Human Umbilical Cord Blood for Transplantation Therapy in Myocardial Infarction

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Abstract

Cell-based therapy is a promising therapy for myocardial infarction. Endogenous repair of the heart muscle after myocardial infarction is a challenge because adult cardiomyocytes have a limited capacity to proliferate and replace damaged cells. Pre-clinical and clinical evidence has shown that cell based therapy may promote revascularization and replacement of damaged myocytes after myocardial infarction. Adult stem cells can be harvested from different sources including bone marrow, skeletal myoblast, and human umbilical cord blood cells. The use of these cells for the repair of myocardial infarction presents various advantages over other sources of stem cells. Among these are easy harvesting, unlimited differentiation capability, and robust angiogenic potential. In this review, we discuss the milestone findings and the most recent evidence demonstrating the therapeutic efficacy and safety of the transplantation of human umbilical cord blood cells as a stand-alone therapy or in combination with gene therapy, highlighting the importance of optimizing the timing, dose and delivery methods, and a better understanding of the mechanisms of action that will guide the clinical entry of this innovative treatment for ischemic disorders, specifically myocardial infarction.

Keywords: Myocardial infarction; Cardiomyocytes; Umbilical cord blood; Angiogenesis; Gene therapy

Abbreviations: MI: Myocardial Infarction; SC: Stem Cells; MSCs: Mesenchymal Stem Cells; EPCs: Endothelial Progenitor Cells; HUCB: Human Umbilical Cord Blood; VSELs: Very Small Embryonic-like Stem Cells; MYHC: Myosin Ventricular Heavy Chain; VEGF: Vascular Endothelial Growth Factor; TF-α: Tumor Necrosis Factor-α; MCP-1: Monocyte/macrophage Chemotactic Protein; IMP: Myocyte Inflammatory Protein; INF-gamma: Interferon-gamma; BMSC: Bone Marrow Mesenchymal SCs; SH: Silk Fibroin/hyaluronic Acid; IV: Intravenous; IC: Intracoronary; USSCs: Unrestricted Human Somatic Stem Cells; LV: Left Ventricular; FS: Fractional Shortening; RWMS: Regional Wall Motion Score; LVEDP: Left Ventricular End Diastolic Pressure; CMCs: Cardiomyocytes; SFD-1: Stromal Cell Derived Factor-1; LVEF: Left Ventricular Ejection Fraction; AAVs: Adeno Associated viral Vectors; 3D: Three-Dimensional; BDNF: Brain Derived Neurotrophic Factor

Introduction

Myocardial infarction (MI) remains one of the leading causes of death. The resulting heart failure from MI is preceded by a pathological cascade of events including the irreversible loss of myocytes, scarring of the myocardial tissue, expansion of the infarct area, concentric hypertrophy, and left ventricular dilation [1,2].

The repair of damaged cardiac tissue or vascular tissue may be achieved along with improved myocardial function [3,4]. However, there is still a gap in clinical therapies for MI. While there are native cardiac cells in the heart, their population levels remain too small to make a therapeutic difference [5-7]. Transplantation for MI was first suggested in 1994 [8]. Although recent studies have indicated that injection of bone marrow mononuclear cells aids in cardiac remodeling and guard against fibrosis [9], additional optimization laboratory studies are warranted prior to initiating large-scale clinical trials of transplantation therapy for MI. The use of adult stem cell (SC) for transplantation therapy has been demonstrated to afford benefits in MI [10]. Accumulating preclinical evidence of safety and efficacy of SC therapy for MI, and the entry of SC therapy to the clinic, provided the impetus for us to update a review of the field [11].

Various types of cells have been discussed and tested as a potential therapy for the repair of damaged myocardium. Hematopoietic progenitor cells have been shown to reduce apoptosis [12,13]. Human amniotic epithelial cells have been demonstrated to differentiate in cardiomyocyte-like cells following transplantation [14]. Mesenchymal stem cells (MSCs) [15-19], skeletal muscle cells [20], skeletal myoblasts [21-24], endothelial precursor cells [25] cardiac progenitor cells [26], and resident cardiac stem cells [27] have been documented to enhance cardiac function and endothelial progenitor cells (EPCs) are being studied for the same result [28]. However, there is disagreement over the optimal cell graft for clinical application. Cultured MSCs from aging bone marrow display a lack of self-renewal, proliferation,
adhesion, and integration into vascular tissue when transplanted to a damaged heart [29-31].

Autologous transplantation is currently a topic of much interest, as this therapy circumvents graft-host immune disease. However, this method is not advantageous in aging and chronically ill populations, who are functional SCs are reduced, limiting any recovery or reparative ability of damaged tissue [29-31].

The limitations of various cells, including bone marrow derived MSCs, prompts exploration of more suitable SC donor sources for transplantation in MI. Human umbilical cord blood (HUCB) cells may overcome these limitations with favorable reparative outcomes, particularly in the aged population where autologous cells are not as beneficial [32-36]. Their supply is much larger than that of the autologous cells, as HUCB cells are present in the blood of umbilical cord, which are in ample supply and can be easily harvested; they can also self-renew, proliferate, and differentiate into varying lineages. Furthermore, HUCB remain viable even after long periods of cryopreservation [13,15,16,21,25]. The risk of losing protein signaling and damaging other protein is minimal in HUCB cells [4,13,15,16,21,25,27-37].

