CellCor MSC CD AOF

First-in-Class Animal Origin Free Chemically Defined Media for the hMSC

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Scope	Mass production, research and drug development of MSC
Superiority	Convenience for culture (ready to use) No Coating material or supplement Superior Proliferation
Application	Adipose derived MSC Bone marrow derived MSC Umbilical cord derived MSC Various origin MSC
competitiveness	Stability of cell Safety, homogeneity

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Comparison of culture test with FBS and Commercial Medium

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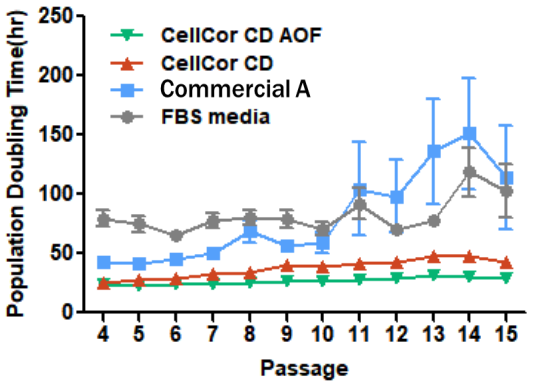
Proliferation

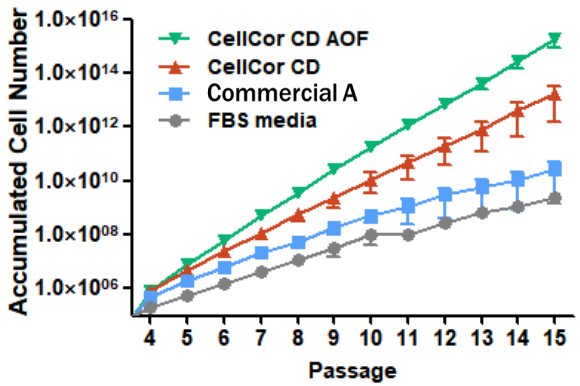
Test
- Test conducted to validate the media's proliferation rate

Methods
- Measure population doubling time (PDT)

PDT



ACN



Experimental Method: 1x10^5 cells /25 T flask seeding, Sub-culture at 85-90% confluency conditions
Measuring and analyzing instrument: NC-250

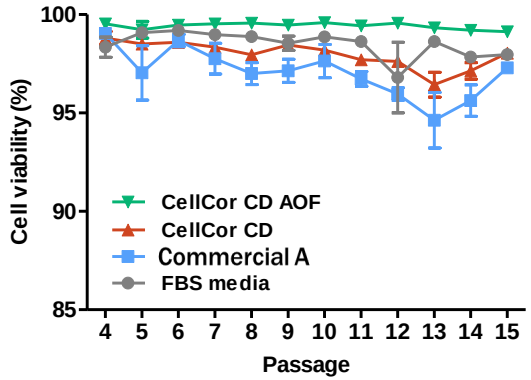
The proliferation rate of MSCs cultured with CellCor CD AOF was faster and more stable across the field compared to fetal bovine serum, serum-free media, and our CellCor CD. In addition, the cumulative number of cells obtained was significantly higher for MSCs cultured with CellCor CD AOF (Donor N=3 or more).

Cell Viability

Test
- AO•DAPI
  (Acridine Orange•4'-6-Diamidino-2-phenylindole)

Methods
- Viability is measured by the ratio of total cells stained with AO and non-viable cells stained with DAPI.

Viability



Experimental Method: 1x10^5 cells /25 T flask seeding, Sub-culture at 85-90% confluency conditions
Measuring and analyzing instrument: NC-250

All mesenchymal stem cells cultured with fetal bovine serum, serum-free medium, CellCor CD, and CellCor CD AOF were confirmed to have stable survival rates across all passages (Donor N=3 or more).

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Comparison of culture test with FBS and Commercial Medium

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Specific Marker

Test
- Purity (CD14, CD34, CD45)
- Identity (CD73, CD90, CD105)
- Immunogenicity (HLA-DR)

Methods
- Purity < 2%
- Identity > 95%
- Immunogenicity < 2%

Cell surface marker

	Surface marker	CellCor CD AOF	CellCor CD	FBS media	Commercial A
Purity (%)	CD14	0.5	0.2	0.7	10.3
	CD34	0.7	1.7	12.9	2.8
	CD45	0.6	0	0.7	0.9
Identity (%)	CD73	100	100	100	99.9
	CD90	100	100	99.9	99.9
	CD105	100	99.7	99.1	99.2
Immunogenicity (%)	HLA-DR	1.0	1.7	0.9	3.1

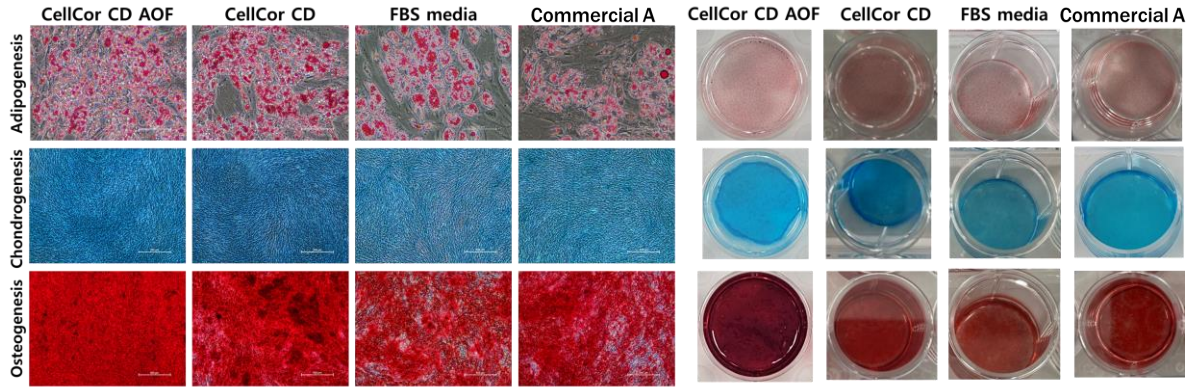
Experimental Method:
- $1x10^5$ cells/25 T flask seeding
- Analysis after 1 hour of staining with CD marker antibody
- Measurement and analysis instrument: Flow Cytometry

The surface markers were compared through flow cytometry after culturing ADSC with CellCor CD AOF, CellCor CD, fetal bovine serum, and serum-free medium. Specific surface markers that cultured with CellCor CD AOF were confirmed to be significantly maintained compared to other media (Donor N=3 or more, passage 10).

Differentiation

Test and Methods
- Adipogenesis : Oil red O Staining
- Chondrogenesis : Alcian blue Staining
- Osteogenesis : Alizarin red S Staining

Stain



Experimental Method:
- Adipogenesis: $2.1x10^5$ cells/12 well, Staining after 2 weeks of differentiation with StemPro™ Adipogenesis Differentiation Kit
- Chondrogenesis: $2.1x10^4$ cells/12 well, Staining after 2 weeks of differentiation with DMEM(High)+Ascorbic acid +Dexamethasone + TGF-β
- Osteogenesis: $2.1x10^4$ cells/12 well, Staining after 4 weeks of differentiation with StemPro™ Osteogenesis Differentiation Kit

Measuring and analysis instrument: NIKON TS2 microscope

When differentiated into Adipogenesis (adipocyte differentiation), Chondrogenesis (chondroblast differentiation), and Osteogenesis (osteoblast differentiation), respectively, after culturing ADSC with CellCor CD AOF, CellCor CD, fetal bovine serum, and serum-free medium, it was confirmed that ADSC differentiation ability was stably maintained compared to fetal bovine and serum-free medium (Donor N=3, passage 7).

