Journal of Applied Pharmaceutical Science
Available online at http://www.japsonline.com

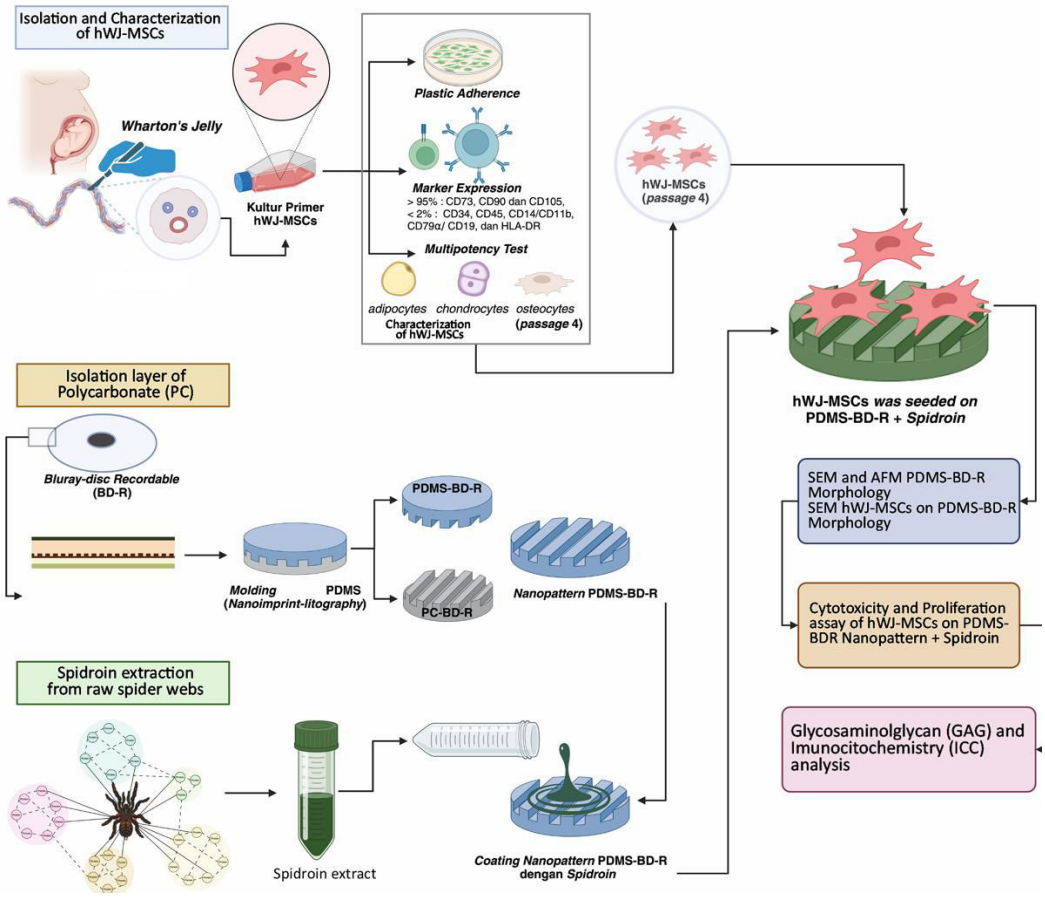
Combination nanopatterns-based Bluray discs and spidroin for directing chondrogenesis of human mesenchymal stem cells

Acep Hendra Punja Unggara[1], Damar Rastri Adhika[2,3], Anggraini Barlian[1,3,4]

[1]Animal Physiology, Development, and Biomedical Sciences Research Group, School of Life Sciences and Technology, Institut Teknologi Bandung, Bandung, Indonesia.
[2]Nano and Quantum Technology Research Group, Engineering Physics, Faculty of Industrial Technology, Institut Teknologi Bandung, Bandung, Indonesia.
[3]Research Center for Nanoscience and Nanotechnology, Institut Teknologi Bandung, Bandung, Indonesia.
[4]Scientific Imaging Center, Institut Teknologi Bandung, Bandung, Indonesia.

Doi: http://dx.doi.org/10.7324/JAPS.2025.238337

SUPPLEMENTARY MATERIAL



Outline Research

Extraction of Spidroin Silk Solution from Argiope appensa Spider Webs

A total of 0.1 g of spider webs was sterilized first using UV light for 15 minutes, then soaked in PBS for 1 hour. The sample was then dissolved in 98% formic acid and homogenized for four hours at 50°C. Perfectly dissolved spider webs are characterized by a dark brownish-yellow colour. The solution is then inserted into the dialysis membrane. Dialysis was carried out every 2 hours, using 5 mM Tris-Cl Buffer solution pH 8 at a temperature of 4°C for 48 hours. After dialysis, the solution was transferred to a 15 mL falcon tube and centrifuged at 5000 rpm for 10 minutes at 4°C. The supernatant was kept, and the particle discarded. Next, the protein concentration was determined using the Bradford Assay technique, and the absorbance was measured at 595 nm. The protein content following dissolution varies from 2 to 2.5 mg/ml.

PDMS-BD-R Nanopattern Fabrication

The BD-R polycarbonate (PC) layer is obtained by peeling the microplastic polymer layer at the bottom of the BD-R optical disc using a razor/cutter. The BD-R PC was previously heated in an oven at 600C for 1 hour. After the stripping process, a shiny layer, called the reflective layer, will be visible. Soak the optical disc in 40% nitric acid solution for 12 hours to remove the reflective layer. Next, the nitric acid solution was discarded in the acid waste, and the BD-R PC was dried. Next is the sonification process using distilled water followed by isopropyl alcohol. Sonication was carried out for one hour each. After the sonication process, the BD-R PC layer was characterized using SEM to determine the presence of nanopatterns on the PC surface before being fabricated using PDMS.

PDMS-BD-R Nanopattern fabrication was carried out by pouring PDMS polymer liquid treated with a curing agent (10:1 w/w) onto the surface of the PC BD-R. To remove the gas in the PDMS elastomer, PC BD-R was placed in a desiccator and vacuumed for 40 minutes. After all the gas is gone, the PC BD-R is heated in an oven at 80°C for two hours. The hardened PDMS elastomer was then peeled off and punched with a 13 mm diameter hollow puncher.

I. Statistical analysis of MTT assay results

Table S1. Overall MTT cytotoxic results

Spidroin Concentration		1000 ug/mL	500 ug/mL	250 ug/mL	100 ug/mL	50 ug/mL	25 ug/mL	Kontrol	Blank	
n	1	0.599	0.626	0.652	0.752	0.785	0.68	0.607	0.038	OD value
	2	0.554	0.672	0.622	0.78	0.83	0.708	0.574	0.038	
	3	0.582	0.593	0.616	0.701	0.745	0.735	0.611	0.023	
	4	0.594	0.616	0.68	0.796	0.807	0.686	0.606	0.016	
Average		0.58225	0.62675	0.6425	0.75725	0.79175	0.70225	0.5995	0.02875	
StDev		0.0201391	0.03318006	0.02954657	0.04167633	0.03617895	0.02493157	0.01713671	0.01105667	

Table S2. Overall MTT cytotoxic result (% viability conversion)

	Control	1000 ug/ml	500 ug/ml	250 ug/ml	100 ug/ml	50 ug/ml	25 ug/ml
OD Value	0.607	0.599	0.626	0.652	0.752	0.785	0.68
	0.574	0.554	0.672	0.622	0.78	0.83	0.708
	0.611	0.582	0.593	0.616	0.701	0.745	0.735
	0.606	0.594	0.616	0.68	0.796	0.807	0.686
	Control	**1000 ug/ml**	**500 ug/ml**	**250 ug/ml**	**100 ug/ml**	**50 ug/ml**	**25 ug/ml**
% Cells Viability	100%	99%	103%	104%	115%	104%	87%
	100%	97%	117%	108%	136%	145%	123%
	100%	95%	97%	101%	115%	122%	120%
	100%	98%	102%	112%	131%	133%	113%
Mean	**100%**	**97%**	**105%**	**106%**	**124%**	**126%**	**111%**

Table S3. Test of Normality Data MTT assay

	1000 ug/mL	500 ug/mL	250 ug/mL	100 ug/mL	50 ug/mL	25 ug/mL	Control
Test for normal distribution							
Shapiro-Wilk test							
W	0.8908	0.9481	0.9158	0.939	0.9814	0.9218	0.7436
P value	0.387	0.704	0.5138	0.648	0.9099	0.5468	0.0336
Passed normality test (alpha=0.05)?	Yes	Yes	Yes	Yes	Yes	Yes	No
P value summary	ns	ns	ns	ns	ns	ns	*

The significance value for every treatment (p-value > 0,05) is more than the α (0.05), meaning that the data is normally distributed.
*) Because the repetition of data used in the MTT test is less than 5, it does not meet the homogeneity test requirements. However, it can be concluded based on the distribution of the data obtained, which appears to have different variations (not homogeneous).

Table S4. Test of Significancy Data MTT Assay

ANOVA summary	
F	4.516
P value	0.004
P value summary	**
Significant diff. among means (P < 0.05)?	Yes
R squared	0.5634

The significance value (p-value) is 0.004, which is smaller than the α (0.05), meaning that there is an effect of spidroin extract use on hWJ-MSCs proliferation.

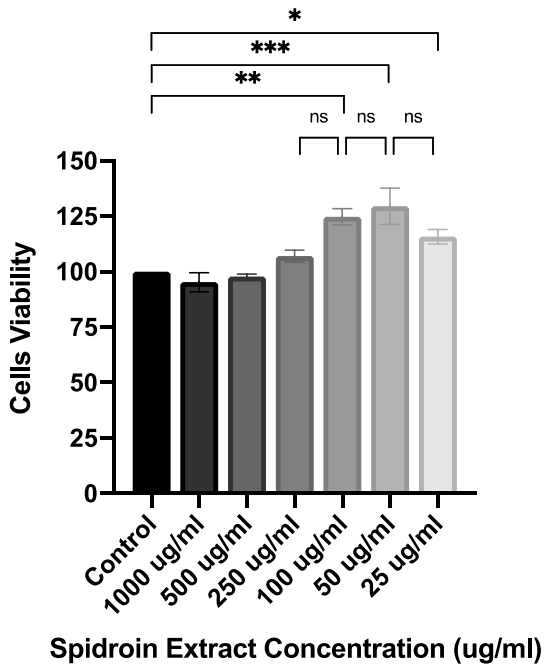


Figure 1. Cytotoxicity test of spider web extract on hWJ-MSC cells as measured by the MTT as-say. Data are mean ± SD with significance markers * (p<0.05), ** (p<0.01), and *** (p <0.001).

Table S5. Post Hoc Test on MTT cytotoxic

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
1000 ug/mL vs. Kontrol	-0.0196	-0.08043 to 0.04123	No	ns	0.944
500 ug/mL vs. Kontrol	0.0182	-0.04263 to 0.07903	No	ns	0.961
250 ug/mL vs. Kontrol	0.0354	-0.02543 to 0.09623	No	ns	0.53
100 ug/mL vs. Kontrol	0.1442	0.08337 to 0.2050	Yes	***	<.001
50 ug/mL vs. Kontrol	0.1806	0.1198 to 0.2414	Yes	***	<.001
25 ug/mL vs. Kontrol	0.107	0.04617 to 0.1678	Yes	***	<.001
100 ug/mL vs. 50 ug/mL	-0.0364	-0.09723 to 0.02443	No	ns	0.498
100 ug/mL vs. 25 ug/mL	0.0372	-0.02363 to 0.09803	No	ns	0.472
250 ug/mL vs. 100 ug/mL	-0.1088	-0.1696 to -0.04797	Yes	***	<.001
250 ug/mL vs. 50 ug/mL	-0.1452	-0.2060 to -0.08437	Yes	***	<.001
500 ug/mL vs. 100 ug/mL	-0.126	-0.1868 to -0.06517	Yes	***	<.001
500 ug/mL vs. 50 ug/mL	-0.1624	-0.2232 to -0.1016	Yes	***	<.001
500 ug/mL vs. 25 ug/mL	-0.0888	-0.1496 to -0.02797	Yes	**	0.001
1000 ug/mL vs. 100 ug/mL	-0.1638	-0.2246 to -0.1030	Yes	***	<.001
1000 ug/mL vs. 50 ug/mL	-0.2002	-0.2610 to -0.1394	Yes	***	<.001
1000 ug/mL vs. 25 ug/mL	-0.1266	-0.1874 to -0.06577	Yes	***	<.001

The significance value (p value) < 0.001 shows in the spidroin concentration range from 25 µg/mL to 100 µg/mL, which is smaller than the α (0.05), meaning that the spidrion extract significantly induced the proliferation of hWJ-MSCs compared with the control. The spidroin concentration range starts from 25 µg/mL up to. 1000 µg/mL produces cell viability >90%. This indicates that spider web extract is not toxic to cells in this concentration range.

II. Statistical analysis of MTT proliferation results (Day 1)

Table S6. Overall MTT proliferation results (O.D 595 nm)

Day	PDMS NP (-)			PDMS NP (+)			PDMS_BD P (-)			PDMS_BD P (+)			Control (No PDMS)		
1	0.325	0.301	0.289	0.329	0.309	0.365	0.429	0.37	0.39	0.401	0.43	0.455	0.295	0.328	0.33
3	0.335	0.322	0.355	0.365	0.335	0.4	0.395	0.42	0.45	0.49	0.54	0.551	0.33	0.341	0.32
5	0.48	0.506	0.422	0.49	0.556	0.558	0.79	0.826	0.828	0.85	0.935	0.9	0.42	0.395	0.455
7	0.552	0.71	0.666	0.89	0.746	0.968	1.098	0.98	1.08	1.152	1.39	1.255	0.45	0.538	0.535
14	0.877	0.922	0.918	1.039	1.117	1.128	1.311	1.355	1.448	1.709	1.775	1.818	0.595	0.505	0.551

Table S7. Overall MTT proliferation result (% viability conversion)

	Day 1				Day 3				Day 5			
Cells Numb	PDMS NP (-)	PDMS NP (+)	PDMS BDR (-)	PDMS BDR (+)	PDMS NP (-)	PDMS NP (+)	PDMS BDR (-)	PDMS BDR (+)	PDMS NP (-)	PDMS NP (+)	PDMS BDR (-)	PDMS BDR (+)
1	110%	112%	145%	136%	102%	111%	120%	148%	114%	117%	188%	202%
2	92%	94%	113%	131%	94%	98%	123%	158%	128%	141%	209%	237%
3	88%	111%	118%	138%	111%	125%	141%	172%	93%	123%	182%	198%

	Day 7				Day 14			
Cells Numb	PDMS NP (-)	PDMS NP (+)	PDMS BDR (-)	PDMS BDR (+)	PDMS NP (-)	PDMS NP (+)	PDMS BDR (-)	PDMS BDR (+)
1	123%	198%	244%	256%	147%	175%	220%	287%
2	132%	139%	182%	258%	183%	221%	268%	351%
3	124%	181%	202%	235%	167%	205%	263%	330%

Table S8. Test of Normality MTT Proliferation Data (Day 1)

	Day 1			
Shapiro-Wilk test	**PDMS NP (-)**	**PDMS NP (+)**	**PDMS BDR (-)**	**PDMS BDR (+)**
W	0.8811	0.7915	0.8639	0.9423
P value	0.3275	0.0944	0.2783	0.5367
Passed normality test (alpha=0.05)?	Yes	Yes	Yes	Yes
P value summary	ns	ns	ns	ns

The significance value for every treatment (p-value > 0,05) is more than the α (0.05), meaning that the data is normally distributed.
*) Because the repetition of data used in the MTT proliferation test is less than 5, it does not meet the homogeneity test requirements.

