Stem Cell Banking and Its Impact on Cardiac Regenerative Medicine

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Abstract

Cardiovascular diseases, including heart failure, are the most frequent cause of death annually, even higher than any other pathologies. Specifically, patients who suffer from myocardial infarction may encounter adverse remodeling processes of the heart that can ultimately lead to heart failure. Prognosis of patients affected by heart failure is very poor with 5-year mortality close to 50 %. Despite the impressive progress in the clinical treatment of heart failure in recent years, heart transplantation is still required to avoid death as the result of the inexorable decline in cardiac function. Unfortunately, the availability of donor human hearts for transplantation largely fails to cover the number of potential recipient requests. From this urgent unmet clinical need the interest in stem cell applications for heart regeneration made its start, and has rapidly grown in the last decades. Indeed, the discovery and application of stem and progenitor cells as therapeutic agents has raised substantial interest with the objective of reversing these processes, and ultimately inducing cardiac regeneration. In this scenario, the role of biobanking may play a remarkable role to provide cells at the right time according to the patient's clinical needs, mostly for autologous use in the acute setting of myocardial infarction, largely reducing the time needed for cell preparation and expansion before administration.

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Keywords

Cardiovascular diseases • Cardiac progenitor cells • Biobanking • Cell storage • Acute setting • Regenerative medicine

Abbreviations

MI	Myocardial Infarction
AMI	Acute Myocardial Infarcion
BMMNCs	Bone Marrow Mononuclear Cells
BMMSCs	Bone Marrow Mesenchymal Stem Cells
CHF	Chronic Heart Failure
SPECT	Single-Photon Emission Computed Tomography
LV	Left Ventricular
MRI	Magnetic Resonance Imaging
PCI	Percutaneous Coronary Intervention

14.1 Heart Regeneration

The human heart is one of the organs which regenerate less in the body, or at least its regenerative potential is clearly lower than the intestine, liver, bone or skin [1]. As a consequence, heart failure and its growing incidence raises many questions from the regenerative medicine point of view, mostly considering the predicition that it will reach epidemic proportions as the population ages.

Unlike humans, many lower vertebrates can regenerate limbs and internal organs after injury. Amphibian heart regeneration is well known. Furthermore, more recently the zebrafish has demonstrated to be a useful experimental model based on its broad regenerative capacity and easiness of genetic manipulation. Of note, the zebrafish heart can fully regenerate following surgical amputation of up to 20 % of the ventricular mass [2, 3]. This remarkable regenerative capacity is missing in the postnatal mammalian hearts, however some degree of cardiomyocyte renewal has to be recognized [4–6]. Despite the fact that proliferative rates are clearly small and quite difficult to detect, they raise the question whether such innate processes could be increased and employed therapeutically. To achieve functional heart recovery, it is critical to address its real cause which is the loss of cardiac tissue. The human left ventricle has 2–4 billion cardiomyocytes, and a myocardial infarction can eliminate up to 25 % of them in a few hours causing cardiomyocyte deficieny in the heart. Disorders of cardiac overload like hypertension or valvular disease slowly eliminate cardiomyocytes over many years, and aging is associated with physiological loss of ~1 g of myocardium per year in the absence of any specific cardiac disease. Given these observations, the main objective of cardiac regenerative medicine is to replace damaged heart cells and, therefore, to restore the physiological structure and function of the organ [7–9]. These innovative cell-based therapeutic applications are of particular interest in the clinical setting of cardiovascular diseases which could significantly benefit from a regenerative approach.

Over the past decades, several stem cell types have been identified for their capacity to regenerate the human heart. Bone marrow, and its subpopulations, including mesenchymal stem cells (MSCs) and endothelial progenitor cells (EPCs), have been thoroughly evaluated [10–14]. In addition, other therapeutically appealing sources are adipose tissue or the umbilical cord blood which seem to harbor a reservoir of suitable stem cells for cardiac regeneration. Furthermore, the more recent identification of endogenous progenitor cells in the human heart pushed the development of next-generation regenerative approaches employing heart-derived stem cells [15–17], as well as stem cells guided toward cardiac lineage *ex vivo* [18–20].

We are on the verge of a remarkable development in the field of cardiac regenerative medicine. Having such valuable biological sources available rapidly moves the question on the

opportunity of storing them according to the patient's clinical needs. Hence the opportunity of storing such reservoirs for specific therapeutic use is progressively under evaluation. For successful clinical application in the setting of regenerative medicine or other therapies, the concept of an off-the-shelf product is desirable. One important feature of such cellular product is its instant availability for the intended applications. Thus, biobanking technology applied to cardiac regenerative medicine will further facilitate clinical application since it allows sufficient time for product qualification, and mostly it can favor the optimal timing for transplantation, when the patient is ready to receive the cells.

