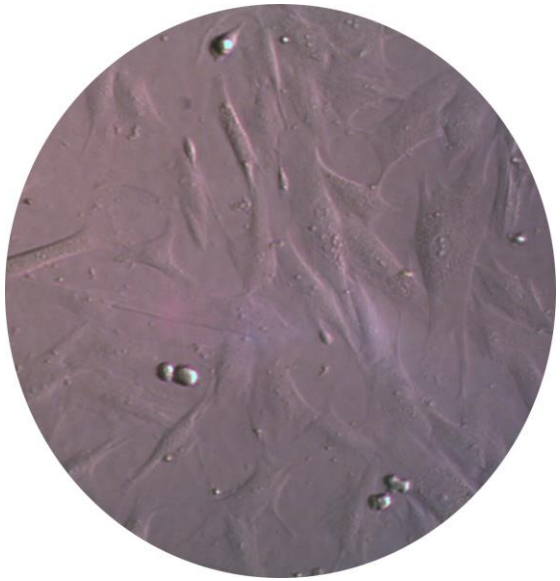
AVANTBIO™

Chemically-defined, Animal Origin-free (cdAOF) Cell Culture Systems for Human Fibroblasts and Other Mesenchymal Cells



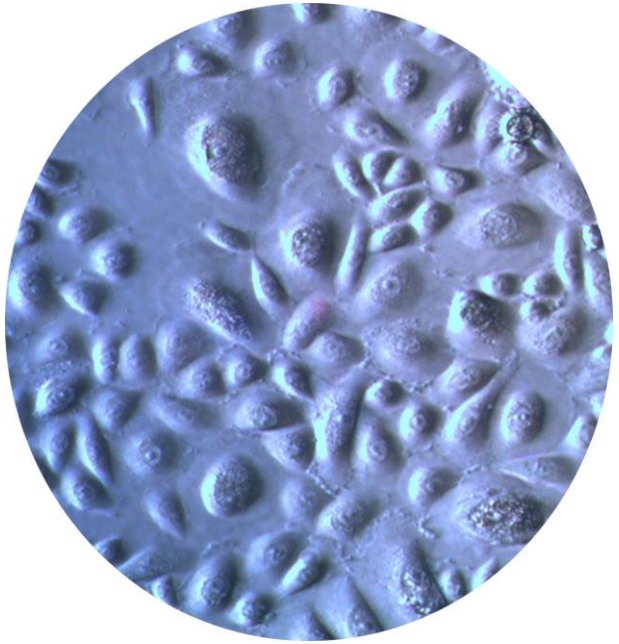


Table of Contents:

Historically, many cell- and tissue-based therapy protocols utilize cell culture media that includes the use of components that are originated from human or non-human animal (e.g. bovine, porcine, rodent) sources. these animal-derived components include:

- *Blood-derived Serum and Plasma*
- *Serum-derived Albumin, Transferrin and Fetuin Protein*
- *Replication Incompetent Feeder Layer Cells (both mortal and immortal cell lines)*
- *Pituitary Gland Tissue Extract*
- *Placental Amniotic Membranes*
- *Extracellular Matrix Proteins*
- *Blood-derived Platelet Lysate*
- *Liver Extract*

Motivation for cell- and engineered tissue-based therapy companies to adopt chemically-defined animal Origin-free (cdAOF) cell culture systems:

- *Lessens the risk of animal component originated adverse events, that include the transfer of animal-originated pathogens (e.g., mad cow/BSE, viruses) to humans as well as inflammatory (allergic) reactions to animal-derived cell culture media components*

- *Reduces the potential for cell and tissue therapy product variability by using quality-tested, controllable, chemically defined cell culture components (i.e. precise control of cell and tissue culture manufacturing conditions)*

- *Pressure from regulatory authorities (FDA, EMA) to pursue cdAOF Cell Culture Options*

- *Can reduce the cost of manufacturing therapeutic cells and tissue, especially in light of recent qualified fetal bovine serum (FBS) shortages as well as the increasing cost for qualified FBS and other animal components*

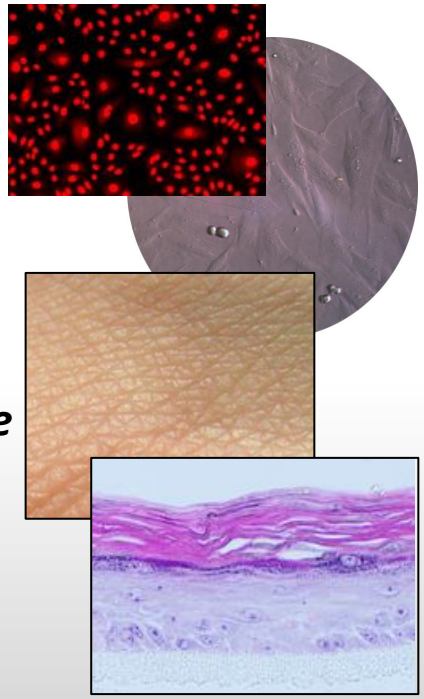
- *Pressure from animal welfare groups and the general public to employ cdAOF cell culture technology, as it is also "Animal Cruelty-free"*

- *Provides a competitive marketing advantage that highlights complete "animal cruelty-free" (e.g. fetal bovine serum-free) cell and tissue culture environment when carrying out the in vitro testing and marketing of OTC cosmeceuticals and other consumer personal care products*

Human Dermal Fibroblasts (HDF)

Potential applications in basic and preclinical laboratory research

- ***2D Human Fibroblast and Other Mesenchymal Cell Cultures***
  - ***Skin-derived Dermal fibroblasts***
  - ***Cornea-derived Stromal Fibroblasts (Keratocytes)***
  - ***Dermal Fibroblasts as Precursor Cells for Induced Pluripotent Stem Cells (iPSCs)***
- ***3D Bioprinted, Automated or Manually Reconstructed Connective Tissue, Using Human Fibroblasts***
  - ***Connective Tissue (e.g., Skin Dermis, Corneal Stroma)***
  - ***Other Connective Tissues***
  - ***Tissue on a Chip (e.g., Skin Dermis, Corneal Stroma)***



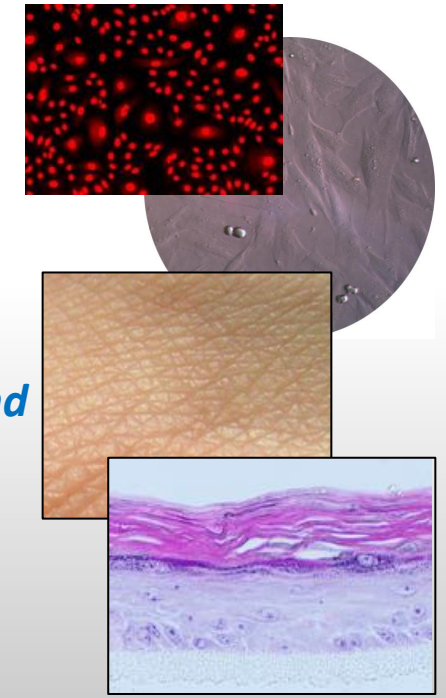
Human Dermal Fibroblasts (HDF)

Potential clinical applications in regenerative and esthetic medicine using dermal fibroblasts and other mesenchymal cells

- ***2D Cell-based Therapies (e.g. Cutaneous, Cornea, Other)***
- ***2D Cell-derived Therapeutic Products (e.g., Conditioned Medium and Extracellular vesicles / EVs)***
- ***3D Reconstructed Connective Tissue Therapies (e.g. Cutaneous, Cornea, Other)***

Potential cosmeceutical applications using dermal fibroblasts and other mesenchymal cells

- ***Cell-derived Cosmeceutical Products (e.g., Conditioned Medium and EVs)***



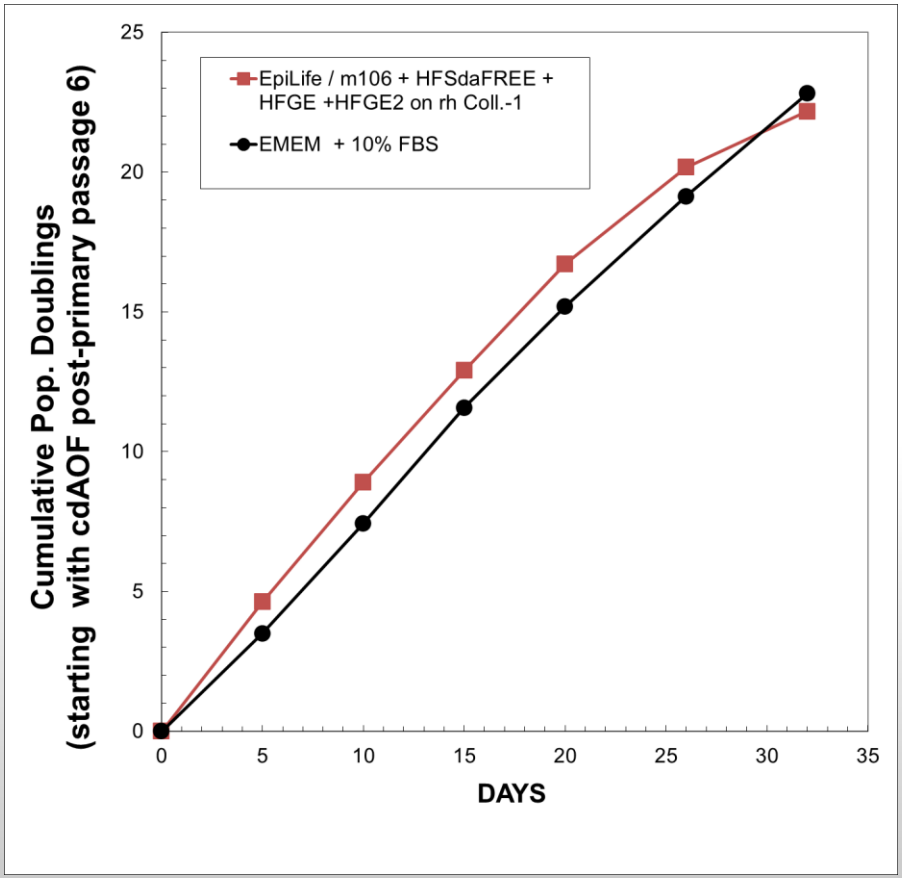
To avoid the use of animal derived components, Avantbio developed an improved, chemically-defined animal origin-free (cdAOF) cell culture system for human dermal fibroblasts (HFSdaFREE), that does not include any human- or other animal-derived products

A 3-component, cdAOF supplement kit

The following slides are a collection of representative research and development results derived from testing AvantBio's cdAOF HFSdaFREE cell culture system

Experimental screening of candidate animal origin-free cell culture components on serum-starved neonatal human dermal fibroblasts (HDFn) yielded a robust, chemically-defined, animal origin-free (cdAOF) cell culture supplement system for HDFn

The HFSdaFREE KIT system supports the efficient, long-term, post-primary serial propagation of serum-starved HDFn under cdAOF conditions, and compares favorably to 10% fetal bovine serum (FBS)

