
Effect of $\pm 5^{\circ}\text{C}$ difference from physiologic temperature on surface area, volume, and surface-to-volume ratio of transformed cells

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Abstract

Information on the relationship between culture temperature and changes in the cell's area, volume and area to volume (S/V) ratio is lacking. Cancerous epithelial-like cell lines SW480, SW742 and T-47D were cultured at 32, 37 and 42°C for 48hr. Digital images of cells were analyzed using software Imaged. Observed surface area, volume changes, and surface area to volume (S/V) ratio were significantly different at 32, 37 and 42°C in the three cell-lines tested ($P < 0.05$). S/V ratios for all three cell lines at 37°C were significantly greater than that of at 42°C ($P = 0.0001$).

Keywords: Temperature; surface area to volume ratio; cell size

1. Introduction

The morphology and surface area of eukaryotic cells depend on adhesion to a suitable substratum and cell flattening [1], cytoskeletal microfilaments [2], actin [3] and temperature [4, 5].

The ratio between the surface area and volume of cells has a significant impact on their biology [5]. An increased surface area to volume (S/V) ratio gives increased exposure to the surrounding environment which may be regarded as important for cellular normality [4].

A low S/V ratio is not necessarily a handicap because cells can also be long and thin (nerve cells) or convoluted (microvilli) [5], however, a decreased surface area can lead to biological problems [4]. Greater contact with the environment through the surface of a cell or an organ (relative to its volume) is a good sign of cell health.

On the other hand, high surface area to volume ratios may present problems like temperature control in unfavorable environments [4]. The effect of temperature changes on Chinese hamster cell morphology [6] has been studied; however, there is scant data in the literature regarding the effect of different culture temperatures of human cells, and the

relation of a 37°C on surface area to volume ratio. An in-depth understanding of the mechanisms underlying regulatory volume behavior in cells has been hampered by the lack of adequate methodology for characterizing this phenomenon.

Literature is lacking information on the relationship between culture temperature and changes in the cell's area, volume and area to volume (S/V) ratio. We, for the first time, measured volume and surface area of three cell lines cultured for 48hr exposed to 32, 37 and 42°C with a simple digital imaging and mathematical calculation.

2. Materials and Methods

2.1. Cellular Heat Treatment

Cancerous epithelial-like cell lines SW480 (colon cancer), SW742 (colorectal cancer) and T-47D (breast cancer) were purchased from National Cell Bank of Islamic Republic of Iran, and were grown in RPMI 1640 (Sigma USA) supplemented with 10% FBS (Sigma USA), non-essential amino acids, 5 mM L-glutamine, 100 IU/ml penicillin, and 100 µg/ml streptomycin.

Before treatment the growth medium was removed and fresh medium added. Each cell line at each temperature was cultured in three different flasks. Heat treatment was carried out by completely immersing the flasks into a water bath

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thermostatically controlled. SW480, SW742 and T-47D cells were cultured at 32, 37 and 42°C for 48hr. Heat treatments were in triplicate and results of pooled data for each cell-line are reported. In each flask, at least thirty cells were observed and the averages were calculated.

2.2. Evaluation of Observed Cell Surface

Digital images of control and heat-treated cells were obtained using stereomicroscope digital camera NIKON S500 and were then analyzed using software Image (1.42l, Wayne Rasband, National Institutes of Health, USA), a public domain Java-based image processing program. Image imported the image as an ordinary format and then the observed surface area of at least 30 cells within the culture medium were selected by user. The software then calculated those areas. This program could measure the observed cell surface and record it in an EXCEL convertible statistical order.

2.3. Cell volume evaluation

Control and heat-treated cell suspensions were carefully obtained by the repeated pipetting of 0.06% trypsin/0.02% EDTA (pH 7.3). The radii of at least 30 suspended cells within the culture medium that had a spherical dimension were measured using Image software. The volume was calculated mathematically via geometric formula of sphere volume.

2.4. Statistical Analyses

All analyses were performed using the Statistical Package for the Social Sciences (SPSS) for Windows, version 13.0 (Chicago, IL, USA). The comparisons of the variables in the three groups, each comprised from at least 30 measured cells were performed with Kruskal-Wallis test, while the comparisons for the two groups were performed with Mann-Whitney U test. Differences were considered as statistically significant at $p < 0.05$.

3. Results

Figure 1 shows the mean observed surface area (mm^2) changes of three cell-lines cultured in 32, 37 and 42°C. Observed surface areas were significantly different at 32, 37 and 42°C in the three cell-lines tested ($p < 0.05$). In SW480 and SW742 cell lines, observed surface area at 42°C was significantly smaller than that of at 37°C ($p < 0.05$). In T47D and SW742 cell lines, observed surface areas at 32°C were significantly greater than that of at 37°C ($p < 0.0001$). Observed surface area of SW480 cell line at 32°C was significantly

smaller than that of at 37°C ($P < 0.0001$). Surface areas of T47D cells were not significant between 37 and 42°C and in SW480 cells between 32 and 42°C.

