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Cardiovascular Disease and Adult Healing Cells: From Bench Top to Bedside

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ABSTRACT

Cardiovascular disease, especially ischemic heart disease resulting from coronary artery disease (CAD), is one of the major causes of death and disability in the United States. Even though the dead myocardial cells can be replaced by scar tissue in the healing process, the resulting myocardium cannot function as well as the pre-infarcted myocardium, because scar tissues cannot contract. This "normal" healing process results in decreased cardiac output, which can lead to heart failure. Moreover, the scar tissue has abnormal electrical properties, which can lead to sometimes fatal arrhythmias. Previous studies demonstrated that when Lac-Z-labeled healing cells were infused into two animal models of myocardial infarction, that these cells were found to be located within the myocardium, the cardiac skeleton, and the vasculature undergoing repair. These results suggested that healing cells have the potential to repair damaged hearts. The current series of studies were undertaken to determine whether healing cells customarily reside in normal non-injured hearts of small and large animals, and whether autologous healing cells could be infused safely into a post-myocardial infarction patient. Adult rats were euthanized following the guidelines of Mercer University's IACUC. Adult pigs were euthanized following the guidelines of Fort Valley State University's IACUC. The human study was performed under the guidance of the Medical Center of Central Georgia's IRB. Animal hearts were harvested, fixed, cryosectioned, and stained with three antibodies: carcinoembryonic antigen-cell adhesion molecule-1 (CEA-CAM-1) for totipotent stem cells, stage-specific embryonic antigen-4 (SSEA-4) for pluripotent stem cells, and smooth muscle alpha-actin (IA4) for smooth muscle in the wall of the accompanying vasculature, thus serving as the positive procedural control. Cells positive for both CEA-CAM-1 and SSEA-4 were found to be located in adult rat and porcine hearts. Infusion of autologous healing cells into a post-myocardial infarcted patient resulted in an increase in their cardiac output after two successive healing cell infusions. Current IRB-approved studies are underway to assess the safety and efficacy of infused healing cells into individuals with cardiovascular disease.

Keywords
Healing cells, Stem cells, Totipotent stem cells, Pluripotent stem cells, SSEA, CEA-CAM-1, Adult rat, Adult pig, Porcine, Human, Heart, Myocardium, Epicardium, Cardiovascular disease, Myocardial infarction, Immunocytochemistry, ELICA-fixative.

Introduction
Cardiovascular disease, especially ischemic heart disease resulting from coronary artery disease (CAD), is one of the major causes of death and disability in the United States [1-4]. Coronary Artery Bypass Graft (CABG) and stenting represent current attempts to prevent the steadily rising number of deaths that result from acute myocardial infarctions (MIs) [5]. These therapies, however, present significant risks of their own. CABG can lead to rupture or required reoperation due to anastomotic stenosis. These complications raise the risk of this therapeutic approach. In addition, stent thrombosis can produce a cerebrovascular accident, leading to severe disability and mortality. Thus, these surgical therapies pose many risks, rendering them less than ideal as a therapeutic approach to the treatment of CAD [6].

Following a myocardial infarction (MI), the myocardium is often replaced with non-functional scar tissue [7]. Such scarring and damage to the myocardium often results in left ventricular systolic dysfunction, ventricular aneurysm, decreased cardiac output, and heart failure [8-10]. Thus, mortality and morbidity following a MI remain high despite current pharmacological treatments including treatment with Angiotensin Converting Enzyme Inhibitors (ACE-I) and/or Angiotensin-Receptor Blockers (ARBs) [9].

Since stem cells can form the functional components of heart tissue and cardiac blood vessels [11,12], they could also form the basis for therapeutic approaches that avoid the disastrous complications of CABG and stenting. The use of stem cells obtained from the human embryo is very controversial, for moral and ethical reasons. Moreover, the use of embryonic stem cells requires immunosuppressive therapy to avoid problems associated with HLA-mismatch and tissue rejection. Such immunosuppressive therapy can itself be a source of increased morbidity and mortality [5]. Stem cell therapy using autologous adult stem cells in place of embryonic stem cells would not only eliminate the moral and ethical controversies, but also avoid the risks of immunosuppressive therapy.

