



Supplementary Information for

The Synthetic Artificial Stem Cell: Shifting the Paradigm of Cell Therapy in Regenerative Engineering

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Supplementary text

Figure S1

Tables S1 to S7 (not allowed for Brief Reports)

Supplementary Information Text**Supplementary Methods****Adipose Derived Stem Cell (ADSC) Characterization by Flow Cytometry**

Isolated ADSCs were characterized by flow cytometry using cell surface markers (Becton–Dickinson LSR II, BD Biosciences, USA) at passage 3 (P3). Cells were washed using sterile PBS, centrifuged and re-suspended in sterile Fluorescence –Activated Cell Sorting (FACS) buffer (PBS, 1% FBS) containing 10 µL of the FITC-conjugated CD29 antibody, Fluorescein isothiocyanate (FITC) conjugated CD90 antibody, FITC conjugated CD34, FITC conjugated CD45, and PE conjugated CD11b for 30 min. Unlabeled cells were used as controls. Cells were then scanned with FACS; acquired and gated using forward scatter (FSC) and side scatter (SSC) parameters to exclude cell debris and aggregates.

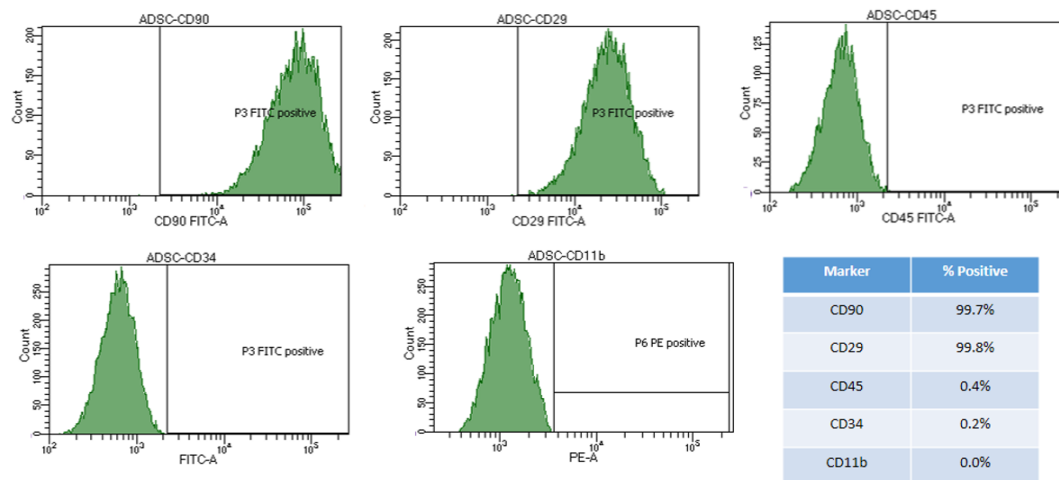


Fig. S1. Flow Cytometry Showing stemness of Isolated ADSCs

Table S1. Nitric Oxide % of IL-1 β ANOVA Summary

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Group	4	533932	133483	974.32	0
Error	251	34387	137		
Total	255	568319			
Grouping Information Using the Tukey Method and 95% Confidence					
Group	N	Mean	Grouping		
ADSC	47	104.69	A		
Blank MS	23	104.12	A		
IL-1B	66	100	A		
SASC	60	76.64		B	
Untreated	60	-10.474			C
Means that do not share a letter are significantly different.					
Difference of Levels	Difference of Means	SE of Difference	95% CI	T-Value	Adjusted P-Value
Blank MS - ADSC	-0.57	2.98	(-8.70, 7.56)	-0.19	1
IL-1B - ADSC	-4.69	2.23	(-10.79, 1.41)	-2.1	0.22
SASC - ADSC	-28.05	2.28	(-34.28, -21.83)	-12.3	0
Untreated - ADSC	-115.16	2.28	(-121.39, -108.94)	-50.51	0
IL-1B - Blank MS	-4.12	2.83	(-11.85, 3.62)	-1.45	0.593
SASC - Blank MS	-27.48	2.87	(-35.32, -19.65)	-9.57	0
Untreated - Blank MS	-114.59	2.87	(-122.43, -106.76)	-39.92	0
SASC - IL-1B	-23.36	2.09	(-29.06, -17.67)	-11.19	0
Untreated - IL-1B	-110.47	2.09	(-116.17, -104.78)	-52.91	0
Untreated - SASC	-87.11	2.14	(-92.94, -81.28)	-40.76	0
Individual confidence level = 99.32%					
Tukey Simultaneous 95% CIs					

