Supporting Information

Bioinspired silk protein modification to develop instant dissolvable microneedle with superior mechanical properties, long-term biomolecule stabilization

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Sample name	composition	silk concentration	Trehalose/sucrose concentration	Model biomolecules/drug
UMS4.5-DMN	Unmodified silk	4.5% w/v	-	
UMS4.5-DMN-RT90		4.5% w/v	-	
MS20-DMN	Modified silk	20% w/v	-	
MS15-DMN		15% w/v	-	
MS10-DMN		10% w/v	-	
UMS4.5-T7.5-DMN	Trehalose/sucrose incorporated	4.5% w/v	7.5% w/v	
UMS4.5-T5-DMN		4.5% w/v	5% w/v	

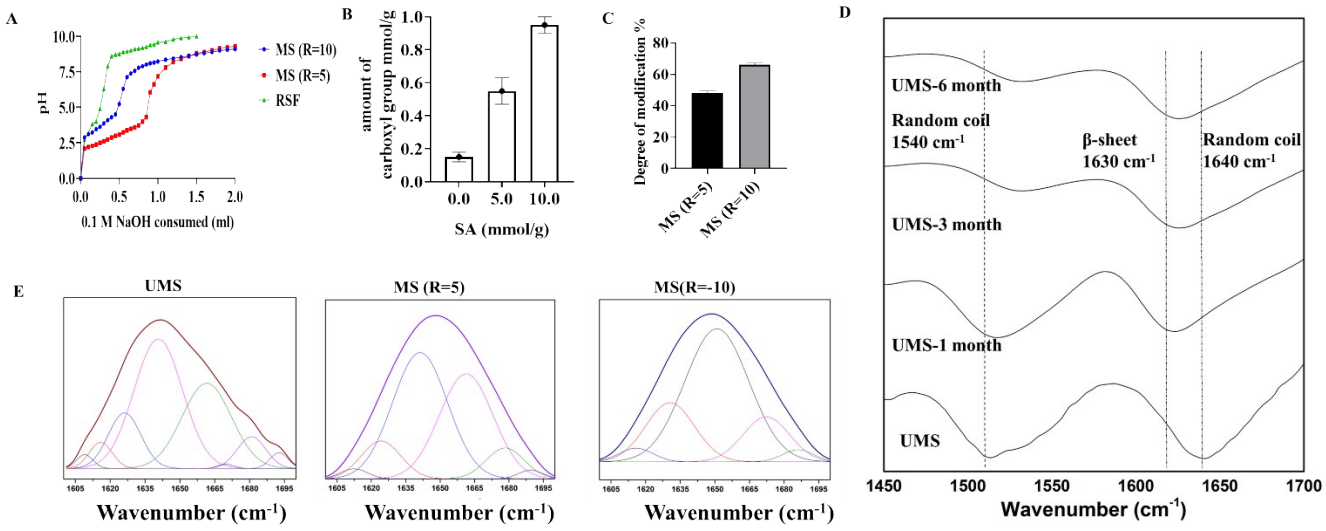
Materials and methods

Determination of water content of UMS-DMN and MS-DMN

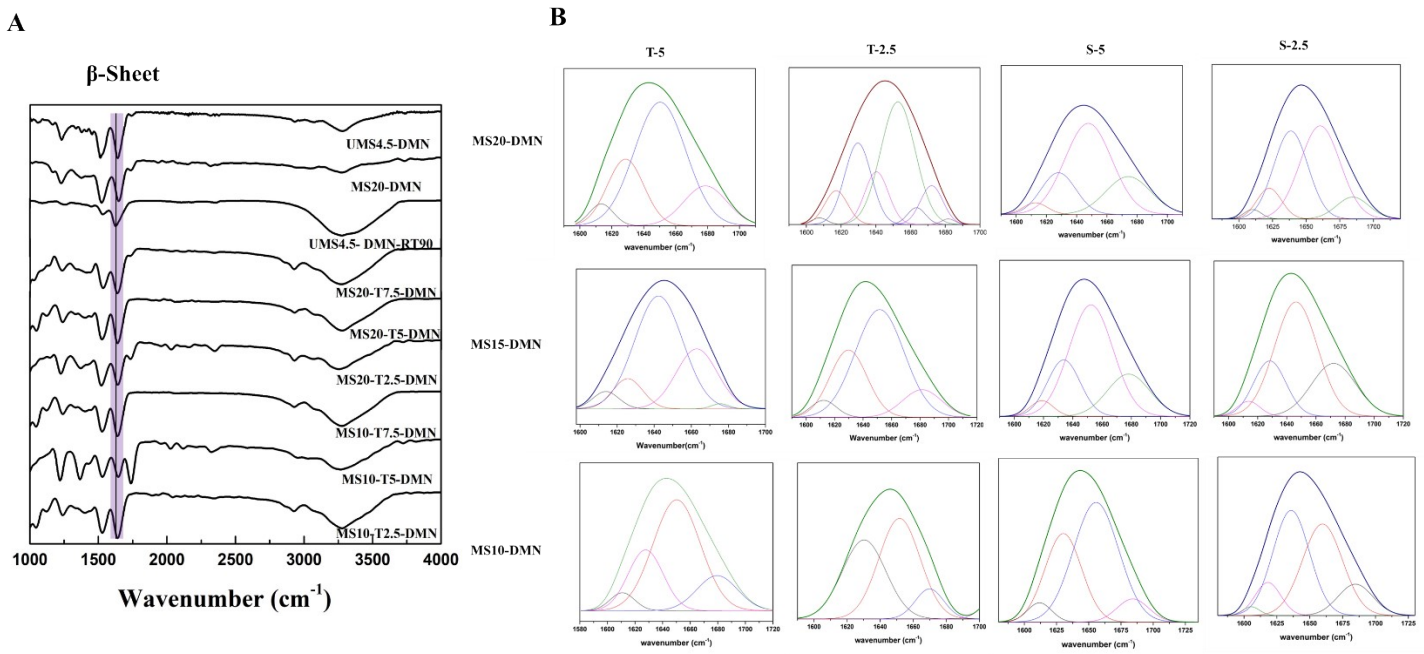
The percentage water content of UMS-DMN and MS-DMN was determined with a Q500 Thermo Gravimetric Analyser (TA Instruments, Elstree, Herts, UK). Samples of 2.0 mg were heated from ambient