Leiden University
Medical Center

Intradermal cancer vaccine:
Engineering an automated nano-droplet dispensing system to fabricate HPV peptide-TLR2 conjugate loaded dissolving microneedles

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&
Division of Biotherapeutics (LU)

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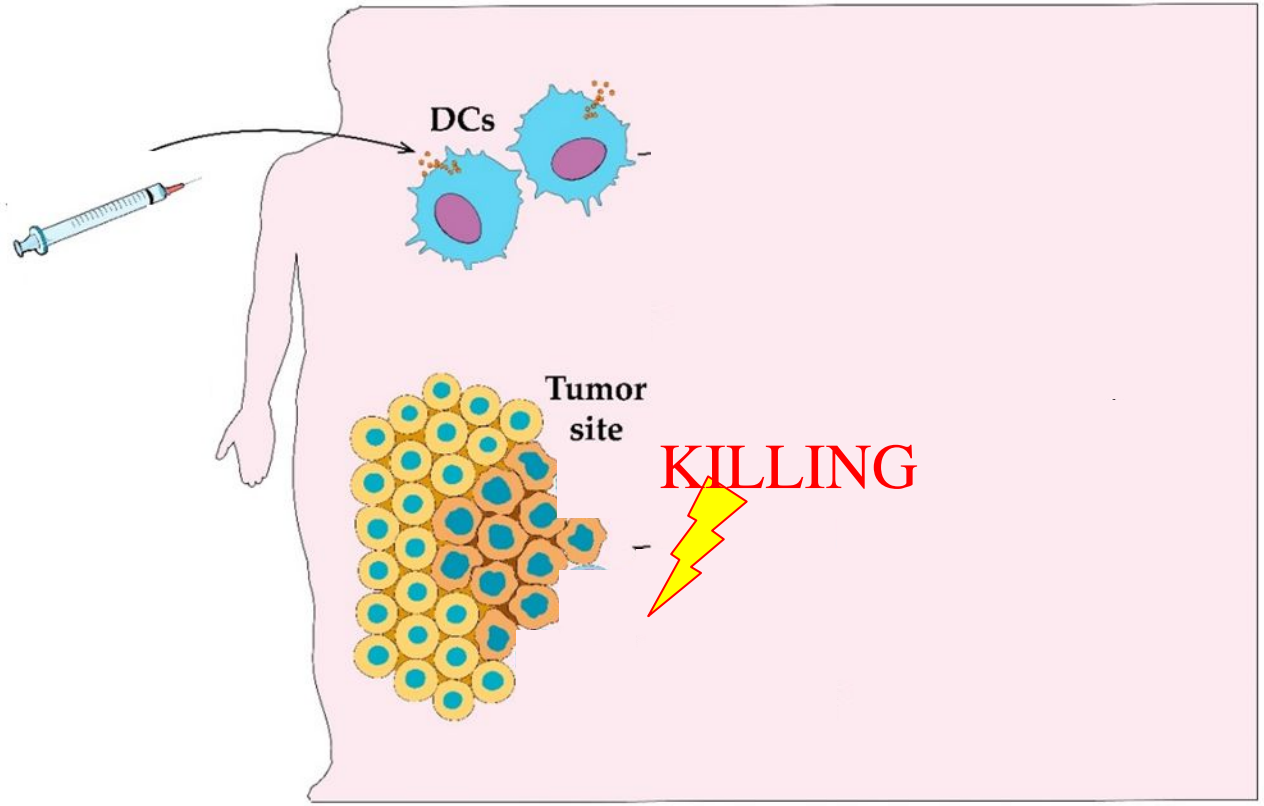
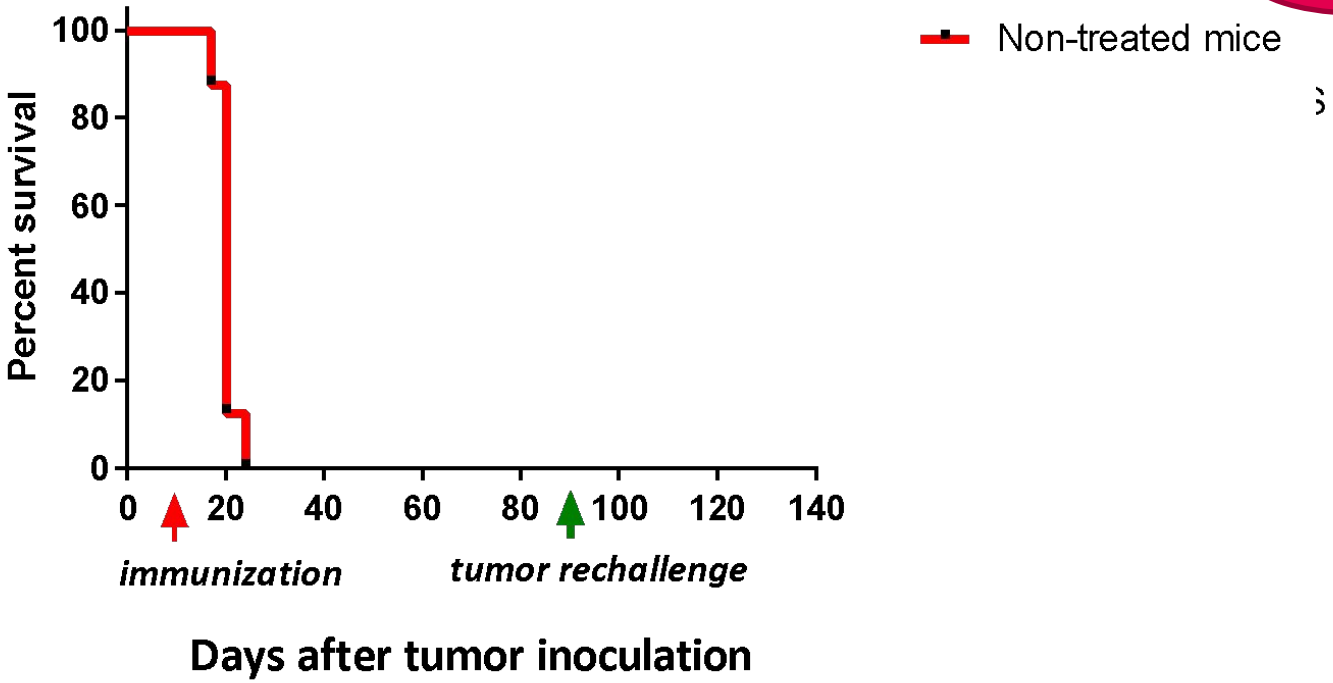
DCs play a crucial role in the induction, activation and proliferation of cancer specific T-cells