Table S2. SOX9 Fold Change ANOVA Summary

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Group	4	6.3515	1.58786	133.93	0
Error	55	0.6521	0.01186		
Total	59	7.0035			
Tukey Pairwise Comparisons					
Grouping Information Using the Tukey Method and 95% Confidence					
Group	N	Mean	Grouping		
Untreated	15	1.0148	A		
SASC	12	0.4038		B	
IL-1B	15	0.2384			C
Blank MS	9	0.2312			C
ADSC	9	0.227			C
Means that do not share a letter are significantly different.					
Tukey Simultaneous Tests for Differences of Means					
Difference of Levels	Difference of Means	SE of Difference	95% CI	T-Value	Adjusted P-Value
Blank MS - ADSC	0.0042	0.0513	(-0.1406, 0.1490)	0.08	1
IL-1B - ADSC	0.0113	0.0459	(-0.1182, 0.1408)	0.25	0.999
SASC - ADSC	0.1768	0.048	(0.0413, 0.3122)	3.68	0.005
Untreated - ADSC	0.7878	0.0459	(0.6583, 0.9173)	17.16	0
IL-1B - Blank MS	0.0071	0.0459	(-0.1224, 0.1367)	0.16	1
SASC - Blank MS	0.1726	0.048	(0.0371, 0.3081)	3.59	0.006
Untreated - Blank MS	0.7836	0.0459	(0.6541, 0.9132)	17.07	0

SASC - IL-1B	0.1654	0.0422	(0.0465, 0.2844)	3.92	0.002
Untreated - IL-1B	0.7765	0.0398	(0.6643, 0.8886)	19.53	0
Untreated - SASC	0.611	0.0422	(0.4920, 0.7300)	14.49	0
Individual confidence level = 99.34%					
Tukey Simultaneous 95% CIs					

Table S3. ADAMTS5 Fold Change ANOVA Summary.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Group	4	77.8	19.4493	19.49	0
Error	51	50.89	0.9978		
Total	55	128.69			
Tukey Pairwise Comparisons					
Grouping Information Using the Tukey Method and 95% Confidence					
Group	N	Mean	Grouping		
Blank MS	3	5.0767	A		
IL-1B	15	3.723	A		
SASC	14	2.593		B	
ADSC	9	2.055		B	C
Untreated	15	1.0045			C
Means that do not share a letter are significantly different.					
Tukey Simultaneous Tests for Differences of Means					
Difference of Levels	Difference of Means	SE of Difference	95% CI	T-Value	Adjusted P-Value
Blank MS - ADSC	3.022	0.666	(1.138, 4.906)	4.54	0
IL-1B - ADSC	1.668	0.421	(0.477, 2.860)	3.96	0.002
SASC - ADSC	0.539	0.427	(-0.668, 1.746)	1.26	0.715
Untreated - ADSC	-1.05	0.421	(-2.242, 0.141)	-2.49	0.108
IL-1B - Blank MS	-1.354	0.632	(-3.141, 0.433)	-2.14	0.218
SASC - Blank MS	-2.483	0.636	(-4.281, -0.686)	-3.91	0.002
Untreated - Blank MS	-4.072	0.632	(-5.859, -2.285)	-6.45	0

SASC - IL-1B	-1.13	0.371	(-2.180, -0.080)	-3.04	0.029
Untreated - IL-1B	-2.719	0.365	(-3.750, -1.687)	-7.45	0
Untreated - SASC	-1.589	0.371	(-2.639, -0.539)	-4.28	0.001
Individual confidence level = 99.33%					
Tukey Simultaneous 95% CIs					

Table S4. PRG4 Fold Change ANOVA Summary

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Group	4	571508	142877	101.63	0
Error	19	26710	1406		
Total	23	598218			
Tukey Pairwise Comparisons					
Grouping Information Using the Tukey Method and 95% Confidence					
Group	N	Mean	Grouping		
ADSC	3	392.9	A		
IL-1B	6	377.39	A		
Blank MS	3	308.1	A		
SASC	6	159.1		B	
Untreated	6	1.0063			C
Means that do not share a letter are significantly different.					
Tukey Simultaneous Tests for Differences of Means					
Difference of Levels	Difference of Means	SE of Difference	95% CI	T-Value	Adjusted P-Value
Blank MS - ADSC	-84.7	30.6	(-176.7, 7.3)	-2.77	0.08
IL-1B - ADSC	-15.5	26.5	(-95.1, 64.2)	-0.58	0.976
SASC - ADSC	-233.8	26.5	(-313.5, -154.1)	-8.82	0
Untreated - ADSC	-391.8	26.5	(-471.5, -312.2)	-14.78	0
IL-1B - Blank MS	69.3	26.5	(-10.4, 149.0)	2.61	0.108
SASC - Blank MS	-149.1	26.5	(-228.7, -69.4)	-5.62	0
Untreated - Blank MS	-307.1	26.5	(-386.8, -227.4)	-11.58	0

SASC - IL-1B	-218.3	21.6	(-283.4, -153.3)	-10.09	0
Untreated - IL-1B	-376.4	21.6	(-441.4, -311.3)	-17.39	0
Untreated - SASC	-158	21.6	(-223.1, -93.0)	-7.3	0
Individual confidence level = 99.27%					
Tukey Simultaneous 95% CIs					