The survival of transplanted HUCB and their differentiation into myocytes or endothelial cells appear necessary, at least acutely, to promote left ventricular remodeling [38-53]. However, the extent and stability of efficacy of HUCB cells for repair of MI require more preclinical investigations, along with the need to elucidate the mechanism through which the cells contribute to myocardial repair [3,54]. Table 1 reviews the literature by dosage and delivery route. Optimizing the HUCB cells transplantation regimen for the amelioration and repair of the failing heart post-MI is a key translational research goal for this evolving area of research. Additionally, this update serves as an evaluation of the mechanisms of action mediating the therapeutic benefits of HUCB cells in MI may reveal insights on the reparative capacity of the cells.

Benefits of Utilizing HUCB

HUCB cells have several properties that make them advantageous for cell transplantation therapies over other sources. Unlike bone marrow and embryonic derived SCs, harvesting HUCB cells is non-invasive and does not put the mother or the infant at risk [55,56]. These cells can be cultured to an unlimited supply, avoiding numerous ethical issues that plague other SCs [57,58]. To harvest the HUCB cells, a physician clamps the umbilical cord and punctures the umbilical vein with a syringe to draw out blood into a bag with anticoagulants and nutrients. The blood is cleaned of infectious agents prior to cryopreservation and finally stored in a blood bank for future use [59]. Once harvested, HUCB cells can easily proliferate, and be indefinitely cultured [57,58,60].

Cryopreservation does not hinder any proliferation potential, making HUCB cells viable and long lasting [59]. Furthermore, cryopreservation results in the amount of retroviral vector mRNA in cord blood increasing its ability to transduce retrovectorial vectors. This enhanced amphotrophic retroviral vector expression facilitates the utility of, gene therapy as these receptors are a central target for transduction of genes of interest [61].

HUCB is also a richer source of hematopoietic stem and progenitor cells with higher proliferation and expansion potential than bone marrow [62-65]. There is approximately 4% higher frequency of CD34+, CD38-, and CD133+ cells in primitive hematopoietic SCs derived from HUCB than in bone marrow [32,66,67]. These findings suggest the higher benefits transplantation of HUCB could yield of bone marrow.

One of the biggest challenges in cell based treatment and transplantation is to overcome graft rejection. HUCB cells have the benefit of having immature immunogenicity, suggesting that these cells will have a lower incidence of graft-versus-host disease as compared to other varieties of SC [31,56,62,68-71]. Recently, researchers discovered that HUCB contains a small percentage of very small embryonic-like SCs (VSELs) another source of pluripotent SCs [72-74]. Additionally, it has been shown that HUCB cells possess the ability to repair muscle cells and endothelial cells due to their myogenic and angiogenic properties, indicating that they would be well suited for repairing damaged myocardium [33-41,43-45,49,55,57]. HUCB cells have a long track record of safety profile in successful clinical transplantation [58,59,75]. Altogether, these advantages support the notion that adult SCs provide a high level of safety and efficacy to the transplant recipient.

HUCB Mechanisms of Cardiac Repair

There is still much uncertainty for the exact mechanism by which HUCB cells ameliorate cardiac deficits or how they reduce infarct volume. The various populations of SCs found in the HUCB highlight multi-pronged mechanisms. Immunophenotyping and analysis of the function properties reveal a close resemblance to bone marrow-derived SC characteristics [76,77], that led to much speculation that HUCB cells resemble bone marrow SCs. However, the exact mechanisms of action underlying the beneficial effects of the HUCB cells are unknown; below are a few of the more common postulated therapeutic pathways.

Cellular Cardiomyoplasty

Cellular cardiomyoplasty may result in improvement and reversion of the adverse hemodynamic and neurohumoral imbalance post MI. HUCB is a rich source for HSCs and MSCs, which specifically are known to differentiate into other cell types such as cardiomyocytes, osteocytes, chondrocytes, and fat cells [4,32,60,78]. That SCs from HUCB can differentiate into cardiomyocytes suggests that cellular cardiomyoplasty is likely involved in the repair damaged myocardium and increase contractile performance after SC transplantation [13,40,41,45,69,76,77,79-84].

HUCB-derived MSCs have been shown to regenerate into cardiomyocytes in vitro. Using a medium of low serum DMEM to form an adherent layer, the expanded HUCB cells were added to a supplemented medium with 5-azacytidine to induce cardiomyocytes. To identify cells similar to cardiomyocytes, cardiogenic specific contractile protein troponin T staining was performed, revealing 70% of the cells had differentiated into cardiomyocyte-like cells [85]. A similar study analyzed the role of HUCB CD133+ cells by culturing them either in medium supporting endothelium-differentiation or cardiomyocyte-differentiation endothelium markers such as VE-cadherin, CD146, KDR, and CD105, as well as morphofunctional features of endothelium in endothelial-supporting cultures of cardiac muscle proteins such as troponin I and myosin ventricular heavy chain alpha/beta; MYHC were discovered in the endothelium-oriented cultures. In the cardiomyocyte-oriented cultures, specific gene expression of GATA 4, NKKX2.5, troponin I, and MYHC were found. Thus, HUCB CD133+ cells have been implicated to promote myogenesis and angiogenesis [86].

