In vitro stem cell assays study

In Vitro Stem Cell Study Assays (colony assays, embryoid bodies, neurosphere, liquid culture)

The introduction of proper methods for the assessment of human embryonic stem cells (hESCs) can lead to the full realization of the potential of these cells in biomedical research and devising strategies for the treatment of human diseases. Currently, the *in vitro* colony-forming cell assay is perhaps the most specific and sensitive procedure to determine the proportion of undifferentiated and differentiated cells in culture. It has been regarded as a superior method in this regard in comparison to any other method used for assessing the phenotypic features of the hESCs. The other routinely used method employed for the purpose of evaluating the pluripotency of the hESC cultures is the embryoid body formation that involves the cell aggregate development. Similarly, the methods such as liquid culture and neurosphere formation are also employed to assess the efficiency of the hESCs *in vitro* . These *in vitro* methods have come up as valuable monitoring tool for assessing hESC culture quality, as well as in the evaluation of quantitative pluripotent cell number changes that can impact differentiation and self-renewal properties of the treated cells following manipulation.

Colony assays

The colony forming cell assay (CFC) since their introduction in 1961, have been routinely used in the evaluation of hematopoietic stem cells used in clinical application and research so as to quantify and assess cell sample progenitor content. The colony assay has also been implicated for the identification of inhibitory and stimulatory growth factors as supportive

diagnostic assays that determine the proliferative potential of the hematopoietic stem cells from cord blood, mobilized peripheral blood and bone marrow in clinical transplantations. Recently, the new lead compounds are assessed for the maximum tolerated dose before the clinical trials with this method. The CFC frequency and content show great variation owing to the source of the samples that requires the appropriate selection of the source cell population depending on the specific application that is needed. Often processing of the cell source is required to procure the specific cell population. If the interest is to identify a specific cell phenotypic CFC frequency, prior to the commencement of the process, enrichment for mononuclear cells using cell purification and ficoll density gradient centrifugation can be employed. Although the procedure can significantly reduce the unwanted cells but is only possible at the expense of losing the progenitors. While others have specifically removed cell populations through moderate processing that can interfere with the colony detection within the matrix. If the number of cells are limiting (e. g., in case of CB transplantation), their use in clinical settings may be compromised significantly with the loss of any progenitors. Therefore, methods involving minimal invasive techniques of processing have been employed, however it can be quite challenging to enumerate and detect the CFCs within the background of red blood cells. Established methods of quantifying CFCs include suspension culturing of MPB, CB or BM supplemented with the required cytokines in a semi solid matrix for 7-14 days. The recent gain in recombinant growth factor knowledge and their appropriate application in the last decade have optimized the CFC yield significantly that makes it easy to quantify hematopoietic progenitors like platelet, myeloid and erythroid

lineages. Now, the use of cytokine cocktails is being contemplated for controlling the differentiation and proliferation of particular type of CFCs that can address a number of previously unsolved questions in stem cell research. The preparation of media formulations without any cytokine can lead to the identification of a potential cytokine or cytokine mimetic. Similarly, cytokine at saturation concentration can be used for the assessment of toxic effects of a compound as depicted by a particular type of progenitor cells while synergistic activity can be assessed through CFU at minimal cytokine concentration. Despite being inherently subjective, the CFC derived colonies are enumerated microscopically for the identification of the progenitors. The correlationship as observed by some groups between the engraftment and progenitor content can be partly explained by the varying CFC measurements between centers [1]. However, such observation has not been supported by others. The CFC assay has remained a very powerful tool in the research to assess specific population of hematopoietic cells across different organs of many animal models. Thus, the technique has provided details of the regulatory molecules controlling hematopoietic cell differentiation and proliferation as well as functional aspects of the different hematopoietic cell population. The assessment of the hematopoietic CFC frequency *in vitro* can help in the determination of using some potential chemotherapeutic agents in clinical trials and determine the feasibility of using certain chemotherapeutics for clinical trials. Simultaneously, the results emanating from such assays have been found to be corroborative with clinical outcome and have lead to hematotoxic compound identification [2]. Therefore, the colony assay has been the standard functional assay of

choice in assessing the ability to differentiate and divide by various hematopoietic progenitor cells.

