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Stem Cell & Regenerative Medicine

Stem Cell and Regenerative Methods for Space Personnel

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ABSTRACT

Significant deterioration of organs and tissues occurs in astronauts as a result of cosmic radiation exposure. This is a major obstacle to the developing space industry involving extended space exploration, commercial asteroid mining and the colonization of Earth's moon and Mars. Cosmic radiation includes galactic cosmic rays, and solar particle events of x-rays and gamma rays that penetrate deeply inducing DNA damage. Potential medical issues for astronauts arising from radiation include cancer, rapid aging, sterility, impaired immune system, re-emergence of pathogenic viruses, neurodegeneration and memory impairment. In summary, there are potentially catastrophic consequences for space personnel from prolonged exposure to radiation. Upregulating the regeneration of tissue using advances in stem cell technology would be a plausible method to counteract radiation damage triggering cellular degeneration. Stem cells are 'survival' cells and are responsible for the ongoing repair and regeneration of the human body. Increasing the number of available stem cells improves the regenerative capacity of the body. Current stem cell technology is not possible in space, as it requires multiple medical specialists using major hospital surgical and laboratory equipment. The authors have developed a portable stem cell biotechnology kit that is compact, lightweight, and simple to operate and could be performed by astronauts in space on a frequent basis to potentially regenerate affected tissue and reverse the effects of cosmic radiation. The technology uses laboratory validated peptide hydrogels for stem cell migration and expansion. The astronaut only requires donating a small amount of blood or adipose tissue. Preliminary analysis showed stem cells have high yield, excellent cell viability, and demonstrate normal stem cell health parameters of autophagy and annexin V. This approach may help to balance the degeneration / regeneration cycle of the body subject to radiation. Space personnel can be trained in a short period of time to perform the technology with safety and efficacy.

Keywords

Asteroid mining, Cosmic radiation, Helium-3 mining, Stem cells, Tissue regeneration.

Introduction

Space exploration and space mining are high priority areas for the future development of many nations. Robotic surveys and extraterrestrial rock and soil sample collection have identified the moon to be rich in helium-3 for proposed nuclear fusion and asteroids having enriched concentrations of minerals. In addition, manned missions for permanent bases on the moon and Mars are well under way with the major space agencies and leading commercial entrepreneurs of Elon Musk and Jeff Bezos. The medical health of space personnel is currently a major obstacle to space progress in two areas. Firstly, microgravity causes fluid distribution to the eyes and brain but developments in pressurized space suit design may reduce and normalize tissue fluid physiology preventing vision impairment. The second major health problem to overcome is cosmic radiation causing detrimental cellular changes and molecular DNA mutations. Due to the damaging effects from cosmic radiation all phases of human space exploration are restricted and include extended missions on the International Space Station (ISS), space walks, planned long duration space flights to Mars and valuable asteroids, and future colonization.

Molecular and cellular effects of cosmic radiation

The most damaging ionizing radiation for astronauts are galactic cosmic rays (GCR) and solar particle events (SPE) of x-rays and gamma rays [1], with excellent current comprehensive reviews of the magnitude of this issue [2,3]. Of particular concern are

the heavier nuclei, known as high charge and energy (HZE) particles, causing disjoining of DNA double strand breaks from deletions, subsequently leading to potential cancer of healthy cells in astronauts [4]. A recent study showed that a combination of microgravity with x-rays can magnify the problem with experiments on lymphoblasts causing chromosomal aberrations [5]. The consequences are an acceleration of aging through cell senescence and altered oxidative metabolism from radiolysis of mitochondria [6]. A potential major concern for space personnel was the effect of ionizing radiation triggering neurodegeneration and significant impairment of the hippocampus-dependent learning and memory in rodents exposed to low doses of HZE ions [7]. Gamma radiation penetrates deeply into organs and ionizes the molecular makeup of tissues by a photoelectric effect, Compton scattering or pair production that results in gene mutations and cancer [8].

Cosmic radiation for astronauts is chronic exposure to numerous highly penetrating radiation forms. Medical knowledge has documented the effects of acute high intensity radiation from manmade nuclear fission events such as Hiroshima and Chernobyl. However, translation of direct effects from the acute to chronic phase is largely from *ex vivo* and *in vitro* experiments. A very limited analysis of the chronic form is based on the single twin Scott Kelly study, and the results were alarming for microgravity and cosmic radiation [9]. An analysis of astronauts on the ISS also showed depletion of the immune system with re-emergence of viruses [10]. Studies on the effects of acute radiation include nausea, vomiting, headache, disorientation, fatigue, internal hemorrhage and hypotension [11]. Any one of these symptoms are potentially catastrophic in the space environment. There is of course the potential that astronauts and space miners will be exposed to acute solar flare episodes, resulting in 'acute on chronic' radiation episodes amplifying the damaging effects on space personnel. This problem could be increased when considering the paucity of knowledge regarding the total number of earth-borne viruses with the potential to inhabit and infect astronauts, and the possible existence of exposure to space-borne viruses. Clearly, there is a critical need to have the astronauts' immune systems functioning to optimum levels on a daily basis in space.

From an experimental perspective for identifying cosmic radiation effects, space medicine research is restricted to terrestrial experimental laboratory radiation on tissue. Unfortunately, earth-based radiation experiments cannot reproduce the entire spectrum of cosmic radiation. In space, there are multiple ionizing and non-ionizing events on the organs that are constantly changing in intensity and temporal nature. Permanent space colonies will also need in-depth knowledge to examine radiation effects on multiple human cell types, plants and insects, and potentially animal cells. Understanding cellular regenerative health mechanisms to improve the human immune system could be applicable to other species. The need to colonize would in the long term include animals, plants and insects to fully replicate the food chain for continued extra-terrestrial human existence.

The physical changes associated with extended space flight has been well documented in the Scott Kelly astronaut twin study. Negative neurological findings from cosmic radiation on his return to earth showed cognitive decline, sleep disturbance, and pain in the extremities. Other immune system issues included re-emergence of dormant herpes viruses in neurological tissue and skin. Current NASA approaches for reducing cosmic radiation exposure are improved monitoring, cargo stowage shielding and less operational time outside the spacecraft [12]. Using an integrative medicine approach, Russian astronauts have used the adaptogenic herb *Rhodiola rosea* to lessen symptom effects [13]. On a positive note, for Scott Kelly, telomere length increased to produce 'younger' cells, and silent genes (on earth) became active in space, suggesting an opportunity for stem cells to also increase their regenerative potential in space [14]. One major direction to maintain health is to have simple repeated 'on demand' stem cell treatments with new portable in-flight biotechnology. This would provide a foundation for normalizing astronaut health cycles of cellular degeneration (cosmic radiation) balanced by tissue regeneration (stem cells).

Regenerative medicine and stem cells

Tissues damaged by cosmic radiation ideally requires replacement by autologous (self) regeneration. Enormous medical breakthroughs have occurred in the last decade in the field of regenerative medicine using stem cells as the basis for comprehensive tissue regeneration. Stem cells are semi-primordial cells found in all plants, insects, animals and humans. They serve the basis of maintaining the survival of the species, and longevity for every human. Cell turnover of the representative tissue / organ types in the human body is phenomenal. It is estimated that the body regenerates a new set of lungs, kidneys, liver and pancreas every 3-4 years, a new heart every 20-25 years, dermis every 6 weeks, and hematopoietic stem cells generate 3-5 million new red cells every second [15]. Essentially humans are complex self-cloning biological mechanisms and a 40 year old astronaut would have already regrown most organs ten times and regenerated two hearts. Two studies have investigated stem cell growth characteristics on the ISS [16,17]. Both studies assessed human mesenchymal stem cells (MSCs) for microgravity effects and confirmed the viability of stem cell expansion and viability in low earth orbit. NASA is continuing to fund basic science stem cell research on the ISS with CubeLab from the University of California San Diego (UCSD) and a $5 million grant to a collaborative UCSD/ Space Tango (USA) project to develop a new lab called the Integrated Space Stem Cell Orbital Research (ISSCOR) [18].