temperature to 300 °C at a heating rate of 10 °C min^{-1}. Nitrogen flow rates of 40 ml min^{-1} (balance purge gas) and 60 ml min^{-1} (sample purge gas) were maintained for all samples. The data from thermogravimetric analysis experiments were analyzed with TA Instruments Universal Analysis 2000 software, version 4.4A (TA Instruments, Elstree, Herts, UK)

UMS4.5-T2.5-DMN		4.5% w/v	2.5% w/v	
MS20-T7.5-DMN	Trehalose/sucrose incorporated modified silk MN	20% w/v	7.5% w/v	
MS20-T5-DMN		20% w/v	5% w/v	
MS20-T2.5-DMN		20% w/v	2.5% w/v	
SSD0.5-UMS-DMN	SSD loaded Unmodified silk MN	20% w/v	5% w/v	0.5 mg
SSD1-UMS-DMN		20% w/v	5% w/v	1 mg
SSD2-UMS-DMN		20% w/v	5% w/v	2 mg
HRP-MS20-T5-DMN	SSD loaded modified silk MN	20% w/v	5% w/v	2 µg
HRP-UMS4.5-T5-DMN		4.5% w/v	5% w/v	2 µg
PRP-UMS4.5-T5-DMN	PRP loaded Unmodified silk MN	4.5% w/v	5% w/v	8 *10^6 cells
PRP-MS20-T5-DMN	SSD loaded modified silk MN	20% w/v	5% w/v	8 *10^6 cells