In this chapter we will review to the best of our knowledge the most updated applications of cryopreserved stem cells sources for human cardiac regeneration.

14.2 Available Sources for Cardiac Regenerative Medicine

In recent years, stem cell biology has become one of the fastest moving areas of biomedical research. Particularly, the heart is one of the human organs on which this type of research is more actively investigating for regenerative purposes. In general and quite simplifying, we can divide the field of cardiac regenerative medicine into two main areas concerning cell type: endogenous and exogenous cells.

Resident stem or progenitor cell populations were identified in postnatal hearts by means of a variety of approaches, including expression of surface markers like c-Kit, Sca-1, Isl-1 or physiological properties like the ability to efflux fluorescent dyes or form multicellular spheres in culture. Initially, little overlap among these progenitor classes was thought to exist. However, recent studies indicate shared markers among cell populations or different stages of maturation of the same cell type. C-kit-positive cells or Cardiac Stem Cells are extensively studied. The tyrosine kinase receptor c-Kit is expressed by hematopoietic stem cells, mast cells and other mature circulating cells, as well as in the thymic epithelium. Small c-Kit-positive cells have been identified in niches throughout the adult heart and, following isolation, they have been reported to give rise to cardiomyocytes, smooth muscle cells and endothelial cells when administered, eventually contributing to the regeneration of the myocardium [21–25]. These observations led to the SCIPIO (Stem Cell Infusion in Patients with Ischemic cardiOmyopathy, ClinicalTrials.gov Indentifier: NCT00474461) Phase I randomised, open-label, single centre trial which tested the safety and feasibility of autologous c-Kit-positive cell administration as an adjunctive treatment to patients undergoing coronary bypass surgery [26, 27].

Cardiosphere-derived cells (CDCs) are the other cardiac progenitor cell population already employed in clinical trials for the treatment of recent myocardial infarctions (CADUCEUS trial, ClinicalTrials.gov Indentifier: NCT00893360). CDCs outgrow from cultured cardiac tissue fragments and form the so-called cardiospheres. These spheres obviously represent a mixture of cells which express stem cell markers like c-Kit, and other markers typical of the stromal-vascular compartment [28, 29]. Patients treated with CDCs showed significant reductions in scar mass, and increase in viable heart mass [30, 31].

Considering stem cell sources outside the heart, considerable interest was focused in the earlier studies on bone marrow-derived cells for cardiac regeneration. This interest was derived by the observation that hematopoietic stem cells could transdifferentiate into cardiomyocytes [10]. However, subsequent reports showed that hematopoietic stem cells do not form cardiomyocytes but become mature blood cells after transplantation [12, 13]. Nevertheless, the administration of hematopoietic stem cells after infarction showed improvement in ventricular function, and paracrine signaling was identified as the principal mechanism of their action. In analogy, bone marrow-derived mesenchymal stromal cells (MSCs) were originally reported to differentiate into cardiomyocytes and, similarly, are now thought to exert their actions through paracrine effect [14]. Clinical trials indicate the

safety and feasibility of bone marrow-derived cells through coronary injection, however the observed benefits are quite modest [32–39]. Bone marrow-derived MSCs are also employed in clinical trials (e.g. Prospective Randomized Study of Mesenchymal Stem Cell Therapy in Patients Undergoing Cardiac Surgery, PROMETHEUS, ClinicalTrials.gov Indentifier: NCT00587990) as well as adipose tissue-derived stromal cells (ClinicalTrials.gov Indentifier: NCT02387723). On the overall, the most updated evidence indicates that these cells produce signals that control the response of cells resident in the myocardium, and therefore regulate cardiac healing [40, 41].

Another very interesting approach involves the "cardiopoietic" guidance of multipotent adult stem cells [19, 20, 42]. This is achieved through a specific cardiogenic cocktail which guides human Mesenchymal Stem Cells towards the cardiac lineage through manipulation of their culture environment as was assessed in the C-CURE trial (ClinicalTrials.gov Indentifier: NCT00810238).

For a comprehensive overview of the different adult stem cell types employed in clinical trials for cardiac regeneration, please refer to Table 14.1.

The inherent characteristics of pluripotent stem cells offer a potential solution to the current epidemics of heart failure by providing human cardiomyocytes to support heart regeneration. These properties apply to embryonic stem cells (ESCs) and the more recently developed induced pluripotent stem cells (iPSCs). Human embryonic stem cells (hESCs) derived from the inner cell mass of the pre-implantation embryo appear to be a promising cell source for regenerative medicine [43, 44]. They are capable of extensive proliferation in and self-renewal and, as pluripotent cells, may be induced to generate more differentiated cell types such as functional cardiomyocyte. ESCs have been differentiated in three-dimensional aggregates, the so-called embryoid bodies (EBs), which typically delivers <1 % cardiomyocytes. Further improvement of the methodology are available through Wnt/β--catenin signaling pathway. Human ESC-derived cardiomyocytes express early cardiac transcription factors like Nkx2.5 and GATA4, and possess functional properties similar to cardiomyocytes in the developing heart [45]. Obviously, also these approaches have their drawbacks: ESCs are derived from the inner cell mass of pre-implantation blastocysts, and this raises the ethical controversy of their use in addition to the potential formation of teratomas [46]. Moreover, ESC-based therapies will be allogeneic and therefore require immunosuppression.