Comparison of culture test with FBS and Commercial Medium

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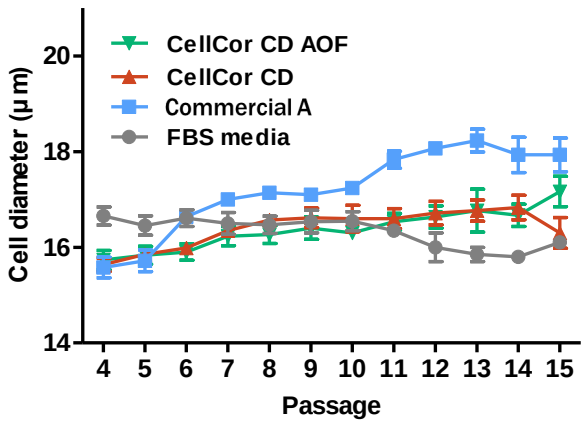
Senescence

Test
- Test that validates changes in cell senescence post sub-culture

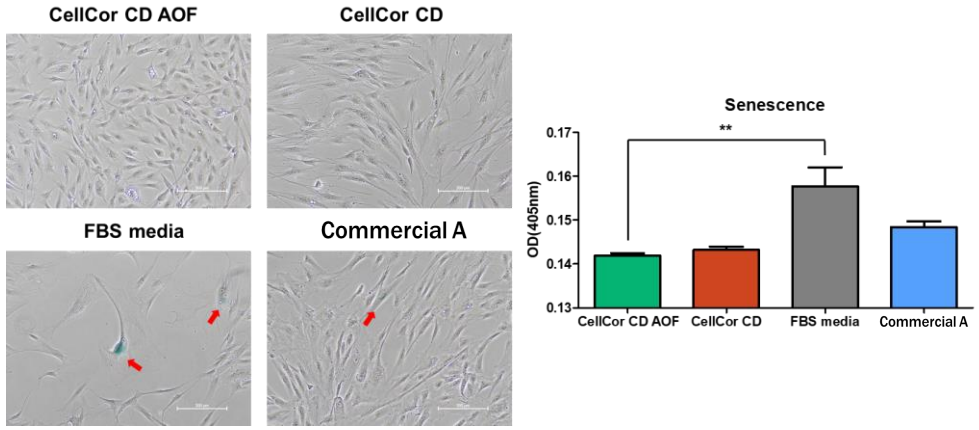
Methods
- Identify dyed senescent cells to measure the amount of senescent cells

Cell size



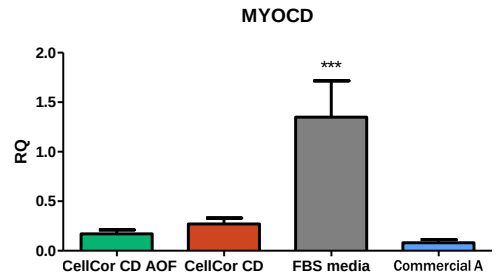
- Experimental Method: 1x10^5 cells/25 T flask seeding, Sub-culture at 85-90% confluency conditions
- Measuring and analyzing instrument: NC-250

Stain



- Experimental Method: 3.8x10^4 cells/6-well seeding, After 3 days, staining with X-gal reagent, absorbance measured at 405nm (**p < 0.01)
- Measuring and analyzing instrument: Microscope, Microplate reader

Senescence gene expression



- Experimental Method: 1x10^5 cells/25 T flask seeding, After 3 days, harvest ADSC and qRT-PCR (*** p<0.001)
- Measuring and analyzing instrument: QuantStudio Real-Time PCR

ADSC cultured with CellCor CD AOF showed the lowest senescence (Donor N=3, passage 7)

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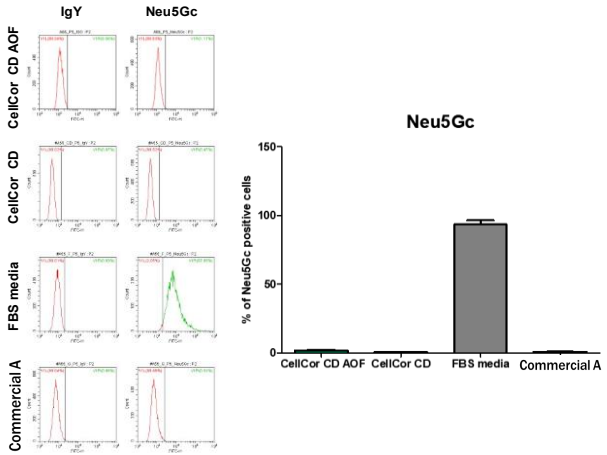
Comparison of culture test with FBS and Commercial Medium

Animal/Serum Derivative Identification Test

Test
- Animal-derived serum (Neu5Gc) Identification
- Human-derived serum (BDKRB1) Identification

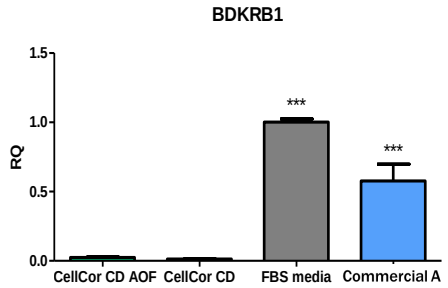
Methods
- Molecular Analysis of Neu5Gc Found in Mammals
- Identify BDKRB1 gene expression, a human-derived serum marker

Animal-derived serum (Neu5Gc) Identification



Experimental Method: Measured after 1 hour of staining with Neu5Gc antibody on mesenchymal stem cells cultured in their respective culture media.
Measurement and analysis instrument: Flow cytometry

Human-derived serum (BDKRB1) Identification



Experimental Method: 1x10^5 cells/25 T flask seeding/3 days later mesenchymal stem cell harvest, qRT-PCR (*** p<0.001)

Measurement and analyzer: QuantStudio Real-Time PCR

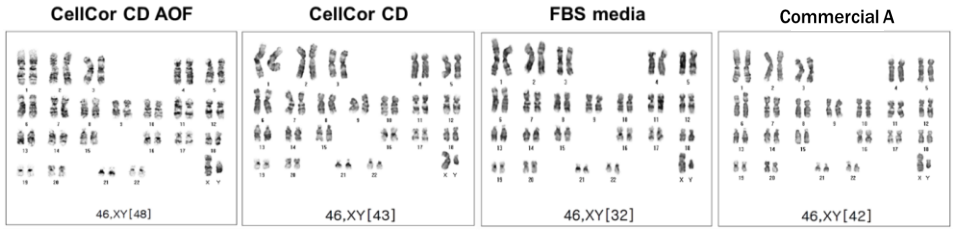
We checked animal-derived serum components and human-derived serum components in CellCor CD AOF and CellCor CD, fetal bovine serum, and serum-free media. We confirmed that CellCor CD AOF and CellCor CD media do not contain animal- or human-derived components (Donor N=3, passage 5).

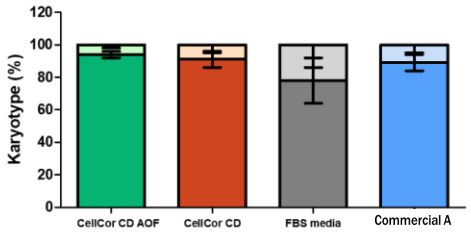
Genetic Stability Comparison Test

Test
- Identify genetic mutations and abnormal DNA regulation during cell division

Methods
- Staining of mesenchymal cells to determine number and morphology

Karyotype





Experimental method: 1x10^5 cells/25 T flask seeding/staining of chromosomes of metaphase cells to check for abnormal mutations such as checking the shape, number, and arrangement of chromosomes.

Measuring and analyzing equipment: Request for Gendix analysis

We confirmed less difference in karyotype between lots in CellCor CD AOF and CellCor CD, Commercial A compared to MSC cultured in FBS media (Donor N=2, passage 7).