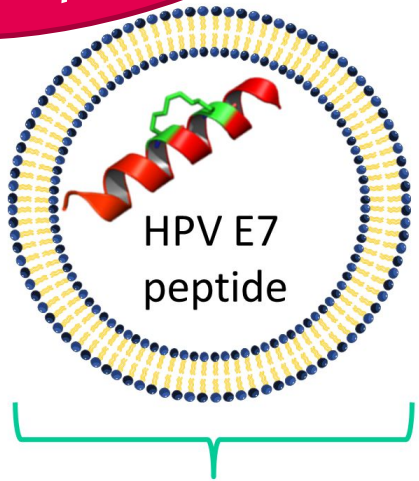


Adapted from: Baldin et al. *Cancers* 2020

Improve tumor control by formulation and administration route

See poster #167





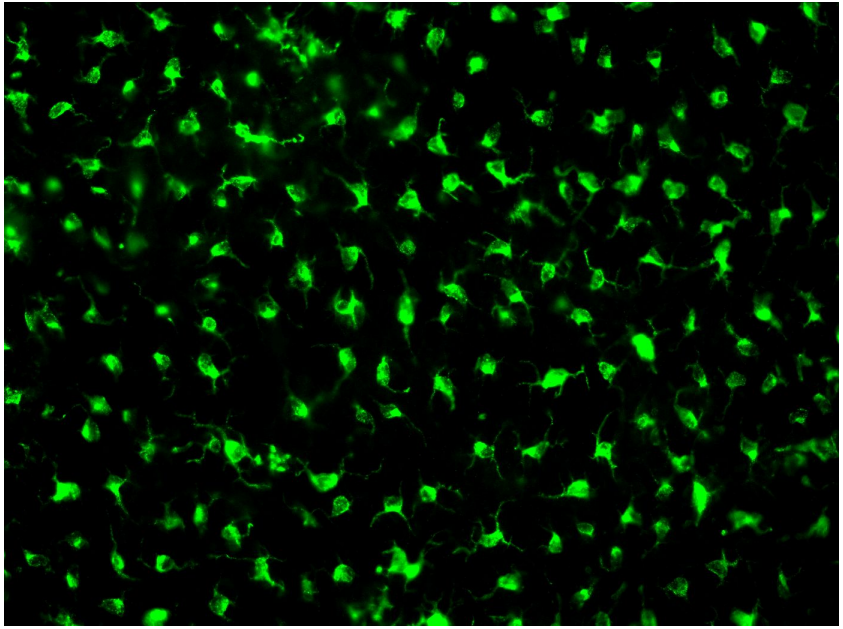
Diameter: **200 nm**
Z-potential: **+27 mV**