Embryoid body formation

The generation of embryoid bodies (EBs) from hiPSCs is an efficient method to assess their functionality *in vitro* besides *in vivo* teratoma formation. An amalgam of three germ layer developmental cells, the EB is a cell aggregate with three dimensional features [3]. This method requires the placement of undifferentiated hiPSCs in suspension thereby inducing the stochastic differentiation of the cells towards all the three germ layer cells. The hiPSC differentiation into cell lineages of different types has been successfully monitored with EB formation. The most important aspect of this procedure lies in the fact that standard *in vitro* tissue culture materials and techniques are employed. This helps in avoiding expenses and ethical issues pertaining to the maintenance of immunodeficient mice. Under laboratory conditions, the EBs are first grown in petriplates and once the optimum conditions are felt to be there, scaled up with very less difficulty. The hiPSCs are readily induced to form EBs and undergo the tri-lineage differentiation by a number of methods thereby providing a means of analysis of the stem cells in a reproducible and controlled manner. This is in sharp contrast to the teratoma formation where many a times it has been observed that despite the presence of all the factors needed for hiPSC mediated teratoma formation it fails to do so for some yet unknown reasons. There are a number of methods suggested for the generation of embryoid bodies and also for a different number of purposes that range from the illustration of candidate pluripotent stem cell potency to the specific tissue type generation and *in vitro*

stochastic germ layer differentiation. The EB formation has been found to be highly useful for specific lineages of tissue in the differentiation initiation and enhancing differentiation into specific lineage types like neural, cardiac and hematopoietic. The EB generating procedures vary in terms of forming uniform sized cell aggregates and the long-term viability maintenance. Typically, controlling the EB growth to uniform size has been advantageous from the point of reproducible specific type of tissue differentiation. On the contrary, under periods of extended culture, EB formation of varied size stimulates diverse tissue formation that represents the differentiation potential. Thus, EB formation is a useful technique for the assessment of the differentiation potential of stem cells.

Neurosphere

The generation of new cell continually argues in favor of the existence of a founder cell population that possess the capacity to self-renew, proliferate and undergo differentiation into a number of different progenies that is what is referred to as the stem cell. Due to poorly characterized morphological features and the lack of definite and specific markers, generally stem cells have been known by their functional attributes rather than the phenotypic features. This has given rise to a number of theoretical and practical problems with the most common being the identification of a cell population depending upon its behavior that is exhibited in an unnatural condition or environment. Such circulating problem looming over the stem cell biology research that has undermined a number of experimental efforts calls for an alternative procedure of stem cell functional assessment. However, notwithstanding this caveat, the continuous genesis of cells within the adult

mammalian CNS requires the establishment of precise defining characteristics and assaying techniques for the manipulation and study of this elusive and rare cell type. Neurosphere formation, the serum-free, defined culture system has been found to allow for small cell population (<0. 1%) responsive to specific growth factors to proliferate, survive and undergo differentiation to form clusters [4]. Following a 7 day incubation in culture medium supplemented with growth factors like EGF, the neurospheres were found to consist nearly 3000-5000 cells, measuring approximately 100-200 mm in diameter. The spheres can be passaged at this stage with a one week interval that can result in increasing the total cell count within the neurospheres. Three CNS phenotypes viz., oligodendrocytes, astrocytes and neurons are differentiated from the stem cell progeny when neurospheres (as dissociated cells or intact clusters) are plated on adhesive substrates without the presence of growth factors. Although the methodology pertaining to the study of neuronal cell differentiation through neurosphere formation seems relatively easier, only a strict adherence to the standard protocols can yield successful results. The neurosphere assay is quite essential in the study of processes of the developmental stages as well as elucidating the role of epigenetic and genetic factors in determining the role of stem cells in CNS phenotype maintenance. Neurosphere formation can be a practical means of assaying system with regard to the functionality of the stem cells to differentiate into the neuronal lineage.

Liquid Culture

A number of assays for hematopoietic stem and progenitor cells have been developed by the liquid culture mode of growing these cells. Generally, the

liquid cultures are polyclonal in nature in contrast to the colony formation assay and hence the individual precursor response is very difficult to detect within the culture. Therefore, in liquid culture an average response is considered to be yielded by the initiating population in the culture for the growth of precursors. It can be successfully employed as for instance in comparing total cell number generated in conditions wherein two different growth conditions have been employed. Flow cytometric analysis has also been sometimes used in enumerating the cell count both at the end of culturing process and also at the beginning so as to detect the type and stage of differentiation. The frequency of precursor response could be estimated combining the techniques of liquid culture and colony formation for the cells grown in liquid culture. The measurement of CFU-C after and before the liquid culture can provide an insight into the effect of the liquid culture growth conditions on the differentiation and growth of the starter cells for the culture. The procedure has been utilized widely in the *ex vivo* study of hematopoietic precursor expansion and the effect of various growth factors on them. This has come up as a potentially powerful tool in the generation of transplantation quality hematopoietic precursors in considerably large numbers [5]. Clonal liquid cultures can be performed in a more direct manner by the application of FACS. The employment of FACS results in the sorting of single cells with great accuracy into individual specific wells of culture plates. Other ways of doing liquid culture involves manual pipetting so as to seed limiting number of cells in culture plates and achieve clonality.