GSC Advanced Research and Reviews
eISSN: 2582-4597 CODEN (USA): GARRC2
Cross Ref DOI: 10.30574/gscarr
Journal homepage: https://gsconlinepress.com/journals/gscarr/

(REVIEW ARTICLE)

Check for updates

Proposed treatment plan for restoration of histoarchitecture and function of severed nerve embedded in scar tissue

Henry E. Young [1, 2, *]

[1] *Dragonfly Foundation for Research and Development, Macon, GA, 31210, USA.*
[2] *Henry E Young PHD Regeneration Technologies, Macon, GA, 31210, USA.*

GSC Advanced Research and Reviews, 2025, 24(01), 323-354

Publication history: Received on 17 June 2025; revised on 26 July 2025; accepted on 29 July 2025

Article DOI: https://doi.org/10.30574/gscarr.2025.24.1.0217

Abstract

The restoration of histoarchitecture and function of a severed peripheral nerve embedded in scar tissue presents a problem with multiple issues, thus requiring a multiple-prong approach. Each issue must be addressed for a successful end result: Wallerian Degeneration/Regeneration; the normal histoarchitecture of a mixed sensory/motor peripheral nerve; identification of stem cell(s) that have the differentiation potential to form neurons and their supportive tissues; isolation of those stem cells in the least invasive method possible; methods to deliver stem cells to the cell bodies and cell processes of a mixed nerve; local non-toxic anesthetics to mix with stem cells for direct injections through soft tissues; pre-address any underlying co-morbidities that may prevent successful restoration of nerve function; provide building blocks for Schwann cell remyelination of nerve fibers; non-surgical removal of existing scar tissue; increase activity of innate immune system to remove fragments of scar tissue; decrease inflammation at the original site of scar tissue formation; resolve platelet rich plasma's inductive influence on stem cells; decrease future scar tissue formation; and resolve disuse atrophy of skeletal muscle by restoring tone in skeletal muscle. The ultimate goal of this treatment plan is to increase quality of life using naturally-occurring autologous endogenous adult telomerase positive stem cells (aTPSCs), combinatorial nutraceutical super pill (CNSP), supplements, and an enzyme to address the issues defined above. The hypothesis is to support Wallerian regeneration given the appropriate cells, supplements, and enzymes to regrow the severed nerve for reinnervation to regain function in associated skeletal muscles.

Keywords: Stem Cells; Nerves; Scar Tissue; Regeneration; Reinnervation

1. Introduction

This is a case study of a 19-year-old male that was involved in car accident with severe trauma to his brachial plexus. The trauma resulted in radial, axillary, and suprascapular nerve damage. The traumatized radial nerve self-repaired after 5 months. At 6 months after the accident, electromyography testing revealed no conduction in the axillary or suprascapular nerves. Surgery was performed on the axillary nerve the following day with 4.7-inch sural nerve graft from his calf. No evidence of a distal portion of the suprascapular nerve was located. The suprascapular nerve was embedded in scar tissue at the site of severance of the nerve.

In the 50+ years I have been doing research in the areas of wound healing; restoration of function; limb regeneration; changes in the extracellular matrix with increasing age of the individual; discovery, isolation, and characterization of endogenous adult telomerase positive stem cells (aTPSCs); morphogenetic proteins derived from demineralized bone matrix; exosome conditioned medium derived from differentiated cells; the use of aTPSCs in animal models of disease; the use of aTPSCs in human clinical studies of disease, expanded human clinical trials, and CNSP to direct activities of endogenous aTPSCs in situ, I have learned a very important lesson. The body has a defined protocol for doing things.

* Corresponding author: Henry E. Young.

And that the body's protocol has been perfected over multiple millennia. If one tries to change the body's protocol, it won't work. The body will supersede anything you try, and in the end will get its own way. So, it is far better to support the body rather than to trying to fight it.

The restoration of the histoarchitecture and function of severed peripheral nerve(s) embedded in scar tissue presents a problem with multiple issues, thus requiring a multiple-prong approach. Each issue must be address for a successful end result. The issues involved are:

- Wallerian Degeneration/Regeneration;
- The normal histoarchitecture of a mixed sensory/motor peripheral nerve;
- Identification of stem cell(s) that have the differentiation potential to form neurons and their supportive Schwann cells, loose fibrous connective tissue, capillaries, arterioles/venioles, small arteries, small veins, lymphatics;
- Isolation of those particular stem cell(s) in the least invasive method possible (do not want to create one problem to fix another);
- Methods to deliver particular aTPSCs to the cell bodies and cell processes of a mixed nerve;
- Local non-toxic anesthetic to mix with aTPSCs for direct injections through soft tissues;
- Address any underlying co-morbidities that may prevent successful restoration of nerve function;
- Provide building blocks for Schwann cell myelin synthesis and secretion for remyelination of nerve fibers;
- Non-surgical removal of existing scar tissue, without creating more scar tissue;
- Increase activity of innate immune system to remove fragments of scar tissue;
- A decrease in inflammation at the original site(s) of scar tissue formation;
- Resolve Platelet Rich Plasma (PRP) inductive influence on aTPSCs;
- Decrease and/or prevent future scar tissue formation at site; and
- Resolve disuse atrophy of skeletal muscle by restoring tone in skeletal muscle to be re-innervated by nerve.

There is preliminary clinical study evidence for using endogenous adult telomerase positive stem cells (aTPSCs) and combinatorial nutraceutical super pill (CNSP) to provide the appropriate cells for repair. The evidence comes from two individuals that were treated for traumatic spinal cord transection injury at the T12 level (1,2). They were both paraplegic below level T12, having lost all body functions, e.g., bladder/bowel function, walking, etc., due to non-functional nerves L1 to S5. One was treated with fresh isolate aTPSCs, activated ex vivo, and then transfused into the individual. This occurred by intranasal infusion of TSCs, injection of PSCs and MesoSCs into the intervertebral foramen under C-arm guidance, and TSCs, PSCs, and MesoSCs given by systemic IV infusion. Following two successful treatments, the individual regained bladder/bowel function (1). Their results suggested reinnervation of those two organ systems. The other individual was treated with CNSP to induce activation of aTPSCs in situ. After two years of CNSP ingestion, the individual regained flexion and extension movements in the anterior (quadriceps) and posterior (hamstring) muscular compartments of the thigh (2). While these two studies provide only preliminary evidence, it does suggest the possibility that restoration of peripheral nerve function is possible.

The ultimate goal for the 20-year-old individual is to increase their quality of life using naturally-occurring endogenous adult telomerase positive stem cells (aTPSCs), combinatorial nutraceutical super pill (CNSP), supplements, and an enzyme to address the issues defined above. The HYPOTHESIS for this treatment plan is to try to allow Wallerian regeneration to occur given the appropriate cells, supplements, and enzymes to non-surgically remove the impeding scar tissue, and thus allow regrowth of the severed nerve for reinnervation to the associated skeletal muscles.

2. Materials and methods

The proposed treatment methods are shown (Fig. 1).

Figure 1 addresses issues 3-7 from the above list, e.g., 3. Identification of stem cell(s) that have the differentiation potential to form neurons and their supportive Schwann cells, loose fibrous connective tissue, capillaries, arterioles/venioles, small muscular arteries, small muscular veins, lymphatics; 4. Isolation of those stem cell(s) in the least invasive method possible (do not want to create one problem to fix another); 5. Method to deliver the stem cells to the cell bodies and cell processes of a mixed nerve; 6. Local anesthetic to mix with aTPSCs for direct injections through soft tissues; and 7. Address any underlying co-morbidities that may prevent successful restoration of nerve function. Additional compounds/methods need to be instituted to cover issues 8-14, e.g., 8. Provide building blocks for Schwann cell myelin synthesis and secretion; 9. Removal of existing scar tissue; 10. increase activity of innate immune system to remove fragments of scar tissue; 11. decrease in inflammation at the original site(s) of scar tissue formation; 12. Resolve

Platelet Rich Plasma (PRP) inductive influence on aTPSCs; 13. Decrease and/or prevent future scar tissue formation at site; and 14. Resolve disuse atrophy of skeletal muscle by restoring tone in skeletal muscle innervated by nerve.

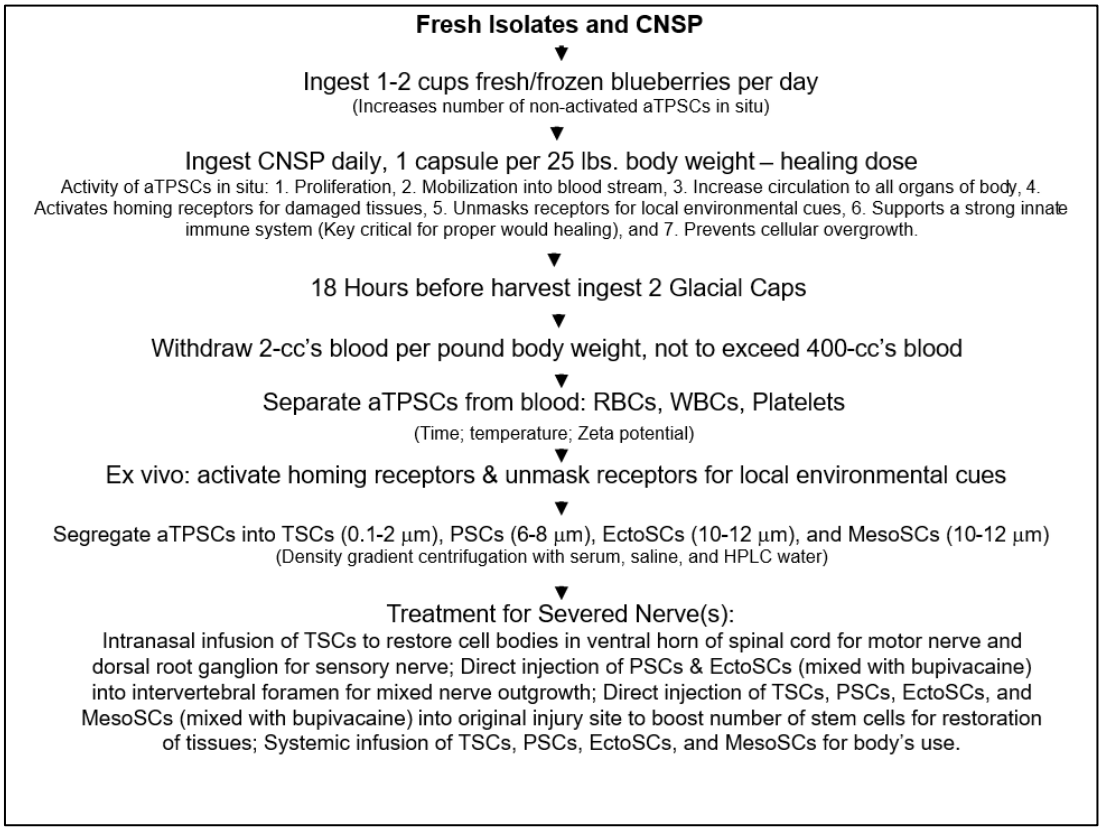


Figure 1 Proposed treatment for restoration of histoarchitecture and function of severed peripheral nerve.

3. Results

No results as yet from this treatment plan since it has yet to be started. This is a PROPOSED treatment plan. I will accept any and all suggestions for improvements to this proposed treatment. Also, if you would be willing to collaborate on this treatment plan, the individual involved and myself would be greatly appreciative. Email: young.hey1@yahoo.com.

4. Discussion

4.1. Wallerian Degeneration/Regeneration

Wallerian Degeneration Regeneration describes a series of events that occur in a peripheral mixed sensory and motor nerve that has been injured or damaged. When this occurs, the portion of the mixed nerve, both proximal and distal to the site of injury undergoes degeneration. The proximal portion of the nerve and its surrounding myelin sheath disintegrate back to the location of the cell bodies (central portion of nerve cell containing the nucleus). For sensory nerve fibers, the cell bodies are located in the dorsal root ganglion. For motor nerve fibers, the cell bodies are located in the ventral horn of the spinal cord. Macrophages and Schwann cells clear the debris field proximal and distal to the site of injury and prepare the environment for regeneration. If the nerve fiber's cell bodies remain intact and the connective tissue sheathes (e.g., endoneurium, perineurium, and epineurium) remain intact, regeneration (outgrowth of cell processes) from the cell bodies can begin. Schwann cells proliferate and form guiding structures within the connective tissue sheathes, e.g., entire nerve fiber within the epineurium, bundles of nerve fibers within the perineurium, and individual nerve fibers within the endoneurium. The Schwann cell within the connective tissue structures direct new outgrowth from the cell bodies to the point of injury. And, if no or minimal scar tissue is present, direct new outgrowth to their target organ (skeletal muscle). As the naked nerve fibers regrow, Schwann cells remyelinate the new segments, allowing the regenerating nerve to potentially regain function. If dense scar tissue is present at site of injury, a physical barrier is formed to prevent outgrowth of the mixed nerve fiber to sensory structures (e.g., Golgi tendon organs, spindle fibers, stretch receptors, etc.) and motor structures (motor end plates) within skeletal muscle (3-7).

4.2. The normal histoarchitecture of a mixed motor/sensory peripheral nerve

The normal histoarchitecture of a mixed motor/sensory peripheral nerve demonstrates a highly organized entity designed to transmit both motor and sensory signals between the central nervous system and the periphery. Each nerve consists of bundles of nerve fibers, that conduct impulses from the ventral horn of the spinal cord toward muscles (motor) and others that carry sensory information from the periphery (sensory) to the dorsal root ganglion, then through interneurons to the spinal cord (anterior horn of spinal cord) to the brain. The outer most connective tissue layer is the epineurium, which surrounds the entire nerve, i.e., bundles of nerve fibers, and also contains small muscular arteries for nutrient supply and waste removal. Each nerve bundle is covered by the perineurium, serving as a semi-permeable barrier that regulates the microenvironment and maintains the blood-nerve barrier. The perineurium contains small arteries and veins. Each individual nerve fiber is surrounded by a connective tissue "tube" termed the endoneurium. This tube consists of loose fibrous connective tissue, capillaries, and telomerase positive pluripotent stem cells and telomerase positive totipotent stem cells. Lining the inside of the endoneurial tubes are Schwann cells, which surround the individual nerve fibers. These nerve fibers may be myelinated or unmyelinated. Myelinated nerve fibers are associated with motor function and fast conducting sensory fibers, such as those for proprioception, and touch. They are wrapped in concentric layers of Schwann cell plasma membrane, in a "jelly roll" fashion. Between myelinating Schwann cells are gaps, termed "Nodes of Ranvier" along the myelinated nerve fibers allow for rapid saltatory propagation signal conduction. Unmyelinated nerve fibers are associated with sensory function, such as those for Golgi tendon organs, muscle spindles, pain, and temperature. They are engulfed by the Schwann cells, but lack the previously described myelination structure (8,9).