Table S9. Test of Significancy MTT Proliferation Data (Day 1)

ANOVA summary					
F	6.763				
P value	0.014				
P value summary	*				
Significant diff. among means (P < 0.05)?	Yes				
R squared	0.7172				
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
eatment (between column	2785	3	928.2	F (3, 8) = 6.763	P=.014
Residual (within columns)	1098	8	137.3		
Total	3883	11			
Data summary					
Number of treatments (columns)	4				
Number of values (total)	12				

The significance value *(p-value)* is 0.014, which is smaller than the α (0.05), meaning that there is an effect of BD-R PDMS *nanopattern* whether coated with spidroin extract (+) or not (-) is able to induce hWJ-MSCs proliferation.

Table S10. Post Hoc Test on MTT Proliferation Data (Day 1)

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
PDMS NP (-) vs. PDMS NP (+)	-9	-39.63 to 21.63	No	ns	0.785
PDMS NP (-) vs. PDMS BDR (-)	-28.67	-59.30 to 1.966	No	ns	0.067
PDMS NP (-) vs. PDMS BDR (+)	**-38.33**	**-68.97 to -7.701**	**Yes**	*	**0.016**
PDMS NP (+) vs. PDMS BDR (-)	-19.67	-50.30 to 10.97	No	ns	0.246
PDMS NP (+) vs. PDMS BDR (+)	-29.33	-59.97 to 1.299	No	ns	0.061
PDMS BDR (-) vs. PDMS BDR (+)	-9.667	-40.30 to 20.97	No	ns	0.748

On day 1, there is the significance value (p-value) is 0.016, which is smaller than the α (0.05), meaning that there is an effect of BD-R (+) PDMS *nanopattern* coated with spidroin (+) is better than the NP PDMS without spidroin (-).

III. Statistical analysis of MTT proliferation results (Day 3)

Table S11. Test of Normality MTT Proliferation Data (Day 3)

	Day 3			
Shapiro-Wilk test	**PDMS NP (-)**	**PDMS NP (+)**	**PDMS BDR (-)**	**PDMS BDR (+)**
W	0.9988	0.9995	0.8547	0.9908
P value	0.9352	0.9592	0.253	0.8168
Passed normality test (alpha=0.05)?	Yes	Yes	Yes	Yes
P value summary	ns	ns	ns	ns

The significance value for every treatment (p-value > 0,05) is more than the α (0.05), meaning that the data is normally distributed.
*) Because the repetition of data (n) used in the MTT proliferation test is less than 5, it does not meet the homogeneity test requirements.

Table S12. Test of Significancy MTT Proliferation Data (Day 3)

ANOVA summary					
F	14.28				
P value	0.001				
P value summary	**				
Significant diff. among means (P < 0.05)?	Yes				
R squared	0.8426				
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	5664	3	1888	F (3, 8) = 14.28	P=.001
Residual (within columns)	1058	8	132.3		
Total	6722	11			
Data summary					
Number of treatments (columns)	4				
Number of values (total)	12				

The significance value *(p-value)* is 0.001, which is smaller than the α (0.05), meaning that there is an effect of BD-R PDMS *nanopattern* whether coated with spidroin extract (+) or not (-) is able to induce hWJ-MSCs proliferation.

Table S13. Post Hoc Test on MTT Proliferation Data (Day 3)

Tukey's multiple comparisons test	**Mean Diff.**	**95.00% CI of diff.**	**Below threshold?**	**Summary**	**Adjusted P Value**
PDMS NP (-) vs. PDMS NP (+)	-9	-39.07 to 21.07	No	ns	0.776
PDMS NP (-) vs. PDMS BDR (-)	-25.67	-55.74 to 4.402	No	ns	0.097
PDMS NP (-) vs. PDMS BDR (+)	**-57**	**-87.07 to -26.93**	**Yes**	**	**0.001**
PDMS NP (+) vs. PDMS BDR (-)	-16.67	-46.74 to 13.40	No	ns	0.35
PDMS NP (+) vs. PDMS BDR (+)	**-48**	**-78.07 to -17.93**	**Yes**	**	**0.004**
PDMS BDR (-) vs. PDMS BDR (+)	**-31.33**	**-61.40 to -1.264**	**Yes**	*	**0.041**

On day 3, there is the significance value (p-value) is less than 0.05, which is smaller than the α (0.05), meaning that there is an effect of BD-R PDMS to induce hWJ-MSCs proliferation, especially for BD-R (+) PDMS *nanopattern* compared to NP (+/-) PDMS and BD-R (-) PDMS *nanopattern*.

IV. Statistical analysis of MTT proliferation results (Day 5)

Table S14. Test of Normality MTT Proliferation Data (Day 5)

	Day 5			
Shapiro-Wilk test	**PDMS NP (-)**	**PDMS NP (+)**	**PDMS BDR (-)**	**PDMS BDR (+)**
W	0.9231	0.9067	0.826	0.8322
P value	0.4633	0.4072	0.1783	0.1939
Passed normality test (alpha=0.05)?	Yes	Yes	Yes	Yes
P value summary	ns	ns	ns	ns

The significance value for every treatment (p-value > 0,05) is more than the α (0.05), meaning that the data is normally distributed.
*) Because the repetition of data (n) used in the MTT proliferation test is less than 5, it does not meet the homogeneity test requirements.

Table S15. Test of Significancy MTT Proliferation Data (Day 5)

ANOVA summary					
F	28.34				
P value	<.001				
P value summary	***				
Significant diff. among means (P < 0.05)?	Yes				
R squared	0.914				
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	17889	3	5963	F (3, 8) = 28.34	P<.001
Residual (within columns)	1683	8	210.4		
Total	19573	11			
Data summary					
Number of treatments (columns)	4				
Number of values (total)	12				

The significance value *(p-value)* is <0.001, which is smaller than the α (0.05), meaning that there is an effect of BD-R PDMS *nanopattern* whether coated with spidroin extract (+) or not (-) is able to induce hWJ-MSCs proliferation.

Table S16. Post Hoc Test on MTT Proliferation Data (Day 5)

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
PDMS NP (-) vs. PDMS NP (+)	**-66**	**-103.9 to -28.07**	**Yes**	**	**0.002**
PDMS NP (-) vs. PDMS BDR (-)	**-85.33**	**-123.3 to -47.41**	**Yes**	***	**<.001**
PDMS NP (-) vs. PDMS BDR (+)	0.6667	-37.26 to 38.59	No	ns	>.999
PDMS NP (+) vs. PDMS BDR (-)	-19.33	-57.26 to 18.59	No	ns	0.414
PDMS NP (+) vs. PDMS BDR (+)	**66.67**	**28.74 to 104.6**	**Yes**	**	**0.002**
PDMS BDR (-) vs. PDMS BDR (+)	**86**	**48.07 to 123.9**	**Yes**	***	**<.001**

On day 5, there is the significance value (p-value) is ranging < 0.001 to 0.002, which is smaller than the α (0.05), meaning that there is an effect of BD-R (+/-) PDMS to induce hWJ-MSCs proliferation compared to NP (+/-) PDMS.

V. Statistical analysis of MTT proliferation results (Day 7)

Table S17. Test of Normality MTT Proliferation Data (Day 7)

	Day 7			
Shapiro-Wilk test	**PDMS NP (-)**	**PDMS NP (+)**	**PDMS BDR (-)**	**PDMS BDR (+)**
W	0.8322	0.9435	0.9597	0.8147
P value	0.1939	0.5418	0.6141	0.1501
Passed normality test (alpha=0.05)?	Yes	Yes	Yes	Yes
P value summary	ns	ns	ns	ns

The significance value for every treatment (p-value > 0,05) is more than the α (0.05), meaning that the data is normally distributed.

*) Because the repetition of data (n) used in the MTT proliferation test is less than 5, it does not meet the homogeneity test requirements.

Table S18. Test of Significancy MTT Proliferation Data (Day 7)

ANOVA summary					
F	15.71				
P value	0.001				
P value summary	**				
Significant diff. among means (P < 0.05)?	Yes				
R squared	0.8549				
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	24860	3	8287	F (3, 8) = 15.71	P=.001
Residual (within columns)	4221	8	527.6		
Total	29081	11			
Data summary					
Number of treatments (columns)	4				
Number of values (total)	12				

The significance value *(p-value)* is 0.001, which is smaller than the α (0.05), meaning that there is an effect of BD-R PDMS *nanopattern* whether coated with spidroin extract (+) or not (-) is able to induce hWJ-MSCs proliferation.

Table S19. Post Hoc Test on MTT Proliferation Data (Day 7)

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
PDMS NP (-) vs. PDMS NP (+)	-46.33	-106.4 to 13.72	No	ns	0.14
PDMS NP (-) vs. PDMS BDR (-)	**-83**	**-143.1 to -22.94**	**Yes**	**	**0.01**
PDMS NP (-) vs. PDMS BDR (+)	**-123.3**	**-183.4 to -63.28**	**Yes**	***	**<.001**
PDMS NP (+) vs. PDMS BDR (-)	-36.67	-96.72 to 23.39	No	ns	0.28
PDMS NP (+) vs. PDMS BDR (+)	**-77**	**-137.1 to -16.94**	**Yes**	*	**0.014**
PDMS BDR (-) vs. PDMS BDR (+)	-40.33	-100.4 to 19.72	No	ns	0.217

On day 7, there is the significance value (p-value) is <0.05, which is smaller than the α (0.05), meaning that there is an effect of BD-R (+/-) PDMS to induce hWJ-MSCs proliferation compared to NP (+/-) PDMS.

Statistical analysis of MTT proliferation results (Day 14)

Table S20. Test of Normality Data MTT Proliferation Data (Day 14)

	Day 14			
Shapiro-Wilk test	**PDMS NP (-)**	**PDMS NP (+)**	**PDMS BDR (-)**	**PDMS BDR (+)**
W	0.9959	0.97	0.8272	0.9621
P value	0.8776	0.6678	0.1812	0.6258
Passed normality test (alpha=0.05)?	Yes	Yes	Yes	Yes
P value summary	ns	ns	ns	ns

The significance value for every treatment (p-value > 0,05) is more than the α (0.05), meaning that the data is normally distributed.
*) Because the repetition of data (n) used in the MTT proliferation test is less than 5, it does not meet the homogeneity test requirements.

Table S21. Test of Significancy MTT Proliferation Data (Day 14)

ANOVA summary					
F	21.17				
P value	<.001				
P value summary	***				
Significant diff. among means (P < 0.05)?	Yes				
R squared	0.8881				
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	41788	3	13929	F (3, 8) = 21.17	P<.001
Residual (within columns)	5263	8	657.8		
Total	47050	11			
Data summary					
Number of treatments (columns)	4				
Number of values (total)	12				

The significance value *(p-value)* is <0.001, which is smaller than the α (0.05), meaning that there is an effect of BD-R PDMS *nanopattern* whether coated with spidroin extract (+) or not (-) is able to induce hWJ-MSCs proliferation.

Table S22. Post Hoc Test on MTT Proliferation Data (Day 14)

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
PDMS NP (-) vs. PDMS NP (+)	-34.67	-101.7 to 32.40	No	ns	0.404
PDMS NP (-) vs. PDMS BDR (-)	**-84.67**	**-151.7 to -17.60**	**Yes**	*	**0.016**
PDMS NP (-) vs. PDMS BDR (+)	**-157**	**-224.1 to -89.94**	**Yes**	***	**<.001**
PDMS NP (+) vs. PDMS BDR (-)	-50	-117.1 to 17.06	No	ns	0.157
PDMS NP (+) vs. PDMS BDR (+)	**-122.3**	**-189.4 to -55.27**	**Yes**	**	**0.002**
PDMS BDR (-) vs. PDMS BDR (+)	-72.33	-139.4 to -5.271	Yes	*	0.035

On day 14, there is the significance value (p-value) is <0.05, which is smaller than the α (0.05), meaning that there is an effect of BD-R (+/-) PDMS to induce hWJ-MSCs proliferation compared to NP (+/-) PDMS

II. Statistical analysis of MTT proliferation results (Day 1-14)

Table S23. Test of Normality MTT Proliferation Data (Day 1-14)

Shapiro-Wilk test					
W	0.912	0.8958	0.9204	0.9366	0.9061
P value	0.48	0.387	0.532	0.642	0.444
Passed normality test (alpha=0.05)?	Yes	Yes	Yes	Yes	Yes
P value summary	ns	ns	ns	ns	ns
Kolmogorov-Smirnov test					
KS distance	0.1999	0.2245	0.2249	0.1925	0.2215
P value	>.100	>.100	>.100	>.100	>.100
Passed normality test (alpha=0.05)?	Yes	Yes	Yes	Yes	Yes
P value summary	ns	ns	ns	ns	ns
Number of values	5	5	5	5	5

The significance value for every treatment (p-value > 0,05) is more than the α (0.05), meaning that the data is normally distributed.

Table S24. Test of Homogeneity MTT Proliferation Data (Day 1-14)

Bartlett's test	
Bartlett's statistic (corrected)	3.551
P value	0.3142
P value summary	ns
Are SDs significantly different (P < 0.05)?	No

The significance value (p-value) is 0.3142, which is bigger than the α (0.05), meaning that the data group comes from a population that has the same variance (homogeneous).

Table S24. Test of Significancy Data MTT proliferation (Day 1-14)

Two-way ANOVA	Ordinary				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	7.808	<.001	***	Yes	
Row Factor	50.71	<.001	***	Yes	
Column Factor	35.3	<.001	***	Yes	
ANOVA table					
Interaction	SS	DF	MS	F (DFn, DFd)	P value
Row Factor	17543	12	1462	F (12, 40) = 4.209	P<.001
Column Factor	113939	4	28485	F (4, 40) = 82.00	P<.001
Residual	79301	3	26434	F (3, 40) = 76.10	P<.001
	13895	40	347.4		
Data summary					
Number of columns (Column Factor)	4				
Number of rows (Row Factor)	5				
Number of values	60				

The significance value (p-value) less than 0.001, which is smaller than the α (0.05), meaning that there is an effect of BD-R PDMS *nanopattern* whether coated with spidroin extract (+) or not (-) is able to induce hWJ-MSCs proliferation.