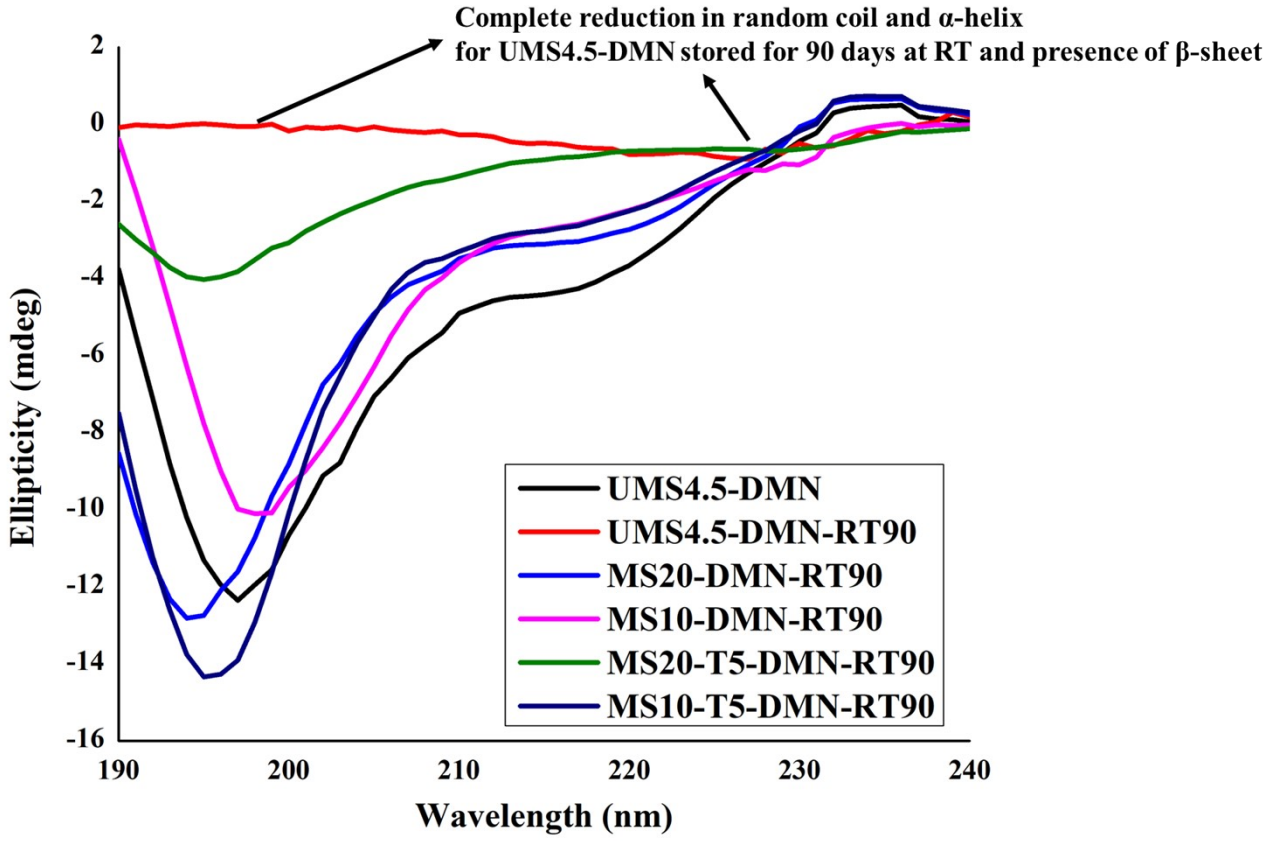
SI Table 1. Detailed formulation of all variants of the unmodified and modified silk microneedles



