Optimation of Hematopoietic Stem Cells' Culture Medium: A Review

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ABSTRACT

Hematopoietic stem cells (HSCs) usage has numerous potential benefits for basic and clinical research. Growing interest in this field is caused by possible HSCs application to cure various hematologic diseases, malignancies, immunodeficiency diseases, and inborn errors of metabolism. Although the demand of HSCs is increasing, ex vivo culture of HSCs has various medium compositions. This article focuses on HSCs' culture medium optimation in different medium supplements, which might overcome non-optimal HSCs culture results. Literature searching was conducted in PubMed and google scholar database. Paper selection processes were done by author separately and studies which met the eligible criteria were included in this review. A total of 53 relevant articles were identified and 11 articles that met the eligible criteria were included in this review. There are two main types of supplement, cytokines and non-cytokines. In cytokines addition, 7 studies generally show that the supplement support HSCs expansion. The addition of non-cytokines supplements has more diverse result in studies. Positive results with various effectivity in $CD34^+$ expansion were shown in 3 studies, while one study shows a negative result. Medium supplementation must be explored more to find best substances which can optimize HSCs culture. Further research using non-cytokines substance shall be conducted regarding its various effect and might give us a big opportunity to find an optimal condition for HSCs culture.

Key words: Hematopoietic stem cells, culture medium supplementation, CD34+

INTRODUCTION

Hematologic diseases such as hematologic malignancies, non-malignant hematologic disease, immunodeficiency, and inborn errors of metabolism have become big problems of global health. Some of these diseases are considered as incurable and need an alternative treatment solution. Hematopoietic stem cells (HSCs) transplantation have enormous potential application in this field regarding its regenerative characteristic. To get a good cells quality and quantity for this purpose, HSCs must be cultured ex vivo in a medium (Park et al., 2015).

Ex vivo culture result of HSCs primarily depends on its medium. In the early development, medium of HSCs culture uses

animal-based serum which mimicking the condition of blood to grow the cells.

However, the variability of the sources, collection, and processing of this serum can produce a variability in HSCs growth product. The indefinite result, which originated from culture medium can be disadvantageous, thus serum-free medium is developed to get a better result. Serum-free medium should be enriched with addition substance to be able to grow the HSCs. Different substances such as cytokines and proteins may produce specific outcome. This addition is called supplementation for the basic medium (Ireland et al., 2006).

This review describes HSCs' culture medium optimization in different medium supplements. Comparison of several substances including cytokines and proteins were made in this review to reach a better understanding about HSCs culture.

MATERIALS AND METHODS

Literature searching was conducted in PubMed and Google Scholar database. We included original publications of HSCs' culture medium supplementation. We specifically included platelet-rich plasma, albumin, and cytokine in our searching. We excluded non-english articles, conference proceedings, abstracts, commentaries and reviews. Literature searches of journal databases retrieved 53 articles and 12 articles which met the eligible criteria were included in this review.

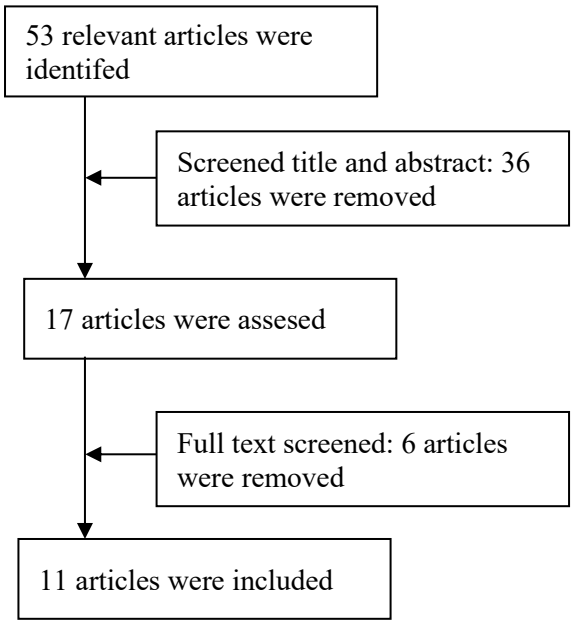

Fig. 1. Flow chart of literature search

RESULTS AND DISCUSSION

Serum-free medium for HSCs culture

The serum-based medium was used in culturing HSCs. Nowadays, it is suspected that serum-based medium might contain substances that could inhibit proliferation and/or differentiation of HSCs, like TGF-β growth factor. The serum, which was used in medium production, has variability in

quantity and quality. Hence, serum-free medium is currently preferred in HSCs culture (Stem Cell Technologies, 2018; Yao et al., 2004).