Stem cells are also responsible for healing injured body parts such as a skin incision, burn, or bone fracture. After an injury, the tissue response is to release proinflammatory messenger molecules at the site of injury that are dispersed by the blood vasculature to form a chemical gradient that attracts stem cells to the site. Stem cells increase in number (termed stem cell expansion or proliferation) and release signaling molecules to local cells initiating repair. Replacement of injured cells and damaged tissue by stem cells is fundamental to maintaining good health and survival. When

mature functioning differentiated cells are persistently damaged (from cosmic radiation) and undergo cell death at a rate faster than normal stem cell regeneration then the body's physiology declines leading to poor health, diseases and ultimately premature death. Enhancing stem cell expansion (i.e. increasing the number of available stem cells) leads to rapid regeneration and the return to normal functional capability. A clinical example of the regenerative potential of stem cells is shown where autologous adipose derived stem cells were injected into the side of a disfigured face resulting in comprehensive multi-tissue regeneration of cranial nerves, arterial blood supply, muscle, adipose tissue and dermis (Figure 1). The long-term functional survival of astronauts and space miners is critical for the success of the space industry and is yet to be realized. Regenerative medicine can play a key role in this area. Interestingly, stem cells have also shown to be impenetrable to infection from SARS-CoV2 (COVID-19) [19] and clinical reports have demonstrated preliminary efficacy to resolve many of the persistent symptoms after COVID-19 infection [20].

The molecular messengers expressed by stem cells are in the form of exosomes; these contain a diverse cargo of anti-inflammatory cytokines, peptides and proteins to signal complex instructional and temporal queues to damaged cells. The multi-staging of stem cell expansion, expression and differentiation can be guided by the use of natural compounds such as vitamins, amino acids, peptides and plant polyphenols. These natural compounds are compact, easily stored and have long shelf life. Combinatorial therapy of autologous stem cells utilizing natural compound formulas is backed by substantial scientific evidence and is suggested by the authors as a safe and innovative method to protect astronauts and space miners to complete missions and commercial space ventures while exposed to galactic radiation.

Methods

The purpose of this study was to design a simple, portable clinical stem cell protocol and kit that could be used by space personnel for repeat 'on demand' regenerative treatments. Aspects were considered in optimizing the current gold standard method for safety, quality assessment and validation, efficacy, robustness, portability, design simplicity and training requirements.

Current scientific and medical method used in clinics

Stem cell treatments are used by many university, hospital and private clinics throughout the world. Randomized controlled trials (RCTs) have established their proof of concept in regenerative medicine particularly in the area of leukemia [21], multiple sclerosis [22] and more recently osteoarthritis [23]. The current gold standard for stem cell therapy in hospital clinics uses autologous (patient's own) adipose or blood derived stem cells, or allogeneic (foreign donor) cells that have been expanded in bioreactors and stored by cryopreservation. Adipose derived stem cells (ADSCs) are mesenchymal cells that have multipotent ability to form several different tissue types and typically require acquisition of 150-300gm tumescent lipoaspirate performed under a general anesthetic by a specialist surgeon [24]. A scientist will then extract the ADSCs by cycles of GMP grade collagenase, saline washing and centrifugation, and is performed in a class 2 biosafety cabinet. The ADSCs are reconstituted in saline, placed in syringes for injection back into the patient at the target site, or via intravenous infusion. This method was used by the author (ERV) to publish the first report on human stem cells to reduce neurological pain and inflammation in humans via direct injection into the head and face (Figure 2) [25]. Blood derived hematopoietic stem cells (HSCs) are obtained from blood, separated and then expanded (increased in number) in a bioreactor over several weeks. During

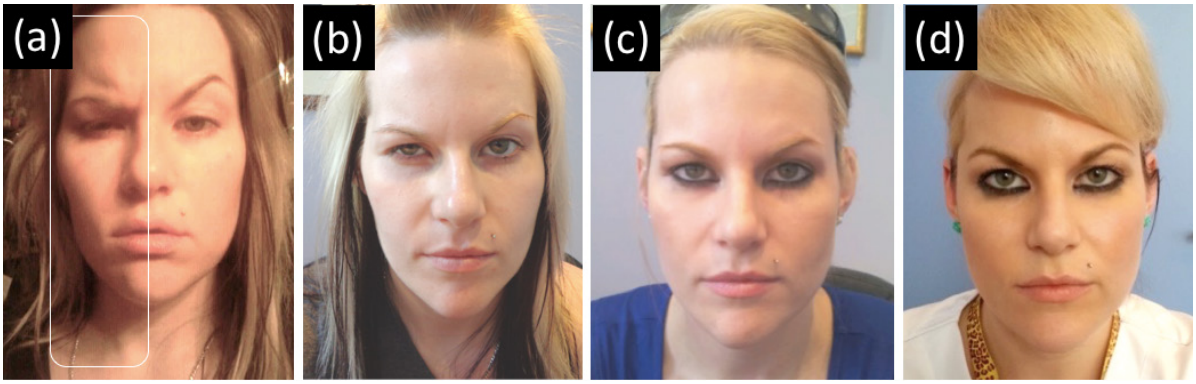

Figure 1: Comprehensive tissue regeneration using a single stage stem cell treatment in a 28-year-old female with substantial facial atrophy (*Vickers ER, Medicine Today 2015*).

(a) baseline image before treatment shows extensive destruction of the 1st, 2nd and 3rd division trigeminal sensory nerve branches, facial arterial supply, jaw muscles, adipose tissue and dermis (white outline box).

(b) stem cells acquired from adipose tumescent lipoaspirate with local anesthetic, cells resuspended in saline and injected back to the damaged trigeminal neurovasculature. Dual combination of herbs *Achillea millefolium* and *Passiflora incarnata* were used to improve the stem cell environment. Image taken at one month after treatment showing 30-40% regeneration of the face.

(c) continued growth of facial tissues to recover 75% of the atrophy. Image taken at four months after stem cell administration.

(d) maturation of all tissues to a functional state from a single treatment. Image taken at 18 months. The side of the face where stem cells were administered has regenerated new tissue resulting in a cosmetic appearance of an 18-20 year old. In contrast, the untreated side has continued to age to a 30-35 year old face with loss of collagen and elastin in the cheek and corner of the mouth.

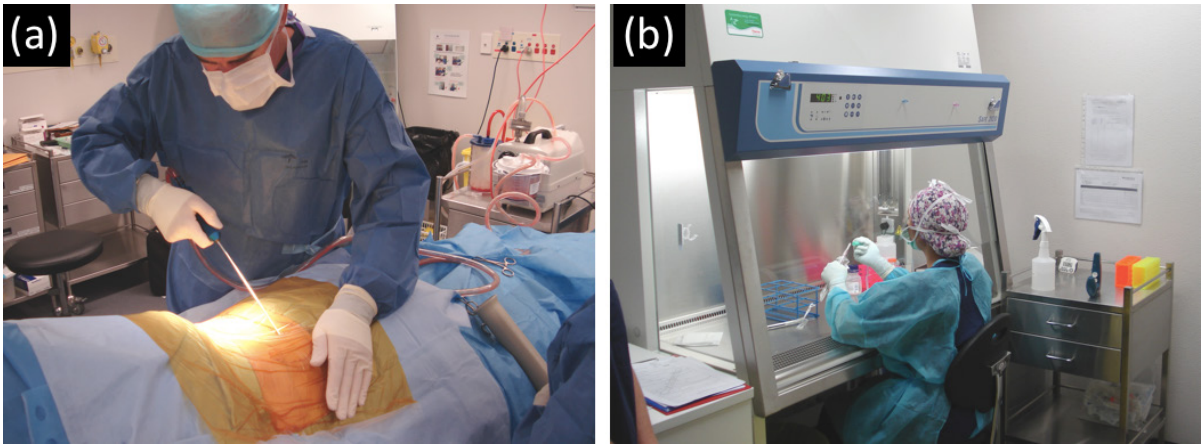

Figure 2: Current clinical stem cell method used in the majority of hospital stem cell clinics.

(a) Adipose stem cell acquisition method of tumescent liposuction employed in most hospitals needing plastic surgeon, anesthesiologist, two theater nurses, general anesthetic. Operating time of one hour to harvest 250-300 gm of lipoaspirate. Patient wears a corset for several weeks to reduce swelling and there is significant pain and bruising for several weeks.

(b) Lipoaspirate given to science team to harvest stem cells. Procedure takes 1-2 hours and uses GMP collagenase enzyme with cycles of washing and centrifugation. Stem cells resuspended in normal saline and given to clinician for injection to injured body part or via intravenous infusion.

stem therapy a small sample of ADSCs and HSCs are sent for validation with direct microscopy and flow cytometry.

