arrays could deliver pDNA and/or NP cargos into the skin, we prepared PEM-coated microneedles carrying Cy3-labeled pLUC DNA (Cy3-pLUC) or DiI-labeled PLGA NPs (DiI-PLGA NPs). These PEM-coated arrays were applied to the ears of live anesthetized MHC II-GFP mice for 1 min, 5 min or 24 hrs, and then both the freshly explanted ear skin and the applied microneedles were examined by CLSM. Interestingly, the cargo delivery properties of these two types of microneedle coatings were quite distinct. Microneedles carrying (poly-1/pDNA) films examined before and after application to skin showed very little loss of DNA from the needle surfaces after applications of 1 or 5 min (Figure 2B and Figures S7A, B, D, and data not shown), and little detectable transferred DNA in the epidermis (Figures 2D and S8A), but arrays applied to skin for 24 hours led to nearly complete loss of pDNA from the microneedles (Figure 2B and Figures S7C, D) with a corresponding pronounced accumulation of DNA in the skin at depths colocalizing with LCs (Figure 2E and S8B). In contrast, microneedles carrying 4 bilayers of spray-deposited (poly-1/PLGA NP) films showed immediate transfer of NPs into the epidermis and coincident loss of NP signal from the microneedles themselves following even a 5 minute application on the skin (Figures 2C, 2F, S9, S10). These disparate results suggest that plasmid DNA-containing PEM thin films remained intact upon microneedle penetration and subsequently release DNA over a period of 24 hours, while PLGA NP-containing PEM thin films are likely deposited in the skin concomitantly with microneedle insertion. It is anticipated that pDNA undergoes some

degree of interpenetration during incorporation in PEMs, consistent with other polyion species. This would lead to molecular entanglements that would not be present in the nanoparticle thin films and could account for the relative ease of removal of these films once inserted into the skin. Thus both PEM multilayer architecture and the nature of the encapsulated components are parameters controlling the delivery properties of PEM-coated microneedles. Notably, arrays coated first with (poly-1/pLUC) followed by 4 bilayers of spray-deposited (poly-1/PLGA NPs) co-delivered DNA and PLGA NPs to the skin of live mice after a 24-hr application (Figures 2G and S11).

Although murine and human skin exhibit a number of structural differences, preclinical mouse studies of transcutaneous vaccine delivery have been remarkably predictive of clinical trial results. [6] In addition, the mouse model permits a detailed functional analysis of biological responses to delivered pDNA or NPs. In order to further evaluate the potential of PEM-coated microneedle arrays for transcutaneous DNA delivery, we assessed the ability of (poly-1/pLUC)-coated PLGA microneedles to transfect cells *in vivo*. PEM-coated microneedles were applied to the dorsal ear skin of C57BL/6 mice, and *in vivo* transfection was quantified over time using whole animal bioluminescence imaging to detect luciferase expression. Mice were treated by application of a 24 bilayer (poly-1/pLUC)-coated microneedle array to ear skin for 5 minutes (Figure 3A), or a 1- (Figure 3B), 5- (Figure 3C) or 24-bilayer (Figure 3D) array for 24 hrs. Bioluminescence was then monitored *in vivo* for 7 days. Successful *in vivo* transfection and expression of firefly luciferase in the ear skin was detected for both 5 minute and 24 hr application times, despite the low level of pDNA detected in skin for the former (Figure 3E). In both cases, luciferase expression was detected for over a week, though pLUC-coated microneedles applied for 24 hours resulted in an increase in the intensity of luciferase expression, as expected from the CLSM results described above. Additionally, the iterative nature of LbL film construction is amenable to robust dosage control. Application of microneedle arrays coated with 1, 5, or 24 bilayers of (poly-1/pLUC) for 24 hours gave luciferase expression levels spanning an order of magnitude (Figure 3F).

In conclusion, as a first step towards the design of a general materials platform for transcutaneous DNA and therapeutic NP delivery, we have demonstrated for the first time the application of LbL self-assembly for the deposition of functional coatings on microneedle arrays. We have shown the versatility of this approach, engineering PEM films containing pDNA and/or degradable polymer NPs, and demonstrating their utility for delivery into the viable epidermis through microneedle application. Finally, we have shown for the first time to our knowledge, successful *in vivo* transfection via DNA released from microneedle-supported PEM films. These findings suggest the potential utility of these materials for DNA vaccine delivery and gene therapy, as well as the possibility of co-delivery of therapeutic-loaded degradable polymer NPs for sustained and controlled release of encapsulated materials *in vivo*.