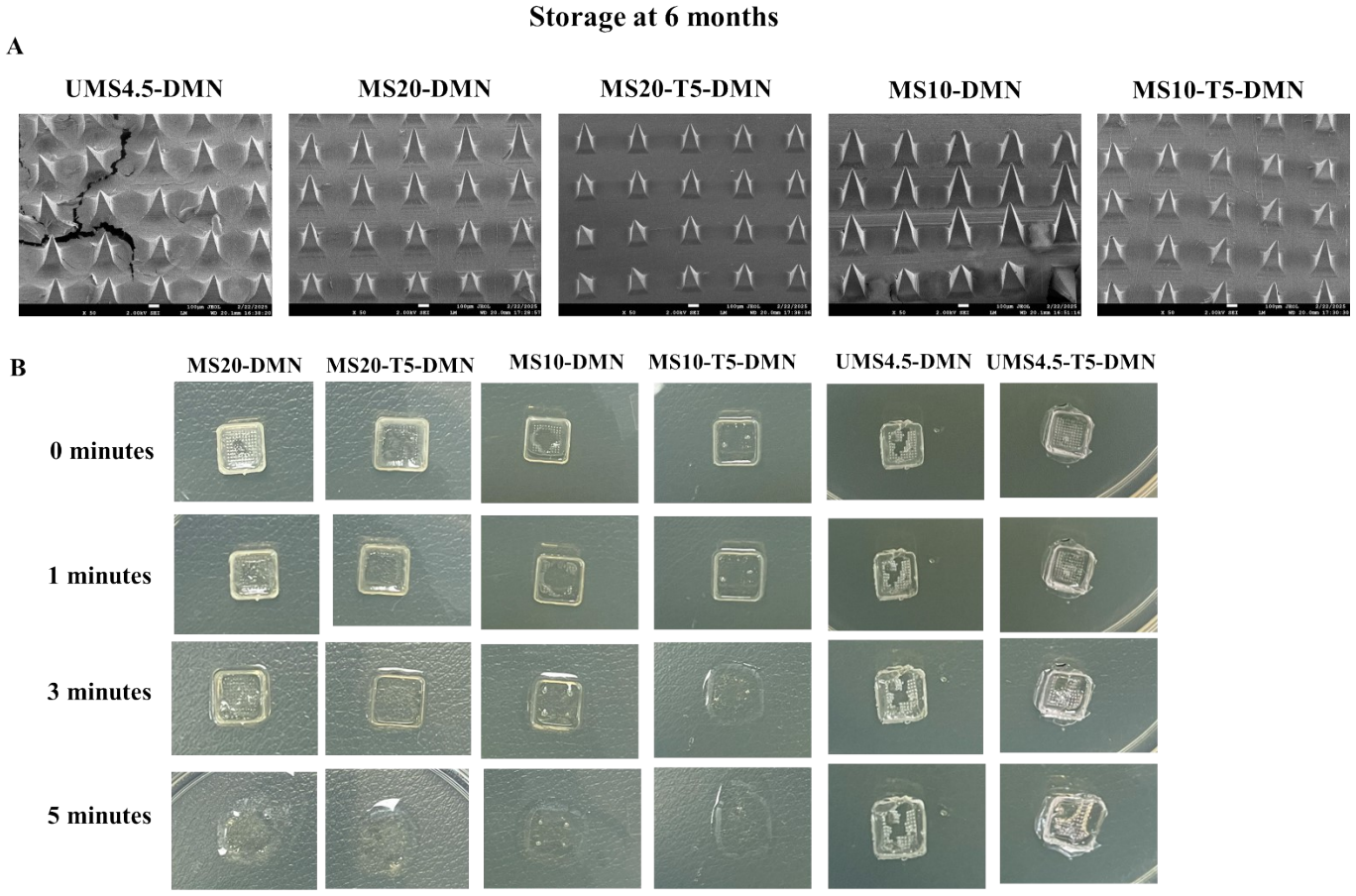
SI Figure 1 (A) The pH vs. NaOH titration curve for the unmodified and modified silk (B) amount of carboxylic group of UMS, MS (R=5 and R=10) with the varying degree of modification with SA. (D) Deconvoluted FTIR spectra of the UMS and MS



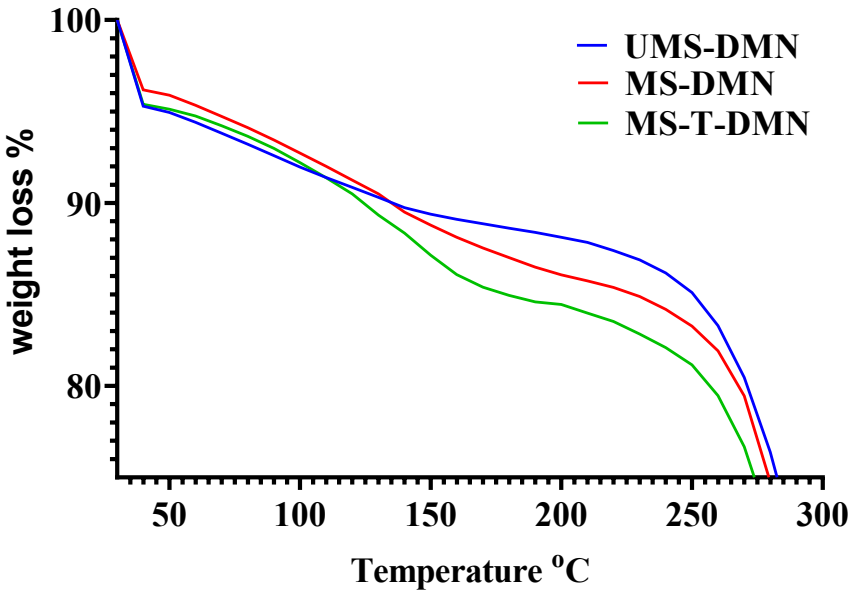
SI Figure 2 (A) The FTIR spectra of UMS and MS with varying concentrations of the stabilizing agents (trehalose 7.5%, 5%, 2.5% w/v). (B) The deconvoluted amide I spectra of the UMS4.5-DMN, MS20-DMN, MS10-DMN incorporated with the different concentration of trehalose (7.5%, 5%, 2.5%)



