3D printed microneedles for the transdermal delivery of NAD+ precursor: Towards Personalisation of Skin Delivery

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Table 1: Various 3D printers used in the literature and their respective geometrical measurements (input and output: base diameter and height, and tip diameter). Data were extrapolated from research articles conducted in the last 10 years.

Name of Printer	# of µNDs/array	Input (µm)		Output (µm)		Output Aspect ratio	Input:output height (%)	Tip diameter (µm)	x-y resolution (µm)	z-resolution (µm)	Ref
		base diameter	Height	base diameter	Height						
Form2	10	200	600	200	360	1 : 1.8	60	40	140	25	34
Form3	225	360	900	360	800	1 : 2.2	88		25	25	35
Phrozen shuffle	36	600	1000		600	1 : 1.3	60	35	47	25	38
Titan1	238	300	900	600	800	1 : 1.3	88	50	100	50	39
Form1	100	1000	1000	1000	1000	1 : 1	100	-	140	50	41
Phrozen shuffle	9	1000	1250	1100	940	1 : 0.9	75	60	47	50	43
XYZ PartPro100xP	49	-	700	-	565	-	80	119	-	50	44
Anycubic Photon	16	400	800	-	-	-	-	-	-	100	48
Omnicure S2000	25	200	1000	180	800	1 : 4.4	80	80	-	70	49, 54
Lumen-X DLP	16	300	800	300	600	1 : 2	75	~100	35	50	49, 50
Haas MiniMill 4X	9	300	1500	200	800	1 : 4	53	-	-	50	51, 55
DLP based 3D printer	144	450	1000	414	711	1 : 1.7	71	40	-	10	51, 52
Formlabs3	49	500	1300	500	~1100	1 : 2.4	84	490	25	25	34, 54
Form2	48	1000	1000	-	-	-	-	-	140	25	55, 56
Form2, MAX X27	49	1000	1000	800	800	1 : 1	80	140	150	50	35, 57, 58
Nanoscribe	25	150	750	208	730	1 : 3.5	97	-	0.2	-	38, 56
Form	182	500	600	300	~400	1 : 1.3	67	-	-	25	50, 59
Form	49	950	1150	994	1125	1 :1.13	98	60	-	25	52, 60
Form 2	100	800	1000	~ 500	710	1 : 1.4	71	~25	140	25	59, 61

Note: Approximated values are denoted as ~. Input to Output ratio was calculated based on the base diameter: height. Input:Output height % was calculated using = (Output height / Input height)*100