IPSCs were originally generated by the reprogramming of adult somatic cells (mainly dermal fibroblasts) via the expression of up to four embryonic stem cell-related transcription factors (Oct-4, Sox2, Klf4 and c-Myc) [47]. These "first-generation" iPSCs were questionable because viruses were used to introduce reprogramming factors, therefore raising concerns about neoplastic transformation. The use of episomal gene delivery, excisable transgenes, or cell-permeant recombinant proteins have been introduced quite recently to overcome this issue. In the most successful experimental setting, cardiac differentiation was obtained by reprogramming mouse embryonic fibroblasts into cardiomyocytes [48, 49] with the so-called Yamanaka factors (Oct-4, Sox2, Klf4 and c-Myc) to initiate reprogramming, subsequently blocking signaling through the JAK-STAT pathway, which is required for pluripotency in the mouse, and by finally adding the cardiogenic factor BMP-4. This method activated the cardiac lineage program and, within 2 weeks, substantial numbers of beating colonies were observed. Despite some challenges, this is an exciting new avenue of research and could be used in autologous cell therapies.

The emergence of personalized medicine has raised especially in complex diseases such as cancer, cardiovascular and neurodegenerative diseases in which individual responses often complicate the development of therapeutic treatement. In the growing field of research on extracellular vesicles (EVs), exosomes (EXOs), a specific type of EVs, have been proposed as an interesting tool. Both healthy and unhealthy cells secrete vesicles into the extracellular space [50, 51]. EVs entrap lipids, proteins and nucleic acids which can mediate different biological functions. The extracellular vesicles (EVs) are mainly clas-

Table 14.1 First generation stem cell-based studies in ischemic heart disease

Name of the study	Acronym, PI	Year	Clinical setting	Stem cell type	Cell harvesting and manipulation	Cell delivery Number of cells and route of administration	Effect on Ejection Fraction (EF)
Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction	TOPCARE-AMI (Assmus, B. et al.)	2002	AMI, Time of cell administration: 3 days after MI	BMMNCs	Density gradient and cell culture	7.3×10^6, intracoronary injection (3 injections of 10 ml each in X-VIVO™ 10 medium)	No change by LV angiography
Bone-marrow-derived cell transfer after ST-elevation myocardial infarction	BOOST (Woller, K. C. et al.)	2004	AMI, Time of cell administration: 5 days after MI	BMMNCs	Density gradient (Ficoll) centrifugation	2.4×10^9, intracoronary injection (4 or five injections in heparinized saline)	Positive by MRI
Reinfusion of enriched progenitor cells and infarct remodeling in acute myocardial infarction	REPAIR-AMI (Schachinger, V. et al.)	2006	AMI, Time of cell administration: 3–6 days after MI	BMMNCs	Density gradient (Ficoll) centrifugation	1.98×10^8, intracoronary injection in X-VIVO™ 10 medium and 20 % autologous serum	Positive by LV angiography
Autologous stem cell transplantation in acute myocardial infarction	ASTAMI (Lunde, K. et al.)	2006	AMI, Time of cell admin–stration: 4–7 days after MI	BMMNCs	Density gradient (Ficoll) centrifugation	6.8×10^7, intracoronary injection in heparin-treated plasma	No change by CT (SPECT)
Effects of intracoronary injection of mononuclear bone marrow cells on left ventricular function, arrhythmia risk profile, and restenosis after thrombolytic therapy of acute myocardial infarction	FINCELL (Lunde, K. et al.)	2008	AMI, Time of cell administration: morning of the day of PCI	BMMNCs	Density gradient (Ficoll) centrifugation	3.6×10^9, intracoronary injection in unspecified medium and 50 % autologous serum	Positive by echocardiography

Table 14.1 (continued)