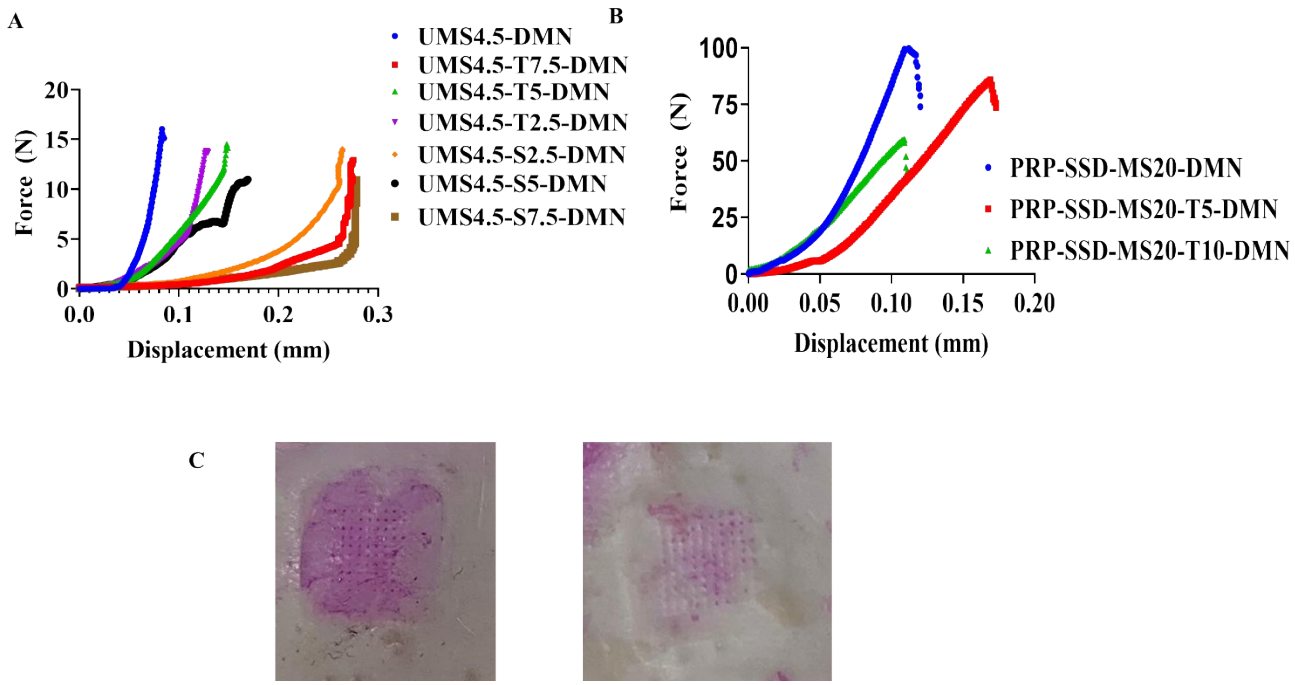
SI Figure 3 CD spectra showing the effect of storage of MS-DMNs, MS-T-DMNs in RT for 90 days showing the presence of higher random coil/α-helix whereas UMS4.5-DMN showed higher β-sheet in the same storage condition