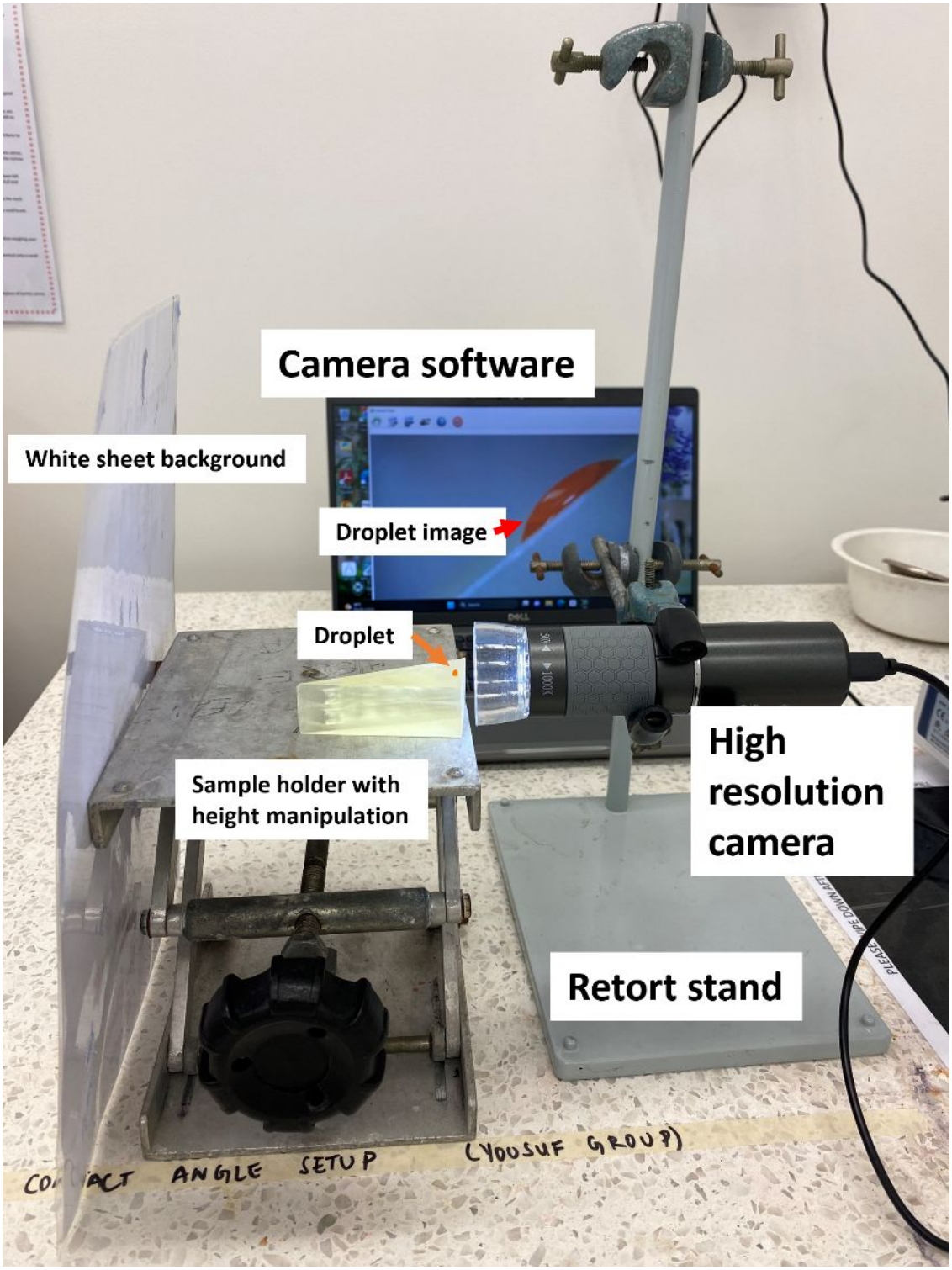

Figure S1: Image showing the setup to measure the contact angle of liquid droplet on the material 3D printed surface. The whole setup was on a vibration free bench (limestone bench). Sample holder with height manipulation was performed using a lab jack. The high-resolution camera was secured on a retort stand. A laminated white sheet was used a background. The high-resolution camera images were taken using an xploviewer app.

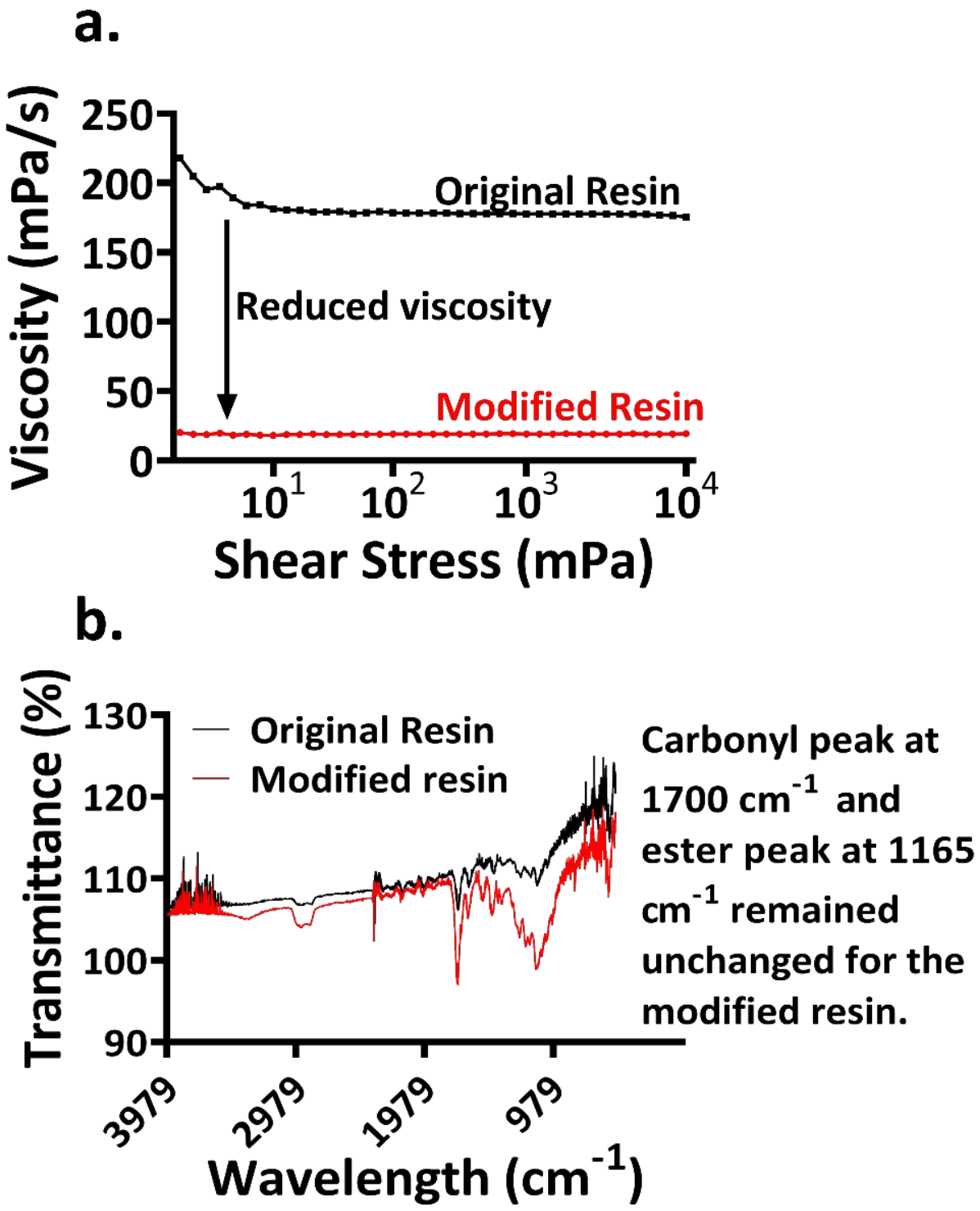

Figure S2: Graphical presentation showing the (a) viscosity changes of the original vs. modified resin respectively, when it is under shear stress, and (b) FTIR of both the original and modified resin. Graphical data were plotted in PRISM.

