



## Interpretation of cell culture phenomena

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**Abstract.** This paper discusses the dilemma of interpreting unusual or abnormal phenomena seen in cell cultures and is not intended to address the statistical design of experiments. Problems that can be encountered when growing cells in experimental situations include low or decreasing cell numbers, abnormal cell morphology, microbial contamination, and

detachment of the cell monolayer. If any of these situations occur, it is not realistic to proceed with data analysis until the problem is corrected. The best policy is to attempt to standardize all types of cultures used for analysis and to avoid using any cultures that display atypical characteristics.

**Key words:** Cell culture, Cell morphology, Contamination, Detachment

### Perspective

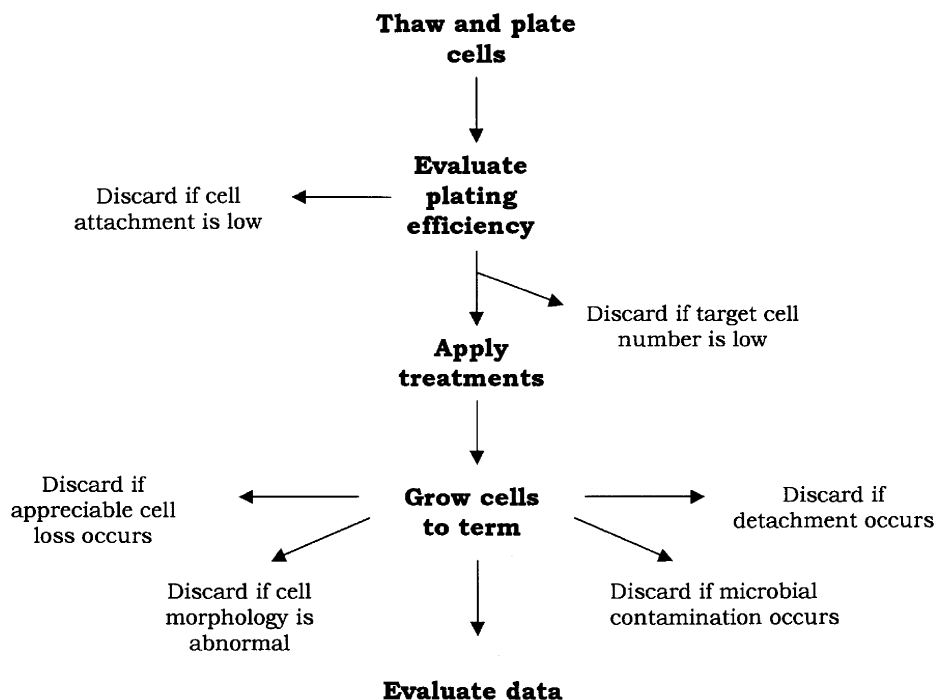
Culturing animal cells is a highly complex, multifaceted process with many variables that may influence the health and response of the cells. When atypical behavior of cells in culture is observed, a cautionary attitude must be adopted concerning the interpretation of any experimental data generated. One of the most perplexing tasks in analyzing manuscripts pertaining to cell culture is determining the health status and purity of the cultured cells. Because different laboratories use such a variety of culture conditions, media formulations and treatment regimens, it is nearly impossible to compare results between laboratories. Further, because few pictures of the cultured cells are included in the results of many of these papers, one is left with simply guessing whether the data may be applicable to his/her situation. Interestingly, while problems exist with cross interpretations of culture results, attempts or suggestions to standardize culture conditions for many cell systems have met with little support.

We suggest that there are a variety of cell culture problems that limit further consideration of the data as being reliable and scientifically valid. The underlying criterion that we use to evaluate all muscle cell cultures is cell health. If the cells within a culture are not decreasing in numbers prior to treatment application or at the end of the treatment period, are maintaining normal morphology throughout the experimental time frame, are not contaminated by mold, yeast or bacteria, and remain attached as an intact monolayer, we consider the data to be reliable and proceed with data analysis (Figure 1). If any of the aforementioned conditions are not met, then we discard the cells and begin again with a new culture.

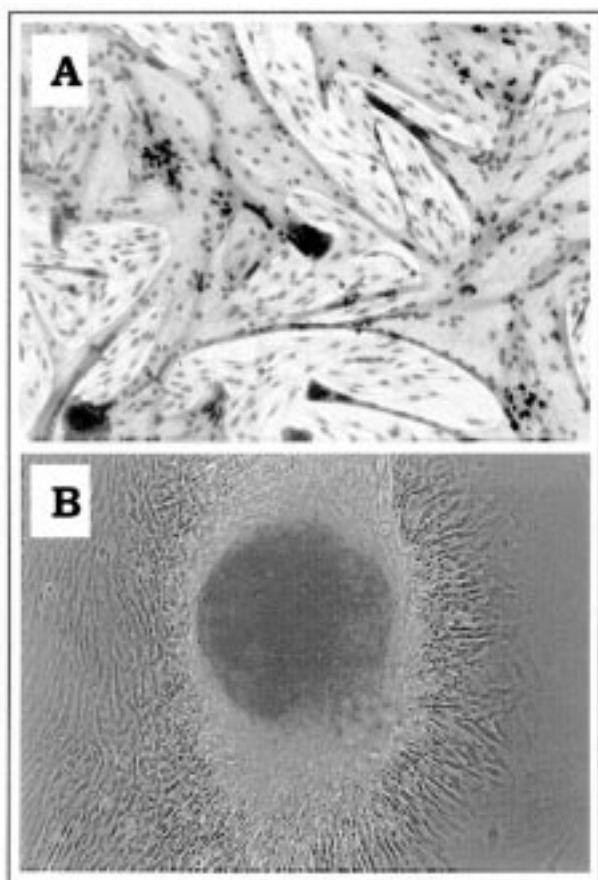
An appreciable decrease in cell number during the course of an experiment is cause for re-evaluation. This excludes some cell loss due to a low plating efficiency, which simply means that some cells characteristically attach better than others. However, when recovering cells from a frozen state, if the number of viable cells is substantially lower than what is expected, a problem with the freezing or thawing process may be indicated, and the cells should be discarded. Similarly, if abnormally low numbers of cells attach when initiating an experiment, it is best to stop the procedure, evaluate possible explanations for the observations, and start over. Sparsely seeded and struggling cell cultures will not accurately represent a healthy population of the same cells. Furthermore, in primary cell culture systems, one must make sure that the cells of interest are not overgrown by another cell type.