Cardiomyocyte differentiation of HUCB has been induced in vitro [85-89]. One novel approach for directing cardiomyocyte differentiation examined the creation of a culture medium containing...
<table>
<thead>
<tr>
<th>HUCB Dose and Delivery Route</th>
<th>Time of Delivery Post AMI</th>
<th>Cell Type</th>
<th>MI Model</th>
<th>Lesion Size</th>
<th>Potential Mechanism of Action</th>
<th>Recovery</th>
<th>Source</th>
</tr>
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<tbody>
<tr>
<td>1X10⁵ IC</td>
<td>1 Week</td>
<td>USSC from HUCB</td>
<td>Swine with balloon occlusion</td>
<td>Increased</td>
<td>USSC survived in the infarct border zone at 5 weeks, did not express cardiomyocyte/endothelial markers. Micro infarctions were found in heart</td>
<td>No difference in global and regional LV function at 5 weeks</td>
<td>[98]</td>
</tr>
<tr>
<td>1.1X10⁵ IM</td>
<td>20 Minutes</td>
<td>USSC from HUCB</td>
<td>Rats with LAD ligation</td>
<td>Decreased</td>
<td>cardiomyocytes generation and vascularization are dose-dependent</td>
<td>LV structural integrity was upheld. No statistical difference between the groups in fractional shortening</td>
<td>111</td>
</tr>
<tr>
<td>1.2X10⁶ IM</td>
<td>24 hours</td>
<td>1. Unseparated CD 34+ cells 2. FACs sorted CD34+KDR+ or CD34-KDR- mouse with LAD ligation</td>
<td>Decreased</td>
<td>CMC apoptosis and fibrosis were decreased and lownumber of HNA+ nuclei within a CMC context was found at 21 days after MI</td>
<td>Improved L VE DP and dp/ dt(max) at 3 and 4 months PT</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>2.1X10⁶ IM</td>
<td>1-2 hours</td>
<td>HUCB mononuclear cells</td>
<td>Mice with LAD ligation</td>
<td>Decreased</td>
<td>Cells were detected only at the 48 hour marker</td>
<td>Higher capillary density, showed less improved myocardial contractility then bone marrow derived cells</td>
<td>42</td>
</tr>
<tr>
<td>1.5X10⁷ IM</td>
<td>1-2 hours 2.24 hours</td>
<td>HUCB mononuclear clear cells</td>
<td>Rats with LAD ligation</td>
<td>Decreased</td>
<td>NA</td>
<td>IM had more improved cardiac function than IV, 4X10⁶ had a greater decrease in infarct volume</td>
<td>109</td>
</tr>
<tr>
<td>1X10⁶ IM</td>
<td>1 hour</td>
<td>HUCB mononuclear progenitor cells</td>
<td>Rats with LAD ligation</td>
<td>Decreased</td>
<td>NA</td>
<td>Improved E F, dp/ dt(max), and anteroseptal wall thickening at 3 and 4 months PT T</td>
<td>40</td>
</tr>
<tr>
<td>1X10⁶ IM</td>
<td>30 minutes</td>
<td>HUCB EP C expressing CD 34+</td>
<td>Rats with transient LAD ligation</td>
<td>NA</td>
<td>Positive human staining for new vascular structures</td>
<td>Vascular structures formed ; left ventricular ejection fraction</td>
<td>45</td>
</tr>
<tr>
<td>1X10⁶ IM</td>
<td>Immediately</td>
<td>HUCB mononuclear cells expressing CD34+ cocultured with adenovirus associate virus</td>
<td>Mice with LAD ligation</td>
<td>Decreased</td>
<td>Cells were integrated into cardiomyocytes. Increased capillary density</td>
<td>Smaller L V activity and higher ejection fraction as well as improved fractional shortening</td>
<td>47</td>
</tr>
<tr>
<td>1.1X10⁷ IM</td>
<td>1.2 hours 2.24 hours</td>
<td>HUCB mononuclear cells</td>
<td>Rats with LAD ligation</td>
<td>1. Decreased 2. Decreased</td>
<td>Limited expression of TNF-alpha, MCP-1, MIP, and INF –gamma in acutely infarcted myocardium</td>
<td>NA</td>
<td>51</td>
</tr>
<tr>
<td>1.5X10⁷ IM</td>
<td>Immediately</td>
<td>HUCB MSC s expressing GATA-4</td>
<td>Rats with LAD ligation</td>
<td>Decreased</td>
<td>MSC survival increased with the expression of GATA-4</td>
<td>Improved L V anterior wall thickness</td>
<td>39</td>
</tr>
<tr>
<td>5X10⁶</td>
<td>1 hour</td>
<td>HUCB mononuclear cells combined with fixation of collagen matrix</td>
<td>Mice with LAD ligation</td>
<td>NA</td>
<td>Increased infarcted area thickness</td>
<td>Improved left ventricular enddiastolic volume at day 45 PT</td>
<td>53</td>
</tr>
<tr>
<td>5X10⁶ IM</td>
<td>2 Weeks</td>
<td>USSCs from HUCB</td>
<td>Rats with left coronary artery ligation</td>
<td>NA</td>
<td>Transplanted cells seen, with some expressing cardiac trophin-T, von Wille brand factor, and smooth muscle actin. Capillary and arteriole density were also markedly increased</td>
<td>Improved LVEF and left ventricular dimension and posterior wall thickness at 2 and 4 weeks PT</td>
<td>49</td>
</tr>
<tr>
<td>1X10⁷ IM</td>
<td>Immediately</td>
<td>HUCB mononuclear cells</td>
<td>Rats with LAD ligation</td>
<td>Decreased</td>
<td>Transplanted cells were detected. Collagen density was decreased. Expression of VEGF and number of micro vessels were increased</td>
<td>Improved left ventricular wall motion, LVE DP and dp / dt (max) at 3 and 4 weeks PT</td>
<td>50</td>
</tr>
<tr>
<td>100X10⁷ IM</td>
<td>4 Weeks</td>
<td>USSC s from HUCB</td>
<td>Pigs occluded by coil</td>
<td>NA</td>
<td>Grafted cells were detected at 4 weeks PT</td>
<td>Improved wall motion, regional perfusion, and EF. Scar thickness at 4 weeks PT</td>
<td>46</td>
</tr>
<tr>
<td>1.0X10⁸ IM</td>
<td>1-2 hours</td>
<td>HUCB mononuclear cells</td>
<td>Rats with LAD ligation</td>
<td>1. Decreased 2. Decreased</td>
<td>NA</td>
<td>Less improvement than IM. 4X10⁶ produced better improvement</td>
<td>109</td>
</tr>
<tr>
<td>1.2X10⁸ IV</td>
<td>7 days</td>
<td>HUCB CD 133+ cells</td>
<td>Rats with LAD ligation</td>
<td>Decreased</td>
<td>Human cells were detected</td>
<td>LVFS and anterior wall thickness were improved at 1 month PT</td>
<td>48</td>
</tr>
<tr>
<td>2X10⁹ IV</td>
<td>20 minutes</td>
<td>HUCB CD 34++ cells</td>
<td>Rats with LAD ligation</td>
<td>NA</td>
<td>CD34+, CD45+, and PCAM-1+cells enhanced neo vascularization at 4 weeks after PT</td>
<td>Improved FS and dp/dt (max) at 4 weeks PT</td>
<td>41</td>
</tr>
</tbody>
</table>
suggesting they are not ripe for infarct repair [100]. A study comparing study showed low frequency levels of differentiation of HUCB MSCs, post MI either via IV injection or IC delivery [56,98]. A more recent is no evidence of cardiomyocyte differentiation of HUCB cells injected between cell grafted hearts and sham hearts [99]. Furthermore, there is no evidence of cardiomyocyte differentiation of HUCB cells injected post MI either via IV injection or IC delivery [56,98]. A more recent study showed low frequency levels of differentiation of HUCB MSCs, suggesting they are not ripe for infarct repair [100]. A study comparing the results of differentiated versus non-differentiated cells vis-à-vis revealed no significant difference in cardiac improvement between the two groups [101]. While these studies have questioned the use of these cells, they also suggest that perhaps the therapy is not entirely dependent on cellular cardiomyoplasty. An in vivo model revealed bone-marrow transplanted cells fused with cardiac muscle [92], suggesting that this fusion of host and transplanted cells may result in genetic transfer and thus rejection. A more recent study analyzed HUCB CD34+ cells co-cultured with neonatal ventricular myocytes for the presence of cardiomyocyte properties using a reporter gene system to determine whether cardiac transformation is due to differentiation of the cells or cellular fusion. Interestingly, this co-culturing system led to cell fusion, and therefore the cells expressed the myocyte features by accumulating the cardiac physiological genetic properties [90]. However, equally compelling evidence has refuted the notion of cell fusion, in that gender-specific bone marrow-derived cell grafts in experimental mouse MI revealed male-originated cells, ruling out cell fusion [93]. Due to these inconsistencies, future studies are warranted to clarify whether cellular cardiomyoplasty truly improves cardiac function following HUCB transplantation into the infarcted myocardium.

