Supplementary information

Osteogenesis Study of 3D Printed of Silver Nanoparticles-based Electroactive and Anti-infection Scaffold Using Human Wharton's Jelly Mesenchymal Stem Cells

Mira Mira[a], Arie Wibowo[*b,c], Gusti Umindya Nur Tajalla[d], Glen Cooper[e], Paulo Jorge Da Silva Bartolo[f], Anggraini Barlian[*c,g].

[a]Master of Biotechnology, School of Life Sciences and Technology, Institut Teknologi Bandung, Bandung, West Java, Indonesia

[b]Material Science and Engineering Research Group, Faculty of Mechanical and Aerospace Engineering, Institut Teknologi Bandung, Bandung, West Java, Indonesia. E-mail: ariewibowo@itb.ac.id

[c]Research Center for Nanoscience and Nanotechnology, Institut Teknologi Bandung, Bandung, West Java, Indonesia

[d]Materials and Metallurgy Engineering, Institut Teknologi Kalimantan, Balikpapan, East Kalimantan, Indonesia

[e]School of Engineering, University of Manchester, Manchester M13 9PL, UK

[f]The Singapore Centre for 3D Printing (SC3DP), Nanyang Technological University, 50 Nanyang Avenue, Block N3.1-B2C-03, Singapore 639798

[g]School of Life Sciences and Technology, Institut Teknologi Bandung, Bandung, West Java, Indonesia. E-mail: aang@sith.itb.ac.id

Corresponding author: ariewibowo@itb.ac.id , aang@sith.itb.ac.id

I. Statistical analysis of proliferation results

Table S1. Overall proliferation results

Day	1			3			5			7			14		
Cell Number	**Control**	**PCL**	**PCL/AgNPs**	**Control**	**PCL**	**PCL/AgNPs**	**Control**	**PCL**	**PCL/AgNPs**	**Control**	**PCL**	**PCL/AgNPs**	**Control**	**PCL**	**PCL/AgNPs**
Cell Number 1	33798	46039	54741	10660	55587	51912	7238	74791	68948	6608	74888	77217	4284	88089	99978
Cell Number 2	34057	43294	49470	7899	47984	51553	4791	67706	70705	4040	65466	78593	2483	78893	92221
Cell Number 3	33139	42547	49017	7545	49263	51297	4793	67609	82169	4161	66874	78077	2572	79251	91356
Cell Number 4	53534	39282	48240	7407	44657	58303	3987	51870	72045	4499	64351	77664	1148	73304	81255
Cell Number 5	42690	39928	48345	7746	43614	61029	4403	55083	73258	4469	64576	58094	1382	79210	82652
Cell Number 6	42838	40152	48281	7740	45506	59805	4456	55110	71567	4484	65628	72906	1351	78962	83126
Average	40009	41874	49682	8166	47769	55650	4945	62028	73115	4710	66964	73759	2203	79618	88431
StDev	7991	2580	2526	1234	4370	4537	1163	9225	4663	949	3984	7942	1188	4751	7339

1.1.Statistical analysis of proliferation day 1

Table S2. Test of normality on proliferation day 1

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
CellNumberDay1	.142	18	.200*	.953	18	.477

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

The significance value (p-value) is 0.477, which is bigger than the α (0.05), meaning that the data is normally distributed.

Table S3. Test of homogeneity of variance on proliferation day 1

Test of Homogeneity of Variances

CellNumberDay1

Levene Statistic	df1	df2	Sig.
6.011	2	15	.012

The significance value (p-value) is 0.012, 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 S4. Test of significance on proliferation day 1

ANOVA

CellNumberDay1

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	316035885.778	2	158017942.889	6.164	.011
Within Groups	384513952.000	15	25634263.467		
Total	700549837.778	17			

The significance value (p-value) is 0.011, which is smaller than the α (0.05), meaning that there is an effect of scaffold use on hWJ-MSCs proliferation.

Table S5. Post Hoc

Multiple Comparisons

Dependent Variable: CellNumberDay1

	(I) ExperimentDay1	(J) ExperimentDay1	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	Control Day 1	PCL Scaffold Day 1	-1864.33333	2923.14120	.802	-9457.1057	5728.4390
		PCL/AgNps Scaffold Day 1	-9673.00000*	2923.14120	.012	-17265.7723	-2080.2277
	PCL Scaffold Day 1	Control Day 1	1864.33333	2923.14120	.802	-5728.4390	9457.1057
		PCL/AgNps Scaffold Day 1	-7808.66667*	2923.14120	.043	-15401.4390	-215.8943
	PCL/AgNps Scaffold Day 1	Control Day 1	9673.00000*	2923.14120	.012	2080.2277	17265.7723
		PCL Scaffold Day 1	7808.66667*	2923.14120	.043	215.8943	15401.4390
Dunnett T3	Control Day 1	PCL Scaffold Day 1	-1864.33333	3428.33083	.926	-12792.0243	9063.3576
		PCL/AgNps Scaffold Day 1	-9673.00000	3421.63087	.079	-20602.8203	1256.8203
	PCL Scaffold Day 1	Control Day 1	1864.33333	3428.33083	.926	-9063.3576	12792.0243
		PCL/AgNps Scaffold Day 1	-7808.66667*	1474.19583	.001	-11979.2821	-3638.0512
	PCL/AgNps Scaffold Day 1	Control Day 1	9673.00000	3421.63087	.079	-1256.8203	20602.8203
		PCL Scaffold Day 1	7808.66667*	1474.19583	.001	3638.0512	11979.2821

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

Because the data is not homogeneous, the Dunnett T3 test was used. The significance value (p-value) is 0.001, which is smaller than the α (0.05), meaning that the PCL/AgNPs scaffold significantly induced the proliferation of hWJ-MSCs better than the PCL scaffold.