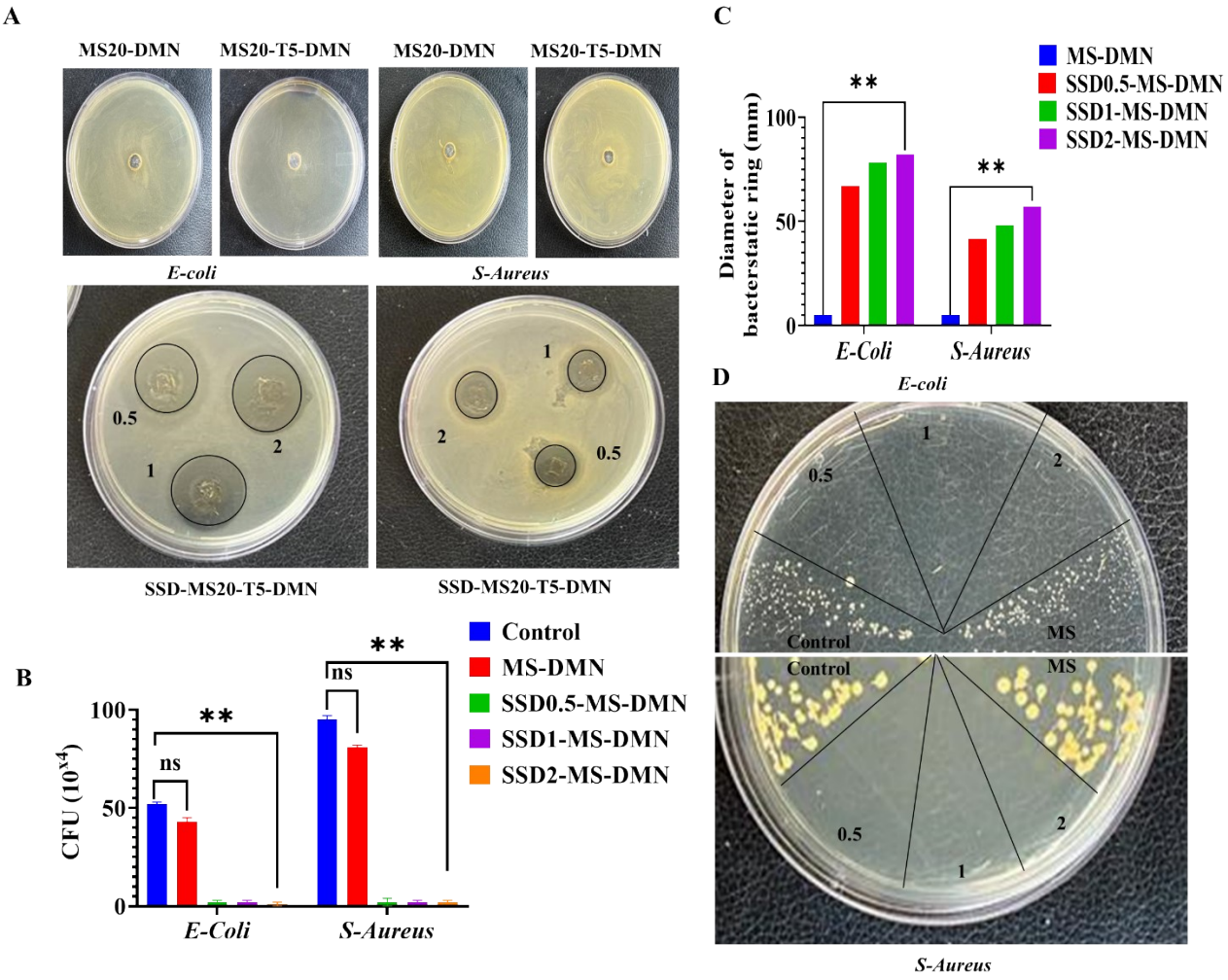
SI Figure 4. (A) SEM images of the stability of the microneedles after storing at 25ºC for 6 months. (B) Representative optical image of the dissolving nature of the MS20-DMN, MS20-T5-DMN, MS10-DMN, MS10-T5-DMN compared with UMS4.5-DMN and UMS4.5-T5-DMN stored room temperature for 6 months.



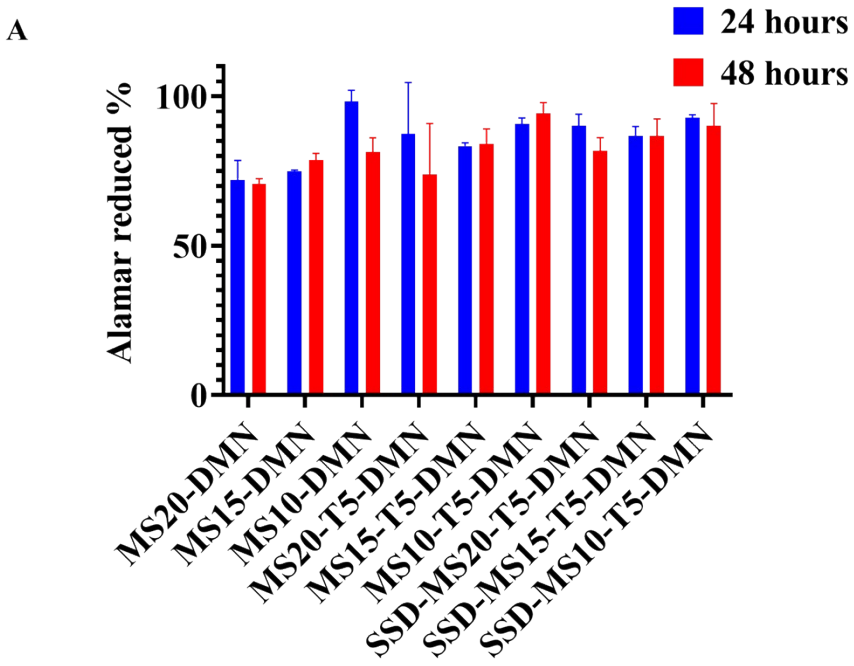
SI Figure 5. TGA analysis of the UMS-DMN, MS-DMN, MS-T-DMN