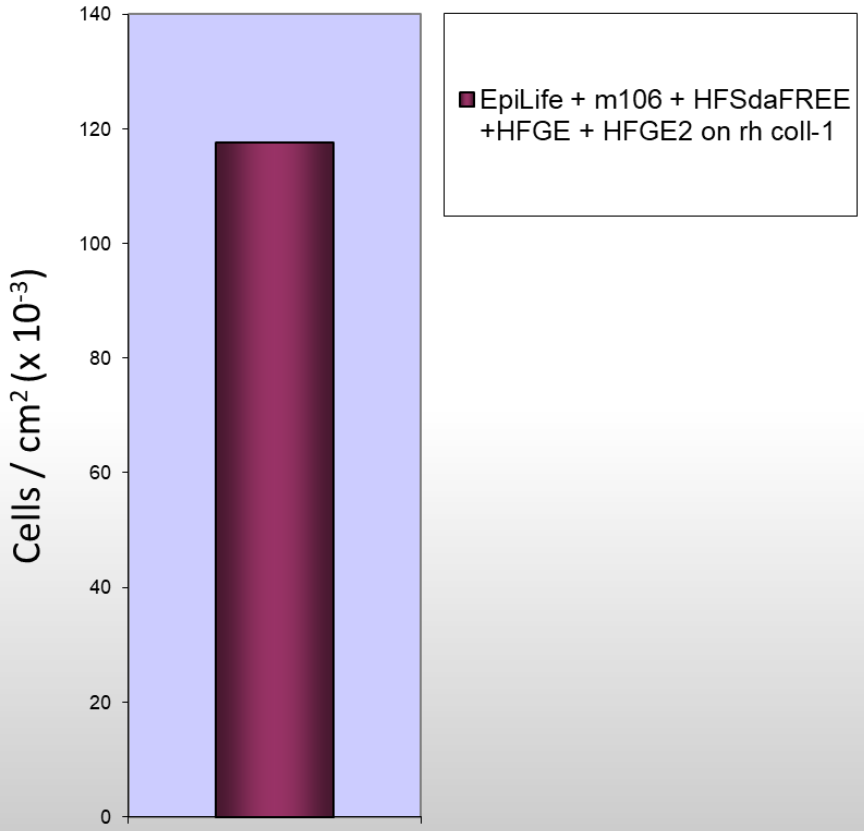


Methods: Cryopreserved post-primary serum-starved passage 1 neonatal human dermal fibroblast (HDFn) reared in medium m106 plus LSGS (Life Technologies; 2% FBS + growth factors), at the primary culture (passage 0) level, were placed into passage 2 cell culture, under chemically defined animal origin-free (cdAOF) cell culture conditions. Briefly, using AvantBio's HFSdaFREE KIT supplements in a 50/50 blend of EpiLife basal Medium / Medium m106, cdAOF HDFn were serially passaged on human recombinant collagen-1 (rh Collagen-1) coated substrata, through passage 5 and then cryopreserved in cdAOF CRYOVIVE 5% DMSO cryomedia. The passage 5 serum starved HDFn were later thawed and plated into passage 6 culture at 2,500 cells/cm2, into rh Collagen-1 precoated 6 well plates, in triplicate, using HFSdaFREE KIT supplements as described above, or control HDFn cell cultures propagated on untreated cell wells, using a conventional animal product containing medium (EMEM + 10% FBS). The HDFn were serially passaged through passage 11, in the cdAOF and control animal product-containing culture conditions, as shown above. Mean cumulative population doublings were calculated at the end of each passage, from triplicate wells, +/- the SEM. LSGS, EpiLife and medium m106 are trademarks of Life Technologies Corporation.

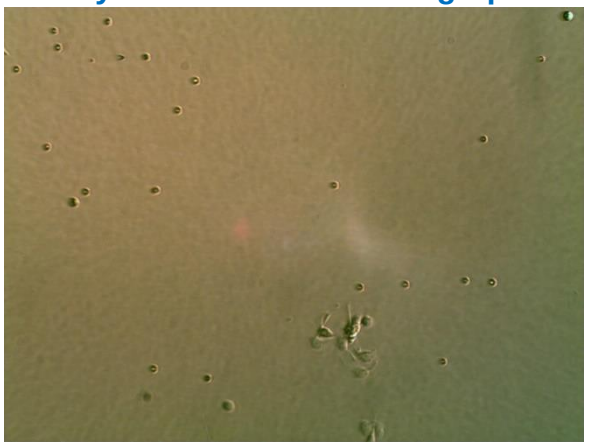
AvantBio's HFSdaFREE kit system supports the efficient isolation and propagation of primary adult* human dermal fibroblasts (HDFa) under chemically defined animal origin-free (cdAOF) conditions

(*53 year old female)

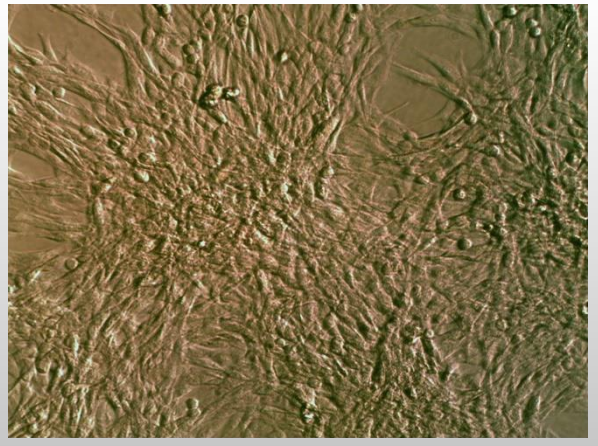
Day 12 Mean Cell Density



Day 1 and 12 Photomicrographs



Primary Culture, Day 1

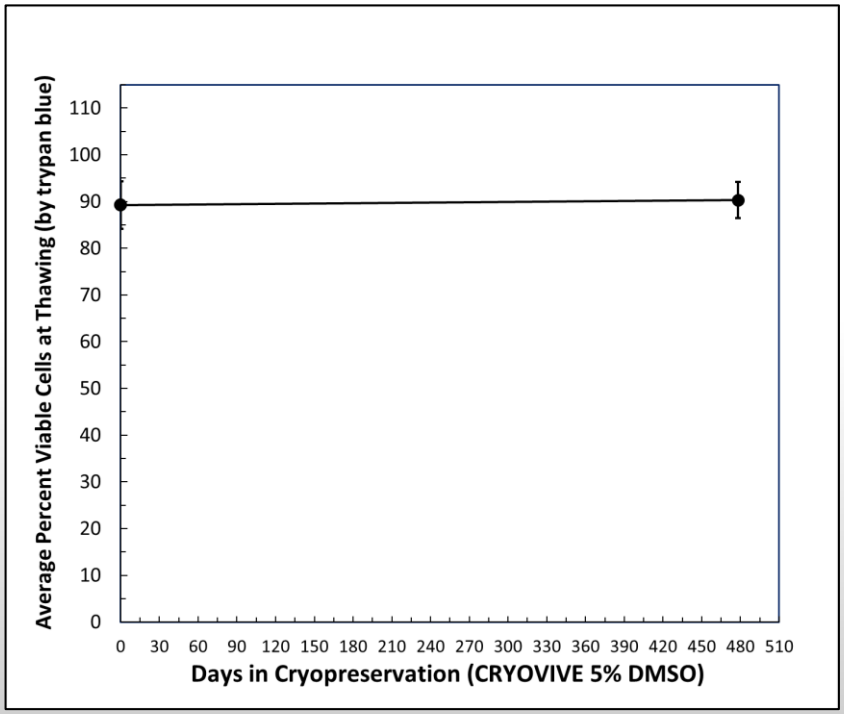


Primary Culture, Day 12

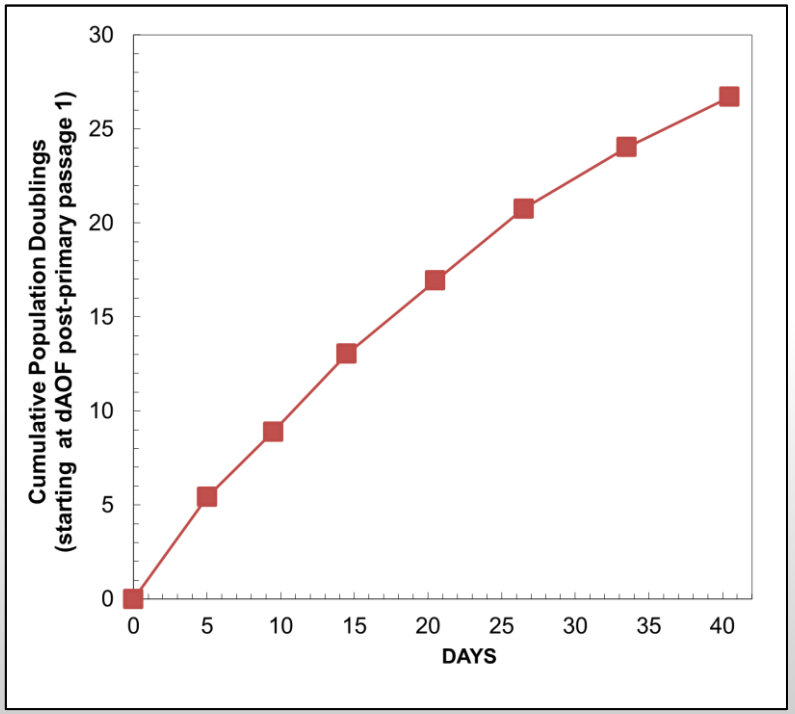
Methods: Adult human dermal-derived cells were collectively isolated from adult skin (1.13 cm2, 53 year old female) under chemically defined, animal origin-free (cdAOF) conditions. Utilizing a protocol employing collagenase treatment of depidermized skin at 37 deg. C., the dermal cells were then collected by centrifugation (718,400 viable cells recovered) at 40 hours post-tissue harvest. The dissociated dermal cells were finally plated into primary culture on recombinant human collagen-1 coated substratum at 15,000 viable cells/cm^2), into cell wells (8 cm^2 total area). The cells were cultured for 12 days, exchanging media every 2 days, using 50% Epilife / 50% m106 basal medium supplemented with HFSdaFREE + HFGE + HFGE2. Cell density was calculated by dissociation with 0.2 ml TrypLE Select per well, for 4 minutes at 37 deg. C, and finally counting the cells. Value represents mean cell density on primary culture day 12. . EpiLife, medium m106 and TrypLE Select are trademarks of Life Technologies Corporation.

Efficient recovery and serial propagation of cryopreserved primary adult* human dermal fibroblasts (HDFa), after 16 months cryopreservation. HDFa were placed into post-primary passage 1 culture, under chemically-defined animal origin-free (cdAOF) cell culture conditions, using the HFSdaFREE KIT system (*53 year old female)

Post-thaw Viability of Cryopreserved Primary HDFa Cultured Under cdAOF Conditions



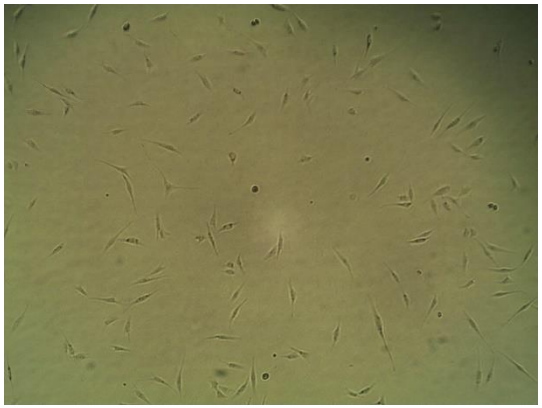
Serial Propagation of Cryopreserved Primary HDFa Under cdAOF Conditions



Methods: Chemically defined animal origin-free (cdAOF) adult human dermal fibroblast (HDFa) primary cell cultures (described in slide 9) were established using HFSdaFREE KIT supplements and 50/50 blend of EpiLife basal / Medium m106, dissociated with TrypLE Select and then cryopreserved in cdAOF CRYOVIVE 5% DMSO cryomedia. After 16 months in liquid nitrogen vapor storage, the primary HDFa were plated into passage 1 culture at 2,500 cells/cm^2, into rh Collagen-1 precoated 6 well plates, in triplicate. Passage 1 HDFa were serially passaged in the cdAOF culture environment using AvantBio's HFSdaFREE KIT supplements in the basal medium described above. At the end of each passage, the cells were dissociated with TrypLE Select and counted using a hemocytometer. Mean cumulative population doublings were calculated at the end of each passage, from triplicate wells, +/- the SEM. EpiLife, medium m106 and TrypLE Select are trademarks of Life Technologies Corporation.