1.2.Statistical analysis of proliferation day 3

Table S6. Test of normality on proliferation day 3

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
CellNumberDay3	.283	18	.000	.774	18	.001

a. Lilliefors Significance Correction

The significance value (p-value) is 0.001, which is smaller than the α (0.05), meaning that the data is not normally distributed.

Table S7. Test of significance on proliferation day 3

Mann-Whitney Test

Ranks

	ExperimentDay3	N	Mean Rank	Sum of Ranks
CellNumberDay3	Control Day 3	6	3.50	21.00
	PCL Scaffold Day 3	6	9.50	57.00
	Total	12		

Test Statistics[a]

	CellNumberDay3
Mann-Whitney U	.000
Wilcoxon W	21.000
Z	-2.882
Asymp. Sig. (2-tailed)	.004
Exact Sig. [2*(1-tailed Sig.)]	.002[b]

a. Grouping Variable: ExperimentDay3

b. Not corrected for ties.

The significance value (p-value) is 0.004, which is smaller than the α (0.05), meaning that the PCL scaffold significantly induced the proliferation of hWJ-MSCs better than the control.

Mann-Whitney Test

Ranks

	ExperimentDay3	N	Mean Rank	Sum of Ranks
CellNumberDay3	Control Day 3	6	3.50	21.00
	PCL/AgNps Scaffold Day 3	6	9.50	57.00
	Total	12		

Test Statistics[a]

	CellNumberDay3
Mann-Whitney U	.000
Wilcoxon W	21.000
Z	-2.882
Asymp. Sig. (2-tailed)	.004
Exact Sig. [2*(1-tailed Sig.)]	.002[b]

a. Grouping Variable: ExperimentDay3

b. Not corrected for ties.

The significance value (p-value) is 0.004, which is smaller than the α (0.05), meaning that the PCL/AgNPs scaffold significantly induced the proliferation of hWJ-MSCs better than the control.

Mann-Whitney Test

Ranks

	ExperimentDay3	N	Mean Rank	Sum of Ranks
CellNumberDay3	PCL Scaffold Day 3	6	4.00	24.00
	PCL/AgNps Scaffold Day 3	6	9.00	54.00
	Total	12		

Test Statistics[a]

	CellNumberDay3
Mann-Whitney U	3.000
Wilcoxon W	24.000
Z	-2.402
Asymp. Sig. (2-tailed)	.016
Exact Sig. [2*(1-tailed Sig.)]	.015[b]

a. Grouping Variable: ExperimentDay3

b. Not corrected for ties.

The significance value (p-value) is 0.016, which is smaller than the α (0.05), meaning that the PCL/AgNPs scaffold significantly induced the proliferation of hWJ-MSCs better than the PCL scaffold.

1.3.Statistical analysis of proliferation day 5

Table S8. Test of normality on proliferation day 5

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
CellNumberDay5	.248	18	.005	.766	18	.001

a. Lilliefors Significance Correction

The significance value (p-value) is 0.001, which is smaller than the α (0.05), meaning that the data is not normally distributed.

Table S9. Test of significance on proliferation day 5

Mann-Whitney Test

Ranks

	ExperimentDay5	N	Mean Rank	Sum of Ranks
CellNumberDay5	Control Day 5	6	3.50	21.00
	PCL Scaffold Day 5	6	9.50	57.00
	Total	12		

Test Statistics[a]

	CellNumberDay5
Mann-Whitney U	.000
Wilcoxon W	21.000
Z	-2.882
Asymp. Sig. (2-tailed)	.004
Exact Sig. [2*(1-tailed Sig.)]	.002[b]

a. Grouping Variable: ExperimentDay5

b. Not corrected for ties.

The significance value (p-value) is 0.004, which is smaller than the α (0.05), meaning that the PCL scaffold significantly induced the proliferation of hWJ-MSCs better than the control.

Mann-Whitney Test

Ranks

	ExperimentDay5	N	Mean Rank	Sum of Ranks
CellNumberDay5	Control Day 5	6	3.50	21.00
	PCL/AgNps Scaffold Day 5	6	9.50	57.00
	Total	12		

Test Statistics[a]

	CellNumberDay5
Mann-Whitney U	.000
Wilcoxon W	21.000
Z	-2.882
Asymp. Sig. (2-tailed)	.004
Exact Sig. [2*(1-tailed Sig.)]	.002[b]

a. Grouping Variable: ExperimentDay5

b. Not corrected for ties.

The significance value (p-value) is 0.004, which is smaller than the α (0.05), meaning that the PCL/AgNPs scaffold significantly induced the proliferation of hWJ-MSCs better than the control.

Mann-Whitney Test

Ranks

	ExperimentDay5	N	Mean Rank	Sum of Ranks
CellNumberDay5	PCL Scaffold Day 5	6	4.33	26.00
	PCL/AgNps Scaffold Day 5	6	8.67	52.00
	Total	12		

Test Statistics[a]

	CellNumberDay5
Mann-Whitney U	5.000
Wilcoxon W	26.000
Z	-2.082
Asymp. Sig. (2-tailed)	.037
Exact Sig. [2*(1-tailed Sig.)]	.041[b]

a. Grouping Variable: ExperimentDay5

b. Not corrected for ties.

