

A NEW ASSAY SYSTEM TO QUANTIFY AND STUDY PROLIFERATION OF ENDOTHELIAL PROGENITOR CELLS (EPC)

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INTRODUCTION

Colony assay methods have been widely used for studying progenitor cell presence in hematopoietic tissues whereas assessed progenitors are not necessarily of hematopoietic origin.

Among hematopoietic and stromal fibroblast cells endothelial cells (EC) and progenitors (EPC) are further known cells residing in the bone marrow. Although EPC have been largely used in experimental *in vitro* and *in vivo* settings, their characterization so far is limited to assays describing phenotypic and morphologic characteristics thus lacking to refer to "proliferation" as a key feature of progenitor cells. There exists no standard assay to evaluate the clonogenicity of EPC, the knowledge of the proliferative potential of these cells however would provide essential information for a future use of EPC in regenerative medicine.

AIM OF EXPERIMENTS

We aimed to develop an assay for the quantitation of the EPC growth kinetics reflecting their proliferative potential *in vitro*.

RESULTS

The cultivation of umbilical cord blood (UCB)-derived test EPC was shown as a starting colony and effective expansion until confluency. Flow cytometry revealed a phenotype that can be assigned to EC/EPC with the expression of CD31, CD13, CD29, CD73, CD105, CD146, HLA Class I, binding of BS-1-lectin and no reactivity with antibodies against CD90 and HLA Class II.

The colony quantification of Colony Forming Units of Endothelial Progenitor Cells (CFU-EPC) after a 12-14 day culture period of 150 HUVEC (passage 3) and 150 HMVECS (passage 2) revealed that HUVEC displayed a global higher proliferative potential compared to HMVEC in this particular assay. The overall colony count did not depend on the absence or presence of erythropoietin, but HPP CFU-EPC (colonies >2 mm diameter) were reduced at a concentration of 10-4 I.U. Epo/mL (compared to 1 or 10-8 I.U.) in HUVEC only. No influence of erythropoietin concentrations on HPP CFU-EPC was observed in HMVEC showing a general smaller proportion of HPP colonies. In colony assays with UCB-EC passage 3 the absolute colony count was higher compared to passage 4 or 5 and no effect of erythropoietin was detected in total colony counts. However, the proportion of HPP colonies increased from 10% at passage 3 to 20 - 50% in later passages. Variations of erythropoietin concentration showed at least a minor influence on the fraction of HPP colonies.

We next determined the EC/EPC proliferation potential in modified media using human platelet lysates (HPL) instead of FBS. HPL supported HPP CFU-EPC development to a higher extent in comparison to FBS.

The absence of human epidermal growth factor, human fibroblast growth factor B and R3-insulin like growth factor-1 resulted in significantly reduced and smaller colonies when compared to fully supplemented HPL cultures and revealed a proliferative potential comparable to FBS cultures with the lack of HPP colony development (no CFU-EPC > 4 mm). The absolute EC/EPC cell count and the x fold increase of cells within 12 days strongly correlated to the number and appearance of CFU-EPC in the colony assays showing a 467 – 604 and a 17 – 71 fold increase of cell number in HPL and HPL/-3 supplemented cultures, respectively (Figure 1).

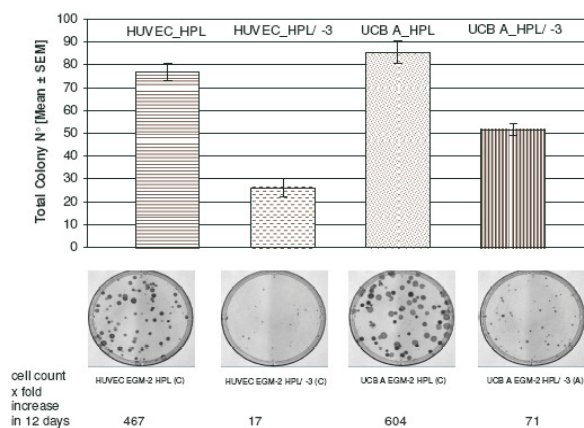


Figure 1. Correlation of the total colony number and size with the increase of the absolute cell number in a CFU-EPC assay performed within 12 days.

CONCLUSION

The new method described allows for a standardized quantification of EPC proliferation *in vitro* using the CFU-EPC assay and possibly delivers a tool for the prediction of the behaviour of somatic or circulating EPC when applied *in vivo*. Information about the cells and the proof for a progenitor quality is provided by an elaborate evaluation of colony number, size and cell number within a defined time period under standardized culture conditions. The use of HPL enhances the proliferative capacity of EPC. Modified culture conditions may be used for drug- or growth factor- testing. It is conceivable that the CFU-EPC method is used for longitudinal evaluation of EPC with serial cultures over more than 10 – 12 passages to assess cellular senescence. The influence of environmental, chemical or physical factors on the proliferative potential of EPC (for instance, high compared to low oxygen levels) can be tested using this particular CFU-EPC method.

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COLLABORATION DETAILS

- Collaboration can be in the form of a license agreement or a research cooperation

POSSIBLE PARTNERS

- blood banks
- stem cell manufacturers
- assay manufacturers
- media manufacturers

DEVELOPMENT STATUS

- *in vitro* assay
- patent pending