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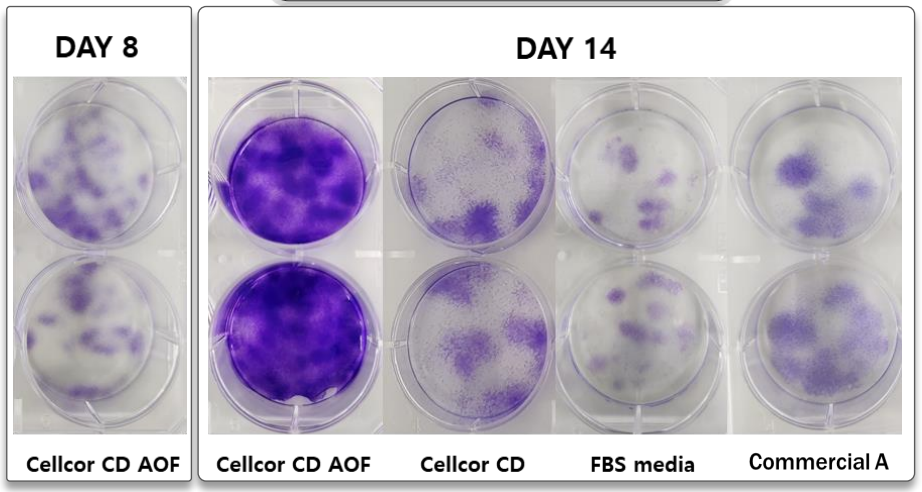
Cell Self-Renewal

Test
- Identify Colony Forming assay

Methods
- Cationic dye, crystal violet, stains negatively charged DNA, proteins, etc. in the cell purple

Colony Forming assay



Experimental method: $3x10^2$ cells/6 wells seeding/2 day medium change/2 weeks later staining with crystal violet

Measurement and analysis instrument: camera shooting

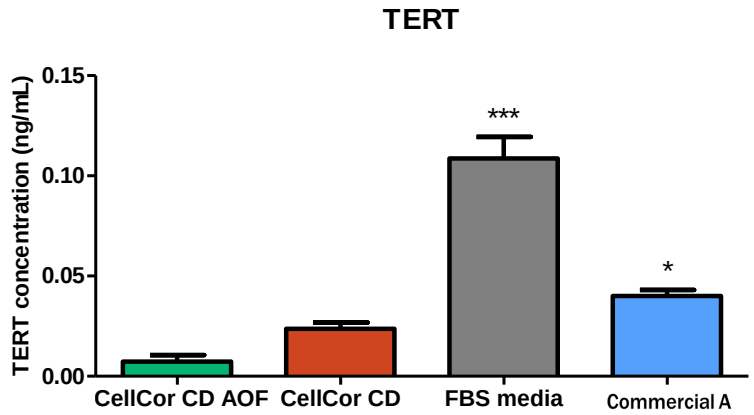
A colony forming assay was performed to determine self-renewal capacity after culturing ADSCs with CellCor CD AOF and CellCor CD, fetal bovine serum, and serum-free media. The highest self-renewal capacity was observed in ADSCs cultured with CellCor CD AOF.

Cell Tumorigenicity

Test
- Telomerase Reverse Transcriptase (TERT) Quantitative Test

Methods
- Sandwich ELISA assay for the quantitative measurement of Telomerase Reverse Transcriptase (450 nm)

TERT assay



Experimental Methods: Quantitative determination of Telomerase Reverse Transcriptase (450 nm) using culture medium after incubation of cells with each medium. (*p < 0.05, ***p < 0.001)

Measurement and analysis equipment: Microplate reader

A quantitative TERT assay was performed to determine the tumorigenicity of ADSCs cultured with CellCor CD AOF and CellCor CD, fetal bovine serum, and serum-free media. TERT expression was highest in ADSCs cultured with fetal bovine serum and lowest when cultured with CellCor CD AOF (N=3, passage 4).

Comparison of culture test with FBS and Commercial Medium

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Genetic Stability

Test
- Identify STR profile

Methods
- Analyze whether STR differences occur due to medium.
- BIONICS outsourcing service

STR profile

Sample	Sample	% Match	Interpretation
1. A90 FBS P2	2. A90 FBS P7	100%	Related (Same donor)
3. A90 Commercial A P2	4. A90 StemPro P7	100%	Related (Same donor)
5. A90 CD P2	6. A90 CD P7	100%	Related (Same donor)
7. A90 AOF P2	8. A90 AOF P7	100%	Related (Same donor)

Experimental method: After genomic DNA extraction, STR locus amplification by PCR and STR pattern analysis

Measurement and analysis instrument: Applied biosystems simpliAmp PCR cycler, Applied biosystems 3730XL DNA analyzer

According to STR analysis, it was confirmed that the STR pattern was not altered even after culturing for 5 passages in all 4 types of media (Donor N=2, passages 2, 7).

	Sample	Sample	Sample	Sample	Result
	AOF	CD	FBS	Commercial A	
Marker	Allele	Allele	Allele	Allele	
D8S1179	11 12	11 12	11 12	11 12	Comparison
D21S11	30	30	30	30	
D7S820	10 12	10 12	10 12	10 12	
CSF1PO	9 10	9 10	9 10	9 10	
D3S1358	15 17	15 17	15 17	15 17	
TH01	6.3 7 8.3 9	6.3 7 8.3 9	6.3 7 8.3 9	6.3 7 8.3 9	
D13S317	11 12	11 12	11 12	11 12	
D16S539	9 10	9 10	9 10	9 10	
D2S1338	17 23	17 23	17 23	17 23	
D19S433	14	14	14	14	
Vwa	14 17	14 17	14 17	14 17	
TPOX	8 11	8 11	8 11	8 11	
D18S51	14	14	14	14	
AMEL	X Y	X Y	X Y	X Y	
D5S818	8 9 12 13	8 9 12 13	8 9 12 13	8 9 12 13	
FGA	22 23 24.2	22 23 24.2	22 23 24.2	22 23 24.2	

Comparison of culture test with FBS and Commercial Medium

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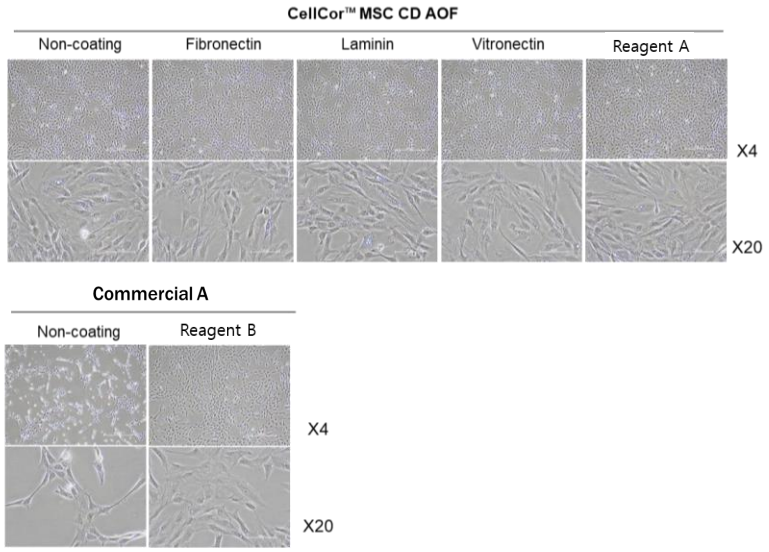
Cell Culture Coating Verification Test

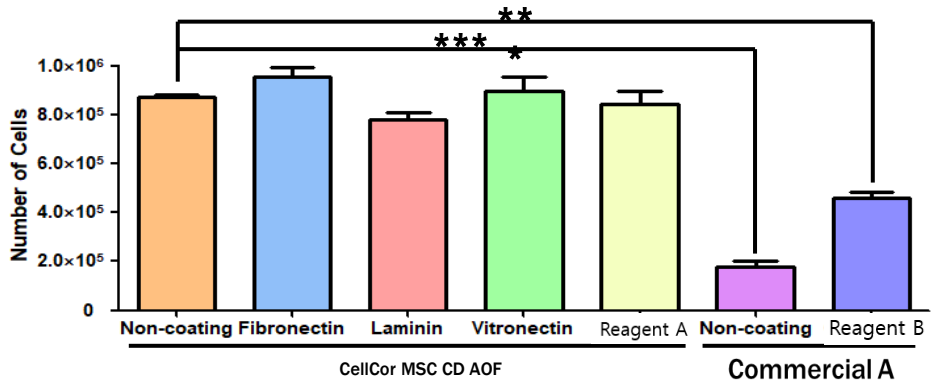
Test
- Verify Cell Proliferation.

Methods
- Verify the number of cells acquired

Cell Morphology



Acquired Cell Number



Experimental Method : 1x10^5 cells/25 T flasks seeded and sub-cultured after 72 hours to determine cell number (*** p<0.001)

Measurement and analyzer : Nikon TS2 Microscope, Chemometec NC-250

We tested for cell culture with CellCor MSC CD AOF and Commercial A media. The Commercial A showed a difference in cell culture with and without coating, but CellCor MSC CD AOF worked well for cell culture regardless of coating (N=2, passage 4).

