NAD+ Modulation with Nicotinamide Mononucleotide Coated 3D Printed Microneedle Implants

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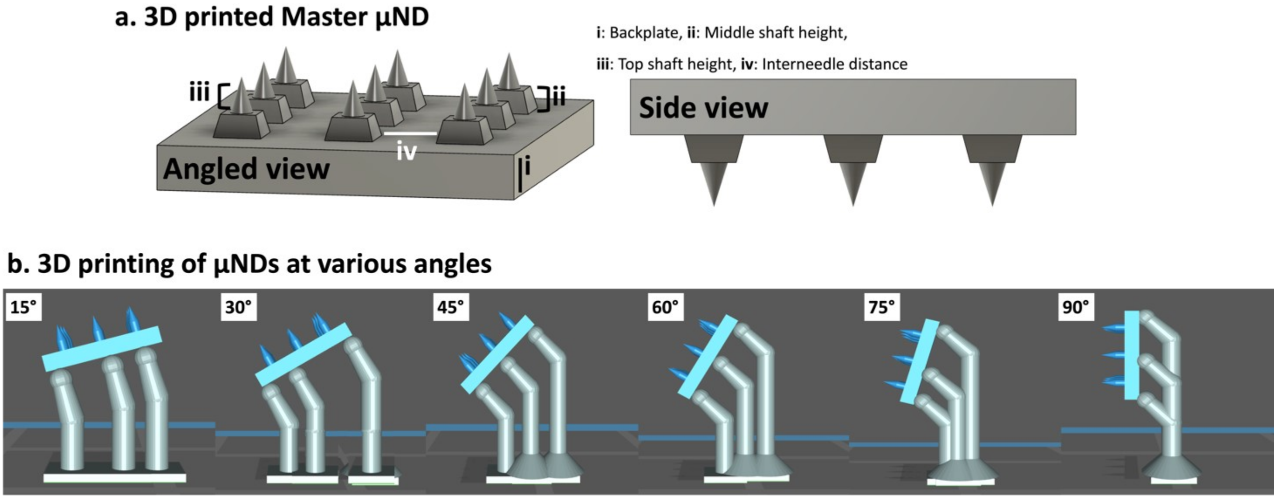

Figure 1: Optimisation of 3D printed master µND arrays. a) 3D printed master µND array with the various parts of the design include the backplate (i), the middle shaft (ii), the top shaft (iii), and the interneedle distance (iv).

Printing high fidelity master µND were studied elsewhere [63-67]. The 3D µND CAD design files (designed in Autodesk Fusion) were exported to STL file format and imported into a print preparation software (ChiTuBox V 1.3.0). The 3D printed master µND arrays were cleaned as per [67] with Methanol (100%) sonication wash for 8 mins, followed by detergent sonication clean for another 8 mins, to remove any residual uncured/cured resin on the µND array. Female µND moulds were then prepared using the 3D printed Master µND arrays, using an established method with some modifications [66]. Once dried, the master µND arrays were dip-coated in a silicone oil (WD-40 Inc, Australia) to protect against cure inhibition when pouring PDMS (Poly-dimethyl-siloxane) solution. The master µND array was placed needle facing upwards inside 15 mL falcon tube cap, polydimethylsiloxane (PDMS; Sylgard® 184, Dow Corning, Midland, MI, USA) with the ratio of 10:1 was then poured on the 3D printed master µND array, covering the entire array. The PDMS-casted 3D printed master µND was then degassed at room temperature for 30 min and cured in the oven at 65 °C for 24 h. The 3D master µND array was slowly peeled off from the PDMS cast. The newly fabricated master female moulds were then stored in nitrogen filled falcon tubes for further use.

