

Assessing Cellular Health in Cell Culture System

Madhurima Ghosh¹, Jhinuk Chatterjee², V. Krishnamurthy³, Arundhati Pal⁴, Parnika Mukherjee⁵, Rahila Rajvada⁶

^{1,4,5,6}Department of Biotechnology, PES Institute of Technology, Bangalore, India

^{2,3}Department of Biotechnology, PES University, Bangalore, India

Email : miss_madhurima_ghosh@yahoo.co.in, jhinukchatterjee@pes.edu, v.krishnamurthy@pes.edu

Abstract - Assessment of cellular health is crucial for any cell culture system. Cell morphology is an important indicative parameter for cellular health. In the present study image analysis was used to assess cellular health in culture system. PBMC culture was maintained in RPMI-1640 and monitored for 96 hours and images were captured every 24 hours using a digital camera at a low magnification of 20X. Images were analyzed using open source software ImageJ. Analysis was done for average circularity. Average circularity was chosen as important morphological parameter for cellular health assessment. Cells lose their circularity with time in cell culture system which is indicative of deteriorating cellular health.

Keywords - PBMC, RPMI-1640, ImageJ, Image analysis.

I. INTRODUCTION

Cell morphology is a crucial indicative parameter for cellular health. A suitable live-cell image contains just enough information to confidently identify the feature of interest. Image analysis is the extraction of meaningful information from images, mainly from digital images. In biological studies, it is used to analyze cell behavior and morphology. Images of living cells are often grainy and poorly composed, but still represent the maximum amount of information that can be extracted from the specimen under the specific experimental conditions. ImageJ is a public domain Java image processing and analysis program [1-4]. It runs on any computer with a Java 1.5 or later virtual machine. Downloadable distributions are available for Windows, Mac OSX and Linux. It can display, edit, analyze, process, save and print 8-bit, 16-bit and 32-bit images. It can read many image

formats including TIFF, GIF, JPEG, BMP and 'raw'. ImageJ has an open architecture that provides extensibility via Java plugins. In the present study PBMC culture was maintained in RPMI-1640 and monitored for 96 hours and images were captured every 24 hours from phase contrast microscope with 20X magnification using a digital SLR camera. For analysis, open source image analysis software ImageJ were used. Analysis was carried out for cell circularity as average circularity is an important morphological parameter for cellular health assessment for nearly circular cells like PBMC.

II. MATERIAL AND METHODS

Human blood was obtained from registered blood bank in the city (Indian Red Cross Society, Race Course Road, Bangalore). All chemicals were obtained from Sigma Aldrich.

Human PBMC (Peripheral Blood Mononuclear Cells) isolation and culture

20 mL of anticoagulated blood obtained from the Blood Bank was used to isolate PBMCs using gradient centrifugation with equal volumes of Histopaque 1077 for 30 min at 1500 rpm. Harvested PBMCs collected from the interface were washed three times with phosphate buffered saline (PBS, pH 7.4) for 10 min at 1000 rpm. For the final wash step, the cells were divided equally in three different tubes and each tube washed with PBS for 10 min at 1000 rpm. Finally cells were resuspended in RPMI-1640. Culture conditions were set in triplicate in 24-well cell culture plate. The cells were incubated at 37°C in

a humidified incubator containing 5% CO₂ for 96 hours using an inoculum of 2X10⁶ cells/mL [5-10].

Image acquisition and analysis

PBMC culture was maintained in RPMI-1640 and monitored for 96 hours and images were captured every 24 hours using a digital SLR (single-lens reflex) camera (Samsung WB650 Optical zoom: 15x Digital zoom: 12x). The objective of the phase contrast microscope was kept at 20X and the eyepiece was kept at 10x. Each well was focused on, and images were captured every 24 hours from each well.