### Angiogenesis

Another possible reparative mechanism is SC-induced angiogenesis in the ischemic area after MI. Numerous studies have shown that transplanted HUCB cells increased the neovascularization in the infarcted myocardial, and improved cardiac function [38,43,45-47,49,58,64,65,102]. This neovascularization is suggested to trigger the native and endogenous cells of the myocardium to proliferate and regenerate, as well as to protect against the apoptosis of the ischemic regions. A major promoter of this neovascularization is HO-1, a known cytoprotective enzyme in angiogenesis, paired with carbon dioxide, which is demonstrated to influence cardiac regeneration post MI [103]. The CO aids in vasculogenesis by activating c-kit+ stem/progenitor cells and increasing the differentiation of SCs to form new arteries and cardiomyocytes through the creation of growth factor HIF-1α, SDF-1α and vascular endothelial growth factor-B (VEGF-B) expression. However, the HO-1 relies on the CO to promote angiogenesis by inducing SDF-1α expression only, indicating that HO-1 and CO have potential to enhance cardiac regeneration [103]. The graft deposition may influence the resulting neovascularization in that HUCB-derived EPCs following transplantation were ingressed in the myocardium wall which was found to display robust neovascularization, suggesting that transplanting the cells into the capillaries could induce revascularization [105]. These studies altogether support that angiogenesis may mediate the improved cardiac function following transplantation of HUCB-derived SCs.

### Paracrine Effects

Paracrine effects refer to communication between adjacent cells mediated by the action of regulatory molecules, such as growth factors and cytokines. These effects may play a crucial role in improving left
ventricular function following SC transplantation. Much evidence supports the idea that paracrine factors from SCs transplanted into the myocardium contribute to left ventricular remodeling and function [39,105,106].

Increased vascular endothelial growth factor (VEGF) mRNA expression was detected at 7 and 27 days post HUCB cell transplantation, which was found to coincide with increased microvasculature near the infarct boundaries [50]. Additional angiogenic factor expression (fibroblast growth factor, VEGF, and SC homing factor SDF-1) was observed in engrafted MSCs two weeks post transplantation, increasing capillary density 40%. The left ventricle exhibited an improved contractile function at eight weeks post transplantation, suggesting that growth factor secretion improved cardiac function [105]. Enhancing the expression of Ang1 and VEGF in HUCB CD34+ cells resulted in a further reduction of infarct volume and robust increment in capillary density, suggesting further the role of paracrine effect in improved cardiac function [49]. This initial paracrine effect was also shown to trigger a multitude of therapeutic pathways, in that by increasing angiogenesis, reducing collagen content and thus changing the extracellular matrix, it culminates with an enhanced recruitment of endogenous myofibroblasts [49].