Previous studies noted that both totipotent and pluripotent healing cells were resident cell populations in the connective tissue stroma of skeletal muscle [13], blood [14,15], bone marrow [16], adipose tissue [17], dermis [17], pancreas [18], and lung [19] of adult mammals. Therefore, we hypothesized that similar healing cells may be present in adult rat and porcine hearts. The first portion of this study, utilizing immunocytochemical staining of sectioned tissues, was undertaken to address this hypothesis. The results demonstrated that both totipotent and pluripotent healing cells were located in adult rat and porcine hearts.

The second portion of this study was based on previous animal studies of the infusion of genomically-labeled healing cells into hearts of adult rat damaged to mimic myocardial infarction. These studies demonstrated the presence of Lac-Z-labeled cells within the myocardium, connective tissue cardiac skeleton, and vasculature tissues undergoing regeneration/repair [11,12]. Therefore, based on location studies in adult mammals and the animal models of myocardial infarction, we hypothesized that autologous healing cells isolated from the blood could be safely infused into a post-myocardial infarcted patient.

Subsequent analysis of cardiac output in this patient demonstrated both safety and efficacy with this technique as well. Current IRB-approved clinical studies with a larger cohort of test subjects are underway to assess the safety and efficacy of infused healing cells into individuals with cardiovascular disease. The discovery and use of these totipotent and pluripotent healing cells could provide an important initial step toward the ultimate goal of successful and safe stem cell therapy for the treatment of cardiovascular disease.

Material and Methods
Animal Studies
The use of animals in this study complied with the guidelines of Mercer University's Institutional Animal Care and Use Committee (IACUC) and Fort Valley University's IACUC. These guidelines reflect the criteria for humane animal care of the National Research Council as outlined in "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (National Academy Press, 1996).

Human Studies
The use of adult humans reported herein complied with the guidelines of the Medical Center of Central Georgia Institutional Review Board. All individuals signed a consent form before participating in this study.

Animal Study
Tissue Harvest – Rat
Postnatal Sprague-Dawley rats (n=10) were euthanized using inhalation of carbon dioxide. The anterior chest wall and abdomen were washed with Betadine solution and incised. Hearts were removed, incised through both right and left ventricles in order to divide the heart into upper and lower portions, and blood removed from the chambers with Dulbecco's Phosphate Buffered Saline (Dulbecco's Phosphate Buffered Saline (DPBS, Invitrogen, GIBCO, Grand Island, NY). The hearts were then placed into ELICA fixative.

Tissue Harvest – Pig
Twenty adult 120 lb. female Yorkshire pigs (n=20) were anesthetized with tiletamine and zolazepam, then prepared for surgery with a Betadine wash and draped in a sterile fashion. Following a mid-line laparotomy incision, the sternum was divided and ribs retracted. The pericardial sac was incised, the heart separated from the great vessels by a transverse incision and the heart removed into ice-cold DPBS. The heart was divided into upper and lower portions,

and blood removed from the chambers with DPBS. The hearts were divided into pieces containing endocardium, myocardium, and epicardium including the overlying coronary vasculature, divided into approximately one-inch pieces, and placed into ELICA fixative.

Further Tissue Processing

The ELICA fixative consisted of aqueous 0.4% v/v glutaraldehyde, 2% w/v paraformaldehyde, and 1% w/v glucose, Ph 7.4, with an osmolality 1.0 [13]. Pieces of rat and pig heart tissue were allowed to remain in the fixative for 1 to 24 weeks at ambient temperature. After fixation, the pieces of heart tissue were transferred and stored in Dulbecco's Phosphate Buffered Saline (DPBS, Invitrogen, GIBCO, Grand Island, NY) at pH 7.4 at ambient temperature. Pieces of heart tissue were removed, placed into Tissue Tek OCT Compound 4583 (Miles Laboratory, Ames Division, Elkhart, IN) and then frozen at -20°C. The frozen pieces were individually cryostat sectioned at seven microns in thickness with a Tissue Tek Cryostat II (GMI, Ramsey, MN), placed on positively charged slides (Mercedes Medical, Sarasota, FL) and refrigerated at -20°C. Immunocytochemical staining was performed following established procedures for ELICA analysis [13,20].