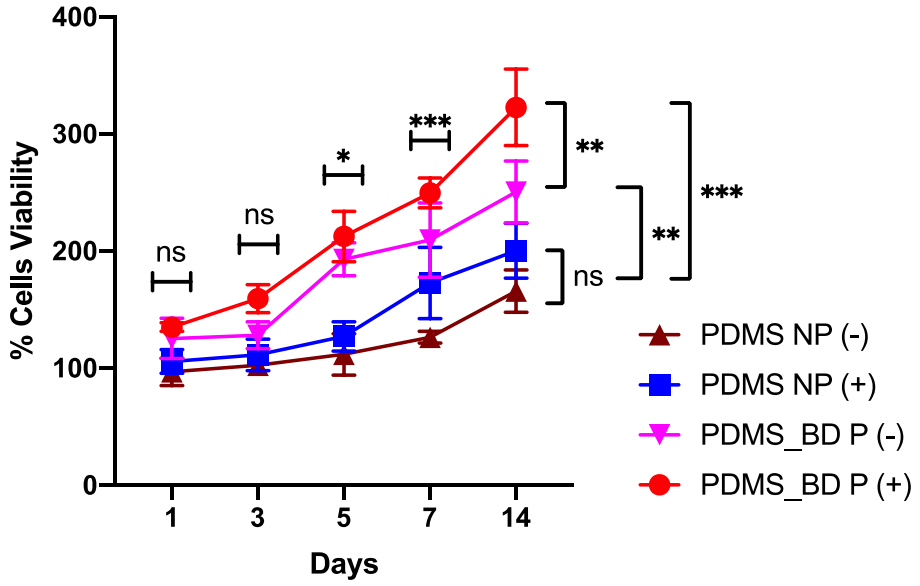


Figure S2. Proliferation graph of hWJ-MSC cells cultured on BD-R Nanopattern PDMS and Un-pattern (NP) PDMS elastomers, whether coated with spidroin extract (+) or not (-). The proliferation of hWJ-MSC cells was measured using the MTT method on days 1, 3, 5, 7, and 14. Data are mean ± SD with significance markers * ($p<0.05$) ** ($p<0.01$) and * ** ($p < 0.001$).

Table S25. Post Hoc Test on MTT proliferation (Day 1-14)

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value	Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
1:PDMS NP (-) vs. 1:PDMS NP (+)	-9	-66.65 to 48.65	No	ns	>.999	3:PDMS NP (+) vs. 14:PDMS NP (-)	-54.33	112.0 to 3.31	No	ns	0.085
1:PDMS NP (-) vs. 1:PDMS_BD P (-)	-28.67	-86.32 to 28.98	No	ns	0.926	3:PDMS NP (+) vs. 14:PDMS NP (+)	-89	146.6 to -31.3	Yes	***	<.001
1:PDMS NP (-) vs. 1:PDMS_BD P (+)	-38.33	-95.98 to 19.32	No	ns	0.577	3:PDMS NP (+) vs. 14:PDMS_BD P (-)	-139	196.6 to -81.3	Yes	***	<.001
1:PDMS NP (-) vs. 3:PDMS NP (-)	-5.667	-63.32 to 51.98	No	ns	>.999	3:PDMS NP (+) vs. 14:PDMS_BD P (+)	-211.3	269.0 to -153.	Yes	***	<.001
1:PDMS NP (-) vs. 3:PDMS NP (+)	-14.67	-72.32 to 42.98	No	ns	>.999	3:PDMS_BD P (-) vs. 3:PDMS_BD P (+)	-31.33	88.98 to 26.3	No	ns	0.858
1:PDMS NP (-) vs. 3:PDMS_BD P (-)	-31.33	-88.98 to 26.32	No	ns	0.858	3:PDMS_BD P (-) vs. 5:PDMS NP (-)	16.33	41.32 to 73.9	No	ns	>.999
1:PDMS NP (-) vs. 3:PDMS_BD P (+)	-62.67	-120.3 to -5.017	Yes	*	0.021	3:PDMS_BD P (-) vs. 5:PDMS NP (+)	1	56.65 to 58.6	No	ns	>.999
1:PDMS NP (-) vs. 5:PDMS NP (-)	-15	-72.65 to 42.65	No	ns	>.999	3:PDMS_BD P (-) vs. 5:PDMS_BD P (-)	-65	122.6 to -7.35	Yes	*	0.014
1:PDMS NP (-) vs. 5:PDMS NP (+)	-30.33	-87.98 to 27.32	No	ns	0.886	3:PDMS_BD P (-) vs. 5:PDMS_BD P (+)	-84.33	142.0 to -26.6	Yes	***	<.001
1:PDMS NP (-) vs. 5:PDMS_BD P (-)	-96.33	-154.0 to -38.68	Yes	***	<.001	3:PDMS_BD P (-) vs. 7:PDMS NP (-)	1.667	55.98 to 59.3	No	ns	>.999
1:PDMS NP (-) vs. 5:PDMS_BD P (+)	-115.7	-173.3 to -58.02	Yes	***	<.001	3:PDMS_BD P (-) vs. 7:PDMS NP (+)	-44.67	102.3 to 12.9	No	ns	0.314
1:PDMS NP (-) vs. 7:PDMS NP (-)	-29.67	-87.32 to 27.98	No	ns	0.904	3:PDMS_BD P (-) vs. 7:PDMS_BD P (-)	-81.33	139.0 to -23.6	Yes	***	<.001
1:PDMS NP (-) vs. 7:PDMS NP (+)	-76	-133.6 to -18.35	Yes	**	0.002	3:PDMS_BD P (-) vs. 7:PDMS_BD P (+)	-121.7	179.3 to -64.0	Yes	***	<.001
1:PDMS NP (-) vs. 7:PDMS_BD P (-)	-112.7	-170.3 to -55.02	Yes	***	<.001	3:PDMS_BD P (-) vs. 14:PDMS NP (-)	-37.67	95.32 to 19.9	No	ns	0.607
1:PDMS NP (-) vs. 7:PDMS_BD P (+)	-153	-210.6 to -95.35	Yes	***	<.001	3:PDMS_BD P (-) vs. 14:PDMS NP (+)	-72.33	130.0 to -14.6	Yes	**	0.004
1:PDMS NP (-) vs. 14:PDMS NP (-)	-69	-126.6 to -11.35	Yes	**	0.007	3:PDMS_BD P (-) vs. 14:PDMS_BD P (-)	-122.3	180.0 to -64.6	Yes	***	<.001
1:PDMS NP (-) vs. 14:PDMS NP (+)	-103.7	-161.3 to -46.02	Yes	***	<.001	3:PDMS_BD P (-) vs. 14:PDMS_BD P (+)	-194.7	252.3 to -137.	Yes	***	<.001
1:PDMS NP (-) vs. 14:PDMS_BD P (-)	-153.7	-211.3 to -96.02	Yes	***	<.001	3:PDMS_BD P (+) vs. 5:PDMS NP (-)	47.67	9.983 to 105.	No	ns	0.218
1:PDMS NP (-) vs. 14:PDMS_BD P (+)	-226	-283.6 to -168.4	Yes	***	<.001	3:PDMS_BD P (+) vs. 5:PDMS NP (+)	32.33	25.32 to 89.9	No	ns	0.825
1:PDMS NP (+) vs. 1:PDMS_BD P (-)	-19.67	-77.32 to 37.98	No	ns	0.998	3:PDMS_BD P (+) vs. 5:PDMS_BD P (-)	-33.67	91.32 to 23.9	No	ns	0.777
1:PDMS NP (+) vs. 1:PDMS_BD P (+)	-29.33	-86.98 to 28.32	No	ns	0.911	3:PDMS_BD P (+) vs. 5:PDMS_BD P (+)	-53	110.6 to 4.65	No	ns	0.104
1:PDMS NP (+) vs. 3:PDMS NP (-)	3.333	-54.32 to 60.98	No	ns	>.999	3:PDMS_BD P (+) vs. 7:PDMS NP (-)	33	24.65 to 90.6	No	ns	0.802
1:PDMS NP (+) vs. 3:PDMS NP (+)	-5.667	-63.32 to 51.98	No	ns	>.999	3:PDMS_BD P (+) vs. 7:PDMS NP (+)	-13.33	70.98 to 44.3	No	ns	>.999
1:PDMS NP (+) vs. 3:PDMS_BD P (-)	-22.33	-79.98 to 35.32	No	ns	0.993	3:PDMS_BD P (+) vs. 7:PDMS_BD P (-)	-50	107.6 to 7.65	No	ns	0.16
1:PDMS NP (+) vs. 3:PDMS_BD P (+)	-53.67	-111.3 to 3.983	No	ns	0.094	3:PDMS_BD P (+) vs. 7:PDMS_BD P (+)	-90.33	148.0 to -32.6	Yes	***	<.001
1:PDMS NP (+) vs. 5:PDMS NP (-)	-6	-63.65 to 51.65	No	ns	>.999	3:PDMS_BD P (+) vs. 14:PDMS NP (-)	-6.333	63.98 to 51.3	No	ns	>.999
1:PDMS NP (+) vs. 5:PDMS NP (+)	-21.33	-78.98 to 36.32	No	ns	0.996	3:PDMS_BD P (+) vs. 14:PDMS NP (+)	-41	98.65 to 16.6	No	ns	0.459
1:PDMS NP (+) vs. 5:PDMS_BD P (-)	-87.33	-145.0 to -29.68	Yes	***	<.001	3:PDMS_BD P (+) vs. 14:PDMS_BD P (-)	-91	148.6 to -33.3	Yes	***	<.001
1:PDMS NP (+) vs. 5:PDMS_BD P (+)	-106.7	-164.3 to -49.02	Yes	***	<.001	3:PDMS_BD P (+) vs. 14:PDMS_BD P (+)	-163.3	221.0 to -105.	Yes	***	<.001
1:PDMS NP (+) vs. 7:PDMS NP (-)	-20.67	-78.32 to 36.98	No	ns	0.997	5:PDMS NP (-) vs. 5:PDMS NP (+)	-15.33	72.98 to 42.3	No	ns	>.999
1:PDMS NP (+) vs. 7:PDMS NP (+)	-67	-124.6 to -9.350	Yes	**	0.01	5:PDMS NP (-) vs. 5:PDMS_BD P (-)	-81.33	139.0 to -23.6	Yes	***	<.001
1:PDMS NP (+) vs. 7:PDMS_BD P (-)	-103.7	-161.3 to -46.02	Yes	***	<.001	5:PDMS NP (-) vs. 5:PDMS_BD P (+)	-100.7	158.3 to -43.0	Yes	***	<.001
1:PDMS NP (+) vs. 7:PDMS_BD P (+)	-144	-201.6 to -86.35	Yes	***	<.001	5:PDMS NP (-) vs. 7:PDMS NP (-)	-14.67	72.32 to 42.9	No	ns	>.999
1:PDMS NP (+) vs. 14:PDMS NP (-)	-60	-117.6 to -2.350	Yes	*	0.034	5:PDMS NP (-) vs. 7:PDMS NP (+)	-61	118.6 to -3.35	Yes	*	0.028
1:PDMS NP (+) vs. 14:PDMS NP (+)	-94.67	-152.3 to -37.02	Yes	***	<.001	5:PDMS NP (-) vs. 7:PDMS_BD P (-)	-97.67	155.3 to -40.0	Yes	***	<.001
1:PDMS NP (+) vs. 14:PDMS_BD P (-)	-144.7	-202.3 to -87.02	Yes	***	<.001	5:PDMS NP (-) vs. 7:PDMS_BD P (+)	-138	195.6 to -80.3	Yes	***	<.001
1:PDMS NP (+) vs. 14:PDMS_BD P (+)	-217	-274.6 to -159.4	Yes	***	<.001	5:PDMS NP (-) vs. 14:PDMS NP (-)	-54	111.6 to 3.65	No	ns	0.089
1:PDMS_BD P (-) vs. 1:PDMS_BD P (+)	-9.667	-67.32 to 47.98	No	ns	>.999	5:PDMS NP (-) vs. 14:PDMS NP (+)	-88.67	146.3 to -31.0	Yes	***	<.001
1:PDMS_BD P (-) vs. 3:PDMS NP (-)	23	-34.65 to 80.65	No	ns	0.991	5:PDMS NP (-) vs. 14:PDMS_BD P (-)	-138.7	196.3 to -81.0	Yes	***	<.001
1:PDMS_BD P (-) vs. 3:PDMS NP (+)	14	-43.65 to 71.65	No	ns	>.999	5:PDMS NP (-) vs. 14:PDMS_BD P (+)	-211	268.6 to -153.	Yes	***	<.001
1:PDMS_BD P (-) vs. 3:PDMS_BD P (-)	-2.667	-60.32 to 54.98	No	ns	>.999	5:PDMS NP (+) vs. 5:PDMS_BD P (-)	-66	123.6 to -8.35	Yes	*	0.012
1:PDMS_BD P (-) vs. 3:PDMS_BD P (+)	-34	-91.65 to 23.65	No	ns	0.764	5:PDMS NP (+) vs. 5:PDMS_BD P (+)	-85.33	143.0 to -27.6	Yes	***	<.001
1:PDMS_BD P (-) vs. 5:PDMS NP (-)	13.67	-43.98 to 71.32	No	ns	>.999	5:PDMS NP (+) vs. 7:PDMS NP (-)	0.6667	56.98 to 58.3	No	ns	>.999
1:PDMS_BD P (-) vs. 5:PDMS NP (+)	-1.667	-59.32 to 55.98	No	ns	>.999	5:PDMS NP (+) vs. 7:PDMS NP (+)	-45.67	103.3 to 11.9	No	ns	0.279
1:PDMS_BD P (-) vs. 5:PDMS_BD P (-)	-67.67	-125.3 to -10.02	Yes	**	0.009	5:PDMS NP (+) vs. 7:PDMS_BD P (-)	-82.33	140.0 to -24.6	Yes	***	<.001
1:PDMS_BD P (-) vs. 5:PDMS_BD P (+)	-87	-144.6 to -29.35	Yes	***	<.001	5:PDMS NP (+) vs. 7:PDMS_BD P (+)	-122.7	180.3 to -65.0	Yes	***	<.001
1:PDMS_BD P (-) vs. 7:PDMS NP (-)	-1	-58.65 to 56.65	No	ns	>.999	5:PDMS NP (+) vs. 14:PDMS NP (-)	-38.67	96.32 to 18.9	No	ns	0.562
1:PDMS_BD P (-) vs. 7:PDMS NP (+)	-47.33	-105.0 to 10.32	No	ns	0.228	5:PDMS NP (+) vs. 14:PDMS NP (+)	-73.33	131.0 to -15.6	Yes	**	0.003
1:PDMS_BD P (-) vs. 7:PDMS_BD P (-)	-84	-141.6 to -26.35	Yes	***	<.001	5:PDMS NP (+) vs. 14:PDMS_BD P (-)	-123.3	181.0 to -65.6	Yes	***	<.001
1:PDMS_BD P (-) vs. 7:PDMS_BD P (+)	-124.3	-182.0 to -66.68	Yes	***	<.001	5:PDMS NP (+) vs. 14:PDMS_BD P (+)	-195.7	253.3 to -138.	Yes	***	<.001
1:PDMS_BD P (-) vs. 14:PDMS NP (-)	-40.33	-97.98 to 17.32	No	ns	0.488	5:PDMS_BD P (-) vs. 5:PDMS_BD P (+)	-19.33	76.98 to 38.3	No	ns	0.999
1:PDMS_BD P (-) vs. 14:PDMS NP (+)	-75	-132.6 to -17.35	Yes	**	0.002	5:PDMS_BD P (-) vs. 7:PDMS NP (-)	66.67	9.017 to 124.3	Yes	*	0.01
1:PDMS_BD P (-) vs. 14:PDMS_BD P (-	-125	-182.6 to -67.35	Yes	***	<.001	5:PDMS_BD P (-) vs. 7:PDMS NP (+)	20.33	37.32 to 77.9	No	ns	0.998
1:PDMS_BD P (+) vs. 14:PDMS_BD P (+	-197.3	-255.0 to -139.7	Yes	***	<.001	5:PDMS_BD P (-) vs. 7:PDMS_BD P (-)	-16.33	73.98 to 41.3	No	ns	>.999
1:PDMS_BD P (+) vs. 3:PDMS NP (-)	32.67	-24.98 to 90.32	No	ns	0.814	5:PDMS_BD P (-) vs. 7:PDMS_BD P (+)	-56.67	14.3 to 0.983	No	ns	0.059
1:PDMS_BD P (+) vs. 3:PDMS NP (+)	23.67	-33.98 to 81.32	No	ns	0.987	5:PDMS_BD P (-) vs. 14:PDMS NP (-)	27.33	30.32 to 84.9	No	ns	0.95
1:PDMS_BD P (+) vs. 3:PDMS_BD P (-	7	-50.65 to 64.65	No	ns	>.999	5:PDMS_BD P (-) vs. 14:PDMS NP (+)	-7.333	64.98 to 50.3	No	ns	>.999
1:PDMS_BD P (+) vs. 3:PDMS_BD P (+	-24.33	-81.98 to 33.32	No	ns	0.983	5:PDMS_BD P (-) vs. 14:PDMS_BD P (-)	-57.33	15.0 to 0.316	No	ns	0.053
1:PDMS_BD P (+) vs. 5:PDMS NP (-)	23.33	-34.32 to 80.98	No	ns	0.989	5:PDMS_BD P (-) vs. 14:PDMS_BD P (+)	-129.7	187.3 to -72.0	Yes	***	<.001
1:PDMS_BD P (+) vs. 5:PDMS NP (+)	8	-49.65 to 65.65	No	ns	>.999	5:PDMS_BD P (+) vs. 7:PDMS NP (-)	86	28.35 to 143.6	Yes	***	<.001
1:PDMS_BD P (+) vs. 5:PDMS_BD P (-	-58	-115.6 to -0.3504	Yes	*	0.047	5:PDMS_BD P (+) vs. 7:PDMS NP (+)	39.67	17.98 to 97.3	No	ns	0.518
1:PDMS_BD P (+) vs. 5:PDMS_BD P (+	-77.33	-135.0 to -19.68	Yes	**	0.001	5:PDMS_BD P (+) vs. 7:PDMS_BD P (-)	3	54.65 to 60.6	No	ns	>.999
1:PDMS_BD P (+) vs. 7:PDMS NP (-)	8.667	-48.98 to 66.32	No	ns	>.999	5:PDMS_BD P (+) vs. 7:PDMS_BD P (+)	-37.33	94.98 to 20.3	No	ns	0.622
1:PDMS_BD P (+) vs. 7:PDMS NP (+)	-37.67	-95.32 to 19.98	No	ns	0.607	5:PDMS_BD P (+) vs. 14:PDMS NP (-)	46.67	10.98 to 104.	No	ns	0.248
1:PDMS_BD P (+) vs. 7:PDMS_BD P (-	-74.33	-132.0 to -16.68	Yes	**	0.002	5:PDMS_BD P (+) vs. 14:PDMS NP (+)	12	45.65 to 69.6	No	ns	>.999
1:PDMS_BD P (+) vs. 7:PDMS_BD P (+	-114.7	-172.3 to -57.02	Yes	***	<.001	5:PDMS_BD P (+) vs. 14:PDMS_BD P (-)	-38	95.65 to 19.6	No	ns	0.592
1:PDMS_BD P (+) vs. 14:PDMS NP (-)	-30.67	-88.32 to 26.98	No	ns	0.877	5:PDMS_BD P (+) vs. 14:PDMS_BD P (+)	-110.3	168.0 to -52.6	Yes	***	<.001
1:PDMS_BD P (+) vs. 14:PDMS NP (+)	-65.33	-123.0 to -7.684	Yes	*	0.013	7:PDMS NP (-) vs. 7:PDMS NP (+)	-46.33	104.0 to 11.3	No	ns	0.258
1:PDMS_BD P (+) vs. 14:PDMS_BD P (	-115.3	-173.0 to -57.68	Yes	***	<.001	7:PDMS NP (-) vs. 7:PDMS_BD P (-)	-83	140.6 to -25.3	Yes	***	<.001
:PDMS_BD P (+) vs. 14:PDMS_BD P (+	-187.7	-245.3 to -130.0	Yes	***	<.001	7:PDMS NP (-) vs. 7:PDMS_BD P (+)	-123.3	181.0 to -65.6	Yes	***	<.001
3:PDMS NP (-) vs. 3:PDMS NP (+)	-9	-66.65 to 48.65	No	ns	>.999	7:PDMS NP (-) vs. 14:PDMS NP (-)	-39.33	96.98 to 18.3	No	ns	0.533
3:PDMS NP (-) vs. 3:PDMS_BD P (-)	-25.67	-83.32 to 31.98	No	ns	0.972	7:PDMS NP (-) vs. 14:PDMS NP (+)	-74	131.6 to -16.3	Yes	**	0.003
3:PDMS NP (-) vs. 3:PDMS_BD P (+)	-57	-114.6 to 0.6496	No	ns	0.056	7:PDMS NP (-) vs. 14:PDMS_BD P (-)	-124	181.6 to -66.3	Yes	***	<.001
3:PDMS NP (-) vs. 5:PDMS NP (-)	-9.333	-66.98 to 48.32	No	ns	>.999	7:PDMS NP (-) vs. 14:PDMS_BD P (+)	-196.3	254.0 to -138.	Yes	***	<.001
3:PDMS NP (-) vs. 5:PDMS NP (+)	-24.67	-82.32 to 32.98	No	ns	0.981	7:PDMS NP (+) vs. 7:PDMS_BD P (-)	-36.67	94.32 to 20.9	No	ns	0.652
3:PDMS NP (-) vs. 5:PDMS_BD P (-)	-90.67	-148.3 to -33.02	Yes	***	<.001	7:PDMS NP (+) vs. 7:PDMS_BD P (+)	-77	134.6 to -19.3	Yes	**	0.001
3:PDMS NP (-) vs. 5:PDMS_BD P (+)	-110	-167.6 to -52.35	Yes	***	<.001	7:PDMS NP (+) vs. 14:PDMS NP (-)	7	50.65 to 64.6	No	ns	>.999
3:PDMS NP (-) vs. 7:PDMS NP (-)	-24	-81.65 to 33.65	No	ns	0.985	7:PDMS NP (+) vs. 14:PDMS NP (+)	-27.67	85.32 to 29.9	No	ns	0.945
3:PDMS NP (-) vs. 7:PDMS NP (+)	-70.33	-128.0 to -12.68	Yes	**	0.005	7:PDMS NP (+) vs. 14:PDMS_BD P (-)	-77.67	135.3 to -20.0	Yes	**	0.001
3:PDMS NP (-) vs. 7:PDMS_BD P (-)	-107	-164.6 to -49.35	Yes	***	<.001	7:PDMS NP (+) vs. 14:PDMS_BD P (+)	-150	207.6 to -92.3	Yes	***	<.001
3:PDMS NP (-) vs. 7:PDMS_BD P (+)	-147.3	-205.0 to -89.68	Yes	***	<.001	7:PDMS_BD P (-) vs. 7:PDMS_BD P (+)	-40.33	97.98 to 17.3	No	ns	0.488
3:PDMS NP (-) vs. 14:PDMS NP (-)	-63.33	-121.0 to -5.684	Yes	*	0.019	7:PDMS_BD P (-) vs. 14:PDMS NP (-)	43.67	13.98 to 101.	No	ns	0.351
3:PDMS NP (-) vs. 14:PDMS NP (+)	-98	-155.6 to -40.35	Yes	***	<.001	7:PDMS_BD P (-) vs. 14:PDMS NP (+)	9	48.65 to 66.6	No	ns	>.999
3:PDMS NP (-) vs. 14:PDMS_BD P (-)	-148	-205.6 to -90.35	Yes	***	<.001	7:PDMS_BD P (-) vs. 14:PDMS_BD P (-)	-41	98.65 to 16.6	No	ns	0.459
3:PDMS NP (-) vs. 14:PDMS_BD P (+)	-220.3	-278.0 to -162.7	Yes	***	<.001	7:PDMS_BD P (-) vs. 14:PDMS_BD P (+)	-113.3	171.0 to -55.6	Yes	***	<.001
3:PDMS NP (+) vs. 3:PDMS_BD P (-)	-16.67	-74.32 to 40.98	No	ns	>.999	7:PDMS_BD P (+) vs. 14:PDMS NP (-)	84	26.35 to 141.6	Yes	***	<.001
3:PDMS NP (+) vs. 3:PDMS_BD P (+)	-48	-105.6 to 9.650	No	ns	0.209	7:PDMS_BD P (+) vs. 14:PDMS NP (+)	49.33	8.316 to 107.	No	ns	0.175
3:PDMS NP (+) vs. 5:PDMS NP (-)	-0.3333	-57.98 to 57.32	No	ns	>.999	7:PDMS_BD P (+) vs. 14:PDMS_BD P (-)	-0.6667	58.32 to 56.9	No	ns	>.999
3:PDMS NP (+) vs. 5:PDMS NP (+)	-15.67	-73.32 to 41.98	No	ns	>.999	7:PDMS_BD P (+) vs. 14:PDMS_BD P (+)	-73	130.6 to -15.3	Yes	**	0.003
3:PDMS NP (+) vs. 5:PDMS_BD P (-)	-81.67	-139.3 to -24.02	Yes	***	<.001	14:PDMS NP (-) vs. 14:PDMS NP (+)	-34.67	92.32 to 22.9	No	ns	0.737
3:PDMS NP (+) vs. 5:PDMS_BD P (+)	-101	-158.6 to -43.35	Yes	***	<.001	14:PDMS NP (-) vs. 14:PDMS_BD P (-)	-84.67	142.3 to -27.0	Yes	***	<.001
3:PDMS NP (+) vs. 7:PDMS NP (-)	-15	-72.65 to 42.65	No	ns	>.999	14:PDMS NP (-) vs. 14:PDMS_BD P (+)	-157	214.6 to -99.3	Yes	***	<.001
3:PDMS NP (+) vs. 7:PDMS NP (+)	-61.33	-119.0 to -3.684	Yes	*	0.027	14:PDMS NP (+) vs. 14:PDMS_BD P (-)	-50	107.6 to 7.65	No	ns	0.16
3:PDMS NP (+) vs. 7:PDMS_BD P (-)	-98	-155.6 to -40.35	Yes	***	<.001	14:PDMS NP (+) vs. 14:PDMS_BD P (+)	-122.3	180.0 to -64.6	Yes	***	<.001
3:PDMS NP (+) vs. 7:PDMS_BD P (+)	-138.3	-196.0 to -80.68	Yes	***	<.001	14:PDMS_BD P (-) vs. 14:PDMS_BD P (+)	-72.33	130.0 to -14.6	Yes	**	0.004