**10^5 TC-1 cells*

Varypataki et al., Cancer Immunol Res 31 (2017) 222–233

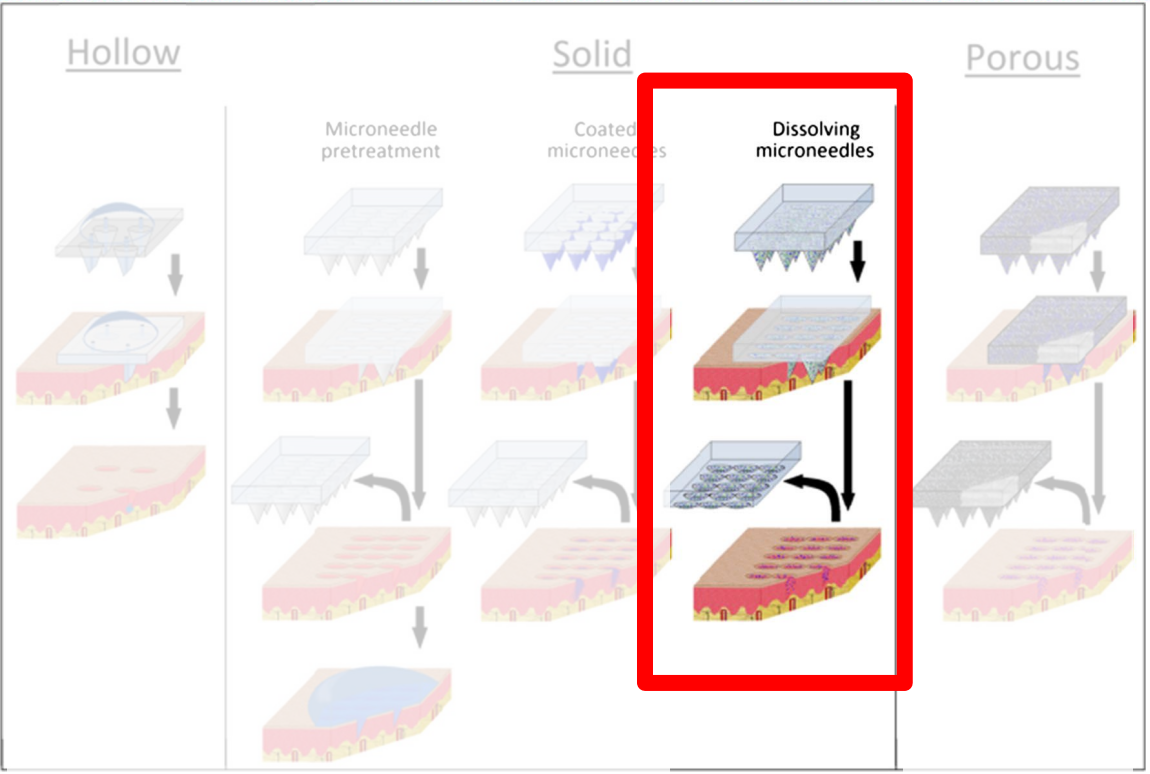
Skin contains a dense network of dendritic cells

- Skin is a potent immune organ
  - *Many APCs (LCs/dDCs) and therefore esp. suitable for T-cell induction/cancer vaccination*
- Superior immune responses
- 3 to 20-fold dose sparing potential



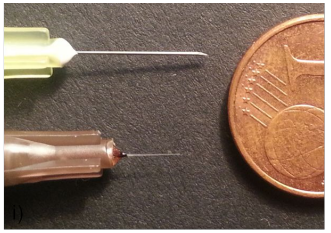
Perpendicular cryosection of human epidermis containing a network of Langerhans cells