The significance value (p-value) is 0.037, which is smaller than the α (0.05), meaning that the PCL/AgNPs scaffold significantly induced the proliferation of hWJ-MSCs better than the PCL scaffold.

1.4.Statistical analysis of proliferation day 7

Table S10. Test of normality on proliferation day 7

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
CellNumberDay7	.299	18	.000	.729	18	.000

a. Lilliefors Significance Correction

The significance value (p-value) is 0.000, which is smaller than the α (0.05), meaning that the data is not normally distributed.

Table S11. Test of significance on proliferation day 7

Mann-Whitney Test

Ranks

	ExperimentDay7	N	Mean Rank	Sum of Ranks
CellNumberDay7	Control Day 7	6	3.50	21.00
	PCL Scaffold Day 7	6	9.50	57.00
	Total	12		

Test Statistics[a]

	CellNumberDay7
Mann-Whitney U	.000
Wilcoxon W	21.000
Z	-2.882
Asymp. Sig. (2-tailed)	.004
Exact Sig. [2*(1-tailed Sig.)]	.002[b]

a. Grouping Variable: ExperimentDay7

b. Not corrected for ties.

The significance value (p-value) is 0.004, which is smaller than the α (0.05), meaning that the PCL scaffold significantly induced the proliferation of hWJ-MSCs better than the control.

Mann-Whitney Test

Ranks

	ExperimentDay7	N	Mean Rank	Sum of Ranks
CellNumberDay7	Control Day 7	6	3.50	21.00
	PCL/AgNps Scaffold Day 7	6	9.50	57.00
	Total	12		

Test Statistics[a]

	CellNumberDay7
Mann-Whitney U	.000
Wilcoxon W	21.000
Z	-2.882
Asymp. Sig. (2-tailed)	.004
Exact Sig. [2*(1-tailed Sig.)]	.002[b]

a. Grouping Variable: ExperimentDay7

b. Not corrected for ties.

The significance value (p-value) is 0.004, which is smaller than the α (0.05), meaning that the PCL/AgNPs scaffold significantly induced the proliferation of hWJ-MSCs better than the control.

Mann-Whitney Test

Ranks

	ExperimentDay7	N	Mean Rank	Sum of Ranks
CellNumberDay7	PCL Scaffold Day 7	6	4.67	28.00
	PCL/AgNps Scaffold Day 7	6	8.33	50.00
	Total	12		

Test Statistics[a]

	CellNumberDay7
Mann-Whitney U	7.000
Wilcoxon W	28.000
Z	-1.761
Asymp. Sig. (2-tailed)	.078
Exact Sig. [2*(1-tailed Sig.)]	.093[b]

a. Grouping Variable: ExperimentDay7

b. Not corrected for ties.

The significance value (p-value) is 0.078, which is bigger than the α (0.05), meaning that the PCL/AgNPs scaffold did not significantly induce the proliferation of hWJ-MSCs better than the PCL scaffold.

1.5.Statistical analysis of proliferation day 14

Table S12. Test of normality on proliferation day 14

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
CellNumberDay14	.327	18	.000	.726	18	.000

a. Lilliefors Significance Correction

The significance value (p-value) is 0.000, which is smaller than the α (0.05), meaning that the data is not normally distributed.

Table S13. Test of significance on proliferation day 14

Mann-Whitney Test

Ranks

	ExperimentDay14	N	Mean Rank	Sum of Ranks
CellNumberDay14	Control Day 14	6	3.50	21.00
	PCL Scaffold Day 14	6	9.50	57.00
	Total	12		

Test Statistics[a]

	CellNumberDay14
Mann-Whitney U	.000
Wilcoxon W	21.000
Z	-2.882
Asymp. Sig. (2-tailed)	.004
Exact Sig. [2*(1-tailed Sig.)]	.002[b]

a. Grouping Variable: ExperimentDay14

b. Not corrected for ties.

The significance value (p-value) is 0.004, which is smaller than the α (0.05), meaning that the PCL scaffold significantly induced the proliferation of hWJ-MSCs better than the control.

Mann-Whitney Test

Ranks

	ExperimentDay14	N	Mean Rank	Sum of Ranks
CellNumberDay14	Control Day 14	6	3.50	21.00
	PCL/AgNps Scaffold Day 14	6	9.50	57.00
	Total	12		

Test Statistics[a]

	CellNumberDay14
Mann-Whitney U	.000
Wilcoxon W	21.000
Z	-2.882
Asymp. Sig. (2-tailed)	.004
Exact Sig. [2*(1-tailed Sig.)]	.002[b]

a. Grouping Variable: ExperimentDay14

b. Not corrected for ties.

The significance value (p-value) is 0.004, which is smaller than the α (0.05), meaning that the PCL/AgNPs scaffold significantly induced the proliferation of hWJ-MSCs better than the control.

Mann-Whitney Test

Ranks

	ExperimentDay14	N	Mean Rank	Sum of Ranks
CellNumberDay14	PCL Scaffold Day 14	6	4.00	24.00
	PCL/AgNps Scaffold Day 14	6	9.00	54.00
	Total	12		

Test Statistics[a]

	CellNumberDay14
Mann-Whitney U	3.000
Wilcoxon W	24.000
Z	-2.402
Asymp. Sig. (2-tailed)	.016
Exact Sig. [2*(1-tailed Sig.)]	.015[b]

a. Grouping Variable: ExperimentDay14

b. Not corrected for ties.

The significance value (p-value) is 0.016, which is smaller than the α (0.05), meaning that the PCL/AgNPs scaffold significantly induced the proliferation of hWJ-MSCs better than the PCL scaffold.