Clinical disadvantages of the current method

The current method is a high cost per stem cell operation approach requiring multiple specialists of surgeon, anesthesiologist, operating theater nurses, recovery ward nurse, and laboratory scientists. The need for operating theater equipment and specialist personnel render the current approach impractical for space medicine. Bioreactor expansion of HSCs requires constant monitoring by specialist technicians, 'clean room' standards and significant expense on the growth medium. Bacterial contamination of the growth medium can have disastrous consequences and result in a toxic infusion of bacteria and stem cells causing death [26]. Evidence has shown ADSCs and HSCs that have been incubated, expanded, stored at -180C and then thawed repeatedly revealed the stem cells to have lost certain 'stemness' qualities [27] causing death in children who had previous chemotherapy [28].

Critique of other types of stem cells

Other types of stem cells have been investigated for clinical therapy. Allogeneic cells (from another person) are inexpensive to produce in bioreactors but problems arise regarding histocompatibility, using complex molecular testing methods (HLA compatibility) and potential development of graft versus host disease (GVHD). In addition, there is the potential misuse and misdiagnosis in legal issues in these human chimeras with two sets of DNA. One example showed the allogeneic stem cells from the younger donor quickly took over the reproductive organs of the host [29]. Another source uses umbilical cord cells that can have a closer HLA compatibility match between donor and host such as daughter and mother but due to limited cell numbers obtained (<600,000 cells) they can only be used for a single application. Embryonic stem cells are totipotent and able to regenerate all tissue types. However, they are obtained by destroying an embryo and are considered an unethical source of stem cells for routine clinical therapies in many countries. Embryonic stem cells (ESCs) can have a higher teratogenic disposition and cause tumor formation [30]. Induced pluripotent stem cells (IPSCs) were developed by the Nobel Laureate Shinya Yamanaka and rely on mature skin cells to be chemically coaxed into their original embryonic form [31]. The initial conversion efficiency rate of mature cells to the IPSC form was very low of 0.0001% with the original Yamanaka factors but has now greatly increased to 90.7% [32]. IPSCs are currently undergoing clinical trials and will have future widespread medical applications, but the method currently require experienced developmental cell biologists and biochemists.

Development of new clinical methods

The authors have performed over 250 stem cell treatments on patients at various international locations including Sydney (Australia), Nanjing and Guangzhou (PR China), and London (UK). Due to the urgency of several patient's conditions and the need to move quickly across multiple locations a new method was developed. Principal outcome requirements were a high margin of patient safety using a minimal clinical surgery and anesthesia technique, and a maximum number of people of two comprising one medical and one technical personnel. In addition, there was the need to design a simplified laboratory equipment approach to yield viable stem cells. The method had to meet the essential criteria of portability, robustness and validation. The equipment had to be easily replaceable and/or repairable.

Results - Development of three new methods

Our continual clinical exposure and further understanding of the mechanisms of stem cells showed them to be very resilient cells and able to stand harsher laboratory separation techniques. This information was coupled on research reports on diverse areas of hydrogels, herbal polyphenols and the actions of endogenous proteins and peptides. Together, the emerging information offered

persuasive evidence that an improved method could be developed. Subsequently, the author ERV has developed three additional methods of stem cell therapy using a simplified collection of adipose tissue combined with the utilization of specific formulas of polyphenols and peptidic hydrogels. All three methods described have potential use in regenerative space medicine to prevent and reverse the damaging effects of cosmic radiation.

Method 1: Minimal collection of lipoaspirate and mechanical digest. This method requires acquisition of only 3-8 gm abdominal lipoaspirate and is performed under local anesthetic. Saline is added to the tumescent lipoaspirate to make a working volume to 8ml in a 10ml syringe. This is connected by a 2-way connector to another syringe and the opposing syringes plunging actions reduce the adipose tissue to an oil in water emulsion consistency. The emulsion is transferred to a vortex mixer for 4 minutes, then centrifuged at 4000 rpm for 10 minutes. There are clear layers of fat, oil, saline, stem cells and red cells (Figure 3). The stem cells are collected, resuspended in saline and injected back into the patient. The entire process takes one hour with very high cell viability (>95%) and typically yields 5-10 million stem cells. There is rapid return to work for the patient and the technique has recorded no complications, causes only low discomfort and occasional slight bruising at the lipoaspirate site. The technique could be explained and taught to astronauts with a very high margin of safety. Other sites of adipose tissue harvesting can be from the thigh and upper arm. Advantages of this technique is use of standardized equipment, small footprint, low potential for contamination and its dual utility for clinical use and scientific investigations (Figure 4). The operative technique uses sterile single use standard needles, syringes and cannulas in a closed loop system and without the need of a biosafety cabinet environment (Figure 4). Moreover, there is no need for collagenase enzyme that has a limited shelf life and collagenase may contain unknown viruses as the product is usually isolated from pigs.

Method 2: ADSCs and HSCs chemoattraction and expansion in peptidic hydrogels.

Chemoattraction is the ability of stem cells to migrate to a site via a chemical gradient. This area has undergone intensive research for clinical applications. For example, a gel with a proven safe chemical could be injected and the patient has no need to undergo surgery to acquire lipoaspirate with stem cells. Two blood proteins with hematopoietic stem cell migration activity are macrophage colony stimulating factor 1 (CSF-1, 522 amino acids) and stromal derived factor 1 (SDF-1, 89 amino acids) but their long amino acid sequences render them impractical for clinical utility due to synthesis cost [33,34]. Several studies were published in 2020 with other compounds. New Zealand researchers (September 2020) developed a novel chemotactic factor (~12 kDa) termed 'MayDay', derived from the N-terminal 31–188 sequence of decorin [35]. 17β-estradiol has demonstrated itself as a cofactor in osteogenic cell migration [36] and agrin has shown local activation for osteogenesis [37]. A recent development by the author ERV has been the identification of a number of small sequence (<20 mer) peptides using low-cost F-moc peptide synthesis [38]. This allows attraction of stem cells from blood into a new medium for rapid growth of stem cells. It is necessary to isolate HSCs from blood breakdown products such as the heme molecule that is toxic (Figure 5). This would permit astronauts to simply obtain a small amount of blood from the tip of the finger using a diabetic lance, then chemoattract the stem cells followed by expansion in a 37^0C incubator. The resultant yield is millions of viable stem cells in 3-8 days from a drop of blood. It is anticipated that stem cell regenerative treatments in space could easily be conducted on a daily basis for astronaut protection against cosmic radiation and tissue renewal using autologous cells.

Method 3: Direct peptidic hydrogel injections

This novel approach would inject a peptide-sodium alginate hydrogel to the injured or diseased site and directly attract large

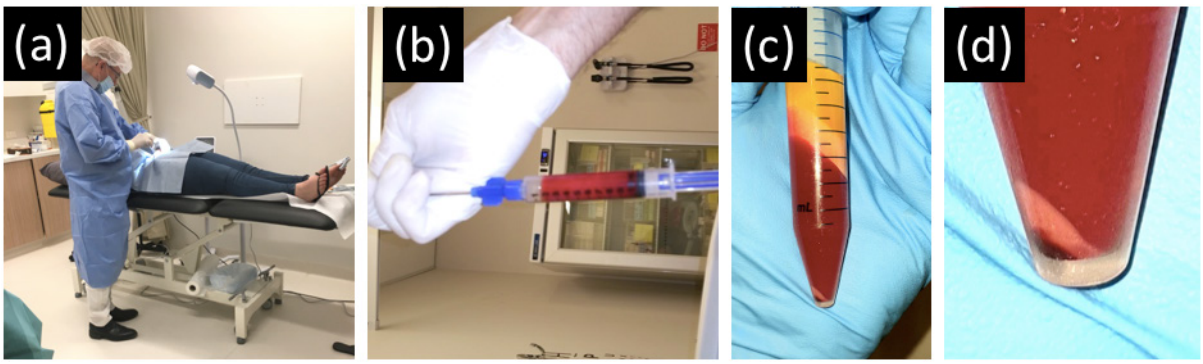

Figure 3: Method 1 using minimal liposuction of 3-8 gm of tumescent lipoaspirate performed under local anesthetic. Operating time is 15 minutes, the patient can communicate and is ambulant at all stages. There is only minor postoperative swelling and discomfort with return to work in 24 hours.

