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Ex-Vivo Expansion of Cardiac Stem Cell Therapy Products for Clinical Use: The Importance of Moving towards the Optimization of Process Development

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Abstract

The interest in stem cell-based therapies is rapidly increasing given the general progressive population ageing and as a consequence of the massive incidence of Heart Failure (HF). This urgent clinical need has prompted innovation in the field of stem cell applications towards the effort of developing new regenerative approaches.

Introduction

Preliminary studies based on stem cells were initially applied to the clinical setting of acute myocardial infarction (AMI) with the purpose of delivering a cardioprotective effect. The field has then rapidly expanded to embrace chronic heart failure as a cardiorestorative therapy [1]. For the easiness of collection as hematologic tissue, Bone Marrow (BM)-derived stem cells were utilized as the primary source of these applied therapies. Specifically, unselected Bone-Marrow Mononuclear Cells (BMMNCs) fuelled the first generation of clinical trials focused on cardiac regeneration. In the setting of AMI, the initial stem-cell based studies showed consistent although modest improvement in cardiac function and scar repair. In the largest European trial REPAIR-AMI, LVEF was significantly increased in the treated group compared to the patients receiving placebo [2]. The BOOST and the FINCELL trials further confirmed the significant improvement in LVEF by angiography in the stem cell treated groups [3,4]. By contrast, the latest HEBE trial completed in 2011, the Swiss-AMI trial and the ASTAMI trial reported similar or reduced effect of BMMNC therapy and the placebo [5-7]. Of note, in the HEBE trial, cells were processed for an undefined period of time and were delivered to patients in a heparin solution containing human serum albumin. Heparin as a reagent used in the harvesting of BM or in BMMNC delivery was later shown to disrupt the CXCR4–SDF-1 axis, thus reducing the chemotactic and functional capabilities of BMMNCs [8].

The different outcomes and the underlined variability in the methodologies employed in these early phase trials prompted the efforts for stem cell process development optimization. On the overall, cell manufacturing before implantation has been demonstrated to be crucial to provide the desired effect of cardiac regeneration.

After BMMNCs, further advances in stem cell therapies included resident adult stem cells in the heart or lineage-specified cells to provide the therapeutic influence on the damaged tissue. Resident cardiac stem cells (CSCs, c-kit-positive cells) isolated from right atrial appendages were employed in the Phase I SCIPIO trial. One-year follow-up outcome suggested beneficial effect of CSC therapy on LVEF and on infarct scar size in the treated patients [9]. The CADUCEUS autologous cardiosphere-based trial also confirmed therapeutic benefit on infarct scar dynamics [10], and the ALLSTAR and the RECONSTRUCT studies will further investigate the efficacy of allogenic cardiosphere-derived cells in AMI. An hybrid approach combining basic fibroblast growth factor (bFGF) together with autologous CSCs was utilized in the ALCADIA study. This combination approach of growth factors and stem cells prompted a new trend in regenerative cardiology. Within this context, an interesting alternative approach includes the methodology to guide adult multipotent stem cells towards cardiac lineage through manipulation of their culture environment as was assessed in the C-CURE trial [11] with Cardiopoietic human Mesenchymal Stem Cells (CP-hMSCs) (Table 1).

Discussion

These studies among others led to the observation that bioprocessing for regenerative cardiology is a new investigation field. More specifically, once the therapeutic cell source is determined for an intended clinical application, the safety and efficacy of the stem cell product has shown to be significantly influenced by cell bioprocessing protocols. As a consequence, the development of robust production processes by optimizing culture variables is critical to manufacture biologics that retain desired regenerative properties while minimizing the potential risks [12,13]. There is not, as yet, consensus as to which will be the best cell for cardiac clinical applications and it is likely that we will not refer to one single cell type. As a direct result of the knowledge obtained through the first generation of clinical applications of stem cell-based therapeutics, a new focus has arisen on the development of reliable and robust culture procedures that will produce identical batches of cells for a given clinical application [14]. Further clarification of dosing and