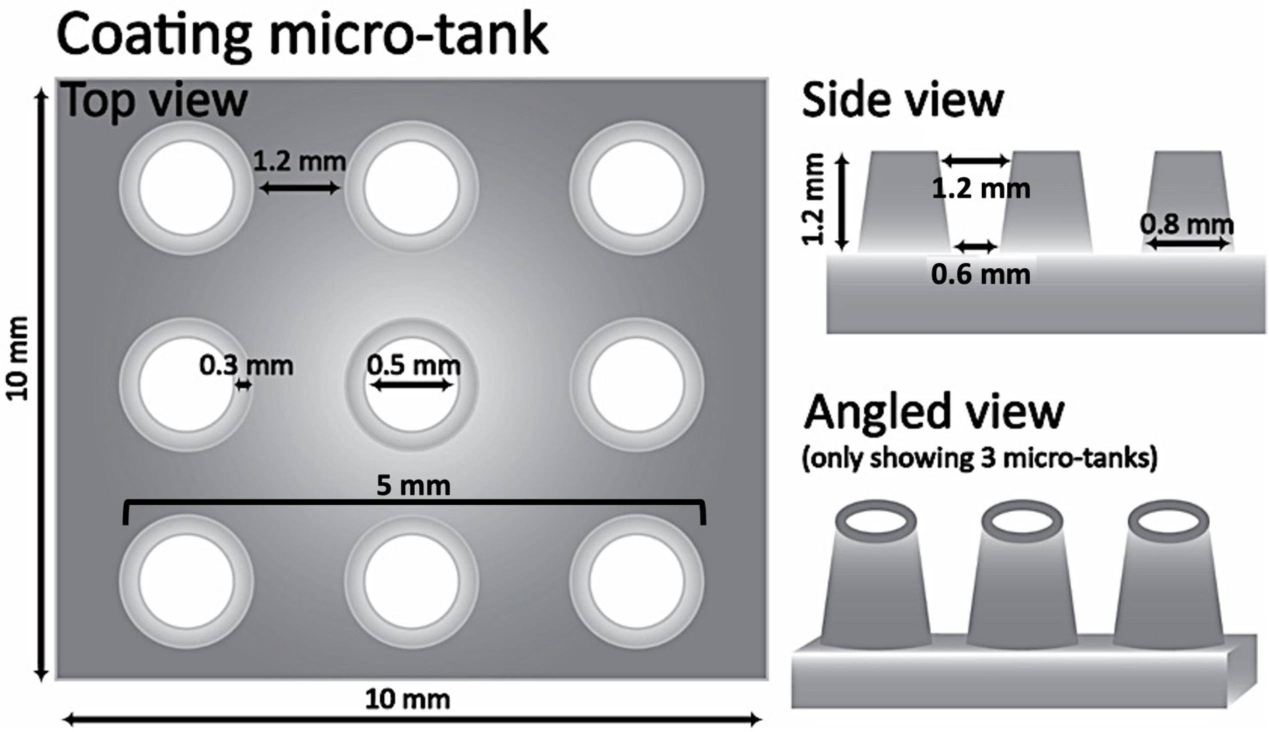

Figure 2: Illustrations of the microtanks prepared for the coating if µND implants in this experiemnt. The dimension of the individual micro-tanks: micro-tank inner diameter approximately 500 µm and height 1.2 mm, with 3 X 3 micro-tank array, with the distance between the micro-tanks set to 5 mm in CAD (Autodesk fusion). The illustration is not up to scale. The micro-tanks are hollow to facilitate the filling from the bottom of the base rather than the top, and secondly, any positive pressure or air bubbles created from the dipping of the µNDs will dissipate from the bottom opening of the base.