Name of the study	Acronym, PI	Year	Clinical setting	Stem cell type	Cell harvesting and manipulation	Cell delivery Number of cells and route of administration	Effect on Ejection Fraction (EF)
Intracoronary infusion of mononuclear cells from bone marrow or peripheral blood compared with standard therapy in patients after acute myocardial infarction treated by primary percutaneous coronary intervention	HEBE (Hirsch, A. et al.)	2011	AMI, Time of cell administration: <8 days after MI	BMMNCs	Density gradient (Lymphoprep™) centrifugation	2.96×10^8, intracoronary injection in sodium heparin and 4 % human serum albumin	No change by MRI
Effect of the use and timing of bone marrow mononuclear cell delivery on left ventricular function after acute myocardial infarction	TIME (Traverse, J. H. et al.)	2012	AMI, Time of cell administration: 3 or 7 days after MI	BMMNCs	Sepax® cell separation system	1.50×10^8, intracoronary injection in normal saline and 5 % Human Serum Albumin	No change by MRI
Effect of intracoronary delivery of autologous bone marrow mononuclear cells 2–3 weeks following acute myocardial infarction on left ventricular function	Late-TIME (Traverse, J. H. et al.)	2011	AMI, Time of cell administration: 2–3 weeks after MI	BMMNCs	Sepax® cell separation system	1.50×10^8, intracoronary injection in normal saline and 5 % Human Serum Albumin	No change by MRI
SWiss multicenter intracoronary stem cells study in acute myocardial infarction	SWISS-AMI (Sürder, D. et al.)	2010	AMI, 5–7 days or 3–4 weeks after MI	BMMNCs	Density gradient (Ficoll) centrifugation	$\geq 5 \times 10^7$, intracoronary injection in 10 ml of X-VIVO 10 with 20 % of autologous serum	No change by Cardiac Magnetic resonance (CMR)

Cardiac stem cells in patients with ischaemic cardiomyopathy	SCIPIO (Bolli, R. et al.)	2011	CHF, infUsed 4 months after CABG surgery	c-kit-positive resident cardiac stem cells	Cell culture and magnetic sorting (MACS)	0.5×10^6–1×10^6, intracoronary injection in PlasmaLyte A	Positive by Echocardiography
Intracoronary cardiosphere-derived cells for heart regeneration after myocardial infarction	CADUCEUS (Makkar, R. R. et al.)	2012		cardiosphere-derived cells	Cell outgrowth from cardiac biopsy, and cardiosphere formation	12.5×10^6–25×10^6, intracoronary injection in a saline solution containing heparin (100 U/mL) and nitroglycerin (50 μg/mL)	Positive by MRI
The prospective randomized study of mesenchymal stem cell therapy in patients undergoing cardiac surgery	PROMETHEUS (Hare, J. M. et al.)	2014	Chronic ischemic left ventricular dysfunction secondary to MI	BMMSCs	Bone marrow aspiration from the iliac crest, isolation and expansion of MSCs	2×10^7–2×10^8 MSCs intramyocardial injection, transepicardial delivery, in PBS buffer or Plasma-Lyte A supplemented with 1 % human serum albumin	Positive by MRI

sified as exosomes (EXOs), microvesicles (MVs), retrovirus-like particles, and apoptotic bodies (APOs) according to their origin. In particular, EXOs are currently defined as cup-shaped nanovesicles about 30–100 nm wide that originate within the endosomal network and can be found in body fluids, including urine, saliva, blood, breast milk, and cerebrospinal fluid. They are of particular interest to the study of complex diseases for their contribution to long-range intercellular communication. Moreover, the experimental observation that the production of EXOs is increased in association with disease, and the fact that their content varies with disease state, led to the evaluation of the potential of EXOs as biomarkers and vehicles to regulate the spread of disease [52, 53]. Current experimental evidence supports a central role of EXOs in cancer development, progression, metastasis, and drug resistance through promotion of carcinogenesis and tumor growth, angiogenesis, modulation of the tumor microenvironment, modulation of immune responses, and induction of mechanisms to acquire therapy resistance. Despite some controversial results found in literature, efforts are being made to resolve the lack of consensus on the methods of isolation and rigorous criteria to characterize them highlighting their potential importance in clinical applications, especially in the cancer field. As our understanding of the biology of EXOs increases, the opportunity of employing EXOs as nanocarriers for immunotherapeutics or vaccines, as angiogenesis modulators and for many other applications is intensely being evaluated.