Microneedle technologies for intradermal vaccination



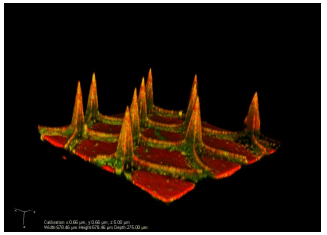
Examples of microneedle technologies

Hollow



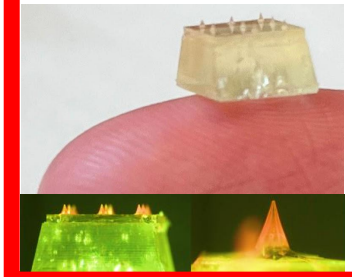
van der Maaden et al., Pharm res 2014

Coated



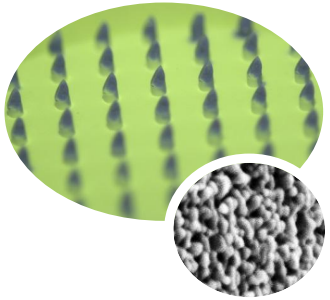
van der Maaden et al., Langmuir 2015

Dissolving



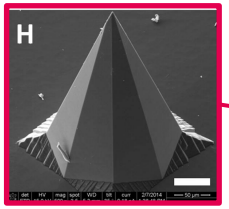
van der Maaden & Lee et al., Int J Pharm 2021