VIII. Statistical analysis of Glycosaminoglycans (GAG) (Day 7)

Table S26. Overall analysis of Glycosaminoglycans (GAG) Data (O.D. 605 nm) Day 7

	Day 7				Day 14				Day 21				
Cells Numb	PDMS BD (+)	PDMS BDR (-)	PDMS NP (+)	PDMS NP (-)	PDMS BD (+)	PDMS BDR (-)	PDMS NP (+)	PDMS NP (-)	PDMS BD (+)	PDMS BDR (-)	PDMS NP (+)	PDMS NP (-)	
1	0.251	0.191	0.175	0.0919	1.155	0.886	0.51	0.435	1.395	1.241	0.602	0.567	
2	0.299	0.28	0.179	0.118	0.805	0.901	0.368	0.455	1.2985	1.353	0.745	0.602	O.D. 605 nm
3	0.231	0.185	0.18	0.145	0.997	0.745	0.435	0.301	1.294	1.155	0.688	0.655	
4	0.242	0.171	0.18	0.133	1.092	1.072	0.499	0.377	1.695	1.098	0.701	0.595	
Mean	0.25575	0.20675	0.1785	0.121975	1.01225	0.901	0.453	0.392	1.420625	1.21175	0.684	0.60475	
StDev	0.02997082	0.049547116	0.002380476	0.022891101	0.15266821	0.133917885	0.065609959	0.069098963	0.18875618	0.11100563	0.05986095	0.036754818	

Table S27. Test of Normality Glycosaminoglycans (GAG) Data Day 7

	Day 7			
Shapiro-Wilk test	PDMS BD (+)	PDMS BDR (-)	PDMS NP (+)	PDMS NP (-)
W	0.8632	0.7824	0.7635	0.967
P value	0.272	0.074	0.051	0.823
Passed normality test (alpha=0.05)?	Yes	Yes	Yes	Yes
P value summary	ns	ns	ns	ns

The significance value for every treatment (p-value > 0,05) is more than the α (0.05), meaning that the data is normally distributed.

Table S28. Test of Homogeneity Glycosaminoglycans (GAG) Data Day 7

Bartlett's test	
Bartlett's statistic (corrected)	12.93
P value	0.005
P value summary	**
Are SDs significantly different (P < 0.05)?	Yes

The significance value (p-value) is 0.005, which is smaller than the α (0.05), meaning that the data group comes from a population that has a different variance (not homogeneous).

Table S29. Test of Significancy Glycosaminoglycans (GAG) Data Day 7

ANOVA summary					
F	12.86				
P value	<.001				
P value summary	***				
Significant diff. among means (P < 0.05)?	Yes				
R squared	0.7627				
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	0.03744	3	0.01248	F (3, 12) = 12.86	P<.001
Residual (within columns)	0.01165	12	0.0009707		
Total	0.04909	15			

The significance value *(p-value)* is <0.001, which is smaller than the α (0.05), meaning that there is an effect of BD-R PDMS *nanopattern* whether coated with spidroin extract (+) or not (-) is able to enhance GAG content.

Table S30. Pos Hoc Test on Glycosaminoglycans (GAG) Data Day 7

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
PDMS NP (-) vs. PDMS_BD P (-)	-60.67	-155.4 to 34.04	No	ns	0.2948
PDMS NP (-) vs. PDMS_BD P (+)	**-95.27**	**-190.0 to -0.5639**	**Yes**	*	**0.0484**
PDMS NP (+) vs. PDMS_BD P (-)	-37.8	-132.5 to 56.90	No	ns	0.67
PDMS NP (+) vs. PDMS_BD P (+)	-72.4	-167.1 to 22.30	No	ns	0.169
PDMS_BD P (-) vs. PDMS_BD P (+)	-34.6	-129.3 to 60.10	No	ns	0.7261

On day 7, there is the significance value (p-value) is 0.0484, which is smaller than the α (0.05), meaning that there is an effect of BD-R (+) PDMS to induce GAG content compared to NP (+/-) PDMS.