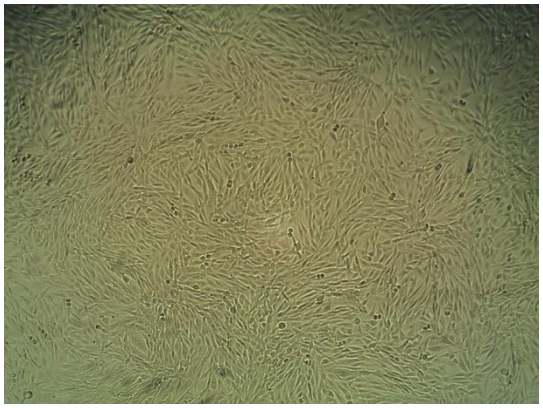
Efficient recovery and serial propagation of cryopreserved primary adult* human dermal fibroblasts (HDFa), after 16 months cryopreservation. HDFa were placed into post-primary passage 1 culture, under chemically-defined animal origin-free (cdAOF) cell culture conditions, using the HFSdaFREE KIT system (*53 year old female)

Day 1, 3 and 5 Photomicrographs

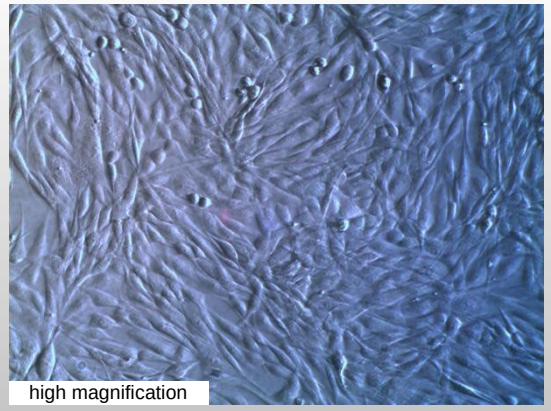


Passage 1, Day 1

Passage 1, Day 3



Passage 1, Day 5

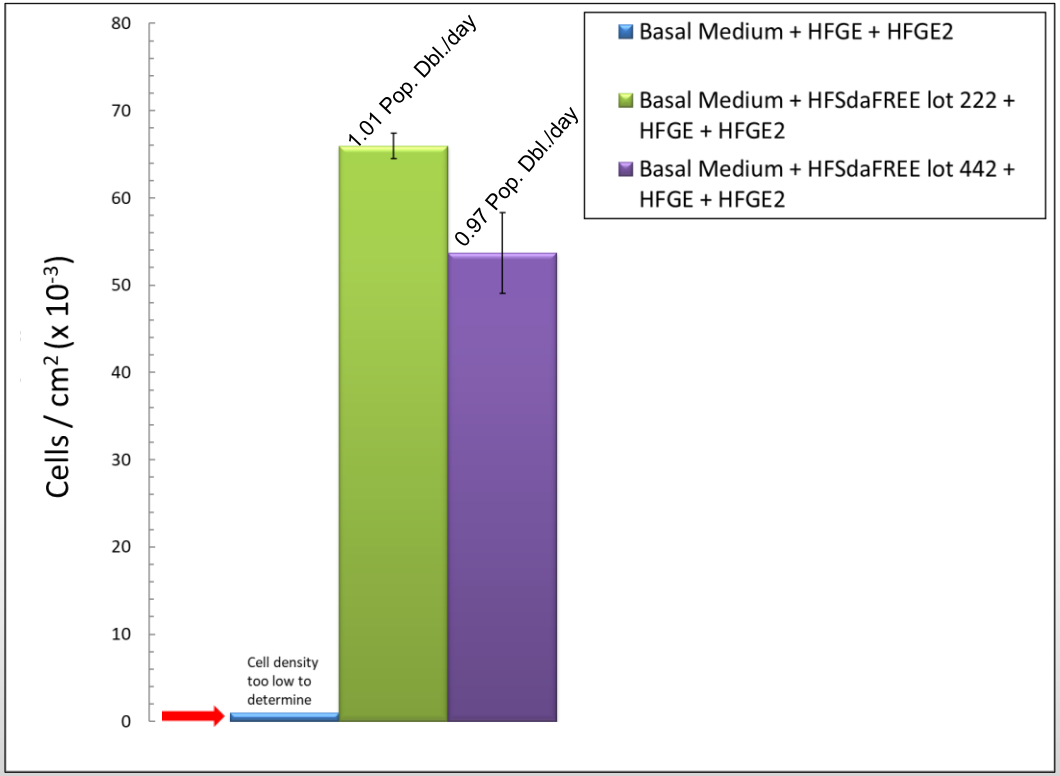


Passage 1, Day 5

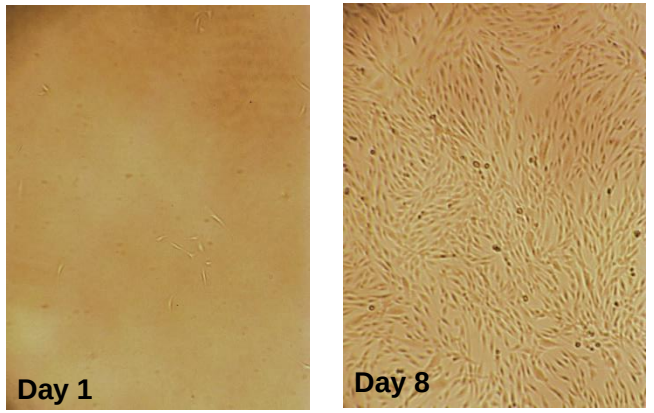
Methods: Chemically defined animal origin-free (cdAOF) adult human dermal fibroblast (HDFa) primary cell cultures (described in slide 9) were established using HFSdaFREE KIT supplements and 50/50 blend of EpiLife basal / Medium m106, dissociated with TrypLE Select and then cryopreserved in cdAOF CRYOVIVE 5% DMSO cryomedia. After 16 months in liquid nitrogen vapor storage, the primary HDFa were plated into passage 1 culture at 2,500 cells/cm^2, into rh Collagen-1 precoated 6 well plates, in triplicate. Passage 1 HDFa were serially passaged in the cdAOF culture environment using AvantBio's HFSdaFREE KIT supplements in the basal medium described above. At the end of each passage, the cells were dissociated with TrypLE Select and counted using a hemocytometer. Mean cumulative population doublings were calculated at the end of each passage, from triplicate wells, +/- the SEM. Representative photomicrographs were taken during passage 1, at days 1,3 and 5. EpiLife , medium m106 and TrypLE Select are trademarks of Life Technologies Corporation.

HFSdaFREE KIT QC performance test on passage 2 serum-starved neonatal dermal fibroblasts (HDFn) under chemically defined animal origin-free (cdAOF) cell culture conditions

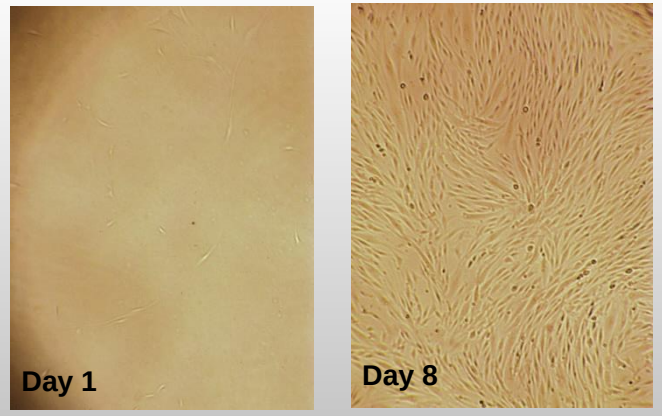
Day 8 Mean Cell Densities



Day 1 and 8 Photomicrographs



HFSdaFREE lot 222 + HFGE + HFGE2: DOM JUL 2022

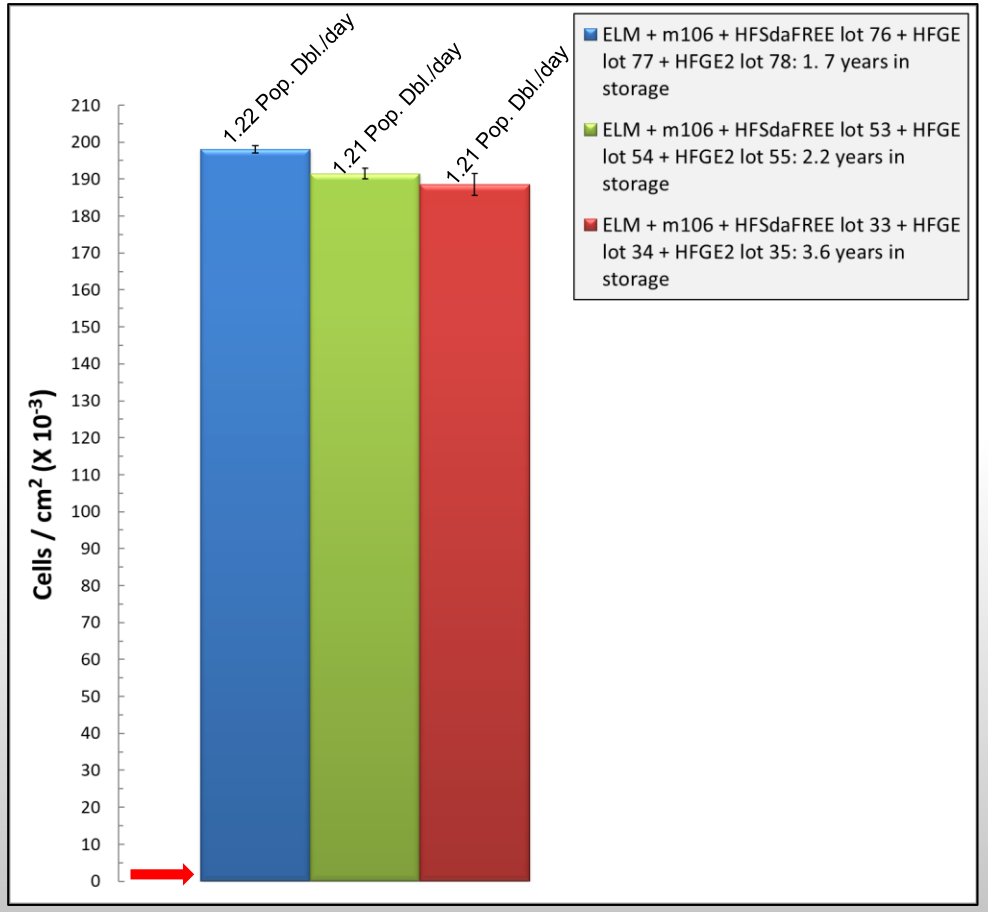


HFSdaFREE lot 442 + HFGE + HFGE2: DOM AUG 2023

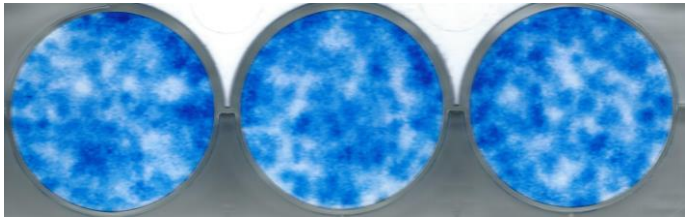
Methods: Serum-starved, post-primary passage 2 human neonatal dermal fibroblast (HDFn) were cultured using the indicated lots of the HFSdaFREE KIT cdAOF supplements and a 50/50 blend of EpiLife basal / Medium m106. HDFn were cultured on Collagen-1 pre-coated cell wells (12 well format). Results are displayed as final mean cell densities after 8 days of culture, +/- the SEM. Seeding density = 250 cells/cm^2 (see red arrow). Representative photomicrograph images were taken at day 1 and 8. Pop. Dbl./day = average population doublings per day (a measure of relative growth rate). EpiLife Medium (ELM) and medium m106 are registered trademarks of Life Technologies Corporation.