Comparison of culture test with FBS and Commercial Medium

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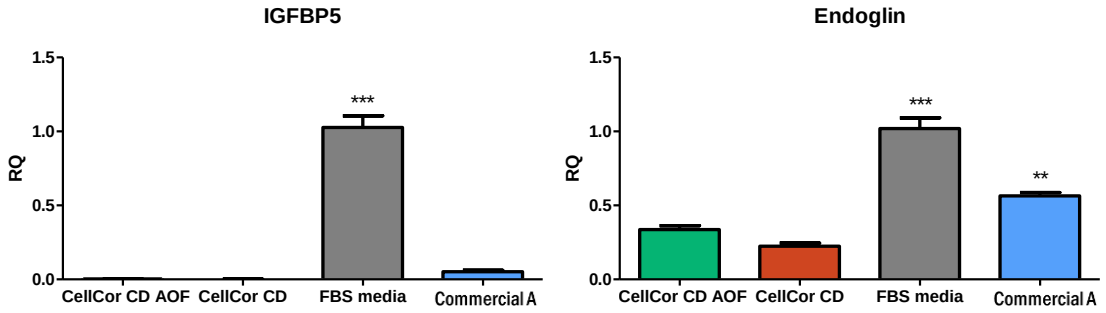
Gene expression analysis and functional validation

Test
- Analysis of senescence related gene expression

Methods
- Identify of expression of senescence-related genes IGFBP5 (Insulin-like growth factor binding protein 5) and Endoglin

Senescence-related gene expression



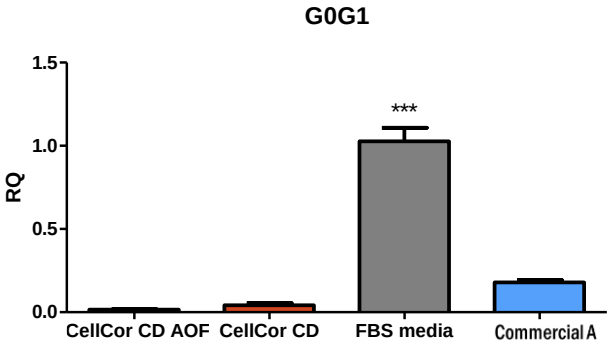
Experimental Method: 1x10^5 cells/25 T flasks seeding/3 days later mesenchymal stem cell harvest, qRT-PCR confirmation, (*p < 0.01, ***p < 0.001)
Measurement and analyzer: QuantStudio Real-Time PCR

Senescence-related gene expression was highest in culture with fetal bovine serum medium and it showed lower expression in mesenchymal stem cells cultured with CellCor CD AOF (Donor N=3, passage 7).

Test
- Analysis of apoptosis related gene expression.

Methods
- Identify of expression of apoptosis-related gene G0G1

Apoptosis-related gene expression



Experimental Method: 1x10^5 cells/25 T flasks seeding/3 days later mesenchymal stem cell harvest, qRT-PCR confirmation, (***p < 0.001)
Measurement and analyzer: QuantStudio Real-Time PCR

Apoptosis-related gene expression was highest in culture with fetal bovine serum medium and it showed lower expression in mesenchymal stem cells cultured with CellCor CD AOF (Donor N=3, passage 7).

Comparison of culture test with FBS and Commercial Medium

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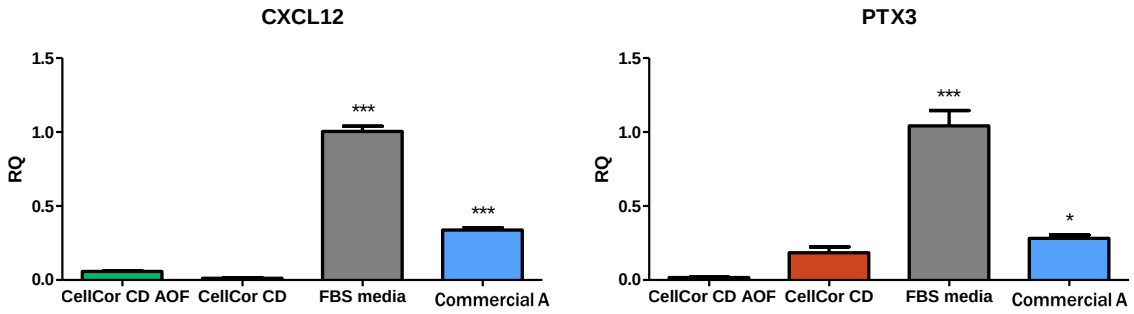
Gene expression analysis and functional validation

Test
- Analyze immune response-related genes

Methods
- Identify expression of immune response-related genes CXCL12 (C-X-C motif chemokine 12), PTX3 (Pentraxin 3)

Immune response related gene expression



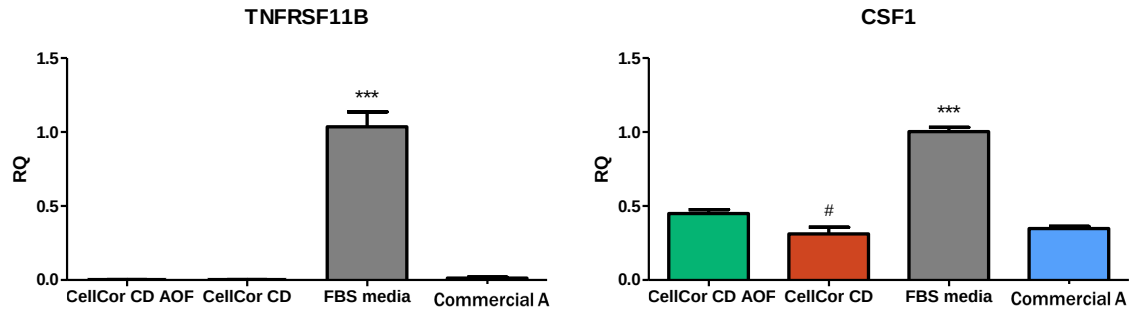
Experimental Method: 1x10^5 cells/25 T flasks seeding/3 days later mesenchymal stem cell harvest, qRT-PCR confirmation, (*p < 0.05, ***p < 0.001)
Measurement and analyzer: QuantStudio Real-Time PCR

Immune response-related gene expression was highest in culture with fetal bovine serum medium and it showed lower expression in mesenchymal stem cells cultured with CellCor CD AOF (Donor N=3, passage 7).

Test
- Analyze inflammatory response-related genes

Methods
- Identify expression of inflammatory response-related genes TNFRSF11B (tumor necrosis factor receptor superfamily member 11b) ,CSF1 (colony-stimulating factor 1).

Inflammatory response related gene expression



Experimental Method: 1x10^5 cells/25 T flasks seeding/3 days later mesenchymal stem cell harvest, qRT-PCR confirmation, (*p < 0.05, ***p < 0.001)
Measurement and analyzer: QuantStudio Real-Time PCR

Inflammatory response-related gene expression was highest in culture with fetal bovine serum medium and it showed lower expression in mesenchymal stem cells cultured with CellCor CD AOF (Donor N=3, passage 7).