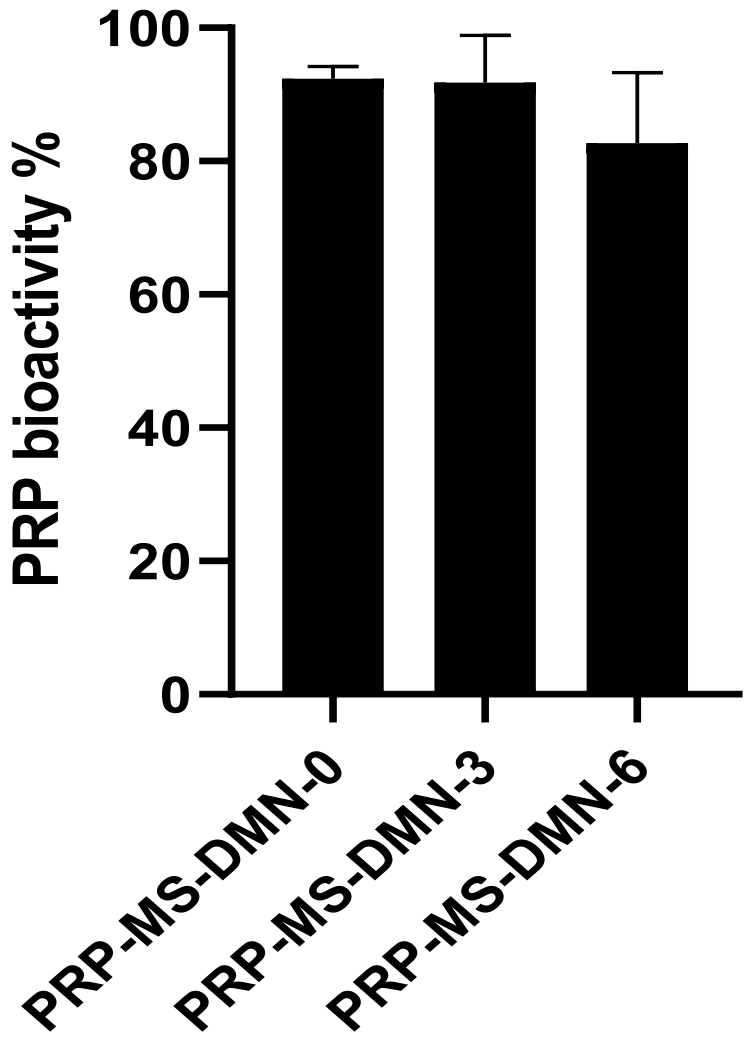
SI Figure 6 (A) The mechanical property of the unmodified silk with the incorporation of the trehalose. (B) The mechanical property of MS20-DMN with varying concentrations of trehalose. (C) The insertion capability of the microneedle (MS20-T5-DMN andUMS4.5-DMN-RT90) was checked the porcine skin.



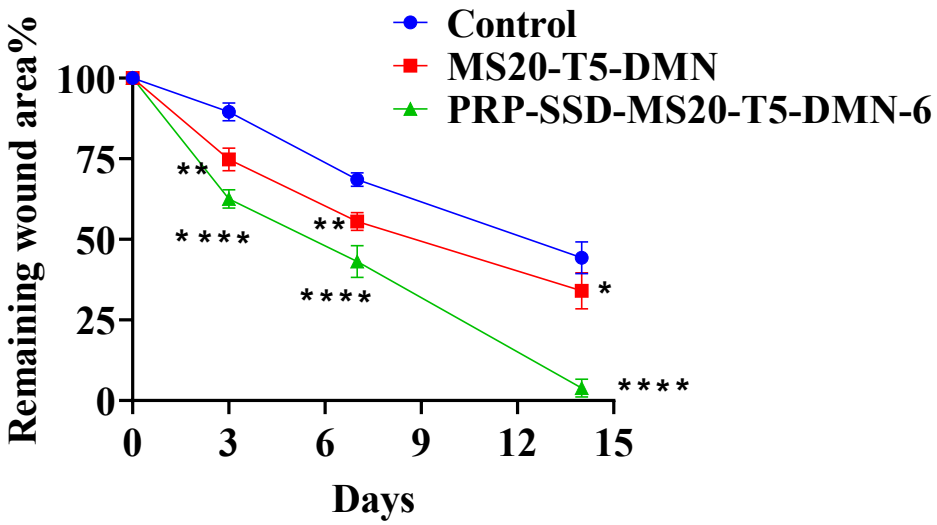
SI Figure 7 (A). Shows the Zone of inhibition of the MS20-DMN, MS20-T5-DMN, and SSD-loaded SSD0.5/1/2-MS20-T5-DMN incubated with *S.aureus and E.coli* for 24 hours. (B) Quantitative analysis of zone of inhibition of various microneedles incubated with S-aureus and E-coli for 24 hours. (C) Shows the time-kill assay of different concentrations of SSD loaded SSD0.5/1/2-MS20-T5-DMN incubated with the *S.aureus and E.coli* for 24 hours. (D) quantitative analysis of the time-kill assay of SSD0.5/1/2-MS20-T5-DMN.