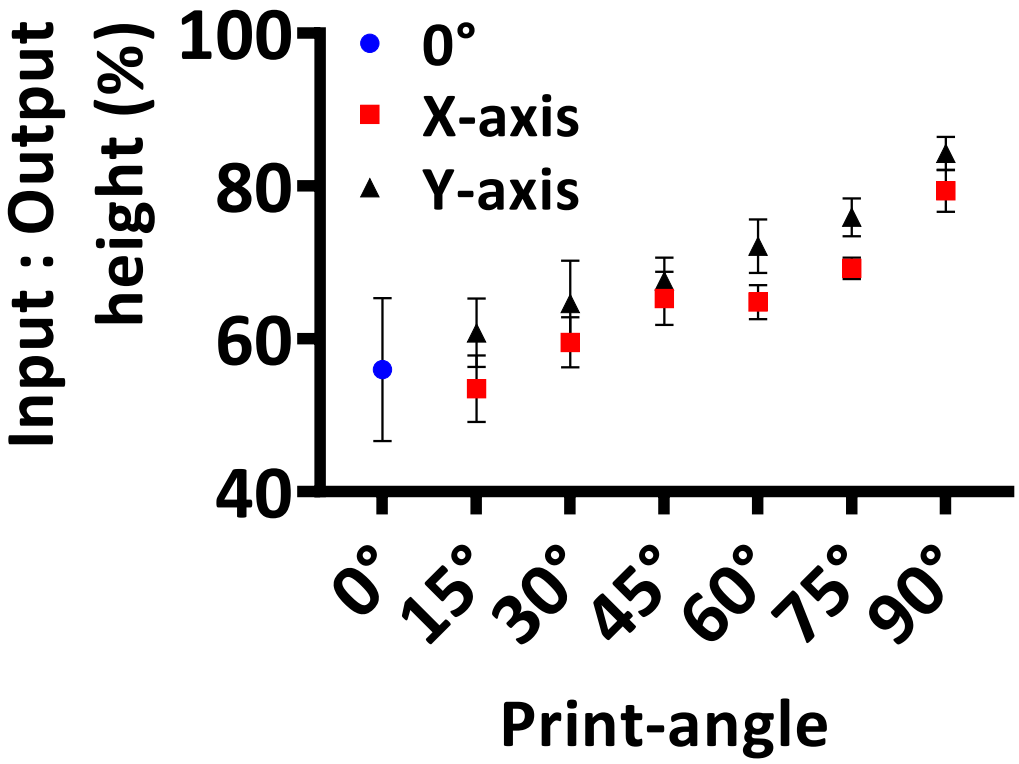

Figure S3: Effect of *print-angle* on the 3D printed µNDs height. The spatial adjustment was performed on the ChiTuBox software by rotating the µND model at x and y axis (represented by the various angles, 0 – 90° at 15° increments). The output µNDs height (n = 3, data shows Av ± SD and plotted in PRISM).

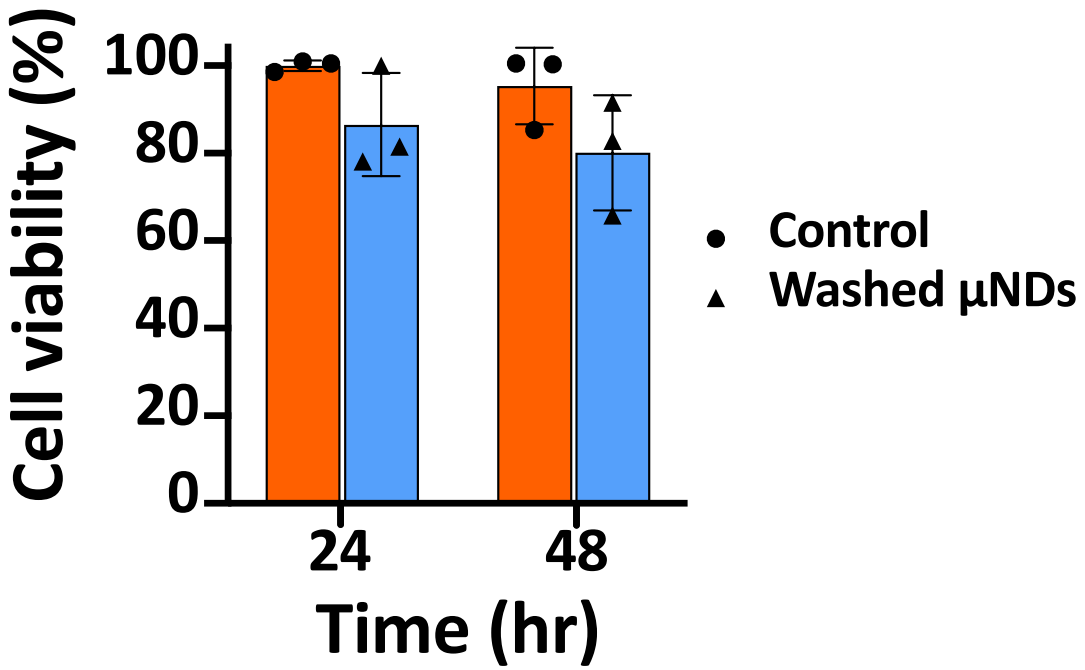

Figure S4: Keratinocytes cell (HaCat) viability studies on washed 3D printed discs (diameter: 5 mm and thickness: 0.5 mm) at 24 and 48 hr, respectively. Control indicates cells without 3D printed discs treatment, whereas washed discs indicate cleaning the discs with methanol followed by detergent (identical to the washing regimen employed for the cleaning of 3D printed µNDs). The resin and the print parameter of 3D printed discs were identical to 3D printed µNDs. As per ISO10993-5, which is an internationally harmonised method for materials acceptance test, the material is considered cytotoxic if the cell viability is below 70 %. Data are non-significant and were calculated using PRISM