A critical issue which emerged from the "first generation" of cell therapy-based clinical trials for cardiovascular regenerative medicine is the overall low retention rate after cell delivery to the myocardium. In order to exploit their full therapeutic potential, cells need to be retained at the site of injury, and their long-term survival is a fundamental characteristic to enable them to fully achieve regeneration. To overcome this problem, the concept of 3D cell culture has been proposed to enhance cell engraftment based on the mimicking of the *in-vivo* tissue microenvironment. The growth of 3D cell culture is essential to consider cells in their comprehensive context because in nature, i.e. within the body, a cell is always surrounded by its niche or microenvironment that has profound infuence on its properties. Most commonly, this involves the use of porous, biodegradable scaffolds onto which cells are seeded, but other approaches include the integration of cells into hydrogels or creating scaffold-free tissues composed only of cells and the matrix they secrete. Of great interest is the use of so-called microtissues or spheroids [54, 55]. Microtissues are composed of 500 up to 10,000 cells and were initially used as a model to elucidate tumor biology; they have recently evolved from their known role as *in vitro* models to a novel role as therapeutic agents. In the setting of cardiovascular applications, microtissues may provide an optimal strategy to implant stem cells into the injured heart since they can be transplanted via transcatheter delivery because of their scaffold-free nature. The cardiomyocytes used in tissue engineering are commonly immature cells or stem cells [56]. In order to sustain an adult workload, these cells will need to organize into the cable-like structure of myocardium and increase in size more than ten-fold. There is an ongoing debate whether this maturation should take place before or after transplantation. Certainly, when myocyte-only constructs are transplanted the tissue survives poorly. When vascular endothelial cells and stromal cells are also included, the mixed population creates a synergic effect: the endothelial cells form networks resembling a primitive vascular system, and the stromal cells create a matrix to provide mechanical integrity. Interestingly, the body mostly reacts to the surface topography of the implanted material (whether it is smooth or contains pores and grooves) rather than to its chemical composition. More complex topographies provide less inflammation, less scarring, and increased angiogenesis. Although this rapidly emerging field of tissue engineering has been extensively studied so far mainly in preclinical models, such as constructs of engineered heart tissue from neonatal cardiomyocytes sutured to the surface of infarcted rat hearts, initial studies are promising [57].

14.3 Biobanking: A Clinical Need for Cardiac Regenerative Applications

As previously mentioned, the importance of stem cell biobanking for cardiovascular diseases lays on the opportunity of delivering an "off-the-shelf" product readily available according to the patient's needs. This applies in particular in the acute clinical setting, when the rapid administration of an autologous stem cell therapy product is critical to beneficially affect the clinical outcome.

The intrinsic nature of biobanking activities related to standardization of protocols, evaluation of isolation yields, factors that affect characterization and storage of samples, argues in favor of biobanks playing a central role in the advancement of stem cell-based research and mostly in the development of stem cell-based clinical applications. Biobanks, intended as institutions applying standardized protocols for the collection, processing, storage and release for administration of human stem cells are the ideal infrastructure acting as a service platform to overcome current research hurdles and mainly to expedite the progression of stem cell-based applications in the treatment of cardiovascular diseases for the upcoming era of precision and personalized medicine [58–60].

In general, ice formation during cryopreservation may be a crucial and critical event because it can produce severe cell damage, both during freezing and thawing processes. Vitrification is the transformation of liquids into a solid state. This can be achieved by rapid cooling in combination with a cryoprotective agent with a high osmolarity which prevents cryoinjury due to ice formation. Commonly, the slow-cooling approach is accompanied by a low concentration of the cryoprotective agent to reduce cytotoxicity. Of note, every cell type has its specific optimal cooling rate. Interestingly, gap junctions propagate intercellular ice formation at a given temperature, and may lead to increased intracellular damage and finally to cryoinjury. In addition to the general problems related to the cryopreservation of single cells, such as cryoinjury caused by ice formation, there are additional considerations for the cryopreservation of 3D tissues [54]. Importantly, the cryoprotective agent should be distributed homogeneously within the entire tissue to protect the cells from intracellular ice formation, which can lead to severe cell damage and cell death. For some cell types, such as embryonic stem cells, freezing in aggregates results in higher cell number recovery after thawing compared to single-cell suspensions. Beneficial effects are attributed to the cryoprotective role of the surrounding extracellular matrix.

Historically, cardiac valve cryopreservation has long been used for the treatment of valvular heart disease. Valvular heart disease is still a significant cause of morbidity and mortality worldwide. In the United States, approximately 60,000 valve replacement operations are performed annually, and valve replacement surgery is efficacious [1, 61]. State-of-the-art valves used in the clinical setting include mechanical valves and biological valves such as glutaraldehyde fixed xenografts (derived from animals) or cryopreserved homografts (derived from human donors). Glutaraldehyde-fixed or cryopreserved biological valves do not require anticoagulation treatment, however, they represent non-viable prostheses. They basically lack the ability to grow, to repair, or to remodel. This raises severe problems specifically related to pediatric patients. Approximately 1 % of all newborns have congenital heart defects, and many of them require surgical heart valve replacement. Since currently available valves cannot grow with the young patients, repeated surgical replacement operations have to be performed with exponentially increased morbidity and mortality. Given these crucial observations, the importance of providing patient-specific cryopreserved cardiomyocytes is imperative. For this important reason, biobanking patient-specific stem cells is of utmost importance [62, 63].

Here we report some specific recent examples in which cardiac stem cell banking has been particularly successful in terms of its therapeutic application.

As an important translational step towards creating an effective clinical therapy, Chong et al.