IX. Statistical analysis of Glycosaminoglycans (GAG) (Day 14)

Table S31. Test of Normality Glycosaminoglycans (GAG) Data Day 14

	Day 14			
Shapiro-Wilk test	**PDMS BD (+)**	**PDMS BDR (-)**	**PDMS NP (+)**	**PDMS NP (-)**
W	0.9398	0.9597	0.9072	0.9311
P value	0.653	0.777	0.468	0.601
Passed normality test (alpha=0.05)?	Yes	Yes	Yes	Yes
P value summary	ns	ns	ns	ns

The significance value for every treatment (p-value > 0,05) is more than the α (0.05), meaning that the data is normally distributed.

Table S32. Test of Homogeneity Glycosaminoglycans (GAG) Data Day 14

Bartlett's test	
Bartlett's statistic (corrected)	2.819
P value	0.42
P value summary	ns
Are SDs significantly different (P < 0.05)?	No

The significance value (p-value) is 0.42, which is bigger than the α (0.05), meaning that the data group comes from a population that has the same variance (homogeneous).

Table S33. Test of Significancy Glycosaminoglycans (GAG) Data Day 14

ANOVA summary					
F	31.09				
P value	<.001				
P value summary	***				
Significant diff. among means (P < 0.05)?	Yes				
R squared	0.886				
ANOVA table	**SS**	**DF**	**MS**	**F (DFn, DFd)**	**P value**
Treatment (between columns)	1.173	3	0.3911	F (3, 12) = 31.09	P<.001
Residual (within columns)	0.151	12	0.01258		
Total	1.324	15			

The significance value *(p-value)* is <0.001, which is smaller than the α (0.05), meaning that there is an effect of BD-R PDMS *nanopattern* whether coated with spidroin extract (+) or not (-) is able to enhance GAG content.

Table S34. Pos Hoc Test on Glycosaminoglycans (GAG) Data Day 14

Tukey's multiple comparisons test	**Mean Diff.**	**95.00% CI of diff.**	**Below threshold?**	**Summary**	**Adjusted P Value**
PDMS BD (+) vs. PDMS BDR (-)	0.1113	-0.1242 to 0.3467	No	ns	0.521
PDMS BD (+) vs. PDMS NP (+)	**0.5593**	**0.3238 to 0.7947**	**Yes**	***	**<.001**
PDMS BD (+) vs. PDMS NP (-)	**0.6203**	**0.3848 to 0.8557**	**Yes**	***	**<.001**
PDMS BDR (-) vs. PDMS NP (+)	**0.448**	**0.2125 to 0.6835**	**Yes**	***	**<.001**
PDMS BDR (-) vs. PDMS NP (-)	**0.509**	**0.2735 to 0.7445**	**Yes**	***	**<.001**
PDMS NP (+) vs. PDMS NP (-)	0.061	-0.1745 to 0.2965	No	ns	0.867

On day 14, there is the significance value (p-value) is <0.001, which is smaller than the α (0.05), meaning that there is an effect of BD-R (+/-) PDMS to induce GAG content compared to NP (+/-) PDMS.

X. Statistical analysis of Glycosaminoglycans (GAG) (Day 21)

Table S35. Test of Normality Glycosaminoglycans (GAG) Data Day 21

	Day 21			
Shapiro-Wilk test	**PDMS BD (+)**	**PDMS BDR (-)**	**PDMS NP (+)**	**PDMS NP (-)**
W	0.7958	0.9858	0.9385	0.9376
P value	0.095	0.935	0.645	0.64
Passed normality test (alpha=0.05)?	Yes	Yes	Yes	Yes
P value summary	ns	ns	ns	ns

The significance value for every treatment (p-value > 0,05) is more than the α (0.05), meaning that the data is normally distributed.

Table S36. Test of Homogeneity Glycosaminoglycans (GAG) Data Day 21

Bartlett's test	
Bartlett's statistic (corrected)	7.71
P value	0.052
P value summary	ns
Are SDs significantly different (P < 0.05)?	No

The significance value (p-value) is 0.052, which is bigger than the α (0.05), meaning that the data group comes from a population that has the same variance (homogeneous).

Table S37. Test of Significancy Glycosaminoglycans (GAG) Data Day 21

ANOVA summary					
F	34.45				
P value	<.001				
P value summary	***				
Significant diff. among means (P < 0.05)?	Yes				
R squared	0.896				
ANOVA table	**SS**	**DF**	**MS**	**F (DFn, DFd)**	**P value**
Treatment (between columns)	1.82	3	0.6067	F (3, 12) = 34.45	P<.001
Residual (within columns)	0.2114	12	0.01761		
Total	2.031	15			

The significance value *(p-value)* is <0.001, which is smaller than the α (0.05), meaning that there is an effect of BD-R PDMS *nanopattern* whether coated with spidroin extract (+) or not (-) is able to enhance GAG content.

Table S38. Pos Hoc Test on Glycosaminoglycans (GAG) Data Day 21

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
PDMS BD (+) vs. PDMS BD (-)	0.2589	-0.01974 to 0.5375	No	ns	0.072
PDMS BD (+) vs. PDMS NP (+)	**0.7366**	**0.4580 to 1.015**	**Yes**	***	**<.001**
PDMS BD (+) vs. PDMS NP (-)	**0.8159**	**0.5373 to 1.094**	**Yes**	***	**<.001**
PDMS BDR (-) vs. PDMS NP (+)	**0.4778**	**0.1991 to 0.7564**	**Yes**	**	**0.001**
PDMS BDR (-) vs. PDMS NP (-)	**0.557**	**0.2784 to 0.8356**	**Yes**	***	**<.001**
PDMS NP (+) vs. PDMS NP (-)	0.07925	-0.1994 to 0.3579	No	ns	0.832

On day 21, there is the significance value (p-value) is <0.001, which is smaller than the α (0.05), meaning that there is an effect of BD-R (+/-) PDMS to induce GAG content compared to NP (+/-) PDMS.

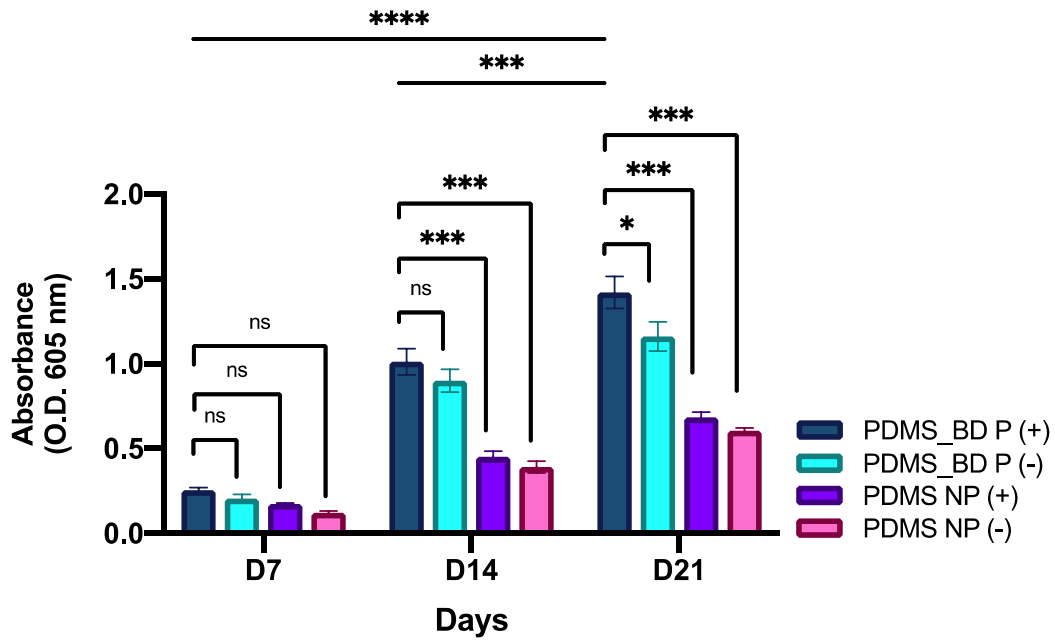


Figure 12. Quantification of matrix GAG abundance with Alcian Blue staining was measured over a period of 21 days at several points (days 7, 14, and 21). Data are mean ± SD with significance markers * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$), and **** ($p < 0.0001$), total $n = 4$.

Table S39. Pos Hoc Test on Glycosaminoglycans (GAG) Two-Way ANOVA Data Day 1- 21

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
D7:PDMS_BD P (+) vs. D7:PDMS_BD P (-)	0.049	-0.2026 to 0.3006	No	ns	>.999
D7:PDMS_BD P (+) vs. D7:PDMS NP (+)	0.081	-0.1706 to 0.3326	No	ns	0.991
D7:PDMS_BD P (+) vs. D7:PDMS NP (-)	0.1338	-0.1179 to 0.3854	No	ns	0.777
D7:PDMS_BD P (+) vs. D14:PDMS_BD P (+)	-0.7565	-1.008 to -0.5049	Yes	***	<.001
D7:PDMS_BD P (+) vs. D14:PDMS_BD P (-)	-0.6453	-0.8969 to -0.3936	Yes	***	<.001
D7:PDMS_BD P (+) vs. D14:PDMS NP (+)	-0.1973	-0.4489 to 0.05439	No	ns	0.25
D7:PDMS_BD P (+) vs. D14:PDMS NP (-)	-0.1363	-0.3879 to 0.1154	No	ns	0.757
D7:PDMS_BD P (+) vs. D21:PDMS_BD P (+)	-1.165	-1.417 to -0.9132	Yes	***	<.001
D7:PDMS_BD P (+) vs. D21:PDMS_BD P (-)	-0.906	-1.158 to -0.6544	Yes	***	<.001
D7:PDMS_BD P (+) vs. D21:PDMS NP (+)	-0.4283	-0.6799 to -0.1766	Yes	***	<.001
D7:PDMS_BD P (+) vs. D21:PDMS NP (-)	-0.349	-0.6006 to -0.09736	Yes	**	0.001
D7:PDMS_BD P (-) vs. D7:PDMS NP (+)	0.032	-0.2196 to 0.2836	No	ns	>.999
D7:PDMS_BD P (-) vs. D7:PDMS NP (-)	0.08478	-0.1669 to 0.3364	No	ns	0.988
D7:PDMS_BD P (-) vs. D14:PDMS_BD P (+)	-0.8055	-1.057 to -0.5539	Yes	***	<.001
D7:PDMS_BD P (-) vs. D14:PDMS_BD P (-)	-0.6943	-0.9459 to -0.4426	Yes	***	<.001
D7:PDMS_BD P (-) vs. D14:PDMS NP (+)	-0.2463	-0.4979 to 0.005387	No	ns	0.06
D7:PDMS_BD P (-) vs. D14:PDMS NP (-)	-0.1853	-0.4369 to 0.06639	No	ns	0.333
D7:PDMS_BD P (-) vs. D21:PDMS_BD P (+)	-1.214	-1.466 to -0.9622	Yes	***	<.001
D7:PDMS_BD P (-) vs. D21:PDMS_BD P (-)	-0.955	-1.207 to -0.7034	Yes	***	<.001
D7:PDMS_BD P (-) vs. D21:PDMS NP (+)	-0.4773	-0.7289 to -0.2256	Yes	***	<.001
D7:PDMS_BD P (-) vs. D21:PDMS NP (-)	-0.398	-0.6496 to -0.1464	Yes	***	<.001
D7:PDMS NP (+) vs. D7:PDMS NP (-)	0.05278	-0.1989 to 0.3044	No	ns	>.999
D7:PDMS NP (+) vs. D14:PDMS_BD P (+)	-0.8375	-1.089 to -0.5859	Yes	***	<.001
D7:PDMS NP (+) vs. D14:PDMS_BD P (-)	-0.7263	-0.9779 to -0.4746	Yes	***	<.001
D7:PDMS NP (+) vs. D14:PDMS NP (+)	-0.2783	-0.5299 to -0.02661	Yes	*	0.02
D7:PDMS NP (+) vs. D14:PDMS NP (-)	-0.2173	-0.4689 to 0.03439	No	ns	0.146
D7:PDMS NP (+) vs. D21:PDMS_BD P (+)	-1.246	-1.498 to -0.9942	Yes	***	<.001
D7:PDMS NP (+) vs. D21:PDMS_BD P (-)	-0.987	-1.239 to -0.7354	Yes	***	<.001
D7:PDMS NP (+) vs. D21:PDMS NP (+)	-0.5093	-0.7609 to -0.2576	Yes	***	<.001
D7:PDMS NP (+) vs. D21:PDMS NP (-)	-0.43	-0.6816 to -0.1784	Yes	***	<.001
D7:PDMS NP (-) vs. D14:PDMS_BD P (+)	-0.8903	-1.142 to -0.6386	Yes	***	<.001
D7:PDMS NP (-) vs. D14:PDMS_BD P (-)	-0.779	-1.031 to -0.5274	Yes	***	<.001
D7:PDMS NP (-) vs. D14:PDMS NP (+)	-0.331	-0.5827 to -0.07939	Yes	**	0.003
D7:PDMS NP (-) vs. D14:PDMS NP (-)	-0.27	-0.5217 to -0.01839	Yes	*	0.027
D7:PDMS NP (-) vs. D21:PDMS_BD P (+)	-1.299	-1.550 to -1.047	Yes	***	<.001
D7:PDMS NP (-) vs. D21:PDMS_BD P (-)	-1.04	-1.291 to -0.7881	Yes	***	<.001
D7:PDMS NP (-) vs. D21:PDMS NP (+)	-0.562	-0.8137 to -0.3104	Yes	***	<.001
D7:PDMS NP (-) vs. D21:PDMS NP (-)	-0.4828	-0.7344 to -0.2311	Yes	***	<.001