HFSdaFREE KIT performance test on passage 1 serum-starved neonatal dermal fibroblasts (HDFn): *the cdAOF HFSdaFREE kit system is stable, when stored at -20 °C, for up to 3.6 years*

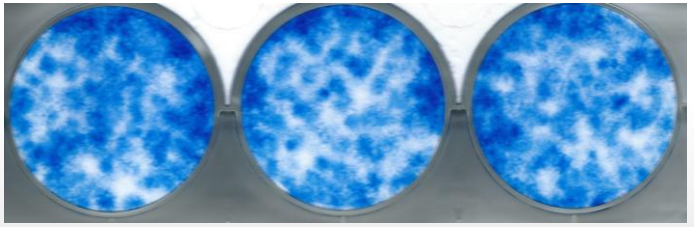
Day 8: Mean Cell Densities



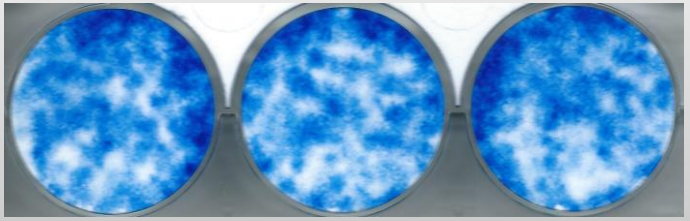
Day 8: Fixed & Stained HDFn



HFSdaFREE lot 76 + HFGE lot 77 + HFGE2 lot 78: 1.7 years in storage



HFSdaFREE lot 53 + HFGE lot 54 + HFGE2 lot 55: 2.2 years in storage



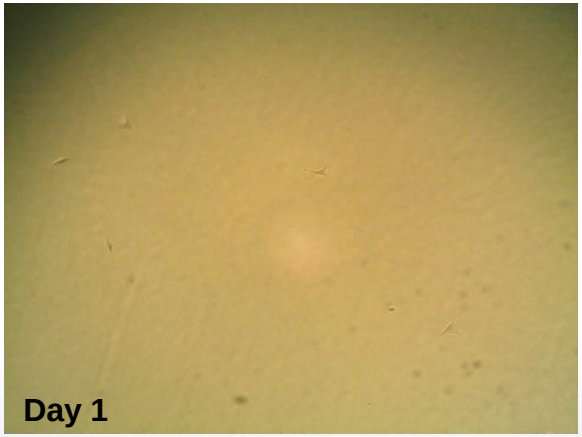
HFSdaFREE lot 33 + HFGE lot 34 + HFGE2 lot 35 : 3.6 years in storage

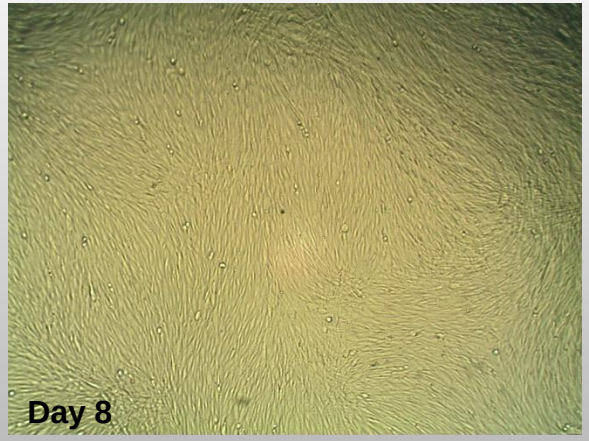
Methods: Serum-starved, post-primary passage 1 human neonatal dermal fibroblast (HDFn) were cultured using the indicated lots of the HFSdaFREE KIT cdAOF supplements and a 50/50 blend of EpiLife basal / Medium m106. HDFn were cultured on human recombinant Collagen-1 pre-coated cell wells (6 well format). Results are displayed as final mean cell densities after 8 days of culture, +/- the SEM. Seeding density = 250 cells/cm^2 (see red arrow). Representative stained cell images were taken at day 8. Pop. Dbl./day = average population doublings per day (a measure of relative growth rate). EpiLife Medium (ELM) and medium m106 are registered trademarks of Life Technologies Corporation.

Performance test on serum-starved neonatal dermal fibroblasts (HDFn): *the cdAOF HFSdaFREE kit system is stable, when stored at -20 °C, for up to 3.6 years*

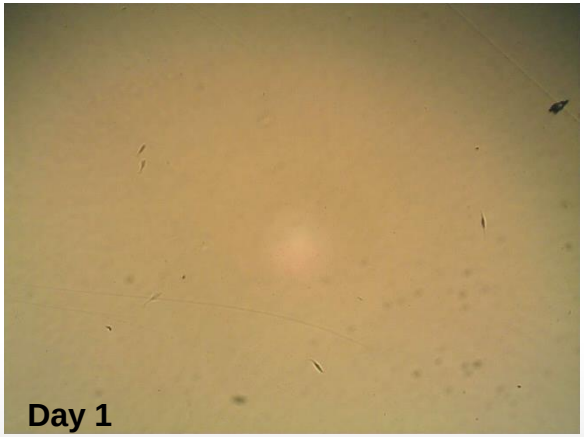
Day 1 and Day 8 Photomicrographs

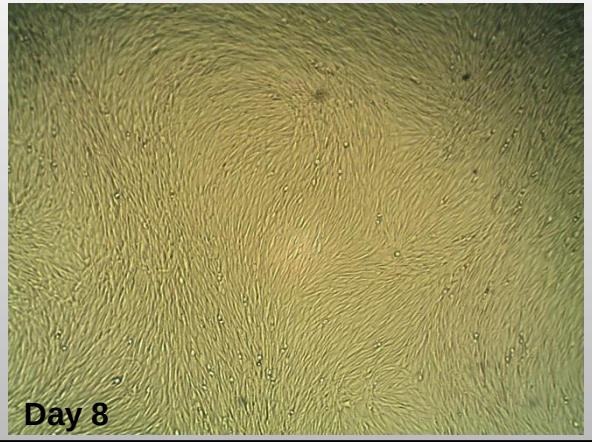
HFSdaFREE lot 76 + HFGE lot 77 + HFGE2 lot 78: 1.7 years in storage



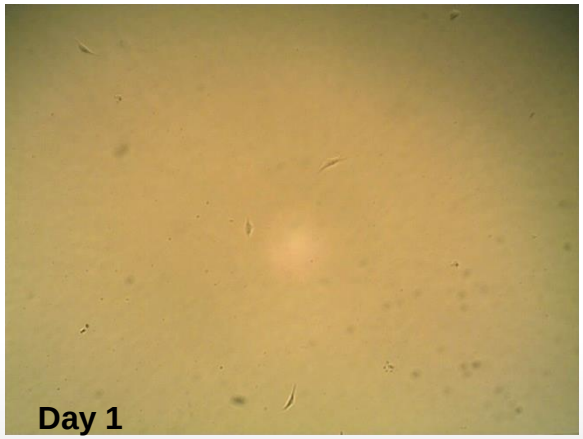


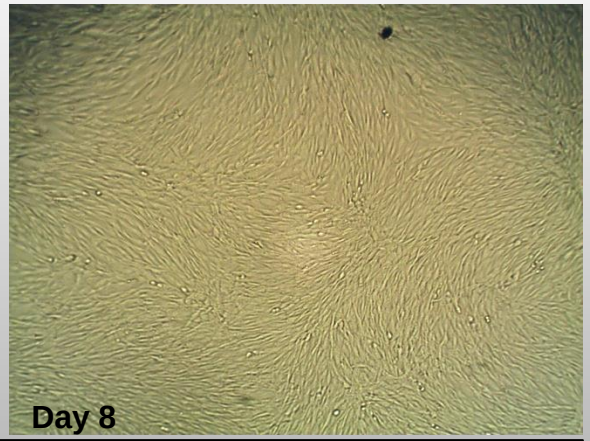
HFSdaFREE lot 53 + HFGE lot 54 + HFGE2 lot 55: 2.2 years in storage





HFSdaFREE lot 33 + HFGE lot 34 + HFGE2 lot 35 : 3.6 years in storage





Methods: Serum-starved, post-primary passage 1 human neonatal dermal fibroblast (HDFn) were cultured using the indicated lots of the HFSdaFREE KIT cdAOF supplements and a 50/50 blend of EpiLife basal / Medium m106. HDFn were cultured on human recombinant Collagen-1 pre-coated cell wells (6 well format). Results are displayed as final mean cell densities after 8 days of culture, +/- the SEM. Seeding density = 250 cells/cm^2 (see red arrow). Representative photomicrograph images were taken at days 1 and 8. EpiLife Medium (ELM) and medium m106 are registered trademarks of Life Technologies Corporation.