[64] investigated the ability of exogenously delivered human Embryonic Stem Cell-derived Cardiomyocytes (hESC-CMs) to engraft and electrically couple to infarcted host myocardium in a non-human primate (NHP) model of myocardial infarction (MI). This model provides a heart size and rate more comparable to the human and required delivery of about 1×10^9 cells. Feasibility of this large-scale hESC-CM delivery required cryopreservation of cells, a method which was previously validated by the authors in an established immunodeficient mouse model of MI. No adverse effects of cryopreservation on hESC-CM were reported Therefore, delivery of cryopreserved hESC-CMs appears to be a sound strategy for large- scale transplantation in large animals or in humans. In particular, seven pigtail macaques (Macaca nemestrina) were used for this study without randomization. MI was created by percutaneous ischemia-reperfusion 2 weeks prior cell administration, and immunosuppression started 5 days prior to hESC-CM delivery. In order to obtain differentiated cultures containing beating cardiomyocytes, Activin A and Bone Morphogenetic Protein 4 (BMP4) were applied to defined, serum-free, monolayer culture conditions. hESC-CMs were collected and cryopreserved after 16–20 days of CM differentiation. One day before collection for delivery, cells were subjected to a pro-survival "cocktail" (PSC) protocol, to enhance engraftment after transplantation. Briefly, cultures were heat-shocked and exposed to RPMI-B27 medium supplemented with IGF1 (100 ng ml^{-1}) and cyclosporine A (0.2 mM). One day later, cultures were collected with Trypsin-EDTA, and neutralized with a Trypsin inhibitor. Cells were washed and resuspended by slowly adding cryopreservation solution CryoStorTM CS-10 (BioLife Solutions Inc.). The final cell concentrations were approximately $5–10 \times 10^6$ cells in 0.25 ml/vial or $4–8 \times 10^7$ cells in 1.5 ml/vial. Cells were frozen using a controlled rate freezer at −1 °C/min before the temperature reached −40 °C, and −5 °C/min from −40 °C to −80 °C. Vials were then transferred to a liquid nitrogen tank after reaching −80 °C. To thaw the cells, the vials were incubated in a water bath at 37 °C until no ice crystals were visible. The cell suspension was slowly diluted with RPMI/B27. The cells were subjected to flow cytometric analysis, cultured on Matrigel-coated plates in RPMI/B27 or prepared for transplantation. Immediately before transplantation, cells were suspended in a 1.5 ml volume (per animal) of modified PSC consisting of 50 % (v/v) growth factor–reduced Matrigel, supplemented with Bcl-XL BH4 (50 nM), cyclosporine A (200 nM), IGF1 (100 ng ml^{-1}) and pinacidil (50 mM). hESC-CMs were delivered into the infarct region and surrounding border zones under direct surgical visualization using a method optimized to improve cell retention. No macroscopic or microscopic evidence of teratoma or other tumor was detected. All hESC-CM- treated monkeys showed significant remuscularization of the infarct areas. Graft size, calculated on the basis of green fluorescence protein (GFP) expression, ranged from 0.7 to 5.3 % of the LV, averaging 40 % of the infarct volume. Greater than 98 % of engrafted human cells expressed the sarcomeric protein α-actinin, indicating that almost all graft cells were cardiomyocytes. Importantly, these hESC-CM displayed increased maturation from 14 d to 84 d, evidenced by increased myofibril alignment, presence of sarcomeres and increase in cardiomyocyte diameter. This remarkable study demonstrates that hESCs can be grown, differentiated into cardiomyocytes and cryopreserved at a scale sufficient to treat a large animal model of MI. With further refinements in manufacturing, scale up to trials in human patients appears feasible. Despite previous studies of the same group in smaller animal models such as mice, rats, and guinea pigs gave no evidence of arrhythmias after hESC-CM engraftment [45], arrhythmias were consistently observed in the non-human primate study. The two most likely reasons appear to be differences in heart size and rate. Regarding size, the larger hearts of adult macaques (37–52 g) compared to hearts of mice (0.15 g), rats (1 g) and guinea pigs (3 g) allows for more hESC-CMs to be delivered, and resultant grafts are approximately ten-fold larger than the largest obtained in other species. Another important factor is the species-specific heart rates (macaques: 100–130 beats/min, vs. guinea-pigs: 230, rats: ~400, mice