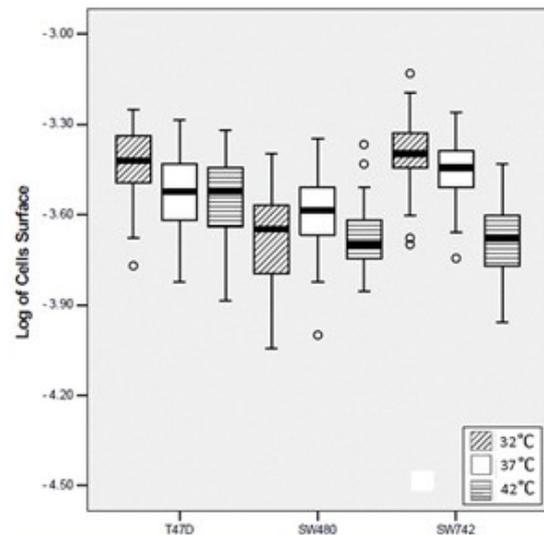


Fig. 1. Log of mean observed surface changes (mm^2) in three cell-lines cultured at 32, 37 and 42°C for 48hr

Figure 2 shows volume changes (mm^3) in three cell-lines cultured at 32, 37 and 42°C for 48hr. Cell Volumes were significantly different in 32, 37 and 42°C ($p < 0.05$), all 2×2 comparisons were significant in the three cell-lines ($p < 0.05$). Measured volume for all three cell lines at 37°C were significantly smaller than that of their volume at 32°C ($p = 0.0001$). The volume at 37°C for all three cell lines was significantly smaller than that at 42°C ($p = 0.0001$).

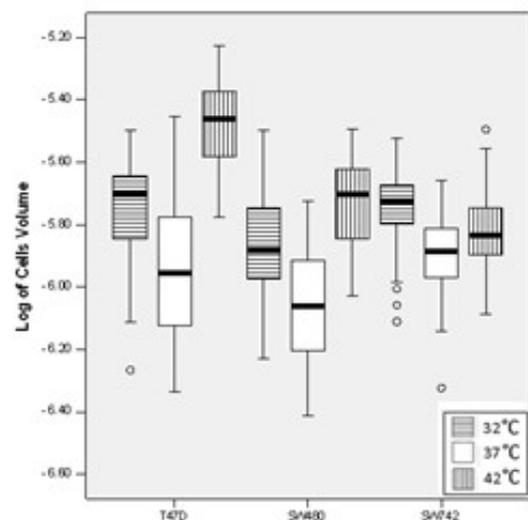


Fig. 2. Log of mean volume (mm^3) in three cell-lines cultured at 32, 37 and 42°C for 48hr

Figure 3 shows the results of changes in "surface area" to "volume" ratio (S/V ratio, mm^{-1}) in the three cell-lines studied.

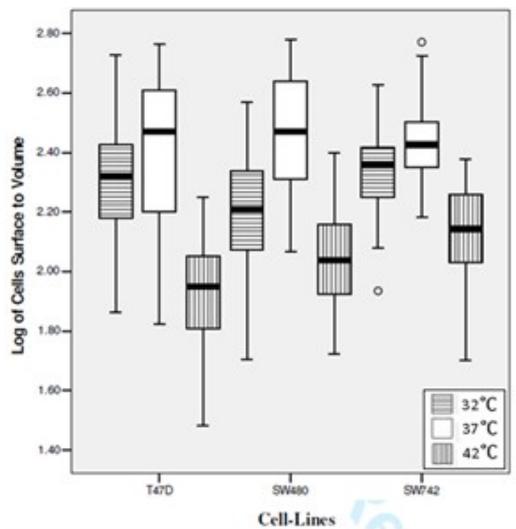


Fig. 3. Log of mean observed surface area to volume ratio (mm^{-1}) in three cell-lines cultured at 32, 37 and 42°C for 48hr

S/V ratios were significantly different at 32, 37 and 42°C ($P < 0.05$). All 2×2 comparisons were significant in the three cell-lines ($P < 0.05$). S/V ratios calculated for all three cell lines at 37°C were significantly greater than that of at 32°C ($P = 0.0001$). S/V ratios for all three cell lines at 37°C were significantly greater than that of at 42°C ($P = 0.0001$).

Figure 4 shows nine digital images of heat-treated cell's obtained using stereomicroscope digital camera. Images include T47D, SW480 and SW742 cells at 32°C, 37°C and 42°C.

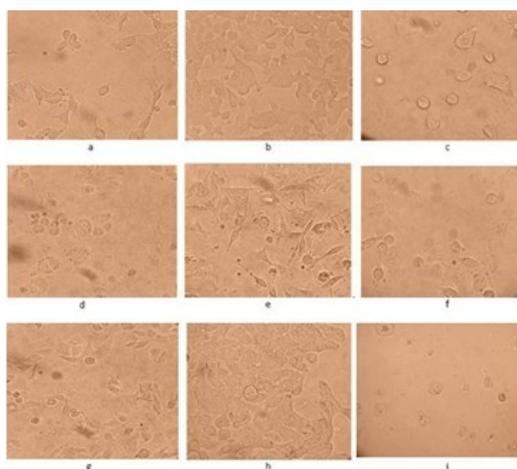


Fig 4. Digital images of heat-treated cell's obtained using stereomicroscope digital camera NIKON S500. T47D at 32°C (a), T47D at 37°C (b), T47D at 42°C (c), SW480 at 32°C (d), SW480 at 37°C (e), SW480 at 42°C (f), SW742 at 32°C (g), SW742 at 37°C (h), SW742 at 42°C (i)

4. Discussion

To the best of our knowledge this is the first report that transformed cell's S/V ratio is dependent on culture temperature and that S/V ratio at 37°C is the highest compared to that of febrile range temperature 42°C and that of low temperature of 32°C.