II. Statistical Analysis of RT-qPCR

Table S14. Overall *RUNX2* gene expression

Treatment	***RUNX2* Gene Expression**														
	Day 7					***Day 14***					***Day 21***				
	1	**2**	**3**	**4**	**Average**	**1**	**2**	**3**	**4**	**Average**	**1**	**2**	**3**	**4**	**Average**
Control	2,24	0,55	0,71	1,14	1,16	1,60	1,35	0,61	0,76	1,08	0,91	0,72	1,08	1,40	1,03
PCL Scaffold	4,03	9,92	5,86	3,48	5,82	3,83	2,46	4,22	2,60	3,28	3,04	1,92	0,77	2,93	2,17
PCL/AgNPs Scaffold	5,97	49,44	21,22	1,92	19,64	5,18	5,75	4,35	6,07	5,34	4,30	2,76	1,97	1,86	2,72

Table S15. Overall *COL1A1* gene expression

Treatment	***COL1A1* Gene Expression**														
	Day 7					***Day 14***					***Day 21***				
	1	**2**	**3**	**4**	**Average**	**1**	**2**	**3**	**4**	**Average**	**1**	**2**	**3**	**4**	**Average**
Control	0,55	0,72	1,64	1,54	1,11	1,17	1,11	0,81	0,95	1,01	1,22	1,46	0,78	0,72	1,05
PCL Scaffold	0,33	2,69	2,76	0,40	1,54	1,00	0,75	0,89	0,98	0,91	2,24	2,25	3,21	1,86	2,39
PCL/AgNPs Scaffold	2,97	0,70	0,79	0,88	1,34	0,22	0,30	0,34	0,37	0,31	6,08	2,84	2,90	1,34	3,29

Table S16. Overall *OPN* gene expression

Treatment	***OPN* Gene Expression**														
	Day 7					***Day 14***					***Day 21***				
	1	**2**	**3**	**4**	**Average**	**1**	**2**	**3**	**4**	**Average**	**1**	**2**	**3**	**4**	**Average**
Control	0,36	1,15	1,61	1,48	1,15	1,240	0,525	1,173	1,310	1,062	1,88	1,22	0,55	0,80	1,11
PCL Scaffold	1,42	0,35	0,24	0,43	0,61	1,098	0,061	1,218	0,719	0,774	1,12	2,20	2,14	0,88	1,58
PCL/AgNPs Scaffold	0,60	1,32	0,71	0,80	0,86	0,146	0,279	0,228	0,172	0,206	2,44	3,07	1,31	4,85	2,92

2.1.Statistical analysis of *RUNX2* gene expression day 7

Table S17. Test of normality on *RUNX2* gene expression day 7

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
RUNX2GeneExpressionDay 7	.331	12	.001	.616	12	.000

a. Lilliefors Significance Correction

The significance value (p-value) is 0.000, which is smaller than the α (0.05), meaning that the data is not normally distributed.

Table S18. Test of significance on *RUNX2* gene expression day 7

Mann-Whitney Test

Ranks

	Treatment	N	Mean Rank	Sum of Ranks
RUNX2GeneExpressionDay7	Control Day 7	4	2.50	10.00
	PCL Scaffold Day 7	4	6.50	26.00
	Total	8		

Test Statistics[a]

	RUNX2GeneExpressionDay7
Mann-Whitney U	.000
Wilcoxon W	10.000
Z	-2.366
Asymp. Sig. (2-tailed)	.018
Exact Sig. [2*(1-tailed Sig.)]	.029[b]

a. Grouping Variable: Treatment

b. Not corrected for ties.

The significance value (p-value) is 0.018, which is smaller than the α (0.05), meaning that PCL scaffold significantly increased *RUNX2* gene expression compared to control on day 7.

Mann-Whitney Test

Ranks

	Treatment	N	Mean Rank	Sum of Ranks
RUNX2GeneExpressionDay7	Control Day 7	4	2.63	10.50
	PCL/AgNPs Scaffold Day 7	4	6.38	25.50
	Total	8		

Test Statistics[a]

	RUNX2GeneExpressionDay7
Mann-Whitney U	.500
Wilcoxon W	10.500
Z	-2.233
Asymp. Sig. (2-tailed)	.026
Exact Sig. [2*(1-tailed Sig.)]	.029[b]

a. Grouping Variable: Treatment

b. Not corrected for ties.

The significance value (p-value) is 0.026, which is smaller than the α (0.05), meaning that PCL/AgNPs scaffold significantly increased *RUNX2* gene expression compared to control on day 7.

Mann-Whitney Test

Ranks

	Treatment	N	Mean Rank	Sum of Ranks
RUNX2GeneExpressionDay7	PCL Scaffold Day 7	4	3.88	15.50
	PCL/AgNPs Scaffold Day 7	4	5.13	20.50
	Total	8		

Test Statistics[a]

	RUNX2GeneExpressionDay7
Mann-Whitney U	5.500
Wilcoxon W	15.500
Z	-.726
Asymp. Sig. (2-tailed)	.468
Exact Sig. [2*(1-tailed Sig.)]	.486[b]

a. Grouping Variable: Treatment

b. Not corrected for ties.

The significance value (p-value) is 0.468, which is bigger than the α (0.05), meaning that the PCL/AgNPs scaffold did not significantly increase *RUNX2* gene expression compared to the PCL scaffold on day 7.