Bio-compatible engineered silk-derived fibroin protein can be fabricated into a variety configurations, in some cases for applications in regenerative medicine, that include the delivery of therapeutic cells or tissue on discs (mats), scaffolds (fibers) and hydrogels

International Journal of *Molecular Sciences*

MDPI

Review

Silk Fibroin as a Functional Biomaterial for Tissue Engineering

Weizhen Sun [1], David Alexander Gregory [1,2], Mhd Anas Tomeh [1] and Xiubo Zhao [1,3,*]

Int. J. Mol. Sci. **2021**, *22*, 1499. https://doi.org/10.3390/ijms22031499

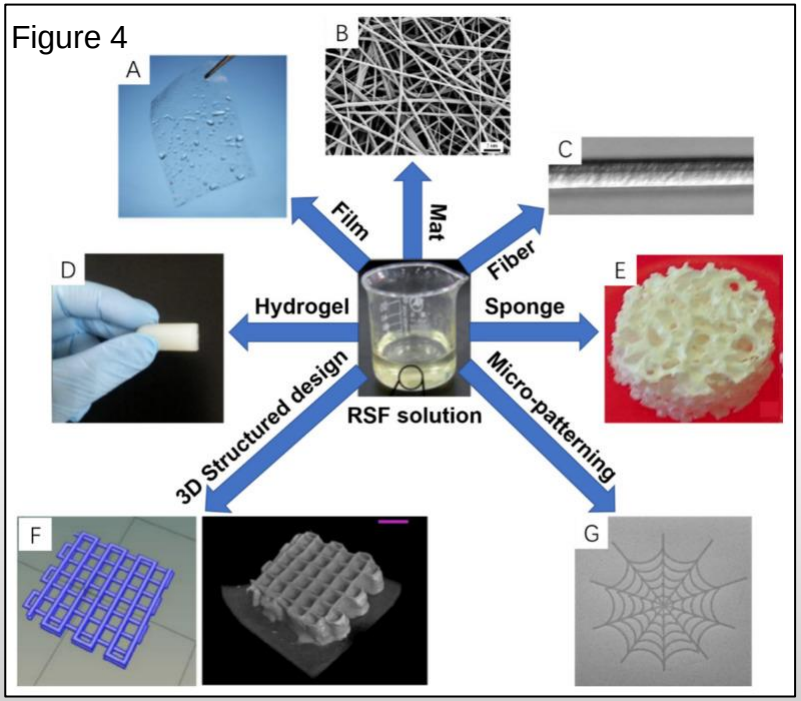
Research Article

Exosomes of adult human fibroblasts cultured on 3D silk fibroin nonwovens intensely stimulate neoangiogenesis

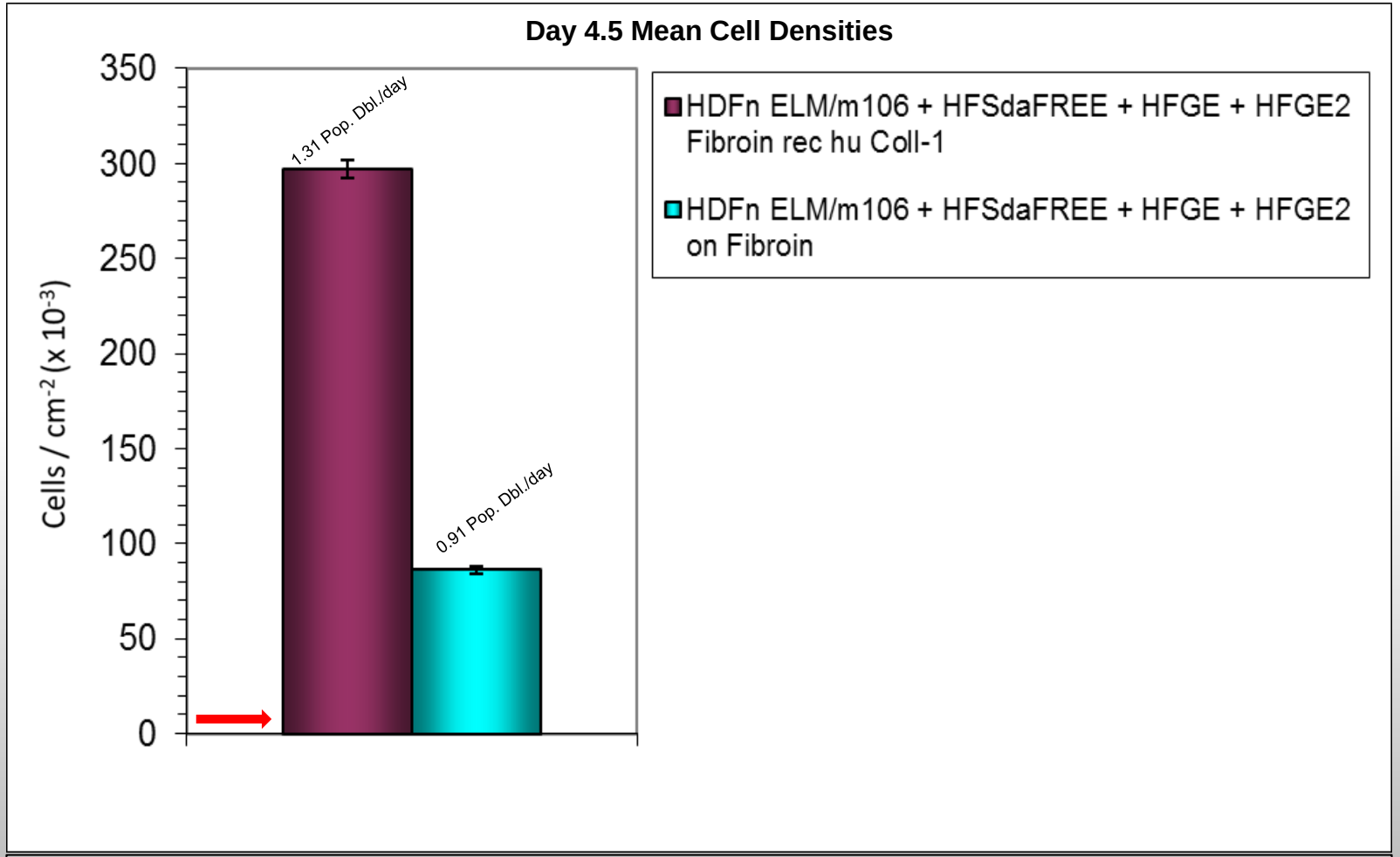
Peng Hu[1,2,†], Anna Chiarini[1,†,*], Jun Wu[1,3], Giuliano Freddi[4], Kaiyu Nie[2], Ubaldo Armato[1,3] and Ilaria Dal Prà[1,3,†,*]

Burns & Trauma, Volume 9, 2021, tkab003, https://doi.org/10.1093/burnst/tkab003

Figure 4



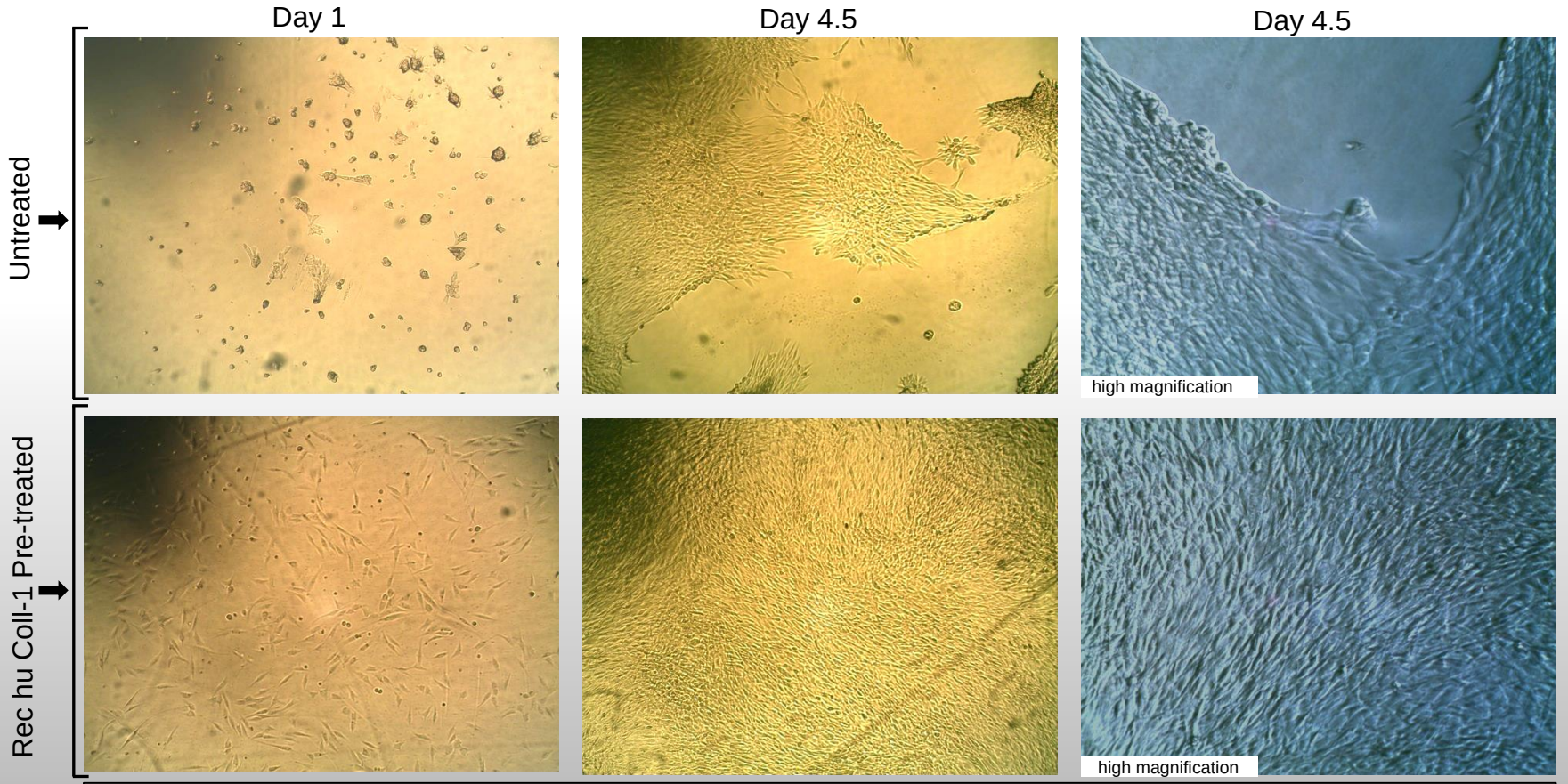
Propagation of serum-starved neonatal human dermal fibroblasts (HDFn) on bio-compatible, silk-derived fibroin discs (mats). HDFn were cultured under chemically defined animal origin-free (cdAOF) cell culture conditions, using AvantBio's HFSdaFREE KIT system, with or without pretreatment with recombinant human collagen-1



Methods: Serum-starved, post-primary passage 2 human neonatal dermal fibroblast (HDFn) were cultured using the indicated lots of the HFSdaFREE KIT cdAOF supplements and a 50/50 blend of EpiLife basal / Medium m106. HDFn were cultured on silk-derived Fibroin discs (mats in 24-well format), in triplicate, both with and without human recombinant Collagen-1 (rec hu Coll-1) pre-coating. Results are displayed as final mean cell densities after 4.5 days of culture, +/- the SEM. Seeding density = 5,000 cells/cm^2 (see red arrow). Pop. Dbl./day = average population doublings per day (a measure of relative growth rate). EpiLife Medium (ELM) and medium m106 are registered trademarks of Life Technologies Corporation.

Propagation of serum-starved neonatal human dermal fibroblasts (HDFn) on bio-compatible, silk-derived fibroin discs (mats). cells cultured under chemically defined animal origin-free (cdAOF) cell culture conditions, using AvantBio's HFSdaFREE KIT system, +/- pretreatment with recombinant human collagen-1

Photomicrographs after 1 and 4.5 days of Culture



Methods: As described for slide 4, **s**erum-starved, post-primary passage 2 human neonatal dermal fibroblast (HDFn) were cultured under chemically defined animal origin-free (cdAOF) conditions using HFSdaFREE KIT supplements (as described in the previous figure). HDFn were cultured on silk-derived Fibroin discs (mats in 24-well format), in triplicate, both with and without human recombinant Collagen-1 (rec hu Coll-1) pre-coating. Results are presented as photomicrographs taken at day 1 and 4.5. EpiLife Medium (ELM) and medium m106 are registered trademarks of Life Technologies Corporation.

