ISSN(Online): 2320-9801
ISSN (Print): 2320-9798

International Journal of Innovative Research in Computer and Communication Engineering

(An ISO 3297: 2007 Certified Organization)

Website: www.ijircce.com

Vol. 5, Special Issue 3, April 2017

Segmentation of Blebs in Human Embryonic Stem Cells

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ABSTRACT: The main aim of this paper is to segment the bleb from the human embryonic stem cells. The health of the human embryonic stem cells (hESC) is determined by the characteristics of the blebs – important biological indicator. With the nature of blebbing, the dynamic and apoptotic blebs can be identified. Region based active contour method is used for segmentation of bleb in human embryonic stem cells. To improve the segmentation accuracy, bio-inspired method is adopted. Two types of bleb formation occur: extraction and retraction. The full bleb formation occurs between the stage of extraction and retraction known as intermediate bleb. Bleb changes their size and image properties dynamically in both process and between frames. Thence, adaptive parameters are needed for each segmentation method, which provides accurate and quick analysis.

KEYWORDS: Active Contour,Bleb, Extraction, Human Embryonic Stem Cells, Intermediate Bleb, Retraction.

I. INTRODUCTION

EMBRYONIC STEM CELLS deduced from the inner cell mass (ICMs) of blastocyst, the sperm in the oocyte can be divided into number of totipotent cells which cannot be differentiated. These cells when enter into the blastocyst it can be differentiated into number of specialized cells. Embryonic stem cells are the pluripotent cells that can be derived from the inner cell of mammalian blastocyst. The specialized cells can be further differentiated to produce more number of stem cells. The word “ES cells” was used to differentiate these embryo-derived pluripotent cells from teratocarcinoma-derived pluripotent embryonal carcinoma (EC) cells.Embryonic stem (ES) cells have been recognized from several mammalian species, including mouse, monkey, and human [16]. It is well established that various signaling pathways, including phosphoinositide 3-kinase (PI3K)/Akt and Wnt/β-catenin signaling in the maintenance of ES cell pluripotency, and is known about the signaling pathways, involved in the derivation ES cells from ICMs.

Embryonic stem (ES) cells go through protracted proliferation while remaining composed for multilineage differentiation. A sole network of transcription factors may illustrate self-renewal and simultaneously suppress differentiation. First, the generation of mature lineage from ES cells in culture delivers access to populations of initial precursors that are difficult, if not impossible, to access in vivo. Evaluates of these mutations in vivo are habitually tricky by the early death of the embryo in utero. Second, the developmental potential of ES cells carrying targeted mutations of genes vital for embryonic development can be formed in culture.Moreover, under well-defined conditions, embryonic stem cells are adept of propagating themselves indefinitely.

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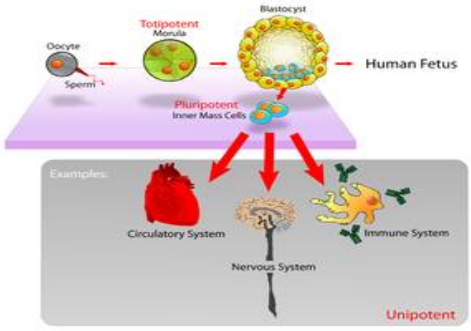

Fig 1. Embryonic Stem cells

This permits embryonic stem cells to be hired as beneficial tools for both research and regenerative medicine, as they can yield vast numbers of themselves for continual research or clinical use. For the reason, of their manipulability and hypothetically boundless capacity for self-renewal, ES cell therapies have been suggested for regenerative medicine and tissue replacement after injury or disease.

II. HUMAN EMBRYONIC STEM CELLS

As stated earlier, human embryonic germ (EG) cells share many of the characteristics of human ES cells, but differ in significant ways. Human EG cells are derived from the primordial germ cells, which occur in a specific part of the embryo/fetus called the gonadal ridge, and which normally develop into mature gametes (eggs and sperm). Gearhart and his collaborators devised methods for growing pluripo-tent cells derived from human EG cells. The process requires the generation of embryoid bodies from EG cells, which consists of an unpredictable mix of partially differentiated cell types [10]. The embryoid body-derived cells resulting from this process have high proliferative capacity and gene expression patterns that are representative of multiple cell lineages. This suggests that the embryoid body-derived cells are progenitor or precursor cells for a variety of differentiated cell types [11]. The characterization of stem cell cultures has two main purposes: monitoring the genomic integrity of the cells and tracking the expression of proteins associated with pluripotency. Genomic analysis is necessary to ensure stem cells maintained in culture have not undergone chromosomal changes through chromosomal loss or duplication, or changes in their epigenetic profiles. Proteomic analysis ensures that the cells are expressing the factors necessary to maintain pluripotency. Confirmation of the differentiated state by analysing key genetic and protein markers ensures identification and propagation of the correct cell type. This section provides an overview of different analysis methods for stem cells including karyotyping, single-nucleotide polymorphism (SNP) analysis, epigenetic profiling, flow cytometry and immunocytochemistry, RT-qPCR, digital PCR, western blotting, biomarker analysis, and teratoma formation. A variety of factors affect stem cell cultures.