Immunocytochemistry

The tissue sections were incubated with 95% ethanol to remove the OTC cryostat embedding medium and then washed under running water for five minutes. The tissue sections were incubated with 5.0% (w/v) sodium azide (Sigma, St. Louis, MO) in DPBS for 60 minutes [13]. They were then washed in running water for five minutes, and incubated with 30% hydrogen peroxide (Sigma, St. Louis, MO) for 60 minutes to irreversibly inhibit endogenous peroxidases [13,20]. Tissue sections were rinsed with running water for five minutes and incubated for 60 minutes with blocking agent (Vectastain ABC Reagent Kit, Vector Laboratories Inc., Burlingame, CA) in DPBS [13]. The blocking agent was removed and the sections rinsed with running water for five minutes. They were then incubated with primary antibody for 60 minutes. The primary antibodies consisted of 0.005% (v/v) carcinoembryonic antigen cell adhesion molecule-1 (CEA-CAM-1) in DPBS for totipotent stem cells [21]; 1 µg per ml of stage-specific embryonic antigen-4 for pluripotent stem cells (SSEA-4, Developmental Studies Hybridoma Bank, Iowa City, IA) in DPBS [11]; and smooth muscle alpha-actin (IA4, Developmental Studies Hybridoma Bank) in DPBS [13]. The primary antibody was removed. The sections were rinsed with running water for five minutes, and incubated with secondary antibody for 60 minutes. The secondary antibody consisted of 0.005% (v/v) biotinylated affinity purified, rat adsorbed anti-mouse immunoglobulin G (H+L) (BA-2001, Vector Laboratories) in DPBS [13]. The secondary antibody was removed. The sections were rinsed with running water for five minutes, and then incubated with avidin-HRP for 60 minutes.

The avidin-HRP consisted of 10 ml of 0.1% (v/v) Tween-20 (ChemPure, Curtain Matheson Scientific, Houston, TX) containing 2 drops reagent-A and 2 drops reagent-B (Peroxidase Standard PK-4000 Vectastain ABC Reagent Kit, Vector Laboratories) in DPBS [13]. The avidin-HRP was removed. The sections were rinsed with running water for five minutes, and incubated with either AEC or DAB substrate (Sigma) for 60 minutes. The AEC and DAB substrates were prepared as directed by the manufacturer. The substrate solution was removed. The sections were rinsed with running water for 10 minutes and then cover-slipped with Aqua-mount (Vector Laboratories) [13].

Positive and negative controls were included to assure the validity of the immunocytochemical staining [13]. The positive controls consisted of adult-derived totipotent stem cells (positive for CEA-CAM-1) [6,15], pluripotent stem cells (positive for SSEA-4) [5,23], and smooth muscle surrounding blood vessels within the tissue (positive for IA4) [15]. The negative controls consisted of the staining protocol with DPBS alone (no antibodies or substrate), without primary antibodies (CEA-CAM-1, SSEA-4, or IA4), without secondary antibody (biotinylated anti-mouse IgG), without avidin-HRP, and without substrate (AEC) [15].

Visual Analysis

Stained sections were visualized using a Nikon TMS phase contrast microscope with bright field microscopy at 40x, 100x, and 200x. Photographs were taken with a Nikon CoolPix 995 digital camera.

Human Study

Endogenous adult healing cells were isolated from blood from one male (n=1) who was diagnosed with a six-year history of post-myocardial infarction with a cardiac output of 25%. In brief, adult human blood was obtained by venipuncture following standard acceptable medical practice. The blood was collected using sterile procedures and placed in 10-ml EDTA hemovac tubes (Beckton-Dickinson), inverted several times to mix and then refrigerated at 4°C for 48 hours until further processing to isolate endogenous stem cells within the peripheral blood plasma fraction [14,15].

Stem Cell Isolation

After 48 hours of gravity separation, the blood had separated into a floating plasma fraction and a sedimented cellular fraction. The cellular fraction contained hematopoietic stem cells, red blood cells, white blood cells, and most mesodermal stem cells [14,15]. The plasma fraction was withdrawn using a sterile pipette, placed in a second sterile tube and refrigerated at 4°C.