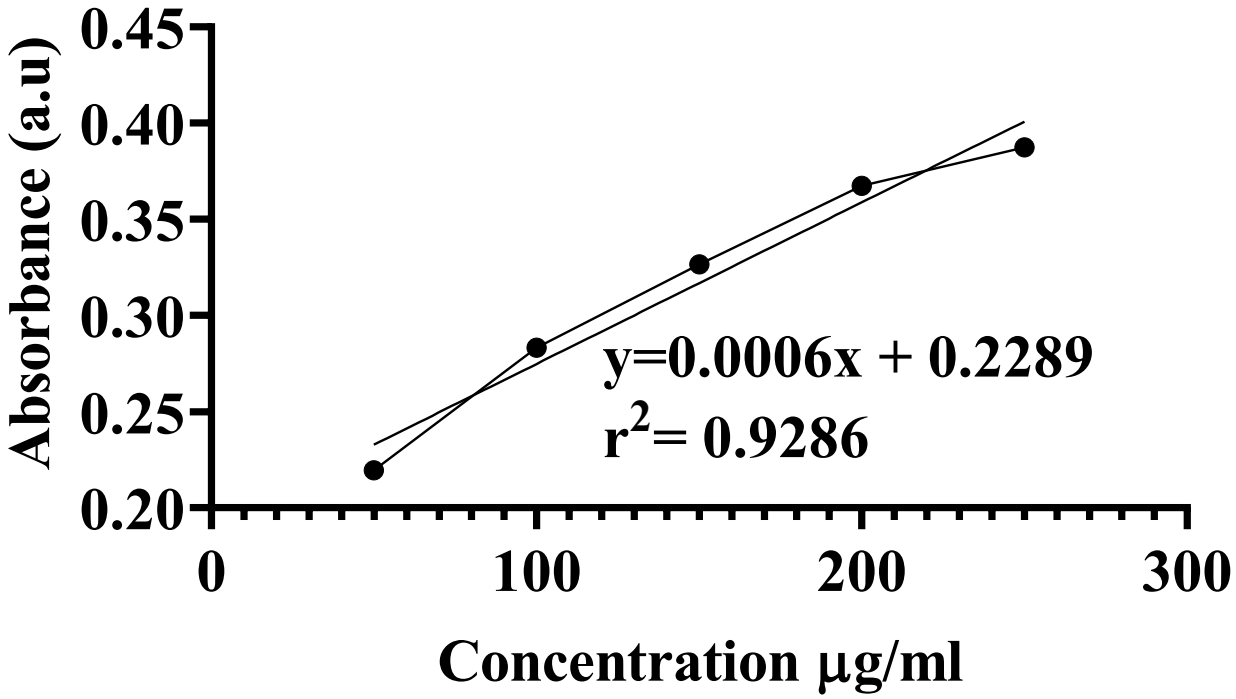
SI Figure 8 (A) The Alamar assay for the various silk microneedles incubated with HEK293 cells



SI Figure 9 *In-vitro* bioactivity of PRP released from the PRP-MS20-T5-DMN stored at 4^0C analyzed in the UMSC



SI Figure 10 Quantitative analysis of the wound closure rate diabetic rats treated with PBS control, PRP-MS20-T5-DMNs-0, and PRP-MS20-T5-DMNs-6.



SI Figure 11 Standard curve graph of the HRP using HRP enzymatic assay.