~600). Faster spontaneous rates will favor ventricular capture from native conduction pathways. These factors are relevant to clinical translation given that the human heart is larger (300 g) with a slower basal rate (70 beats/min) than macaques used in this study. Since ventricular arrhythmias can be life-threatening, they need to be understood mechanistically and managed to guarantee safe clinical translation. Nevertheless, the extent of remuscularization and electromechanical coupling documented in this study with cryopreserved ESC-derived cardiomyocyes encourage further development of human cardiomyocyte transplantation as a clinical therapy for heart failure. Of note, this methodology does not require feeder cells or conditioned medium for the maintenance of undifferentiated hESCs, and cardiomyocyte differentiation is achieved by treatment of adherent cultures with only two growth factors, activin A and BMP-4, in a defined serum-free medium. The differentiated cells express appropriate cardiomyocyte markers and display electrophysiological phenotypes expected for cardiomyocytes. Furthermore, to our knowledge, this is the first time that efficient cryopreservation of hESC-derived cardiomyocytes is performed. The cryopreserved cardiomyocytes were shown to survive both *in vitro* and *in vivo* and have the ability to form viable grafts. The cryopreserved cells are capable of surviving even in the central regions of the infarct region for as long as 4 weeks after the transplantation. Together, this differentiation and cryopreservation technology will offer significant advantages to support a robust process for the production of cardiomyocytes on a scale suitable for cellular therapy. Mainly, highly specific and scalable cardiomyocyte differentiation of hESCs maintained in serum-free medium has been demonstrated, the resulting differentiated cells possess the characteristics of human cardiomyoyctes and have shown successful engraftment of the cryopreserved cardiomyocytes after transplantation. Further optimization of the process, such as the use of completely xeno-free reagents, will be key for safer clinical applications [65–69].

Umbilical Cord Blood (UCB) stored in biobanks has now been quite frequently used to treat hematopoietic malignancies, marrow failure, metabolic diseases, and immunodeficiency disorders. The plasticity of UCB cells and its readily availability as stored material have also encouraged its broader regenerative applications, such as the treatment of spinal cord injury and chronic wounds, including clinical trials on other non-hematopoietic diseases, such as epidermolysis bullosa, and neonatal hypoxic ischemic encephalopathy [70]. More recently, the potentiality of UCB has been explored in animal models of cardiac infarction, diabetes, and various neurological diseases. Specifically, in animal models of MI, direct injection of CD34+, CD133+, or mononuclear cells (MNCs) from UCB into the necrotic myocardium or into the border zone after coronary artery ligation, resulted in reduced infarction size and improved left ventricular (LV) function. In addition to the CD34+ or CD133+ populations and MNCs of UCB, UCB-derived Unrestricted Somatic Stem Cells (USSCs) have also been applied to treat MI in animal models. USSC is the first identified UCB stem cell population with intrinsic pluripotent differentiation potential. These $CD45^{neg}/CD34^{neg}$ cells were isolated from UCB based on their outgrowth in the presence of dexamethasone. USSCs could be differentiated *in vitro* into bone, cartilage, adipocytes, hematopoietic cells, and neural cells, and *in vivo* they might form myocardial cells. In a chronic porcine model of myocardial infarction (MI), direct injection of USSCs to the border of infarction 4 weeks post-MI resulted in reduction of infarct size, reduction in end-diastolic volume, and improvement in LV ejection fraction. The engrafted USSCs could be detected 4 weeks after transplantation. In contrast, in an acute porcine model of MI, injection of USSCs led to a surprising complete prevention of scar formation and functional improvement. However, long-term survival of the transplanted cells and cell-related regeneration of the myocardium were not observed. In contrast, in a similar porcine model of MI, Moelker and colleagues injected USSCs into the infarct-related coronary artery 1 week post-MI. Magnetic resonance imaging failed to document any improvement in regional or global LV function. The transplanted cells survived only

in the infarct border zone at 5 weeks and did not express any cardiomyocyte or endothelial markers. Additionally, intracoronary administration of USSCs was accompanied by increases in inflammatory cell infiltration and calcification in the infarct zone, as compared to control animals [71–73]. Although encouraging results have been obtained in many preclinical studies of UCB cell therapy, challenges remain to be overcome. Of note, UCB stem cells offer many practical advantages, such as relative ease of collection, minimal risk to the donors, and mostly the opportunity to bank carefully screened samples for administration. Furthermore, there has been accumulating evidence for the existence in UCB of very primitive stem cells that share ESC properties but do not present a risk for teratoma formation upon transplantation. Therefore, these primitive UCB-derived stem cells could be a suitable alternative to ESCs for cardiac tissue regeneration.

A small pilot study was performed to test the safety and feasibility of freeze-controlled cryopreserved bone marrow-derived mesenchymal stromal cells (BM-MSC) to treat severe dilated ischemic cardiomyopathy [74]. MSCs were isolated from BM aspiration, expanded, harvested, and cryopreserved using 90 % autologous plasma and 10 % dimethyl sulfoxide (DMSO). The cells were frozen gradually using a rate-controlled freezer to –90 °C, and then transferred into vapor phase liquid nitrogen for storage. Three consecutive patients with end-stage ischemic heart failure were enrolled in the study. Patients underwent concurrent coronary artery bypass graft (CABG) with intramyocardial MSC injection. On the day of injection, MSCs were thawed in a water bath, washed once and then resuspended in sterile saline solution. MSC suspension was transferred into a 10 mL luer-lock syringe for injection. The whole duration of cryopreservation to the day of surgery was 5–16 days. The viability of the cells in the final suspension was higher than 90 % in all three cases. There was significant improvement in cardiac function, scar reduction and increased wall thickness for all patients on cardiac magnetic resonance imaging at 6 months compared with baseline. No arrhythmias were reported. The preliminary data of this small pilot study seem to suggest that intramyocardial injection of cryopreserved BM-MSCs is feasible and safe for ischemic cardiomyopathy. Cryopreserving autologous MSC in a rate-controlled manner does not lead to significant cell death and is an important practical opportunity to circumvent the timing of preparation of stem cells for injection.