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Table 1: First generation stem cell-based studies in ischemic heart disease

Study name	Study ID, PI	Year	Stem Cell Type	Cell manipulation	Cell Number and route of administration	Effect on Ejection Fraction (EF)
Reinfusion of Enriched Progenitor Cells And Infarct Remodeling in Acute Myocardial Infarction	REPAIR-AMI Schachinger V. et al. [2]	2006	BMMNCs	Density gradient (Ficoll) centrifugation	1.98 × 10^8, intracoronary injection in X-VIVO™ 10 medium and 20% autologous serum	Positive by LV angiography
Bone-marrow-derived cell transfer after ST-elevation myocardial infarction	BOOST Woller K. C. et al. [2]	2004	BMMNCs	Density gradient (Ficoll) centrifugation	2.4 × 10^9, intracoronary injection (4 or five injections in heparinized saline)	Positive by MRI
Effects of intracoronary injection of mononuclear bone marrow cells on left ventricular function.	FINCELL Huikuri, H. V. et al. [3]	2008	BMMNCs	Density gradient (Ficoll) centrifugation	3.6 × 10^9, intracoronary injection in unspecified medium and 50% autologous serum	Positive by echocardiography
Autologous Stem Cell Transplantation in Acute Myocardial Infarction	ASTAMI Lunde K. et al. [6]	2006	BMMNCs	Density gradient (Ficoll) centrifugation	6.8 × 10^7, intracoronary injection in heparin-treated plasma	No change by CT (SPECT)
Intracoronary infusion of mononuclear cells from bone marrow or peripheral blood compared with standard therapy in patients after acute myocardial infarction treated by primary PCI	HEBE Hirsch A. et al. [5]	2011	BMMNCs	Density gradient (Lymphoprep) centrifugation	2.96 × 10^8, intracoronary injection in sodium heparin and 4% Human Serum Albumin (HSA)	No change by MRI
SWiss multicenter Intracoronary Stem cells Study in Acute Myocardial Infarction	SWISS-AMI, Sürder D. et al. [7]	2010	BMMNCs	Density gradient (Ficoll) centrifugation	≥ 5 × 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. [8]	2011	c-kit-positive resident Cardiac Stem Cells	Cell culture and Magnetic sorting (MACS)	0.5 × 10^6 to 1 × 106, intracoronary injection in PlasmaLyte A	Positive by Echocardiography
Intracoronary cardiosphere-derived cells for heart regeneration after myocardial infarction	CADUCEUS Makkar R.R. et al. [10]	2012	Cardiosphere-derived cells	Cell culture of primary explant to obtain 3D cardiospheres.	1.3 × 10^7 to 2.5 × 10^7, intracoronary injection in in normal saline, heparin (100 U/ml) and nitroglycerin (50 µg/ml)	No change by cMRI. (Further advancement in ALLSTAR and RECONSTRUCT trials)
Cardiopoietic stem Cell therapy in heart failURE	C-CURE, Bartunek, J. et al. [11]	2013	Cardiopoietic hMSCs	Cell culture guidance and lineage specification	6.0 × 10^8 to 1.2 × 10^9, NOGA guided injections of 5% HAS in lactated Ringer's solution.	Positive by Echocardiography. (Further advancement in CHART-1 and CHART-2 trials)

Abbreviations: MI = Myocardial Infarction; AMI = Acute Myocardial Infarction; BMMNCs= Bone Marrow Mononuclear Cells; SPECT = Single-Photon Emission Computed Tomography; LV= Left ventricular; MRI = Magnetic Resonance Imaging; PCI = Percutaneous Coronary Intervention; hMSCs = human Mesenchymal Stem Cells; HSA = Human Serum Albumin.

comprehensive characterization of stem cells is of utmost importance, as several trials have highlighted an inverse relationship between cell dose and clinical outcomes [15].