Experimental

PLGA Microneedle Fabrication

PDMS molds (Sylgard 184, Dow Corning) were fabricated by laser ablation using a Clark-MXR, CPA-2010 micromachining system. PLGA pellets (50:50wt lactide: glycolide, 46 kDa, Lakeshore Biomaterials) were melted over the molds under vacuum (-25 in. Hg) at 145°C for 40 minutes, and then cooled at -20°C before separating the cast microneedle arrays. Arrays were characterized using a JEOL 6700F FEG-SEM.

PLGA Nanoparticle Preparation

PLGA nanoparticles were prepared as previously described. [32] Briefly, PLGA (30 mg), DOPC/DOPG lipids (4:1 mol ratio, 5 mg, Avanti Polar Lipids), and DiI or DiD (6.4 ng, Invitrogen) were co-dissolved in dichloromethane (1 mL). PBS (200 μ L) was added, the emulsion was sonicated (7W, 1 minutes) using a Microson cell disruptor, added to Milli-Q (MQ) water (4 mL), and sonicated again (12W, 5 minutes), followed by incubation for 12 hours at 25°C. The resulting particles were purified on a sucrose gradient and analyzed using a BIC 90+ light scattering instrument (Brookhaven Instruments Corp).

Polymer Multilayer Film Preparation

All LbL films were assembled using a Carl Zeiss HMS DS50 slide stainer. Films were constructed on silicon wafers, quartz slides, and PLGA microneedle arrays following treatment with O₂ plasma. To build (PS/SPS) baselayers, substrates were dipped alternatively into PS (2 mg/mL, 100 mM NaOAc, Sigma-Aldrich) and SPS (5 mM, 20 mM NaCl, Sigma-Aldrich) solutions for 10 minutes separated by two sequential 1 minute rinses in MQ water. [30] (Poly-1/pLUC) multilayers were deposited similarly, alternating 5 minute dips in Poly-1 (2 mg/mL in 100 mM NaOAc, synthesized according to previous literature [24, 26]) and pLUC (1 mg/mL, 100 mM NaOAc, a gift from Dr. Daniel Barouch, Beth Israel Deaconess Medical Center) solutions separated by two sequential rinsing steps in NaOAc (100 mM, pH 5.0). Fluorescent pLUC was prepared using Cy3 Label-IT reagent (Mirus Bio Corporation). All solutions (except pLUC) were adjusted to pH 5.0 and filtered (0.2 μ m) prior to dipping.

Particle Multilayer Film Preparation

Films were assembled using a previously described spray LbL technique. [33] Briefly, microneedle arrays were coated with atomized spray solutions using modified air-brushes. Poly-1 (2 mg/mL, 100 mM NaOAc) and PLGA NP (20 mg/mL in MQ water) solutions were sprayed alternatively for 3 seconds (0.2 mL/s, 15 cm range) separated by 6 second rinses with NaOAc (100 mM). Film thickness was measured using a Tencor P-16 surface profilometer. Film delivery was characterized through CLSM imaging of microneedle arrays using a Zeiss LSM 510 and data analysis using Image J. [35]

In Vivo Transcutaneous Delivery

Animals were cared for in the USDA-inspected MIT Animal Facility under federal, state, local, and NIH guidelines for animal care. Microneedle application experiments were performed on anesthetized C57BL/6 mice (Jackson Laboratories) and MHC II-GFP transgenic mice (a gift from Prof. Hidde Ploegh) [34]. Ears were rinsed briefly with PBS on the dorsal side and dried before application of microneedle arrays by gentle pressure. Microneedles were then removed or secured in place using Nexcare medical tape (3M). Mice were sacrificed and excised ears were stained with trypan blue before imaging for needle penetration. Ears collected from mice treated with Cy3-pLUC- and/or DiI-PLGA-NP-coated microneedle arrays were mounted on glass slides and imaged by CLSM. Transfection in mice treated with pLUC-coated arrays was measured using an IVIS Spectrum 200 (Caliper Lifesciences) to detect bioluminescence, following IP injection of luciferin.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported in part by the Dept. of Defense (DAAD19-02-D-0002), and by the Ragon Institute of MGH, MIT, and Harvard. P.C.D was supported by the NIH Biotechnology Training Program at MIT. We thank Elizabeth Horrigan for technical assistance in animal studies and Robert Parkhill and Mike Nguyen (VaxDesign, Inc.) in microneedle array fabrication. D.J.I is an investigator of the Howard Hughes Medical Institute.