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
D14:PDMS_BD P (+) vs. D14:PDMS_BD P (-)	0.1113	-0.1404 to 0.3629	No	ns	0.918
D14:PDMS_BD P (+) vs. D14:PDMS NP (+)	0.5593	0.3076 to 0.8109	Yes	***	<.001
D14:PDMS_BD P (+) vs. D14:PDMS NP (-)	0.6203	0.3686 to 0.8719	Yes	***	<.001
D14:PDMS_BD P (+) vs. D21:PDMS_BD P (+)	-0.4084	-0.6600 to -0.1567	Yes	***	<.001
D14:PDMS_BD P (+) vs. D21:PDMS_BD P (-)	-0.1495	-0.4011 to 0.1021	No	ns	0.643
D14:PDMS_BD P (+) vs. D21:PDMS NP (+)	0.3283	0.07661 to 0.5799	Yes	**	0.003
D14:PDMS_BD P (+) vs. D21:PDMS NP (-)	0.4075	0.1559 to 0.6591	Yes	***	<.001
D14:PDMS_BD P (-) vs. D14:PDMS NP (+)	0.448	0.1964 to 0.6996	Yes	***	<.001
D14:PDMS_BD P (-) vs. D14:PDMS NP (-)	0.509	0.2574 to 0.7606	Yes	***	<.001
D14:PDMS_BD P (-) vs. D21:PDMS_BD P (+)	-0.5196	-0.7713 to -0.2680	Yes	***	<.001
D14:PDMS_BD P (-) vs. D21:PDMS_BD P (-)	-0.2608	0.5124 to -0.00911	Yes	*	0.037
D14:PDMS_BD P (-) vs. D21:PDMS NP (+)	0.217	-0.03464 to 0.4686	No	ns	0.147
D14:PDMS_BD P (-) vs. D21:PDMS NP (-)	0.2963	0.04461 to 0.5479	Yes	*	0.01
D14:PDMS NP (+) vs. D14:PDMS NP (-)	0.061	-0.1906 to 0.3126	No	ns	>.999
D14:PDMS NP (+) vs. D21:PDMS_BD P (+)	-0.9676	-1.219 to -0.7160	Yes	***	<.001
D14:PDMS NP (+) vs. D21:PDMS_BD P (-)	-0.7088	-0.9604 to -0.4571	Yes	***	<.001
D14:PDMS NP (+) vs. D21:PDMS NP (+)	-0.231	-0.4826 to 0.02064	No	ns	0.097
D14:PDMS NP (+) vs. D21:PDMS NP (-)	-0.1518	-0.4034 to 0.09989	No	ns	0.623
D14:PDMS NP (-) vs. D21:PDMS_BD P (+)	-1.029	-1.280 to -0.7770	Yes	***	<.001
D14:PDMS NP (-) vs. D21:PDMS_BD P (-)	-0.7698	-1.021 to -0.5181	Yes	***	<.001
D14:PDMS NP (-) vs. D21:PDMS NP (+)	-0.292	-0.5436 to -0.04036	Yes	*	0.012
D14:PDMS NP (-) vs. D21:PDMS NP (-)	-0.2128	-0.4644 to 0.03889	No	ns	0.166
D21:PDMS_BD P (+) vs. D21:PDMS_BD P (-)	0.2589	0.007238 to 0.5105	Yes	*	0.039
D21:PDMS_BD P (+) vs. D21:PDMS NP (+)	0.7366	0.4850 to 0.9883	Yes	***	<.001
D21:PDMS_BD P (+) vs. D21:PDMS NP (-)	0.8159	0.5642 to 1.068	Yes	***	<.001
D21:PDMS_BD P (-) vs. D21:PDMS NP (+)	0.4778	0.2261 to 0.7294	Yes	***	<.001
D21:PDMS_BD P (-) vs. D21:PDMS NP (-)	0.557	0.3054 to 0.8086	Yes	***	<.001
D21:PDMS NP (+) vs. D21:PDMS NP (-)	0.07925	-0.1724 to 0.3309	No	ns	0.993

XI. Statistical analysis of *Water Contact Angel*

Table S40. Data WCA analysis

	PDMS	PDMS + Plasma treatment	PDMS + Plasma treatment + Spidroin
WCA	82.45	49.08	14.54
	90.14	51.9	13.18
	84.67	48.98	14.09
	84.89	49.55	12.55
Mean	85.54	49.88	13.59
StDEV	3.260239	1.37104279	0.89483704

Table S41. Test of Normality WCA analysis

Tests of Normality

	Perlakuan	Kolmogorov-Smirnov[a] Statistic	df	Sig.	Shapiro-Wilk Statistic	df	Sig.
Sudut Kontak	PDMS (no treatment)	.329	4	.	.888	4	.374
	PDMS + Plasma treatment	.345	4	.	.770	4	.059
	PDMS + Plasma treatment + Spidroin	.249	4	.	.915	4	.511

The significance value for every treatment (p-value > 0,05) is more than the α (0.05), meaning that the data is normally distributed.

Table S42. Test of Homogeneity WCA analysis

Tests of Homogeneity of Variances

		Levene Statistic	df1	df2	Sig.
Sudut Kontak	Based on Mean	1.700	2	9	.236
	Based on Median	.624	2	9	.558
	Based on Median and with adjusted df	.624	2	4.361	.578
	Based on trimmed mean	1.503	2	9	.273

The significance value (p-value) is > 0.05, which is bigger than the α (0.05), meaning that the data group comes from a population that has the same variance (homogeneous).

Table S43. Test of Significancy WCA analysis

ANOVA

Sudut Kontak

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	10294.813	2	5147.407	1122.484	<.001
Within Groups	41.272	9	4.586		
Total	10336.085	11			

The significance value *(p-value)* is <0.001, which is smaller than the α (0.05), meaning that there is an effect of plasma treatment and PDMS coating can reduce value of water contact angle.

Table S44. Pos Hoc Test on WCA analysis

Post Hoc Tests

Multiple Comparisons

Dependent Variable: Sudut Kontak

Tukey HSD

(I) Perlakuan	(J) Perlakuan	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	Upper Bound
PDMS (no treatment)	PDMS + Plasma treatment	35.65750*	1.51422	<.001	31.4298	39.8852
	PDMS + Plasma treatment + Spidroin	71.74500*	1.51422	<.001	67.5173	75.9727
PDMS + Plasma treatment	PDMS (no treatment)	-35.65750*	1.51422	<.001	-39.8852	-31.4298
	PDMS + Plasma treatment + Spidroin	36.08750*	1.51422	<.001	31.8598	40.3152
PDMS + Plasma treatment + Spidroin	PDMS (no treatment)	-71.74500*	1.51422	<.001	-75.9727	-67.5173
	PDMS + Plasma treatment	-36.08750*	1.51422	<.001	-40.3152	-31.8598

*. The mean difference is significant at the 0.05 level.

The significance value *(p-value)* is <0.001, which is smaller than the α (0.05), meaning that there is an effect of plasma treatment and PDMS coating can reduce value of water contact angle.

Figure Supplementary Information

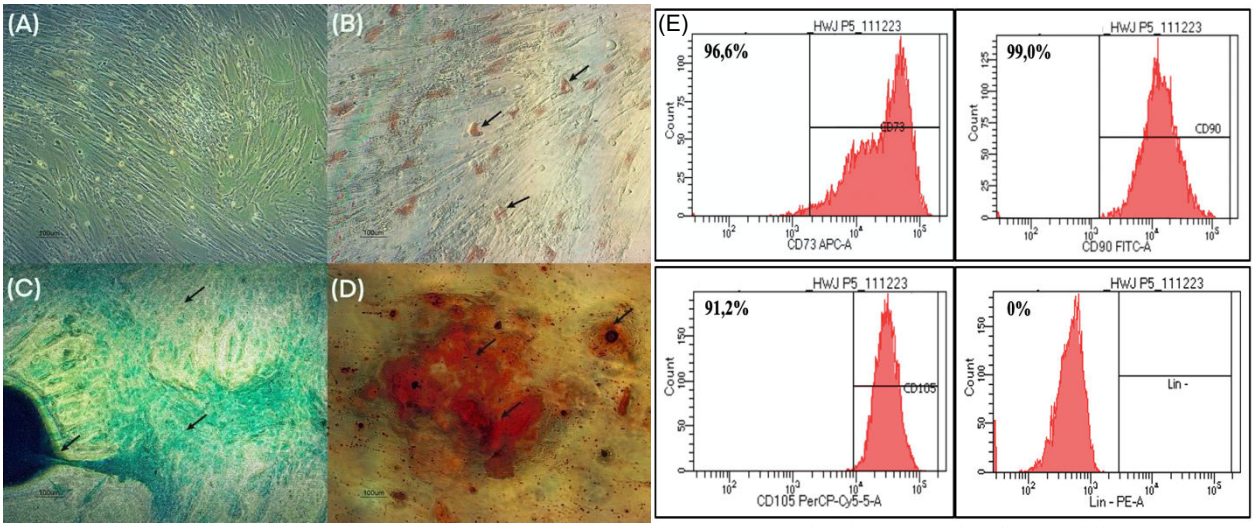
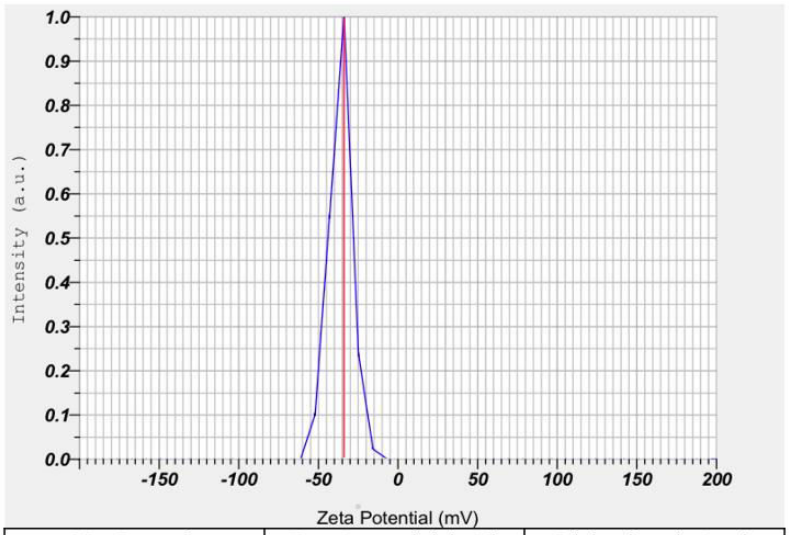


Figure S4. Culture characterization of hWJ-MSCs. (A) Control group (non-staining) (B) Oil Red O staining for adipocyte differentiation (C) Alcian Blue staining for chondrogenic differentiation and (D) Alizarin Red staining for osteocyte differentiation induced with specific differentiation medium. The black arrow indicates a positive result for the marker of each staining group. (E) CD73 APC-A positive marker (96.6%); CD90 FITC-A (99.0%) CD105 PerCP-Cy5-5-A (91.2%) and negative markers Lin–PE-A (CD34, CD45, CD11B, CD19 and HLA-DR PE (0%).



No. Puncak	Zeta Potensial (mV)	Viskositas (mPa.s)
1	-34,56	0.896

Figure S5. Characterization of the spidroin *Argiope modesta*. The viscosity and zeta potential values of spidroin show a range of values indicating good and relatively stable physicochemical properties.

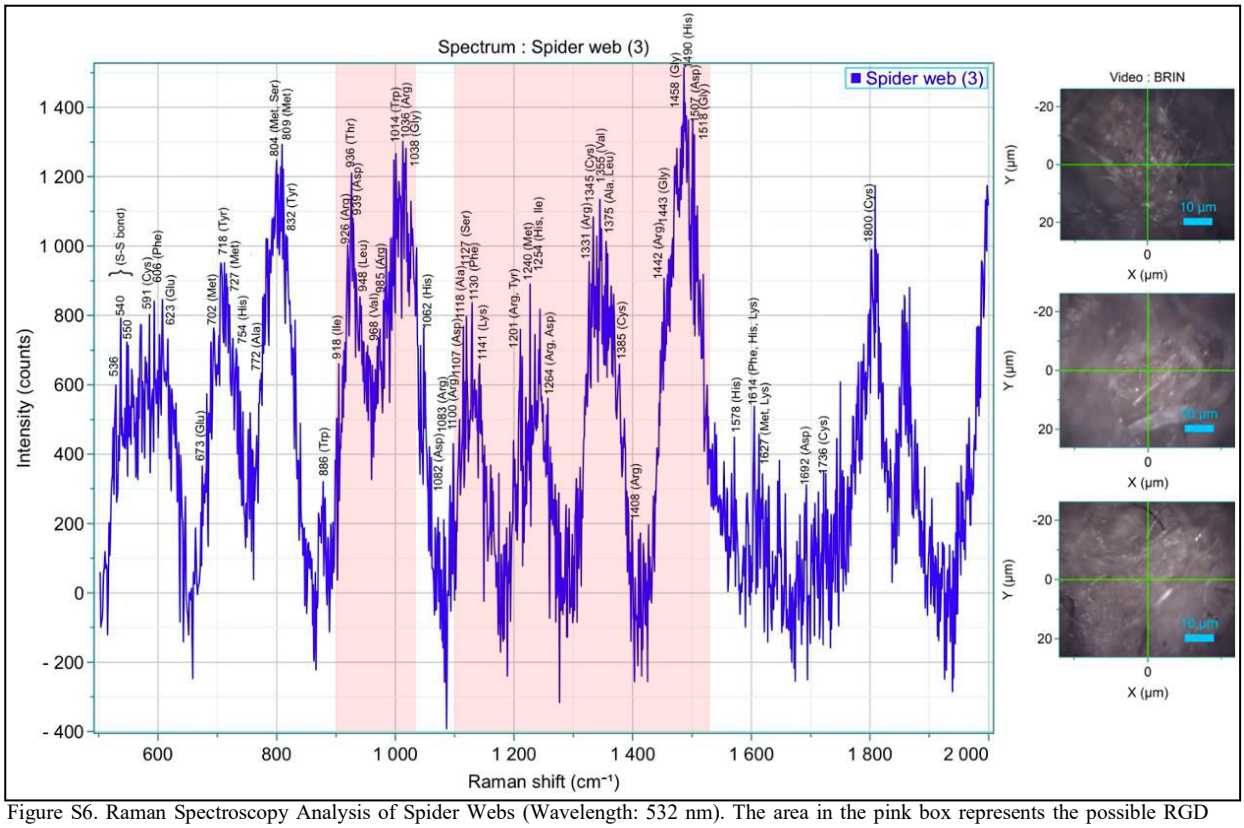


Figure S6. Raman Spectroscopy Analysis of Spider Webs (Wavelength: 532 nm). The area in the pink box represents the possible RGD sequences.

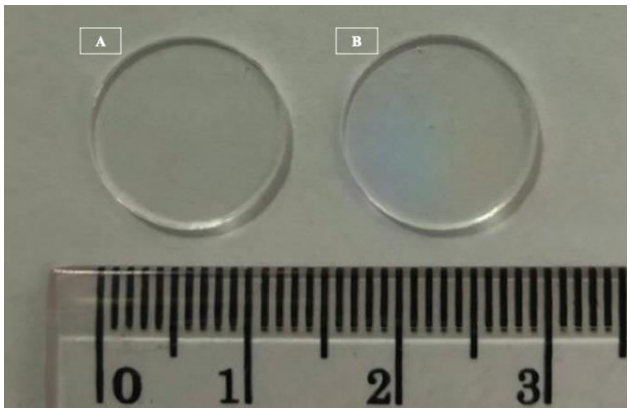


Figure S7. PDMS Nanopattern Morphology. (A) Grafting resulting from PDMS unpattern (flat) molding. (B) Grafting resulting from PDMS BD-R Nanopattern molding.