(a) surgical personnel of one clinician.

(b) standard sterile single use syringes and cannulas in a closed loop system.

(c) nonenzymic method of emulsification of fat followed by vortex mixing and centrifugation. From the top of the centrifuge tube there are layers of oil, fat, saline with red cells, pinkish white layer of stem cells, and small amount of compacted red cells. Laboratory procedure can be completed by the clinician or technician.

(d) higher magnification image of the stem cell layer. Stem cells removed and resuspended in saline for clinical administration. A small aliquot is taken for laboratory confirmation.

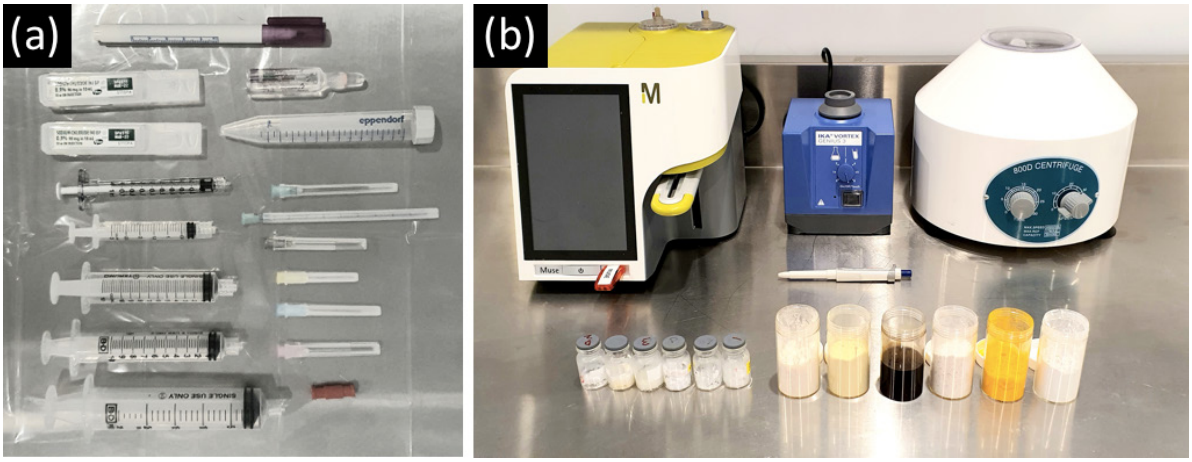

Figure 4: Method 1 showing equipment required for minimal liposuction procedure and acquisition of stem cells.

(a) Surgical pack contains local anesthetic, saline, disposable syringes 1ml-50ml, standard needles, liposuction cannula, 2-way connector, 15ml Eppendorf centrifuge tube and skin marking pen. The kit is lightweight, compact, sterile and single use to prevent cross infection.

(b) Hardware contains compact flow cytometer, vortex mixer and centrifuge. Examples of six small vials of peptides and six vials of polyphenols with long shelf life and each vial can contain enough powders for approximately 20 stem cell procedures. There is an enormous range of peptides and polyphenols (5,000) and specific formulas have been developed by author ERV to potentially reverse the effects of cosmic radiation on different human tissues and organs.

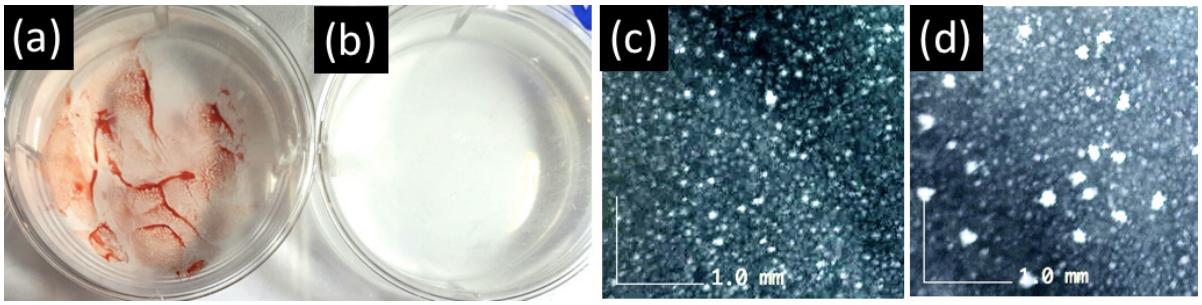

Figure 5: Method 2 using peptidic hydrogels for blood stem cell chemoattraction and cell expansion.

(a) 1-2 drops of blood obtained by a diabetic lance to the fingertip is added to the hydrogel and incubated at 37^0C for 3-8 days.

(b) The adjacent connected well contains the chemoattractive peptide in the hydrogel. Hematopoietic stem cells migrate into this well and away from the original well containing the toxic heme metabolic product from red cells.

(c) image of the peptidic well at 24 hours shows thousands of small developing colony forming units (CFUs) under darkfield microscopy

(d) CFUs at 5 days show increased proliferation

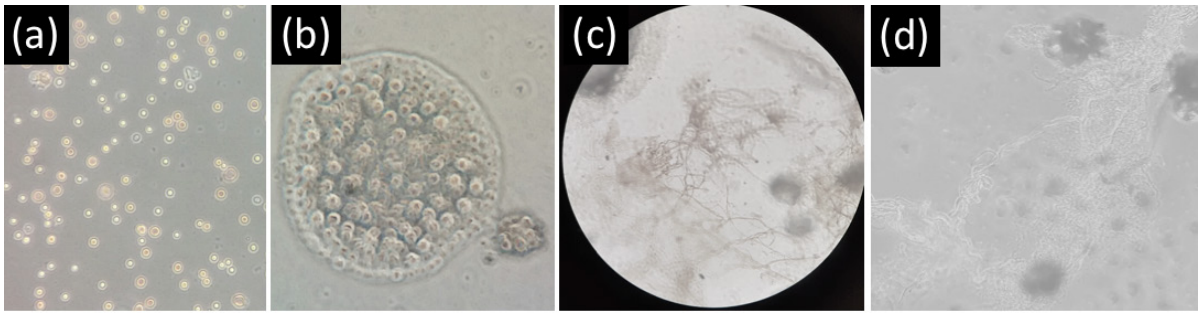

Figure 6: Validation of Method 1 of stem cell proliferation to tissue. Stem cells were kept in a 37^0C incubator for 8 weeks. Stem cells were grown in phosphate buffered saline pH 7.4 and images taken with an inverted microscope 100-400 X.

(a) immediate plating of aliquot with a mixture of red cells and smaller white stem cells.

(b) stem cell expansion at one week showing a single cell has proliferated to a stem cell colony forming unit (CFU). Budding to develop another CFU is seen.

(c) early development of perivasculature between CFUs at 4 weeks.

(d) maturing of vasculature and CFU differentiation for early tissue development at 8 weeks.

numbers of circulating stem cells. Alginate hydrogels have very high safety margins and form the main compound ingested to prevent stomach acid reflux. Alginates are used as thickeners in many food products and numerous publications on their suitability for biodegradable materials for drug delivery. Moreover, the gel is extremely versatile as it can be cross-linked with calcium, zinc and magnesium to yield a 3D solid construct for the repair of bone and cartilage. The injection of a low viscosity hydrogel requires standard needles and syringes. Where the gel needs to be injected for deep placement into the tissue and prevent intravascular injection then the use of portable 3MHz and 7-15MHz ultrasound transducer heads coupled to a laptop is required. Training on ultrasound-guided placement is well within the scope of astronauts. The technique uses standard syringes and needles. Typically, a 21–23-gauge needle permits deposition of the low viscosity alginate hydrogel imparting a further margin of safety in its delivery into deeper tissues and organs. The authors are currently undertaking preliminary clinical studies with this method.

Method Validation

The confirmation of stem cell identity is accomplished by several validated laboratory protocols. Direct viewing of single cells, colony formation and perivasculature is achieved under light microscopy 400X (inverted) – 1000X (oil) (Figure 6), fluorescence microscopy, adherence to plastic during proliferation and flow cytometry analysis of CD markers [39]. Flow cytometers can be small and lightweight (single laser with two channels) to a larger footprint of 3-4 lasers with 8-12 channel detectors. Multiple cell health parameters on the flow cytometer can measure a range of variables of autophagy, annexin, H2A.X, Ki67, nitric oxide and reactive oxygen species (Figure 7).