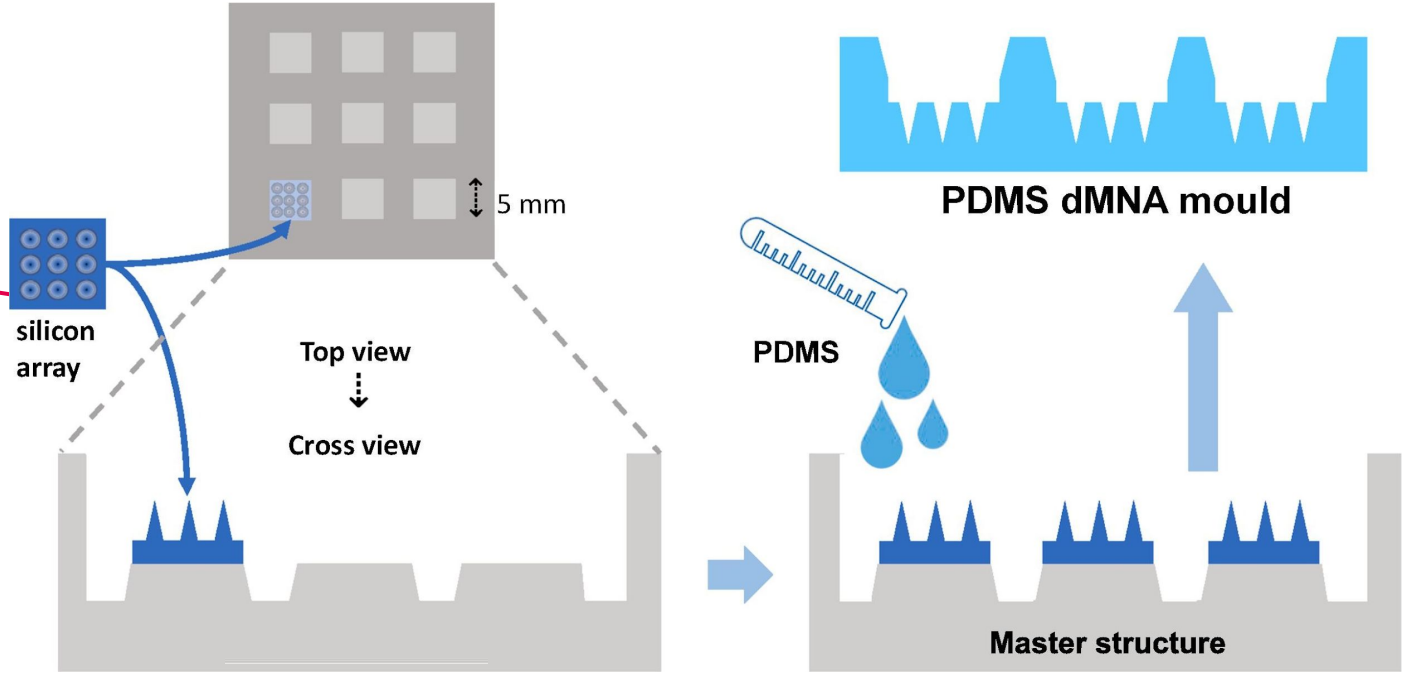
Nanoporous



van der Maaden et al., Front Immu 2017

Preparation of inverse microneedle molds





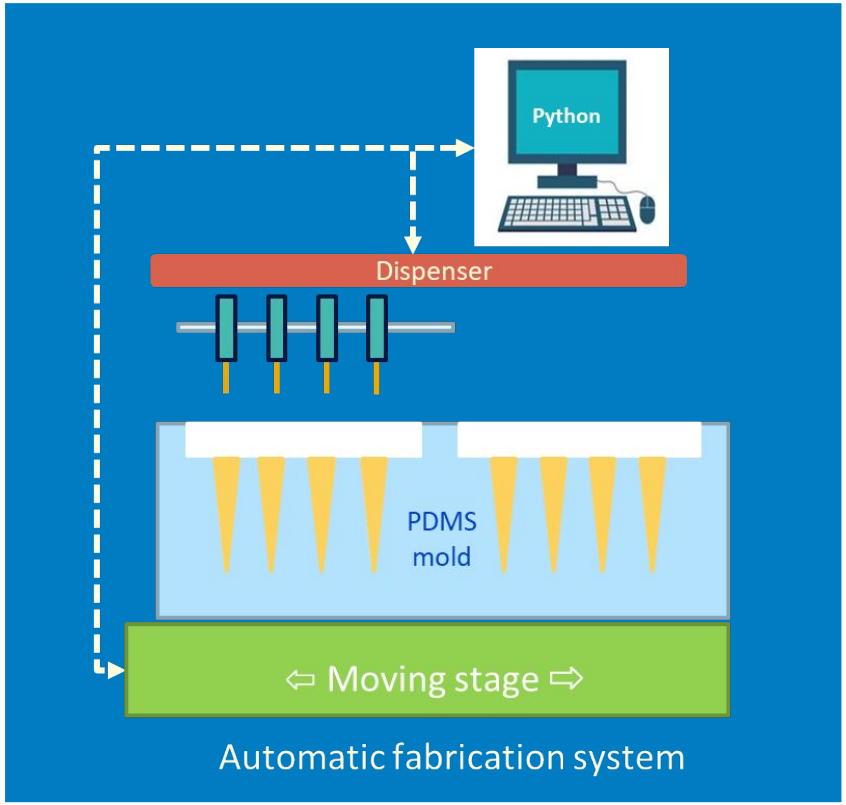
Lee & van der Maaden et al., Int. J. Pharm. 600 (2021) 120473

Preparation of dissolvable microneedles via centrifugation method

- Inefficient:
  - > 99% of antigen is not in the microneedles
- Expensive/not suitable:
  - Antigen is generally most expensive part of vaccine; &
  - Personalized cancer antigen is limited available
- Time consuming:
  - Preparation time is > 3 days

Need for an improved microneedle fabrication method

Engineering: Automatic fabrication system using nanodroplet dispensing

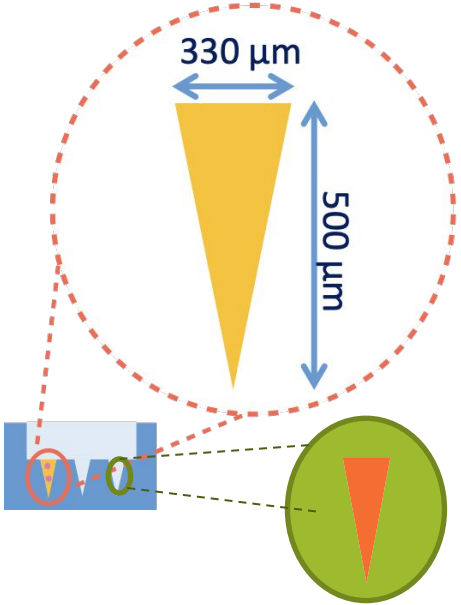


Components:

- Dispenser
- Moving stages
- Camera
- Vacuum chamber
- Computer

Lee & van der Maaden et al., Int. J. Pharm. 600 (2021) 120473

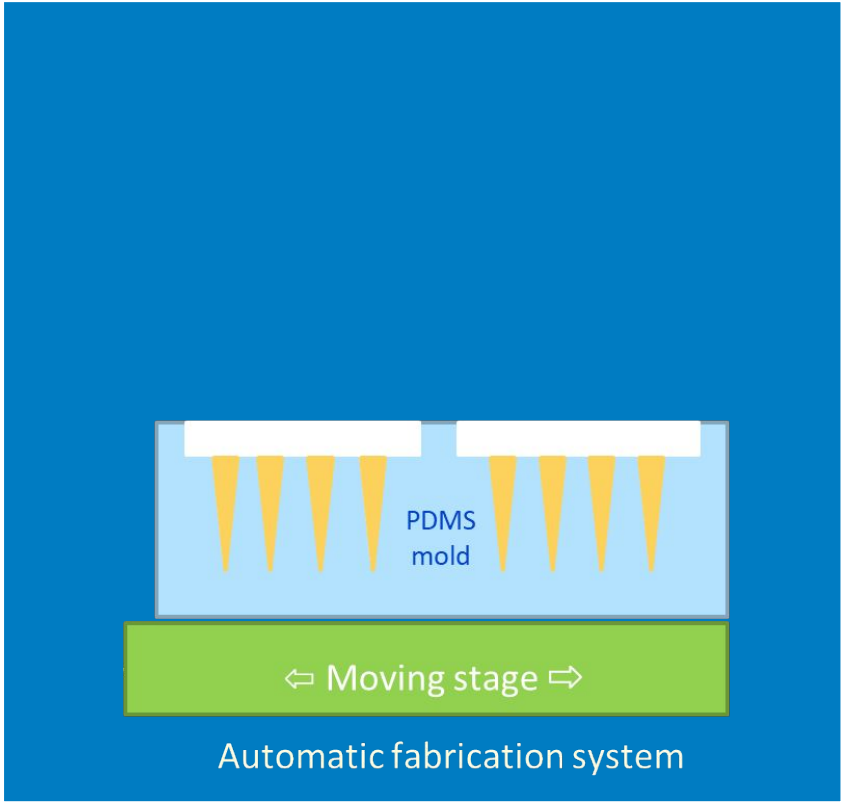
Engineering an automatic fabrication system using nanodroplet dispensing



- **Optimizing nano-dispenser**
  - **Generation of droplets down to 1.2 nL (132 nm)**

Lee, van der Maaden & Bouwstra et al., Int. J. Pharm. 600 (2021) 120473

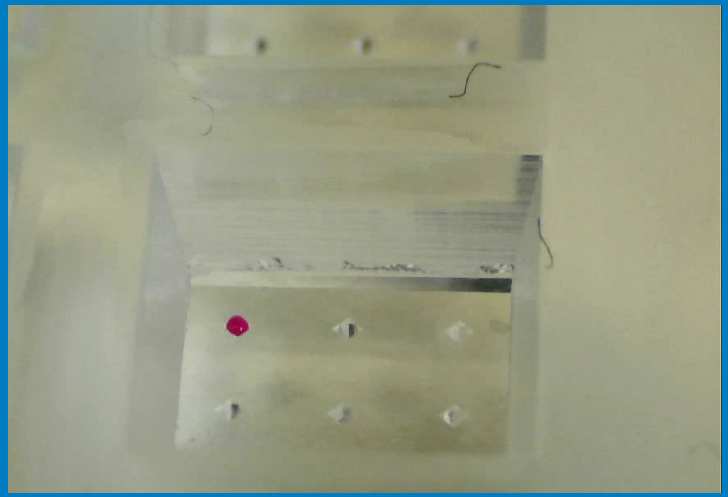
Engineering an automatic fabrication system using nanodroplet dispensing



- Optimizing nano-dispenser
  - Generation of droplets down to 1.2 nL (132 nm)
- **Add x-y microstepper-stages**
  - **High resolution steps of 0.2 mm/step**

Lee, van der Maaden & Bouwstra et al., Int. J. Pharm. 600 (2021) 120473

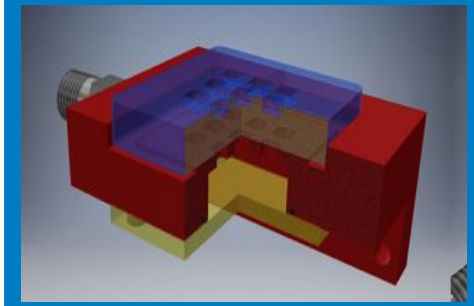
Engineering an automatic fabrication system using nanodroplet dispensing



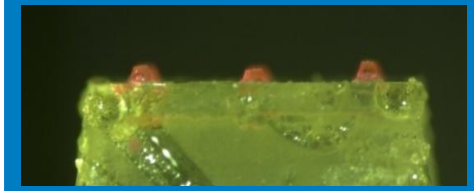
- Optimizing nano-dispenser
  - Generation of droplets down to 1.2 nL (132 nm)
- Add x-y microstepper-stages
  - High resolution steps of 0.2 mm/step
- **Include microscopic camera vision**
  - **Follow nano-dispensing**

