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IN-VITRO PROLIFERATION AND ENRICHMENT OF CD34+ AUTOLOGOUS HAEMATOPOIETIC STEM CELLS, OBTAINED FROM PATIENTS WITH END-STAGE LEFT VENTRICULAR FAILURE

P M Athauda arachchi [1,2] and AI Kuruppu [2]

General Sir John Kotelawala Defence University[1] and Durdans Hospital, Colombo[2]

ABSTRACT

With a rising global prevalence of end-stage heart failure and a limited availability of cardiac transplantation programmes or left ventricular assist devices in many parts of the world, there is a renewed interest in potential alternative treatment options.

Along with other groups, we have previously demonstrated the beneficial effect of intracoronary autologous bone marrow derived peripheral haemopoietic stem cell transplantation, to improve myocardial contractility in end-stage heart failure.

We now assess the ability of these cells to expand in numbers in-vitro, to achieve enrichment of stem cell counts, using standard cell culture methods and flow- cytometric analysis.

We demonstrate that with in-vitro culturing (within 3 passages post-harvest), the CD34+ stem cell fraction increases in standard culture media, across multiple samples. However, optimal culture conditions to achieve near pure stem cell populations with rapid cell proliferation, still needs to be defined.

KEYWORDS: *End-stage heart failure, Intra-coronary Transplantation, Autologous bone-marrow-derived peripheral haematopoietic stem cells.*

ABBREVIATIONS: *DMEM: Dulbecco's Modified Eagle Medium, FCS: Foetal Calf Serum, CD34+: Cluster of Differentiation 34 positive, FACS: Fluorescence-activated cell sorting, WBC: white blood cells.*

Corresponding Author: P M Athauda arachchi, Email: *athaudaarachchipm@kdu.ac.lk*

https://orcid.org/0000-0001-5401-5548

1. INTRODUCTION:

The global prevalence of end-stage heart failure is rising, despite the presence of successful reperfusion strategies for acute myocardial infarction, improved pharmacological and device therapy for heart failure. The changing demographics and disease onset at a young age and longer survival may paradoxically be contributing to this observation in both developed and developing countries. The limited availability of cardiac transplantation programmes and absence of left ventricular assist devices in many parts of the world, has renewed interests in potential alternative options, for treating patients with end stage heart failure (Athauda-arachchi 2019).

Haematopoetic stem cells derived from bone marrow have been used for autologous transplantation since (Barnes et al 1956) for haematological cancers. Many groups, including ours (Athauda arachchi 2022) have previously demonstrated the beneficial effect of intracoronary autologous bone marrow derived peripheral haemopoietic stem cell transplantation, to improve myocardial contractility post-MI (Schächinger 2004) or in end-stage heart failure (Schächinger et al 2006).The harvesting techniques and utility of these cells in many other conditions have been widely described (Snowden et al 2018, Mahla 2016 and Burt et al 2008).

However, it is not known whether these cells when taken in-vitro, could exhibit rapid proliferation and yield enriched stem cell populations, using standard cell culture media.

With this initial study, we aimed to assess the potential for in-vitro expansion of peripherally derived autologous haematopoietic stem cells.

2. METHODS

Two 1 ml samples of bone marrow derived peripheral haemopoietic stem cells, obtained as voluntary donations from patients with severe heart failure, undergoing autologous intra-coronary stem cell infusion, was transported on ice, and used for culture in DMEM, 1% penicillin/streptomycin, 20% FCS. The nutrients the medium includes glucose, L-glutamine and sodium pyruvate (contained in DMEM) and mixture of macromolecules, including hormones, transport proteins, growth factors, lipids, minerals, elements, and detoxifying factors conferred by 20% FCS.Cells were seeded at densities of approximately 1million per ml in T25 flasks. They were incubated in 5% CO_2 and at 37^{o} C and passaged three times into new media every 48 hours. Cells were analysed morphologically, by Trypan blue exclusion assay and by flowcytometry, for the presence of CD34 surface marker (with external validation of result at Sri Jayewardenepura Hospital, Colombo), indicative of "stemness" of the hematopoietic cells, before (passage 1) and after culturing (passages 2 & 3).