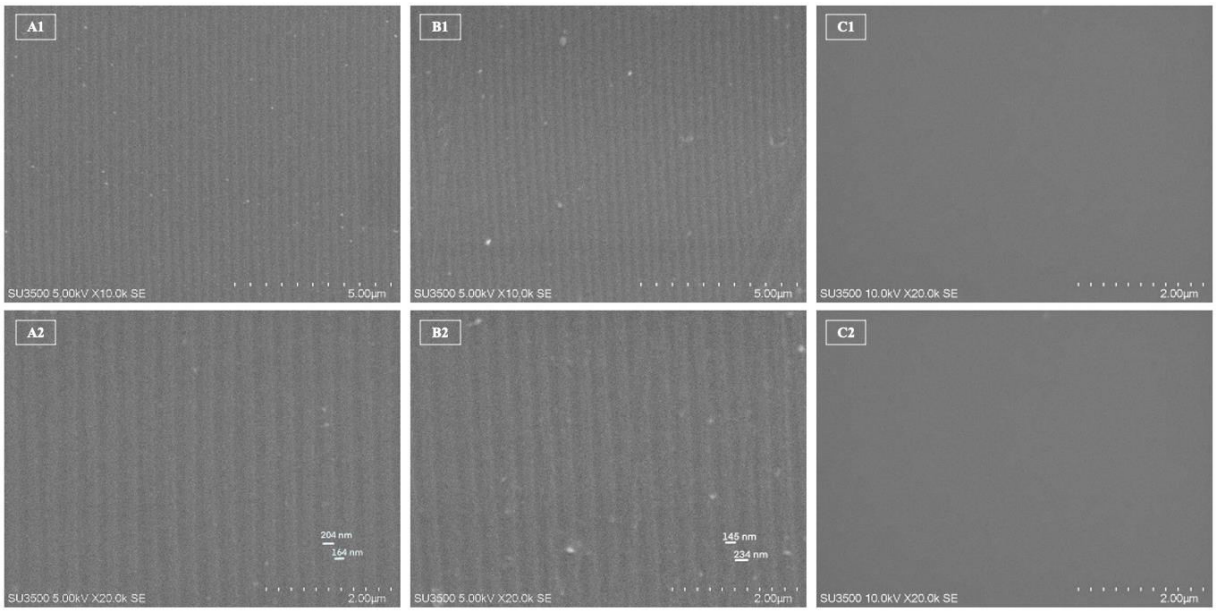


Figure S8. Nanopattern Substrate Surface Characteristics using SEM. Nano patterns on PC Bluray optical discs using Scanning Electron Microscopy (SEM) after pre-processing at 10,000x (A1) and 20,000x (A2) magnification. Nano patterns printed on PDMS fabricated from PC Bluray optical discs at 10,000x (B1) and 20,000x (B2) magnification. PDMS print without a pattern (control) at 20,000x magnification (C1 and C2). Inset scale = 2 µm and 5 µm.

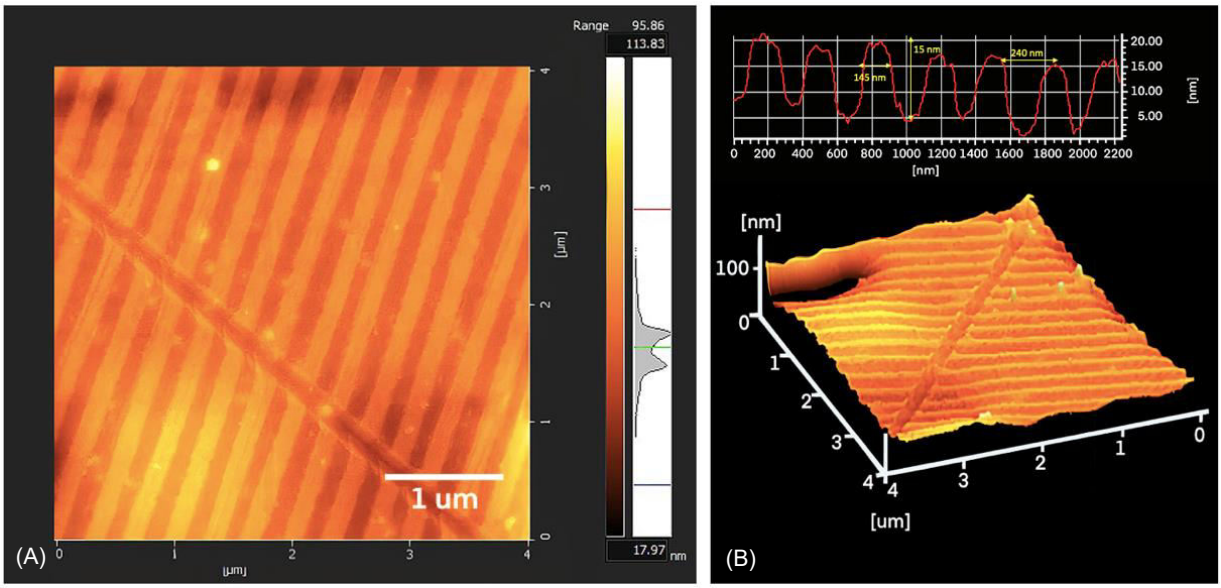


Figure S6. Characterization of BD-R PDMS Nanopatterns using Atomic Force Microscopy (AFM). (A) 2D imaging of PDMS BD-R using AFM (inset scale: 1 µm). (B) 3D imaging of PDMS BD-R using AFM.

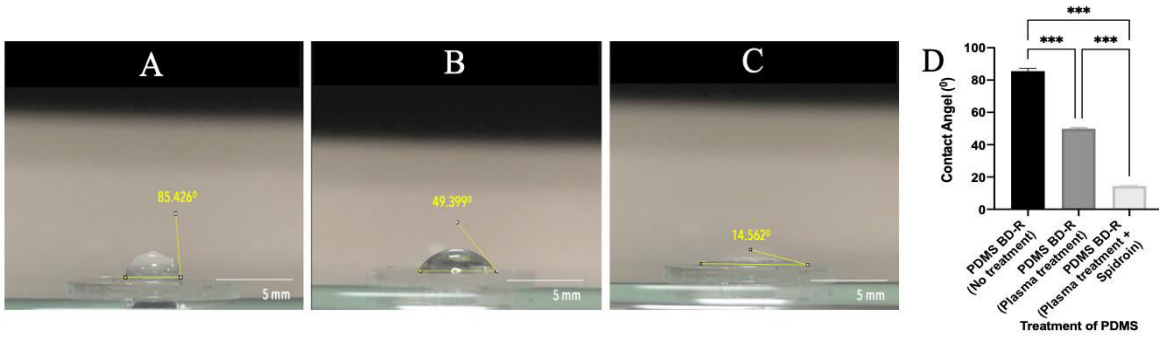


Figure S9. A contact angle was formed on PDMS material coated with spider web extract. (A) BD-R PDMS material before plasma treatment. (B) BD-R PDMS material after plasma treatment. (C) After plasma treatment, PDMS BD-R material was coated with spidroin (spider web extrct)

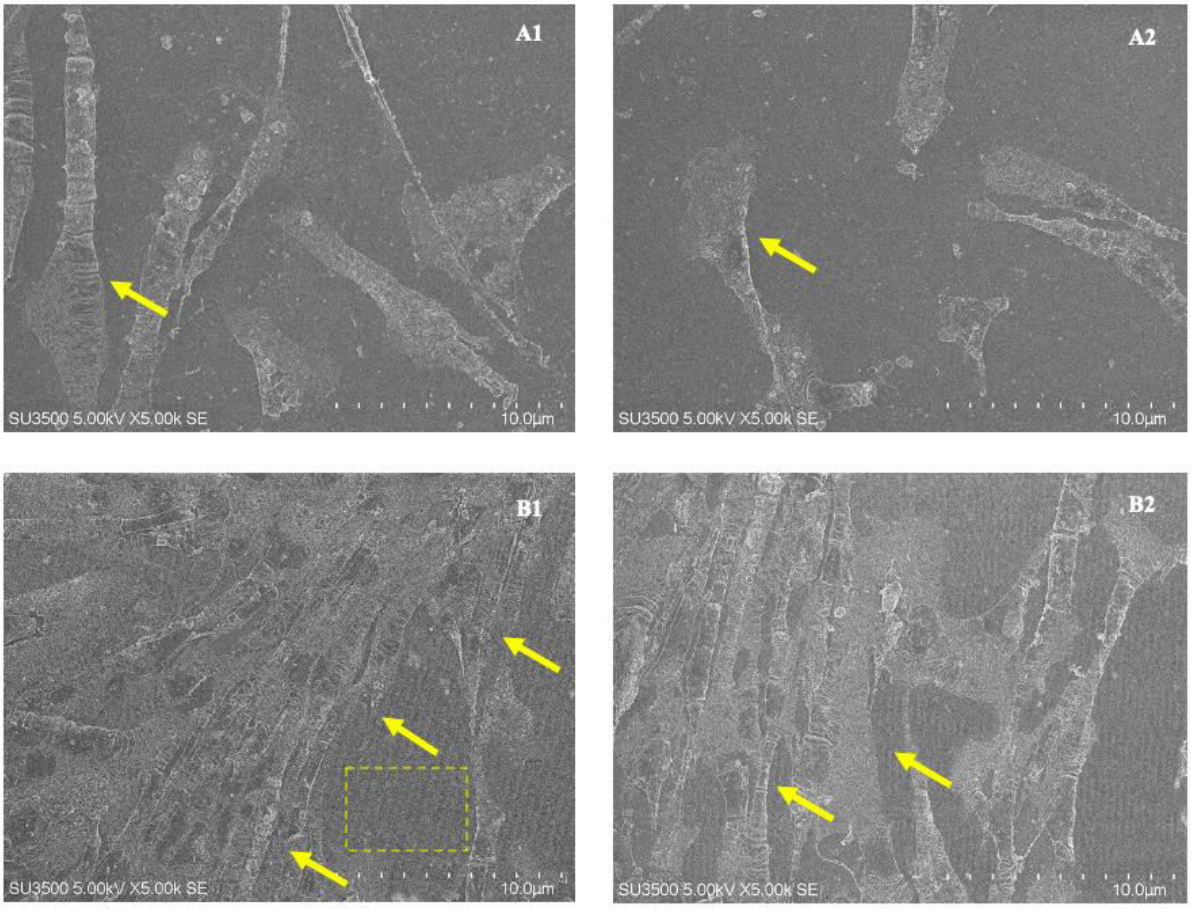


Figure S10. Morphology of hWJ-MSCs on PDMS-BDR and unpatterned PDMS using SEM. (A1-2) Morphology of hWJ-MSCs cells cultured on Unpattern PDMS coated with spidroin extract at 5000x magnification. (B1-2) Morphology of hWJ-MSCs cells cultured on BD-R PDMS Nanopatterns coated with spidroin extract at 5000x magnification.

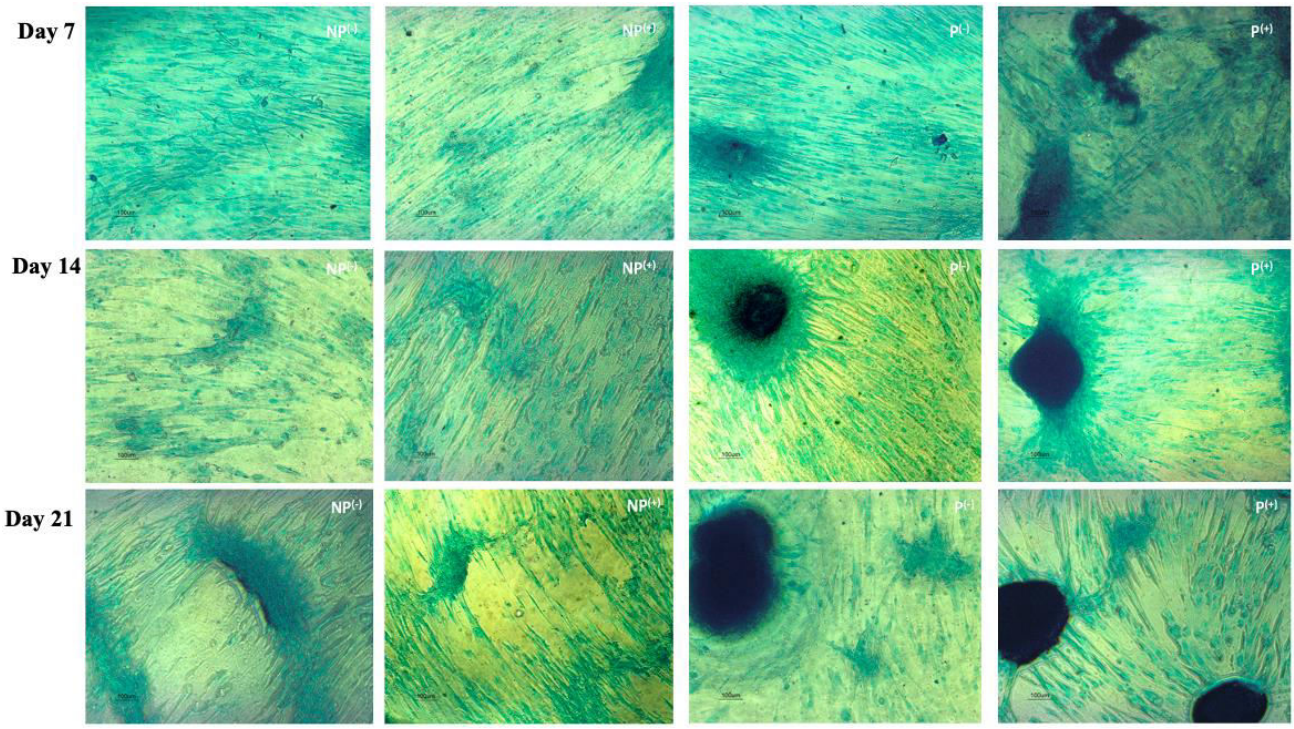


Figure S11. Morphology of hWJ-MSC cells with Alcian Blue staining cultured on PDMS elastomer substrate coated with spidroin extract (+) and without spidroin extract (-) on days 7, 14, and 21. NP = Unpattern PDMS. P = PDMS BD-R Nanopattern. Inset scale = 100 µm, magnification 20x.