Lee, van der Maaden & Bouwstra et al., Int. J. Pharm. 600 (2021) 120473

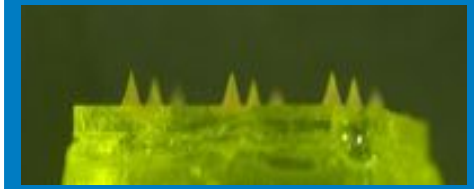
Engineering an automatic fabrication system using nanodroplet dispensing



Vacuum chamber design



Production without vacuum



Production with vacuum

- Optimizing nano-dispenser
  - Generation of droplets down to 1.2 nL (132 nm)
- Add x-y microstepper-stages
  - High resolution steps of 0.2 mm/step
- Include microscopic camera vision
  - Follow nano-dispensing
- **Design vacuum chamber**
  - **Ensure reproducible tip filling**

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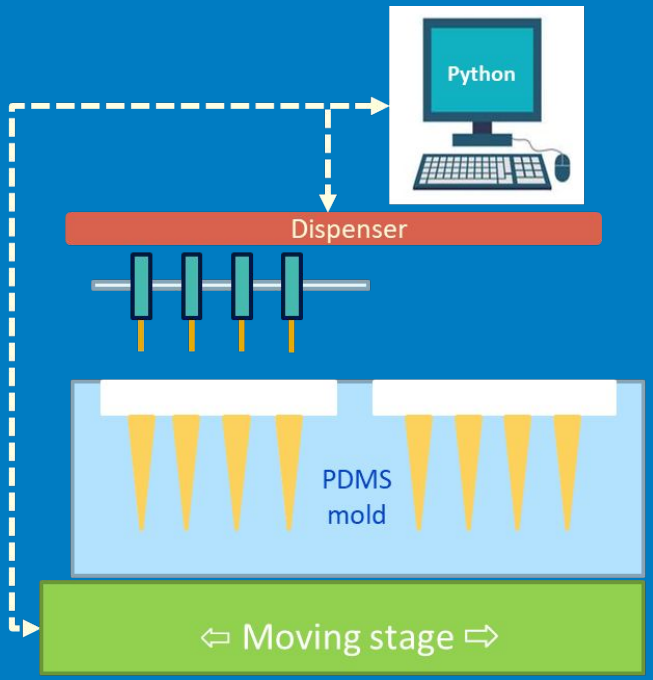
Engineering an automatic fabrication system using nanodroplet dispensing



Automatic fabrication system

- Optimizing nano-dispenser
  - Generation of droplets down to 1.2 nL (132 nm)
- Add x-y microstepper-stages
  - High resolution steps of 0.2 mm/step
- Include microscopic camera vision
  - Follow nano-dispensing
- Design vacuum chamber
  - Ensure reproducible tip filling
- **Incorporate all components in Python**

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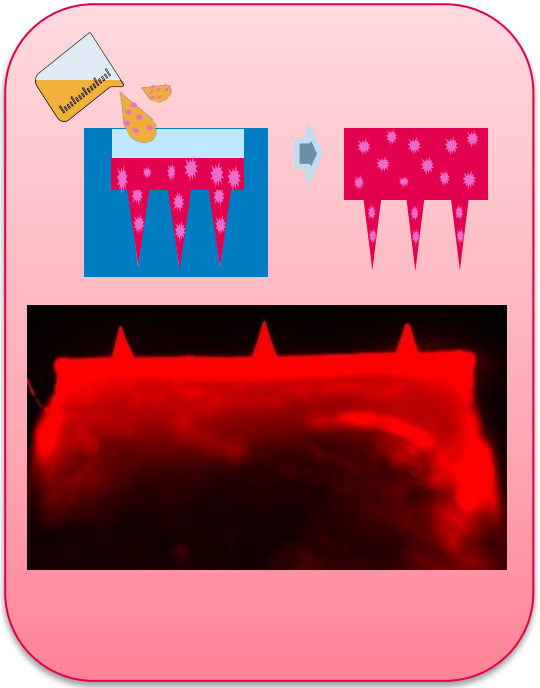
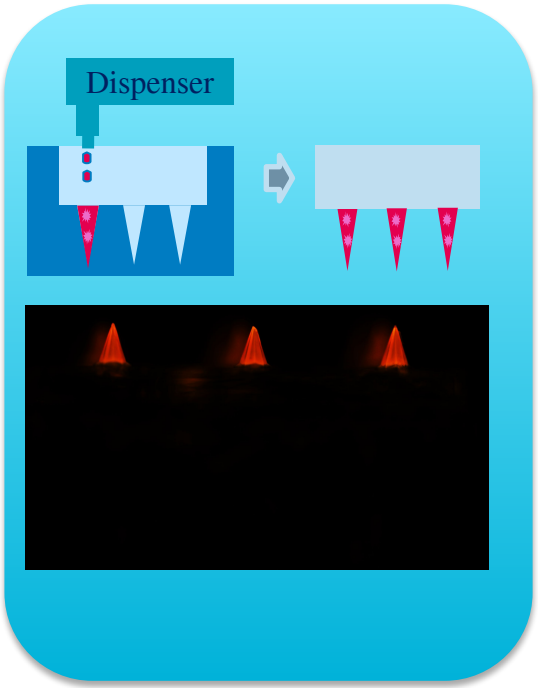
Improved fabrication method for dissolvable microneedles