References

1. Giudice EL, Campbell JD. *Adv Drug Delivery Rev.* 2006; 58:68.
2. Donatus U, Bruce G, Robert T. *Bull World Health Organ.* 2002; 80:859. [PubMed: 12481207]
3. Pruss-Ustun, ERA.; Hutin, Y. WHO Environmental Burden of Disease Series. World Health Organization; 2003.
4. Quan FS, Kim YC, Yoo DG, Compans RW, Prausnitz MR, Kang SM. *PLoS One.* 2009; 4:e7152. [PubMed: 19779615]
5. Kim YC, Quan FS, Yoo DG, Compans Richard W, Kang SM, Prausnitz Mark R. *J Infect Dis.* 2010; 201:190. [PubMed: 20017632]
6. Glenn GM, Kenney RT, Ellingsworth LR, Frech SA, Hammond SA, Zoetewij JP. *Expert Rev Vaccines.* 2003; 2:253. [PubMed: 12899576]
7. Prausnitz MR, Langer R. *Nat Biotechnol.* 2008; 26:1261. [PubMed: 18997767]
8. Gill HS, Prausnitz MR. *J Controlled Release.* 2007; 117:227.
9. Prausnitz MR. *Adv Drug Delivery Rev.* 2004; 56:581.
10. Kim BS, Park SW, Hammond PT. *ACS Nano.* 2008; 2:386. [PubMed: 19206641]
11. Lynn DM. *Adv Mater.* 2007; 19:4118.
12. Su X, Kim BS, Kim SR, Hammond PT, Irvine DJ. *ACS Nano.* 2009; 3:3719. [PubMed: 19824655]
13. Jessel N, Oulad-Abdelghani M, Meyer F, Lavalle P, Haikel Y, Schaaf P, Voegel JC. *Proc Natl Acad Sci U S A.* 2006; 103:8618. [PubMed: 16735471]
14. Boudou T, Crouzier T, Ren K, Blin G, Picart C. *Adv Mater.* 2010; 22:441. [PubMed: 20217734]
15. Cavalieri F, Postma A, Lee L, Caruso F. *ACS Nano.* 2009; 3:234. [PubMed: 19206271]
16. Dimitrova M, Affolter C, Meyer F, Nguyen I, Richard DG, Schuster C, Bartenschlager R, Voegel JC, Ogier J, Baumert TF. *Proc Natl Acad Sci U S A.* 2008; 105:16320. [PubMed: 18922784]
17. Zhang J, Chua LS, Lynn DM. *Langmuir.* 2004; 20:8015. [PubMed: 15350066]
18. Jewell CM, Zhang J, Fredin NJ, Lynn DM. *J Controlled Release.* 2005; 106:214.
19. Pearton M, Allender C, Brain K, Anstey A, Gateley C, Wilke N, Morrissey A, Birchall J. *Pharm Res.* 2008; 25:407. [PubMed: 17671832]
20. Mikszta JA, Alarcon JB, Brittingham JM, Sutter DE, Pettis RJ, Harvey NG. *Nat Med.* 2002; 8:415. [PubMed: 11927950]
21. Gill HS, Soederholm J, Prausnitz MR, Saellberg M. *Gene Ther.* 2010 No pp yet given.
22. Park JH, Allen MG, Prausnitz MR. *J Controlled Release.* 2005; 104:51.
23. Haq MI, Smith E, John DN, Kalavala M, Edwards C, Anstey A, Morrissey A, Birchall JC. *Biomed Microdevices.* 2009; 11:35. [PubMed: 18663579]
24. Lynn DM, Langer R. *J Am Chem Soc.* 2000; 122:10761.
25. Greenland JR, Liu H, Berry D, Anderson DG, Kim WK, Irvine DJ, Langer R, Letvin NL. *Mol Ther.* 2005; 12:164. [PubMed: 15963932]
26. Akinc A, Anderson DG, Lynn DM, Langer R. *Bioconjugate Chem.* 2003; 14:979.
27. Wood KC, Boedicker JQ, Lynn DM, Hammond PT. *Langmuir.* 2005; 21:1603. [PubMed: 15697314]
28. Kim BS, Smith RC, Poon Z, Hammond PT. *Langmuir.* 2009; 25:14086. [PubMed: 19630389]
29. Jewell CM, Lynn DM. *Adv Drug Delivery Rev.* 2008; 60:979.
30. Samuel, RE.; Hammond, PT. MIT Invention Disclosure (provisional patent), Case No 14323, 'Protamine-based surface mediated gene transfer devices'.