2.2.Statistical analysis of *RUNX2* gene expression day 14

Table S19. Test of normality on *RUNX2* gene expression day 14

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
RUNX2GeneExpressionDay 14	.165	12	.200*	.901	12	.164

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

The significance value (p-value) is 0.164, which is bigger than the α (0.05), meaning that the data is normally distributed.

Table S20. Test of homogeneity of variance on *RUNX2* gene expression day 14

Test of Homogeneity of Variances

RUNX2GeneExpressionDay14

Levene Statistic	df1	df2	Sig.
1.421	2	9	.291

The significance value (p-value) is 0.291, which is bigger than the α (0.05), meaning that the data group comes from a population that has the same variance (homogeneous).

Table S21. Test of significance on *RUNX2* gene expression day 14

ANOVA

RUNX2GeneExpressionDay14

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	32.000	2	16.000	23.040	.000
Within Groups	6.250	9	.694		
Total	38.250	11			

The significance value (p-value) of 0.000 is smaller than α (0.05), meaning that there is a significant influence of scaffold use on *RUNX2* gene expression on day 14.

Table S22. Post Hoc

Multiple Comparisons

Dependent Variable: RUNX2GeneExpressionDay14
Tukey HSD

(I) TreatmentDay14	(J) TreatmentDay14	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control Day 14	PCL Scaffold Day 14	-2.00000*	.58926	.020	-3.6452	-.3548
	PCL/AgNPs Scaffold Day 14	-4.00000*	.58926	.000	-5.6452	-2.3548
PCL Scaffold Day 14	Control Day 14	2.00000*	.58926	.020	.3548	3.6452
	PCL/AgNPs Scaffold Day 14	-2.00000*	.58926	.020	-3.6452	-.3548
PCL/AgNPs Scaffold Day 14	Control Day 14	4.00000*	.58926	.000	2.3548	5.6452
	PCL Scaffold Day 14	2.00000*	.58926	.020	.3548	3.6452

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

- The significance value (p-value) is smaller than the α (0.05), meaning that PCL scaffold and PCL/AgNPs scaffold significantly increased *RUNX2* gene expression compared to control on day 14.
- The significance value (p-value) is smaller than the α (0.05), meaning that PCL/AgNPs scaffold significantly increased *RUNX2* gene expression compared to PCL scaffold on day 14.

2.3.Statistical analysis of *RUNX2* gene expression day 21

Table S23. Test of normality on *RUNX2* gene expression day 21

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
RUNX2GeneExpressionDay 21	.247	12	.041	.846	12	.033

a. Lilliefors Significance Correction

The significance value (p-value) is 0.033, which is smaller than the α (0.05), meaning that the data is not normally distributed.

Table S24. Test of significance on *RUNX2* gene expression day 21

Mann-Whitney Test

Ranks

	Treatment	N	Mean Rank	Sum of Ranks
RUNX2GeneExpressionDay21	Control Day 21	4	3.00	12.00
	PCL Scaffold Day 21	4	6.00	24.00
	Total	8		

Test Statistics[a]

	RUNX2GeneExpressionDay21
Mann-Whitney U	2.000
Wilcoxon W	12.000
Z	-2.000
Asymp. Sig. (2-tailed)	.046
Exact Sig. [2*(1-tailed Sig.)]	.114[b]

a. Grouping Variable: Treatment

b. Not corrected for ties.

The significance value (p-value) is 0.046, which is smaller than the α (0.05), meaning that PCL scaffold significantly increased *RUNX2* gene expression compared to control on day 21.

Mann-Whitney Test

Ranks

	Treatment	N	Mean Rank	Sum of Ranks
RUNX2GeneExpressionDay21	Control Day 21	4	2.50	10.00
	PCL/AgNPs Scaffold Day 21	4	6.50	26.00
	Total	8		

Test Statistics[a]

	RUNX2GeneExpressionDay21
Mann-Whitney U	.000
Wilcoxon W	10.000
Z	-2.477
Asymp. Sig. (2-tailed)	.013
Exact Sig. [2*(1-tailed Sig.)]	.029[b]

a. Grouping Variable: Treatment

b. Not corrected for ties.

The significance value (p-value) is 0.013, which is smaller than the α (0.05), meaning that PCL/AgNPs scaffold significantly increased *RUNX2* gene expression compared to control on day 21.

Mann-Whitney Test

Ranks

	Treatment	N	Mean Rank	Sum of Ranks
RUNX2GeneExpressionDay21	PCL Scaffold Day 21	4	4.00	16.00
	PCL/AgNPs Scaffold Day 21	4	5.00	20.00
	Total	8		

Test Statistics[a]

	RUNX2GeneExpressionDay21
Mann-Whitney U	6.000
Wilcoxon W	16.000
Z	-.607
Asymp. Sig. (2-tailed)	.544
Exact Sig. [2*(1-tailed Sig.)]	.686[b]

a. Grouping Variable: Treatment

b. Not corrected for ties.

The significance value (p-value) is 0.544, which is bigger than the α (0.05), meaning that the PCL/AgNPs scaffold did not significantly increase *RUNX2* gene expression compared to the PCL scaffold on day 21.

2.4.Statistical analysis of *COL1A1* gene expression day 7

Table S25. Test of normality on *COL1A1* gene expression day 7

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
COL1A1GeneExpressionDay7	.261	12	.023	.837	12	.026

a. Lilliefors Significance Correction

The significance value (p-value) is 0.026, which is smaller than the α (0.05), meaning that the data is not normally distributed.