Stem Cell Identification

Totipotent stem cells are Trypan blue-positive very small spherical-shaped cells that are less than 2.0 microns in size [14,15,21-23]. Transitional-totipotent stem cell/pluripotent stem cells display a peripheral rim that stains with Trypan blue and a central core that does not. They are more than 2.0 but less than 6.0 microns in size [14,15]. Pluripotent stem cells do not stain with Trypan blue and are 6.0 to 8.0 microns in size [5,13]. Germ layer lineage stem cells do not stain with Trypan blue and are more than 8.0 but less than 20.0 microns in size [22,23].

Treatment

The healing cells were segregated into two populations: totipotent

stem cells in the first group and pluripotent stem cells and mesodermal stem cells in the second group. Both groups were diluted in sterile 0.9% normal intravenous saline (SNS) and processed for intravenous infusion as a systemic delivery system to the heart. The totipotent stem cells were diluted in 1000-ml of SNS and infused over the course of two hours. The pluripotent stem cells and mesodermal stem cells were diluted in 250-ml of SNS and infused over the course of 30 min. The cardiac output was measured to assess heart function. This had been performed by the patient's cardiologist bi-annually beginning with his myocardial infarction six years previously.

Results

Adult Rat

Two populations of stem cells were visualized in the heart. SSEA-4-positive cells were located in the visceral layer of serous pericardium (epicardium) of the heart, and thus outside of the heart (Figure 1A), whereas CEA-CAM-1-positive cells were located in the endomysial and perimysial connective tissues of the myocardium inside of the heart (Figure 1B). IA4-positive cells containing smooth muscle alpha-actin were located in the tunica media of blood vessels within the heart (Figure 1C), whereas negative controls demonstrated absence of any immunocytochemical staining (Figure 1D).

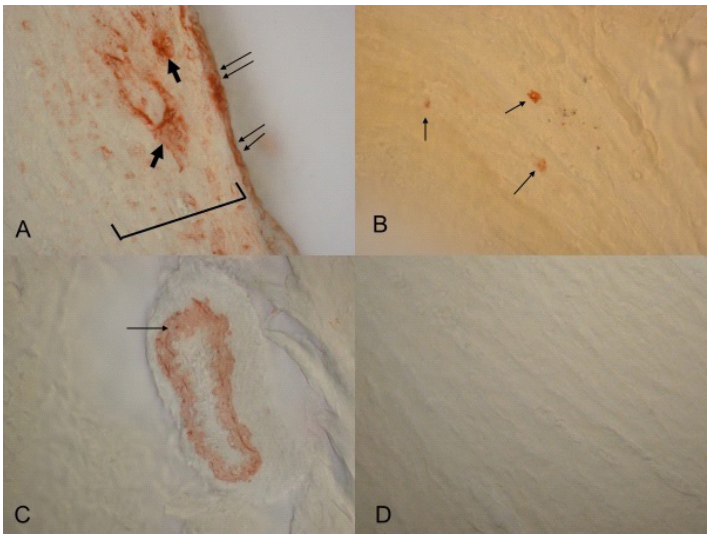

Figure 1: Cryosectioned and immunocytochemically stained (AEC) left ventricle of adult rat heart.

A: SSEA-4-positive cells (red) located within the epicardial connective tissues (double arrows) and within the outer portion of the myocardium (bracket). Intense areas of SSEA-4-positive cells are located surrounding blood vessels (bold arrows) along the outermost portion of the myocardium. Mag x100.
B: CEA-CAM-1-positive clusters of cells (red) within the inner portion of the myocardium (single arrows). Mag x100.
C: IA4-positive smooth muscle alpha-actin (red) within the tunica media of a coronary blood vessel within the outer portion of the myocardium (single arrow). Mag x100.
D: Absence of any positive staining, the negative procedural control. Mag x100.