Comparison of culture test with FBS and Commercial Medium

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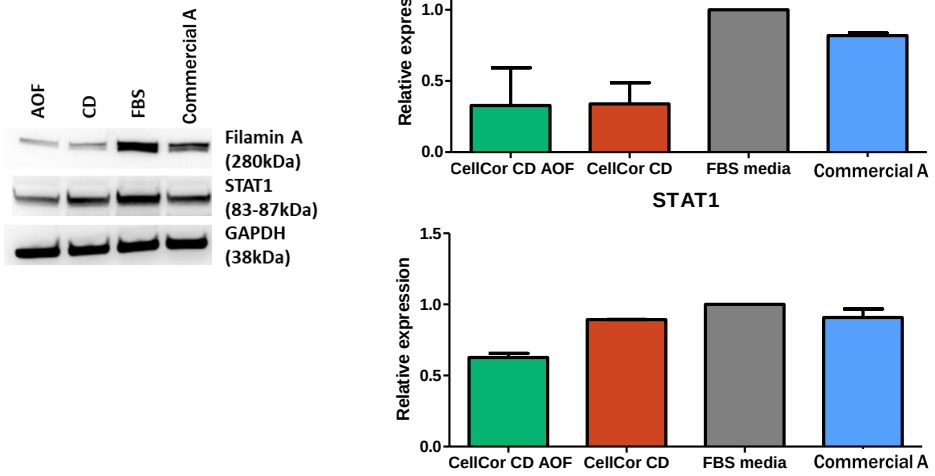
Protein expression analysis and functional validation

Test
- Apoptosis-related protein analysis

Methods
- Identify expression of apoptosis-related proteins filamin A and STAT1 (signal transduction and activator of transcription 1)

Apoptosis-related protein analysis



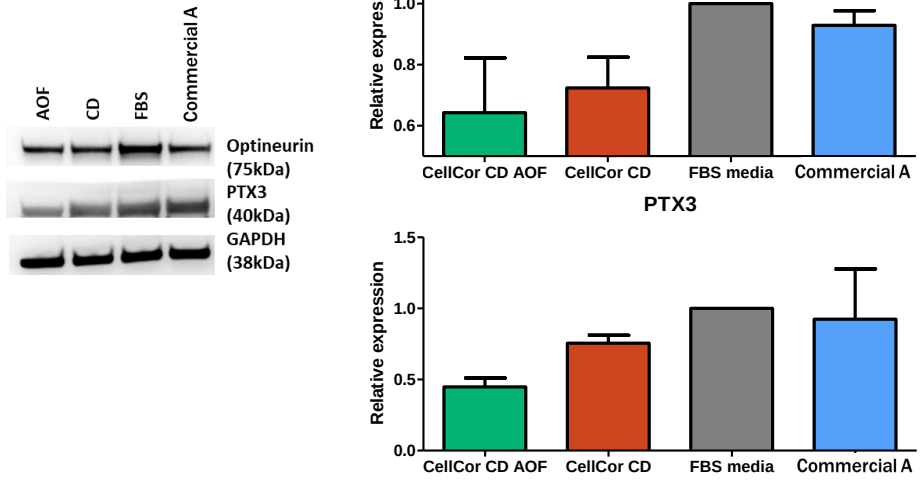
Experimental Method: 1x10^5 cells/25 T flasks seeded/3 days later, mesenchymal stem cells harvested, protein expression confirmed by Western blot.
Measurement and analyzer : Image J, iBright CL1000

Apoptosis-related protein expression was higher in ADSCs cultured in fetal bovine serum medium and lower in ADSCs cultured in CellCor CD AOF (Donor N=2, passage 7).

Test
- Inflammatory response-related protein analysis

Methods
- Identify the expression of PTX3 (Pentraxin 3), an inflammatory response-related protein, and Optineurin

Inflammatory response-related protein analysis



Experimental Method: 1x10^5 cells/25 T flasks seeded/3 days later, mesenchymal stem cells harvested, protein expression confirmed by Western blot.
Measurement and analyzer : Image J, iBright CL1000

Inflammatory response-related protein expression was higher in ADSCs cultured with fetal bovine serum medium and lower in ADSCs cultured with CellCor CD AOF. (Donor N=2, passage 7)

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Comparison of culture test with FBS and Commercial Medium

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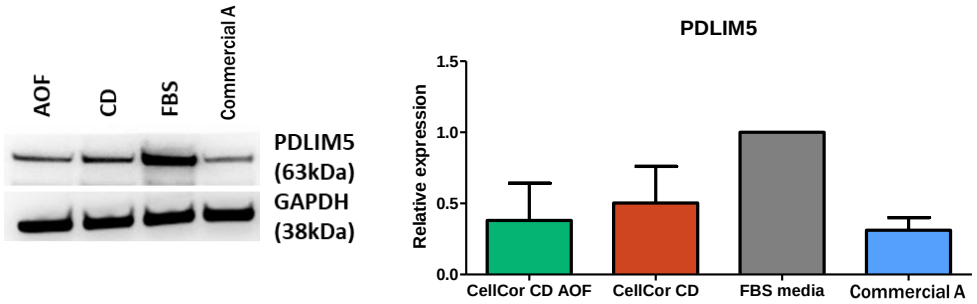
Protein expression analysis and functional validation

Test
- Oncogene candidate-related protein analysis

Methods
- Identify expression of oncogene candidate-related protein PDLIM5 (PDZ and LIM domain protein 5)

Oncogene candidate-related protein analysis



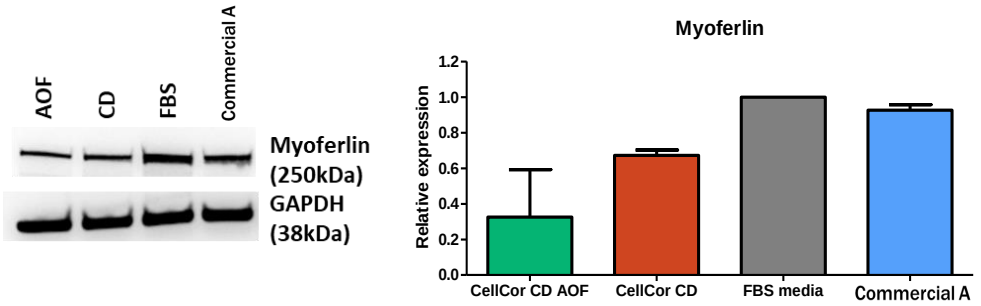
Experimental Method: 1x10^5 cells/25 T flasks seeded/3 days later, mesenchymal stem cells harvested, protein expression confirmed by Western blot.
Measurement and analyzer : Image J, iBright CL1000

Oncogene candidate-related protein expression was higher in ADSCs cultured in fetal bovine serum medium and lower in ADSCs cultured in CellCor CD AOF (Donor N=2, passage 7).

Test
- Cancer overexpression-related protein analysis

Methods
- Identify expression of cancer overexpression-related protein MYOF (Myoferlin)

Cancer overexpression-related protein analysis



Experimental Method: 1x10^5 cells/25 T flasks seeded/3 days later, mesenchymal stem cells harvested, protein expression confirmed by Western blot.
Measurement and analyzer : Image J, iBright CL1000

Cancer overexpression-related protein expression was higher in ADSCs cultured in fetal bovine serum medium and lower in ADSCs cultured in CellCor CD AOF (Donor N=2, passage 7).

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Comparison of culture test with FBS and Commercial Medium

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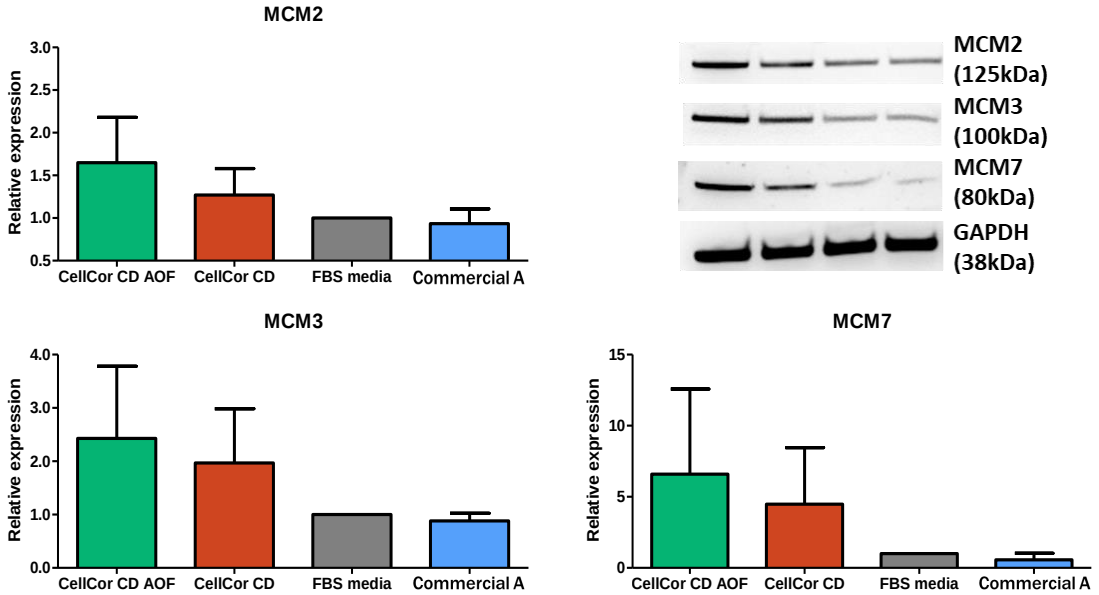
Protein expression analysis and functional validation