Similarly, the HUCB-mediated paracrine effect is exerted by bone marrow-derived MSCs co-injected with adenovirus associated virus (AAV) expressing VEGF, which led to improved therapeutic effects characterized by reduced infarct volume, recovery of cardiac function, neovascularization, and increased MSC survival 50-fold [106]. However, MSC differentiation into cardiomyocytes was not detected, and only a few surviving MSCs were observed when singularly injected [106]. Nonetheless, despite this low MSC differentiation potential and graft persistency, infarct size was still reduced, suggesting that the MSC-secreted paracrine factors is likely the alternative mechanism of functional repair in MI. Indeed, GATA-4 increased MSC survival, promoted neovascularization, and enhanced cardiac recovery by upregulating IGF-1 and VEGF in the MSCs [39].

The overexpression of the angiogenic factors not only promoted neovascularization, but also improved several parameters of cardiac function including fractional shortening, tissue velocity, and wall motion score index [94]. In tandem with increased neovascularization, elevated angiogenic factors promoted myogenesis, vasculosgenesis, and anti-apoptotic effects within the injured myocardium, the major deposition site of the transplanted SCs. The latter is indicative that both migration and paracrine secretory properties of the SCs may interact to produce therapeutic benefit in MI. This combined therapeutic pathway involving cell migration and paracrine secretion is also shown to rescue the scarred tissue as evidenced by improved cardiac function at 4 weeks post MSC injection. However, 6 weeks post injection, no benefits of myogenic differentiation were observed [7], suggesting that cell migration at the early stage is important for treating MI.

Anti-Inflammation

Transplanted HUCB cells have the ability to attenuate the ischemic-induced inflammatory/immune response in the infarcted heart, representing another intriguing potential repair mechanism [51,105,108]. Increasing evidence indicates that HUCB-derived MSCs secrete a variety of pro- and anti-inflammatory cytokines that directly act to limit deleterious and permanent endogenous inflammation of the heart [105]. Similarly, injection of HUCB cells into infarcted myocardium of non-immunosuppressed rats, within 2h or at 24h following left anterior descending coronary artery (LAD) occlusion, resulted in reduction of infarct sizes 1 month later [51], concomitant with a significant change in myocardial concentrations of tumor necrosis factor-alpha (TNF-alpha), monocyte/macrophage chemoattractant protein (MCP-1), monocyte inflammatory protein (MIP), and interferon-gamma (INF-gamma) as compared to control animals at 2, 6, 12, and 24h after coronary occlusion [51].

More recently, an investigation of the immunological/inflammatory responses by the host to implanted bone marrow mesenchymal SCs (BMSC), cultured on silk fibroin/hyaluronic acid (SH) patches [108], suggests that modulation of inflammatory responses is achievable through transplantation of HUCB-MSC, which display similar stem cell phenotypic and functional properties as BMSC. In response to BMSCs, expression of CD68 (macrophage marker) was not detected in the MI zones exposed to the SH patches when compared to non-SH patch-exposed MI zones. The SH patches provided an anti-inflammatory effect, and application of SCs with SH significantly improved wall thickness of LV, had a high viability of delivery of BMSC, largely reduced apoptosis, and significantly promoted neo-vascularization and stimulated VEGF secretions and various other paracrine factors [108]. That HUCB-MSC may also modulate inflammatory responses could attenuate the secondary wave of ischemic damage after the MI.

While these represent some of the more widely accepted MI mechanisms, either a singular or combination of known and unknown factors, identifying the exact mode of action underlying the functional effects of cell therapy in MI requires more investigations. Future experiments should consider these therapeutic pathways in designing HUCB transplantation therapy for MI.

Delivery Routes and Preclinical Outcomes

Although published data about transplantation of HUCB cells into the heart is still in its early stages, animal models of MI have already demonstrated that several delivery routes can be used to successfully transplant these cells effectively and safe. Among the most common delivery methods for transplantation are intramyocardial, intravenous (IV), and intracoronary (IC) injections [38,46,51,52,54,109].

Intramyocardial injection

Intramyocardial injection are injection performed directly into the myocardium [38,46,51,52]. This direct administration of cells into the damage heart muscle has proven to be more effective than indirect methods. Comparing indirect and direct delivery methods, intramyocardial injection significantly reduced the infarct size area as compared to indirect methods of HUCB cell delivery [110]. Although this method is preferred, there are some disadvantages that need to be taken into consideration before delivering the cells. This procedure only allows a very small amount of cell to be delivered, and it is an invasive procedure. Intramyocardial injections require open heart surgery in order to deliver the cells directly to the infarcted heart [54]. Additionally, there is the risk for possible arrhythmogenicity.

Even though this delivery method has some disadvantages, preclinical studies have shown promising results for myocardial repair utilizing this method. Improved diastolic pressure and cardiac function were achieved in an animal model of intramyocardial injections of HUCB cells of different populations, such as CD34+KDR+ or CD34+KDR- cells on non-obese diabetic-severe combined immunodeficiency mice or NOD-SCID mice at 24h after LAD. About 200,000 cells of CD34+KDR+ significantly improved left ventricular diastolic pressure after MI relative to control injection of PBS or mononuclear cells. Histology analyzes reveal limited number of
newly formed cardiomyocytes in the area of injury. Overall, this study was able to successfully use direct method of delivery in identifying the therapeutic subfraction within the CD34+ population [38].

Similar studies have supported the therapeutic role of transplanting HUCB cells directly into the heart, showing improvements of ventricular function following intramyocardial injection of HUCB cells after MI. Using different immunofluorescent tags, HUCB cells injected directly into the heart survived in the myocardium, increased neovascularization and improved cardiac function at 4 weeks after transplantation. These results suggest the large therapeutic potential of HUCB cells when delivered directly to the damaged myocardium [41,46].