Table S26. Test of significance on *COL1A1* gene expression day 7

Kruskal-Wallis Test

Ranks

	TreatmentDay7	N	Mean Rank
COL1A1GeneExpression Day7	Control Day 7	4	6.25
	PCL Scaffold Day 7	4	6.00
	PCL/AgNPs Scaffold Day 7	4	7.25
	Total	12	

Test Statistics[a,b]

	COL1A1GeneExpressionDay7
Chi-Square	.269
df	2
Asymp. Sig.	.874

a. Kruskal Wallis Test

b. Grouping Variable: TreatmentDay7

The significance value (p-value) is 0.874, which is bigger than the α (0.05), meaning there are no different groups.

2.5.Statistical analysis of *COL1A1* gene expression day 14

Table S27. Test of normality on *COL1A1* gene expression day 14

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
COL1A1GeneExpressionDay14	.197	12	.200*	.882	12	.094

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

The significance value (p-value) is 0.094, which is bigger than the α (0.05), meaning that the data is normally distributed.

Table S28. Test of homogeneity of variance on *COL1A1* gene expression day 14

Test of Homogeneity of Variances

COL1A1GeneExpressionDay14

Levene Statistic	df1	df2	Sig.
2.643	2	9	.125

The significance value (p-value) is 0.125, which is bigger than the α (0.05), meaning that the data group comes from a population that has the same variance (homogeneous).

Table S29. Test of significance on *COL1A1* gene expression day 14

ANOVA

COL1A1GeneExpressionDay14

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.148	2	.574	39.138	.000
Within Groups	.132	9	.015		
Total	1.280	11			

The significance value (p-value) of 0.000 is smaller than α (0.05), meaning that there is a significant influence of scaffold use on *COL1A1* gene expression on day 14.

2.6.Statistical analysis of *COL1A1* gene expression day 21

Table S30. Test of normality on *COL1A1* gene expression day 21

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
COL1A1GeneExpressionDay21	.171	12	.200*	.845	12	.032

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

The significance value (p-value) is 0.032, which is smaller than the α (0.05), meaning that the data is not normally distributed.

Table S31. Test of significance on *COL1A1* gene expression day 21

Mann-Whitney Test

Ranks

	TreatmentDay21	N	Mean Rank	Sum of Ranks
COL1A1GeneExpressionDay21	Control Day 21	4	2.50	10.00
	PCL Scaffold Day 21	4	6.50	26.00
	Total	8		

Test Statistics[a]

	COL1A1GeneExpressionDay21
Mann-Whitney U	.000
Wilcoxon W	10.000
Z	-2.309
Asymp. Sig. (2-tailed)	.021
Exact Sig. [2*(1-tailed Sig.)]	.029[b]

a. Grouping Variable: TreatmentDay21

b. Not corrected for ties.

The significance value (p-value) is 0.021, which is smaller than the α (0.05), meaning that PCL scaffold significantly increased *COL1A1* gene expression compared to control on day 21.

Mann-Whitney Test

Ranks

	TreatmentDay21	N	Mean Rank	Sum of Ranks
COL1A1GeneExpression Day21	Control Day 21	4	2.75	11.00
	PCL/AgNPs Scaffold Day 21	4	6.25	25.00
	Total	8		

Test Statistics[a]

	COL1A1Gene ExpressionDay21
Mann-Whitney U	1.000
Wilcoxon W	11.000
Z	-2.021
Asymp. Sig. (2-tailed)	.043
Exact Sig. [2*(1-tailed Sig.)]	.057[b]

a. Grouping Variable: TreatmentDay21

b. Not corrected for ties.

The significance value (p-value) is 0.043, which is smaller than the α (0.05), meaning that PCL/AgNPs scaffold significantly increased *COL1A1* gene expression compared to control on day 21.

Mann-Whitney Test

Ranks

	TreatmentDay21	N	Mean Rank	Sum of Ranks
COL1A1GeneExpression Day21	PCL Scaffold Day 21	4	4.00	16.00
	PCL/AgNPs Scaffold Day 21	4	5.00	20.00
	Total	8		

Test Statistics[a]

	COL1A1Gene ExpressionDay21
Mann-Whitney U	6.000
Wilcoxon W	16.000
Z	-.577
Asymp. Sig. (2-tailed)	.564
Exact Sig. [2*(1-tailed Sig.)]	.686[b]

a. Grouping Variable: TreatmentDay21

b. Not corrected for ties.

The significance value (p-value) is 0.564, which is bigger than the α (0.05), meaning that the PCL/AgNPs scaffold did not significantly increase *COL1A1* gene expression compared to the PCL scaffold on day 21.

2.7.Statistical analysis of *OPN* gene expression day 7

Table S32. Test of normality on *OPN* gene expression day 7

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
OPNGeneExpressionDay7	.149	12	.200*	.903	12	.173

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

The significance value (p-value) is 0.173, which is bigger than the α (0.05), meaning that the data is normally distributed.

Table S33. Test of homogeneity of variance on *OPN* gene expression day 7

Test of Homogeneity of Variances

OPNGeneExpressionDay7

Levene Statistic	df1	df2	Sig.
.520	2	9	.611

The significance value (p-value) is 0.611, which is bigger than the α (0.05), meaning that the data group comes from a population that has the same variance (homogeneous).

Table S34. Test of significance on *OPN* gene expression day 7

ANOVA

OPNGeneExpressionDay7

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.587	2	.294	1.243	.334
Within Groups	2.127	9	.236		
Total	2.714	11			

The significance value (p-value) of 0.334 is bigger than α (0.05), meaning that there is no significant influence of scaffold use on *OPN* gene expression on day 7.