31. Liang JF, Yang VC, Vaynshteyn Y. *Biochem Biophys Res Commun.* 2005; 336:653. [PubMed: 16139792]
32. Bershteyn A, Chaparro J, Yau R, Kim M, Reinherz E, Ferreira-Moita L, Irvine DJ. *Soft Matter.* 2008; 4:1787. [PubMed: 19756178]
33. Krogman KC, Lowery JL, Zacharia NS, Rutledge GC, Hammond PT. *Nat Mater.* 2009; 8:512. [PubMed: 19377464]
34. Boes M, Cerny J, Massol R, Opden Brouw M, Kirchhausen T, Chen J, Ploegh Hidde L. *Nature.* 2002; 418:983. [PubMed: 12198548]
35. Abramoff MD, Magelhaes PJ, Ram SJ. *Biophotonics International.* 2004; 11:36.

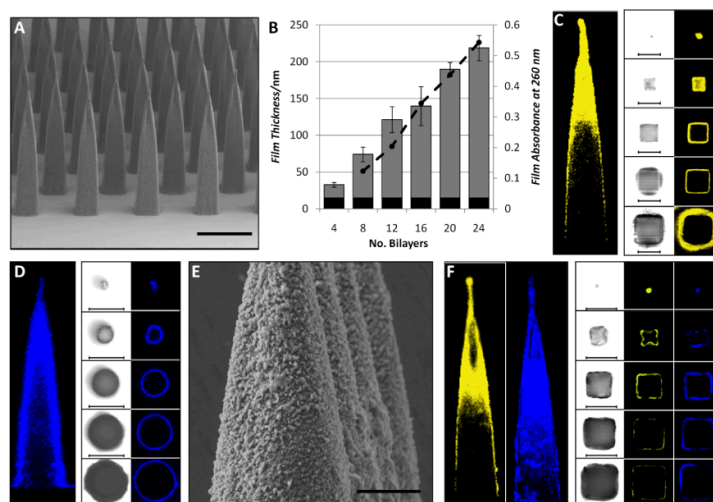


Figure 1. (A) SEM micrograph of uncoated PLGA microneedle arrays of pyramidal geometry (scale - 500 μm). (B) Film growth (left axis) and absorbance (right axis) for (Poly-1/pLUC)_n multilayers assembled on silicon/quartz substrates bearing a (PS/SPS)₂₀ initiating layer (black bar - (PS/SPS)₂₀, grey bar - (Poly-1/pLUC)_n, dashed line - Ab-260 nm). (C, D) Representative confocal micrographs showing a (C) (PS/SPS)₂₀-(Poly-1/Cy3-pLUC)₂₄ coated microneedle and a (D) (PS/SPS)₂₀-(Poly-1/DiI-PLGA NP)₄ coated microneedle (left - transverse section, right - lateral sections, 200 μm intervals, scale - 200 μm). (E) SEM micrograph showing a (PS/SPS)₂₀-(Poly-1/PLGA NP)₄ coated microneedle array (scale - 50 μm). (F) Representative confocal micrographs showing a (PS/SPS)₂₀-(Poly-1/Cy3-pLUC)₂₄-(Poly-1/DiD-PLGA NP)₄ co-coated microneedle (transverse and lateral sections, left - Cy3-pLUC, right - DiD-PLGA NP, 200 μm intervals, scale - 200 μm).

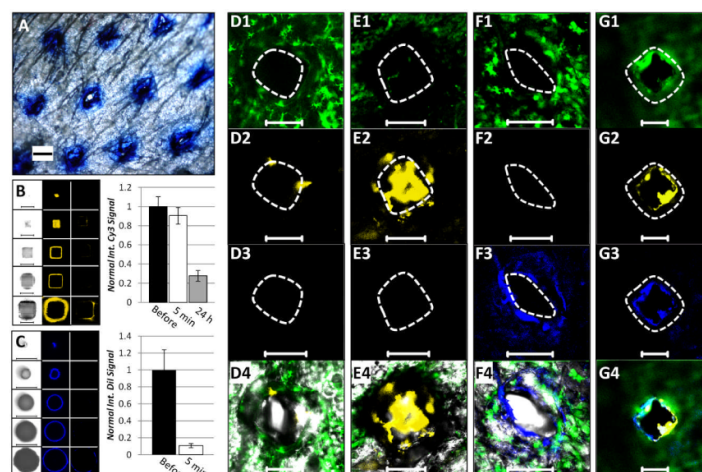


Figure 2.