4.3. Normal Embryogenesis

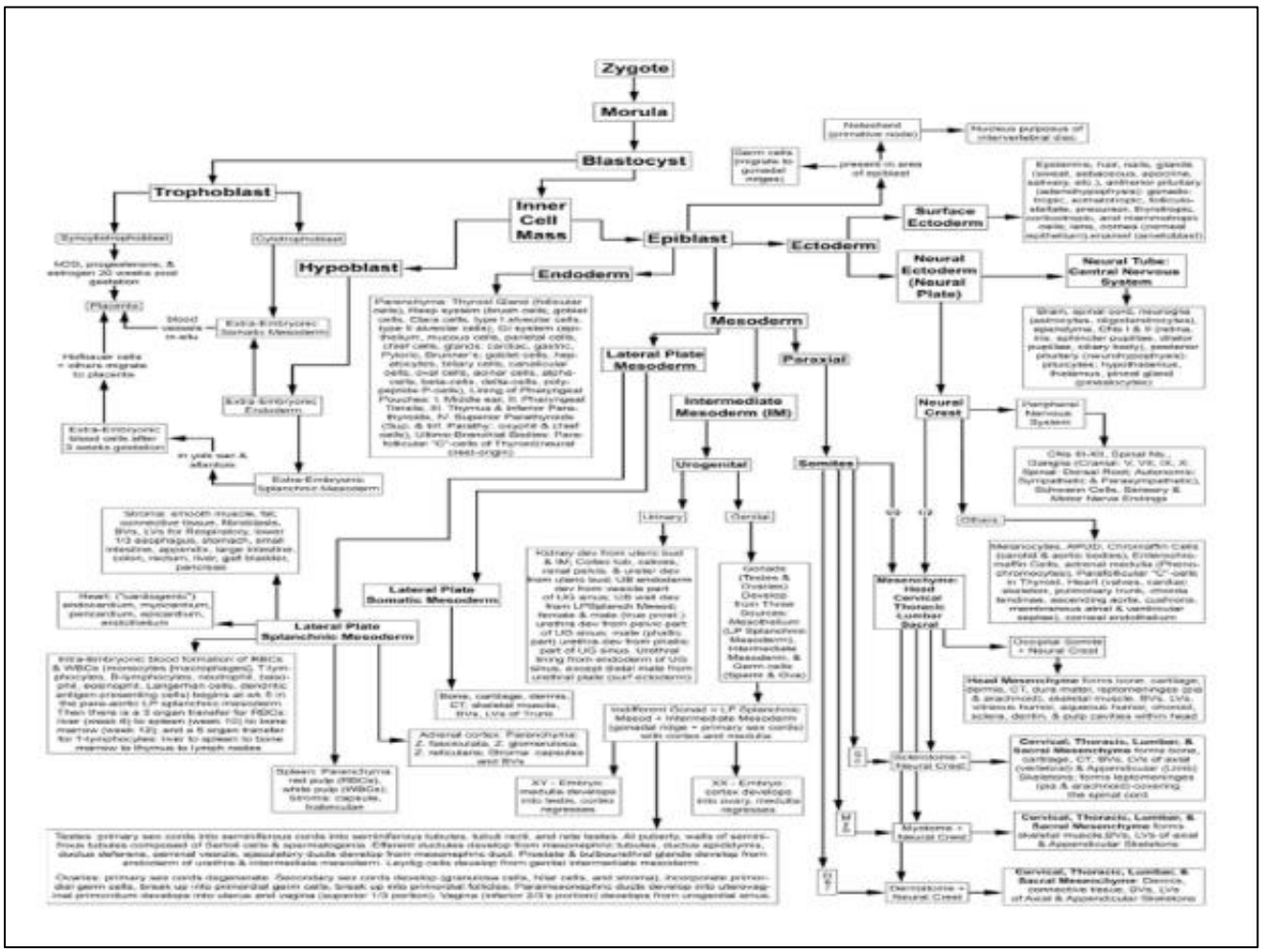


Figure 2 Lineage Map of unidirectional cell differentiation pathway for cells derived from the embryonic zygote (10). Reprinted with permission from Young HE, Black, Jr AC. Adult stem cells. Anatomical Record Part A 2004; 267A:75-102. Wiley-Liss, Inc

Knowledge of normal embryogenesis and subsequent differentiation potential (10) is paramount for anyone attempting to use any type of stem/progenitor cell for regenerative medicine, especially for individuals with neurological, cardiovascular, pulmonary, hepatic, autoimmune, pancreatic, and/or renal chronic diseases, terminal diseases, acute and chronic trauma, and/or those with severe burns. Figure 2 is a Lineage Map detailing the pattern of normal developmental embryogenesis from zygote to adult differentiated cell types. In the flow diagram, differentiation from a primitive cell type (zygote) to a mature (differentiated) cell type is in a unidirectional direction from least primitive cell type to most differentiated cell type (10).

5. Adult Telomerase Positive Stem Cells (aTPSCs)

Five major categories of telomerase positive stem cells were originally identified that were located within the connective tissue matrices of adult animals as normally quiescent hibernating cells. These categories were totipotent stem cells (TSCs), pluripotent stem cells (PSCs), ectodermal stem cells (EctoSCs), mesodermal stem cells (MesoSCs), and endodermal stem cells (EndoSCs). These five major categories were further subdivided into 8 subcategories based on size, Trypan blue staining, cell surface markers, culture conditions, and differentiation capabilities [Table 1, Figs. 4-7], (11).

The eight categories, named in their respective developmental sequence, are totipotent stem cells (TSCs), halo-like stem cells (HLSCs), corona-like stem cells (CLSCs), pluripotent stem cells (PSCs), germ layer lineage stem cells (GLSCs), ectodermal stem cells (EctoSCs), mesodermal stem cells (MesoSCs), and endodermal stem cells (EndoSCs). All eight categories of these stem cells retain the telomerase enzyme until they begin to differentiate, at which point they lose the telomerase enzyme and assume the proliferative abilities of progenitor cells, e.g., 6-8 population doublings for rodents and 70 population doublings for humans before programmed senescence and death (10-15).

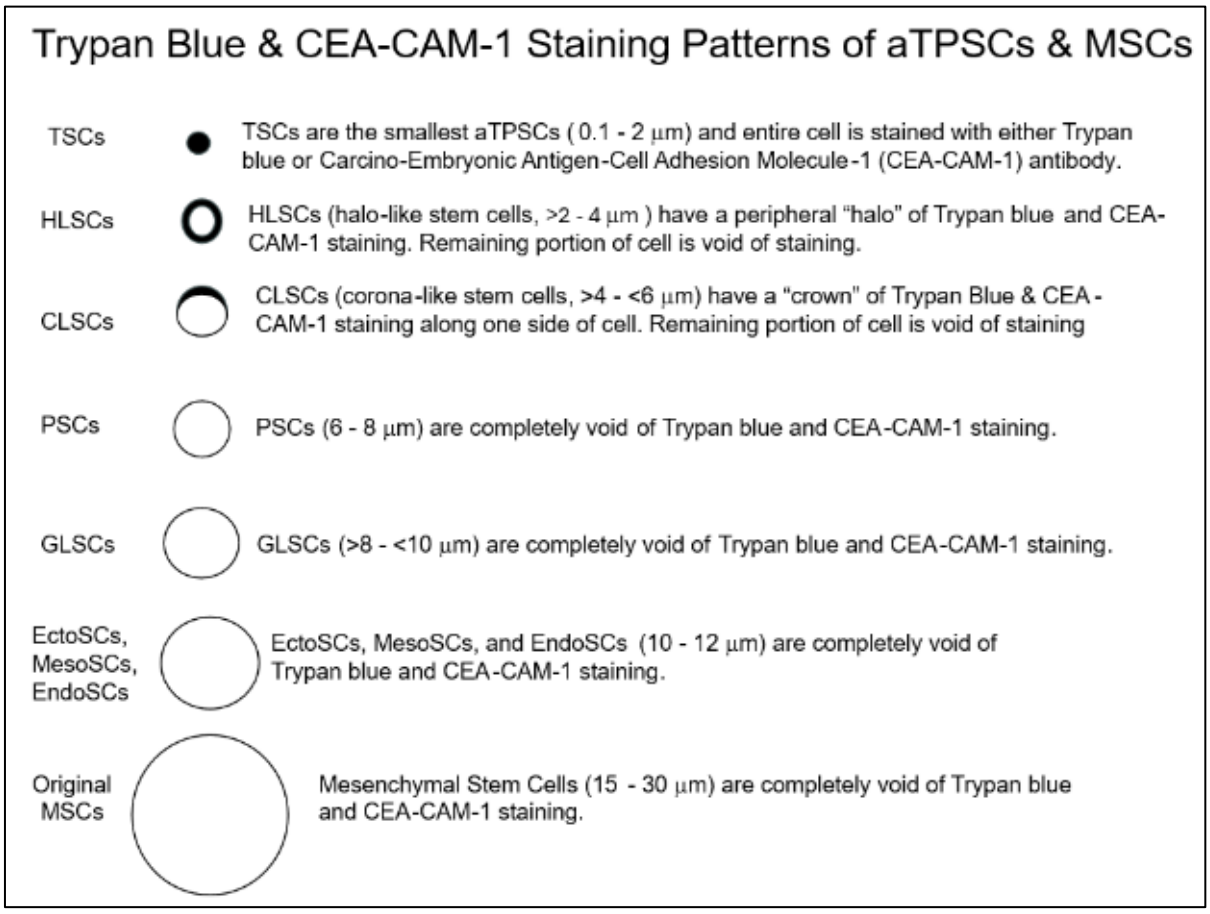


Figure 3 Diagrammatic representation of size comparison and visual staining patterns viewed by brightfield microscopy of aTPSCs (e.g., TSCs, HLSCs, CLSCs, PSCs, GLSCs, EctoSCs, MesoSCs, and EndoSCs) and Mesenchymal Stem Cells (MSCs) when stained with either 0.4% Trypan Blue or Carcino-Embryonic Antigen-Cell Adhesion Molecule-1 (CEA-CAM-1)

Table 1 Attributes of Endogenous Adult Telomerase Positive Stem Cells

Attributes	**TSCs[1]**	**HLSCs[2]**	**CLSCs[3]**	**PSCs[4]**	**GLSCs[5]**	**EctoSCs[6]**	**MesoSCs[7]**	**EndoSCs[8]**
Size, ⍰m	0.1-2.0	>2-4	>4-<6	6-8	>8-<10	10-12	10-12	10-12
Trypan blue	Entire Cell Positive[9]	Halo[10] Positive, Negative	Corona[11] Positive, Negative	Entire Cell Negative[12]	Entire Cell Negative	Entire Cell Negative	Entire Cell Negative	Entire Cell Negative
Cell Surf Markers Animals	CEA-CAM-1[13]	CEA-CAM-1^{high} SSEA-4^{low}	CEA-CAM-1^{low} SSEA-4^{high}	SSEA-4[14]	SSEA-4, Thy-1[15]	Thy-1	Thy-1	Thy-1
Cell Surf Markers Human	CD66e[16]	CD66e^{high} CD10^{low}	CD66e^{low} CD10^{high}	CD10[17]	CD10 CD90[18]	CD56, CD90 MHC-1[19]	CD13, CD90 MHC-1	??? CD90 MHC-1
Culture Conditions	Suspen[20] & Substrate Adhesion[21]	Substrate Adhesion	Substrate Adhesion	Substrate Adhesion	Substrate Adhesion	Substrate Adhesion	Substrate Adhesion	Substrate Adhesion
Differen Cap[22]	All Cells[23]	Somatic Cells only[24]	Somatic Cells only	Somatic Cells only	Somatic Cells only	Ectoderm Lineage Only[25]	Mesoderm Lineage Only[26]	Endoderm Lineage Only[27]
Maximum PDs[29] To Date	>300 Rat PDs	>300 Rat PDs	>300 Rat PDs	>400 Rat PDs	>400 Rat PDs	>400 Rat PDs	>690Human PDs	>400 Rat PDs

Table 1. TSCs[1], totipotent stem cells; HLSCs[2], halo-like stem cells; CLSCs[3], corona-like stem cells; PSCs[4], GLSCs[5], germ layer lineage stem cells; EctoSCs[6], ectodermal stem cells; MesoSCs[7], mesodermal stem cells; EndoSCs[8], endodermal stem cells; entire cell demonstrating Positive[9] staining for 0.4% Trypan blue; Halo[10], complete peripheral rim of positive trypan blue staining with central area of cell negative for Trypan blue staining; Corona[11], crown of positive Trypan blue staining, remainder of cell is negative for Trypan blue staining; entire cell demonstrating Negative[13]staining for 0.4% Trypan Blue; CEA-CAM-1[13], carcinoembryonic antigen-cell adhesion molecule-1; SSEA-4[14], stage-specific embryonic antigen-4; Thy-1[15], N-glycosylated glycophosphatidylinositol (=CD90); CD66e[16], carcinoembryonic antigen; CD10[17], common acute lymphoblastic leukemia antigen (CALLA); CD90[18], N-glycosylated glycophosphatidylinositol (=Thy-1); MHC-1[19], self-recognition molecule Major Histocompatibility Complex-Class-1; Suspension[20], cells grow in suspension culture only; Substrate Adhesion[21], only grows attached to a substrate; Differen Cap[22], differentiation capabilities; All Cells[23], will form all somatic cells of the body, the gametes (spermatogonia and oogonia), and the nucleus pulposus of the intervertebral disc (the only tissue derived from the notochord in adults); Somatic Cells Only[24], will only form somatic cells of the body, will not form gametes, will not form nucleus pulposus of the intervertebral disc; Ecto Lineage Only[25], will only form cells of the ectodermal germ layer lineage, will NOT form cells of either the mesodermal or the endodermal germ layer linages; Meso Lineage Only[26], will only form cells of the mesodermal germ layer lineage, will NOT form cells of either the ectodermal or the endodermal germ cell lineages; Endo Lineage Only[27], will only form cells of the endodermal germ layer lineage, will NOT form cells of either to ectodermal or mesodermal germ layer lineages; NYD[28] , not yet determined; PDs[29], population doublings (11). Reprinted with permission from Young HE, Speight MO. Characterization of endogenous telomerase-positive stem cells for regenerative medicine, a review. Stem Cell Regen Med 2020; 4(2):1-14.

5.1. Identification and Phenotypic Expression Markers to Determine Differentiation Potential

Adult telomerase positive stem cells were identified by immunocytochemical and histochemical staining of phenotypic expression markers [Table 2, Figs. 4-7], flow cytometry [Table 3, Figs. 8,9], and isolation and characterization studies [Tables 4,5] from 15 species of animals, including humans, from multiple tissue sites to ascertain their respective differentiation potentials [Table 6].

5.2. Phenotypic Expression Markers for Cell Identification

One hundred and twelve immunocytochemical and histochemical staining procedures [Table 2] were used to objectively verify the identity and identifiable phenotypic expression markers for 75 separate cell types, in 33 organs and 46 tissues of the body, across 15 species of animals, including humans [Table 3] (11,12,14,15).

Table 2 Antibodies, Immunocytochemistry, & Histochemistry for Phenotypic Expression Markers

Antibody	Antigen	Embryological Origin
CEA-CAM-1	Carcinoembryonic antigen-cell adhesion molecule-1	Totipotent
HCEA	Human Carcinoembryonic antigen	Totipotent
CEA	Carcinoembryonic antigen	Totipotent
CD66e	Carcinoembryonic antigen	Totipotent
DH-TuAg1	Spermatogonia	Totipotent
MC-480	SSEA-1	Pluripotent
MC-631	SSEA-3	Pluripotent
MC-813	SSEA-4	Pluripotent
CD10	Neutral endopeptidase	Pluripotent
AlkPhos	Alkaline Phosphatase	Pluripotent
CD56	Neural cell adhesion molecule	Ectoderm
Pax-6	Neurogenic lineage	Ectoderm
FORSE-1	Neuronal precursor cells	Ectoderm
Vimentin	Cells of neurogenic lineage	Ectoderm
Nestin	Cells of neurogenic lineage	Ectoderm
R401	Nestin-neuronal lineage	Ectoderm
HNES	Nestin-neuronal lineage	Ectoderm
MAB353	Nestin-neuronal lineage	Ectoderm
RT-97	Neurofilaments = neurons	Ectoderm
NF68	Neurofilament-68 = neurons	Ectoderm
S-100	Neurofilaments-100 = neurons	Ectoderm
NF-145	Neurofilaments-145 = neurons	Ectoderm
N-200	Neurofilaments-200 = neurons	Ectoderm
8A2	Neurons	Ectoderm
NG2	Neurons	Ectoderm
TH	Tyrosine hydroxylase, precursor to neural transmitters	Ectoderm
SV2	Synaptic vesicles	Ectoderm
DOPA	Dopamine, transmitter of dopaminergic neurons	Ectoderm
T8660	Beta-tubulin-III	Ectoderm
Tuj1	Beta-tubulin	Ectoderm
GFAP	Glial-fibrillary acidic protein	Ectoderm
CNPase	Glial cells = oligodendrocytes & astrocytes	Ectoderm

Rip	Oligodendrocytes	Ectoderm
MOSP	Oligodendrocyte specific proteins	Ectoderm
MAB	Oligodendrocyte marker	Ectoderm
40E-C	Radial cells and radial glial cells	Ectoderm
VM-1	Keratinocytes	Ectoderm
CD13	Amino endopeptidase	Mesoderm
OP-137	MyoD	Mesoderm
F5D	Myogenin = skeletal muscle	Mesoderm
MF-20	Sarcomeric myosin = skeletal muscle	Mesoderm
ALD58	Myosin heavy chain	Mesoderm
A4.74	Myosin fast chain	Mesoderm
IA4	Smooth muscle alpha actin = smooth muscle	Mesoderm
Calp	Calponin	Mesoderm
MAB-3252	Cardiotin = cardiac myocytes	Mesoderm
MAB1548	Myosin heavy chain of cardiac muscle	Mesoderm
WV1D1	Bone sialoprotein II = bone	Mesoderm
MP111	Osteopontine = bone	Mesoderm
Von Kossa	Stain calcium in bone	Mesoderm
CIIC1	Type-II collagen	Mesoderm
II-4CII	Type-II collagen	Mesoderm
HC-II	Human type-II collagen	Mesoderm
Alcian Blue	Stains anions on carbohydrate groups	Mesoderm
AB 1.0	Alcian Blue, pH 1.0 stains sulfate groups on GAGs	Mesoderm
AB 2.5	Alcian Blue, pH 2.5 stains carboxyl groups on GAGs	Mesoderm
Alcec Blue	Stains anions on carbohydrate groups	Mesoderm
AcB 1.0	Alcec Blue, pH 1.0 stains sulfate groups on GAGs	Mesoderm
AcB 2.5	Alcec Blue, pH 2.5 stains carboxyl groups on GAGs	Mesoderm
Safranin-O	Stains anions on carbohydrate groups	Mesoderm
SO 1.0	Safranin-O, pH 1.0 stains sulfate groups on GAGs	Mesoderm
SO 2.5	Safranin-O, pH 2.5 stains carboxyl groups on GAGs	Mesoderm
D1-9	Type-IX collagen = cartilage	Mesoderm
9/30	Cartilage link protein	Mesoderm
12/21	Cartilage proteoglycan hyaluronate binding region	Mesoderm
12C5	Versican hyaluronate binding region	Mesoderm
H-DC34	Sialomucin-containing hematopoietic/endothelial cells	Mesoderm
CD31	PECAM, Peripheral endothelial cell adhesion molecule	Mesoderm
P1H12	Human endothelial cell surface marker	Mesoderm
P2B1	Peripheral endothelial adhesion molecule	Mesoderm