Stem and progenitor cells are plastic by definition and they constitute a live and functional reagent to be manufactured and administered to patients to exert a therapeutic effect. The successful process development of current laboratory-based protocols to the clinics requires the establishment of methods to achieve control, reproducibility, equivalence and safety of the stem cell product. Towards this objective, our group is focusing on the comprehensive approach of translating stem cell-based products to the clinics.

Cell culture variables are represented by the formulation of the media (basal media and their supplements), adhesion substrates, cell seeding density, together with the physiochemical environment (dissolved oxygen and carbon dioxide concentrations, temperature, pH, osmolality). Obviously, the defined nature of media optimized for isolation and expansion of stem cell therapy products facilitates the development of robust, clinically acceptable cells. Furthermore, in order to culture cells long-term the defined basal medium must be supplemented with several factors. Serum, of animal or human origin, is the most widely used among them. In particular, the development of cultures free of Fetal Bovine Serum (FBS) is warmly recommended from the ethical and scientific point of view. FBS composition is complex, its batch-to-batch variation, and the likelihood of contamination causes phenotypical differences in cell cultures and therefore variations in the derived cell therapy products [16,17]. To efficiently translate cell culture products to the clinics, cells should possibly be seeded in serum-free media just after isolation from the tissue of origin as a primary culture and then, according to the different protocols, expanded in the same serum-free culture medium or further supplemented with specific components, normally for a limited number of passages (on the average ≤ P5) to limit the risk of senescent or transformed phenotype. In order to develop serum-free cultures, cell adhesion is supported by the use of defined substrates. Well-defined recombinant substrates which are widely used such as Vitronectin or Laminin might be used to sustain cell attachment in serum-free conditions [18]. However, another valuable approach is represented by more specific animal component-free adhesion substrates which mimic the cardiac extracellular matrix, such as Fibronectin or Hyaluronic acid. Besides, given that each cell type has its own requirements for *ex vivo* expansion, further optimization of media formulation with defined specific growth factors is achieved to increase cell proliferation and activate or maintain specific cellular functions, for example their stemness profile [19]. Most growth factors are highly cell type specific. IGF-1 or Hepatocyte Growth Factor (HGF) have been described to exert a favorable effect on human Cardiac Stem Cells rejuvenation and mobilization in the damaged or senescent heart [20,21]. Other growth factors are known to be more pleiotropic and therefore can provide positive effects on several different cell types such as Fibroblast Growth Factor-2 or Stem Cell Factor (SCF). In general, a combination of animal component-

free recombinant widely distributed and more specific growth factors might be taken into consideration for the development of a stem cell therapy product. In addition, to fine tune the design of a specific cell therapy product, the supplementation of lipids, antioxidants and/or specific vitamins might be needed to obtain the desired cell type. Retinoic acid (vitamin A) is an additive required in cell culture media for a number of epithelial cell types. Vitamin E and Ascorbate (vitamin) are acting as antioxidants. Other antioxidants commonly used in serum-free media formulations are β-mercaptoethanol and selenium [22]. The importance of the conditioned medium produced by stem cells should also be considered in the development of serum-free media: stem cells in culture may release growth factors thereby stimulating their own proliferation and that of nearby cells.

Conclusion

The development of tailored well-defined serum-free media provides further advantages in the enrichment of a desired cell type in primary cultures therefore producing a more homogeneous outcome and preventing potential undesired differentiation of multipotent progenitors. On the overall, it is clear that this approach will increase clinical efficacy and will provide the highest degree of safety for the patient. This is to underline the fact that Quality Assurance is becoming increasingly important. Good laboratory practice (GLP) and Good Manufacturing Practice (GMP) are now established standards for the process development of stem cell therapy products. Good Cell Culture Practice (GCCP) is now an attempt to develop a common standard for *in vitro* methods. In this view, the implementation of the use of chemically defined, animal component-free media is part of the GCCP [23]. In conclusion, as we move to trials based on next-generation stem cell-based technologies for cardiac regeneration, a comprehensive optimization of cell process development is needed to ensure optimal efficacy and increased equivalence in the clinical setting.

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