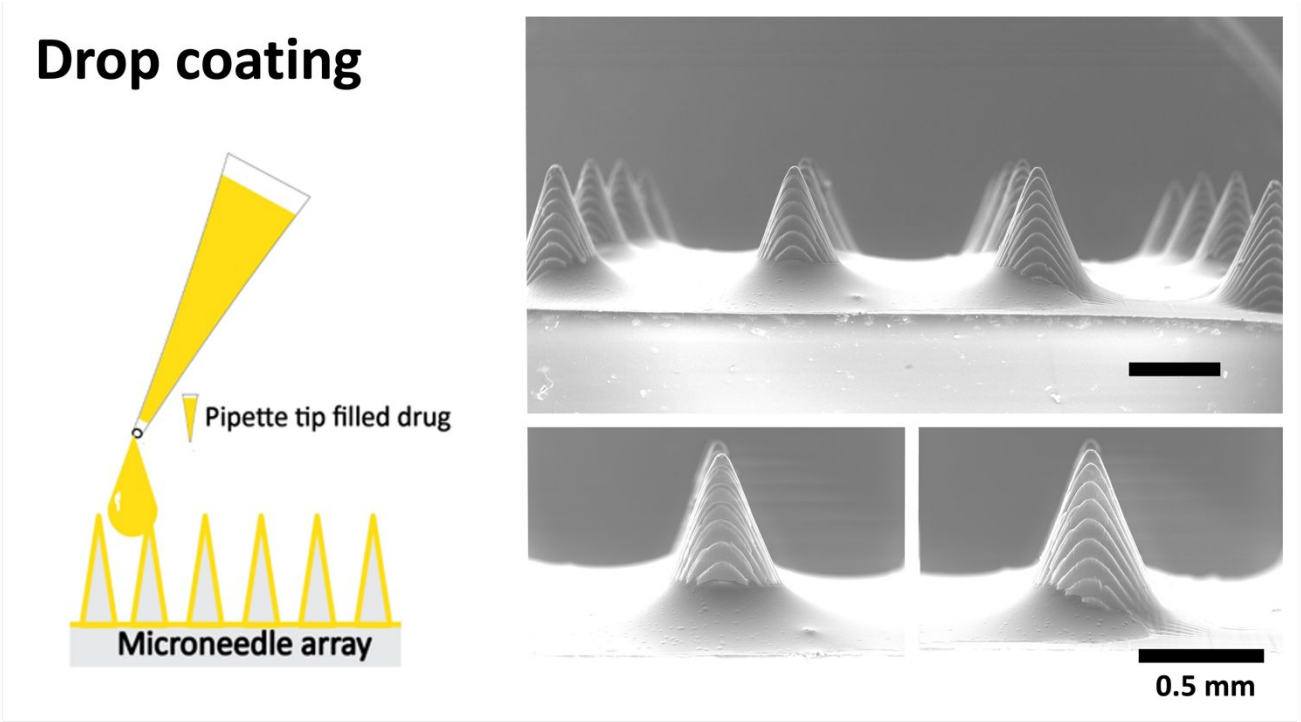

Figure S5: Drop coating illustrating technique (left) and the µNDs with the drug settled at the base of the µND (right). The top scanning electron micrograph (SEM) image shows the drug formulation settled at the base of the µND array, the bottom SEM image shows the magnified outline of two needles (separately) further illustrates the settled drug layer at the base. This experiment was conducted as a qualitative assessment to investigate whether drop coating technique can form a uniform coating layer at the tip of the µNDs only (n = 3).