P8B1	VCAM, vascular cell adhesion molecule	Mesoderm
P2H3	CD62e, E-selectin (vasculature)	Mesoderm
H-endo	CD146, Endothelial cells	Mesoderm
H5A4	CD11b, granulocytes, monocytes, NK-cells	Mesoderm
H4C4	CD44, hyaluronate receptor	Mesoderm
Hermes-1	CD44, hyaluronate receptor	Mesoderm
H5A5	CD45, all hematopoietic cells except RBCs	Mesoderm
H5C6	CD63, macrophages, monocytes, platelets	Mesoderm
HFSP	Human fibroblast specific protein	Mesoderm
1B10	Fibroblast-specific protein	Mesoderm
Sudan Black-B	Stains fat (adipocytes)	Mesoderm
Oil Red-O	Stains fat (adipocytes)	Mesoderm
H-AFP	Human alpha-fetoprotein = fetal liver	Endoderm
R-AFP	Rat alpha-fetoprotein = fetal liver	Endoderm
DESMO	Endodermal epithelial marker of liver	Endoderm
LAP	Canalicular cell surface protein of liver	Endoderm
151-Ig	Liver epithelial growth factor	Endoderm
HA4c19	Bile canalicular cells of liver	Endoderm
OC2	Progenitor cells, oval cell, & biliary cells of liver	Endoderm
OC3	Progenitor cells & biliary cells of liver	Endoderm
OC4	Progenitor cells & biliary cells of liver	Endoderm
OC5	Progenitor cells & biliary cells of liver	Endoderm
OC10	Progenitor cells & biliary cells of liver	Endoderm
H.4	Intracellular staining of liver hepatocytes	Endoderm
H.1	Liver hepatocytes cell surface marker	Endoderm
DPPIV	Progenitor, canalicular, and biliary cells of the liver	Endoderm
OV6	Biliary and oval cells of liver; biliary cells of liver	Endoderm
HESA	Human GI (Gastrointestinal) Epithelium	Endoderm
YM-PS087	Glucagon-secreting cells of endocrine pancreas	Endoderm
YM-PS5088	Insulin-secreting cells of endocrine pancreas	Endoderm
11180	Somatostatin-secreting cells of the endocrine pancreas	Endoderm
CK-19	Ductal cells of the exocrine pancreas	Endoderm
CD90	Germ Layer Lineage & Progenitor Cells	Transition Cell
Thy-1	Germ Layer Lineage & Progenitor Cells	Transition Cell
Telom	Telomerase positive cells	aTPSCs
PI	Propidium Iodide, measure of live cells, flow cytometry	Live Cells
CD95	Apoptosis, measure of apoptotic cells	Dead Cells
DAPI	Fluorescent marker to visualize living and fixed DNA	Live & Dead Cells

Gal-19	Insect beta-galactosidase genomic marker	Cell tracking marker

Table 2. Antibodies (left column) used to identify specific phenotypic expression markers (center column) for particular cell types (right column). Reprinted with permission from Young HE, Black AC. Pluripotent Stem Cells, Endogenous versus Reprogrammed, a Review. MOJ Orthop Rheumatol 1(4): 00019, 2014.

Following IACUC-approved guidelines, animals were euthanized, and organs and tissues harvested for cryosectioning and immunocytochemistry and/or histochemistry to determine location of TSCs and PSCs within the organs and tissues various species. The harvested organs and tissues were immersed in ELICA fixative, frozen, cryosectioned, and stained with cell surface antibodies: CEA-CAM-1 for TSCs and SSEA-4 for PSCs. Antibody to smooth muscle alpha actin (IA4), within the tunica media of muscular arteries, was used as the positive control to ensure that the protocols for antibody staining of cryosectioned tissues was operating properly. Multiple negative controls were run to make sure that there was not any non-specific binding of the reagents to the tissue. The negative controls consisted of no primary probe, with the remaining probes and substrate intact. Intact primary probe, no secondary probe, with all others remaining intact. Intact primary probe, intact secondary probe, no tertiary probe, with use of the horseradish peroxidase substrate. And lastly, primary probe intact, secondary probe intact, tertiary probe intact, but no horseradish peroxidase substrate used (11). Below are representative organs and tissues subjected to these procedures.

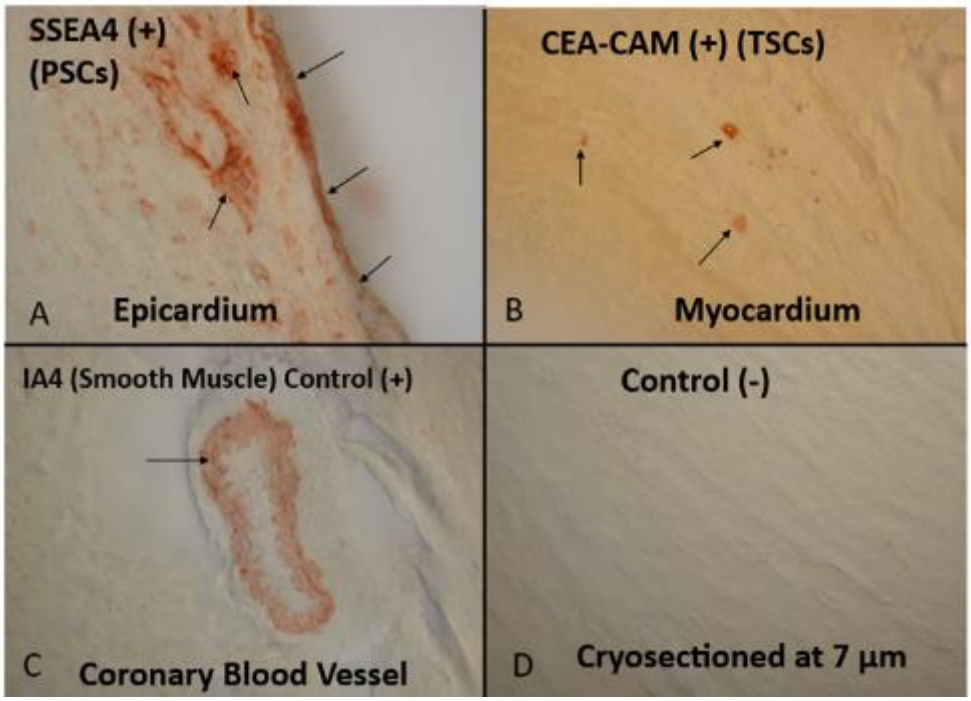


Figure 4 ELICA fixative preserved, frozen, and cryosectioned adult rat heart stained with antibodies for: A, PSCs (SSEA4+); B, TSCs (CEA-CAM-1+); C, smooth muscle (IA4+) positive procedural control, and D, unstained negative procedural control. SSEA4+ PSCs were located in the epicardial connective tissues covering the outside of the myocardium, whereas CEA-CAM-1+ (TSCs) were located within the endomysial connective tissues of the myocardium of the heart. Antibody to smooth muscle alpha actin (IA4), within the tunica media of muscular arteries, was used as the positive control to ensure that the protocols for antibody staining of cryosectioned tissues was operating properly. Multiple negative controls were run to make sure that there was not any non-specific binding of the reagents to the tissue. The negative controls consisted of no primary probe (Fig. 14D), with the remaining probes and substrate intact. Intact primary probe, no secondary probe, with all others remaining intact. Intact primary probe, intact secondary probe, no tertiary probe, with use of the horseradish peroxidase substrate. And lastly, primary probe intact, secondary probe intact, tertiary probe intact, but no horseradish peroxidase substrate used (13). Reprinted with permission from Stout CL, McKenzie J, Long G, et al. Discovery of pluripotent and totipotent stem cells in the heart of the adult rat. Amer Surg 73: S63, 2007

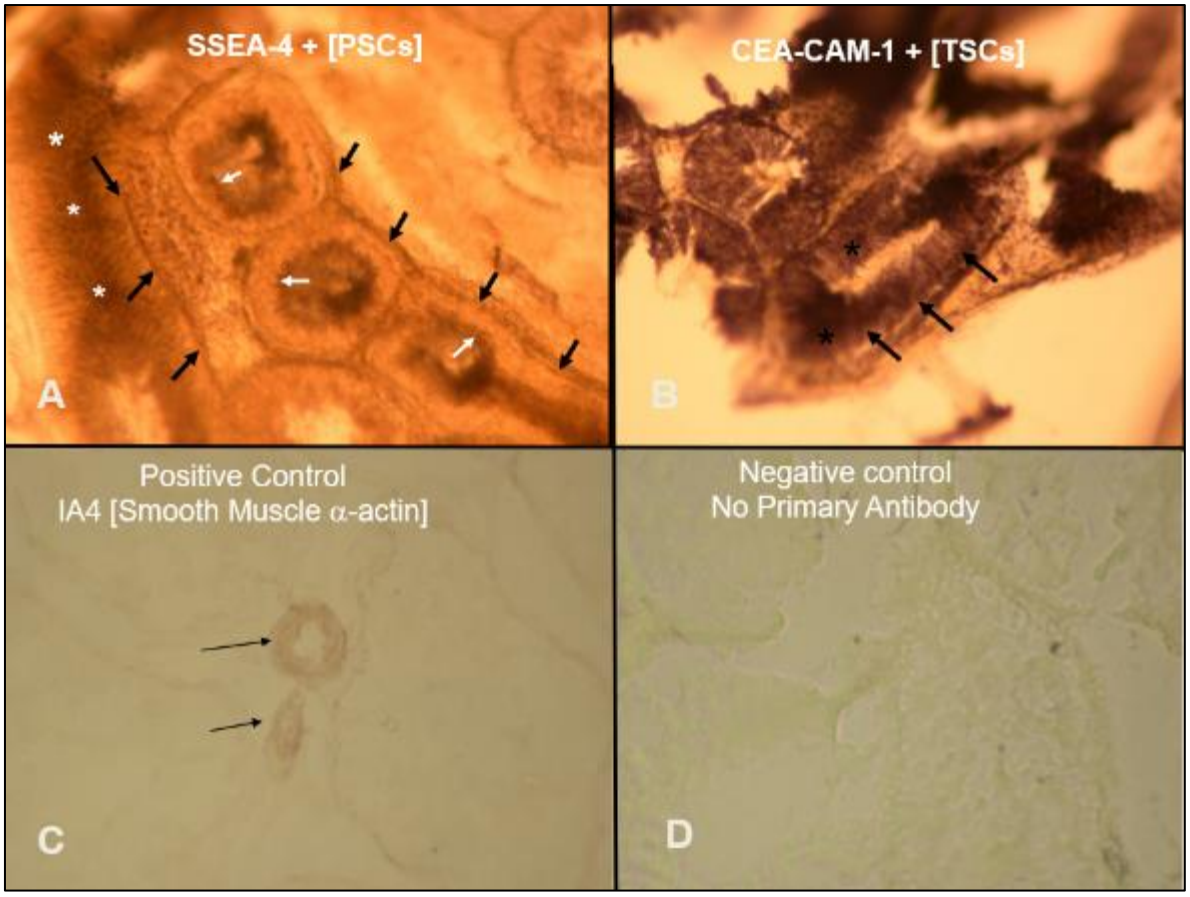


Figure 5 Adult rat testis (RM1021), tissue harvested, fixed in ELICA fixative, vibratome-sectioned at 25 microns, mounted on glass slides, and stained with appropriate antibodies. A. SSEA4+ cells (PSCs) surrounding outside of seminiferous tubules (black arrows) as well as within the innermost layer of seminiferous tubules (white arrows and asterisks), 200x. B. CEA-CAM-1+ staining (TSCs) were present within of seminiferous tubules, with heaviest staining along the inside border of the tubules, 200x. C. IA4+ staining of smooth muscle alpha actin within the tunica media of blood vessels within the testis, positive procedural control, 400x. D. No primary antibody, negative procedural control, 400x. Reprinted with permission from Young HE. Carcinoembryonic antigen-cell adhesion molecule-1 and stage-specific embryonic antigen-4 are present in the reproductive organs of adult mammals (14). GSC Advanced Research and Reviews. 2025; 23(03): 149-147. DOI: https

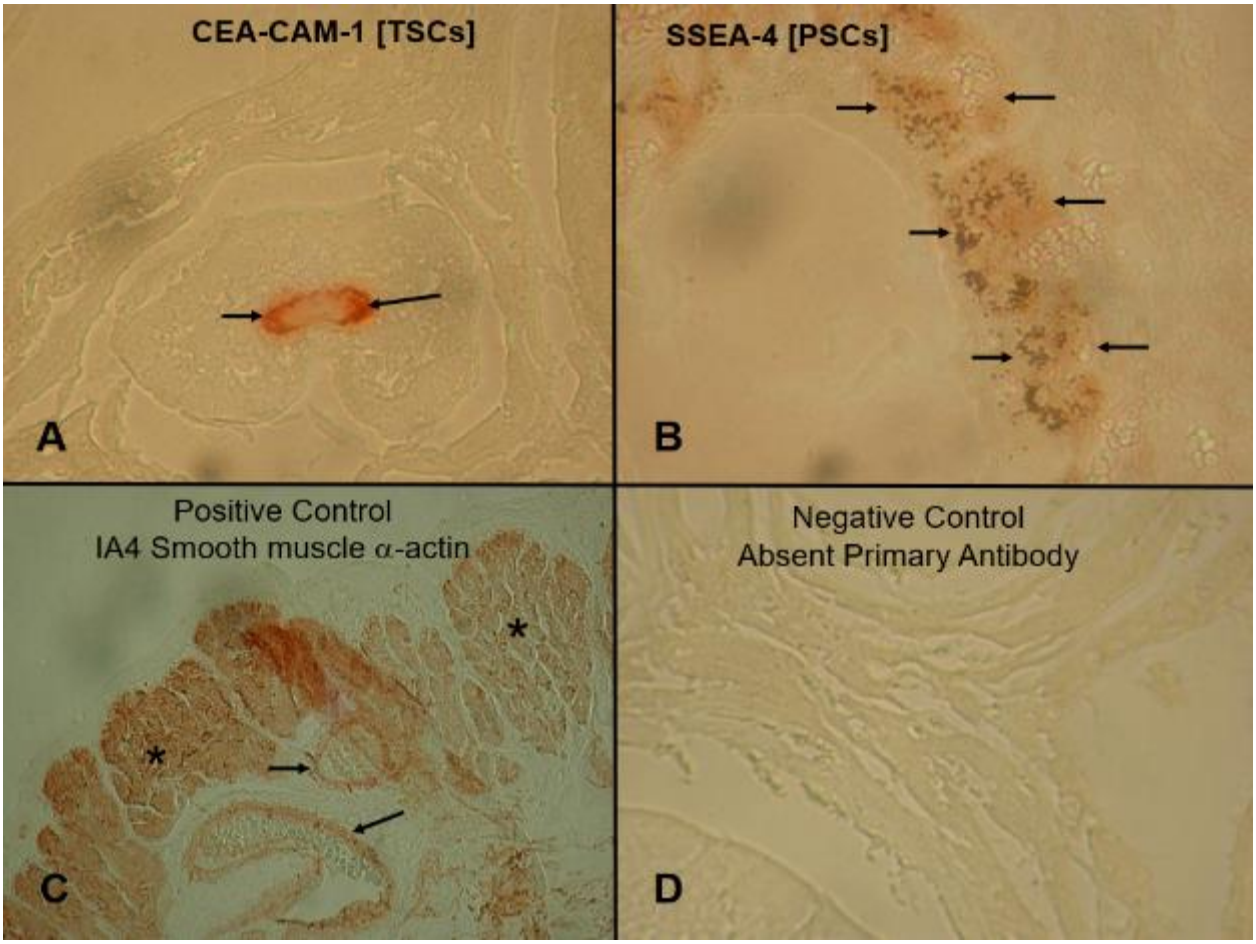


Figure 6 Adult female rat (RF1022) ovary with attached fallopian tube harvested from euthanized rat, fixed in ELICA fixative, cryosectioned at 7 microns, and stained with appropriate antibodies. A. CEA-CAM-1+ staining in area of zona pellucida of ovum inside tertiary follicle (arrows), 400x. B. SSEA-4+ cells interspersed among granulosa cells of Graafian follicle (arrows), 400x. C. IA4+ staining for smooth muscle alpha-actin in both the muscular wall (asterisks) and tunica media of blood vessel (arrows) of the fallopian tube. This particular slide was used in this staining series as the positive procedural control because of the amount of smooth muscle present in the wall musculature and the vascular supply, 200x. D. No primary antibody, no staining present within ovary, used as the negative procedural control, 400x (14). Reprinted with permission from Young HE. Carcinoembryonic antigen-cell adhesion molecule-1 and stage-specific embryonic antigen-4 are present in the reproductive organs of adult mammals. GSC Advanced Research and Reviews. 2025; 23(03): 149-147. DOI: https