Another study injecting HUCB cells directly into the damaged myocardium was found to improve left ventricular function in a rat model of MI [40]. About 10^6 HUCB mononuclear progenitor cells were injected into the myocardium 1 hour post LAD ligation. There were no significant differences in ejection fraction after 1 month between the group injected with HUCB and PBS. However, after 3 and 4 months, the anteroseptal wall, from HUCB-treated rats, was significantly thicker relative to control rats. In addition, a significantly robust reduction in infarct size was achieved in the heart of HUCB injected rats [40], showing the long term effects of HUCB transplantation.

Arteriole and capillary density increased at 4 weeks after transplantation of unrestricted human somatic stem cells (USSCs) derived from the HUCB into the myocardium. To determine whether these cells truly enhanced regeneration through differentiation, markers like cardiac troponin-T, smooth muscle actin, and von Willebrand factor were used for analysis. USSCs were shown to express each marker, indicative of cellular differentiation into cardiomyocytes, smooth muscle cells, and endothelial cells respectively. Using the direct delivery of USSCs, this study supports the theory of cardiomyoplasty [49].

Additionally, transplanting HUCB cells, using direct delivery method of intramyocardial injection, a study was able to support the angiogenic potential of HUCB cells after MI as a possible repair mechanism. In this study, HUCB cells were transplanted immediately after MI. After 4 weeks from transplantation, there was a significant increase of the vascular endothelial growth factor or VEGF 164 and VEGF 188 [50]. Intramyocardial injections of HUCB cells were also found to attenuate the inflammatory immune response after MI [51]. To further augment the host inflammation associated with MI, a collagen matrix with HUCB cells grafted directly onto the infarcted area improved survival as well as cardiomyoplasty [53].

Studies that are more recent further support the intramyocardial injection as an effective cell delivery system. HUCB cells were injected into one or two positions of the myocardium near the edge of the infarct area in rats [110]. Three weeks after implantation, HUCB cells were detected using nuclear staining primarily in the border of the infarct area, suggesting that the cells have the potential to survive for at least three weeks following implantation. As shown in earlier studies, these results also reveal amelioration of cardiac functioning after direct transplantation of HUCB cell into the myocardium after [42-44].

Moreover, in order to find an optimal dose for the direct delivery method, an MI rat model was used. Rats were injected with HUCB cells into the peri-infarct zone in a dose-dependent manner in a series of 6 X 10 μL injections of 1 x 10^5 (considered the low dose, or LD), and 1 x 10^6 (considered the high dose, or HD) of HUCB cells [59]. The effects of the cells were analyzed from 5 to 28 days following transplantation. At day 5, there were no differences across the groups. However, the cells considerably contributed to the maintenance of left ventricular (LV) structure based on percent of fibrosis, and a number of other measurements. On day 28, capillary density related to myocardial neovascularization was enhanced in both dosage groups, as was left ventricular wall motion in comparison to the non-treated group. On day 23, fractional shortening (FS) was higher in the HD group, but not significantly different than the LD group. In contrast, a lower regional wall motion score (RWMS) was observed in the LD and HD groups indicating a better protection in the treatment groups. Analysis of +dP/dt to assess left ventricular contractility revealed that the HD group levels were significantly greater than the LD group, with even lower levels found among the untreated groups [112]. After four weeks, left ventricular end diastolic pressure (LVEDP) was lower in both HD and LD groups. In addition to suggesting the cells improved cardiac functioning, the study found that HUCBs differentiated into human cardiomyocytes (CMCs) in a dose-dependent manner [112].

Altogether, these studies using the direct delivery method to transplant HUCB cells demonstrated promising results for the treatment and repair of the failing heart after MI.

**Intravenous injection**

Intravenous injection of HUCB cells offers a less invasive cellular delivery system than intramyocardial injection. Studies using animal models of stroke have revealed that transplanted cells through, systemic administration, are able to migrate to the ischemic site of injury, and may contribute to the improvement of behavioral deficits [33-36]. However, systemic administration may cause these SCs to aggregate in different organs before reaching the injured site. In fact, it has been shown that only a fraction of these cells reach the site of injury due to the aggregation of the cells within the microvasculature of the liver, lungs, and lymphoid tissue [54]. Additionally, shortness of breath and death due to pulmonary embolisms has been noted with this procedure [112]. Despite some controversy, IV administration of HUCB cells is still studied by many pre-clinical scientific groups to further asses its beneficial effects as an indirect route of delivery and to further improve its outcomes [42,43,48,51].

In a study injecting HUCB cells into the tail vein of mice induced with MI from an LAD ligation, cell migration, cell survival and infarct size were characterized in order to assess the efficacy of IV delivery [42]. Organ analysis of mice showed detectable levels of hDNA after 24 hours, 1 day, and 3 weeks following transplantation: no sham animals were observed with hDNA. However, hDNA was not completely detected in all mice with MI (only 10/19). MI mice showed an abundance of HUCB cells in the perivascular interstitium, while having a reduced infarct volume compared to the sham animals. Furthermore, there was significant infarct reduction in the MI as well as 20% higher capillary density around the infarct area border. There was no decrease in collagen deposition between the two groups. Co-localization of HNA or HLA-1 with GATA-4 or Connexin 43 showed no evidence of HUCB mononuclear cells differentiation into cardiomyocytes. The expression of SDF-1 mRNA on the MI+ mouse was approximately 7-fold higher than the MI- group [42]. In a parallel study, the migration and survival of HUCB mononuclear cells following IV transplant were tracked and revealed cell aggregation, but it is not consistent in all injected mice [43]. Cell migration to the heart was detected only in MI mice and not sham mice, proposing a signal-induced migration by damaged or injured tissue [43].