Factors
Media composition
Cell density
Feeder cell type/density
Growth factors/additives
Feeder free culture
Passage method
Number of passages
Freezing and thawing protocols
Microbial contamination

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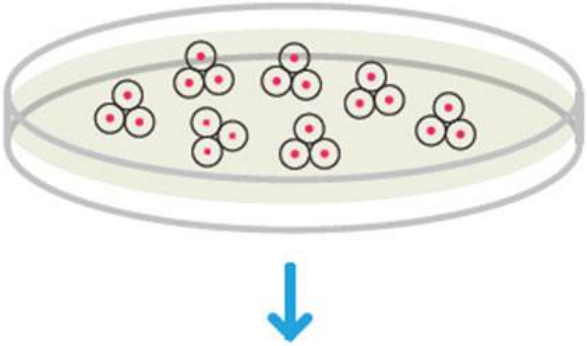

Possible changes
Chromosomal
Phenotype/morphology
Differentiation
Pluripotency loss
Epigenetic changes
Tumorigenesis
Loss of self-renewal ability

Stem cell cultures must be periodically assessed for changes in morphology, karyotype, and ability to differentiate. Adaptation to culture conditions and maintenance in culture of any cell type will change the population. As cells divide and are passaged, the composition of the population changes due to selective pressures on the cells. Additionally, cells that grow and divide more rapidly will become dominant, gradually changing the composition of stem cell populations in culture. Mosaic populations often develop, with several distinct populations evolving. Therefore, keeping stocks of early passages of cells and continual monitoring are important. A number of techniques are used for genetic characterization of stem cells: karyotyping, fluorescence in situ hybridization (FISH), comparative genomic hybridization, single nucleotide polymorphism (SNP) analysis, and epigenetic profiling. Some types of stem cells, particularly embryonic stem cells (ESCs)[13] and induced pluripotent stem cells (iPSCs), are more prone to being genetically unstable and should be observed frequently for chromosomal changes. Stem cells express both unique and specific combinations of transcription factors, cell surface proteins, and cytoplasmic proteins. Techniques used for stem cell analysis and characterization include flow cytometry, array-based analysis of the transcriptome, immunocytochemistry, western blots, and biomarker analysis. Different classes and types of stem cells are characterized by different combinations of markers. In addition to confirming that stem cell lines are stable, markers can be used for screening during the reprogramming of somatic (adult) cells into induced pluripotent stem cells (iPSCs) or to follow the progression of stem cell differentiation[7,5,3].

III. BLEBBING OF HUMAN EMBRYONIC STEM CELLS

Blebs are bulge like protrusion that seems and vanishes from the surface of the cells [12]. Bleb formation consists of two processes: 1) expansion; 2) retraction [12], [14]. Bleb expands sporadically; they grow to the size of ~2µm over ~30s; while cell body size decrease, bleb size rises. During retraction, bleb shrinks over ~120s; while bleb size reduces, cell body size increases. Complete retraction takes place in the healthy bleb, partial retraction or retraction occurs slowly leads to cell death. In between expansion and retraction, there is a full bleb formation at the end of the expansion stage is known as an intermediate bleb. The intermediate stage has the maximum size of bleb [2], [12], [14]. Inhibition of Rho-associated coiled-coil kinase (ROCK) activity enhances the clonal growth of hES cells without harming their capability to form teratomas [16]. The difficulty in recovering the hES cells from cryopreserved stock can be significantly minimized [15]. Blebbing is related to the signaling pathway which is useful for the biologist to determine that the inhibitors can alter the behavior of bleb through the Rho-Rock pathway [2].