WI-38 and its close relative, MRC-5, are normal diploid human fetal lung fibroblast lines that have been utilized for decades to produce a variety of human vaccines. WI-38 and MRC-5 are still utilized today, to produce these human vaccines.

Over a billion vaccine units have been produced in WI-38

Examples include: poliomyelitis, measles, mumps, rubella, varicella (chicken pox), herpes zoster, adenovirus, rabies, Hepatitis A

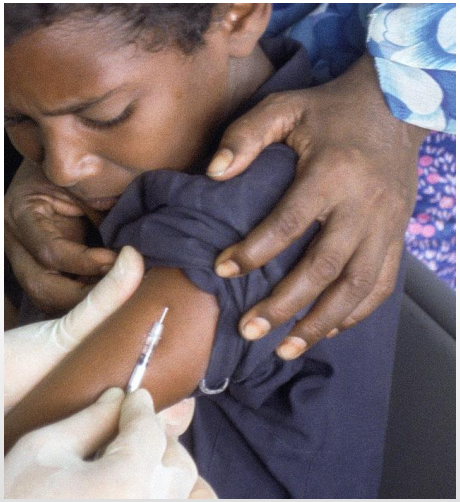
The Role of the WI-38 Cell Strain in Saving Lives and Reducing Morbidity
S. J. Olshansky and L. Hayflick
AIMS Public Health. 2017; 4(2): 127–138.Published online 2017 Mar 2.

"Here we illustrate how the discovery and use of a single cell strain used to grow most viral vaccines in use today (WI-38 [8] and a later derivative [9]), has already had a powerful impact on human life on an order of magnitude that is unprecedented in the history of public health[2]."

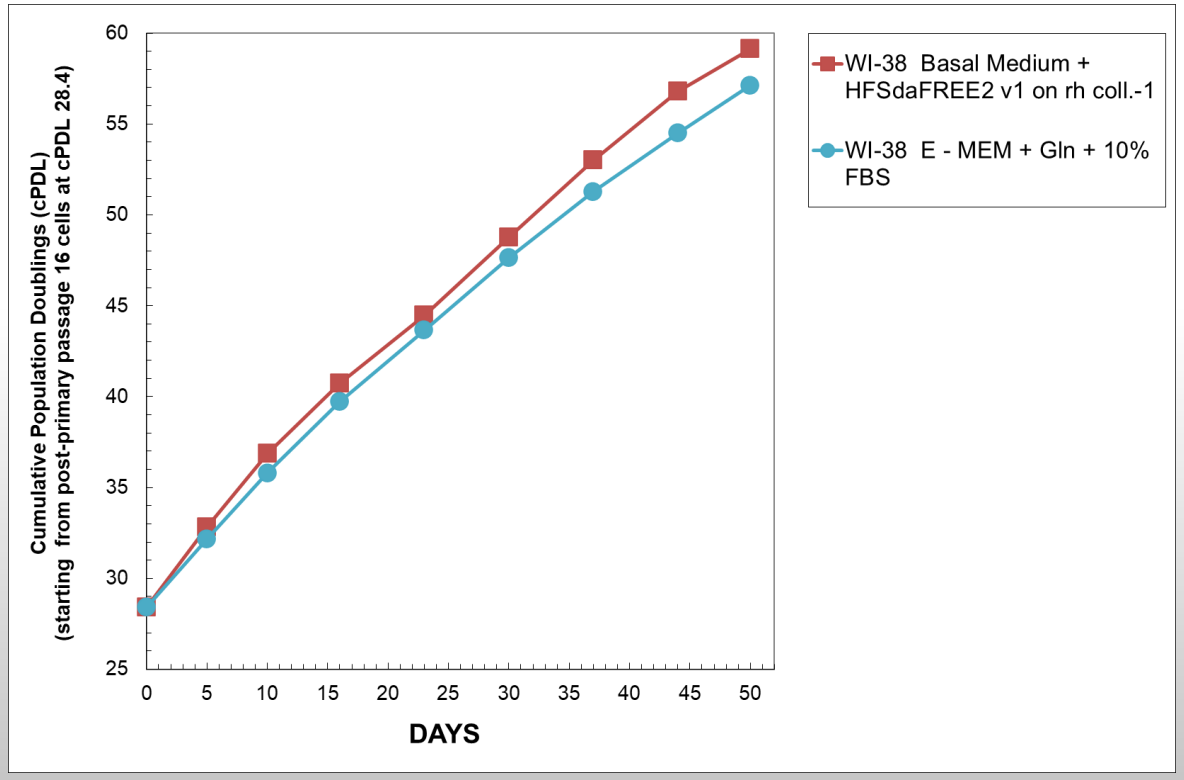
"WI-38 and its derivatives are still in use for producing many viral vaccines that are distributed worldwide today."

Normal diploid human fetal lung fibroblast cell lines (WI-38, MRC-5): *potential for the production of human vaccines under chemically defined animal origin-free (serum-free) cell culture conditions*

Photo Credit: James Gathany



Efficient serial propagation of cryopreserved, serum-starved WI-38 human diploid fetal lung fibroblasts, under chemically defined animal origin-free cell (cdAOF) culture conditions, using the experimental HFSdaFREE2 kit system: *AvantBio's HFSdaFREE2 KIT system compares favorably to the conventional animal-originated 10 % FBS cell culture system*

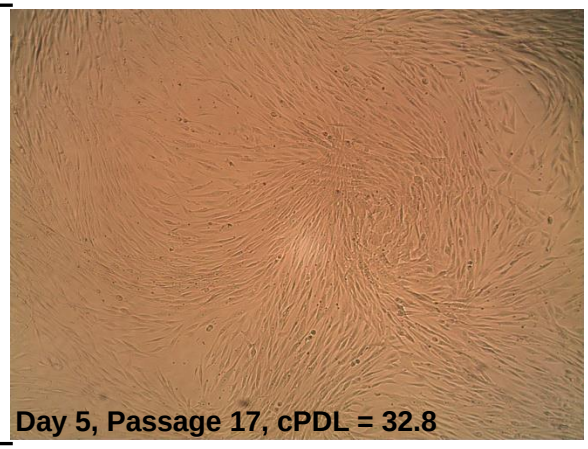


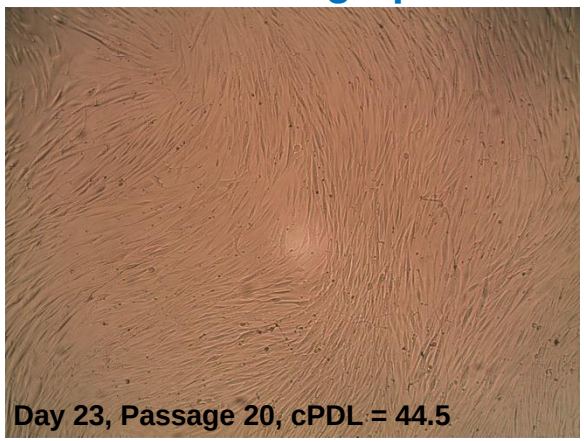
Methods: Cryopreserved, passage 15, population doubling level 23, WI-38 fetal human lung fibroblasts from ATCC (ATCC CCL-75), were thawed and then placed into post-primary passage 16 cell culture using EMEM + 10% FBS (from ATCC). Cells were plated into 3 T-75 flasks, at 3,000 viable cells/cm2 and subsequently cultured, with 2 feedings, for 6 days. On day 6, the cells were washed 2 times over 30 minutes, with 50 mls unsupplemented EMEM basal medium (serum-starved WI-38). The WI-38 cell cultures were dissociated using chemically-defined animal origin-free (cdAOF) TrypLE Select (Life Technologies) and then cryopreserved in cdAOF CRYOVIVE 5% DMSO cryofluid. The serum-starved passage 16 WI-38 cells were later thawed and directly plated into passage 17 culture at 2,500 cells/cm2 using EMEM + 10% FBS (control) or HFSdaFREE2 KIT supplements (as described in the figure) on rh Collagen-1 precoated 6 well plates, in triplicate. WI-38 were serially passaged through passage 24. Mean cumulative population doublings were calculated at the end of each passage, from triplicate wells, +/- the SEM. EpiLife and Medium (m106) are trademarks of Life Technologies Corporation.

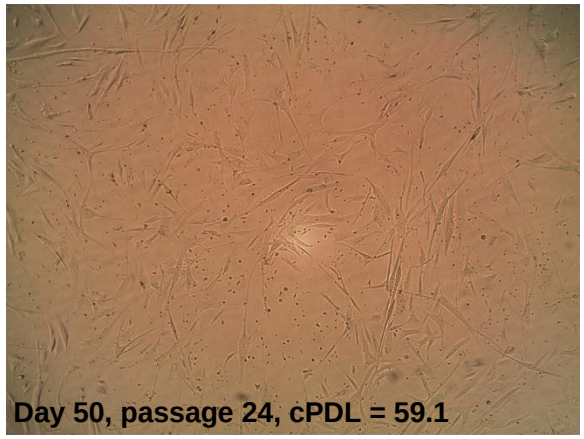
Efficient serial propagation of cryopreserved, serum-starved WI-38 human diploid fetal lung fibroblasts, under chemically defined animal origin-free cell (cdAOF) culture conditions, using the experimental HFSdaFREE2 kit system: ***AvantBio's HFSdaFREE2 KIT system compares favorably to the conventional animal-originated 10 % FBS cell culture system***

Photomicrographs

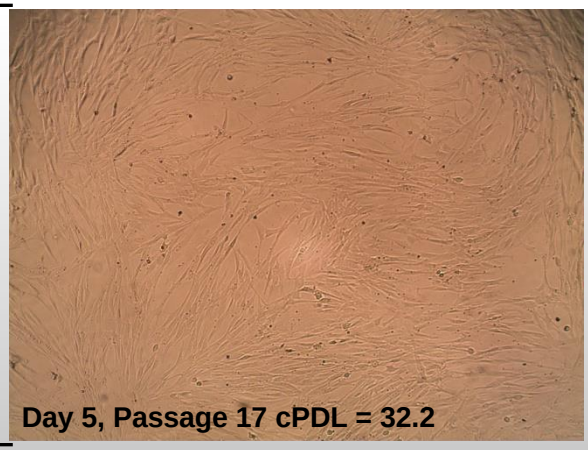
HFSdaFREE2 v1 KIT Supplements + Basal Medium on hr Coll. -1 Pre-coated Substratum