On the overall, biobanking can be defined as a long-term storage and preservation activity that includes and standardizes collection, processing, and release of high-quality biological specimens needed for future scientific investigation or for therapeutic administration. Each sample deposited in a biobank possesses two main features: the biological material (collected, processed, and stored), and the database that contains information regarding demographic and clinical data for every sample in the inventory. Peripheral blood, plasma, serum, blood-derived cell types, solid tissues, urine, saliva, RNA, and DNA are among the most common biological samples being processed and stored in biobanks. The increasing relevance of extracellular vescicles (EVs), and in particular of EXOs in cancer, metabolic diseases, and other complex diseases strongly suggests the incorporation of EXOs in biobanks to provide a significant advance in the knowledge, diagnoses and treatment of these particular diseases in clinical research [58]. Most clinical studies on EXOs have a diagnostic purpose. The number and wide variety of clinical trials in this area indicate the high impact of EXOs mostly in clinical cancer research. In a clinical setting, and due to the plasticity of EXOs landscape to environmental and pathological conditions, investigators envision that their specific markers could be used to obtain a patient Exogram (particular combination of EXO-associated markers for a particular individual at a determined moment) with the purpose of precisely diagnosing or monitoring the response to treatments as part of precision medicine programs. In the context of associating patient clinical data with relevant Exogram markers, biobanks can provide the EXO-based research field an excellent opportunity to achieve its maximum development by providing the ideal platform connecting researchers and clinicians.

Biobanking of EXOs could have a relevant role in the growing field of clinical research [58, 75]. Currently, there are no strictly defined conditions for storing and also isolating EXOs. The only established guidelines are those identified for the isolation of platelets-derived EVs. There is no specific information regarding the collection and storage of EXOs, optimal time, temperature, storage period, freezing-thaw cycles, thawing conditions, or other variables. Preliminary studies included the evaluation of the stability of EXOs at different temperatures finding that there was an advantage in storing EXOs at − 80 °C. However, this may vary according to EXOs prepared from different sources and by different isolation procedures. In addition, the particular lipid composition of EXOs, which is also source-dependent, is expected to have an impact on optimal cryopreservation. Biobanking relies on standardization of conditions that result in sample quality homogeneity and allows proper sample characterization for release [58, 76]. The current difficulty to define EXOs and the hurdles to establish and implement standardized protocols for their isolation and storage, let us understand that there is an urgent need for biobanks to assume an active role also in this research area.

14.4 Conclusions

Many decades of remarkable research activity have greatly increased the understanding and the potentiality of the field of stem cells biology. The availability of different cardiac stem cell sources and stem cell types are giving us the opportunity of regenerating the diseased myocardium. First generation clinical trials on cardiovascular diseases have made an attempt to establish techniques for cell isolation, expansion, delivery, and for determining safety, feasibility, and preliminary efficacy in humans. We are also aware that in this exciting new era of regenerative medicine many challenges still have to be overcome. To name a few, adult progenitor cells still need to provide higher yields of functional cardiomyocytes, pluripotent stem cells have to demonstrate to be employed without the risk of tumorigenesis; additionally, the question of allogeneic vs. autologous use remains open. The need of expanding autologous stem cells to clinically relevant numbers at the time needed to be administered as cell therapy products precludes their use in any acute setting. Allogeneic cells will then provide the only "off the shelf" product, but we need to learn how to properly manage the immune response to prevent their immune rejection. All of these efforts will be advanced by implementation of biobanking activities which promote readily available and high quality controlled products. In this environment, biobanks represent a key resource both for research-based studies, and mostly for the manufacturing of readily available cell therapeutic products. Therefore, it is not surprising that clinics or academic and industrial counterparts have demonstrated an increasing interest in biobanking in the recent years. Concerning the field of cardiac regenerative medicine, the search for innovative treatment options to prevent adverse ventricular remodelling following AMI has been at the forefront of clinical research in cardiology. Evidence from the published clinical trials indicates that adult cell-based therapies seem to be safe. Hence the ability to bank autologous stem cells for later use has the potential to become a significant keystone in the development and implementation of regenerative and personalized medicine strategies. Regenerative medicine applications will most probably benefit from biobanking in order to allow this clinical effort to reach its fullest potential and serve those with the greatest and most immediate cardiovascular need.

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