Raw image was opened in ImageJ interface and brightness and contrast was adjusted to make sure there is substantial difference between the cells and medium colour. Threshold of the image was adjusted to select the areas we desire to measure. RGB image was changed to binary image and median filter was used to filter the image. Image was dilated and then watershed algorithm was used [11-15]. In the final step cells were analyzed by providing the desirable range for circularity. ImageJ calculates circularity of particles using the following formula:

Circularity = $4\pi \cdot \text{area} / \text{perimeter}^2$ which quantifies the roundness of the cell assessed, where a value approaching 1.0 is equivalent to a cell with a cellular profile nearing circularity.

The result page provided by ImageJ after “Analyze Particles” can be saved as an excel sheet.

III. RESULTS & DISCUSSION

PBMC culture was maintained in RPMI-1640 and monitored for 96 hours and images were captured every 24 hours as specified in methods section. The images had low contrast and substantial noise. Images were processed using ImageJ and cells were identified. Following set of figures (Fig.1) show sequential steps of image processing with the output image and histogram. Raw image had low contrast along with lots of background noise. After changing the brightness and contrast there was substantial difference between cells and background. Changing the image threshold and binarization followed by image dialation was able to select cells for further analysis. Finally watershed algorithm was used to carve out cells from debris.

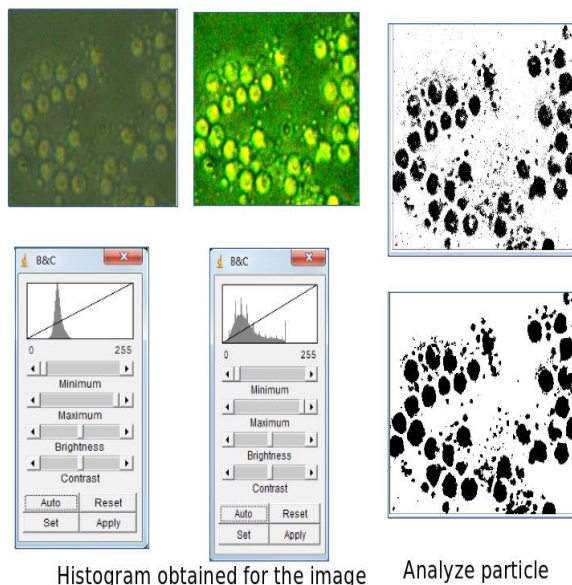


Fig.1. Sequential steps of image processing and analysis in ImageJ

After analyzing the set of images for circularity it was observed that PBMC cells till 48th hours showed average circularity more than 0.6. Around 72 hours cell circularity was 0.6. At 96th hour, majority of the cells have circularity between 0.4- 0.5 which is indicative of deteriorating cellular health. Usually cells lose their circularity according to their microenvironment condition [16-17]. Following figure shows time vs. avg circularity for PBMC cells maintained in RPMI-1640.

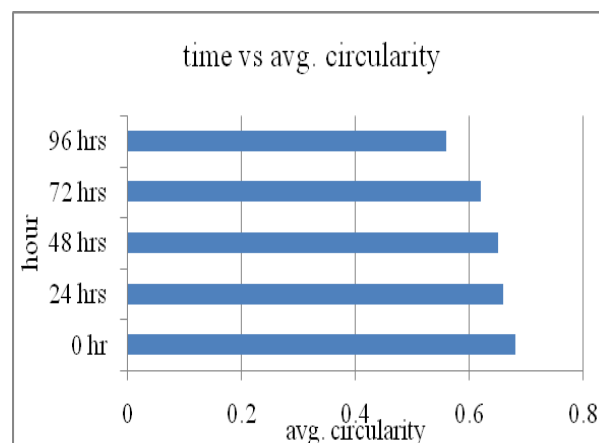


Fig 2. Time vs. avg circularity for PBMC in RPMI-1640

IV. CONCLUSION

The result demonstrates that ImageJ can be efficiently used to analyze cellular circularity and PBMC lose their circularity with time in cell culture system which is indicative of deteriorating cellular health.

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