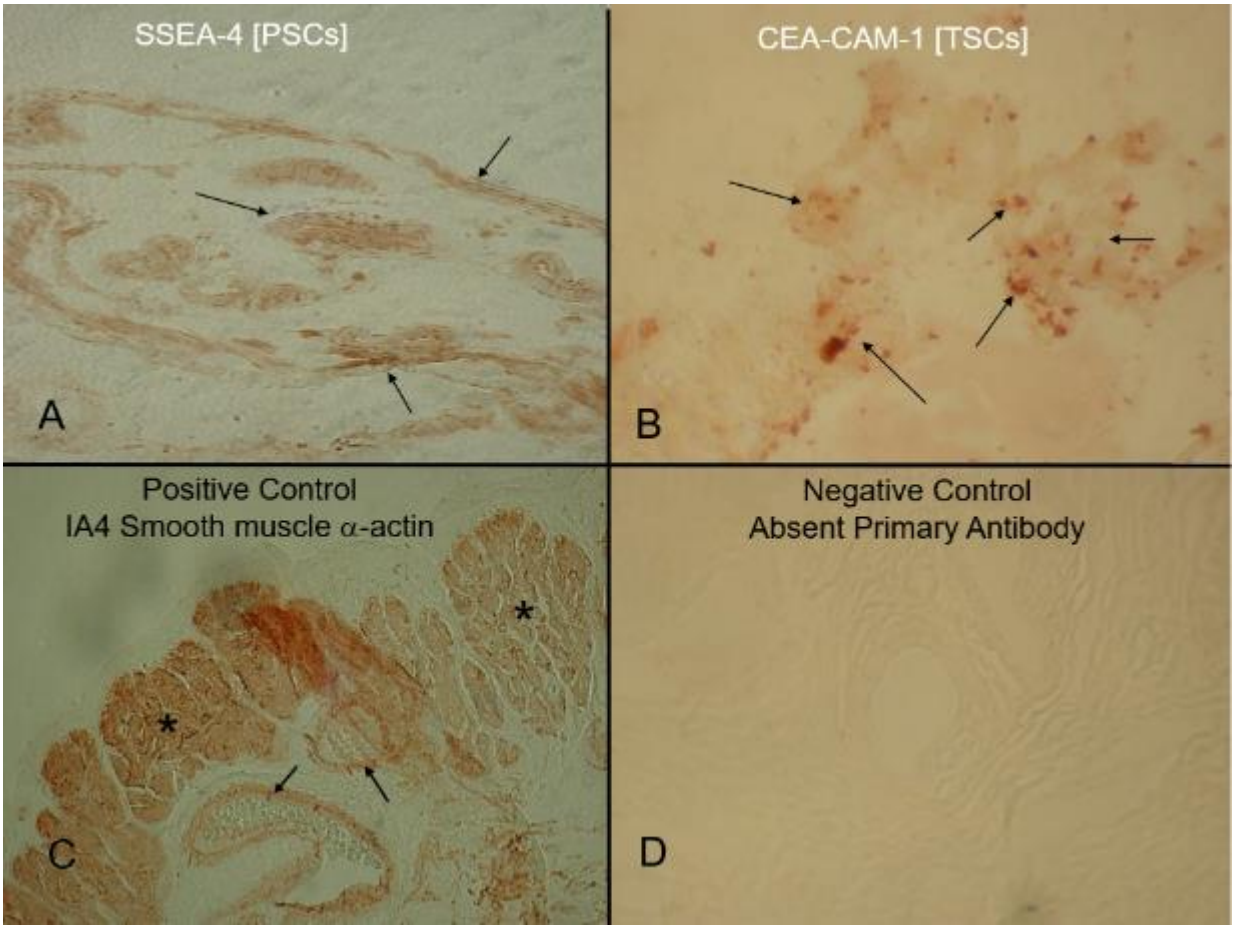


Figure 7 Adult female rat (RF1022) fallopian tube harvested from euthanized rat, fixed in ELICA fixative, cryosectioned at 7 microns, and stained with appropriate antibodies. A. SSEA-4+ PSCs within area of ampulla of fallopian tube (arrows), 200x. B. CEA-CAM-1+ TSCs in the connective tissues of ampulla region of the fallopian tube (arrows), 400x. C. IA4+ smooth muscle alpha-actin in the smooth muscle wall (asterisks) and blood vessels (arrows) of the fallopian tube, as the positive procedural control, 200x. D. No primary antibody, negative procedural control, 400x (14). Reprinted with permission from Young HE. Carcinoembryonic antigen-cell adhesion molecule-1 and stage-specific embryonic antigen-4 are present in the reproductive organs of adult mammals. GSC Advanced Research and Reviews. 2025; 23(03): 149-147. DOI: https

5.3. Flow Cytometry

Differential CD (cluster of human differentiation) marker staining was used to ascertain the cell surface "fingerprint" unique for each subcategory of aTPSCs and the telomerase-negative-mesenchymal stem cells (TN-MSCs). This was initially accomplished using a battery of human CD-markers [Table 3].

Table 3 CD-Marker Fingerprints and Flow Cytometry

Attributes	**TSCs**	**HLSCs**	**CLSCs**	**PSCs**	**GLSCs**	**EctoSCs**	**MesoSCs**	**EndoSCs**	**MSCs**
Positive CD Markers	CD66e	CD66e^{high} CD10^{low}	CD66e^{low} CD10^{high}	CD10	CD90	CD56, CD90, MHC-Class-1	CD13, CD90, MHC-Class-1	??, CD90, MHC-Class-1	CD90 CD105 CD117 CD123 CD166 MHC-Class-1
Negative CD Markers	CD1a, CD2, CD3, CD4, CD5, CD7, CD8, CD9, CD10, CD11b, CD11c, CD13, CD14, CD15, CD16, CD18, CD19, CD20, CD22, CD23, CD24, CD25, CD31, CD33, CD34, CD36, CD38, CD41, CD42b, CD45, CD49d, CD55, CD56, CD59, CD61, CD62e, CD65, CD68, CD69, CD71, CD83, CD90, CD95, CD105, CD117,	CD1a, CD2, CD3, CD4, CD5, CD7, CD8, CD9, CD11b, CD11c, CD13, CD14, CD15, CD16, CD18, CD19, CD20, CD22, CD23, CD24, CD25, CD31, CD33, CD34, CD36, CD38, CD41, CD42b, CD45, CD49d, CD55, CD56, CD59, CD61, CD62e, CD65, CD68, CD69, CD71, CD83, CD90, CD95, CD105, CD117, CD123,	CD1a, CD2, CD3, CD4, CD5, CD7, CD8, CD9, CD11b, CD11c, CD13, CD14, CD15, CD16, CD18, CD19, CD20, CD22, CD23, CD24, CD25, CD31, CD33, CD34, CD36, CD38, CD41, CD42b, CD45, CD49d, CD55, CD56, CD59, CD61, CD62e, CD65, CD68, CD69, CD71, CD83, CD90, CD95, CD105, CD117, CD123,	CD1a, CD2, CD3, CD4, CD5, CD7, CD8, CD9, CD11b, CD11c, CD13, CD14, CD15, CD16, CD18, CD19, CD20, CD22, CD23, CD24, CD25, CD31, CD33, CD34, CD36, CD38, CD41, CD42b, CD45, CD49d, CD55, CD56, CD59, CD61, CD62e, CD65, CD66e, CD68, CD69, CD71, CD83, CD90, CD95, CD105, CD117,	CD1a, CD2, CD3, CD4, CD5, CD7, CD8, CD9, CD10, CD11b, CD11c, CD13, CD14, CD15, CD16, CD18, CD19, CD20, CD22, CD23, CD24, CD25, CD31, CD33, CD34, CD36, CD38, CD41, CD42b, CD45, CD49d, CD55, CD56, CD59, CD61, CD62e, CD65, CD66e, CD68, CD69, CD71, CD83, CD95, CD105, CD117,	CD1a, CD2, CD3, CD4, CD5, CD7, CD8, CD9, CD10, CD11b, CD11c, CD13, CD14, CD15, CD16, CD18, CD19, CD20, CD22, CD23, CD24, CD25, CD31, CD33, CD34, CD36, CD38, CD41, CD42b, CD45, CD49d, CD55, CD59, CD61, CD62e, CD65, CD66e, CD68, CD69, CD71, CD83, CD95, CD105, CD117, CD123,	CD1a, CD2, CD3, CD4, CD5, CD7, CD8, CD9, CD10, CD11b, CD11c, CD14, CD15, CD16, CD18, CD19, CD20, CD22, CD23, CD24, CD25, CD31, CD33, CD34, CD36, CD38, CD41, CD42b, CD45, CD49d, CD55, CD56, CD59, CD61, CD62e, CD65, CD66e, CD68, CD69, CD71, CD83, CD95, CD105, CD117, CD123,	CD1a, CD2, CD3, CD4, CD5, CD7, CD8, CD9, CD10, CD11b, CD11c, CD13, CD14, CD15, CD16, CD18, CD19, CD20, CD22, CD23, CD24, CD25, CD31, CD33, CD34, CD36, CD38, CD41, CD42b, CD45, CD49d, CD55, CD56, CD59, CD61, CD62e, CD65, CD66e, CD68, CD69, CD71, CD83, CD95, CD105, CD117,	CD1a, CD2, CD3, CD4, CD5, CD7, CD8, CD9, CD10, CD11b, CD11c, CD13, CD14, CD15, CD16, CD18, CD19, CD20, CD22, CD23, CD24, CD25, CD31, CD33, CD34, CD36, CD38, CD41, CD42b, CD45, CD49d, CD55, CD56, CD59, CD61, CD62e, CD65, CD66e, CD68, CD69, CD71, CD83, CD95, CD135, Glycopho

	CD123, CD135, CD166, Glycophorin-A, MHC-I, HLA-DR-II, FMC-7, Annexin-V, and Lin	CD135, CD166, Glycophorin-A, MHC-I, HLA-DR-II, FMC-7, Annexin-V, and Lin	CD135, CD166, Glycophorin-A, MHC-I, HLA-DR-II, FMC-7, Annexin-V, and Lin	CD123, CD135, CD166, Glycophorin-A, MHC-I, HLA-DR-II, FMC-7, Annexin-V, and Lin	CD123, CD135, CD166, Glycophorin-A, MHC-I, HLA-DR-II, FMC-7, Annexin-V, and Lin	CD135, CD166, Glycophorin-A, HLA-DR-II, FMC-7, Annexin-V, and Lin	CD135, CD166, Glycophorin-A, HLA-DR-II, FMC-7, Annexin-V, and Lin	CD123, CD135, CD166, Glycophorin-A, HLA-DR-II, FMC-7, Annexin-V, and Lin	rin-A, HLA-DR-II, FMC-7, Annexin-V, and Lin

Table 3. Human Cluster of Differentiation markers (CD) were utilized to ascertain specific cell surface "fingerprints" to discern particular cell types (11,15,16). Reprinted with permission from Young HE, et al. Adult reserve stem cells and their potential for tissue engineering. Cell Biochem Biophys. 2004; 40:1-80.

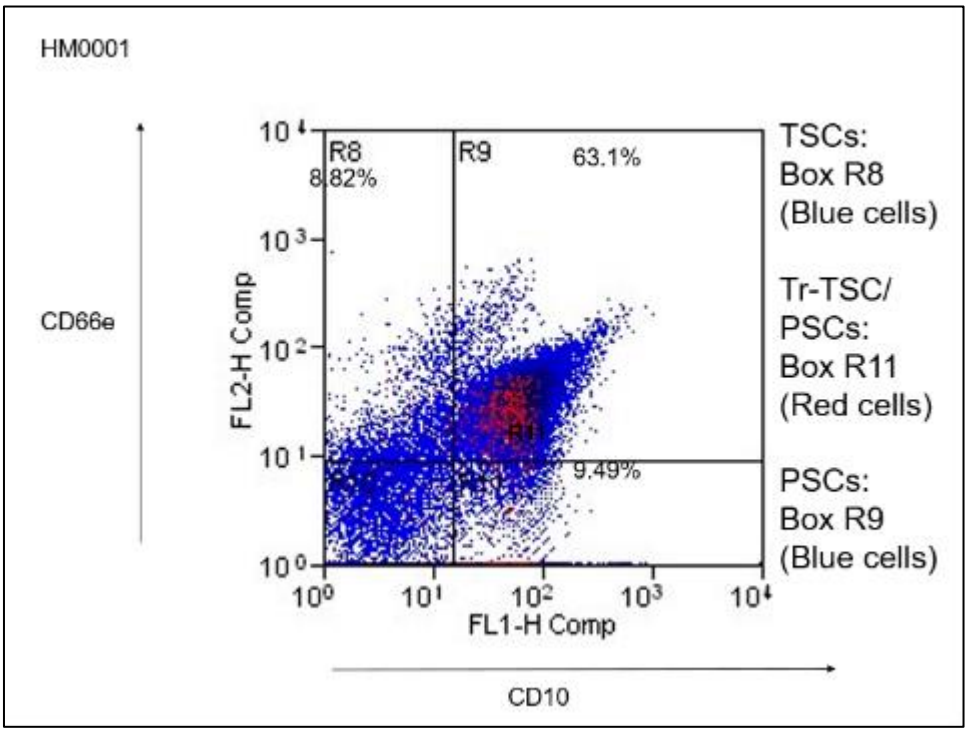
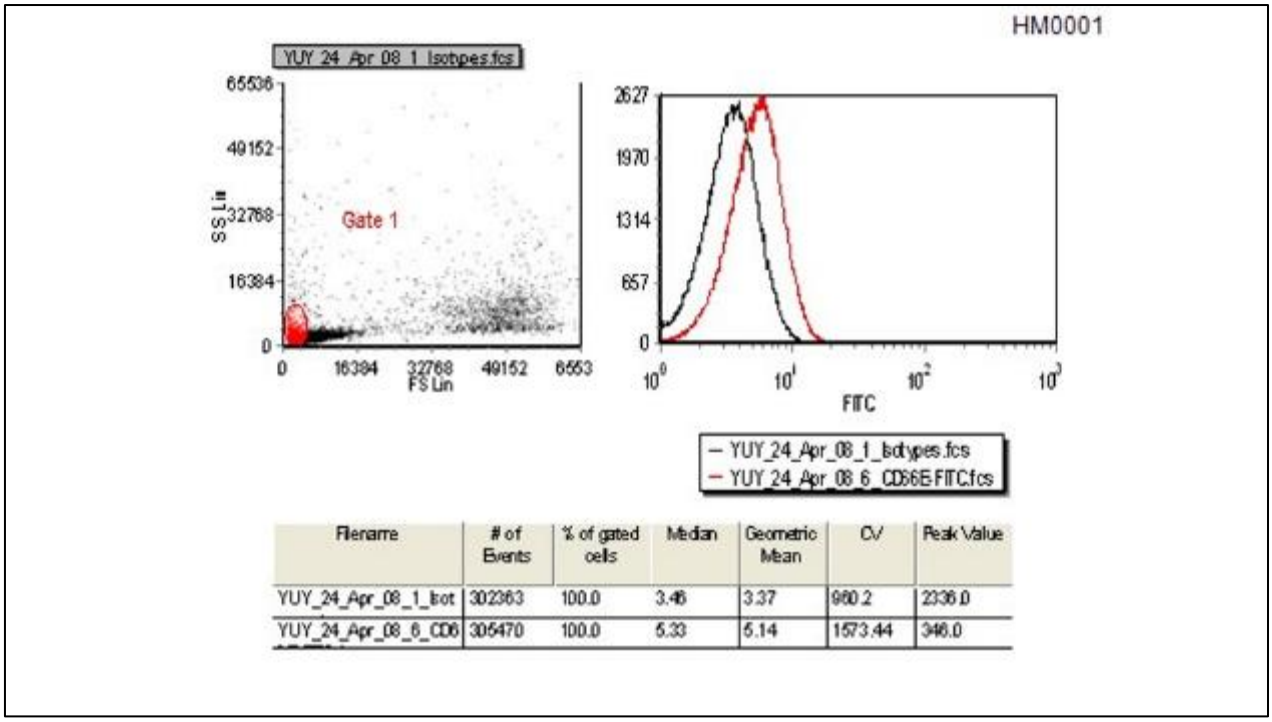


Figure 8 Transitional-totipotent stem cells/pluripotent stem cells, e.g., HLSCs and CLSCs (Tr-TSC/PSCs, red) gate R9, CD66e+/CD10+, represent an intermediate population of cells between the totipotent stem cells (TSCs, blue) gate R8, CD66e+/CD10-, and the pluripotent stem cells (PSCs, blue) gate R11, CD66e-/CD10+ (15). Reprinted with permission from Young HE. Endogenous Adult Telomerase Positive Stem Cells and Combinatorial Nutraceuticals, 50 Years in the Making, 50 Years of Discovery GSC Advanced Research and Reviews. 2025; Submitted

Figure 8 is an example of results from flow cytometry of human (HM0001) totipotent stem cells (TSCs), transitional totipotent stem cells to pluripotent stem cells (HLSCs & CLSCs), and pluripotent stem cells (PSCs). Adult human TPSCs were also sorted using CD antibodies, CD66e and CD10. As shown, TSCs (CD66e) were found in the grid R10 (blue cells), while PSCs (CD10) were found in grid R9 (blue cells). Those cells that were double labeled with CD66e and CD10 (HLSCs and CLSCs) (red) were originally named transitional-totipotent stem cells/pluripotent stem cells (Tr-TSC/PSCs).