The appearance of abnormal cell morphology is another reason for using caution in interpreting experimental results. On occasion, in our muscle cell cultures, we have observed the formation of dense clumps of cells which appear to pile up on each other in three dimensional formations (Figure 2). Reasons underlying these morphological anomalies are unclear, although it is thought that these structures may occur when culture or environmental conditions are not optimal for the cells of interest. Any time we observe the appearance of such atypical changes in cell morphology *in vitro*, we terminate the cultures and discard the results.

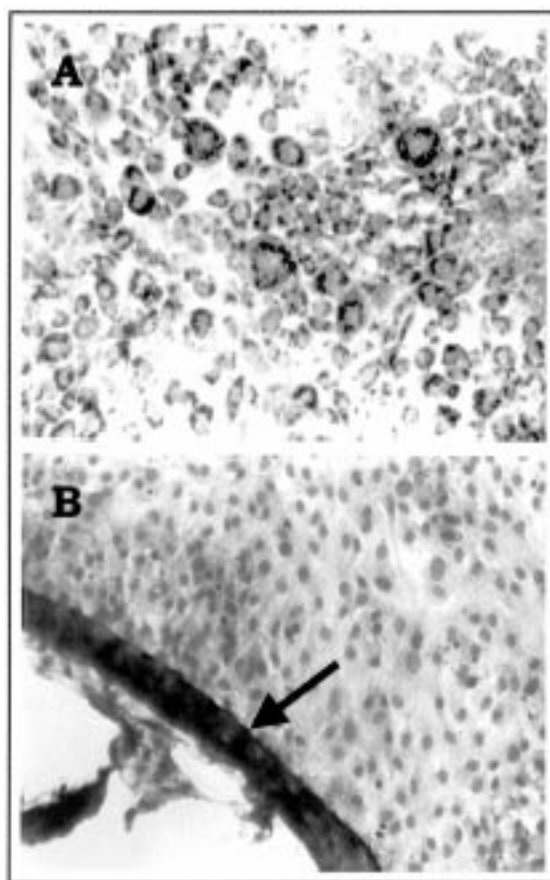
Another phenomenon warranting termination of an experiment is the emergence of any degree of microbial contamination in a culture (see Vierck et al., this issue). Once established, contamination is extremely difficult to eliminate in cell cultures even with the



**Figure 1.** Flow diagram depicting the stages for evaluation of the health of cells in culture and the reasons for termination of the cultures. Cell health must be monitored every day by microscopic observation throughout the entire length of the experiment, from thawing and plating until the final endpoint.



**Figure 2.** Photomicrographs (10×) showing ovine satellite cells with: (A) normal morphology of myotube formation. (B) abnormal morphology of dense clump of cells.



**Figure 3.** Photomicrographs (A = 20×, B = 10×) showing 3T3-L1 preadipocytes with: (A) normal morphology of differentiating cells. (B) abnormal detachment and folding back of cell monolayer (arrow points to folded monolayer).

application of antibiotics. Because of the potential for the pervasive spreading of the microbial invaders to other cultures in the laboratory, the best option is to abort the experiment, apply disinfectants to kill the contaminating organisms, discard all reagents and media used with the contaminated cultures, and reclean and disinfect equipment and the laboratory facility.

One other problem sometimes encountered in cell culture is that of lifting off of the cell monolayer (Figure 3). This event can be attributed to several factors: high cell density; lack of, or depletion of, attachment factors in the medium; overly vigorous changing of the media, mycoplasmal infection; nicking or scraping of the monolayer with a pipet; or attachment of the cells to their own extracellular matrix rather than to the plastic of the culture dish. When detachment phenomena occur in cell culture, we commonly discard the experiment and analyze and reassess culture conditions.

Because of the aforementioned problems, we suggest that if one is using muscle cell cultures as a research model, a significant amount of time should

be spent validating that the cells are performing appropriately. This requires devotion of a technician's time, extra cultureware and other supplies, and storage space for stockpiles of cells at different passage numbers. Cells that have been stored for lengthy periods of time should be re-evaluated, and new cell preparations should be routinely generated. Through careful monitoring of the behavior of cells in culture, abnormal or atypical phenomena may be detected and assessed to ensure that reported laboratory data are scientifically valid. While such critical evaluation of experiments may result in costly repeats and may slow overall research progress, we propose that ultimately more data of a higher quality will be generated for potential publication.

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