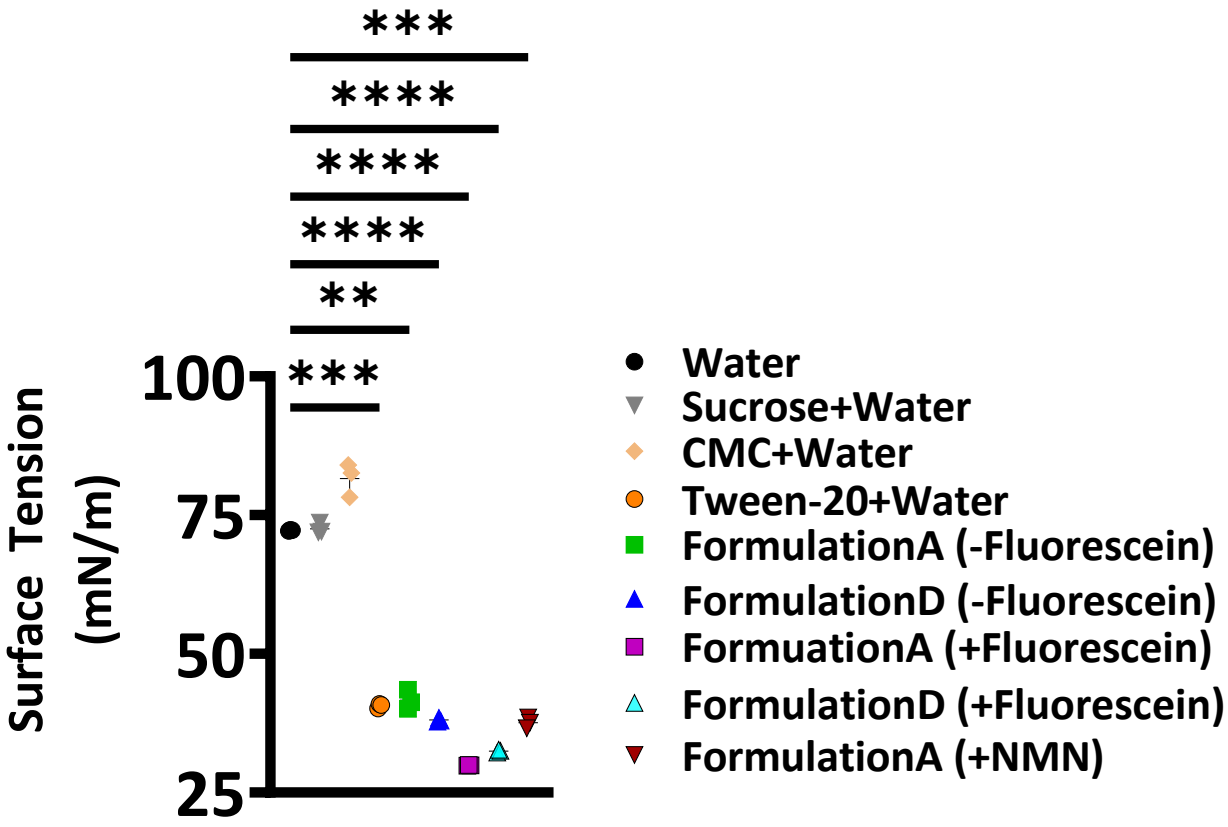

Figure S6: Surface tension measurement of water, Sucorse+water, CMC+water, Tween-20+water, and formulations A and D (without/with Fluorescein) and formulation A with NMN. The surface tension was measured using axisymmetric drop shape analysis with a pendant drop tensiometer (OCA-15EC, DataPhysics Instruments, Germany) using the Young-Laplace fitting. **** Statistical significance ($p<0.0001$) was determined using the one-way ANOVA.