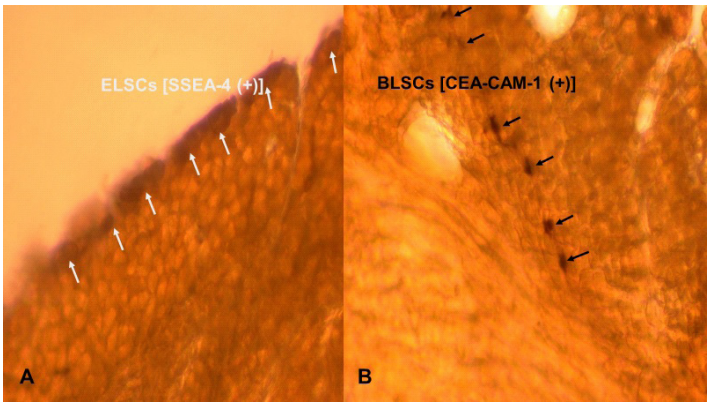

Figure 2: Cryosectioned and immunocytochemically stained (DAB) right ventricle of adult rat heart.

A: SSEA-positive cells (dark cells, white arrows) along outer border of epicardium. Mag x200.
B: CEA-CAM-1-positive clusters of cells (dark cell clusters, black arrows) along interface at junction of epicardium and myocardium. Mag x200.

Adult Pig

Pluripotent stem cells positive for stage-specific antigen-4 (SSEA-4) and totipotent stem cells positive for carcinoembryonic antigen-cell adhesion molecule-1 (CEA-CAM-1) were identified within the intramural myocardium of the adult porcine heart at some distance from the epicardium of the heart. Clusters of cells positive for CEA-CAM-1 were noted next to longitudinal cardiac myofibers (Figure 3A) and also within the connective tissues between cardiac myofibers (Figure 3B). Groups of cells positive for SSEA-4 were identified within the connective tissues between cardiac myofibers (Figure 3C and 3D). In addition, cells positive for CEA-CAM-1 and SSEA-4 were also located within the connective tissue layers (tunica intima and tunica adventitia) of the intramural coronary blood vessels (Figure 3A-D).

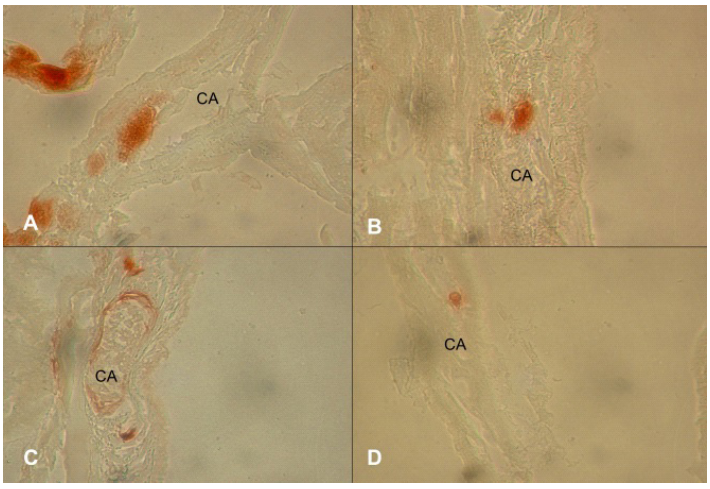

Figure 3: Cryosectioned and immunocytochemically stained (AEC) porcine septal coronary arteries.

A: Clusters of cells positive for CEA-CAM-1 were noted next to longitudinal cardiac myofibers. Mag, x100.

B: Clusters of cells positive for CEA-CAM-1 were noted within the connective tissue stroma between cardiac myofibers. Mag, x100.
C: Groups of cells positive for SSEA-4 were identified within the connective tissues between cardiac myofibers and within the wall structure of a coronary artery. Mag, x100.
D: Groups of cells positive for SSEA-4 were identified within the lumen of a coronary artery. Mag, x100.

An unexpected and apparently spontaneous repair of cardiac myofibers was observed within several of the adult porcine hearts. This apparent repair was located within cardiac myofibers of the interventricular septum of the adult porcine heart surrounding the left anterior descending (LAD) coronary artery. Clusters of CEA-CAM-1 positive cells and SSEA-4-positive cells were located surrounding the left anterior descending (LAD) coronary artery (Figure 4A-D). However, no CEA-CAM-1-positive or SSEA-4-positive cells were located within the lumen of the LAD or within the wall structure of the LAD (Figure 4A-C).