Advantages of the peptide protocols allows concurrent *ex vivo* research to be conducted at the same time of actual treatments, underpinning real time safety and validation of the protocol. A further advantage is incubating the HSCs in a peptide hydrogel with novel drug compounds and the established herbal polyphenols. This may contribute to a further enhancement of personalized cosmic radiation protection. These results could potentially lead to a stem cell 'vaccine' application against radiation during extended space flight. Comprehensive analysis of stem cell exosomes and the proteome expression profile in microgravity could be conducted by portable fluorescence immunoassay or new developments in electrospray mass spectrometry.

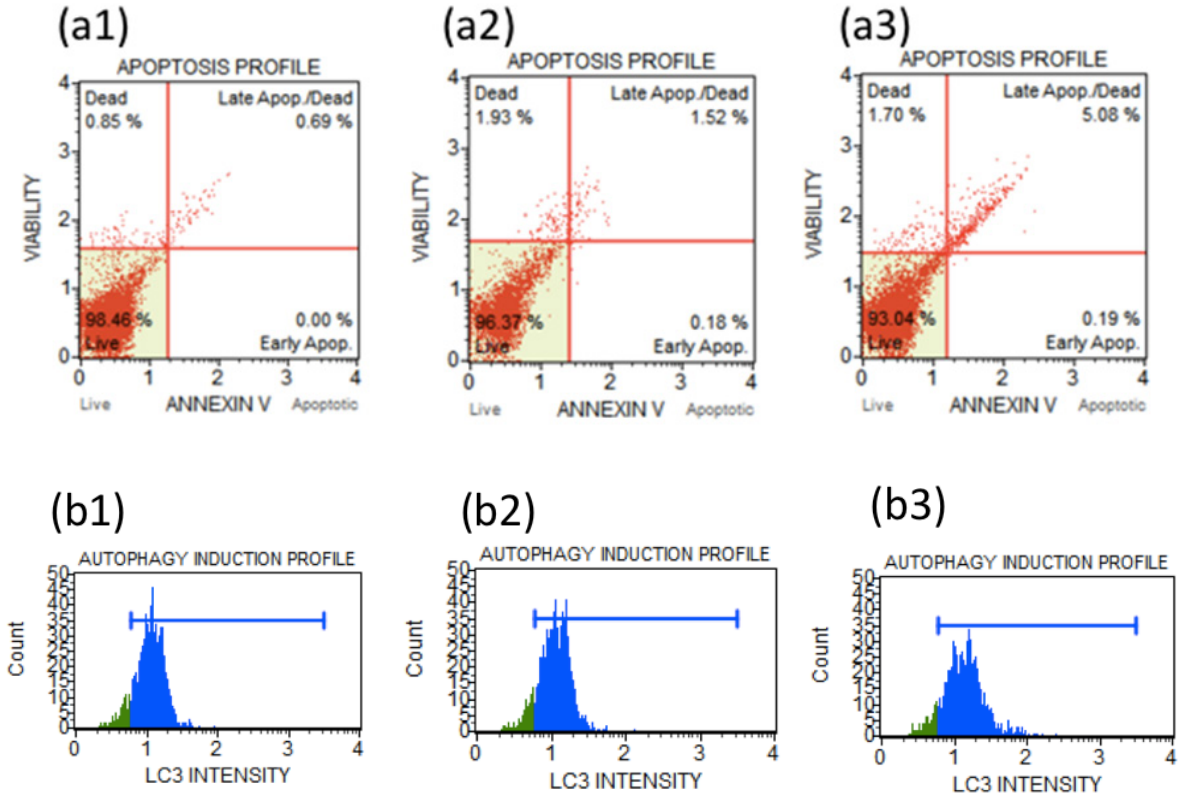

Figure 7: Validation of Method 2 showing hematopoietic stem cell growth from 9 to 20 days in the hydrogel in three subjects: (1) 66 year old male with 9 days gel incubation, (2) 25 year old male with 20 days gel incubation and (3) 59 year old female with 9 days incubation. Live stem cell growth density was (1) 5.97 million cells per gm of gel, (2) 16.5 million cells per gram of gel and (3) 4.83 million cells per gram of gel.

(a) Annexin V apoptosis assay shows excellent live stem cell viability of (1) 98.46%, (2) 96.37% and (3) 93.04%

(b) Autophagy (LC3 intensity) plays a critical role in stem cell quiescence, activation, differentiation, and self-renewal and reveals a count activation (1) 13.1, (2) 13.5 and (3) 18.6

Table 1: List of abbreviations.

ADSC	Adipose (fat) derived stem cell
BMP	Bone morphogenetic protein
BRASH	Biomedical Research Advances for Space Health
CD marker	Cluster of differentiation marker
CFU	Stem cell colony forming unit
COVID-19	Coronavirus disease 2019
CRISPR-Cas9	Clustered regularly interspaced short palindromic repeats (binds to Cas9 enzyme)
CSF	Colony stimulating factor
DNA	Deoxyribonucleic acid
ERV	Author ER Vickers
ESC	Embryonic stem cell
FGF	Fibroblast growth factor
GCR	Galactic cosmic rays
GMP	Good manufacturing practice
GVHD	Graft versus host disease
H2A.X	Histone protein family member X from H2A family
HLA	Human leukocyte antigen
HPLC	High performance liquid chromatography
HSC	Hematopoietic (blood) derived stem cell
HW	Author H Wen
HZE	High charge and energy particles
IPSC	Induced pluripotent stem cell
ISS	International Space Station
ISSCOR	Integrated Space Stem Cell Orbital Research
LC3	Microtubule-associated protein 1A/1B-light chain 3
MAPK	Mitogen-activated protein kinase
n-mer	Monomeric unit of n amino acids
MS	Mass spectrometry (single quadrupole)
MS/MS	Mass spectrometry / mass spectrometry (triple quadrupole)
MS^n	Ion trap mass spectrometry
MSC	Mesenchymal stem cell
NASA	National Aeronautics and Space Administration
Pi3	Phosphoinositide 3-kinase
RCT	Randomized controlled trial
SARS-CoV2	Severe acute respiratory syndrome coronavirus 2
SDF	Stromal derived factor
TCM	Traditional Chinese Medicine
UCSD	University of California San Diego

Enhancing stem cell expansion

As previously mentioned, stem cells are the principal reparative cells for recovery when a body is injured. The greater number of stem cells available lead to an increase in the rate of the cellular regeneration process. Several therapies have been shown to increase stem cell migration, proliferation and differentiation and include vitamin D3 [40], pulsed electromagnetic fields [41], ultrasound [42] and hyperbaric oxygen [43]. In addition, there is substantial published evidence to show that the number of stem cells can be increased (expanded) and rapid healing encouraged by co-administration of natural compounds such as polyphenols derived from plants (herbal medicine) and synthesized peptides that can be incorporated in biological scaffolds.

Herbal medicine

Herbs have been used to improve the healing response of injured tissue for thousands of years. Examples of herbal medicines used by indigenous cultures include Traditional Chinese Medicine (TCM), Indian Ayurvedic Medicine, North American Indian Medicine, Eurasian and Australian Aboriginal plant knowledge and methods. This heuristic approach has underpinned human health and therapy very successfully for hundreds of generations. In the case of the Australian Aboriginal race, they have successfully maintained their culture for 70,000 years using medicines from plants. Comprehensive herbal combinations are still used with great efficacy by Traditional Chinese Medicine doctors. The western approach has used scientific analytical methods to identify the bioactive molecules of individual herbs. The method uses high performance liquid chromatography (HPLC) or gel chromatography to separate and concentrate the herb's constituents. The HPLC is coupled to a mass spectrometer that identifies the molecular weight of the parent molecule (single quadrupole termed MS). Rigorous analysis of the parent molecule determines the chemical group makeup, and then absolute confirmation of molecular identity is performed by triple quadrupole (MS/MS) and ion trap (MS^n) mass spectrometry. Herbs contain many different bioactive molecules termed polyphenols numbering over 8,000. They are classed into subcategories by their chemical formula regarding the number of phenolic rings and structural groups, such as flavonoids, flavonols, flavones, polyphenolic amides, lignans and stilbenes.