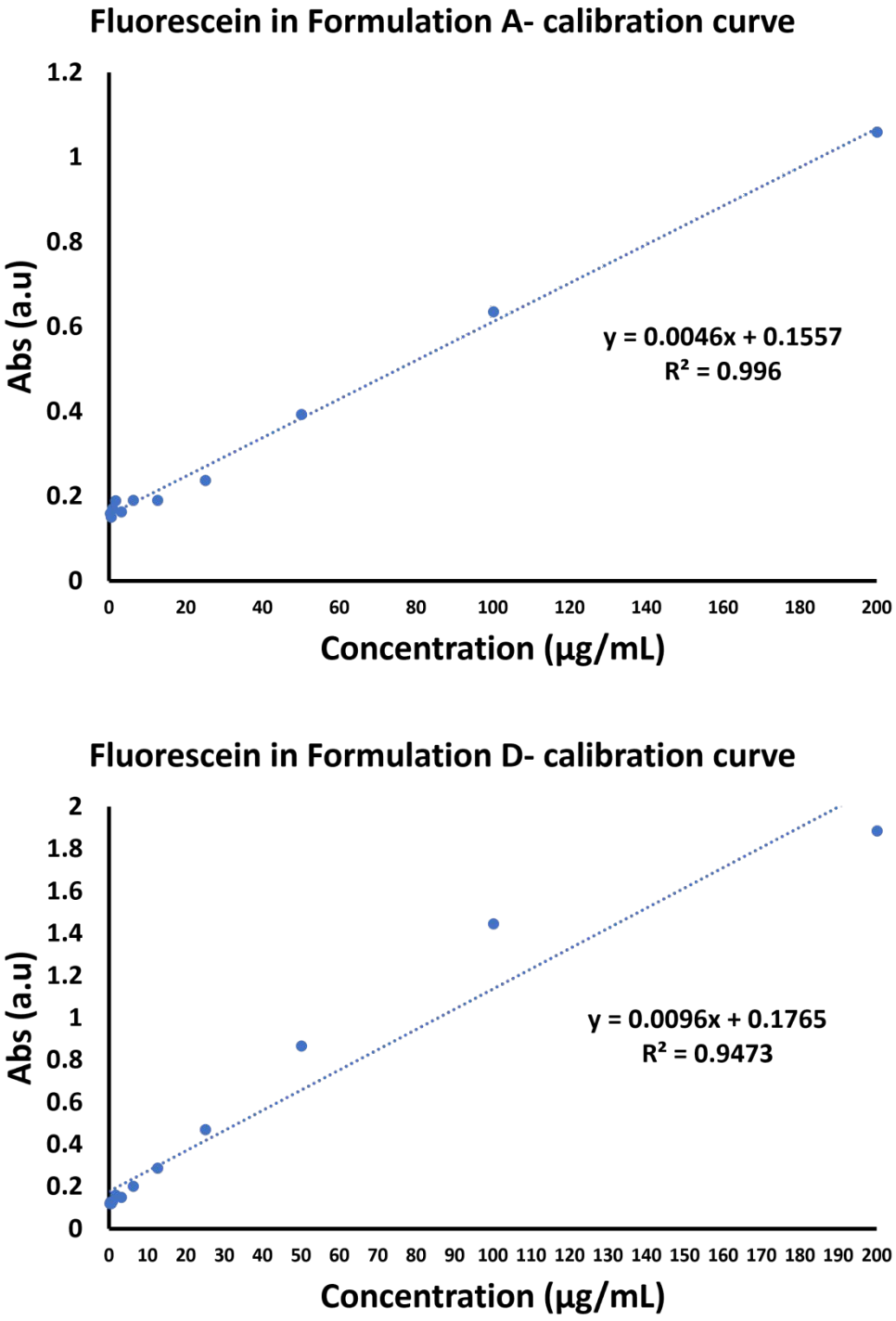

Figure S7: Fluorescein spectroscopy data in Formulations A and D, respectively. Data showing calibration curves of fluorescein in Formulation A (Sucrose and Tween-20) and Formulation D (CMC and Tween-20). The data shows a linear absorbance response with increasing fluorescein concentration. Data was plotted in Microsoft Excel, showing linear trendline and displaying equation and R-squared value on chart.

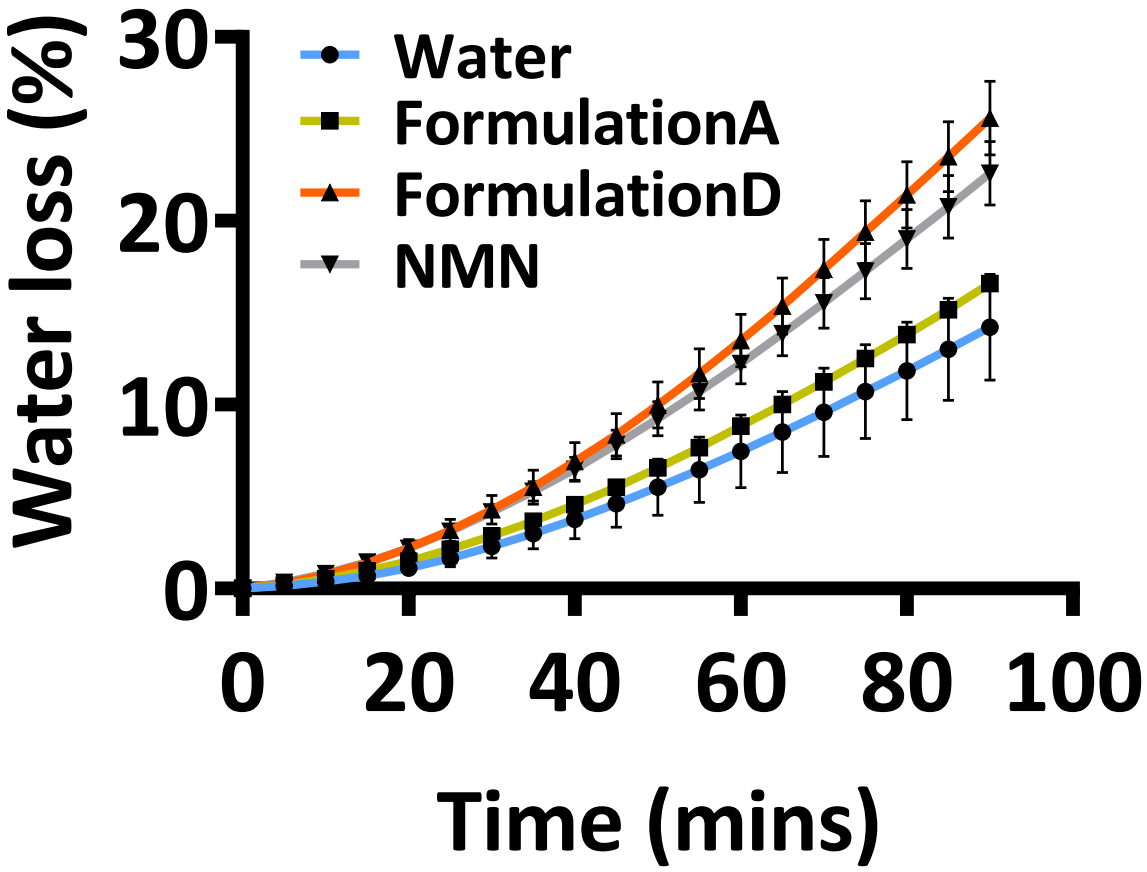

Figure S8: Evaporation rate of water and the three formulations (A, D and A-NMN) used in this experiment. The evaporation rate was determined using the water loss for a duration of 90 mins, using the gravimetric analysis. The total time it took to perform the whole coating experiment was 90 mins, therefore, water loss experiment was conducted for that long. Graph data shows Av. ± SD.