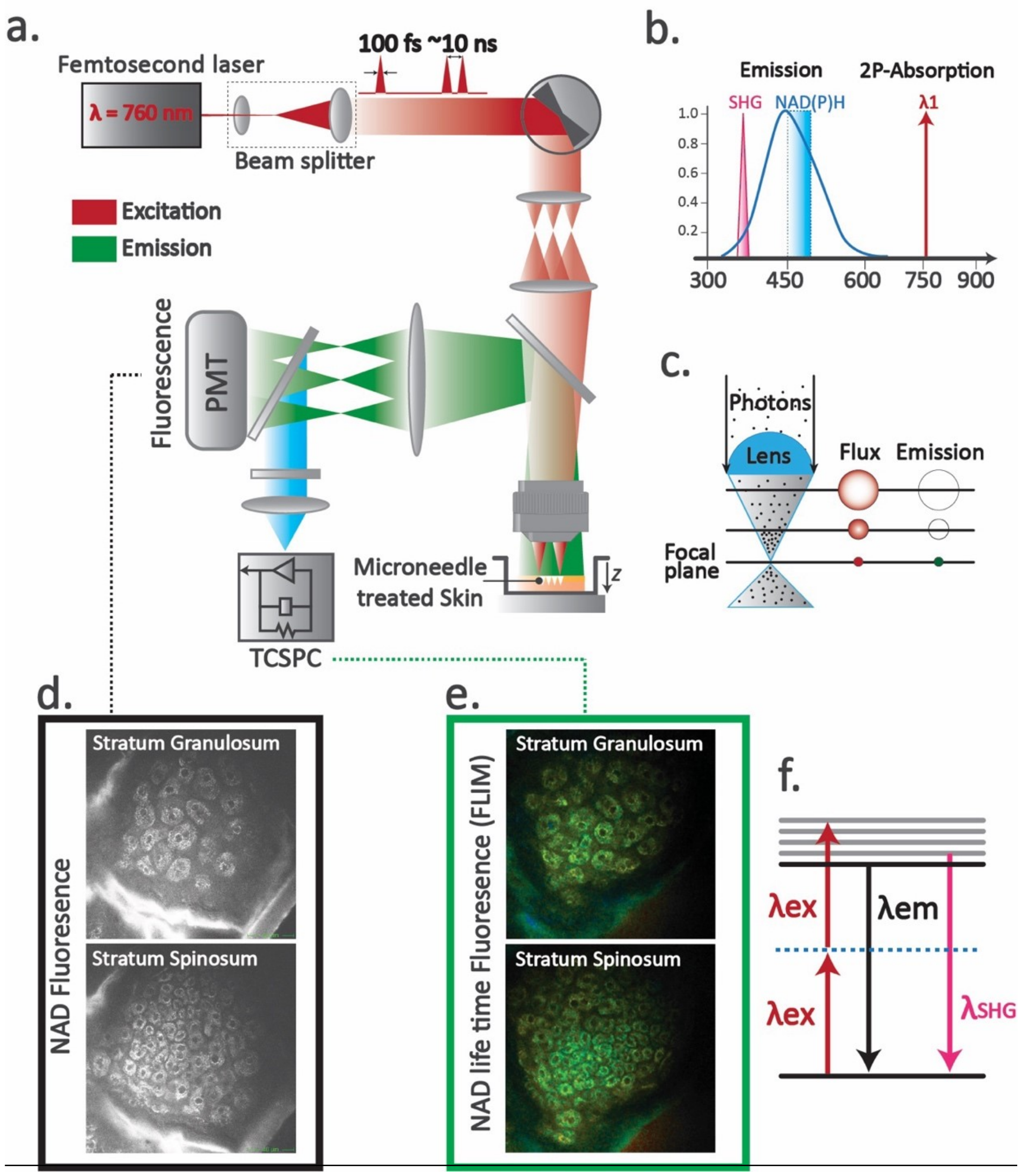

Figure 3: Multicolour two-photon excitation of NAD(P)H. (a.) Schematic of a synchronised pulse trains from single-output femtosecond laser used to generate one colour non-linear signals epi-detected in multi-channel spectral detectors. (c.) Two-photon irradiation with NIR illustrating the total flux in each z section in red colour (circle), but excitation only occurs at the focus (green circle). Therefore, fluorescence emission can be seen through pin-point for two-photon. (d.) Gray-scale NAD fluorescence image output from fluorescence detector showing cells from two distinctive skin layer, Stratum granulosum and stratum spinosum. (e.) multi-colour NAD lifetime fluorescence (FLIM) images of the same layers. d. & e. shows fluorescence of excited NAD(P)H molecules within the cells. (f). Jablonski energy level diagram illustrating two-photon (NIR, λex) exciting a fluorophore molecule to an excited state and the visible fluorescence emitted during relaxation (λem), as well as generation of single harmonic generation (SHG).