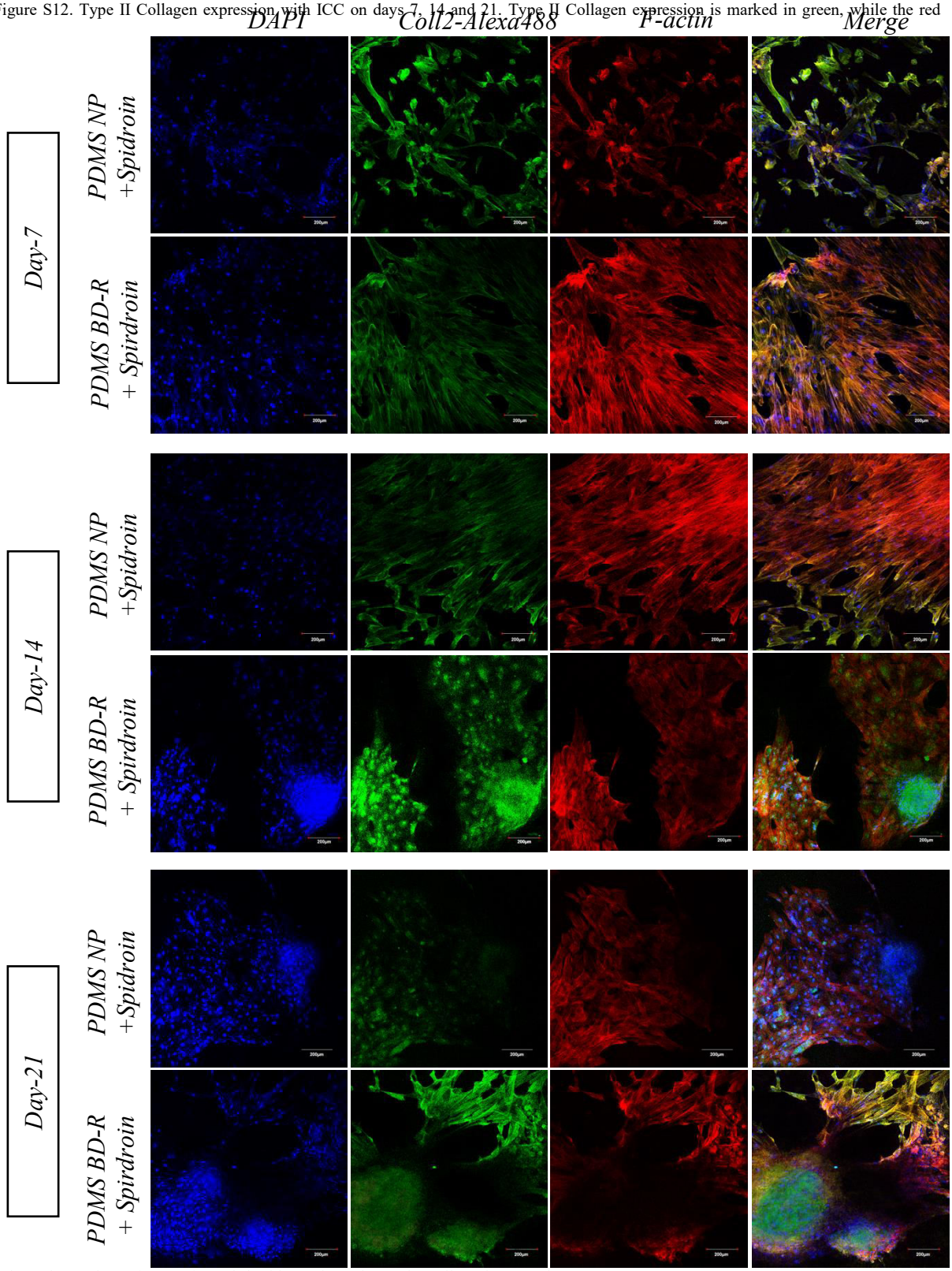


Figure S12. Type II Collagen expression with ICC on days 7, 14 and 21. Type II Collagen expression is marked in green, while the red colour shows the actin protein and the blue colour shows the cell nucleus. Scale: 200 µm. PDMS BD-R; PDMS nanopattern substrate. PDMS NP; PDMS substrate without a pattern (un-pattern).

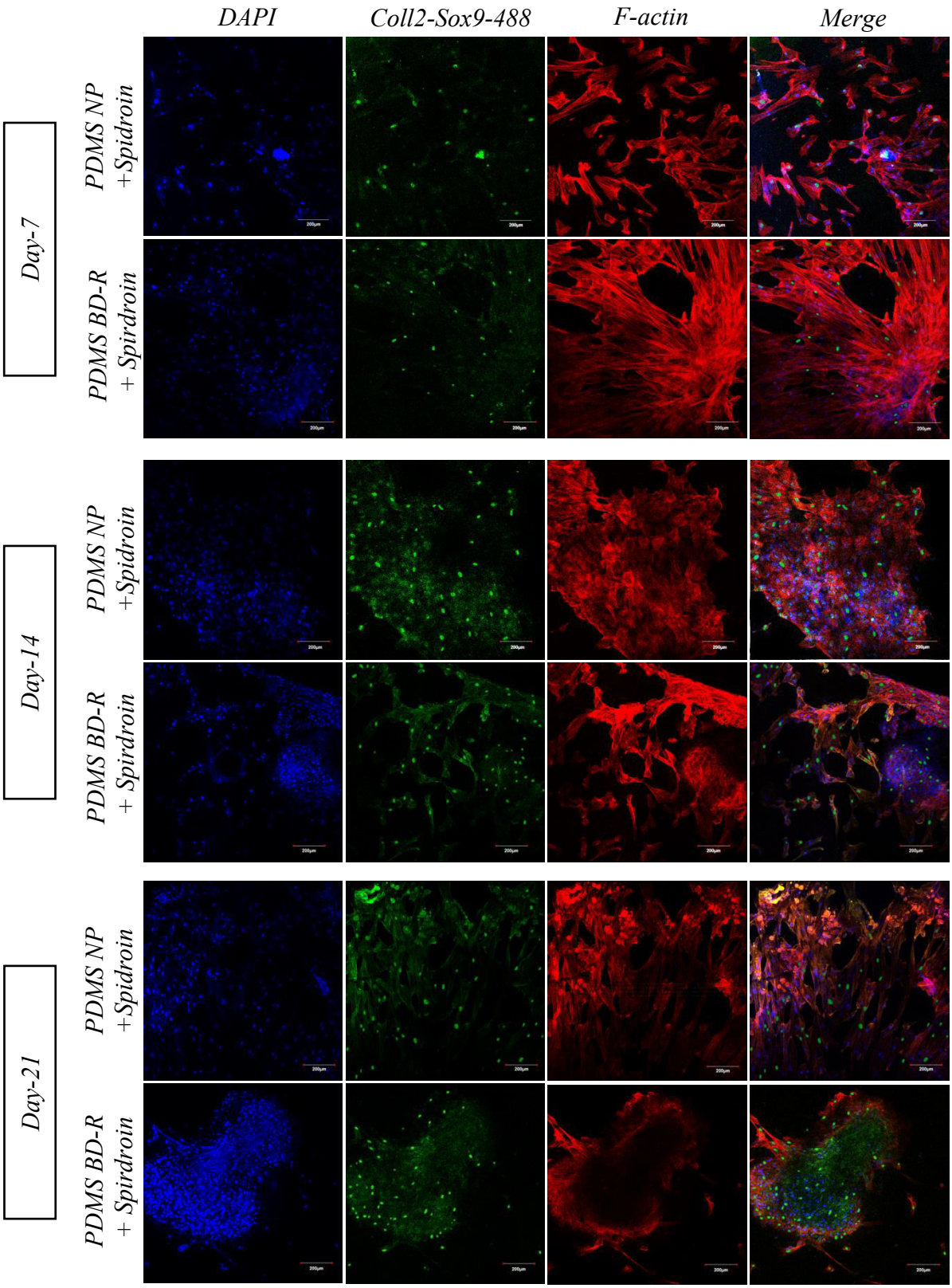


Figure S13. Sox9 expression with ICC on days 7, 14 and 21. Sox9 expression is marked in green, while the red colour shows the actin protein and the blue colour shows the cell nucleus. Scale: 200 µm. PDMS BD-R; PDMS nanopattern substrate. PDMS NP; PDMS substrate without a pattern (un-pattern).

Table S45. Spidroin Peak Raman Shift based on Raman Spectroscopy assay

Raman Shift experiment	Raman Shift literature	Amino acids possibility	Vibration Bond (Potential Energy Distribution)	Type of Primary Structure	Type of Secondary Structure
536, 540, 550	455-613	Cystine	Str S–S bond (dialkyl disulfides)	Amide VI	
623, 673	623, 669	Glutamic acid	COOH def vib		
702	700	Methionine	C-S asym str		
718	715	Tyrosine	Para-substituted benzene (The-ring O asym)	Amide IV, V	β-sheet
727	721	Methionine	C-S-C asym str; H2O rock		
804	805	Methionine, Serine	C–O–H def (the alcoholic hydroxyl); (C–C–N) sym str vib		
832	830	Tyrosine	Para-substitutedbenzene (The-ring O asym)	N–Cα-C, Val N–Cα-C, C-C stretch	
886	879	Tryptophan	H-bend (pyrrole ring); H-scss (indole ring)		
918	918	Isoleucine	Cα-Ct; Cg1-asym bend		
926	930	Arginine	Nh1-Cx-Nh2 sym st; Cd-Ne; Cb-Cg; Ne-Cx		
936	932	Threonine	C-C-N str vib		
939	939	Aspartic acid	g(OH) bend		α-helix, β-sheet
948	948	Leucine	C–C; C–N str vib		
968	967	Valin	C–C str vib		
985	980	Arginine	Cα-Cb; Nt-Cα		
1036	1036	Arginine	Cg-Cd		
1038	1038	Glycine	N- Cα	C-N	
1062	1062	Histidine	Nt-Cα, Cα-Cb		
1082	1084	Aspartic acid	NH3 wag vib		
1083	1083	Arginine	Cx- Nh1-H2 asym bend		
1100	1100	Arginine	Cx- Nh1-H2 asym bend		
1107	1100-1121	Arginine, Aspartic acid	NH3 wag vib		
1118	1115	Alanine	Cb-asym bend; Cα-Cb		
1127	1127	Serine	C–O–H def		
1130	1130	Phenylalanine	δ(X–H), δριvγ		
1141	1141	Lysine	NtH3+ asym rock; Ct- Cα-Hα		
1201	1205, 1201	Arginine, Tyrosine	Para-substituted benzene (The-ring O asym)		β-sheet
1240	1245	Methionine	CH2 twist		
1254	1252	Histidine, Isoleucine	Cb-bend; Hα-Cα-Ct; Hb-Cd-Cg1; Cd-asym rock	Amide III	
1264	1264, 1262	Arginine, Aspartic acid	C-H bend vib		

1331	1331	Arginine, Aspartic acid	Cb-twist; CtOO- sym st; Cg-rock; Cb-Cα-Hα; CH bend vib.	Trp, Cα-H (def)	
1345	1343	Cystein	Str S–S bond (dialkyl disulfides)		
1355	1354	Valin	CH3; d(CH3)		
1375	1378	Alanine, Leucin	Cb-sym rock; Cb-sym bend	Cα-H (def)	
1385	1385	Cystein	Str S–S bond (dialkyl disulfides)		
1408	1408	Arginine	Cb-rock; Cg-wag; CtOO- sym st; Nt-Cα-Hα		
1442	1442	Glycine	Hα-Cα-H; N-Cα-Hα		
1443	1443	Arginine	Nh1- Cx- N2 sym str; Cd-Ne; He; Cx-Ne-He		
1458	1458	Glycine	Hα-Cα-H; N-Cα-Hα		
1490	1500	Histidine	Ne-Ce; Nd-Ce-H; Ne-Ce-H	Amide II	α-helix
1507	1507	Aspartic acid	NH3 -sym bend; NH3-sym rock		
1518	1515	Glycine	NH3-sym bend;NH3-sym rock		
1578	1573	Histidine	Cg-Cd; Cb-Cg; Nd-Cg-Cd		
1614	1607-1616	Phenylalanine, Histidine	ν(C=O); Cg-Cd; Cb-Cg; Nd-Cg-Cd	Amide I	
1627	1629	Methionine	CH3 asym def		
1692	1695	Aspartic acid	NH2 shear vib; COO− str vib		
1736, 1800	1733, 1803	Cysteine	Str S–S bond (dialkyl disulfides)	Amide VI	

Abbreviations:

*asym, asymmetric; str, stretch; sym, symmetric; def, deformation; i.p., in-plane; rock, rocking; sciss, scissoring; tor, torsion; wag, wagging.

*Ct and Nt refer to the carbon and nitrogen atoms of the terminal COO^- and $NH3^+$ groups, respectively.

*ν, stretching; δ, deformation, or in-plane bending; γ, out-of-plane bending.

KEMENTERIAN PENDIDIKAN, KEBUDAYAAN,
RISET DAN TEKNOLOGI
UNIVERSITAS PADJADJARAN
KOMITE ETIK PENELITIAN
RESEARCH ETHICS COMMITTEE
Jl. Prof. Eyckman No. 38 Bandung 40161
Telp. & Fax. 022-2038697 email: kep@unpad.ac.id, website: kep.unpad.ac.id

No. Reg.: 2302040207

PERSETUJUAN ETIK
ETHICAL APPROVAL

Nomor: 316/UN6.KEP/EC/2023

Komite Etik Penelitian Universitas Padjadjaran Bandung, dalam upaya melindungi hak asasi dan kesejahteraan subjek penelitian serta menjamin bahwa penelitian yang menggunakan formulir survei/registrasi/surveilens/ Epidemiologi/Humaniora/Sosial Budaya/Bahan Biologi Tersimpan/Sel Punca dan non klinis lainnya berjalan dengan memperhatikan implikasi etik, hukum, sosial dan non klinis lainnya yang berlaku, telah mengkaji dengan teliti proposal penelitian berjudul:

The Research Ethics Committee Universitas Padjadjaran Bandung, in order to protect the rights and welfare of the research subject, and to guaranty that the research using survey questionnaire/registry/surveillance/ epidemiology/humaniora/social-cultural/archived biological materials/stem cell/other non clinical materials, will carried out according to ethical, legal, social implications and other applicable regulations, has been throughly reviewed the proposal entitled:

"REKAYASA KONDROGENIK *HUMAN WHARTON'S JELLY-DERIVED MESENCHYMAL STEM CELLS (HWJ-MSC)* MENGGUNAKAN *NANOPATTERN* CAKRAM OPTIK *DIGITAL VERSATILE DISC RECORDABLE (DVD-R) DAN BLURAY-DISC RECORDABLE (BD-R)* DILAPISI SUTRA SPIDROIN ASAL LABA-LABA *ARGIOPE MODESTA*"

Nama Peneliti Utama *Principal Researcher*	:	Acep Hendra Punja Unggara
Pembimbing/Peneliti Lain *Supervisor/Other Researcher*	:	Prof.. Dr. Anggraini Barlian, M.Sc.
Nama Institusi *Institution*	:	Program Magister Program Studi Bioteknologi Sekolah Ilmu Teknologi Hayati Institut Teknologi Bandung

proposal tersebut dapat disetujui pelaksanaannya.
hereby declare that the proposal is approved.

Ditetapkan di : Bandung
Issued in
Tanggal : 13-03-2023
Date

Ketua,
Chairman,

Nur Atik, dr, M.Kes., PhD
NIP. 19811010 200801 1 019

Keterangan/*notes*:
Persetujuan etik ini berlaku selama satu tahun sejak tanggal ditetapkan.
This ethical clearance is effective for one year from the due date.
Pada akhir penelitian, laporan pelaksanaan penelitian harus diserahkan ke Komite Etik Penelitian.
In the end of the research, progress and final summary report should be submitted to the Research Ethics Committee.
Jika ada perubahan atau penyimpangan protokol dan/atau perpanjangan penelitian, harus mengajukan kembali permohonan kajian etik penelitian.
If there be any protocol modification or deviation and/or extension of the study, the Principal Investigator is required to resubmit the protocol for approval.
Jika ada kejadian serius yang tidak diinginkan (KTD) harus segera dilaporkan ke Komite Etik Penelitian.
If there are Serious Adverse Events (SAE) should be immediately reported to the Research Ethics Committee