2.8.Statistical analysis of *OPN* gene expression day 14

Table S35. Test of normality on *OPN* gene expression day 14

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
OPNGeneExpressionDay14	.215	12	.130	.850	12	.037

a. Lilliefors Significance Correction

The significance value (p-value) is 0.037, which is smaller than the α (0.05), meaning that the data is not normally distributed.

Table S36. Test of significance on *OPN* gene expression day 14

Kruskal-Wallis Test

Ranks

	TreatmentDay14	N	Mean Rank
OPNGeneExpressionDay 14	Control Day 14	4	9.50
	PCL Scaffold Day 14	4	6.50
	PCL/AgNPs Scaffold Day 14	4	3.50
	Total	12	

Test Statistics[a,b]

	OPNGeneExpressionDay14
Chi-Square	5.538
df	2
Asymp. Sig.	.063

a. Kruskal Wallis Test

b. Grouping Variable: TreatmentDay14

The significance value (p-value) is 0.063, which is bigger than the α (0.05), meaning there are no different groups.

2.9.Statistical analysis of *OPN* gene expression day 21

Table S37. Test of normality on *OPN* gene expression day 21

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
OPNGeneExpressionDay21	.179	12	.200*	.879	12	.085

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

The significance value (p-value) is 0.085, which is bigger than the α (0.05), meaning that the data is normally distributed.

Table S38. Test of homogeneity of variance on *OPN* gene expression day 21

Test of Homogeneity of Variances

OPNGeneExpressionDay21

Levene Statistic	df1	df2	Sig.
1.424	2	9	.290

The significance value (p-value) is 0.290, which is bigger than the α (0.05), meaning that the data group comes from a population that has the same variance (homogeneous).

Table S39. Test of significance on *OPN* gene expression day 21

ANOVA

OPNGeneExpressionDay21

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7.013	2	3.507	3.516	.074
Within Groups	8.977	9	.997		
Total	15.991	11			

The significance value (p-value) of 0.074 is bigger than α (0.05), meaning that there is no significant influence of scaffold use on *OPN* gene expression on day 21.

III. Statistical analysis of Alkaline Phosphatase Detection

Table S40. Overall alkaline phosphatase detection results

Day	Day 7			Day 14			Day 21		
ALP Activity (U/L)	**Control**	**PCL**	**PCL/AgNPs**	**Control**	**PCL**	**PCL/AgNPs**	**Control**	**PCL**	**PCL/AgNPs**
ALP1	482	285	406	483	430	570	554	602	760
ALP2	272	194	373	380	480	568	513	546	799
ALP3	400	176	364	436	483	596	487	585	745
Average	385	218	381	433	464	578	518	578	768
Stdev	106	59	22	51	30	16	34	29	28

Table S41. Test of normality on alkaline phosphatase detection results

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
ALPActivity	.105	27	.200*	.972	27	.664

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

The significance value (p-value) is 0.664, which is bigger than the α (0.05), meaning that the data is normally distributed.

Table S42. Test of homogeneity of variance on alkaline phosphatase detection results

Test of Homogeneity of Variances

ALPActivity

Levene Statistic	df1	df2	Sig.
2.152	8	18	.084

The significance value (p-value) is 0.084, 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 significance on alkaline phosphatase detection results

ANOVA

ALPActivity

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	580029.333	8	72503.667	30.159	.000
Within Groups	43272.667	18	2404.037		
Total	623302.000	26			

The significance value (p-value) is 0.000, which is smaller than the α (0.05), meaning that there was a significant effect of scaffold use on the ALP activity of hWJ-MSCs.

Table S44. Post Hoc

Multiple Comparisons

Dependent Variable: ALP Activity

Tukey HSD

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control day 7	PCL Scaffold day 7	166.33333*	40.03363	.013	26.0610	306.6056
	PCL/AgNPs Scaffold day 7	3.66667	40.03363	1.000	-136.6056	143.9390
	Control day 14	-48.33333	40.03363	.945	-188.6056	91.9390
	PCL Scaffold day 14	-79.66667	40.03363	.568	-219.9390	60.6056
	PCL/AgNPs Scaffold day 14	-193.33333*	40.03363	.003	-333.6056	-53.0610
	Control day 21	-133.33333	40.03363	.070	-273.6056	6.9390
	PCL Scaffold day 21	-193.00000*	40.03363	.003	-333.2723	-52.7277
	PCL/AgNPs Scaffold day 21	-383.33333*	40.03363	.000	-523.6056	-243.0610
PCL Scaffold day 7	Control day 7	-166.33333*	40.03363	.013	-306.6056	-26.0610
	PCL/AgNPs Scaffold day 7	-162.66667*	40.03363	.016	-302.9390	-22.3944
	Control day 14	-214.66667*	40.03363	.001	-354.9390	-74.3944
	PCL Scaffold day 14	-246.00000*	40.03363	.000	-386.2723	-105.7277
	PCL/AgNPs Scaffold day 14	-359.66667*	40.03363	.000	-499.9390	-219.3944
	Control day 21	-299.66667*	40.03363	.000	-439.9390	-159.3944
	PCL Scaffold day 21	-359.33333*	40.03363	.000	-499.6056	-219.0610
	PCL/AgNPs Scaffold day 21	-549.66667*	40.03363	.000	-689.9390	-409.3944
PCL/AgNPs Scaffold day 7	Control day 7	-3.66667	40.03363	1.000	-143.9390	136.6056
	PCL Scaffold day 7	162.66667*	40.03363	.016	22.3944	302.9390
	Control day 14	-52.00000	40.03363	.919	-192.2723	88.2723
	PCL Scaffold day 14	-83.33333	40.03363	.513	-223.6056	56.9390
	PCL/AgNPs Scaffold day 14	-197.00000*	40.03363	.003	-337.2723	-56.7277
	Control day 21	-137.00000	40.03363	.059	-277.2723	3.2723
	PCL Scaffold day 21	-196.66667*	40.03363	.003	-336.9390	-56.3944
	PCL/AgNPs Scaffold day 21	-387.00000*	40.03363	.000	-527.2723	-246.7277
Control day 14	Control day 7	48.33333	40.03363	.945	-91.9390	188.6056
	PCL Scaffold day 7	214.66667*	40.03363	.001	74.3944	354.9390
	PCL/AgNPs Scaffold day 7	52.00000	40.03363	.919	-88.2723	192.2723
	PCL Scaffold day 14	-31.33333	40.03363	.996	-171.6056	108.9390
	PCL/AgNPs Scaffold day 14	-145.00000*	40.03363	.040	-285.2723	-4.7277
	Control day 21	-85.00000	40.03363	.489	-225.2723	55.2723
	PCL Scaffold day 21	-144.66667*	40.03363	.040	-284.9390	-4.3944
	PCL/AgNPs Scaffold day 21	-335.00000*	40.03363	.000	-475.2723	-194.7277
PCL Scaffold day 14	Control day 7	79.66667	40.03363	.568	-60.6056	219.9390
	PCL Scaffold day 7	246.00000*	40.03363	.000	105.7277	386.2723
	PCL/AgNPs Scaffold day 7	83.33333	40.03363	.513	-56.9390	223.6056