The therapeutic delivery of the plant is typically achieved in its traditional form of the entire herb in a powder or liquid ethanol extract. When applying western technology by chromatographic extraction then the individual bioactive compounds can be obtained. These are typically high potency polyphenol powder extracts. Examples of individual polyphenols are curcumin derived from turmeric, and epigallocatechin gallate from green tea. Both of these polyphenols have been shown to have anticancer and antiviral activities suggesting their use by astronauts would be beneficial [44]. The Indian herb *Gingko biloba* has existed for 280-290 million years and has been 'attacked' by thousands of viruses during this time. Subsequently the plant has developed multiple chemical antiviral agents in order to survive. Astronauts have revealed a high prevalence of recurrent viral infections. The antiviral properties of polyphenols and their synergistic combinations can be employed against viruses and their mutations.

Rhodiola rosea is an herb that has already been used by space personnel. It is an adaptogenic herb from Norway and contains multiple polyphenols [45]. Originally used by the Vikings it was subsequently used by Russian cosmonauts [46]. This herb is located in the Arctic environment and consequently has been exposed to higher levels of ionizing radiation and very low temperatures in its habitat, and for its survival has developed a repertoire of antiradiation and antioxidant molecules. The range of potential polyphenols that may have therapeutic utility for space personnel is immense and would number in the thousands of individual bioactive molecules. A trained herbal medicine specialist (author ERV) can then combine specific bioactives to yield highly effective polyphenol formulas with great synergy. A major advantage for space medicine is that these formulas are low weight, very stable in the dried form with a long shelf life and can personalized for the astronaut's metabolism.

Peptides

Peptides are vital components in our body to maintain cellular health. Peptides have direct signaling mechanisms between stem cells and the differentiated cells found in functional tissues. Peptides are composed of specific chains and forms of amino acids. They are found in plants, insects, animals and humans often with a conserved domain of an amino acid sequence between species. The number of peptides in the central nervous system is over 1200 neuropeptides. A recent comprehensive HPLC/MS/MS analysis of human blood identified over 1.9 million peptides, and yet current science would have a reasonable knowledge of the function and structure in <5000 peptides. Peptides are typically synthesised and expressed from the cell as larger parent proteins that are then cleaved into smaller peptides by enzymes. Specific peptides can have antimicrobial, antiviral, anti-inflammatory, anticancer and mood enhancing properties such as the defensin peptide family [47,48] and the endogenous opioid family of endorphins, enkepahlins and endomorphins [49]. A major advantage of peptides is that they can be synthesized in a laboratory and stored in a lyophilised form providing an extremely long shelf life. They are potent in the pico-nanomolar range, and when combined with stem cells they can direct the lineage into designated tissue forms such as bone (bone morphogenetic protein, (BMP)) or cartilage (fibroblast growth factor 2 (FGF-2)) [50,51]. Similar to the plant polyphenols they are can be stored in the space environment with ease as they are very compact and have low mass.

Bioscaffolds

Bioscaffolds are resorbable structures where stem cells, polyphenolic compounds, drugs and peptides can be incorporated within a 3D matrix. The bioscaffold substantially increases the structural support for tissue regeneration. The bioscaffolds are being extensively researched and can be fabricated from materials sourced in food derivatives such as alginate (seaweed) to form a hydrogel. Further hydrogel optimisation for improving the mechanical properties occurs by adding natural cross-linking agents of calcium, magnesium and zinc [38]. Other materials include medical grade surgical suture material (polyglycolic and polylactic acid), polycaprolactone used in circuit board repairs, or sophisticated mineral hybrid materials [52]. Many of the materials have excellent precision for replacing anatomical structures with a high degree of accuracy using 3D printers and scanners [53].

Training

A critical component to the regenerative method in the space environment is the ability to master the various protocols with knowledge and safety, and detailed guidelines have been published by the author (ERV) [54]. The laboratory equipment required is standard, compact and lightweight with a small footprint. The author's current stem cell kit fits into a flight suitcase. Consumables are lightweight, sterile, single use syringes and needles. Polyphenols and peptides are in individual sterilized and lyophilized vials with long shelf life. It is anticipated that training requirements for the astronauts would be one week. Ultimately, with training, the astronaut or space miner is able to regenerate their body with autologous stem cells on demand using compact, low mass technology in order to reverse the damaging effects of cosmic radiation.

Discussion

The stem cell methods described are suitable for astronauts in extended space flight and within the ISS, and commercial asteroid mining. The methodology can be further applied to settlement personnel on the moon and Mars. NASA has identified the health of astronauts as a major impediment to its mission progress. In the past NASA has accomplished its goals by internal scientists and specialists. However, they have acknowledged that the cosmic radiation health issue requires external research efforts with the recently published Biomedical Research Advances for Space Health (BRASH) notification [55]. Specifically, NASA is asking that external research centers use "disruptive technologies to protect health in deep space through manipulation of human metabolism and homeostasis at the cellular or whole organism level". This suggests the use of advanced technology such as CRISPR-Cas9 gene editing or similar innovative but clinically untested methods in space. Over the last decade, in a move to understand cancer (i.e. cancer stem cells) at the molecular level many laboratories have developed humanized animal models where human gene sequences are inserted into rodent DNA. In turn, experiments have been conducted to transinfect animal DNA into human cells. One study inserted tardigrade genes to assess protection against cosmic radiation [56]. This did offer some increased protection to human cells from laboratory induced cosmic radiation. However, a major health concern is that once the genes are inserted, they are impossible to remove in a clinical setting should unexpected catastrophic cellular, tissue or organ changes occur in the human host. Moreover, to obtain sufficient 'animalized' human stem cells requires extended bioreactor cell expansion and potential contamination. Further uncertainty would be the increased proliferation of allogeneic cells in microgravity with the possible consequence of a rapid takeover of the astronaut cell makeup. In addition, GVHD issues are potentially real and disastrous using allogeneic cells in space.

An analysis of the risk of stem cell types was considered in developing our method parameters. Our described method uses autologous mesenchymal stem cells from body fat (ADSCs) or blood (HSCs). The HSCs method is particularly useful with astronauts and space miners for several reasons of simplicity, frequent use, safety and stem cell viability (Figure 8). HSCs have a very low risk of medical side effects. Where complications have been reported in the media, the reason is clinical error and poorly trained personnel. For example, one press report uncovered the fact that untrained nurses had injected stem cells into eyes causing blindness [57]. In most stem cell clinics in the world the preferred cell type is adipose MSCs with an established margin of safety and increasing publications of their efficacy for treating different medical conditions. The three innovative methods described by the authors (ERV/HW) have very low risk of microbial contamination, and cell health can be evaluated prior to use. There is also no need for high maintenance and complex equipment that is required for stem cell banking, long term cell expansion or monitoring with