Filename	# of Events	% of gated cells	Median	Geometric Mean	CV	Peak Value
YUY_24_Apr_08_1_Isot	302363	100.0	3.46	3.37	960.2	2336.0
YUY_24_Apr_08_6_CD6	305470	100.0	5.33	5.14	1573.44	346.0

Figure 9 Flow cytometry of human (HM0001) ultra-small totipotent stem cells (us-TSCs), red; small totipotent stem cell (s-TSCs), black, both ultra-small and small, are located in an area normally assumed to contain cell debris. Yet when this material was stained with propidium iodide, 95% of the material stained for intact nucleated cells. Size range in this area is 0.1 to 1.0 microns (15). Reprinted with permission from Young HE. Endogenous Adult Telomerase Positive Stem Cells and Combinatorial Nutraceuticals, 50 Years in the Making, 50 Years of Discovery. GSC Advanced Research and Reviews. 2025; Submitted

Figure 9 shows flow cytometry of human (HM0001) demonstrating totipotent stem cells (TSCs). The TSCs are located in an area of the flow diagram normally assumed to contain cell debris (X-axis, 10^{o} to 10^{1} and Y-axis 10^{o} to 10^{1}). Yet when this material was stained with propidium iodide, 95% of the material stained for intact nucleated cells. Size range in this area is 0.1 to 1.0 microns [Fig. 9].

5.4. Characterization Studies: Function of Biological Agents on aTPSCs and MSCs

Clonal populations of adult telomerase positive stem cells, e.g., TSCs, PSCs, EctoSCs, MesoSCs, EndoSCs, and a clone of telomerase negative progenitor cells, MSCs, all derived by repetitive single cell serial dilution clonogenic analysis. Ninety-six well plates were utilized to devise a high throughput screening assay, ELICA, for determining biological function of the cells. The ELICA assay used immunocytochemical and histochemical phenotypic expression markers to determine function [Table 2] (11).

One thousand cells were plated per well on a 1% type-1 collagen substratum for the clonal populations of TSCs, PSCs, EctoSCs, MesoSCs, EndoSCs and on uncoated bare plastic for the clonal population of MSCs. Human recombinant proteins, morphogenetic proteins derived from demineralized bone matrices, and exosome-containing conditioned medium from differentiated cells were incubated with these aTPSCs and MSCs and used to identify four categories of biological activities, e.g., anti-differentiating agents, proliferative agents, progression agents, and inducing agents[15], and to determine the differentiation potentials of the aTPSCs and MSCs [Table 4] (16,17).

Table 4 Biological Agents Incubated with TSCs, PSCs, EctoSCs, MesoSCs, EndoSCs, and MSCs To Determine their Respective Function

Agent	Name	Activity
LIF	Leukemia Inhibitory Factor	Inhibits differentiation of TSCs, PSCs, EctoSCs, MesoSCs, and EndoSCs. Has slight inhibitory effect on MSCs
ADF	Anti-Differentiation Factor	Inhibits differentiation of TSCs, PSCs, EctoSCs, MesoSCs, and EndoSCs. Has slight inhibitory effect on MSCs
CAF	Caffeine	Inhibits differentiation of TSCs, PSCs, EctoSCs, MesoSCs, and EndoSCs. Has NO inhibitory effect on MSCs
SIF	Scar Inhibitory factor	Inhibits differentiation of TSCs, PSCs, and MesoSCs from scar tissue formation (abnormal fibroblast/fibrocyte and abnormal ECM), both in vitro and in vivo. No effect on EctoSCs, EndoSCs, or MSCs.
PDGF-AA	Platelet-Derived Growth Factor-AA	Induces proliferation in TSCs, PSCs, EctoSCs, MesoSCs, EndoSCs, and MSCs
PDGF-AB	Platelet-Derived Growth Factor-AB	Induces proliferation in TSCs, PSCs, EctoSCs, MesoSCs, EndoSCs, and MSCs
PDGF-BB	Platelet-Derived Growth Factor-BB	Induces proliferation in TSCs, PSCs, EctoSCs, MesoSCs, EndoSCs, and MSCs
IGF-1	Insulin-Like Growth Factor-1	Acts as a progression agent to accelerate differentiation with subsequent expression of phenotypic markers in MSCs, indicative of fat, cartilage, and bone. Has NO effect on TSCs, PSCs, EctoSCs, MesoSCs, and EndoSCs.
IGF-2	Insulin-Like Growth Factor-2	Acts as a progression agent to accelerate differentiation with subsequent expression of phenotypic markers in MSCs, indicative of fat, cartilage, and bone. Has NO effect on TSCs, PSCs, EctoSCs, MesoSCs, and EndoSCs.
INS	Insulin	Acts as a progression agent to accelerate differentiation with subsequent expression of phenotypic markers in MSCs, indicative of fat, cartilage, and bone. Has NO effect on TSCs, PSCs, EctoSCs, MesoSCs, and EndoSCs.
BMP-2	Bone morphogenetic protein-2	Induces endochondral ossification in TSCs, PSCs, MesoSCs, MSCs; no inductive effect on EctoSCs or EndoSCs.
OsMP	Osteogenic morphogenetic protein	Induces membranous ossification in TSCs, PSCs, MesoSCs; no inductive effect on EctoSCs, EndoSCs, or MSCs.
OsCM	Osteogenic Conditioned Medium	Induces membranous ossification in TSCs, PSCs, MesoSCs; no inductive effect on EctoSCs, EndoSCs, or MSCs.
CarMP	Cartilage morphogenetic protein	Induces cartilage formation in TSCs, PSCs, MesoSCs, and MSCs (hyaline cartilage); no inductive effect on EctoSCs or EndoSCs.
CarCM	Cartilage Conditioned Medium	Induces cartilage formation in TSCs, PSCs, MesoSCs, and MSCs (hyaline cartilage); no inductive effect on EctoSCs or EndoSCs.
AdipMP	Adipocyte morphogenetic protein	Induces formation of white adipose tissue (fat) formation in TSCs, PSCs, MesoSCs, MSCs; no inductive effect on EctoSCs or EndoSCs.
AdipCM	Adipocyte Conditioned Medium	Induces formation of white adipose tissue (fat) formation in TSCs, PSCs, MesoSCs, MSCs; no inductive effect on EctoSCs or EndoSCs.
BMP-4	Bone morphogenetic protein-4	Induces endothelial cell formation in TSCs, PSCs, MesoSCs; no inductive effect on EctoSCs, EndoSCs, or MSCs. Slight proliferative effect on MSCs

a-FGF	Acidic fibroblast growth factor	Induces endothelial cell formation in TSCs, PSCs, MesoSCs; no inductive effect on EctoSCs, EndoSCs, or MSCs. Slight proliferative effect on MSCs
ECGF	Endothelial cell growth factor	Induces endothelial cell formation in TSCs, PSCs, MesoSCs; no inductive effect on EctoSCs, EndoSCs, or MSCs. Slight proliferative effect on MSCs
VEGF	Vascular endothelial growth factor	Induces endothelial cell formation in TSCs, PSCs, MesoSCs; no inductive effect on EctoSCs, EndoSCs, or MSCs. Slight proliferative effect on MSCs
BvMP	Blood Vessel Morphogenetic protein	Induces vasculogenesis in TSCs, PSCs, and MesoSC; no inductive effect on EctoSCs, EndoSCs, or MSCs. Slight proliferative effect on MSCs.
BvCM	Blood Vessel Conditioned Medium	Induces vasculogenesis in TSCs, PSCs, and MesoSC; no inductive effect on EctoSCs, EndoSCs, or MSCs. Slight proliferative effect on MSCs.
FMP	Fibroblast morphogenetic protein	Induces normal fibroblast/fibrocyte formation in TSCs, PSCs, MesoSCs; no inductive effect on EctoSCs, EndoSCs, or MSCs. Slight proliferative effect on MSCs.
FCM	Fibroblast Conditioned Medium	Induces normal fibroblast/fibrocyte formation in TSCs, PSCs, MesoSCs; no inductive effect on EctoSCs, EndoSCs, or MSCs. Slight proliferative effect on MSCs.
ScFMP	Scar Fibroblast morphogenetic protein	Induces abnormal fibroblast/fibrocyte formation with aberrant extracellular matrix, equivalent in all respects to scar tissue formation in vivo in TSCs, PSCs, MesoSCs; no inductive effect on EctoSCs, EndoSCs, or MSCs.
TGF-b	Transforming growth factor beta	Induces scar tissue formation in TSCs, PSCs, MesoSCs; no inductive effect on EctoSCs, EndoSCs, or MSCs. Slight proliferative effect on MSCs
b-FGF	Basic fibroblast growth factor	Induces scar tissue formation in TSCs, PSCs, MesoSCs; no inductive effect on EctoSCs, EndoSCs, or MSCs. Slight proliferative effect on MSCs
EPO	Erythropoietin	In conjunction with c-Kit & IL-6, induces formation of RBCs in TSCs, PSCs, MesoSCs; no inductive effect on EctoSCs, EndoSCs, or MSCs. Slight proliferative effect on MSCs
c-Kit	c-Kit	In conjunction with EPO & IL-6, induces formation of RBCs in TSCs, PSCs, MesoSCs; no inductive effect on EctoSCs, EndoSCs, or MSCs. Slight proliferative effect on MSCs
IL-6	Interleukin-6	In conjunction with EPO & c-Kit, induces formation of RBCs in TSCs, PSCs, MesoSCs; no inductive effect on EctoSCs, EndoSCs, or MSCs. Slight proliferative effect on MSCs
NGF	Nerve growth factor	Induces formation of neuronal lineage cells (neurons, oligodendrocytes, astrocytes, Schwann cells, ganglion cells) in TSCs, PSCs, EctoSCs; no inductive effect on MesoSCs, EndoSCs, or MSCs
BrnMP	Brain Morphogenetic Protein	Induces formation of neurons, astrocytes, and oligodendrocytes in TSCs, PSCs, and EctoSCs; no inductive effect on MesoSCs, EndoSCs, or MSCs.
BrnCM	Brain Conditioned Medium	Induces formation of neurons, astrocytes, and oligodendrocytes in TSCs, PSCs, and EctoSCs; no inductive effect on MesoSCs, EndoSCs, or MSCs.
HGF	Hepatocyte growth factor	Induces formation of liver lineage cells (oval cells, canalicular cells, biliary cells) in TSCs, PSCs, EndoSCs; no inductive effect on MesoSCs, EctoSCs, or MSCs
LivMP	Liver Morphogenetic Protein	Induces formation of multiple liver cells (oval cells, canalicular cells, biliary cells) in TSCs, PSCs, and EndoSCs; no inductive effect on EctoSCs, MesoSCs, or MSCs
LivCM	Liver Conditioned Medium	Induces formation of multiple liver cells (oval cells, canalicular cells, biliary cells) in TSCs, PSCs, and EndoSCs; no inductive effect on EctoSCs, MesoSCs, or MSCs
LngMP	Lung Morphogenetic Protein	Induces formation of type-1 alveolar cells in TSCs, PSCs, and EndoSCs; no inductive effect on EctoSCs, MesoSCs, or MSCs

LngCM	Lung Conditioned Medium	Induces formation of type-1 alveolar cells in TSCs, PSCs, and EndoSCs; no inductive effect on EctoSCs, MesoSCs, or MSCs
PanMP	Pancreatic Morphogenetic Protein	Induces formation of pancreatic ductal cells, alpha-cells, beta-cells, and delta-cells in TSCs, PSCs, and EndoSCs; no inductive effect on EctoSCs, MesoSCs, or MSCs.
PanCM	Pancreatic Conditioned Medium	Induces formation of pancreatic ductal cells, alpha-cells, beta-cells, and delta-cells in TSCs, PSCs, and EndoSCs; no inductive effect on EctoSCs, MesoSCs, or MSCs.
KerMP	Keratinocyte Morphogenetic Protein	Induces keratinocytes in TSCs, PSCs, and EctoSCs; no inductive effect on MesoSCs, EndoSCs, or MSCs.
KerCM	Keratinocyte Conditioned Medium	Induces keratinocytes in TSCs, PSCs, and EctoSCs; no inductive effect on MesoSCs, EndoSCs, or MSCs.
SpMP	Spermatogonia Morphogenetic Protein	Induces spermatogonia in TSCs; no inductive effect on PSCs, MesoSCs, or MSCs.
SpCM	Spermatogonia Conditioned Medium	Induces spermatogonia in TSCs; no inductive effect on PSCs, MesoSCs, or MSCs.
SkMMP	Skeletal Muscle Morphogenetic Protein	Induces skeletal muscle cells in TSCs, PSCs, and MesoSCs; no inductive effect on EctoSCs, EndoSCs, or MSCs.
SkMCM	Skeletal Muscle Conditioned Medium	Induces skeletal muscle cells in TSCs, PSCs, and MesoSCs; no inductive effect on EctoSCs, EndoSCs, or MSCs.
SmMP	Smooth muscle morphogenetic protein	Induces smooth muscle cells in TSCs, PSCs, and MesoSCs; no inductive effect on EctoSCs, EndoSCs, or MSCs.
SmCM	Smooth muscle Conditioned Medium	Induces smooth muscle cells in TSCs, PSCs, and MesoSCs; no inductive effect on EctoSCs, EndoSCs, or MSCs.
CdMMP	Cardiac muscle morphogenetic protein	Induces cardiac muscle cells in TSCs, PSCs, and MesoSCs; no inductive effect on EctoSCs, EndoSCs, or MSCs.
CdMCM	Cardiac muscle Conditioned Medium	Induces cardiac muscle cells in TSCs, PSCs, and MesoSCs; no inductive effect on EctoSCs, EndoSCs, or MSCs.
TenMP	Tendon morphogenetic protein	Induces tendon formation in TSCs, PSCs, MesoSCs; no inductive effect on EctoSCs, EndoSCs, or MSCs
TenCM	Tendon Conditioned Medium	Induces tendon formation in TSCs, PSCs, MesoSCs; no inductive effect on EctoSCs, EndoSCs, or MSCs
LigMP	Ligament morphogenetic protein	Induces ligament formation in TSCs, PSCs, MesoSCs; no inductive effect on EctoSCs, EndoSCs, or MSCs
LigCM	Ligament Conditioned Medium	Induces ligament formation in TSCs, PSCs, MesoSCs; no inductive effect on EctoSCs, EndoSCs, or MSCs

Table 4. Reprinted with permission from Young, et al. Adult reserve stem cells and their potential for tissue engineering. Cell Biochem Biophys, 40(1):1-80, 2004. Young HE. Carcinoembryonic antigen-cell adhesion molecule-1 and stage-specific embryonic antigen-4 are present in the reproductive organs of adult mammals. GSC Advanced Research and Reviews. 2025; 23(03): 149-147. DOI: https://doi.org/10.20574/gscarr.2025.23. 3.0171.