Test
- DNA repair-related protein analysis

Methods
- Identify expression of DNA repair-related protein MCM (minichromosome maintenance) 2, 3, 7

DNA repair-related protein analysis



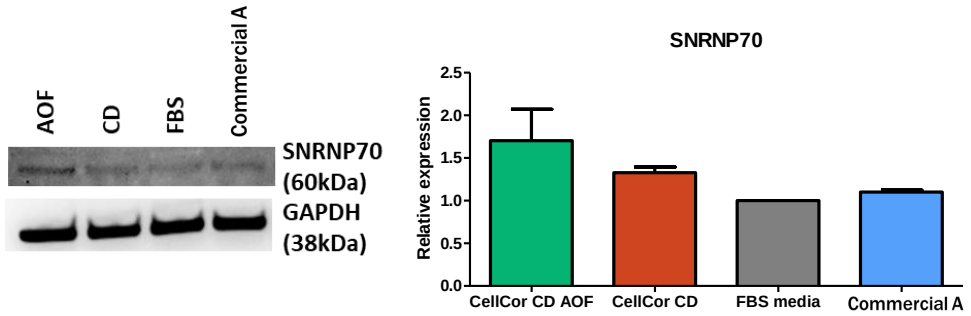
Experimental Method: 1x10^5 cells/25 T flasks seeded/3 days later, mesenchymal stem cells harvested, protein expression confirmed by Western blot.
Measurement and analyzer : Image J, iBright CL1000

DNA repair-related protein expression was higher in ADSCs cultured in CellCor CD AOF and lower in ADSCs cultured in fetal bovine serum medium (Donor N=2, passage 7).

Test
- Stabilization of mRNA-related protein analysis

Methods
- Identify expression of stabilization of mRNA-related protein SNRNP70 (Small nuclear ribonucleoprotein 70 kDa)

Stabilization of mRNA-related protein analysis



Experimental Method: 1x10^5 cells/25 T flasks seeded/3 days later, mesenchymal stem cells harvested, protein expression confirmed by Western blot.
Measurement and analyzer : Image J, iBright CL1000

Stabilization of mRNA-related protein expression was higher in ADSCs cultured in CellCor CD AOF and lower in ADSCs cultured in fetal bovine serum medium (Donor N=2, passage 7).

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Comparison of culture test with FBS and Commercial Medium

Protein expression analysis and functional validation

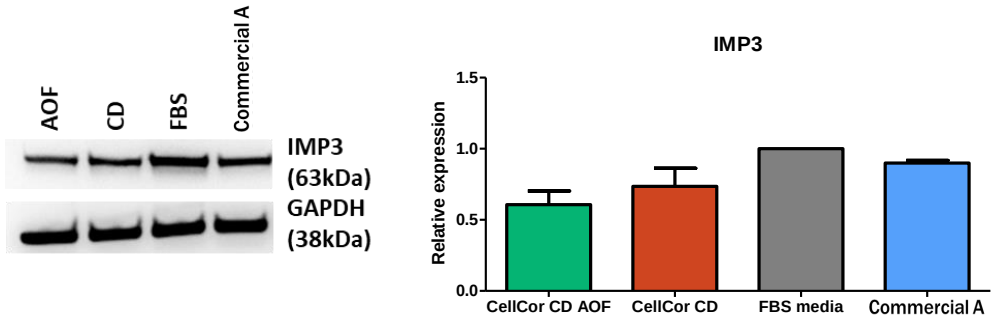
Test

- Malignancy-related protein analysis

Methods

- Identify expression of malignancy-related protein IMP3 (Insulin-like growth factor II mRNA binding protein 3)

Malignancy-related protein analysis



Experimental Method: 1x10^5 cells/25 T flasks seeded/3 days later, mesenchymal stem cells harvested, protein expression confirmed by Western blot.
Measurement and analyzer : Image J, iBright CL1000

Malignancy-related protein expression was higher in ADSCs cultured in fetal bovine serum medium and lower in ADSCs cultured in CellCor CD AOF (Donor N=2, passage 7).

Comparison of culture test with Commercial CD medium

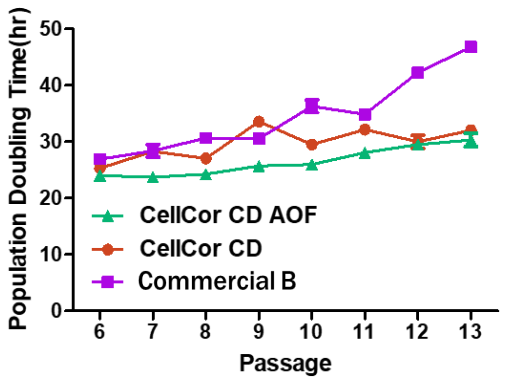
Distributed by: CliniSciences Group

Proliferation

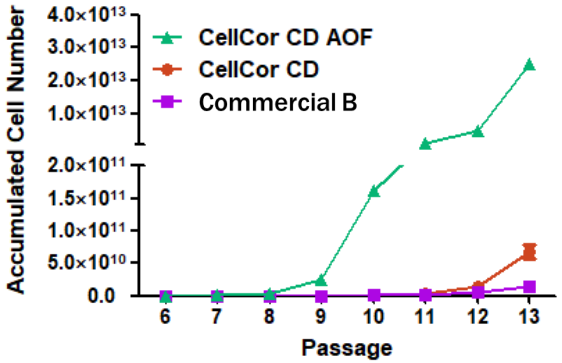
Test
- Test conducted to validate the media's proliferation rate

Methods
- Measure population doubling time (PDT)

PDT



ACN



Experimental Method: 1x10^5 cells /25 T flask seeding, Sub-culture at 85-90% confluency conditions
Measuring and analyzing instrument: NC-250

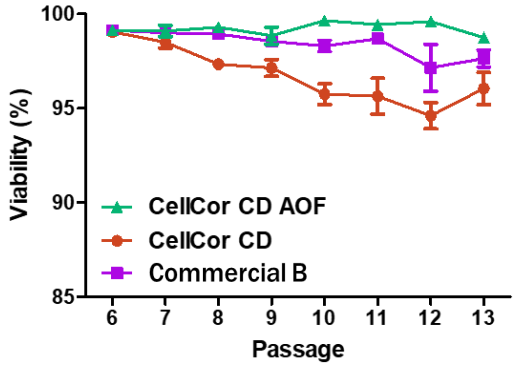
The proliferation rate of MSCs cultured with CellCor CD AOF was faster and more stable across the field compared to Commercial B, and our CellCor CD. In addition, the cumulative number of cells obtained was significantly higher for MSCs cultured with CellCor CD AOF.

Viability

Test
- AO•DAPI
  (Acridine Orange•4'-6-Diamidino-2-phenylindole)

Methods
- Viability is measured by the ratio of total cells stained with AO and non-viable cells stained with DAPI.

Viability



Experimental Method: 1x10^5 cells /25 T flask seeding, Sub-culture at 85-90% confluency conditions
Measuring and analyzing instrument: NC-250

All mesenchymal stem cells cultured with CellCor CD AOF, CellCor CD and Commercial B were confirmed to have stable survival rates across all passages.