(A) Optical micrograph of ear skin showing microneedle penetration pattern stained using trypan blue (scale – 100 μ m). (B) Representative confocal z-stacks and quantification (n = 6) of (PS/SPS)₂₀-(Poly-1/Cy3-pLUC)₂₄-coated microneedle arrays (left – brightfield, middle – before application, right – after 24 hour application, 200 μ m interval, scale - 200 μ m). (C) Representative confocal z-stacks and quantification (n = 6) of (PS/SPS)₂₀-(Poly-1/DiI-PLGA-NP)₄ coated microneedle arrays (left – brightfield, middle – before application, right – after 5 minute application, 200 μ m interval, scale – 200 μ m). Representative confocal micrographs (1 – MHC-GFP II, 2 – Cy3-pLUC, 3 – DiI/D-PLGA NP, 4 – overlay, scale – 200 μ m) showing dorsal ear skin following (D) 5 minute and (E) 24 hour application of a (PS/SPS)₂₀-(Poly-1/Cy3-pLUC)₂₄ coated microneedle array, (F) 5 minute (PS/SPS)₂₀-(Poly-1/DiI-PLGA-NP)₄ coated microneedle application, and (G) 24 hour (PS/SPS)₂₀-(Poly-1/Cy3-pLUC)₂₄-(Poly-1/DiI-PLGA NP)₄ coated microneedle application.

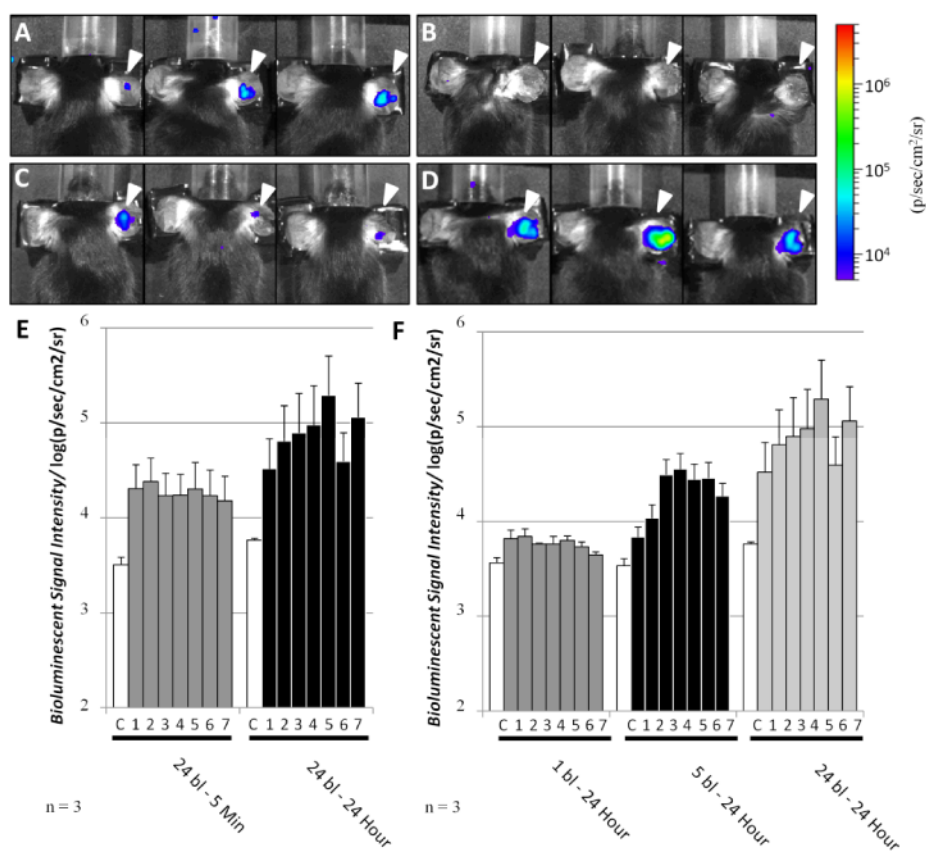


Figure 3. *In vivo* bioluminescent signal observed in C57BL/6 mice ($n = 3$) following treatment with a $(\text{PS/SPS})_{20}\text{-(Poly-1/pLUC)}_n$ – coated microneedle array to the right ear (denoted by arrow): (A) 24 bilayers for 5 minutes, (B) 1 bilayer for 24 hours, (C) 5 bilayers for 24 hours, and (D) 24 bilayers for 24 hours. The bioluminescent results following treatment are summarize in (E, F) for 7 days together with the negative control signal (denoted C) collected from the untreated ear, with (E) demonstrating the effect of application time and (F) showing the result of increasing pLUC dosage.