	Control day 14	31.33333	40.03363	.996	-108.9390	171.6056
	PCL/AgNPs Scaffold day 14	-113.66667	40.03363	.170	-253.9390	26.6056
	Control day 21	-53.66667	40.03363	.905	-193.9390	86.6056
	PCL Scaffold day 21	-113.33333	40.03363	.173	-253.6056	26.9390
	PCL/AgNPs Scaffold day 21	-303.66667*	40.03363	.000	-443.9390	-163.3944
	Control day 7	193.33333*	40.03363	.003	53.0610	333.6056
	PCL Scaffold day 7	359.66667*	40.03363	.000	219.3944	499.9390
	PCL/AgNPs Scaffold day 7	197.00000*	40.03363	.003	56.7277	337.2723
PCL/AgNPs	Control day 14	145.00000*	40.03363	.040	4.7277	285.2723
Scaffold day 14	PCL Scaffold day 14	113.66667	40.03363	.170	-26.6056	253.9390
	Control day 21	60.00000	40.03363	.842	-80.2723	200.2723
	PCL Scaffold day 21	.33333	40.03363	1.000	-139.9390	140.6056
	PCL/AgNPs Scaffold day 21	-190.00000*	40.03363	.004	-330.2723	-49.7277
	Control day 7	133.33333	40.03363	.070	-6.9390	273.6056
	PCL Scaffold day 7	299.66667*	40.03363	.000	159.3944	439.9390
	PCL/AgNPs Scaffold day 7	137.00000	40.03363	.059	-3.2723	277.2723
Control day 21	Control day 14	85.00000	40.03363	.489	-55.2723	225.2723
	PCL Scaffold day 14	53.66667	40.03363	.905	-86.6056	193.9390
	PCL/AgNPs Scaffold day 14	-60.00000	40.03363	.842	-200.2723	80.2723
	PCL Scaffold day 21	-59.66667	40.03363	.846	-199.9390	80.6056
	PCL/AgNPs Scaffold day 21	-250.00000*	40.03363	.000	-390.2723	-109.7277
	Control day 7	193.00000*	40.03363	.003	52.7277	333.2723
	PCL Scaffold day 7	359.33333*	40.03363	.000	219.0610	499.6056
	PCL/AgNPs Scaffold day 7	196.66667*	40.03363	.003	56.3944	336.9390
PCL Scaffold day	Control day 14	144.66667*	40.03363	.040	4.3944	284.9390
21	PCL Scaffold day 14	113.33333	40.03363	.173	-26.9390	253.6056
	PCL/AgNPs Scaffold day 14	-.33333	40.03363	1.000	-140.6056	139.9390
	Control day 21	59.66667	40.03363	.846	-80.6056	199.9390
	PCL/AgNPs Scaffold day 21	-190.33333*	40.03363	.004	-330.6056	-50.0610
	Control day 7	383.33333*	40.03363	.000	243.0610	523.6056
	PCL Scaffold day 7	549.66667*	40.03363	.000	409.3944	689.9390
	PCL/AgNPs Scaffold day 7	387.00000*	40.03363	.000	246.7277	527.2723
PCL/AgNPs	Control day 14	335.00000*	40.03363	.000	194.7277	475.2723
Scaffold day 21	PCL Scaffold day 14	303.66667*	40.03363	.000	163.3944	443.9390
	PCL/AgNPs Scaffold day 14	190.00000*	40.03363	.004	49.7277	330.2723
	Control day 21	250.00000*	40.03363	.000	109.7277	390.2723
	PCL Scaffold day 21	190.33333*	40.03363	.004	50.0610	330.6056

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

The significance value (p-value) is smaller than the α (0.05), meaning that ALP activity in hWJ-MSCs grown on PCL/AgNPs scaffold was significantly higher than ALP activity in hWJ-MSCs grown on PCL scaffold.