3. RESULTS

The morphology of the cells under microscope is demonstrated in figure 1. Immediately after counting in haemocytometer, seeding into the T25 flasks (passage 1), they appear somewhat aggregated, becoming less dense clusters and individual cells

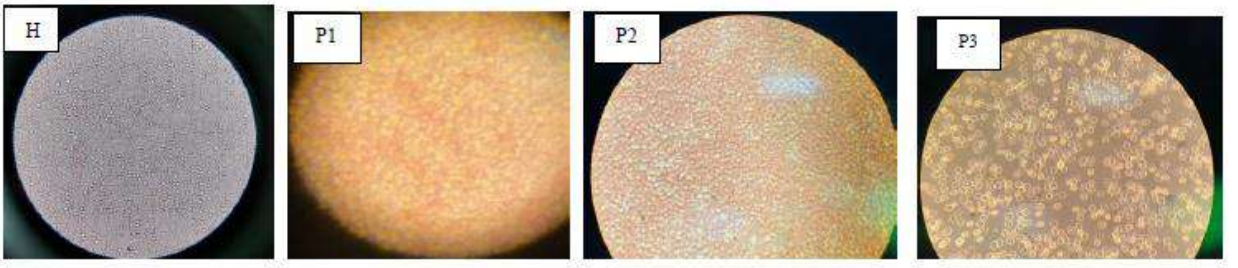

Figure1: Microscopic appearance of hematopoietic stem cells at haemocytometry [H], initial seeding to culture [p1] medium, and follow up of passages [p2 & p3].

later (passage 3). Trypan blue exclusion assay demonstrated >80% live cells after 3rd passage.

At the point of collection, total WBC numbers was approximately $219.5X10^3$ and $203x10^3$ cells per microlitre and contained approximately 1.8% and 8.2% CD34+ cells. Following serial passaging in tissue culture media, the CD34+ve cell counts could be increased to 12.7% after second passage and up to 59.1% by third passage, in one sample and up to 15% in the other, albeit the overall cell number decreased, indicating a preferential survival of the stem cell progeny in the cultures (Table 1) and loss of other cells. It also highlights, a difference in the hematopoietic stem cell numbers collected between individuals, and variabilities in the potential to enrich CD34+ stem cells by culture.

Table 1: Comparison of CD 34+ve cell counts by flow cytometry (FACS)- initial sample [p1], and of passages [p2 & p3], showing differences between the samples of the two subjects

	Subject	Initial counts(P1) cells /µl	Passage 2 cells /µl	Passage 3 cells /µl
Total WBC	1	$219.5X10^3$	$1.34x10^3$	$0.8X10^3$
	2	$203X10^3$	-	$0.27x10^3$
CD45 -ve %	1	1.8%	16.1%	59.6%
	2	8.2%	-	21.8%
CD34+ve% (out of CD45-ves)	1	75.1%	78.8%	99.3%
	2	48.1%	-	69%
CD34+ve% (out of total WBC)	1	1.35%	12.69%	59.18%
	2	3.9%	-	15.04%

4. DISCUSSION AND CONCLUSIONS

Peripherally derived autologous haematopoietic stem cells, hitherto harvested from patients with end-stage heart failure used for intracoronary infusion, may also be successfully passaged in tissue culture flasks using simple culture conditions. Here we described conditions, devoid of added growth factors or cytokines, to maintain the progenitor identity over several passages. The CD 34+ cell percentage in this simple culture technique rises due to stem cell survival/proliferation, with loss of other cells. It is also not certain how many passages the CD34+ autologous haematopoietic stem cells can be enriched this way, without terminal differentiation.

Many contemporary culture media different to ours have been described with different conditions to propagate hematopoetic stem cells (Yadav et al 2020), noting that the final lineage specification can substantially depend on the culture conditions.

The challenge is to standardize a culture system that can predictably enrich the CD34+ hematopoietic stem cell population, with the ideal composition of growth and survival factors. Chemically defined media (CDM) Joannides et al (2007) has previously been described for embryonic stem cell proliferation, but it is not certain whether such culture media which are morphogen free, may be more suitable for achieving higher cell survival and enrichment of CD 34+ hematopoetic stem cells. Further studies are indicated to test such media.

Refining these methods in future, and testing samples from more donors, enables to identify best methods for ex-vivo expansion and storage of peripherally derived autologous haematopoietic stem cells for potential "individualised cell therapy" of end-stage left ventricular failure for a given patient.

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