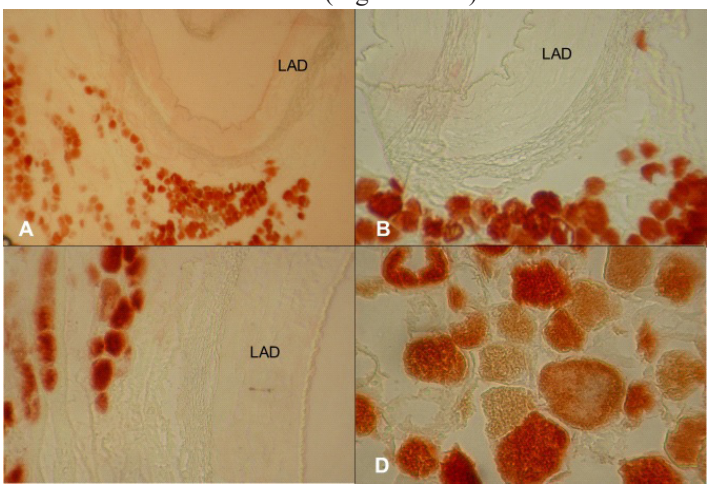

Figure 4: Cryosectioned and immunocytochemically stained (AEC) of spontaneous regeneration of damaged adult porcine heart muscle directly inferior to left anterior descending coronary artery (LAD).

A: Clusters of CEA-CAM-1-positive cells located inferior to LAD. Mag, x100
B: Clusters of CEA-CAM-1-positive cells located inferior to LAD. Mag, x200
C: Clusters of SSEA-4-positive cells located inferior to LAD. Mag, x200
D: Higher power image of clusters of SSEA-4-positive cells located inferior to LAD. Mag, x400

Adult Human

Following a diagnosed myocardial infarction, the single patient in this study had a steady 25% cardiac output of six years duration (Figure 4). His first healing cell infusion consisted of a slow infusion with autologous totipotent stem cells followed a quicker infusion of pluripotent stem cells and mesodermal stem cells. Six months following his first cell infusion his cardiac output rose to 35% (Figure 4), a 40% increase in cardiac output. He was transfused with autologous healing cells a second time following the same protocol as his first infusion, i.e., slow infusion with totipotent stem cells followed by a quicker infusion with pluripotent stem cells and mesodermal stem cells. Six months following his second cell infusion his cardiac output had risen to 45% (Figure 5), an additional increase of approximately 28%.

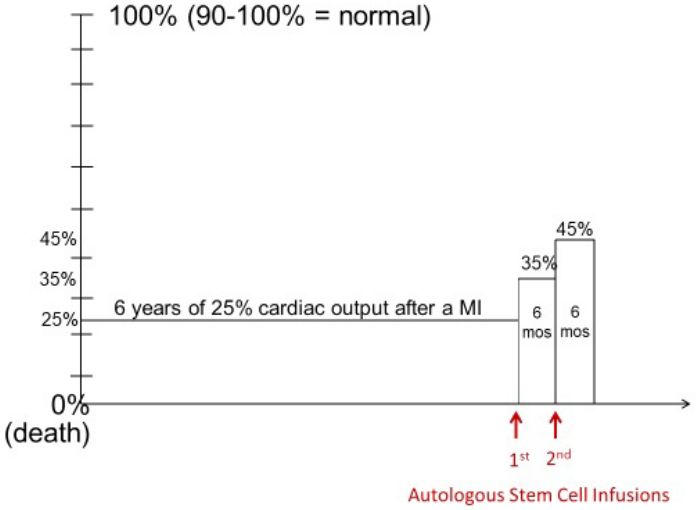

Figure 5: Histogram of cardiac output in a patient (n=1) suffering from a myocardial infarction of six years duration before initial healing cell transplant. From the time of myocardial infarction to first autologous stem cell infusion his cardiac output was 25%, measured bi-annually by his cardiologist. At his bi-annual check-up six months after his first stem cell transplant his cardiac output had risen to 35%. He was transplanted a second time. Six months following his second transplant, at his bi-annual check-up, his cardiac output had risen to 45%.