A serum-free medium has several benefits, some of which are: a) it could reduce the variability component of the culture medium, b) it could eliminate potential source of contamination, and c) mostly it could reduce the usage of FBS, which has been an ethical issue for quite a long time now. Serum-free medium usually also produces cell-specific product, providing the exact stimulation and differentiation needed for each type of cells. Therefore, it is better for basic research in cell physiology (Yao et al., 2004).

Many researches of hematopoietic stem cell cultures using serum-free medium have been conducted. The results show that there were no significant differences in either the number or the type of colonies formed between the two types of medium. Contrary, several studies that use serum-free medium have higher cell number result. Additional measurement needs to be done to develop more serum-free medium for more cell types and/or cell lines, which including review published literature, scan commercial catalogs, and contact other laboratories which have the same interest in a similar field to determine whether a suitable media formulation for a specific cell has already existed (Stem Cell Technologies, 2018; Yao et al., 2004; Gstraunthaler and Gerhard, 2003; Kwok et al., 2007).

Effect of medium supplements on HSC culture

Serum in a medium provides the cells with all the needed ingredients to proliferate and differentiate, but serum-free medium does not have that. Various types of substances were studied in order to find the perfect supplementation for culturing all different kind of cells. Cytokines like IL-3, SCF, IL-16, TPO have often been opted for culturing hematopoeitic stem cells (Lansdrop, 1992).

HSCs cultured in medium which supplemented with combination of FLT3-ligand, SCF, TPO and IL-6 was successfully transplanted to patients without any complications. Switching IL-6 with IL-3 resulted in even better cells expansion. But IL-3 also affected the cultured cells negatively by reducing expanded CD34+ cell's potency to repopulate (Piacibello et al., 2000).

In another research, 50ng/mL stem cell factor (SCF) was given to HSC at culture day 0 continued by supplementation of TPO and FLT3-ligand until day 4. Under the circumstances, ex vivo expansion of HSCs is better and more cost effective (Du et al., 2013).

Interleukin-16 (IL-16) has proved its ability to optimize ex vivo expansion of HSCs. During 14 days of culture, plate which supplemented with IL-16 showed almost twofold of CD34+ cells expansion. IL-16 supplementation also resulted in higher long-term culture-initiating cell (LTC-IC) (Rofani et al., 2009).

Research using TPO concluded that TPO may be favorable in MK progenitor expansion

within hematopoietic cell populations and also for progenitor cell expansion within other lineages. Added in dose 10 or 100 ng/mL, TPO could increase not only the stem cell activity, but also the percentage of MK lineage cells. It is confirmed that using TPO instead of megakaryocyte growth and development factor (MDGF) has no negative effect on progenitor cells expansion (Young et al., 1996; Angchaisusiri et al., 1996; Qu et al., 2016).

Besides cytokines, another supplementation could be added to enhance $CD34^+$ cell culture. Endothelial progenitor cells (EPCs) when combined with cytokines improved population of CD34+ cells compared with those supplemented with cytokines alone. However EPCs did not affect $CD34^+$ cell's repopulating efficacy (Duchez et al., 2010).

$CD34^+$ stem cells cultured in serum-free medium supplemented with PIXY321 and cytokines produced higher percentage of $cd34^+$ cell in S-phase. But it is said that TPO/SCF/FLT3-ligand supplementation was necessary for better results (Wu et al., 2001).

Another research studied for the effects of lipoxygenase metabolites of arachidonic acid on the growth of human blood $CD34^+$ progenitors. Additional 12-HETE and 15-HETE were given on day 3 and day 8 of culture. It was then discovered that HETE could enhance the proliferation and differentiation of $CD34^+$ cells, mostly towards erythroid lineage (Desplat et al., 2000). Caffeic acid phenethyl ester (CAPE) added to medium with cytokines actually stimulates expansion and increase total number of CFU and HPP CFU. CAPE upregulates SCF and HO-1, both of which have important roles in controlling HSPC functions. However, there was no significant difference in total cell number but those stimulated with CAPE showed much higher percentage of $CD34^+CD38$ (Liu et al., 2014).

CONCLUSION

The optimization of HSCs culture in a serum-free medium has lead us to understand various effect of substance on the culture result. Moreover, the effective supplement for serum-free HSCs culture medium now permits researchers to develop a maximum quality of HSCs ex vivo. Further research defining the role of another potential substance as supplement will be necessary to fully understand its role and effectiveness.

Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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