Another cell tracking study demonstrated the migration of HUCB-derived CD133+ cells when IV delivered at seven days after permanent...
coronary artery ligation in rats [48]. One month post transplant, lateral ventricle fractional shortening improved relative to control mice. Only control animals presented thinning of the anterior wall of the heart. Following tracking the migration of the cells, it was revealed they colonized and survived in the infarcted myocardium. The cells in the nearby vessel walls were determined to be of human origin, while scar tissue indicated autologous myofibroblasts and alpha-smooth muscle. This study supports IV administration as an adequate strategy for HUCB cell transplantation, allowing effective migration of the cells to the area of injury where they subsequently induce autologous differentiation for repair of infarcted myocardium [48].

Another important factor for IV delivery of HUCB cells is timing, notably that endogenous signals are able to guide the migration of the cells to the infarct area. An in vivo study found that the greatest migration of IV administered cells to MI region was between 2 hours to 24 hours after LAD occlusion [51]. Protein characterization revealed increased cytokine and chemokine production in this time. In particular, stromal cell derived factor-1 (SDF-1) was highly up-regulated in the infarcted area of the myocardium. SDF-1 is a chemokine that attracts circulating SCs via CXC4R4, integrating activation of integrins in the vasculature [78].

Accumulating preclinical studies have shown the significant clinical relevance that IV administration of HUCB cells has for the treatment and repair of the infarcted heart. In a recent study, the effectiveness of IV delivery method for HUCB cell base therapy has been analyzed at different points in time following MI [113]. Four transplants of equal amounts were IV administered at days 1, 5, 10, and 30 following the MI, and the effects of the cells were analyzed using echocardiographic assessment. It was found that in 5 and 10-days following transplants, rats had significantly increased left ventricular ejection fraction (LVEF) as compared to the control group, whereas the LVEDD and LVESD levels were significantly smaller in the treatment group. Moreover, left ventricular wall thickening was most notable and significant in the 10-day transplantation group. Scar tissue area was reduced in the 5- day group and in the 10-day group relative to the PBS control group. At both time points, microvascular density was larger than the control group, with the 10 day point having the larger area. VEGF levels were higher in the 10 day group than any other as well. At this 10 day time point, the largest concentration of HUCB cells within the infarct area was found, which correlated with the higher VEGF levels [113]. Future studies are warranted to assess the long term potential of the reparative capacity of HUCB cells.

Intravenous delivery of different types of cells derived from HUCB was also examined; in particular comparing the efficacy of injecting post MI expanded HUCB cells with that of non-expanded HUCB cells. Two days post MI, 10^8 expanded and non-expanded HUCB cells were injected into the tails of rats. No detectable differences between the groups were observed at two days post injection, and there was no significant difference in cardiac function at two weeks (analyzed using LVEF). (61 ± 5.9% and 64 ± 4.1%, respectively). Four weeks post IV administration, cardiac function appeared to be improved, but there was still no statistical difference [114]. This study suggests that there were no functional differences between expanded versus non-expanded HUCBcs. Although more studies are needed to further test the efficacy of expanded SCs, these results showed that HUCB cells can be expanded in vitro without losing their functional activity [114, 115].

Despite negative controversial results, especially the formation of embolus using IV administration of HUCB cells, these studies support the concept that the minimally invasive IV administration facilitated HUCB-derived SCs to migrate to infarcted area and ameliorate cardiac function [78].

Intracoronary delivery

SC transplantation can also be achieved using the IC delivery method. This method allows delivery of SCs directly into the damaged myocardium without passing through systemic circulation. However, the possibility of cell aggregation is very high in IC injection, especially if a large amount of cells are delivered in the catheter [60,116] Yet, over the last decade, several studies have shown a good safety profile of IC injection of bone marrow and peripheral blood-derived mononuclear cells [42,44,54,116-119].

The IC route of SC delivery is the least commonly used in MI animal models. After 5 weeks from treatment, it was concluded that LV was not ameliorated, infarcted area was not reduced, and surviving cells did not express cardiomyocytes or endothelial markers [98]. Histological analyses revealed that IC injection caused micro-infarctions due to obstruction of blood vessels likely due to the large amount of cells injected (10^9) [98]. More pre-clinical research needs are warranted to evaluate the efficacy and safety of IC delivery of HUCB for transplantation in MI.

HUCB, Gene Therapy, and Other Novel Techniques

As noted above, HUCB cells for myocardial repair and revascularization following MI offers a glimpse of hope as an alternative therapeutic option. A major caveat in realizing the successful outcome of HUCB transplantation for MI is overcoming delivery of the cells or the cells’ nutritive substances (angiogenic, trophic and anti-inflammatory factors) to the non-conductive environment of the ischemic heart. Genetically modifying SCs may circumvent the technical problems of cell delivery and hostile environment associated with ischemic diseases [95,120-123].

Previous studies on SC therapy for MI reveal potential for the combined use of gene therapy with HUCB cells. Adeno associated viral vectors (AAVs) were used to transduce angiogenic factors to the heart. Human ang1-alone, VEGF (165) alone or a combination with AAVs were transduced to CD34+ cells and injected intramyocardially immediately after ligation of the left anterior descending coronary artery in male SCID mice to infarcted ventricles [47]. Four weeks following gene delivery, protein analysis confirmed the upregulation of ang-1 and VEGF or both in the CD34+ transduced groups. The results showed a significant decrease of the infarct size, and a significant increase in capillary density relative to control (treatment with CD34+ alone) in all treatment groups (AAV-ang-1, AAV-VEGF, or AAV-ang-1+VEGF). In terms of cardiac functioning, echocardiography assessment showed significant amelioration on cardiac performance [47]. The results demonstrated the utility of viral vectors and SCs for the repair of myocardial infarcted hearts.