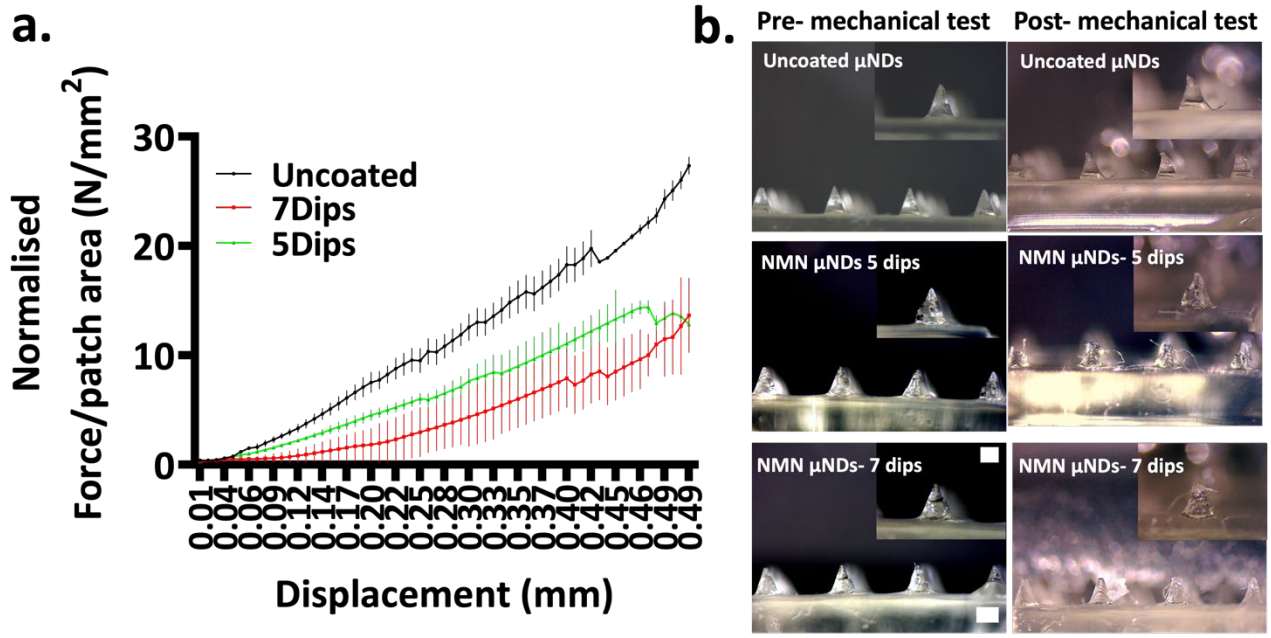

Figure S9: Normalised force vs displacement graph of Uncoated and NMN coated 3D printed µND (5 and 7 dips) under a compression test. The normalised force was calculated by dividing force (N) by the µND backplate area. A lower mechanical structural stiffness was demonstrated with increased NMN amount coating on 3D printed µND. (b) Representative images of Uncoated and NMN coated 3D printed µND (5 and 7 dips) Pre and Post mechanical test. Main image shows 4 µNDs with scale bar of 0.5 mm, and inset image shows one µND with scale bar of 0.2 mm.

Pharmaceutical analysis of NMN from NMN coated μNDs

The retention time (Rt) of the pharmaceutical grade NMN standard was 3.0 mins (**Figure S10a**). This matches with the Rt of NMN in the dissolved from the coated μNDs (**Figure S10c**). **Figure S10b** shows the chromatogram of the coating solution injection minus NMN. In the absence of any quantifiable peak at Rt 3.0 mins, it confirms that the excipients added into the coating solution, sucrose, and Tween-20, doesn't elute at 3.0 mins, and therefore will not affect the quantification of NMN for subsequent experiments. However, a small peak is observed at 3.0 mins, but it is considered noise since the signal to noise ratio was <1, which is an indication of background noise.

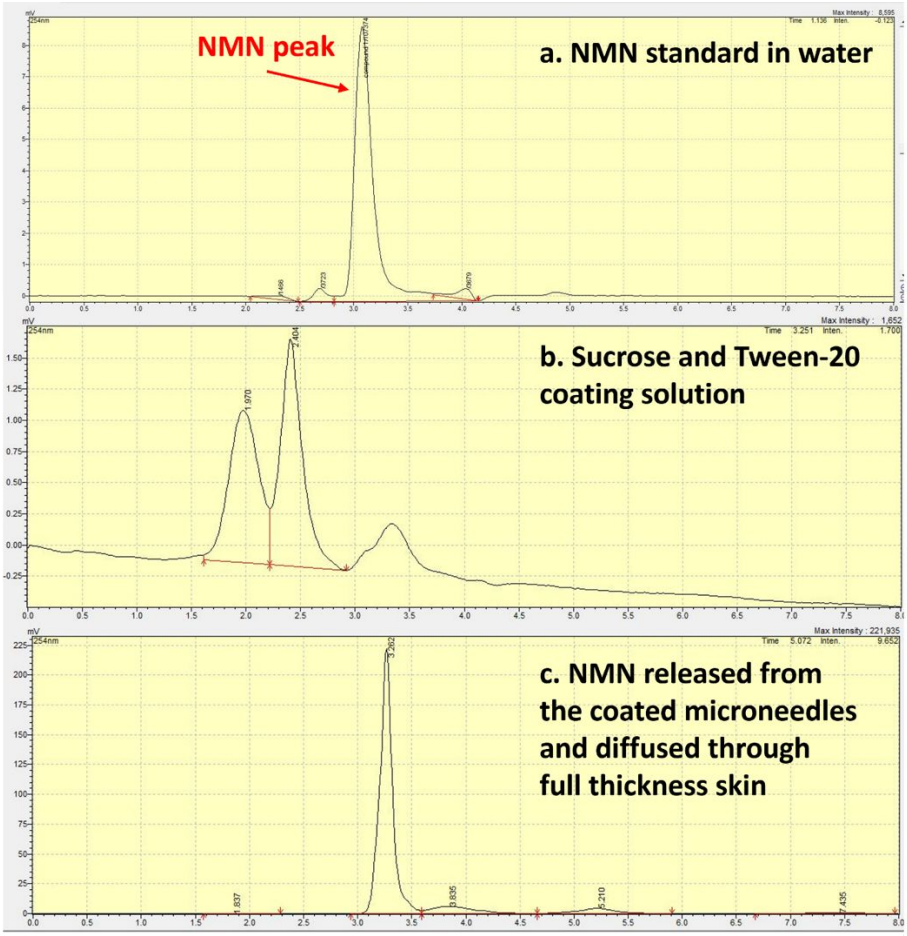

Figure S10: Chromatographic analysis of NMN release from coated μND arrays. HPLC-UV chromatograms of: (a) pharmaceutical grade NMN standard (also. Indicated by the red arrow), (b) of the coating solution containing sucrose and Tween-20, (c). NMN released from the coated μND arrays. The chromatograms were taken from Shimadzu HPLC system.