Discussion

Cardiac disease and its consequences

For decades, the broad class of cardiovascular diseases (CVD) has been the leading cause of mortality worldwide, responsible for 30% of all deaths (about 17 million annually). CVD is responsible for more than 7.5 million inpatient cardiovascular (CV) procedures in the US. CVD places a significant economic burden on patients and health care systems. In 2010, the direct medical costs of CVD totaled US $272 billion in the US alone (2008 USD). In the European Union (EU), direct medical costs of CVD are estimated to reach €106 billion annually. The health care burden of CVD continues to grow with an aging population as well as the contribution of clinical risk factors, such as obesity, high-lipid levels, and consequent atherosclerosis and hypertension [3]. Heart disease, including myocardial infarction, represents an enormous burden on the health care systems of the developed world. However, the incidence of increase of myocardial infarction and other cardiac diseases is also rising sharply in the less well-developed areas of the third world [1,2].

Myocardial infarction and the consequent loss of fully functional myocardium is a major factor in the etiology of heart failure [24]. Heart failure is a common, disabling, and lethal condition [4,25]. The prognosis of patients with heart failure is poor, with a failure rate approaching 50%. The loss of cardiac tissue underlies heart

failure, but current treatments do not address this problem.

Once the myocardium dies in a myocardial infarction, it is replaced by scar tissue over several weeks. The size, location, composition, structure and mechanical properties of the healing scar are all critical determinants of the fate of patients who survive the initial infarction [7]. An additional complication is that scar tissue can disrupt the electrical properties of the myocardium, predisposing the patient to arrhythmias, which can prove to be fatal. Ventricular arrhythmias are particular likely to require cardioversion in order to prevent fatal events [10]. Such scarring and damage to the myocardium often results in left ventricular systolic dysfunction, ventricular aneurysm, and heart failure [8-10]. Thus, mortality and morbidity following myocardial infarction remain high despite current pharmacological treatments including treatment with Angiotensin Converting Enzyme Inhibitors (ACE-I) and/or Angiotensin-Receptor Blockers (ARBs) [9].

Functional Cells, Healing Cells, and Maintenance Cells

There are three basic categories of cells within animals, i.e., functional cells, maintenance cells, and healing cells. The functional cells comprise the majority of the cell types and are composed of both stroma and parenchyma [26]. A few examples are of functional cells are adipocytes, fibrocytes, and cardiac myocytes. Maintenance cells support the functional cells on a daily basis by replacing functional cells as they wear out and die as well as providing trophic factors for their function and survival. Examples of maintenance cells include adipoblasts, fibroblasts, myoblasts, mesenchymal stem cells, medicinal secreting cells, and progenitor cells [26-31].

Healing cells are normally dormant and can be found hibernating within the stromal connective tissues of the body [16,32]. Their function in replacing functional cells and maintenance cells that have been lost due to trauma and/or disease. Examples of healing cells are totipotent stem cells [16,21,26,31], pluripotent stem cells [11,14,26,31,33], ectodermal stem cells [26,31], mesodermal stem cells [26,31,34], and endodermal stem cells [26,31]. Healing cells comprise approximately 10% of all the cells of the body and are ubiquitous throughout all organs and tissues of the body. More specifically, totipotent stem cells comprise approximately 0.1%, pluripotent stem cells approximately 0.9%, and the ectodermal stem cells, mesodermal stem cells, and endodermal stem cells approximately 9% of all cells of the body [12,26,31].

Previous studies by Young and colleagues demonstrated the isolation, repetitive single cell serial dilution clonogenic analysis, characterization, and genomic labelling with Lac-Z (beta-Galactosidase) of 58 clones of rat healing cells. These clones consisted of totipotent stem cells, transitional-totipotent stem cell/pluripotent stem cells, pluripotent stem cells, germ layer lineage stem cells, ectodermal stem cells, mesodermal stem cells, and endodermal stem cells [11,12,21]. Totipotent stem cells have the capability of forming all cell types of the body, including the germ cells, sperm and ova [21]. Pluripotent stem cells (i.e., cells designated as transitional-totipotent stem cell/pluripotent stem cells, pluripotent stem cells, and germ layer lineage stem cells) can form any cell type of the body, but cannot form the germ cells, sperm and ova [11,12]. Ectodermal stem cells can form any cell type of the embryonic ectodermal germ layer lineage [12,35]. Mesodermal stem cells can form any cell type of the embryonic mesodermal germ layer lineage [12,35]. And endodermal stem cells can form any cell type of the embryonic endodermal stem cell lineage [12,35].