5.5. Differentiation Capabilities of aTPSCs

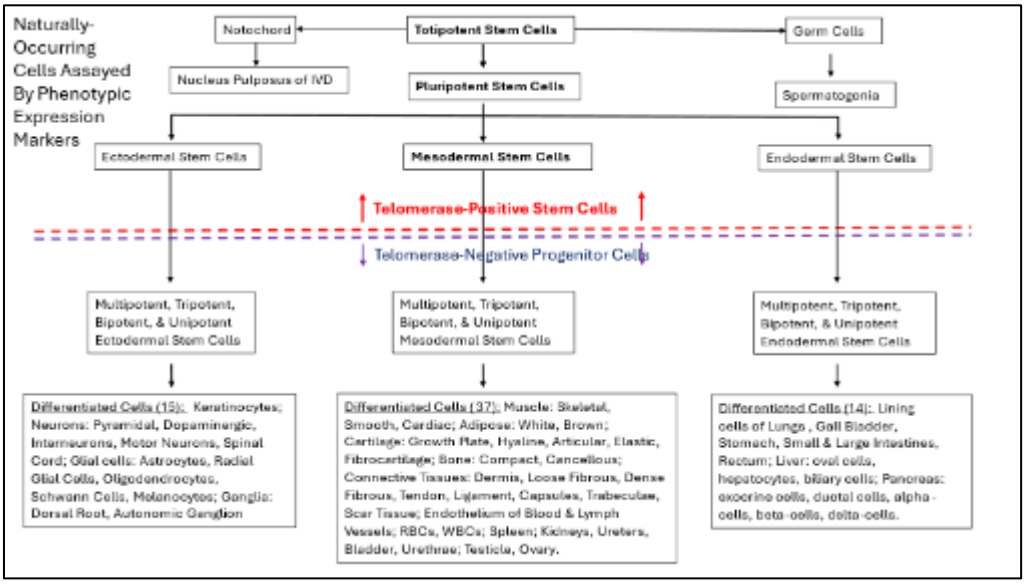


Figure 10 Unidirectional differentiation of primitive undifferentiated telomerase positive totipotent stem cells, through subsequent differentiation steps, i.e., telomerase positive pluripotent stem cells, telomerase positive ectodermal stem cells, telomerase positive mesodermal stem cells, and telomerase positive endodermal stem cells, to lose the telomerase enzyme and become telomerase negative multipotent, tripotent, bipotent, and unipotent progenitor cells, which then differentiate into terminally differentiated adult cells (11). Reprinted with permission from Young HE, Speight MO. Characterization of endogenous telomerase-positive stem cells for regenerative medicine, a review. Stem Cell Regen Med 2020; 4(2):1-14

5.6. Characterization Parameters

Table 5 Comparison/Contrast of Telomerase-Positive Stem Cells with Telomerase-Negative Progenitor Cell

Char[1]	**us-TSC**[2]	**s-TSC**[3]	**HLSC**[4]	**CLSC**[5]	**PSC**[6]	**GLSC**[7]	**MesoSC**[8]	**MSC**[9]
Size, microns	0.1-1	1-2	2-4	4-6	6-8	8-10	10-12	15-30
Try Blue[10]	Pos[11]	Pos	Pos/Neg [12]	Neg/Pos	Neg	Neg	Neg	Neg
Telomerase	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg
With Aging	Constant	Constant	Constant	Constant	Constant	Constant	Constant	Decrease
Viab PM[13]	40+ days	30+ days	>2 days	>2 days	>7 days	>5 days	3 days	1 day
Viab T[14]	4°C	4°C	4°C	4°C	4°C	4°C	4°C	4°C
Sol Tiss[15]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
In CTs[16]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
B Mar[17]	Yes	Yes	NYD[18]	NYD	Yes	Yes	Yes	Yes
Adipose	Yes	Yes	NYD	NYD	Yes	Yes	Yes	Yes
Blood	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
Species Utilized[19]	Av, KD, M, Rt, Rb, F, Cn, O, Cp, P, B, SpB, E, H	Av, KD, M, Rt, Rb, F, Cn, O, Cp, P, B, SpB, E, H	F, Cn, O, Cp, P, H	F, Cn, O, Cp, P, H	Av, KD, M, Rt, Rb, F, Cn, O, Cp, P, B, SpB, E, H	H	Av, KD, M, Rt, Rb, F, Cn, O, Cp, P, B, SpB, E, H	Rt

Clone[20]	Rat-Scl-44β, Rat-Scl-4β	Rat-Scl-9β	NYD	NYD	Rat-Scl-40β	NYD	Rat-$A_2A_2β$	Rt-MSC[9] Rt-Adip[21], Rt-Chon[22], Rt-Os[23]
Contact Inhibition[24]	No	No	No	No	No	Yes	Yes	Yes
Survive Contact Inhibition	NA	NA	NA	NA	NA	Yes	Yes	No
Growth[25]	Suspension[26]	Adherent [27]	Adherent	Adherent	Adherent	Adherent	Adherent	Adherent
Substrate	None	Collag-I[28]	Collag-I	Collag-I	Collag-I	Collag-I	Collag-I	None
SFM[29]	Quiescent	Quiescent	Quiescent	Quiescent	Quiescent	Quiescent	Quiescent	Quiescent
No F[30]	Quiescent	Quiescent	Quiescent	Quiescent	Quiescent	Quiescent	Quiescent	Quiescent
Inhib F[31]	Respond	Respond	Respond	Respond	Respond	Respond	Respond	Respond
Prolif F[32]	Prolif[33]	Prolif	Prolif	Prolif	Prolif	Prolif	Prolif	Prolif
Prog F[34]	No	No	No	No	No	No	No	Yes
Induc F[35]	Yes	Yes	Yes	Yes	Yes	Yes	Meso-dermal Lineage[36] Only	Fat, Cart[37], Bone Only
Commit[38]	s-TSC	HLSC	CLSC	PSC	GLSC	EctoSCs[39] MesoSCs, EndoSCs [40]	MesoPCs [41]	Fat, Cartilage, Bone
# Cells ID[42]	75	74	70	68	66	66	37	3
Lineages[43]	3 + Sp[44]	3 + Sp	3	3	3	3	1	1
Prolif Rt[45]	12-14 hours	12-14 hours	12-14 hours	12-14 hours	12-14 hours	14-18 hours	18-24 hours	Days to weeks
Pop Dbl & Species[46]	>300 Rat	>300 Rat	>300 Rat	>400 Rat	>400 rat	>400 Rat	>690 Human	50-70 Human
Cryo Ag[47]	DMSO	DMSO	DMSO	DMSO	DMSO	DMSO	DMSO	DMSO
Con Ag[48]	7.5%	7.5%	7.5%	7.5%	7.5%	7.5%	7.5%	10%
# Cs Cry[49]	1-10 B[50]	1-10 B	1-10 B	1-10 M[51]	1-10 M	1-10 M	1-10 M	1-10 M
Op Fr T[52]	-80°C	-80°C	-80°C	-80°C	-80°C	-80°C	-70°C	-196°C
Fr Prot[53]	Slow	Slow	Slow	Slow	Slow	Slow	Slow	Flash
Op St T[54]	-80°C	-80°C	-80°C	-80°C	-80°C	-80°C	-70°C	-196°C
Thaw T[55]	37°C	37°C	37°C	37°C	37°C	37°C	37°C	37°C
Recovery	>98%	>98%	>98%	>98%	>98%	>98%	>98%	>95%
Karyotype	Normal	Normal	NYD	NYD	Normal	NYD	Normal	Normal
Expressed	Telom[57],			Telom,				

Genes[56]	Bcl-2, Nanog, Nanos, CXCR4	NYD	NYD	Oct3/4[58], Sonic-hedge hog[59]	NYD	NYD	NYD	NYD
Cell Surf Markers[60]	CEA[61] HCEA[62] CD66e[63]	CEA[61] HCEA[62] CD66e[63]	SSEA^{low} CD10^{low} CEA^{high} CD66e^{high}	SSEA^{high} CD10^{high} CEA^{low} CD66e^{low}	SSEA[64], CD10[65]	SSEA^{high} CD10^{high} CD90^{low} Thy-1^{low}	CD90[66] Thy-1[67] CD13[68] MHC-I[69]	CD105[70] CD117[71] CD123[72] CD166[73] MHC-I

Table 5. Char[1], characteristics tested; us-TSC[2], ultra-small totipotent stem cell; s-TSC[3], small totipotent stem cell; HLSC[4], halo-like stem cell; CLSC[5], corona-like stem cell; ELSC[6], epiblast-like stem cell; GLSC[7], germ layer lineage stem cell; MesoSC[8], mesodermal stem cell – will form all cell types of the mesodermal germ layer lineage; MSC[9], mesenchymal stem cell; Try Blue[10], 0.4% Trypan Blue staining; Pos[11], Positive; Neg[12], Negative; Viab PM[13], viability post mortem (after removal from the animal); Viab T[14], viability temperature; Sol Tiss[15], presence in solid tissues; B Mar[17], bone marrow; NYD[18], not yet determined; Species[19]: Av (chicken, Waddle Crane), KD (Komodo Dragon), M (mouse), Rt (rat), Rb (rabbit), F (feline, cat), Cn (canine, dog), O (ovine, sheep), Cp (caprine, goat), P (porcine, pig), B (bovine, cow), SpB (Speckled Bear), E (equine, horse), H (human); Clone[20], clonal cell populations generated by repetitive single cell clonogenic analysis; Rt-Adip[21], rat-adipogenic progenitor cell; Rt-Chon[22]; rat-chondrogenic progenitor cell; Rt-Os[23], rat-osteogenic progenitor cell; Con Hib[24], contact inhibition of cells at confluence of growth in culture; Growth[25], growth attachment capabilities in culture; Suspension[26], ability to grow in culture suspended in the medium without any attachment to a substratum; Adherent[27], ability to grow in culture when attached to a substratum; Collag-I[28], collagen type-I substratum needed to be provided for cell attachment to occur; SFM[29], serum-free defined medium; No F[30], no growth factors added to the medium; Inhib F[31], inhibitory factors added to the medium to prevent differentiation of the cells, e.g., LIF, leukemia inhibitory factor or ADF, anti-differentiation factor; Prolif F[32], proliferation factor added to the medium, i.e., platelet-derived growth factor BB (PDGF-BB) to stimulate cellular proliferation; Prolif[33], proliferation of cells tested for increased content of DNA per well as assessed by the DNA portion of the ELICA procedure; Prog F[34], progression factor (2 ng/ml insulin) added to the medium to accelerate the phenotypic expression of lineage-committed progenitor cells; Induc F[35], induction factor added to the medium to induce the expression of multiple phenotypes in culture. We routinely used dexamethasone at 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9}, and 10^{-10} molar concentrations as a non-specific induction agent for mesodermal lineage cells. We also used specific induction agents to induce specific cellular phenotypes, e.g., SkM-MP for skeletal muscle, Sm-MP for smooth muscle, Cm-MP for cardiac muscle, Adip-MP for adipocytes, Ch-MP for cartilage, Os-MP for bone, BMP-2 for bone, VEGF for endothelial cells, EPO for erythrocytes, HGF for hepatocytes, BDGF for neurons and glial cells, NGF for neurons, and conditioned medium (exosomes) from differentiated cells and tissues to induce differentiation into pancreatic islets, spermatogonia, etc. [Table 5]; Mesodermal[36], mesodermal germ layer lineage cells; Cart[37], cartilage; Commit[38], commitment into a specific cell type as defined by its unique characteristics of cell surface markers, pattern of phenotypic expression markers, and differentiation capabilities; EctoSCs[39], ectodermal stem cells – will form all cell types of the ectodermal germ layer lineage; EndoSCs[40], endodermal stem cells – will form all cell types of the endodermal germ layer lineage; MesoPCs[41], telomerase-negative mesodermal progenitor cells; # Cs ID[42], number of cells identified by unique phenotypic expression markers. The number of cell types identified was not dependent on the differentiation capabilities of the cells, but rather the limited number of objective assays at our disposal to identify different cell types; Lineages[43], refers to embryonic germ layer lineages, e.g., ectoderm, mesoderm, and endoderm; Sp[44], spermatogonia; Prolif Rt[45], proliferation rate of the cells, assessed during log phase growth; Pop Dbl[46], population doublings; Cryo Ag[47], cryogenic agents used to freeze the cells; Con Ag[48], percent concentration of the cryogenic agent used to freeze the cells; # Cs Cry[49], maximum number of cells that could be frozen in a single aliquot of cells; B[50], billion; M[51], million; Op Fr T[52], optimum freezing temperature; Fr Prot[53], particular freezing protocol used; Op St T[54], optimal storage temperature; Thaw T[55], optimal thawing temperature; Genes[56], genes expressed in the naïve undifferentiated state; Telom[57], telomerase; Oct-3/4[58], genetic expression of the Oct-3/4 gene; Sonic-hh[59], expression of the sonic hedge-hog gene; Cell Surf Markers[60], cell surface markers used for FACS (fluorescent-activated cell sorting) analysis included CD1a, CD2, CD3, CD4, CD5, CD7, CD8, CD9, CD10, CD11b, CD11c, CD13, CD14, CD15, CD16, CD18, CD19, CD20, CD22, CD23, CD24, CD25, CD31, CD33, CD34, CD36, CD38, CD41, CD42b, CD45, CD49d, CD55, CD56, CD59, CD61, CD62e, CD65, CD66e, CD68, CD69, CD71, CD83, CD90, CD95, CD105, CD117, CD123, CD135, CD166, Glycophorin-A, MHC-I, HLA-DR-II, FMC-7, Annexin-V, and Lin; CEA[61], carcinoembryonic antigen-cell adhesion molecule-1; HCEA[62], human carcinoembryonic antigen; CD66e[63], carcino-embryonic antigen, human variant "e"; SSEA^{low}, stage specific embryonic antigen-4 at low levels of detection by FACS analysis; CD10^{low}, CLLA antigen at low levels of detection by FACS analysis; CEA^{high}, carcinoembryonic antigen at high

levels of detection; CD66e^{high}, CD66e at high levels of detection by FACS analysis; SSEA[64], stage-specific embryonic antigen-4; CD10[65], cell surface enzyme with neutral metalloendopeptidase activity; CD90[66] (or Thy-1), heavily N-glycosylated, glycophosphatidylinositol anchored conserved cell-surface protein with a single V-like immunoglobulin domain present in adult stem cells, cancer cells, and MSCs; Thy-1[67](or CD90), heavily N-glycosylated, glycophosphatidylinositol anchored conserved cell-surface protein with a single V-like immunoglobulin domain; CD13[68], aminopeptidase; MHC-I[69], major histocompatibility marker-class-I; CD105[70], transmembrane glycoprotein, endoglin; CD117[71], receptor tyrosine kinase protein; CD123[72], interleukin-3 receptor alpha chain; CD166[73], a transmembrane glycoprotein that is a member of the immunoglobulin superfamily of proteins (11). Reprinted with permission from Young HE, Speight MO. Characterization of endogenous telomerase-positive stem cells for regenerative medicine, a review. Stem Cell Regen Med 2020; 4(2):1-14.

The TSCs and PSCs were located in multiple species of animals, including humans, from multiple tissues and organs [Table 6]. This conservation of adult telomerase positive cell types suggested the possibility that preliminary IACUC-approved animal studies could be used to test these cells within animal models of human afflictions (11-18).

Table 6 Species, Age, and Location of Precursor Healing Stem Cells

Loca[1]	**Sal** [2]	**Re** [3]	**Av** [4]	**Mo** [5]	**Rt** [6]	**Rb** [7]	**Fe** [8]	**Ca** [9]	**Ov** [10]	**Cp** [11]	**Pr** [12]	**Bo** [13]	**SB** [14]	**Eq** [15]	**Hu** [16]
Pre[17]			+ [18]												+
Mor[19]															Sem [20]
Psn[21]	+ H [22]	+ Is[23]	+ Is	+ Is	+ Is H Hc [24]	+	+ Im [25]	+ Im	+ Im	+ Im	+ Is Im	+ Is	+ Is	+ Is	+ Is Hc
Nb[26]				Is	Is										Is
Ad[27]				Is	Is										Is
SM[28]	H Hc			Is	Is	Is	Is	Is	Is	Is	Is	Is	Is	Is	Is
Ge[29]		Is	Is	Is								Is	Is	Is	Is
SkM[30]	H Hc		Is	Is	Is Im	Is	Is	Is	Is	Is	Is	Is		Is	Is
Der[31]	H Hc		Is,	Is Im	Is Im						Is Im				
Hrt[32]			Is, Im	Is	Is Im						Is Im				
GT[33]					Is						Im				
Pos[34]	H Hc		Is	Is	Is Im						Im				Is
Pch[35]	H Hc		Is	Is	Is Im						Im				Is
Ns[36]	H Hc		Is	Is	Is Im						Im				Is

Adip[37]	H Hc		Is	Is	Is Im						Im				Is
Lig[38]	H Hc		Is	Is	Is Im						Im				Is
Ten[39]	H Hc		Is	Is	Is Im						Im				Is
Bv[40]	H Hc		Is	Is	Is Im						Im				Is
BoM[41]	H Hc		Is	Is Im	Is Im						Im				Is
Bld[42]		Is	Is	Is	Is	Is	Is	Is	Is	Is	Is		Is	Is	Is
Tra[43]					Is Im						Im				
Lng[44]					Is Im						Im				
Eso[45]					Is Im						Im				
Stm[46]					Is Im						Im				
Liv[47]					Is Im						Im				
SmI[48]					Is Im						Im				
LgI[49]					Is Im						Im				
Spl[50]					Is Im						Im				
Brn[51]					Is Im						Im				
Men[52]					Is Im						Im				
SpC[54]											Im				
PanEn[55]					Is Im						Im				
PanEx[56]					Is Im						Im				
Kid[57]					Is Im						Im				
Ub[58]					Is Im						Im				
Thy[59]					Is Im						Im				

Tng[60]					Is Im						Im				
Tes[61]					Is Im										
Ft[62]					Is Im										
Ov[63]					Is Im										
Kar[64]				Dip 40 [65]	Dip 42						Dip38			Dip 64	Dip 46

Table 6. Loca[1], Location; Sal[2], adult terrestrial salamanders: *Ambystoma annulatum, maculatum, texanum, tigranum;* Re[3], reptile – Komodo Dragon; Av[4], Avians – chicken, Wadel Crane; Mo[5], mice; Rt[6], rat; Rb[7], rabbits; Fe[8], felines; Ca[9], canines; Ov[10], ovine; Cp[11], caprines; Pr[12], porcine; Bo[13], bovine; SB[14], Spectacled Bear; Eq[15], equine; Hu[16], humans (male & female); Pre[17], prenatal; +[18], presence; Mor[19], morula; Sem[20], scanning electron microscopy; Psn[21], post-natal (adult); H[22], Histology; Is[23], isolation; Hc[24], histochemistry; Im[25], immunocytochemistry; Nb[26], newborn; Ad[27], adolescent; SM[28], sexually mature; Ge[29], geriatric; SkM[30], skeletal muscle; Der[31], dermis; Hrt[32], heart (epicardium, myocardium); GT[33], granulation tissue; Pos[34], periosteum; Pch[35], perichondrium; Ns[36], nerves; Adip[37], adipose tissue (white fat); Lig[38], ligaments; Ten[39], tendons; Bv[40], blood vessels; BoM[41], bone marrow (hematopoietic cells, stromal cells); Bld[42], blood; Tra[43], trachea; Lng[44], lung; Eso[45], esophagus (lamina propria, submucosa, adventitia); Stm[46], stomach (submucosa, serosa); Liv[47], liver; SmI[48], small intestine (lamina propria, submucosa, serosa); LgI[49], large intestine (lamina propria, submucosa, mesocolon); Spl[50], spleen (capsule, trabeculae, interstitial tissue); Brn[51], brain (white mater, gray mater); Men[52], meninges (dura mater, arachnoid mater, pia mater); SpC[53], spinal cord (white mater, gray mater); PanEn[54], endocrine portion of pancreas (pancreatic islets); PanEx[55], exocrine portion of pancreas; Kid[57], kidney (capsule and interstitium); Ub[58], urinary bladder; Thy[59], thyroid gland; Tng[60], tongue; Tes[61], testis; Ft[62], fallopian tube; Ov[63], ovary; Kar[64], karyotypic analysis; Dip[65], diploid number of chromosomes correct for respective species [2]. Reprinted with permission from Young HE, Black AC. Pluripotent Stem Cells, Endogenous versus Reprogrammed, a Review. MOJ Orthop Rheumatol 1(4): 00019, 2014; Young HE. Carcinoembryonic antigen-cell adhesion molecule-1 and stage-specific embryonic antigen-4 are present in the reproductive organs of adult mammals. GSC Advanced Research and Reviews. 2025; 23(03): 149-147. DOI: https://doi.org/10.20574/gscarr.2025.23.3.0171. Young HE. Totipotent stem cells and pluripotent stem cells are present in the reproductive organs of an adult mammal. GSC Advanced Research and Reviews. 2025; 23(03): 158-180. DOI: https://doi.ord/10.30574/gscarr.2025.23.3.0172.