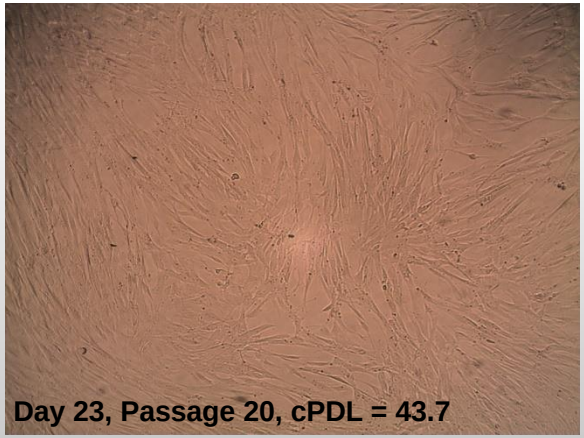


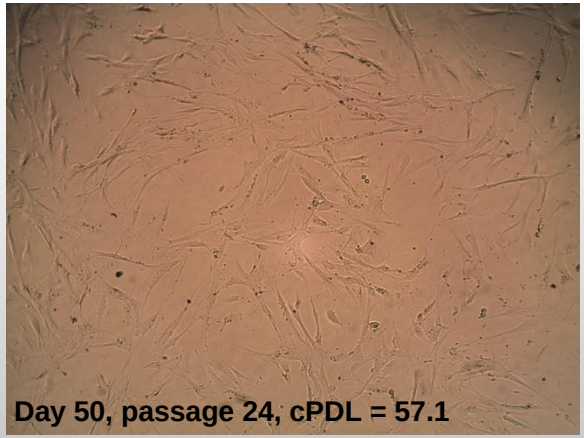




10% FBS + Gln-Supplemented MEM







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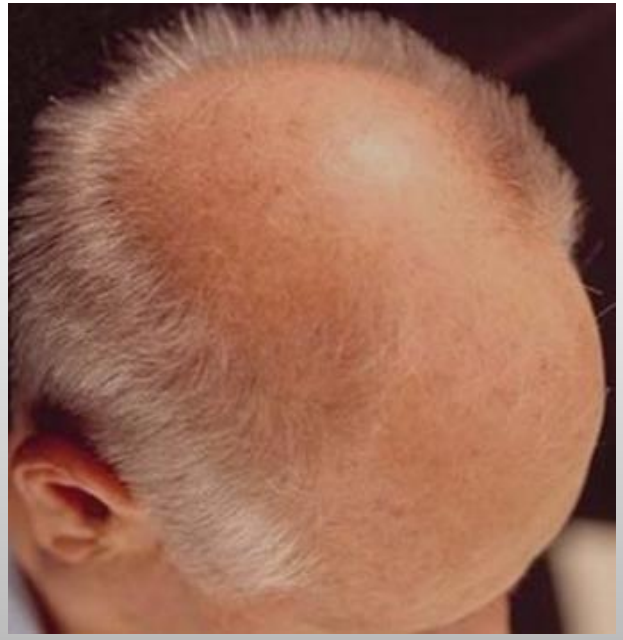
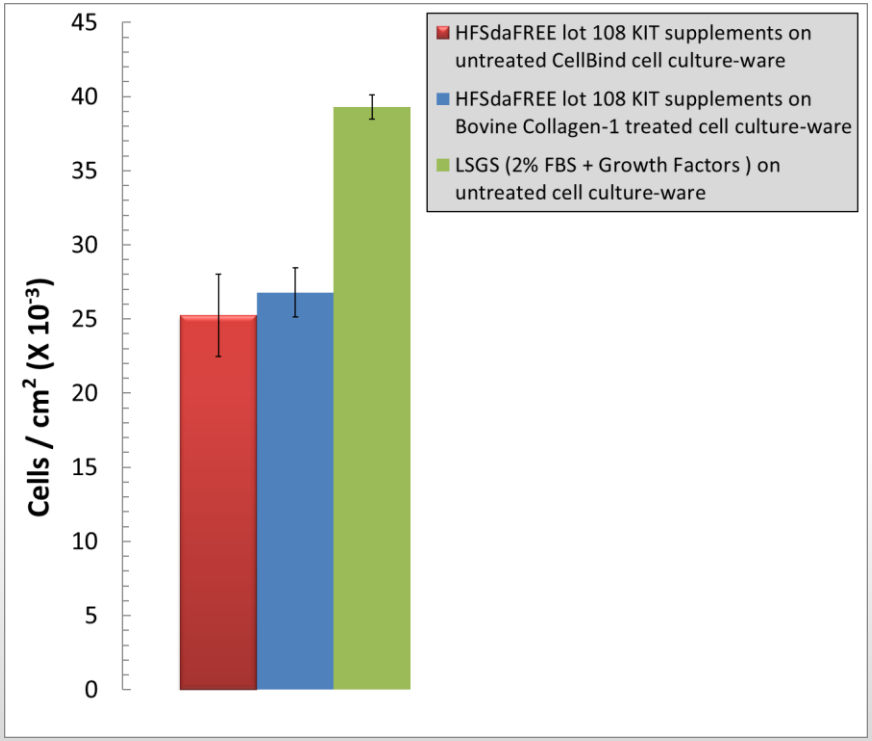
Human hair follicle dermal papilla Cells (HFDPC):

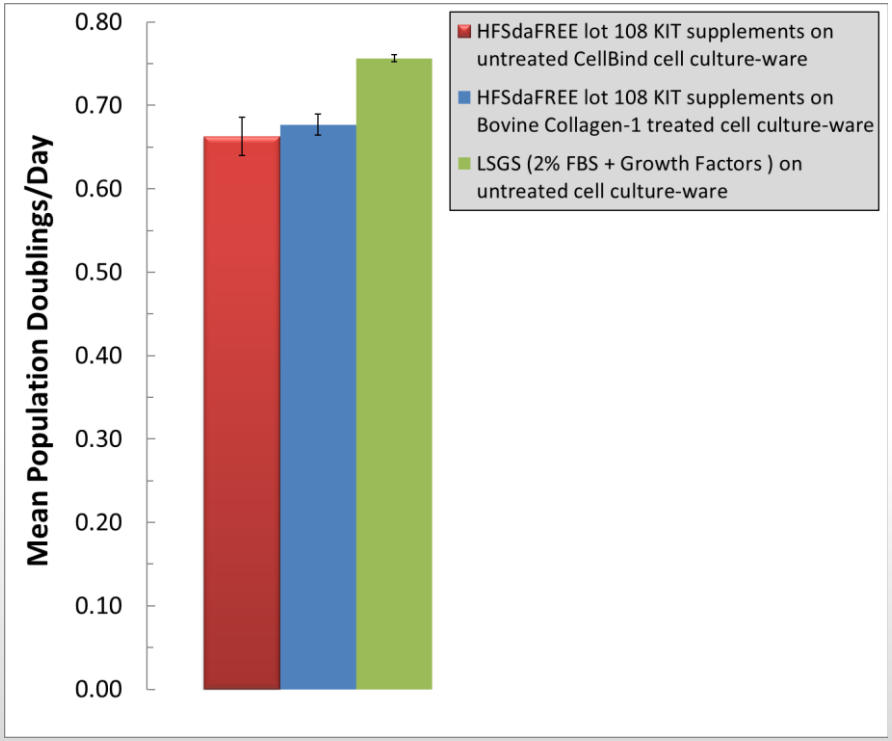
potential use for hair regeneration / restoration under chemically-defined animal origin-free cell culture conditions



Propagation of serum-starved, late-passage adult* human hair follicle dermal papilla cells (HFDPC) using Avantbio's chemically-defined animal origin-free (cdAOF) HFSdaFREE KIT system: *comparison of the HFSdaFREE KIT system to an animal-originated cell culture (2%FBS) system, on two different substrates*

(*53 year old female temporal scalp hair, from Cell Applications, Inc.)





Methods: Post-primary passage 2, cryopreserved adult human dermal papilla cells (HFDPC), from Cell Applications Inc., originally established in Papilla Cell Growth Medium (FBS + growth factors), were thawed and placed directly into media using AvantBio's HFSdaFREE KIT supplement system. Briefly, cryopreserved HFDPC were plated into passage 3 culture at 2,000 viable cells per cm^2, into human recombinant collgen-1 precoated T-25 flasks, using the HFSdaFREE KIT supplements in a 50/50 blend of EpiLife medium and medium m106 (Life Technologies). HFDPC were cultured for 5 days, with feedings, then dissociated with TrypLE select. The dissociated cells were then cryopreserved as passage 3 serum-starved HFDPC cells, using AvantBio's cdAOF CRYOVIVE 5% DMSO cryomedia. After 8 years storage in liquid nitrogen vapor, the passage 3 serum-starved HFDPC were plated into passage 4 culture, at 1,000 viable cells/cm^2, into 12- well plates, in triplicate. Cell-well substrata consisted of untreated CellBind (Corning) cell culture-ware, bovine collagen-1 pretreated conventional cell culture-ware or untreated conventional cell culture-ware, as indicated in the figure. The Passage 4 serum-starved HFDPC were cultured for 7 days in the presence of the indicated supplements. Day 7 mean cell densities and mean population doublings/day were calculated from triplicate wells, +/- the SEM. EpiLife, medium m106, TrypLE select and LSGS are trademarks of Life Technologies Corporation. CellBind is a trademark of Corning Inc.

Propagation of serum-starved, late-passage adult* human hair follicle dermal papilla cells (HFDPC) using Avantbio's chemically-defined animal origin-free (cdAOF) HFSdaFREE KIT system: *comparison of the HFSdaFREE KIT system to an animal-originated cell culture (2% FBS) system, on two different substrates*

(*53 year old female temporal scalp hair, from Cell Applications, Inc.)

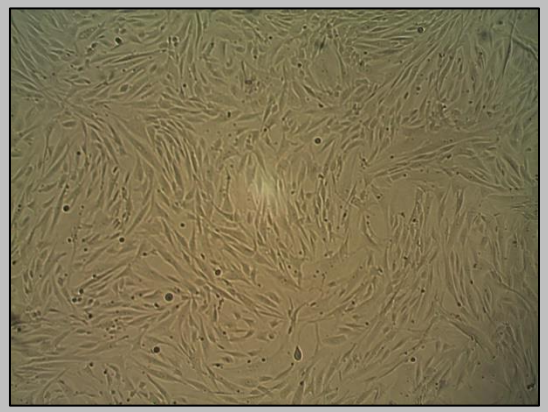
Day 7 Stained Cells

HFSdaFREE lot 108 KIT supplements on untreated CellBind Cell Culture-ware

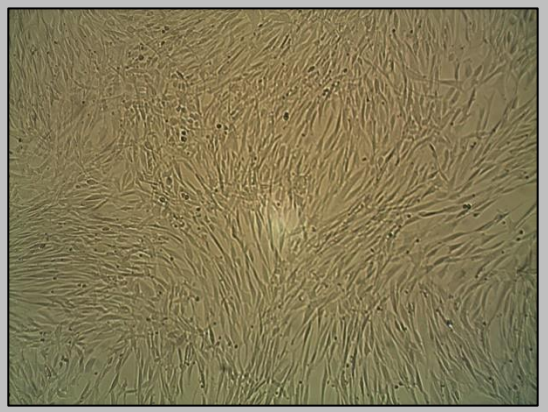
HFSdaFREE lot 108 KIT supplements on Bovine Collagen-1 treated Cell Culture-ware