LC-MS was also performed on NMN standard, Sucrose, Tween-20, coating solution (with NMN 400 µg/mL) and NMN coated µNDs (5 dipped coating) to ensure the molecular mass of NMN doesn't change in the coating solution, as well as it doesn't change in the coated µNDs. NMN standards' molecular mass is 334.39 g/mol, which appeared as a peak in the mass spectrogram (**Figure 5a**). Neither Sucrose (**Figure S11b,** Mw: 342.3 g/mol) [87] nor Tween-20 (**Figure S11c**, Mw: 1227.54 g/mol) [88] showed a peak in that region. NMN was conserved in both the coating solution (**Figure S11d**) and in the coated µNDs (**Figure S11e**). This confirmed that, in the presence of sucrose and tween-20, NMN molecules are not compromised.

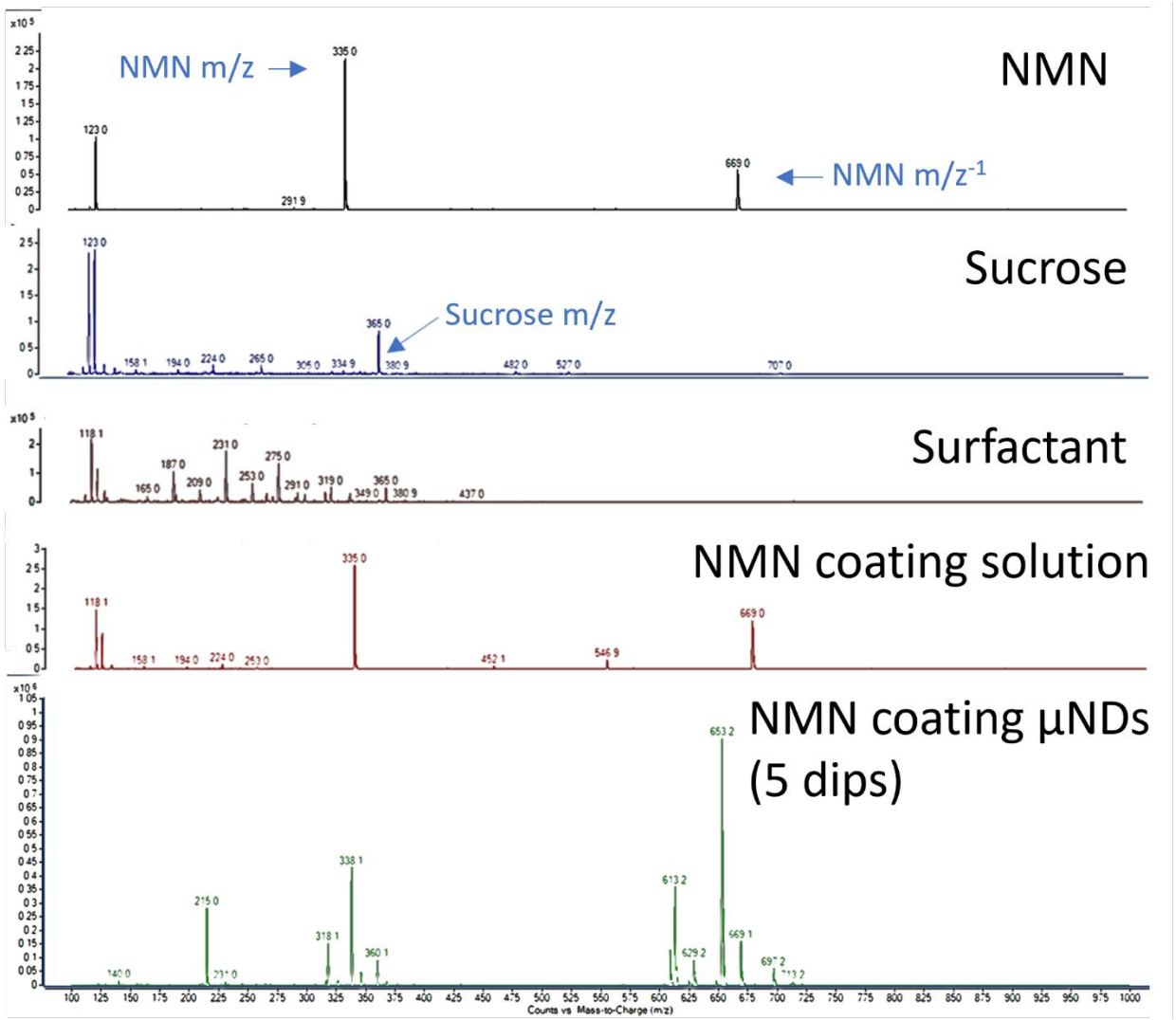

Figure S11: Mass-spectrograms of NMN standard (a), Sucrose (b), Tween-20 (c), coating solution (d, with NMN 400 µg/mL) and NMN coated µNDs (e, 5 dipped coating).