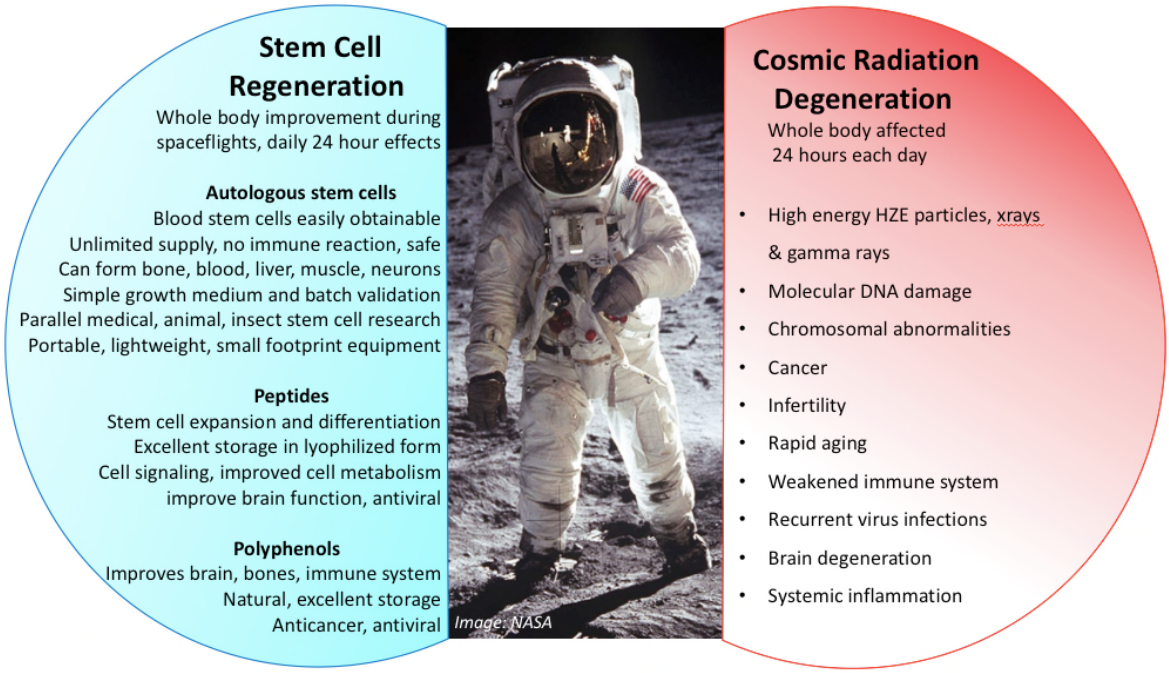

Figure 8: Summary of the possible effects of stem cell regeneration to reverse the damaging effects of cosmic radiation (high-energy particle, gamma rays and x-rays) on the human body. The stem cell hydrogel technique is individualized by using autologous stem cells combined with peptides and polyphenol compounds. The method demonstrates preliminary safety, high efficiency for live stem cell viability and can be used on a daily basis to potentially regenerate damaged tissues.

dedicated laboratory scientists. Moreover, our methods specifically use autologous cells to avoid GVHD. A further advantage of our method is the simple duplication of test batches alongside the treatment batch. Test batches can undergo cell viability analysis without compromising the treatment batch. All that is required is another drop of blood in a parallel test batch. The use of parallel assays would be advantageous in the space environment to explore stem cell growth aspects using combinations of different formulas of polyphenols and peptides. *Ex vivo* stem cell batches can be employed to safely identify the duration of time for an astronaut outside a space capsule, space station or mining base. A further application of the batch method could test variables of the different forms of background radiation, shielding requirements, temperature, pressure and oxygen / carbon dioxide / nitrogen gas mixtures in a planned colony prior to landing. The described peptidic hydrogel method can be used for organoid research and to analyse the peptides and polyphenols found in the stem cells of animals, insects and plants in a cosmic radiation environment. A successful extraterrestrial human colony will, in time, utilize earth's fauna and flora to replicate a harmonious natural environment to augment human settlement.

The mining of space has enormous benefits for earth and its spectrum of inhabitants. Helium-3 on the moon and the introduction of nuclear fusion energy output could feasibly save the planet from the irreversible effects of uncontrolled climate change. It is estimated the moon's surface down to several meters contains over 1 million tonnes of helium-3, and just 25 tonnes would power the entire USA with clean energy for one year [58]. The current cost of energy in the USA is $1.3 trillion in 2018 ($52 million / kg helium-3) [59] and an expected minimum moon return payload of 100kg worth $5.2 billion. In addition, there are asteroids with mineral rich concentrates that would reduce earth mining and the resultant environmental disasters that can occur. Examples of valuable asteroids in close proximity to the earth include asteroid 153201 (diameter 500 meters) with a value of $17.4 billion, Phaethon (diameter 5 km) worth $103 trillion and Heracles (diameter 4.8km) worth $372 trillion [60]. International space treaties permit nations and companies to freely mine the moon and outer space [61]. Uncontrolled mass migration, famine and wars with massive tragic human loss have occurred over regional earthbound shortages of food, oil and gas. The use of asteroids and moon materials could be a critical factor to long-term human survival on earth. A risk analysis comparison is necessary for space mining in selecting robotic (nil health risk) versus human mining personnel (possible health risk). Public opinion can weigh heavily on expensive mission failures and astronaut deaths. This is a fundamental issue with any project, more so in space because of worldwide publicity and the expectation of success. There is of course, risk with space travel and a small risk of using new medical technology of stem cells. However, in a comparative analysis of terrestrial risk of injury and death, space is a relatively safe environment. For example, on earth, between 250,000 and 440,000 Americans die each year as a result of human medical errors alone and is the third leading cause of death in the USA [62]. Approximately two million people die each year from work related

accidents [63] and over 340 million injuries from work each year at a cost of $3 trillion. On the key issue of mining safety, there are approximately 12,000 deaths annually associated with terrestial mining [64]. Space, by comparison, appears as a relatively well-controlled safe environment if cosmic radiation issues can be resolved.

An expert think tank has compared the relative merits and problems facing asteroid robotic mining versus human miners and two major points were identified. For robotic asteroid and moon mining there is the very high developmental cost of designing a robotic miner, yet one minor component failure can lead to complete failure. In contrast, the expert committee stated there were enormous advantages with human space miners if health can be maintained "flexibility, adaptability, creativity that translates into problem-solving, decision-making, and troubleshooting capabilities. These traits have the potential to dramatically improve the performance and success of a mission." [65].

The exploration of space is a principal motivating force of many people for reasons of human curiosity and destiny. Advances in stem cell knowledge and technology could help to overcome the major obstacle imposed by cosmic radiation on astronaut health. Stem cell therapies have extensive published documentary evidence of phase 1-3 trials attesting to their safety and preliminary efficacy in many medical conditions. Translational research suggests physical homeostasis can be achieved by a balance of stem cell regeneration with cosmic radiation degeneration. Our technique has a very high margin of safety and is coupled with equipment that is non-sophisticated, portable, lightweight, small footprint and can be used by all space personnel. Due to the simplicity of the technology, it is envisaged that only short-term training is necessary to achieve confidence and reproducibility by space personnel. The proposed stem cell methods provide an opportunity to maintain health in the space environment against the damaging effects of cosmic radiation.

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References

1. NASA https://www.nasa.gov/analogs/nsrl/why-space-radiation-matters
2. Maalouf M, Durante M, Foray N. Biological effects of space radiation on human cells: history, advances and outcomes. J Radiat Res. 2011; 52: 126-146.
3. Furukawa S, Nagamatsu A, Nenoi M, et al. Space radiation biology for living in space. BioMed Res Intern. 2020; 4703286.
4. Li Z, Jella KK, Jaafar L, et al. Exposure to galactic cosmic radiation compromises DNA repair and increases the potential for oncogenic chromosomal rearrangement in bronchial epithelial cells. Sci Reports. 2018; 8: 11038.
5. Yamanouchi S, Rhone, Mao J-H, et al. Simultaneous exposure of cultured human lymphoblastic cells to simulated microgravity and radiation increases chromosome aberrations. Life. 2020; 10: 187.
6. Giovanetti A, Tortolici F, Rufini S. Why do the cosmic rays induce aging? Front Physiol. 2020; 11: 955.
7. Britten RA, Jewell JS, Davis LK, et al. Changes in the hippocampal proteome associated with spatial memory impairment after exposure to low (20 cGy) doses of 1 GeV/n 56Fe radiation. Radiat Res. 2017; 187: 287-297.
8. https://www.arpansa.gov.au/understanding-radiation/what-is-radiation/ionising-radiation/gamma-radiation
9. Garrett-Bakelman FE, Darshi M, Green SJ, et al. The NASA twins study: a multidimensional analysis of a year-long human spaceflight. Science. 2019; 364: 6436.
10. SK Mehta, ML Laudenslager, RP Stowe, et al. Latent virus reactivation in astronauts on the international space station. NPJ Microgravity. 2017; 12: 11.
11. https://www.mayoclinic.org/diseases-conditions/radiation-sickness/symptoms-causes/syc-20377058
12. NASA https://www.nasa.gov/feature/goddard/2019/how-nasa-protects-astronauts-from-space-radiation-at-moon-mars-solar-cosmic-rays.
13. Li Y, Pham V, Bui M, et al. Rhodiola rosea L.: an herb with anti-stress, anti-aging, and immunostimulating properties for cancer chemoprevention. Curr Pharmacol Rep. 2017; 3: 384-395.
14. Welsh J, Bevelacqua J, Keshavarz M, et al. Is telomere length a biomarker of adaptive response? controversial findings of NASA and residents of high background radiation areas. J Biomed Phys Engineer. 2019; 9: 381-388.
15. Drapeau C. Cracking the Stem Cell Code. 2010, Sutton Heart Publishers. Portland, Oregon, USA.
16. Blaber E, Sato K, Almeida EA. Stem cell health and tissue regeneration in microgravity. Stem Cells and Dev. 2014; 23: 73-78.
17. Huang P, Russell AL, Lefavor R, et al. Feasibility, potency, and safety of growing human mesenchymal stem cells in space for clinical application. NPJ Micr. 2020; 6: 16.
18. https://www.washingtonpost.com/science/stem-cells-in-space/2020/12/04/8915f700-2471-11eb-a688-5298ad5d580a_story.html
19. Schäfer R, Spohn G, Bechtel M, et al. Human mesenchymal stromal cells are resistant to SARS-CoV-2 infection under steady-state, inflammatory conditions and in the presence of SARS-CoV-2-infected cells. Stem Cell Rep. 2020; 16: 419-427.
20. Musial C, Gorska-Ponikowska M. Medical progress: stem cells as a new therapeutic strategy for COVID-19. Stem Cell Res. 2021; 52: 102239.
21. Dessie G, Molla MD, Shibabaw T, et al. Role of stem-cell transplantation in leukemia treatment. Stem Cells Cloning. 2020; 13: 67-77.