XCELL

Comparison of culture test with Commercial CD medium

Distributed by: CliniSciences Group

Specific Marker

Test
- Purity (CD14, CD34, CD45)
- Identity (CD73, CD90, CD105)
- Immunogenicity (HLA-DR)

Methods
- Purity < 2%
- Identity > 95%
- Immunogenicity < 2%

Cell surface marker

	Surface marker	CellCor CD AOF	CellCor CD	Commercial B
Purity (%)	CD14	0.5	0.01	0.05
	CD34	0.7	3.83	15.77
	CD45	0.6	0.01	0.08
Identity (%)	CD73	100	99.88	99.55
	CD90	100	99.69	98.30
	CD105	100	98.75	99.33
Immunogenicity (%)	HLA-DR	1.0	0.01	0.02

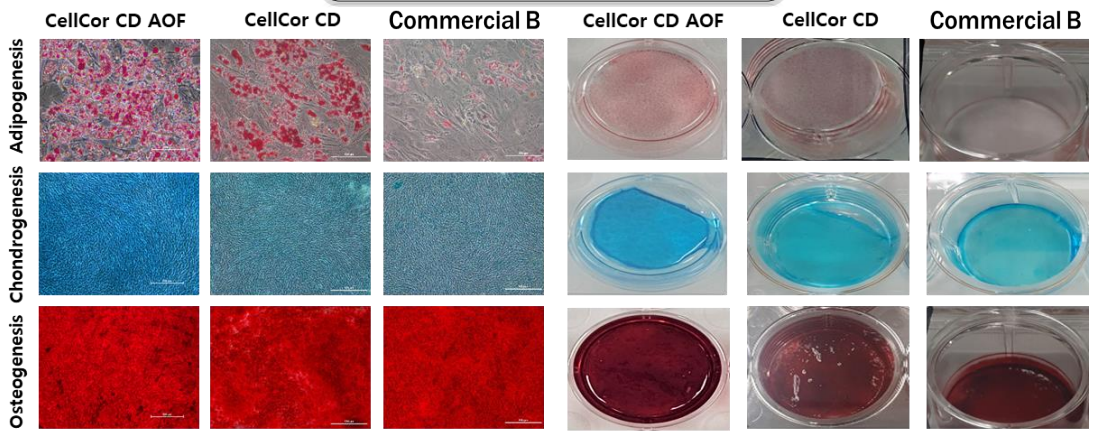
Experimental Method: $1x10^5$ cells/25 T flask seeding, Analysis after 1 hour staining with CD marker antibody
Measuring and analyzing instrument: Flow Cytometry

After culturing ADSC with CellCor CD AOF, CellCor CD and Commercial B, the surface markers were compared through flow cytometry. Specific surface markers that cultured with CellCor CD AOF were confirmed to be significantly maintained compared to other media (Passage 10).

Differentiation

Test and Methods
- Adipogenesis : Oil red O Staining
- Chondrogenesis : Alcian blue Staining
- Osteogenesis : Alizarin red S Staining

Stain



Experimental Method:
- Adipogenesis: $2.1x10^5$ cells/12 well, Staining after 2 weeks of differentiation with StemPro™ Adipogenesis Differentiation Kit
- Chondrogenesis: $2.1x10^4$ cells/12 well, Staining after 2 weeks of differentiation with DMEM(High)+Ascorbic acid +Dexamethasone + TGF-β
- Osteogenesis: $2.1x10^4$ cells/12 well, Staining after 4 weeks of differentiation with StemPro™ Osteogenesis Differentiation Kit

Measuring and analyzing instrument: NIKON TS2 microscope

When differentiated into Adipogenesis (adipocyte differentiation), Chondrogenesis (chondroblast differentiation), and Osteogenesis (osteoblast differentiation), respectively, after culturing ADSC with CellCor CD AOF, CellCor CD and Commercial B. It was confirmed that ADSC differentiation ability was stably maintained compared to Commercial B (Passage 10).

XCELL

Comparison of culture test with Commercial CD medium

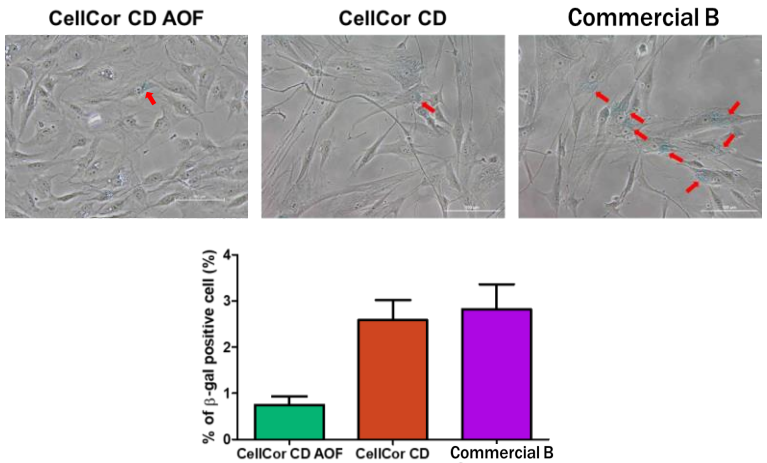
Distributed by: CliniSciences Group

Senescence

Test
- Test that validates changes in cell senescence post sub-culture

Methods
- Identify dyed senescent cells to measure the amount of senescent cells

Stain



Experimental Method: 3.8x10^4 cells/6-well seeding, After 3 days, staining with X-gal reagent
Measuring and analyzing instrument: Microscope

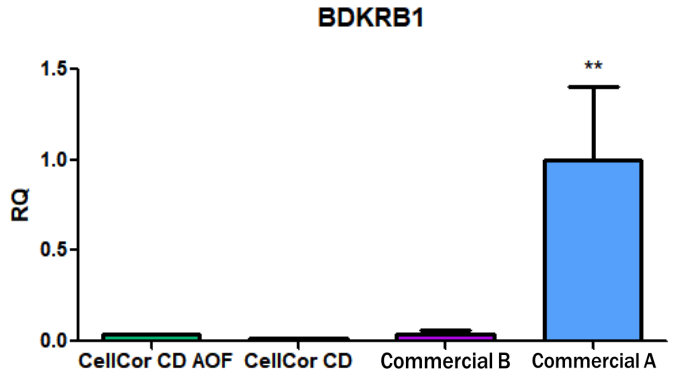
ADSC cultured with CellCor CD AOF showed the lowest senescence (Passage 6).

Human Serum Derivative Identification Test

Test
- Human-derived serum (BDKRB1) Identification

Methods
- Identify BDKRB1 gene expression, a human-derived serum marker

Human-derived serum (BDKRB1) Identification



Experimental Method: 1x10^5 cells/25 T flask seeding/3 days later mesenchymal stem cell harvest, qRT-PCR (*** p<0.001)

Measurement and analyzer: QuantStudio Real-Time PCR

We checked human-derived serum components in CellCor CD AOF, CellCor CD, Commercial A and Commercial B media. We confirmed that CellCor CD AOF, CellCor CD and Commercial B media do not contain animal-derived or human-derived components (Passage 6).

XCELL

Comparison of culture test with Commercial CD medium

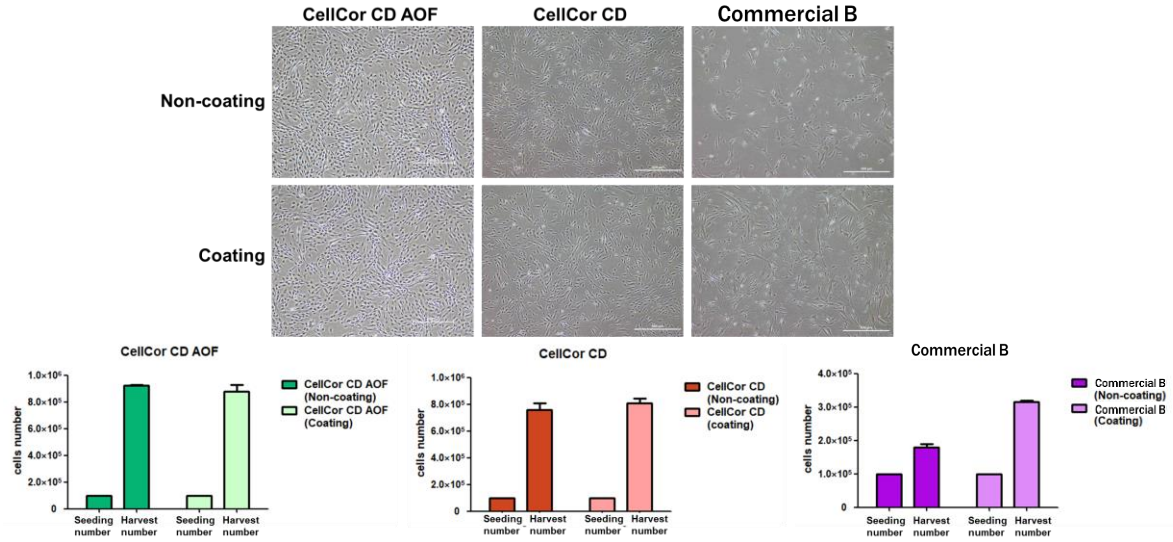
Distributed by: CliniSciences Group

Cell Culture Coating Verification Test

Test
- Verify of cell attachment depending on coating material.