Additional gene-based techniques have been explored to improve the therapeutic potential of HUCB MSCs [123]. In order to effectively engraft these cells, spherical three-dimensional (3D) bullets made of cultured cells in anchored-deprived media were created to deliver MSCs to the heart. This treatment was shown to improve left ventricular contractility, lessen fractional shortening, and decrease and prevent pathologic left ventricular dilation when compared to single cell treatment [123]. The efficacy of MSCs increased once the spherical bullets formed, allowing for cell to cell interaction, inducing E-cadherin, which is essential to the bullet formation, activating and initiating the cascade of proliferative angiogenic pathways and increasing the
endogenic potential of the cells. Overexpression of E-cadherin revealed secretion of VEGF, which probably induces the angiogenic pathways. The same concept was used for core-shell bodies, where MSCs are combined with endothelial cells from the umbilical cord vein. Results revealed MSCs differentiated into smooth muscles and there was a robust excretion of VEGF [124]. Both of these concepts represent a new field of genetic manipulation for enhancing the therapeutic effects of HUCB-derived cells.

Microporation has also been employed to increase efficacy of the MSCs. The technique transduces plasmid DNA into the HUCB-derived cells. Minimal cell damage occurred when brain derived neurotrophic factor (BDNF) was successfully transduced via microporation, wherein immunophenotype, proliferation, and differentiation activity of HUCB-MSCs was not affected when migrating toward brain cancer cells [95]. The study highlights the use of a reliable transduction technique, which further studies could use to transfer trophic factors to muscle tissue of the failing heart ventricles without altering the beneficial effects of SC transplantation therapy.

Although the translational potential of genetic manipulation of HUCB cells is in its infancy, it stands as an innovative approach in overcoming limitations of cell delivery. Further studies are warranted to test the safety and efficacy of combined gene and cell therapy.

**Conclusion**

Cell-based remains an experimental treatment for MI. Historically, the use of HUCB cells is circumvents the ethical concerns associated with embryonic SCs use due to their source and method of acquisition. Animal models of hearing loss, Parkinson’s disease, Alzheimer’s disease, stroke, and Huntington’s disease have also evaluated the therapeutic medical value of HUCB cells. Due to the uniqueness of the diseases, tailored cell therapies to target each disorders may be required to achieve clinical improvement [34-36,38,40,122]. Several pre-clinical studies strongly support the use of HUCB cells for the therapeutic treatment of MI. However, additional research is still necessary to establish HUCB cells as a safe and effective cell–based approach to use in MI patients.

Many studies emphasize the importance of the optimal timing of HUCB administration, as this timing assures higher rates of engraftment, survival, and differentiation compared. Transplantation acutely after the initial injury could decrease cell survival due to the release of inflammatory cytokines, while transplantation at the chronic stage could mean rampant scarring that may prevent graft-host signaling pathways necessary for directed cell migration and differentiation, as well as appropriate paracrine secretion. A careful examination of the literature reveals that transplanting HUCB cells as early as 24 hours after MI ameliorates ventricular function and contractility [38,40-42,47,49,50], and, on the other side, cell transplantation even at 4 weeks post MI has been shown to afford a general improvement of heart function [46,49]. Therefore, it is necessary to conduct vis-à-vis comparative studies in order to find an optimal time frame with the most therapeutic benefit that has direct clinical application.

Disagreement also exists over the optimal number of transplanted cells: the studies show both a variety of doses, and quantity of transplanted cells [38,40-43,45-53]. It is essential to determine an optimal dose response in an effort to standardize the HUCB dosage, which should coincide with high therapeutic value for MI and ventricular repair in cardiac failure. Table 1 organizes the current studies by dosage.

Although HUCB has less immunogenicity issues, graft rejection needs to be monitored to ensure successful transplant outcome. In most studies, HUCB cell transplantation revealed a very attractive option as the treatment was effective in MI rat models without the need for immunosuppression [40-43,45,47,48]. However, studies tended to only follow the fate of the HUCB grafts for very short time periods, from 2 weeks to 4 months being the average time points, suggesting the need to observe the cells under longer-time periods in order to fully determine the need for immunosuppression and the presence of functional recovery. An additional concern and important study that needs to be performed is a long-term follow up of HUCB migration as the cells could move through the heart vasculature to other organs.

Finally, while SC engineering may enhance tissue repair capabilities, their ability to migrate to the target tissue and their capacity to differentiate or exert paracrine effects require elucidation to harness cellular and molecular pathways of exogenous and endogenous repair mechanisms. Although still a novel technique, studies support the notion that gene therapy and HUCB cells could overcame many transplantation challenges or improve the HUCB potential by either enhancing or ameliorating the delivery of trophic factors or by increasing their differentiation potential for the treatment of ischemic diseases as MI and stroke [47,94,95,106,123]. However, gene therapy itself may pose a novel set of safety and efficacy issues that require similar optimization and standardization preclinical studies.

HUCB cells continue to garner preclinical data furthering our basic science of stem cell biology but also providing insights into the translation of cell-based therapeutics for the amelioration of MI and other ischemic disorders.

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**Authors’ contribution**

Conceived the theme of the paper: CVB. Analyzed and interpreted the literature: SAA, NF, MS, NLW, MB, JP, NM, AJ, AS, MC, MP, GF, MF, LS, CGP, TD, KS, NT, PRS, YK LWM, CVB. Wrote the paper: SAA, NF, MS, NLW, LWM, CVB.

**Conflicts of Interest**

CVB and PRS serve as consultants of Saneron-CCEL Therapeutics, Inc. and Cyo-Cell International, Inc., and PRS is a co-founder of Saneron-CCEL Therapeutics, Inc., and CVB and PRS have patent and patent applications in this area.

**References**