22. Rahim F, Arjmand B, Tirdad R, et al. Stem cell therapy for multiple sclerosis. Cochrane Database of Syst Rev. 2018; CD013049.

23. Whittle SL, Johnston RV, McDonald S, et al. Stem cell injections for osteoarthritis of the knee. Cochrane Database Syst Rev. 2019; 5; CD013342.

24. Carstens M, Haq I, Martinez-Cerrato J, et al. Sustained clinical improvement of Parkinson's disease in two patients with facially transplanted adipose-derived stromal vascular fraction cells. J Clin Neurosci. 2020; 81: 47-51.

25. Vickers ER, Karsten E, Flood J, Lilischkis R. A preliminary report on stem cell therapy for neuropathic pain in humans. J Pain Res. 2014; 8: 255-263.

26. https://www.washingtonpost.com/national/health-science/miraculous-stem-cell-therapy-has-sickened-people-in-five-states/2019/02/26/c04b23a4-3539-11e9-854a-7a14d7fec96a_story.html?noredirect=on&noredirect=on

27. Moll G, Alm JJ, Davies LC, et al. Do cryopreserved mesenchymal stromal cells display impaired immunomodulatory and therapeutic properties? Stem Cells. 2014; 32: 2430-2442.

28. https://www.bbc.com/news/health-30194504

29. https://www.nytimes.com/2019/12/07/us/dna-bone-marrow-transplant-crime-lab.html

30. Blum B, Benvenisty N. The tumorigenicity of human embryonic stem cells. Adv Cancer Res. Academic Press. 2008; 100: 133-158.

31. Yamanaka S, Blau HM. Nuclear reprogramming to a pluripotent state by three approaches. Nature. 2010; 465: 704-712.

32. Kogut I, McCarthy SM, Pavlova M, et al. High-efficiency RNA-based reprogramming of human primary fibroblasts. Nat Commun. 2018; 9: 745.

33. Markey KA, Hill GR. Cytokines in hematopoietic stem cell transplantation, In: Foti M, Locati M. Eds., Cytokine Effector Functions in Tissues. 2017; Academic Press, Cambridge: 219-236.

34. Tang T, Hammond HK. Gene transfer for clinical congestive heart failure. In: Laurence J, Franklin M. Eds., Translating Gene Therapy for the Clinic: Techniques and Approaches. 2015; Academic Press, Cambridge.

35. Dempsey SG, Miller CH, Schueler J, et al. A novel chemotactic factor derived from the extracellular matrix protein decorin recruits mesenchymal stromal cells in vitro and in vivo. PloS One. 2020; 15.

36. Zhang W, Li X, Li H, et al. 17β-estradiol promotes bone marrow mesenchymal stem cell migration mediated by chemokine upregulation. Biochem Biophys Res Commun. 2020; 530: 381-388.

37. Eldridge SE, Barawi A, Wang H, et al. Agrin induces long-term osteochondral regeneration by supporting repair morphogenesis. Sci Transl Med. 2020; 2: 559.

38. Vickers ER. Peptidic alginate-based hydrogels demonstrate chemotaxis and expansion to adipose tissue derived and blood derived stem cells. J Biomater Nanobiotechnol. 2020; 11: 245-259.

39. Camilleri ET, Gustafson MP, Dudakovic A, et al. Identification and validation of multiple cell surface markers of clinical-grade adipose-derived mesenchymal stromal cells as novel release criteria for good manufacturing practice-compliant production. Stem Cell Res Ther. 2016; 7: 107.

40. Cortes M, Chen MJ, Stachura DL, et al. Developmental vitamin D availability impacts hematopoietic stem cell production. Cell Rep. 2016; 17: 458-468.

41. Yin Y, Chen P, Yu Q, et al. The effects of a pulsed electromagnetic field on the proliferation and osteogenic differentiation of human adipose-derived stem cells. Med Sci Monit. 2018; 24: 3274-3282.

42. Liu DD, Ullah M, Concepcion W, et al. The role of ultrasound in enhancing mesenchymal stromal cell-based therapies. Stem Cells Transl Med. 2020; 9: 850-866.

43. Fosen KM, Thom SR. Hyperbaric oxygen, vasculogenic stem cells, and wound healing. Antioxid Redox Signal. 2014; 21: 1634-1647.

44. Zhang S, Shan L, Li Q, et al. Systematic analysis of the multiple bioactivities of green tea through a network pharmacology approach. Evid Based Complement Alternat Med. 2014; Article ID 512081.

45. Gupta P. A review on Rhodiola rosea L.: an herb with anti-stress, anti-oxidant and anti-carcinogenic properties. J Med Plants. 2019; 7: 278-281.

46. Suntar I. Rhodiola (Rhodiola rosea L.). In: Nonvitamin and Nonmineral Nutritional Supplements, Academic Press. 2019; 1: 383-400.

47. Ganz T. Defensins: antimicrobial peptides of innate immunity. Nat Rev Immunol. 2003; 3: 710-720.

48. Goebel C, Mackay LG, Vickers ER, et al. Determination of defensin HNP-1, HNP-2, and HNP-3 in human saliva by using LC/MS. Peptides. 2000; 21: 757-765.

49. Fricker LD, Margolis E, Gomes I, et al. Five decades of research on opioid peptides: current knowledge and unanswered questions. Mol Pharmacol. 2020; 98: 96-108.

50. Beederman M, Lamplot JD, Nan G, et al. BMP signaling in mesenchymal stem cell differentiation and bone formation. J Biomed Sci Engineer. 2013; 6: 32-52.

51. Handorf AM, Li WJ. Fibroblast growth factor-2 primes human mesenchymal stem cells for enhanced chondrogenesis. PLoS One. 2011; 6: e22887.

52. Gandolfi MG, Gardin C, Zamparini F, et al. Mineral-doped poly(l-lactide) acid scaffolds enriched with exosomes improve osteogenic commitment of human adipose-derived mesenchymal stem cells. Nanomater. 2020; 432: 1-20.

53. Wang C, Huang W, Zhou Y, et al. 3D printing of bone tissue engineering scaffolds. Bioact Mater. 2020; 5: 82-91.

54. Shparberg RA, Braunsteiner N, Vickers ER. Laboratory training guidelines for clinicians undertaking stem cell therapy. J Stem Cell Res Ther. 2018; 8: 1-10.
55. https://www.bcm.edu/academic-centers/space-medicine/translational-research-institute/funding
56. Jönsson KI. Radiation tolerance in tardigrades: current knowledge and potential applications in medicine. Cancers (Basel). 2019; 11: 1333.
57. https://www.sun-sentinel.com/health/fl-stem-cell-injections-court-complaint-20170322-story.html
58. https://www.explainingthefuture.com/helium3.html
59. http://css.umich.edu/factsheets/us-energy-system-factsheet
60. https://www.bloomberg.com/graphics/2018-asteroid-mining/
61. https://space.nss.org/settlement/nasa/spaceresvol4/spacelaw.html
62. https://www.cnbc.com/2018/02/22/medical-errors-third-leading-cause-of-death-in-america.html
63. https://www.weforum.org/agenda/2017/03/workplace-death-health-safety-ilo-fluor/
64. https://www.bbc.com/news/world-latin-america-11533349
65. https://isulibrary.isunet.edu/doc_num.php?explnum_id=73