Four populations of aTPSCs were identified from the above studies that could fulfill a portion of the above criteria for 3. Identification of stem cell(s) that have the differentiation potential to form neurons and their supportive Schwann cells, loose fibrous connective tissue, capillaries, arterioles/venioles, small arteries, small veins, lymphatics.

Thus, TSCs and PSCs could form neurons and their supportive Schwann cells, loose fibrous connective tissue, capillaries, arterioles/ venioles, small arteries, small veins, lymphatics; whereas EctoSCs will form neurons and their supportive Schwann cells; and MesoSCs will form loose fibrous connective tissue, capillaries, arterioles/venioles, small arteries, small veins, lymphatics. Therefore, it was also decided to add the EctoSCs and MesoSCs to the TSCs and PSCs to maximize cell numbers for transplant, depending on the route of administration (11,15).

5.7. Isolation of those stem cell(s) using the least invasive method possible (do not want to create one problem to fix another)

The aTPSCs normally reside in connective tissue resident niches (14,16,17). Following trauma to the body, stem cells are released from their connective tissue niches to migrate towards damaged tissues as well as mobilized into the bloodstream by reverse diapedesis (Fig. 11) (16-20).

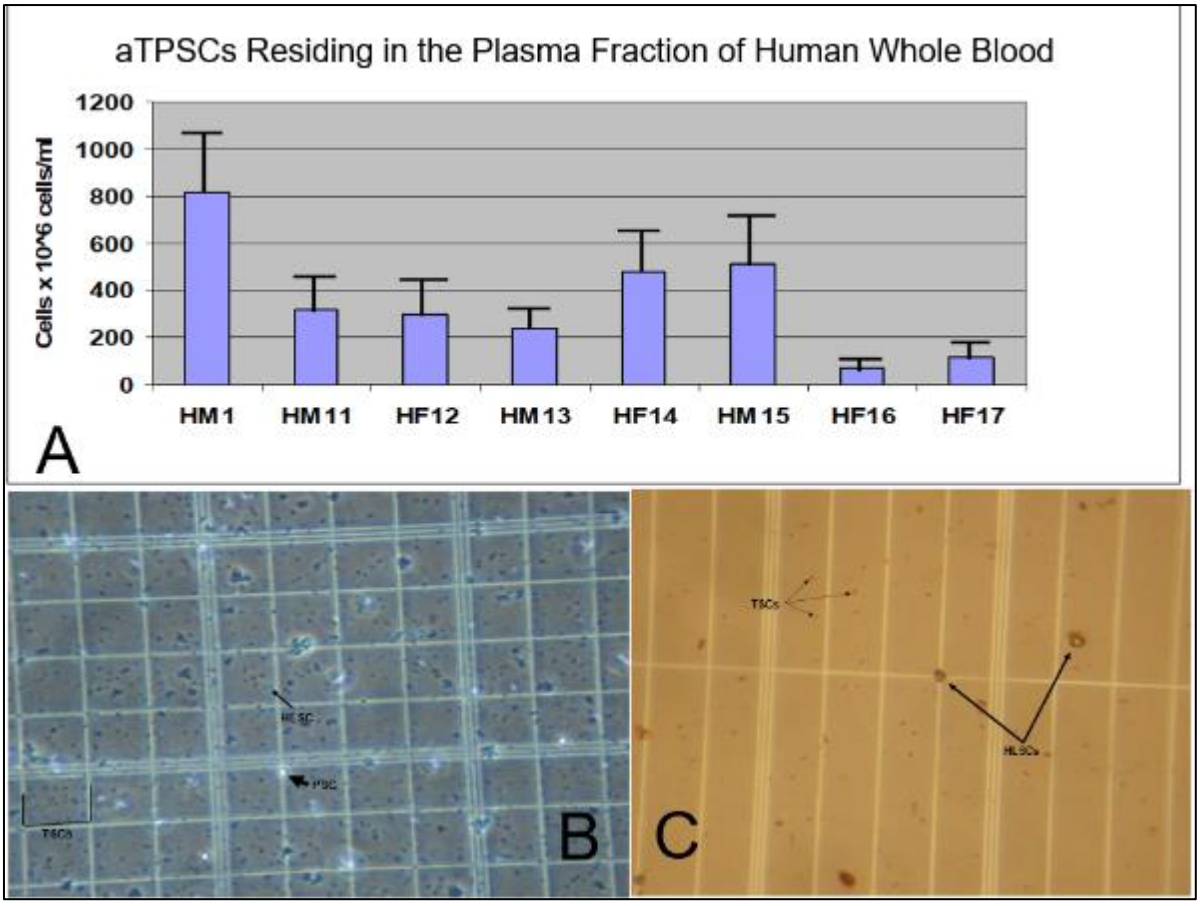


Figure 11 With IRB-approval, 5cc's of blood was removed from each non-primed individual and processed. Whole blood elements were separated from aTPSCs by time, gravity, and zeta potential, separating whole blood into a hematocrit. All blood elements (RBCs, WBCs, platelets) were located in the "blood pellet" whereas the aTPSCs (TSCs, HLSCs, CLSCs, PSCs, GLSCs, EctoSCs, MesoSCs, and EndoSCs) were located in the plasma fraction. The plasma fraction from each individual was withdrawn and placed into separate polypropylene tubes. Fifty microliters of cell suspension were removed to polypropylene microcentrifuge tube. Fifty microliters of 0.4% Trypan blue were added to cell suspension, triturated 5 to 6 times, and 50 microliters of Trypan blue-stained cell suspension placed on a hemocytometer and counted. HM1 = HM0001 (systemic lupus erythematosus); HM11 = HM0011 (rheumatoid arthritis); HF12 = HF0012 (type-1 diabetes); HM13 = HM0013 (osteoarthritis); HF14 = HF0014 (avian tuberculosis); HM15 = (asthma); HF16 = HR0016 (no health problems); and HF17 = HF0017 (no health problems). A, blood draw from eight individuals. Note that the individual with the most severe chronic disease of those examined had the highest number of non-activated aTPSCs in their blood. B, processed plasma from HM0001 and stained with Trypan blue dye. Note small dots (lower left corner of photograph (TSCs); cells with a of dark-stained peripheral rim Trypan blue dye staining (HLSCs); C. Processed plasma from HM0001 and stained with antibody to CEA-CAM-1 (HLSCs). Note a dense peripheral rim around the cells (HLSCs) (19). Reprinted with permission from Young HE, Lochner F, Lochner D, Lochner D, Black GF, Coleman JA, Young VE, McCommon G, Black Jr AC. Primitive stem cells in adult human peripheral blood. J Stem Cell Res. 2017; 1(2) 001:1-8

Isolation of the aTPSCs for therapeutic use is shown in Figure 1. One to two cups of fresh or frozen blueberries stimulate the proliferation of non-activated aTPSCs within its resident connective tissue niches. The body can and will activate the aTPSCs on its own, but it is a very slow process unless there is major trauma to the body. We developed two protocols for aTPSCs activation. Activation defined herein means unmasking homing receptors for chemokines released by damaged tissues and unmasking of receptors for local environmental cues (exosomes) to direct differentiation. The two protocols are 1. isolation of the aTPSCs with their activation ex vivo (outside the body), termed "fresh isolates" (18) and 2. their activation in situ (inside the body), utilizing "CNSP" (20).

Utilizing the patented fresh isolate technologies, it was noted that by giving Glacial Caps™ (DFRD), we could mobilize the normally quiescent hibernating connective tissue-resident aTPSCs into the vascular system (blood stream). Then by simple venipuncture, the aTPSCs suspended in whole blood could be withdrawn from the body to allow for easy

access for the fresh isolate technologies with minimal damage to exiting tissues. Patented technologies were developed to separate the aTPSCs from the whole blood for their subsequent segregation into five populations: TSCs, PSCs, EctoSCs, MesoSCs, and EndoSCs. Then these populations of aTPSCs, chosen for the proposed treatment plan to restore function in severed nerves, are TSCs (any somatic cell type in the body), PSCs (any somatic cell type in the body), EctoSCs (any somatic cell of the ectodermal lineage, e.g., neurons, glial supporting cells, Schwann cells), and MesoSCs (any somatic cell of the mesodermal lineage, e.g., loose fibrous connective tissue, capillaries, arterioles/venioles, small muscular arteries, small muscular veins, lymphatics) (Fig. 10) (11,15).

5.8. Methods to deliver the aTPSCs to a mixed nerve

Three methods were chosen to deliver the aTPSCs to the mixed nerve, e.g., intranasal infusion, direct injection into intervertebral foramina, and direct injection into site of scar tissue. To address issues of potential cell body death of motor neurons in ventral horn of spinal cord and/or sensory neurons in the dorsal root ganglion, TSCs were chosen for intranasal delivery of the cells to these areas via the central canal of the spinal cord (1,21-27). The TSCs are very small in size (0.1-2 microns) which make them an ideal population for this delivery technology, without the use of mannitol to shrink the olfactory epithelium that can create permanent channels for access to brain and spinal cord. Preliminary studies examining the speed of travel of the intranasally delivered TSCs to the cauda equina of the spinal cord, noted an average time of 45 minutes in human volunteers (15). This speed of delivery was utilized for the spinal cord transection clinical study (1) where TSCs were delivered by intranasal delivery to the CNS; and PSCs were delivered by direct injection into the intervertebral foramen of the effected mixed nerves under C-arm guidance (1). To address issues of restoration of connective tissue sheathes and Schwann cells, TSCs, PSCs, MesoSCs, and EctoSCs will be directly injected into the area of the transected nerve fibers (15,27,28).

5.9. Local anesthetics to mix with aTPSCs for direct injections through soft tissues

The local anesthetic routinely given to perform superficial treatments is lidocaine. Unfortunately, lidocaine KILLS dividing aTPSCs. A series of studies were performed to address the kill ratio of various local anesthetics on the aTPSCs. It was noted, that of all the anesthetics tested, lidocaine had a 100% kill ratio, whereas bupivacaine had a 0% kill ratio on the aTPSCs 29). This anesthetic has been incorporated in all subsequent clinical trials where anesthetizing a local environment was necessary to perform the treatment (15).

5.10. Address any underlying co-morbidities that may prevent successful restoration of nerve function; Several things were noticed during the clinical trials of fresh isolates and CNSP

If the individual has a life-threatening condition their body will use the activated aTPSCs there first, no matter where the stem cells are placed. Two best examples. Treatment of a Parkinson patient with fresh isolates (intranasal infusion of TSCs) did nothing for his Parkinson disease, but treated his heart instead. Two fresh isolate treatments raised his cardiac output from 25% to ~45% (30). Second example, SLE patient was set to have a three-way directed treatments for brain (intranasal infusion of TSCs), heart (slow TSC IV infusion for Thebesian system), and lungs (nebulization of TSCs and PSCs) from two allogeneic donors and his own autologous aTPSCs. Unfortunately, two weeks before scheduled transplants he was bitten by brown recluse spider. Two puncture wounds expanded into quarter-sized necrotic tissue at site of bite by time of transplants (overlying tibialis anterior tendon at ankle region). Within one week after stem cell treatment the spider bite completely healed with new skin and underlying tissues to a diameter of about 3" around the original bite area. However, there were no observable effect of neurological, cardiac, or pulmonary issues from their respective treatments (31).

If there are no life-threatening condition(s), the body will heal damaged tissues in reverse order of appearance, most recent damage healed first and then so on and so forth backwards until all damage is healed. Two examples, an individual with long standing COPD wanted treatment for his COPD, with TSCs and PSCs nebulized into the lungs. He had been avid runner, averaging 10K per day until his COPD worsened, where he had to stop running. Following his transplant (same day) he ran 10K. Then he could not figure out why his transplant did not work. He stated his COPD did not improve, but he felt stronger and his knees did not hurt as much (32). Presumably, running caused micro tears in skeletal muscle and micro fractures in bone and cartilage, which his body repaired first. Second example, another individual with COPD wanted treatment, nebulization of TSCS and PSCs into his lungs. He was an avid golfer, but because of his COPD he hadn't played golf in over five years. Following his transplant (same day) he played three 18-hole rounds of golf back-to-back-to-back. As before, he could not figure out why his transplant did not work. He stated his COPD did not improve, but he felt stronger and had more energy (31). Presumably, running caused micro tears in skeletal muscle and micro fractures in bone and cartilage, which his body repaired first (32).

To address pre-existing conditions that may impede successful restoration of nerve function, the individual being treated will ingest CNSP at least for two months or more before fresh isolate treatment. CNSP was designed to stimulate proliferation of aTPSCs in situ, making the person their own sterile bioreactor; mobilize the daughter aTPSCs into the blood stream; increase systemic circulation to all organs and tissues of the body; activation steps: unmask receptors for damaged tissue agents and unmask receptors for local environmental (exosome) cues; support a strong innate immune system (key critical step for successful wound healing); and to prevent tissue overgrowth by the activated aTPSCs (20). CNSP has been shown in 20+ IRB-approved clinical studies to assist in the repair of pre-existing conditions (20). It is hoped that any pre-existing conditions will be resolved before attempting fresh isolate delivery of bolus of stem cells: TSCs via intranasal infusion; TSCs and PSCs via C-arm directed guidance into the intervertebral foramina of associated nerve rootlets; and a mixed population of TSCs, PSCs, EctoSCs, and MesoSCs directly injected into site of transected nerve(s) embedded within scar tissue

5.11. Provide building blocks for Schwann cell myelin synthesis and secretion

The SLE individual with autoimmune-induced type-1 diabetes had ongoing problems with diabetic neuropathy, e.g., pain, referred pain, tingling, “electric shocks”, tingling, “pins and needles” sensations, etc. He noted that treatment with CNSP to provide activated stem cells and taking the supplements: Alpha-Lipoic Acid, Coconut Oil Capsules, and Vitamin-B12 (methylcobalamin), caused the systemic neuropathy to disappear. However, any lapse of ingesting either one or all of the supplements (Alpha-Lipoic Acid, Coconut Oil capsules, and/or Vitamin-B12) caused his peripheral neuropathy to return. If he resumed ingestion of those three supplements along with the CNSP, the polyneuropathy would disappear. This suggested that these compounds were involved in the remyelination of the nerve fibers (9,15).

5.12. Removal of existing scar tissue without surgical intervention

Serrapeptidase is an enzyme that will disintegrate existing scar tissue in situ (15). However, there are disadvantages and advantages to using it. If used systemically, it will disintegrate scar tissue systemically (15). Unfortunately, if the individual has an autoimmune disease, such as SLE, and their already hyperactive adaptive (antibody producing) immune system has the potential to generate antibodies against the foreign partially degraded tissue, fragmented cells, fragmented nuclei, etc. Continued use with destruction of the systemic scar(s) could cause a massive inflammatory response, resulting in an autoimmune flare. An out-of-control autoimmune flare causes collateral damage in surrounding healthy tissue which results in increased scarring in functional tissues and organs (15,31). Therefore, serrapeptidase, suspended in bupivacaine (29), will be injected locally under ultrasound guidance via multiple injections into the site where the nerve fibers are embedded in scar tissue. This should reduce the scar tissue sufficiently to allow nerve fiber penetration and outgrowth to the denervated muscles.