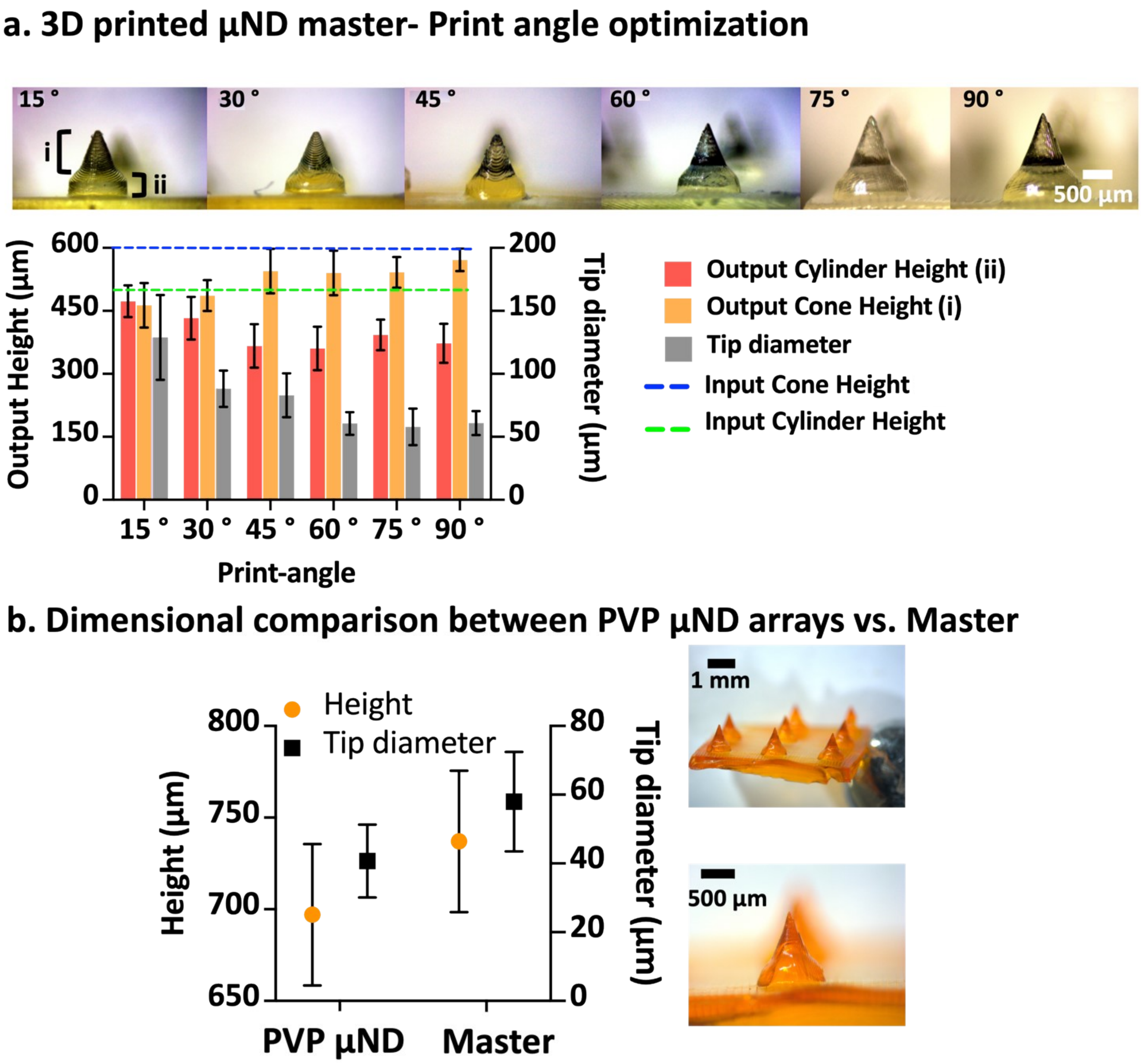

Figure 4: 3D master µND printing optimisation. (a) The µND master were 3D printed at various angles (15 – 90°), evaluating the three main design factors: output cylinder height (ii), output cone height (i) and tip diameter. (b) Comparison of the dimensional features between PVP µNDs made from master µND 3D printed at 90°. The two main dimensional parameters investigated were the µND height and the tip diameter. The microscopy images show PVP µNDs made from the master female mould using solvent casting method. Data was generated using PRISM showing values as AV ± SD.

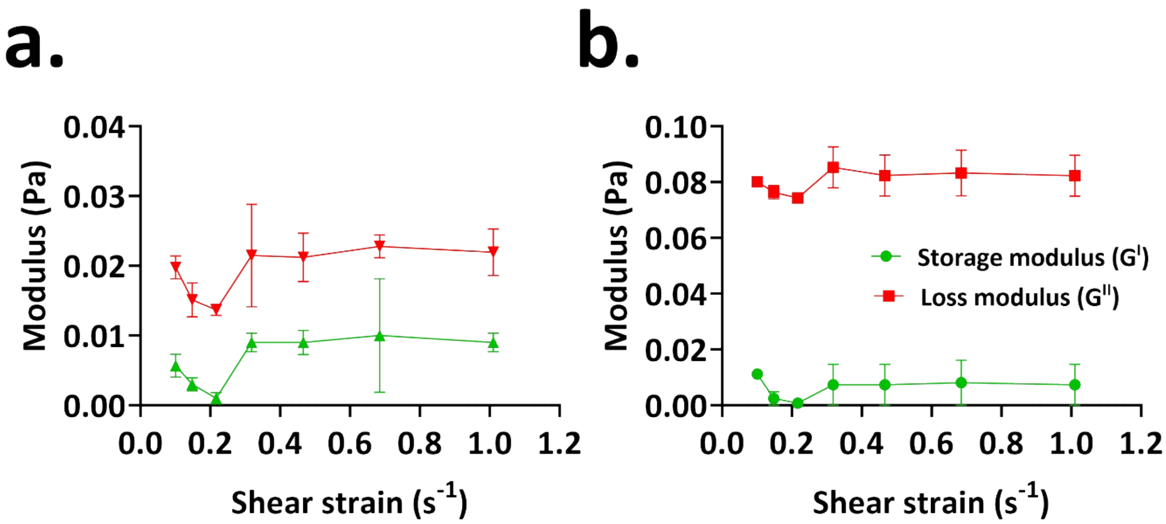

Figure 5: Determining the Linear Viscoelastic region (LVR) of (a) coating formulation A (NMN+Sucrose+Tween 20) and (b) coating formulation B (NMN+CMC+Tween 20). Amplitude sweep test was performed to determine the storage modulus (G”), and loss modulus (G’) at frequency (1 Hz) at room temperature (21–24 °C) collecting 25 points with strain sweep of 0.1-1 % to determine the Linear Viscoelastic Range (LVR). Data were plotted showing Av ± SD in PRISM. All measurements were carried out in triplicates.