Table S5. % Degenerated Area ANOVA Summary

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Group	4	3169	792.17	9.63	0
Error	20	1645	82.24		
Total	24	4814			
Tukey Pairwise Comparisons					
Grouping Information Using the Tukey Method and 95% Confidence					
Group	N	Mean	Grouping		
OA	5	33.75	A		
Blank	5	30.61	A		
ADSC	5	10.62		B	
SASC	5	10.03		B	
Healthy	5	7.53		B	
Means that do not share a letter are significantly different.					
Tukey Simultaneous Tests for Differences of Means					
Difference of Levels	Difference of Means	SE of Difference	95% CI	T-Value	Adjusted P-Value
Blank - ADSC	19.99	5.74	(2.84, 37.15)	3.49	0.018
Healthy - ADSC	-3.09	5.74	(-20.25, 14.06)	-0.54	0.982
OA - ADSC	23.13	5.74	(5.98, 40.29)	4.03	0.005
SASC - ADSC	-0.6	5.74	(-17.75, 16.56)	-0.1	1
Healthy - Blank	-23.09	5.74	(-40.24, -5.93)	-4.03	0.005
OA - Blank	3.14	5.74	(-14.02, 20.29)	0.55	0.981
SASC - Blank	-20.59	5.74	(-37.74, -3.43)	-3.59	0.014
OA - Healthy	26.23	5.74	(9.07, 43.38)	4.57	0.002

SASC - Healthy	2.5	5.74	(-14.66, 19.65)	0.44	0.992
SASC - OA	-23.73	5.74	(-40.88, -6.57)	-4.14	0.004
Individual confidence level = 99.28%					
Tukey Simultaneous 95% CIs					

Table S6. Tibial Young's Modulus Linear Mixed Effects Model Summary

Term	DF Num	DF Den	F-Value	P-Value		
Group	4	627.05	35.81	0		
Tukey Pairwise Comparisons: Group						
Grouping Information Using the Tukey Method and 95% Confidence						
Group	N	Mean	Grouping			
Healthy	131	1.79277	A			
SASC	132	1.41259		B		
ADSC	132	1.35554		B		
Blank	132	1.05128			C	
OA	131	0.96371			C	
Means that do not share a letter are significantly different.						
Tukey Simultaneous Tests for Differences of Means						
Difference of Group Levels	Difference of Means	SE of Difference	DF	Simultaneous 95% CI	T-Value	Adjusted P-Value
Blank - ADSC	-0.3043	0.0774	627.022	(-0.5161, -0.0924)	-3.93	0.001
Healthy - ADSC	0.4372	0.0776	627.059	(0.2249, 0.6495)	5.63	0
OA - ADSC	-0.3918	0.0776	627.059	(-0.6041, -0.1795)	-5.05	0
SASC - ADSC	0.0571	0.0774	627.022	(-0.1548, 0.2689)	0.74	0.948
Healthy - Blank	0.7415	0.0776	627.059	(0.5292, 0.9538)	9.56	0
OA - Blank	-0.0876	0.0776	627.059	(-0.2999, 0.1247)	-1.13	0.791
SASC - Blank	0.3613	0.0774	627.022	(0.1494, 0.5732)	4.67	0
OA - Healthy	-0.8291	0.0778	627.105	(-1.0418, -0.6163)	-10.66	0
SASC - Healthy	-0.3802	0.0776	627.059	(-0.5925, -0.1679)	-4.9	0
SASC - OA	0.4489	0.0776	627.059	(0.2366, 0.6612)	5.78	0

Individual confidence level = 99.36%						
Tukey Simultaneous 95% CIs						

Table S7. Femoral Young's Modulus Linear Mixed Effects Model Summary

Term	DF Num	DF Den	F-Value	P-Value		
Group	4	320.09	28.86	0		
Tukey Pairwise Comparisons: Group						
Grouping Information Using the Tukey Method and 95% Confidence						
Group	N	Mean	Grouping			
Healthy	72	3.61372	A			
ADSC	68	3.00305		B		
SASC	70	2.34487			C	
OA	65	1.69795				D
Blank	65	1.69002				D
Means that do not share a letter are significantly different.						
Tukey Simultaneous Tests for Differences of Means						
Difference of Group Levels	Difference of Means	SE of Difference	DF	Simultaneous 95% CI	T-Value	Adjusted P-Value
Blank - ADSC	-1.313	0.224	320.763	(-1.928, -0.698)	-5.85	0
Healthy - ADSC	0.611	0.218	319.596	(0.012, 1.210)	2.8	0.043
OA - ADSC	-1.305	0.224	320.812	(-1.921, -0.690)	-5.82	0
SASC - ADSC	-0.658	0.22	319.848	(-1.262, -0.055)	-2.99	0.025
Healthy - Blank	1.924	0.221	320.143	(1.317, 2.530)	8.7	0
OA - Blank	0.008	0.227	320.66	(-0.614, 0.630)	0.03	1
SASC - Blank	0.655	0.223	320.552	(0.044, 1.266)	2.94	0.029
OA - Healthy	-1.916	0.221	319.751	(-2.522, -1.310)	-8.67	0
SASC - Healthy	-1.269	0.217	319.013	(-1.863, -0.675)	-5.86	0
SASC - OA	0.647	0.222	319.855	(0.037, 1.257)	2.91	0.032

Individual confidence level = 99.36%						
Tukey Simultaneous 95% CIs						