Subsequent studies, utilizing a transitional-totipotent stem cell/pluripotent stem cell clone, designated as Scl-40β, and a totipotent stem cell clone, designated as Scl-44β, were utilized to encase allogeneic islets to form a pancreatic islet organoid as a proposed treatment modality for Type-I diabetes [18]. Scl-40β also demonstrated its ability to incorporate into and replace damaged cells and tissues in a Parkinson's disease model [36,37] and in two animal models of myocardial infarction [11,12]. One myocardial infarction model consisted of freezing the apex of the heart, while the other model consisted of transiently ligating the left anterior descending coronary artery. In both instances, genomically-labeled cells could be found in the regenerating myocardium, regenerating cardiac skeleton, and regenerating vasculature [11,12].

The genomically-labeled clones of healing cells were derived from the connective tissues of skeletal muscle from outbred Sprague-Dawley. Stout et al. [38] demonstrated that with trauma, connective tissue resident healing cells within the skeletal muscle would migrate into the vasculature and home to the site of damage, whether that site was skeletal muscle or not. These results suggested the possibility that similar types of stem cells resident in one tissue would affect repair in a second dissimilar tissue.

These results also suggested that similar types of healing cells, i.e., totipotent stem cells, pluripotent stem cells, were located in various tissues and organs throughout the body. Young and colleagues have been addressing that issue with normal uninjured adult rat tissues and organs utilizing cryosectioning and staining with antibodies unique for totipotent stem cells and pluripotent stem cells. Thus far, they have noted that totipotent healing cells and pluripotent healing cells exist within skeletal muscle [13], blood [14,15], bone marrow [16], adipose tissue [17], dermis [17], pancreas [18], and lung [19] of adult animals. Due to the lack of ability of the heart to heal itself, the current study addressed whether healing totipotent cells and pluripotent cells were natural residents within the heart.

Young et. al. have previously reported the presence of primitive pluripotent epiblast-like stem cells (positive for SSEA-4) and primitive totipotent blastomere-like stem cells (positive for CEA-CAM-1) in a variety of species of post-natal mammals [13-19]. Recent research by Stout et al. [38] in the adult pig has demonstrated that primitive pluripotent stem cells exist not only in the peripheral blood but also as a resident population of stem cells located within the skeletal muscle. They also demonstrated an increase in the number of primitive pluripotent stem cells passing into the peripheral blood from the skeletal muscle after only 90 minutes of trauma. Since native pluripotent stem cells are

located both in the blood and in the skeletal muscle of adult pigs [38], we hypothesized that similar native primitive pluripotent stem cells may be present in the adult porcine heart where they may contribute in some fashion to myocardial repair. The results demonstrate that populations of cells positive for CEA-CAM-1 or SSEA-4 were located within the connective tissues (endomysium and perimysium) between the intramural myocardial fibers as well as within the connective tissue layers (tunica intima and tunica adventitia) of the intramural coronary vessels. Their presence within the intramural tissues of the adult porcine heart suggests a role in the normal maintenance and repair of damaged or senescent tissues. The possibility exists that these endogenous primitive pluripotent stem cells could assist in the repair of damaged myocardium following a myocardial infarction, although further research will be required to examine this hypothesis.

Regeneration of the patient's own blood vessels could permit the replacement of the patient's own stenotic coronary artery with an autologous clone of the artery. Such advanced regenerative therapy could eliminate some of the risks associated with current therapeutic approaches to the treatment of coronary artery disease and myocardial infarction. The use of autologous adult stem cells provides promise in the treatment of patients who have suffered a myocardial infarction, as well as those in the earlier stages of CAD.

Some reports concerning the use of stem cells to attempt to repair the myocardium have appeared [25,40,41]. This work is in its infancy, and often has revolved around the use of mesenchymal stem cells. There is data indicating that more primitive cells such as very small, embryonic-like (VSEL) stem cells are more effective in the treatment of lesions than the more differentiated mesenchymal stem cells, at least in the lung [42]. The issue of which cells are most effective in healing the tissues of the body such as the myocardium, will certainly require much further study.

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