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Major Concerns with the Cryostorage of Mammalian Cell Cultures

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Abstract:

Cell cultures are of 3 types, viz. animal/human, bacterial and plant. In the laboratory, storage protocols are used to keep, preserve or regenerate these cells. However, the situation in all 3 cell types, with regard to the reason for storage, is different. It's known that when using mammalian (animal/human) cells in tissue culture experiments, not only is the factor of contamination a problem (like in plant tissue culture and micropropagation), but cryostorage is also a major concern. Freeze fractures, uncontrollable cell death, untimely cell proliferation and the formation of ice-crystals are possibilities that exist during the cryofreezing of stored mammalian cell cultures. In this article, the major concerns pertaining to cryostorage in mammalian cells will be discussed. Although not much emphasis would be placed on treatment protocols, this paper provides important emphasis on the need to use 'constituent-designed' (the correct proportions) storage protocols.

Keywords: storage, cryofreezing, regeneration, contamination, oxygen, air-bubbles, exponential, ice-crystals, trypsan, cryostorage.

MAMMALIAN CELL CULTURES

Mammalian cell cultures are either human or animal cells that have been stored in media that is specifically prepared for the storage of cells. In the laboratory, mammalian cells that are stored are retrieved for daily experiments. The initiation, or start, of these experiments aren't difficult, however, getting stored mammalian cell cultures to the exponential phases of growth can become problematic, particularly if the storage protocols weren't optimum. This means that mammalian cell cultures are sensitive not only to the treatment protocols, or treatments administered (be it drug, plant compounds, or combination therapies), but also for the storage medium they are frozen in (Singh and Reddy, 2012).

CRYOFREEZING OF MEDIUM SUSPENDED CULTURES

The cryofreezing of medium suspended cultures are a major concern when working with mammalian cell cultures. This is the case because freezing can impart some type/kind of dormancy to mammalian cells. Also, cryofreezing means that cells are exposed to temperature that could be unfavourable to suspended cultures (Singh, 2018). This implies that the regeneration times of different cryostorage medium suspended cultures would differ considerably amongst each other (Singh and Reddy, 2012). However, this is an assumption because, perhaps the times of regeneration of different cryofreezed mammalian cell cultures could be the same, but differ considerably when treated with the same plant

compound, or fraction (Singh and Reddy, 2012; Hanelt et al., 1994; read Dagne and Yenesew, 1994). This means that intracellular signalling events of cryofreezed cells of the same lineage may differ, perhaps due to cellular mutational events that have occurred prior, during, post-storage and regeneration in medium specifically for exponential growth (Singh, 2018).

SETBACKS AND PROBLEMATICS OF CRYOSTORAGE PROTOCOLS

There are many setbacks and problems concerning cryostorage protocols. One of the main problems with the cryostorage of mammalian cell cultures is that it's difficult to minimise contamination from being stored with frozen mammalian cell cultures (Singh and Reddy, 2012). This is a main problem because if one isn't experienced working with mammalian cells, then experiments would easily be conducted on contaminated cells. This indicates a possibility that cell culture experiments cannot be taken seriously always, since all cell types are prone to contamination. This also indicates that more time is required to optimise cryostorage protocols (Singh, 2018). In additions to the latter statement, another problem is the fact that air-bubbles and the entry of oxygen into cryotubes prior to cryostorage can cause mammalian cells to burst (Singh, 2018). This is a setback because the cells that are to be experimented on, upon exponential growth establishment, would have been of a much higher density that after regeneration (Singh, 2018; read Loguercio and Federico, 2003). A third confounder with mammalian cell culture protocols is the fact that cryofreezing can cause cell fractures. Thus during experiments, particularly during microscopy viewing of stained cell cultures, cells may appear to be dead. This is a setback due to cryostorage protocols because these cells would often be reported as being due to cell treatments, and, thus, processes such as artificial cell death, or apoptosis, may be reported (read Schwartz, 1993; Chinkwo, 2005). Furthermore, cryostorage with the incorrect amounts of constituents in freezing solution (medium, Bovine Calf Serum (or BCS), antibiotics, e.t.c.), may cause uncontrollable cell death, the formation of ice-crystals in the medium, and perhaps even proliferation of the cells (Singh and Reddy, 2012). Another problem is that trypsan solution often contaminates the storage solution, since trypsan-suspended pellets are used during storage (after centrifugation) (Singh and Reddy, 2012).

CONCLUSIONS AND PERSPECTIVES

Trypsan, Bovine Calf Serum, antibiotics and cell growth medium are the least of problems during the cryostorage of mammalian cell cultures. However, these components are a major concern during cryofreezing, since the use of incorrect proportions of these chemicals, may interrupt cell growth. Furthermore, although cryotubes are parafilmed to prevent bacterium contamination, contaminants may in fact be introduced into the cryofreezing solution after performing decontamination protocols. Therefore, contamination, freeze fractures and uncontrollable cell death are major concerns during cryostorage of mammalian cell cultures.

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