5.13. Increase activity of innate immune system to remove fragments of scar tissue

There are two independent and dependent immune systems, e.g., adaptive/humoral and innate. Independently, the adaptive immune system (T-cells, CD4+, CD8+, B-cells) generates antibodies to foreign entities, such as viruses, bacteria, mold, fungi, cells lacking self-recognition molecules for self, nuclear fragments, tissue fragments, etc. The innate immune system (monocytes, macrophages, NK-cells, neutrophils, PMNs, etc.) engulf foreign entities, digest, and either remove the digested material or, if the foreign material overwhelms the innate system, present the digested material with complement for antibody generation (33).

The adaptive immune system can be stimulated using the natural compounds found in Echinacea (15). Whereas, the innate immune system can be stimulated with compounds found in elderberries (15). Therefore, to stimulate the innate immune system, without stimulating the adaptive immune system, elderberries will be ingested daily.

5.14. Decrease in inflammation at the original site(s) of scar tissue formation

There are two very powerful naturally-occurring anti-inflammatory agents, e.g., curcumin (turmeric + black pepper extract) (33-38) and Boswellia serrata (39-42). Both will be given systemically before, during, and after treatment, to minimize any massive inflammatory response by the body (33-42).

5.15. Interaction of Platelet Rich Plasma (PRP) and aTPSCs

Whomever designed platelets was a genius. Platelets are a non-nucleated “bag” of proliferation agents, cell-specific progression agents, and inductive agents. When lysed and its components run on an SDS-PAGE, 46 bands were noted. Two of the most prominent bands on the gel (based on antibody binding) were transforming growth factor-beta (TGF-b) and basic-fibroblast growth factor (b-FGF) (43,44). Other bands present were identified as proliferative and/or progressive, e.g., platelet-derived growth factors, insulin-like growth factor-1, etc. (44,45).

When human recombinant proteins TGF-b and b-FGF were applied singly or in combination to clonal populations of TSCs, PSCs, EctoSCs, MesoSCs, EndoSCs, and MSCs (Table 4), TGF-b and b-FGF induced scar tissue formation in TSCs, PSCs, and MesoSCs; there was no scar tissue inductive effect on EctoSCs, EndoSCs, or MSCs; but there was a slight proliferative effect on MSCs [Table 4] (10,14).

These results would readily explain the formation of scar tissue at any site where lysed platelets come into contact with aTPSCs at a wound site. A wound is created eliciting damage to connective tissues and severing capillaries. The damaged connective tissues release chemokines that activate the TSCs to home to the wound site due to their extraordinary migratory abilities. Platelets also arrive at the wound site as well from severed capillaries. There they lyse releasing their contents at the wound site. Released PDGFs stimulate the TSCs to proliferate. At the wound site metalloproteinases activate the TSCs to respond to local cues, where the released TGF-b and b-FGF induce the non-proliferating TSCs to form scar tissue (15).

5.16. Decrease and/or prevent future scar tissue formation at regeneration site

Therefore, it is IMPERATIVE that platelets and/or their lysed contents are NOT released at the site where nerve regeneration should be occurring to prevent overt scar tissue formation. Additionally, the SLE participant noted that daily ingestion of bromelain apparently inhibited and/or decreased scar tissue formation in his lungs due to his ongoing SLE-induced pulmonary fibrosis, since there was no reduction in his FEV1s during ingestion of bromelain (15,46). Therefore, the individual in this study should also ingest bromelain daily to help prevent any new scar tissue formation at the lesioned site.

5.17. Resolve disuse atrophy of skeletal muscle by restoring tone in skeletal muscle to be innervated by nerve

Skeletal muscle can be stimulated to function by exogenous electrical stimulation (e.g., TENS unit). This procedure can maintain muscle tone (47) as well as helping release homing signals for nerve re-innervation (48-52).

6. Conclusions

The ultimate goal of this proposed treatment is for the individual's body to complete the Wallerian Regeneration process, with the assistance of appropriate activated stem cells, supplements involved in remyelination of nerve fibers, and without the interference of scar tissue, to re-innervate the previously denervated muscle(s).

If you would like to collaborate, we will need IRB-approval from your institution under the FDA's Right to Try criteria. I will write the rough draft of the IRB application with respect to aTPSCs, CNSP, local anesthetic, re-myelinization, and removal of scar tissue without surgical intervention. You will need to fill out the necessary additions based on your Institution's criteria for an IRB-application.

The equipment necessary to isolate, segregate, and activate aTPSCs from whole blood are a HEPA-filtered Class-2 Biosafety Cabinet certified for use with human tissues; a Refrigerated centrifuge with >17,000 RCF capabilities; and 4^{o}C refrigeration. Access to a "C"-arm will be needed for guidance to inject cells into intervertebral foramina. Other specific supply needs, plasticware, syringes, tubes, heparin, IV saline, etc. will be shared at a later date. The Dragonfly Foundation for Research and Development will supply the CNSP, pro bono. The supplements & enzyme listed can be purchased from Swanson Vitamins.

References

[1] Young HE, Speight MO. Traumatic spinal cord injury treated with autologous telomerase-positive stem cells. Stem Cells Regen Med. 2021; 5(1):1-13.

[2] Young HE. Combinatorial Nutraceutical Supplement Pill (CNSP) Stimulates Naïve Adult Telomerase Positive Stem Cells *In-Situ* to Heal Cardiomyopathies. GSC Advanced Research and Reviews. 2024; 20(02), 047-056.

[3] Conforti L, Gilley J, Coleman MP. Wallerian degeneration: an emerging axon death pathway linking injury and disease. Nature Reviews Neuroscience. 2014 Jun;15(6):394-409.

[4] Gaudet AD, Popovich PG, Ramer MS. Wallerian degeneration: Gaining perspective on inflammatory events after peripheral nerve injury. J of Neuroinflammation.2011 Available from https://jneuroinflammation.biomedcentral.com/articles/10.1186/1742-2094-8-110 (accessed on 06/04/2021)

[5] Medical & Exercise Physiology School. Wallerian degeneration/ regeneration process of nerve fiber/axon cut and progressive response. Available from https://www.youtube. com/watch?v=kbzYML05Vac (last accessed 5 March 2021)

[6] The Young Orthopod. Nerve Regeneration. Available from https://www.https://www. youtube.com/watch?v=P02ea4jf50g&t=192s (last accessed 6 March 2021)

[7] Hsu M, and Stevenson FF. Wallerian Degeneration and Recovery of Motor Nerves after Multiple Focused Cold Therapies. US National Library of Medicine. National Institutes of Health. 2015 ; 51(2): 268–275.https://www.ncbi.nlm.nih.gov/pmc/articles/ PMC4315870/ (accessed on 6/04/2021)

[8] Fauwcett DW. Bloom and Fawcett A Textbook of Histology. 11th Edition, WB Saunders Company, 1986, pgs. 311-344, 362-364.

[9] Kierszenbaum AL. Histology and Cell Biology An Introduction to Pathology. Mosdby, 2002, pgs. 199-225.

[10] Young HE, Black, Jr AC. Adult stem cells. Anatomical Record Part A 2004; 267A:75-102. Wiley-Liss, Inc.

[11] Young HE, Speight MO. Characterization of endogenous telomerase-positive stem cells for regenerative medicine, a review. Stem Cell Regen Med 2020; 4(2):1-14.

[12] Young HE, Black AC. Pluripotent Stem Cells, Endogenous versus Reprogrammed, a Review. MOJ Orthop Rheumatol 1(4): 00019, 2014.

[13] Stout CL, McKenzie J, Long G, Henson N, Hawkins KC, Ashley DW, Collins J, Hixson D, Black Jr AC, Young HE. Discovery of pluripotent and totipotent stem cells in the heart of the adult rat. Amer Surg. 2007; 73: S63.

[14] Young HE. Carcinoembryonic antigen-cell adhesion molecule-1 and stage-specific embryonic antigen-4 are present in the reproductive organs of adult mammals. GSC Advanced Research and Reviews. 2025; 23(03): 149-147. https://doi.org/10.20574/ gscarr.2025.23.3.0171.

[15] Young HE. Endogenous Adult Telomerase Positive Stem Cells and/or Combinatorial Nutraceuticals, 50 Years in the Making, 50 Years of Discovery. GSC Advanced Research and Reviews. 2025; Submitted.

[16] Young HE, Duplaa C, Romero-Ramos M, Chesselet M-F, Vourc'h P, Yost MJ, Ericson K, Terracio L, Asahara T, Masuda H, Tamura-Ninomiya S, Detmer K, Bray RA, Steele TA, Hixson D, El-Kalay M, Tobin BW, Russ RD, Horst MN, Floyd JA, Henson NL, Hawkins KC, Groom J, Parikh A, Blake L, Bland LJ, Thompson AJ, Kirincich A, Moreau C, Hudson J, Bowyer III FP, Lin TJ, Black Jr AC. Adult reserve stem cells and their potential for tissue engineering. Cell Biochem Biophys, 40(1):1-80, 2004.

[17] Young HE. Totipotent stem cells and pluripotent stem cells are present in the reproductive organs of an adult mammal. GSC Advanced Research and Reviews. 2025; 23(03): 158-180. DOI: https://doi.ord/10.30574/gscarr.2025.23.3.0172.

[18] Young HE. Fresh Isolate Adult Telomerase Positive Stem Cells: An addition to Embryonic Stem Cells (ESCs), Induced Pluripotent Stem Cells (iPSCs), and/or Mesenchymal Stem Cells (MSCs) for Regenerative Medicine. GSC Advanced Research and Reviews. 2023; 16(1):066-081.

[19] Young HE, Lochner F, Lochner D, Lochner D, Black GF, Coleman JA, Young VE, McCommon G, Black Jr AC. Primitive stem cells in adult human peripheral blood. J Stem Cell Res. 2017; 1(2) 001:1-8.

[20] Young HE. Combinatorial Nutraceutical Supplement Pill (CNSP) Stimulates Naïve Adult Telomerase Positive Stem Cells In-Situ to Heal Cardiomyopathies. GSC Advanced Research and Reviews. 2024; 20(02), 047-056.

[21] Young HE and Black Jr AC. Naturally occurring adult pluripotent stem cells. In: Stem Cells: From Biology to Therapy, Advances in Molecular Biology and Medicine. 1st Ed, R.A. Meyers, Ed, WILEY-BLACKWELL-VCH Verlag GmbH & Co. KGaA. Chap 3, pp. 63-93, 2013. http://onlinelibrary.wiley.com/doi/10.1002/3527600906.mcb.201200017/ abstract.

[22] Young HE, Hyer L, Black AC Jr, Robinson Jr JS. Adult stem cells: from bench-top to bedside. In: Tissue Regeneration: Where Nanostructure Meets Biology, 3DBiotech, North Brunswick, NJ Chap 1, pp 1-60, 2013a.

[23] Young HE, Hyer L, Black AC Jr, Robinson Jr JS. Treating Parkinson disease with adult stem cells. J Neurological Disorders, 2:107-109, 2013b. http://doi.org/10.4172/jnd. 100121.

[24] Young HE, Speight MO. Age-related macular degeneration treated with autologous telomerase-positive totipotent stem cells. Stem Cells Regen Med. 2020; 4(3):1-9. JSCR-20-055.

[25] Young HE, Speight MO. Blunt force trauma-induced total bilateral vision impairment of 13 years duration treated with autologous telomerase positive stem cells. Stem Cells Regen Med. 2021; 5(1):1-22.

[26] Young HE, Speight MO. Chronic inflammatory demyelinating polyneuropathy treated with autologous telomerase-positive stem cells. Stem Cells Regen Med. 2021; 5(2):1-16.

[27] Young HE, Speight MO. Treating Parkinson Disease with Autologous Telomerase-Positive Stem Cells, Update 2021. Stem Cells & Regenerative Medicine. 2021; 5(1): 1-13.

[28] Young HE, Speight MO. Osteoarthritis Treated with Telomerase-Positive Adult Stem Cells in Animals and Humans. Stem Cells Regen Med. 2020; 4(2):1-11. JSCR-20-049.

[29] Young HE, Speight MO. Local Anesthetics Can Affect the Efficacy of Telomerase-Positive Stem Cells. J Regen Med. Biol Res 2020; 1(1):1-9.

[30] Young HE, Speight MO. Cardiovascular disease treated with telomerase-positive stem cells. Stem Cells Regen Med. 2020; 4(2):1-8 JSCR-20-051.

[31] Young HE, Speight MO. Allogeneic and autologous telomerase-positive stem cells as a potential treatment for systemic lupus erythematosus. Stem Cells Regen Med. 2020; 4(2):1-9. JSCR-20-054.

[32] Young HE, Speight MO. Potential treatment of chronic obstructive pulmonary disease with allogeneic and autologous telomerase-positive stem cells. Stem Cells Regen Med. 2020; 4(3):1-11. JSCR-20-056.

[33] Kierszenbaum AL. Histology and Cell Biology An Introduction to Pathology. Mosby, 2002, pgs. 267-298.

[34] Gupta SC, Kismali G, Aggarwal BB. Curcumin, a component of turmeric: from farm to pharmacy. Biofactors. 2013;39(1):2-13.

[35] Bandyopadhyay D. Farmer to pharmacist: curcumin as an anti-invasive and antimetastatic agent for the treatment of cancer. Front Chem. 2014; 2:113.

[36] Sharma RA, Gescher AJ, Steward WP. Curcumin: The story so far. Eur J Cancer. 2005;41(13):1955-1968.

[37] Anand P, Kunnumakkara AB, Newman RA, Aggarwal BB. Bioavailability of curcumin: problems and promises. Mol Pharm. 2007; 4(6):807-818.

[38] Maheshwari RK, Singh AK, Gaddipati J, Srimal RC. Multiple biological activities of curcumin: a short review. Life Sci. 2006; 78(18):2081-2087.

[39] Baum L, Lam CW, Cheung SK, et al. Six-month randomized, placebo-controlled, double-blind, pilot clinical trial of curcumin in patients with Alzheimer disease. J Clin Psychopharmacol. 2008; 28(1):110-113.

[40] Ammon HPT. Boswellic acids and their role in chronic inflammatory diseases. Adv Exp Med 2016; 928:291-327. DOI: https//10.1007/978-3-319-41334-1_13.

[41] Ammon HPT. Modulation of the immune system by Boswellia serrata extracts and boswellic acids. Phytomedicine 2010; 17(11):862-867. Doi: https://10.1016/j.phymed.2010.03.003.

[42] Ammon HPT. Boswellic acids in chronic inflammatory diseases. Planta Med 2006; 72(12): 1100-1116. Doi: https://10.1055/s-2006-947227.

[43] Assoian RK, Komorya A, Meyers CA, Miller DM, Sporn MB. Transforming growth gactor-beta in human platelets. Identification of a major storage site, purification, and characterization. J Biol Chem. 1983; 258(11): 7155-7160.

[44] Pierce GF, Tarpley JE, Yanagohara D, Mustoe THA, Fox GM, Thomason A. Platelet-derived growth factors (BB homodimer), transforming growth factor-beta 1, and basic fibroblast growth factor in dermal wound healing. Neovessel and matrix formation and … Amer J Path. 1992; 140(6): 1375.

[45] Frechette J-P, Martineau I, Gagnon G. Platelet- rich plasmas: growth factor content and roles in wound healing. J Dent Res. 2005; 84(5): 434-439.

[46] Young HE, Speight MO. Telomerase-positive stem cells as a potential treatment for idiopathic pulmonary fibrosis. Stem Cells Regen Med. 2020; 4(2):1-11.

[47] Nussbaum EL, Houghton P, Anthony J, Rennie S, Shay BL, Hoens AH. Neuromuscular electrical stimulation for treatment of muscle impairment: critical review and recommendations for clinical practice. Physiother Can. 2017; 69(5): 1-76. Doi: https://10.3138/ptc.2015-88.

[48] Gordon T. Peripheral nerve regeneration and muscle reinnervation. Int J Mol Sci. 2020; 21(22): https://doi.org/:103390/ijms21228652.

[49] Jiang L, Jones S, Jia X. Stem cell transplantation for peripheral nerve regeneration: current options and opportunities. 2017; 2017: 18(1), 94. https//doi.org/:10.3390/ijms1810094

[50] Khuu S, Fernandez JW, Handsfield GG. Delayed skeletal muscle repair following inflammatory damage in simulated agent-based models of muscle regeneration. PLos Comput Biol. 2023; 19(4): e1011042.

[51] Tu H, Li Y-L. Inflammation balance in skeletal muscle damage and repair. Front Immunol 2023; 14: https://doi.org/10.3389/fimmu.2023.1133355.

[52] Forcina L, Cosentino M, Musaro A. Mechanisms regulating muscle regeneration: insights into interrelated and time-dependent phases of tissue healing. Cells. 2020; 9(5): 1297. https://doi.org/10.3390/cells9051297.