Despite the inconsistent pattern of changes in cell's surface area (Fig. 1), changes in cell volumes showed a homogeneous pattern (V at 42°C and 32°C is greater than 37°C) (Fig. 2). What determines this temperature dependency of cell volume is unclear but changes in $[\text{Na}^+]_i$, $[\text{K}^+]_i$ and $[\text{Cl}^-]_i$ are associated cell volume control. Hyperthermia induces transient increase in $[\text{Na}^+]_i$ and $[\text{Cl}^-]_i$ in Chinese hamster ovary cells heated at 42°C or 45°C for 30 min. $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ co-transporter, Cl^- channels, $\text{Cl}^-/\text{HCO}_3^-$ exchanger, may be involved in the heat-induced increase in $[\text{Cl}^-]_i$ [7]. K^+ and Cl^- channels parallel to K^+ , Cl^- channel and K^+/Cl^- co-transporter activity are important mediators of regulatory volume decrease (RVD), whereas regulatory volume increase (RVI) is temperature-dependent and is essentially mediated by the $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ cotransporter and the Na^+/K^+ pump [8].

In Chinese hamster ovary cells and in normal and transformed rat embryonic fibroblasts, survival as a function of time at 42.4°C was dependent upon the rate of heating from 37°C to 42.4°C. Herman et al suggested that if cells are heated to hyperthermic temperatures over sufficiently long intervals, mechanisms have time to develop, which protects the cell membrane against changes associated with cell death in rapidly heated cells [9]. The protective effect of slow rates of heating may partially explain the relative lack of success thus far observed with the use of whole-body hyperthermia in which heating from 37 degrees to 42°C often requires 2 to 3 h [10].

Changes in S/V ratio in cells exposed to different temperatures might be partially explained by blebbing. This is suggested to be initiated by a combination of events that involve local disruption of membrane-actin cortex interactions, leading to rapid protrusion of the plasma membrane as a result of the cell internal hydrostatic pressure [11, 12].

In Chinese hamster ovary cells heated for 20 min at 45.5°C in different conditions, cellular membrane blebbing was determined for cells maintained at 25°C and 37°C after hyperthermia [13].

The effect of a rapid temperature increase on the volume of yeast cells, yeast spheroplasts and human leukaemia cells has also been investigated. The thermal shift was found to induce a rapid increase in cell volume for cells lacking a cell wall (yeast spheroplasts and human leukemia cells) [14].

In 3T3 cells, temperatures higher than physiological stimulated amino acid transport activity in a dose-dependent manner up to 44°C. However, the temperature increase did not induce widespread transport increase of all other nutrients tested [15].

In human, promonocytic U937 heat-treated cells displayed a lower intracellular pH (pHi) than untreated cells [14]. On the other hand, hypertonicity induced shedding of L-selectin from the surface of human neutrophils. This process is triggered by a decrease of cell volume, and mediated by a RO 31-9790-sensitive protease (shedase), and involves tyrosine kinase activation. Although several candidate tyrosine kinases appear to be osmo-sensitive, it is shown that p38 stress kinase plays an important role in L-selectin shedding in response to hypertonicity [15].

Although we did not measure L-selection or tyrosin kinase activation in this study, it might be hypothesized that the changes in changes in S/V ratio may in part be related to concomitant changes in the tyrosine kinase activation.

As shown at Fig. 3, cell's S/V ratio at 37 is higher than that of 32 and 42. Also, decrease in S/V ratio between 42 and 37 is more pronounced compared to that of between 32 and 37. A possible explanation for this observation is that cells at 32 and 42 decrease their S/V ratio to maintain morphology as close as possible to a spherical dimension. Thus, it might be speculated that the increase of cell volume in non physiological temperatures might serve as a mechanism for cell survival. It is possible that these systems are more affected by higher temperatures, since a larger response is evident at fever range temperatures (42°C) than lower temperature of 32°C. The highest cell S/V ratio at 37°C may be indicative of physiological condition compared to that of critical temperatures of 32°C, and 42°C. As shown in Fig. 4, cell's morphology seems more intact at 37°C compared to that of 32°C, and 42°C. But this difference could not be judged without data acquired from S/V ratio analysis.

This study is a proposal for a simplistic method of calculating cell health via an easy and non destructive test. Cells that are exposed to different non-physiologic conditions could be tested with this surface area to volume ratio calculating method. This study is a pioneer research in introducing cell morphologic parameter (SA/V ratio) as a carat of understanding cell health and status. Of course, further study is needed to strengthen and further discuss this method, but its promising value should be noticed.

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