Centrifugation



- Antigen is only in microneedle tips
- 99% less antigen required
- Increased reproducibility
- Fabrication time decreased to <1 day

Automatic fabrication



Cancer antigen-conjugate: Simultaneous delivery of antigen and adjuvant to DC

Identified tumor antigen
(viral, neoepitope..)

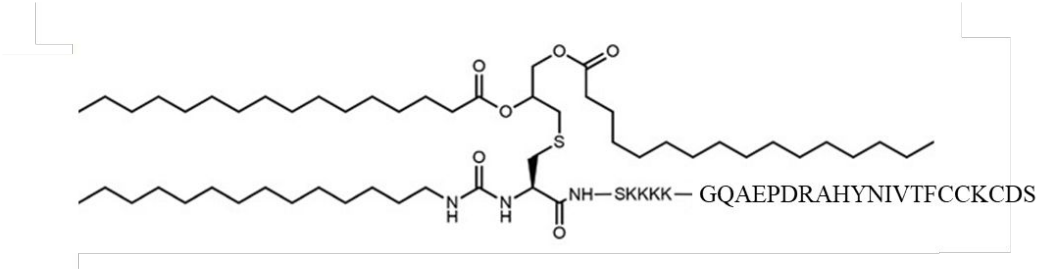
HPV-E7

Adjuvant

synthetic TLR2 ligand

HPV-peptide-TLR2 conjugate

HPV-E7



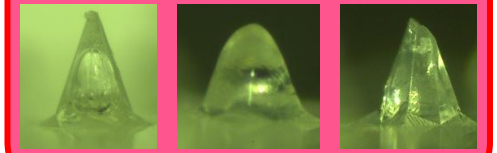
Development of cancer antigen-conjugate microneedle-based vaccine

Formulation screening:
(iterative process)

<u>HPV-conjugate</u>
- Solubility
- Stability
- Compatibility
- Functionality & integrity

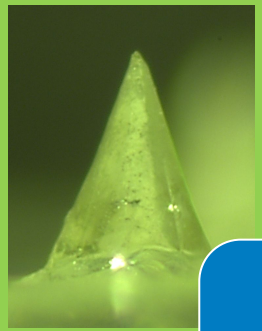
<u>Polymer composition</u>
- Form microneedles
- Sharp/complete tips
- Ability to pierce skin
- Dissolve upon piercing

Examples of failed microneedles



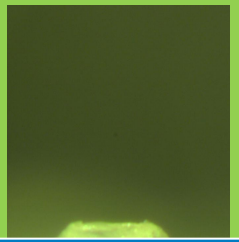
Conclusion:
HPV-TLR2-conjugate microneedles

Form solid and sharp microneedles

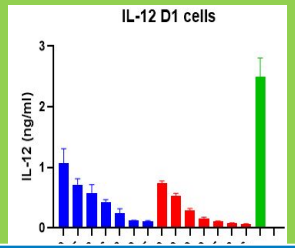


Pierce skin upon dermal application

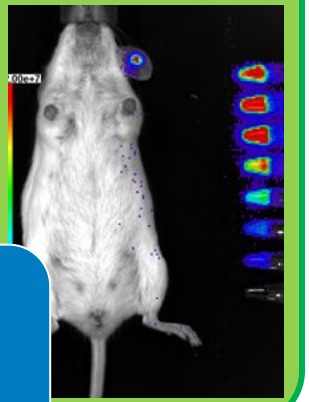
Dissolve in the skin <20 min



Retain functionality



Effective delivery in vivo



A promising technology for fabricating intradermal cancer vaccines

Currently being tested for T-cell induction & tumor control

Acknowledgements & Collaborators

Tumor immunology group

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