Methods
- After cell culturing 72 hours, analysis of the adhesion rate depending on coating materials.

Cell attachment



Experimental Method : 1x10^5 cells/25 T flasks seeded and sub-cultured after 72 hours to determine cell number (*** p<0.001)
Measurement and analyzer : NC-250

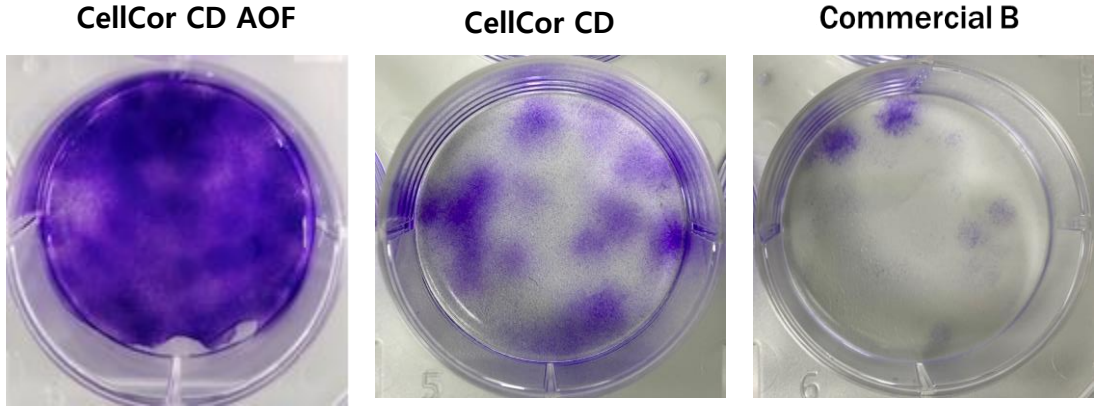
While there was no difference in cell culture between CellCor CD AOF and CellCor CD depending on the presence or absence of coating on the culture dish, we found a difference in cell culture of Commercial B on the presence or absence of coating (Passage 10).

Self-renewal

Test
- Identify Colony Forming assay

Methods
- Cationic dye, crystal violet, stains negatively charged DNA, proteins, etc. in the cell purple

Colony Forming assay



Experimental method: 3x10^2 cells/6 wells seeding/2 day medium change/2 weeks later staining with crystal violet
Measurement and analysis instrument: camera shooting

After culturing ADSCs with CellCor CD AOF, CellCor CD and Commercial B medium, a colony forming assay was performed to determine self-renewal capacity. The highest self-renewal capacity was observed in ADSCs cultured with CellCor CD AOF (Passage 2).

Comparison of culture test with various tissue origin

Distributed by: CliniSciences Group

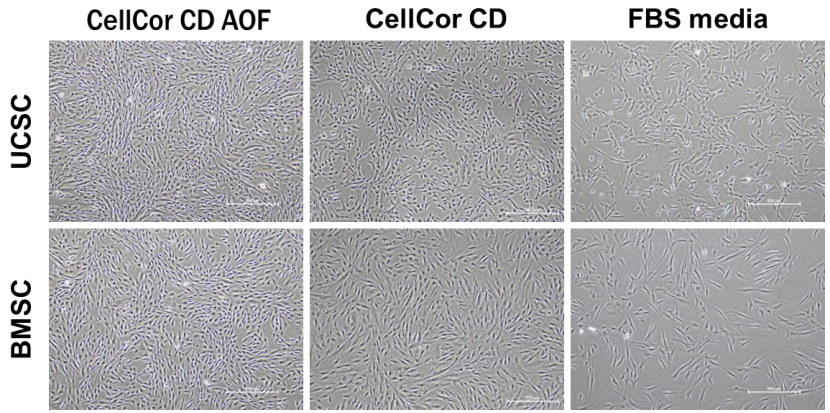
Cell morphology

Test
- Identify stem cell shapes by tissue origin

Methods
- Verify morphology after stem cell culture by tissue origin

Identify stem cell shapes by tissue origin

	Stem cell	Tissue
UCSC	Umbilical Cord-derived Stem Cell (UCSC)	Umbilical Cord
BMSC	Bone Marrow-derived Stem Cell (BMSC)	Bone Marrow



Experimental Method: 1x105 cells/25 T flask seeding , cell shape imaging (4X) under 85-90% confluency conditions
Measurement and analysis instrument : Microscope

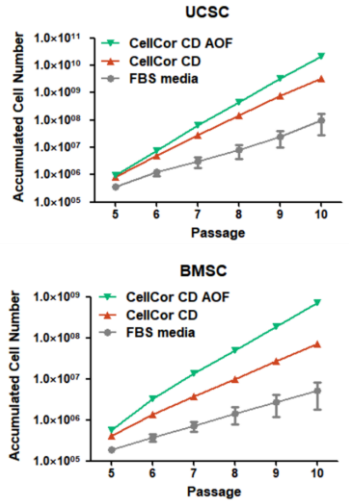
We checked stem cell shape by tissue origin in CellCor CD AOF and CellCor CD MSC. We confirmed that stem cells cultured in CellCor CD AOF and CellCor CD MSC were smaller in size and maintained a more spindle-like shape compared to stem cells cultured in fetal bovine serum medium (passage 5).

Cell proliferation and viability assays

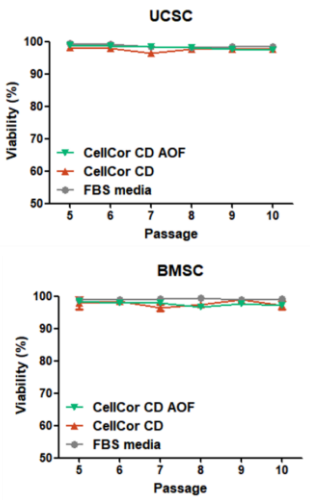
Test
- ACN (Accumulated Cell Number)
- AO•DAPI (Acridine Orange•4'-6-Diamidino-2-phenylindole)

Methods
- (cumulative cell count/cells seeded) x number of cells obtained
- Measures viability as the ratio of total cells stained with AO to non-viable cells stained with DAPI

ACN



Viability



Experimental Method: 1x105 cells/25 T flask seeding , sub-culture at 85-90% confluency conditions
Measuring and analyzing instrument : NC250

CellCor CD AOF and CellCor CD MSC were observed to have a higher proliferation rate compared to fetal bovine serum medium. The cell viability was stable across the field (UCSC, BMSC Donor N=2).

XCELL

Comparison of culture test with various tissue origin

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Specific Marker Expression

Test
- Purity (CD14, CD34, CD45)
- Identity (CD73, CD90, CD105)
- Immunogenicity (HLA-DR)

Methods
- Purity < 2%
- Identity > 95%
- Immunogenicity < 2%

Cell surface marker

UCSC	Surface marker	CellCor CD AOF	CellCor CD	FBS media
Purity (%)	CD14	0.47	0.06	0.1
	CD34	0.86	0.23	0.27
	CD45	0.25	0.12	0.1
Identity (%)	CD73	99.99	99.82	99.71
	CD90	99.99	99.79	99.67
	CD105	99.77	99.42	99.73
Immunogenicity (%)	HLA-DR	0.14	0.52	0.15

BMSC	Surface marker	CellCor CD AOF	CellCor CD	FBS media
Purity (%)	CD14	0.35	0.02	0.28
	CD34	1.25	0.19	0.2
	CD45	0.24	0.02	0.24
Identity (%)	CD73	99.98	99.9	98.64
	CD90	99.99	99.98	97.89
	CD105	99.93	99.74	97.21
Immunogenicity (%)	HLA-DR	0.15	0	29

Experimental Method: $1x10^5$ cells/25 T flask seeding, Analysis after 1 hour staining with CD marker antibody
Measuring and analyzing instrument: Flow Cytometry

After culturing UCSC and BMSC with CellCor CD AOF, CellCor CD and fetal bovine serum, the surface markers were compared through flow cytometry. Specific surface markers that cultured with CellCor CD AOF were confirmed to be significantly maintained compared to other media (UCSC, BMSC Donor N=2, Passage 7)

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