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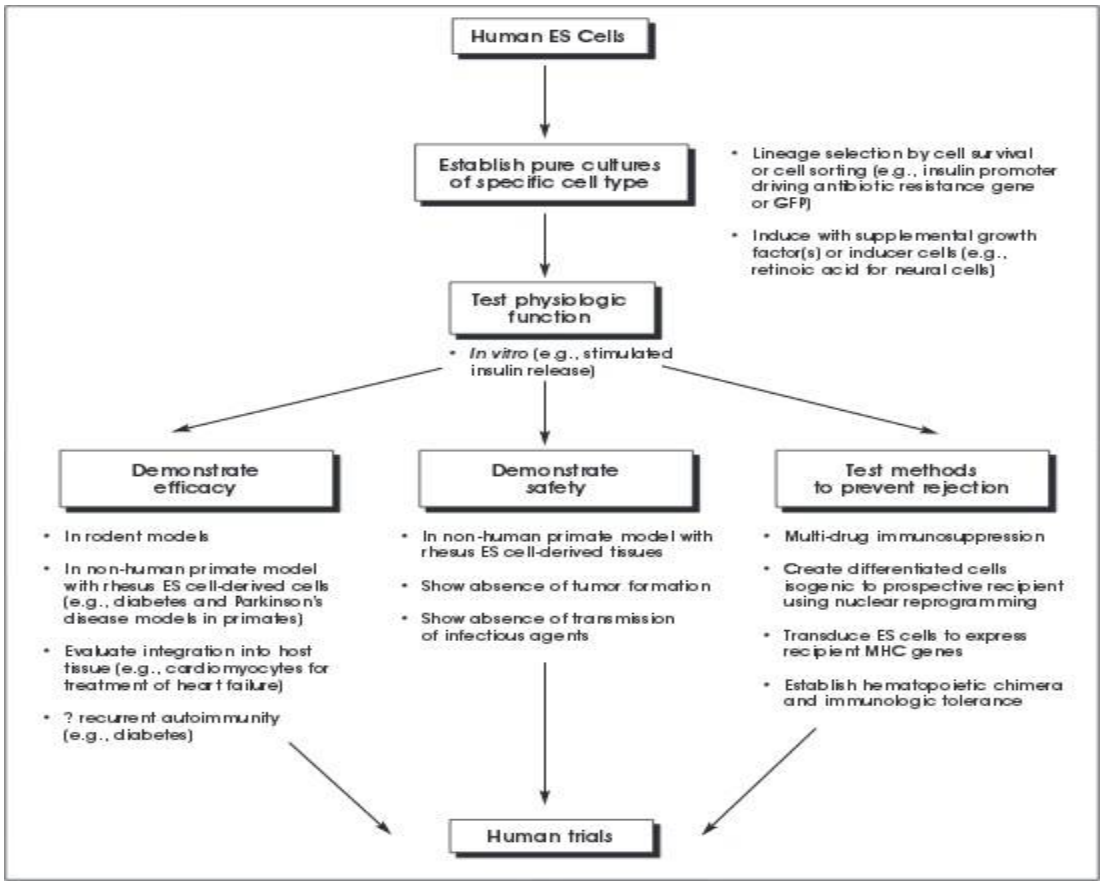
IV. ACTIVE CONTOUR

Active contours or snakes are used broadly for image processing and segmentation applications, mainly to trace object boundaries.It can attain the segmentation results near to the object contours, which can be appropriately used for analysis and recognition of the object shape. The active contours exploit numerous types of previous knowledge, such as intensity distribution information of the image, information about the boundary shape, and texture information, to attain accurate results for object boundaries in image analysis.It can be characterized as region-based models or edge-based models. Edge-based segmentation seems for breaks in the image intensity. Region-based segmentation seems for homogeneity within a sub-region; under desired property, e.g. intensity, color, and texture. Instinctive interpretation of images is a very tough problem in computer vision. Numerous ways are developed in past decade to enhance the segmentation performance in computer vision. Our approach is based in the active contour algorithm with the region based segmentation.

The following energy functional for calculating the snake energy:

$$E_{snake} = E_{internal} + E_{external} + E_{constraint}$$

Snake energy (E_{snake}) contains of three terms. Eint denotes the internal energy of the snake while Eimg represents the image forces, Econ represents external constraint forces.



Internal Energy (Eint) hinges on the inherent properties of the curve and is the addition of elastic energy and bending energy, which is given in equation

$$E_{int} = E_{elastic} + E_{bending}$$

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$E_{int}=\int \frac{1}{2}(\alpha|v_s|^2+\beta|v_{ss}|^2)\,ds$

Where α(s) and β(s) are user defined weights.
External energy (Eext) of the contour is derived from the image.

$E_{ext} = \int E_{image}(v(s))ds$

Image energyis function of the features of the image.

$E_{image} = w_{line}E_{line}+w_{edge}E_{edge}+ w_{term}E_{term}$
$w_{line}, w_{edge}, w_{term}$ are the weight of salient features.

V. IMPLEMENTATION RESULT

The video frames are phase contrast images. Each video was captured using 2 x objectives with 512x512 resolutions. Each video frame is acquired at 0.1 seconds time interval. The input video is shown in figure.