LSGS (2% FBS + Growth Factors) on untreated Cell Culture-ware

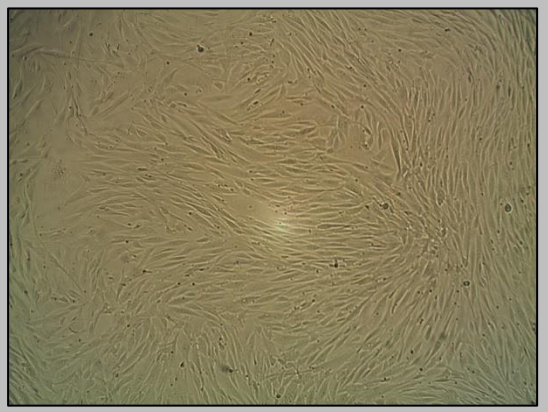
Day 7 Photomicrographs



HFSdaFREE lot 108 KIT supplements on untreated CellBind Cell Culture-ware



HFSdaFREE lot 108 KIT supplements on Bovine Collagen-1 treated Cell Culture-ware

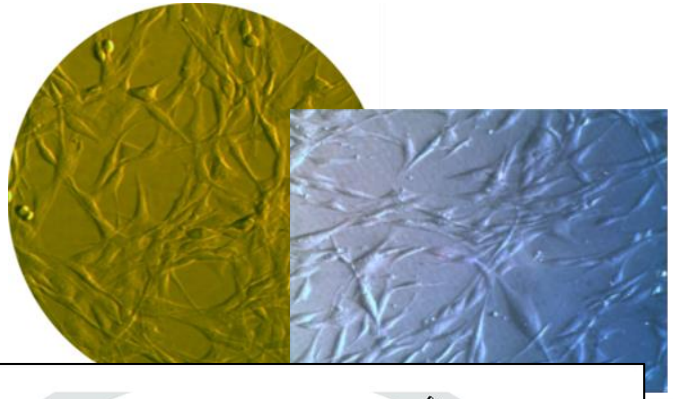


LSGS (2% FBS + Growth Factors) on untreated Cell Culture-ware

Methods: Post-primary passage 2, cryopreserved adult human dermal papilla cells (HFDPC), from Cell Applications Inc., originally established in Papilla Cell Growth Medium (FBS + growth factors), were thawed and placed directly into media using AvantBio's HFSdaFREE KIT supplement system. Briefly, cryopreserved HFDPC were plated into passage 3 culture at 2,000 viable cells per cm^2, into human recombinant collgen-1 precoated T-25 flasks, using the HFSdaFREE KIT supplements in a 50/50 blend of EpiLife medium and medium m106 (Life Technologies). HFDPC were cultured for 5 days, with feedings, then dissociated with TrypLE select. The dissociated cells were then cryopreserved as passage 3 serum-starved HFDPC cells, using AvantBio's cdAOF CRYOVIVE 5% DMSO cryomedia. After 8 years storage in liquid nitrogen vapor, the passage 3 serum-starved HFDPC were plated into passage 4 culture, at 1,000 viable cells/cm^2, into 12- well plates, in triplicate. Cell-well substrata consisted of untreated CellBind (Corning) cell culture-ware, bovine collagen-1 pretreated conventional cell culture-ware or untreated conventional cell culture-ware, as indicated in the figure. The Passage 4 serum-starved HFDPC were cultured for 7 days in the presence of the indicated supplements. Day 7 mean cell densities and mean population doublings/day were calculated from triplicate wells, +/- the SEM.. Representative-stained cells as well as photomicrographs were taken at day 7. EpiLife, medium m106, TrypLE select and LSGS are trademarks of Life Technologies Corporation. CellBind is a trademark of Corning Inc.

Human mesenchymal stem cells (HMSC): *potential for use under chemically-defined animal origin-free cell culture conditions*

Clinical applications in regenerative and esthetic medicine using human mesenchymal stem cells

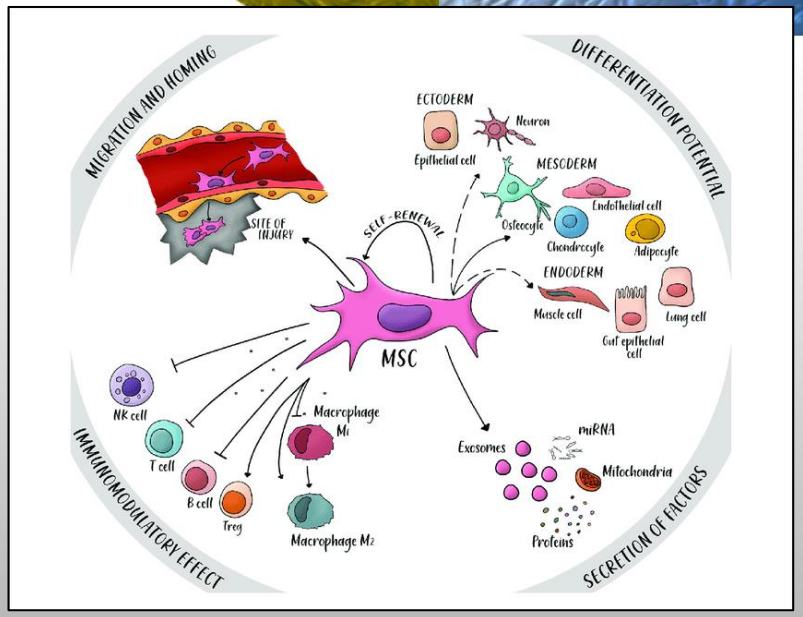


2D Cell-based Therapies

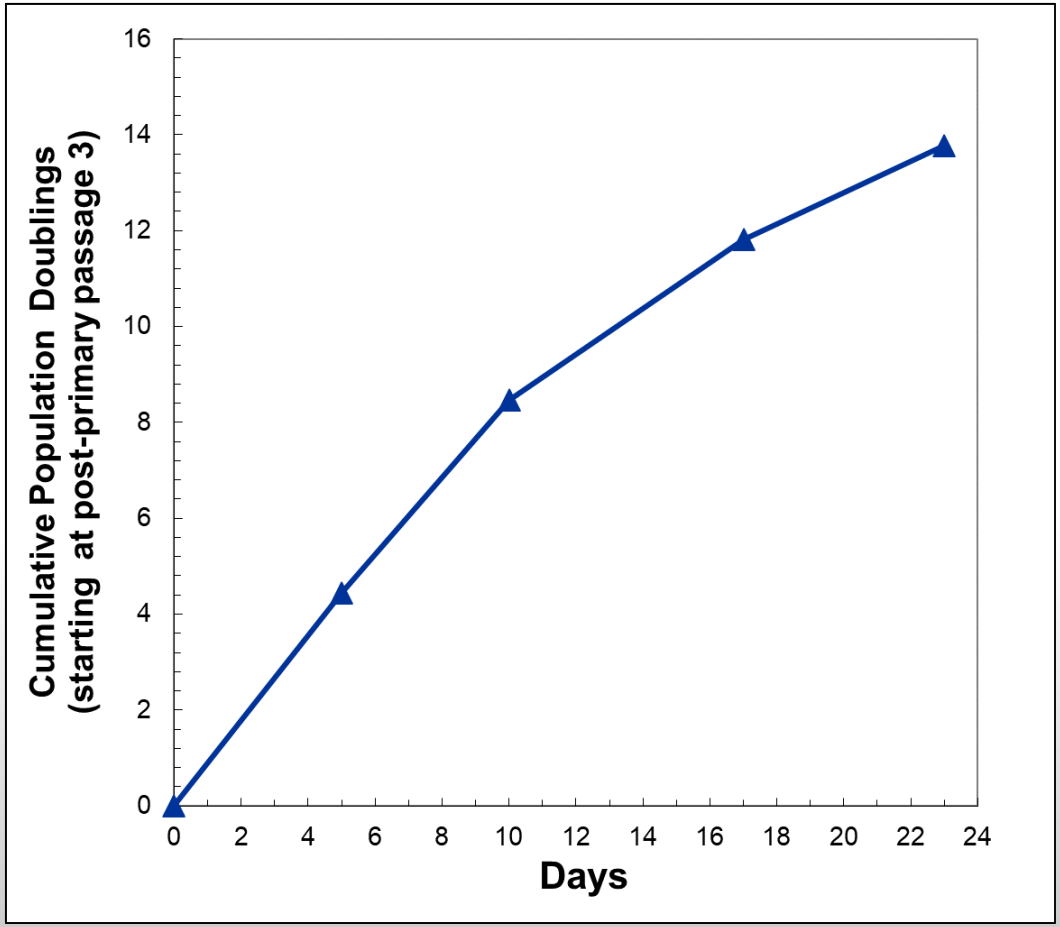
- ***2D Cell-derived Therapeutic Products (e.g., Conditioned Medium and Extracellular Vessicles (EVs))***
- ***3D Reconstructed Tissue Therapies***

Cosmeceutical applications using human mesenchymal stem cells

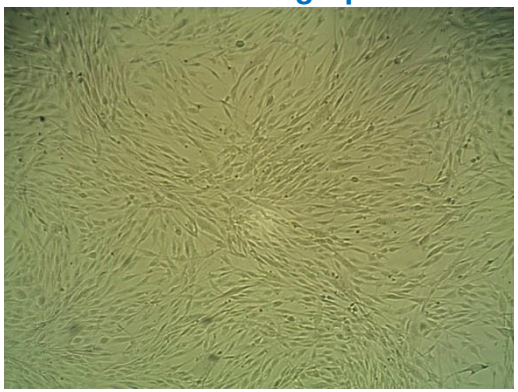
- ***Cell-derived Products (e.g., Conditioned Medium and EVs)***



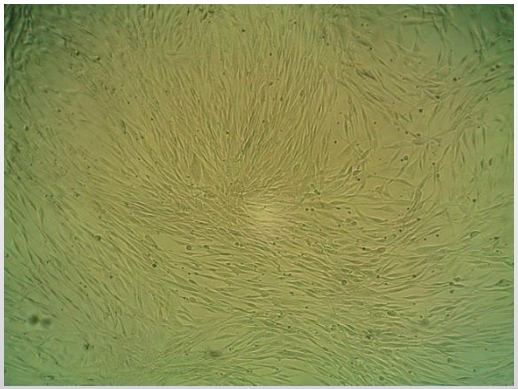
A modified HFSdaFREE - KIT supplement system (HFSdaFREE2 KIT) efficiently supports the chemically-defined animal origin-free (cdAOF) serial propagation of serum-starved adult adipose-derived human mesenchymal stem cells (HMSCs)



Photomicrographs



passage 3, day 5 photomicrograph



passage 4, day 10 photomicrograph

Methods: Cryopreserved post-primary passage 1 STEMPRO Adipose-derived Human Mesenchymal Stem Cells (HMSCs), reared in MesenPro RS Medium (2% FBS + growth factors + basal medium) were purchased from Life Technologies. HMSCs were thawed and plated directly into passage 2 culture using MesnePro RS Medium. At the end of passage 2, the cells were washed 3 times with chemically-defined animal origin- free (cdAOF) FBM basal medium (LONZA), dissociated with the cdAOF TrypLE Select reagent (Life Technologies) and then cryopreserved as serum-starved HMSC, using AvantBio's cdAOF CROVIVE 5% DMSO cryopreservation media. Briefly, using AvantBio's experimental cdAOF HFSdaFREE2 v1 KIT supplements (HFSdaFREE2 v1, HFGE, HFGE2), in a 50/50 blend of cdAOF KBM and FBM basal medium (LONZA), the serum-starved passage 2 HMSCs were thawed and plated into passage 3 culture at 2,500 cells/cm^2. The HMSCs were serially passaged through passage 6, as shown in the figure above. Mean cumulative population doublings were calculated at the end of each passage, from triplicate wells, +/- the SEM. Representative photomicrographs at the end of passage 3 (day 5) and passage 4 (day 10). STEMPRO cells, MesenPro RS medium and TrypLE Select are trademarks of Life Technologies Corporation. FBM and KBM basal medium are trademarks of LONZA Corporation.

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