Cell Image Processing Methods for Automatic Cell Pattern Recognition and Morphological Analysis of Mesenchymal Stem Cells
- An Algorithm for Cell Classification and Adaptive Brightness Correction -

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Abstract

Purpose: The present study aimed at image processing methods for automatic cell pattern recognition and morphological analysis for tissue engineering applications. The primary aim was to ascertain the novel algorithm of adaptive brightness correction from microscopic images for use as a potential image analysis. Methods: General microscopic image of cells has a minor problem which the central area is brighter than edge-area because of the light source. This may affect serious problems to threshold process for cell-number counting or cell pattern recognition. In order to compensate the problem, we processed to find the central point of brightness and give less weight-value as the distance to centroid. Results: The results presented that microscopic images through the brightness correction were performed clearer than those without brightness compensation. And the classification of mixed cells was performed as well, which is expected to be completed with pattern recognition later. Beside each detection ratio of hBMSCs and HeLa cells was 95% and 92%, respectively. Conclusions: Using this novel algorithm of adaptive brightness correction could control the easier approach to cell pattern recognition and counting cell numbers.

Keywords: Image processing, Cell pattern recognition, Morphological analysis, Mesenchymal stem cells, Tissue engineering applications

Introduction

Stem cells have attracted tremendous interest in recent times due to their promise in providing innovative treatments for debilitating some disease. This is due to their potential ability to regenerate and repair damaged tissues. In particular, human bone marrow-derived mesenchymal stem cells (hBMSCs) are one type of adult stem cells. Despite the attractive properties of hBMSCs, there is presently no quick and easy way to find out the various cells. Until now, a lot of time is needed in order to search for abnormal cells, and it is easy to be subject to various time-consuming assays. Hence there is a great need for innovative new ways to assess the cell classification, as well as the quality of cell cultures for potential clinical application. The research presented in the paper investigates the use of digital image processing and pattern recognition techniques to provide a quick and simple method for the assessment of hBMSCs. Above all, the aim of this work is to ascertain whether it is possible, through the use of cell image processing and pattern recognition techniques.
Several researchers have been developing automated methods for segmenting and counting cells in microscopic images (Spencer et al., 1996; Garrido et al., 2000; Shiotani et al., 1994). Some approaches are based on machine learning. Long et al. (2005) and Zheng et al. (2004) proposed methods based on neural network and Markiewicz et al. (2006) proposed a method to cell recognition and count using Support Vector Machine. In this kind of approach, the major task is to create the learning set, which is usually done manually by an independent expert for cell type. With the discovery of the potential of stem cells, many researches have been dealing with this kind of cell. Althoff et al. (2005) and Tang et al. (2005) proposed a method for segmentation and tracking of neural stem cells. Both approaches are based on classical segmentation methods and use the information about the cells’ previous position to decide which blobs correspond to real cells. Also working with neural stem cells, Korzynska (2007) presented a method for automatic counting of neural stem cells growing in cultures which is performed in two steps: 1) segmentation step: the image is separated in several regions and; 2)counting step: each homogeneous region is counted separately. Kachouie et al. (2007) proposed a deconvolution method in the form of an optimized ellipse fitting algorithm to locate individuals hematopoietic stem cells. The methods proposed in (Kachouie et al., 2006; Kachouie et al., 2005) uses the cell morphologic information (e.g. cell size, boundary brightness, interior brightness and boundary uniformity or symmetry) to locate and track hematopoietic stem cells. Importantly the works cited above handled with only one type of stem cell in their images.

Morphological cell analysis has been integrated in new methods for biomedical applications, such as automatic segmentation and analysis of histological tumor sections (Reyes-Aldasoro et al., 2011; Cheng et al., 2010; Schildkraut et al., 2010), boundary detection of cervical cell nuclei considering overlapping and clustering (Plissiti et al., 2011), the granules segmentation and spatial distribution analysis (Diaz et al., 2010), and morphological characteristics analysis of specific biomedical cells (Brun et al., 2011; Amini et al., 2010; Xiong et al., 2010).

Especially, morphological feature quantification for grading cancerous or precancerous cells is widely researched in literatures, such as nuclei segmentation based on marker-controlled watershed transform and snake model for hepatocellular carcinoma feature extraction and classification, which is important to prognosis and treatment planning (Huang et al., 2010), nuclei feature quantification for cancer cell cycle analysis (Li et al., 2010), and using feature extraction include image morphological analysis, wavelet analysis and texture analysis for automated classification of renal cell (Chaudry et al., 2009) Computerized/automated early cancer or abnormalities detection provides a basis for reducing deaths and morbidity, especially for cervical cancer, which is reported to be the most preventable disease through early detection, provision of prompt advice and opportunities for follow-up treatments (Chang et al., 1996).

The scope of this paper is restricted to morphological cell analysis by image processing in the field of tissue engineering research. As such, the goal of this study was to investigate the image processing methods for automatic cell pattern recognition and morphological analysis for tissue engineering applications. The primary aim is to ascertain the novel algorithm of adaptive brightness correction from microscopic images for use as a potential image analysis. In addition, the ability to automatic cell pattern recognition and morphological analysis was investigated and established an adaptive calibration model in an attempt to indicate a possibility of image processing techniques.

Materials and Methods

Culture of hBMSCs and HeLa cells

hBMSCs were obtained from the Intellectual Bio-interface Engineering Center, Dental Research Institute, College of Dentistry, Seoul National University. Also, HeLa cells, cancer cell line, were grown on Petri dishes. Cells were cultured in alpha-minimum essential medium (α-MEM, LM 008-01, Welgene Inc., South Korea) containing 10% fetal bovine serum (FBS, Welgene Inc., South Korea), 10 mM ascorbic acid (L-ascorbic acid), antibiotics, and sodium bicarbonate at 37 °C in a humidified atmosphere of 5% CO₂ (Steri-Cycle 370 Incubator, Thermo Fisher Scientific, USA). Cell culture was initiated with a cell seeding density of 3.0 x 10⁴ viable cells per well. Representative cell images of the two types were prepared in this study (Fig. 1). In the cause of brightness correction, the novel normalization method for microscopic image was developed. Continuously, threshold process was applied to the next step such as cell number counting and specific pattern recognition of various cells.