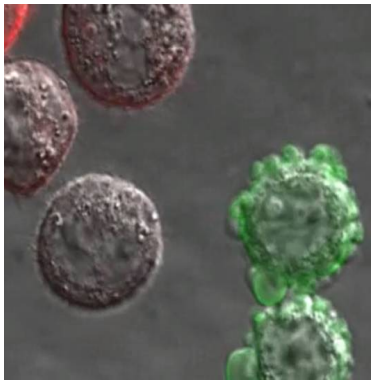

Fig 3. Input stem cells

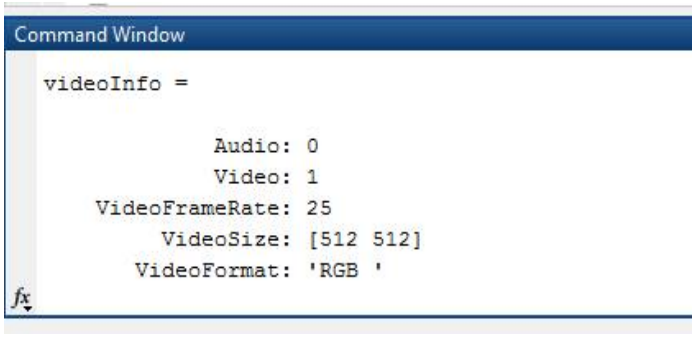

Fig 4. Video information in command window

The command window gives information about the video format; here 25 frames were obtained per interval. The video is acquired in color format which is not effective for image processing. HSV is very useful in image processing applications. The color information is typically noisy than the HSV information. Henceforth RGB can be altered into HSV.

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Median filter is applied to clean the image from acquisition noise.

v(m,n) = median{y(m-k,n-l),(k,l)∈ W}

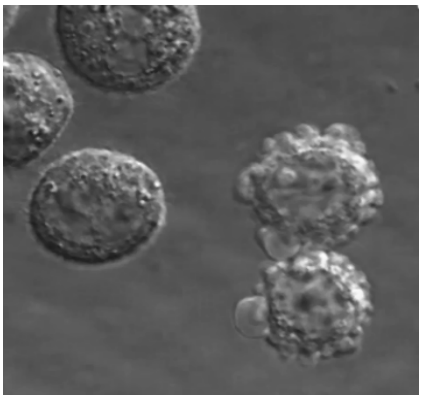

Fig 5. Preprocessed output

Region based active contour is used for extraction of blebs from human embryonic stem cells. The automatic changes in the topology of the image during the progression of the curve can be efficiently deal by this method. The image displays the thin white line which indicates the area of bleb region.

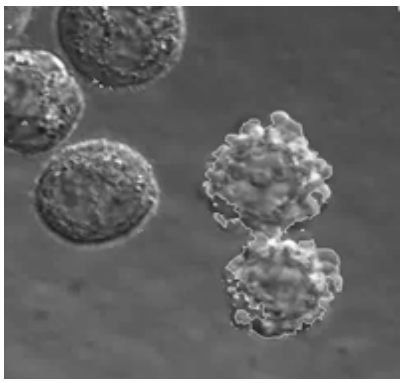

Fig 6. Segmented output

VI. COMPARISON OF RESULTS

Specificity (SPC) measures the proportion of actual negative which are correctly identified.

$$SPC = \frac{TN}{FP+TN}$$

The Jaccard (JAC) is the measure of the similarity between experimental results and the ground truth.

$$JAC = \frac{TP}{TP+FP+FN}$$

True Positive (TP): indicates the overlapped region of the detected bleb`s binary mask and the bleb ground truth`s binary mask.
True Negative (TN): is the overlapped region of the detected background`s binary mask and the background ground-truth`s binary mask.
False Positive (FP): is the detected background`s binary mask that is falsely identified as a part of the bleb region.
False Negative (FN): is the detected bleb`s binary mask that is falsely identifies as part of the background.

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METHOD	PROPOSED			
	MIN	MAX	STD	MEAN
SPC	8.92	16.78	12.17	34.34
JAC	9.57	13.27	12.07	33.80

VII. CONCLUSION

The bio-optimized segmentation methods have better performances than their conventional counterparts. With the bio-inspired optimization metric, low performance due to over-segmentation is reduced. A new concept that the bleb formation/retraction process can be used as a biological indicator of cell health. Healthy cells retract their blebs back to the cell body, while non healthy cells do not retract them or retract them slowly. Bleb area detection by active contour segmentation followed by cuckoo search optimization is implemented. The method yields high true positive rate white it gives low false positive rate. The method also matches the trend of the ground truth experiment closely. To improve the accuracy further, it is necessary to investigate into getting more frames per second to establish inter frame relationship for detecting small tiny bleb regions.

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