Development of Passaging Protocols for Suspension Bioreactor Culture of Human Embryonic Stem Cells.

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Millions of Canadaians struggle with the effects of long term diseases such as osteoarthritis and osteoporosis. Current treatment options focus on symptoms and limiting progression but do not look at treating the underlying cause. Recent research involves a promising new approach using embryonic stem cells (ESCs) to regenerate damaged or diseased tissue. ESCs are promising as they readily proliferate, self-renew and can be driven to differentiate into a variety of cell types. Current small-scale culture methods are labour intensive and yield significantly smaller volumes of cells than are required for clinical applications. This issue of supply may be addressed through the use of suspension bioreactors. Bioreactors have been successfully utilized to culture large numbers of pluripotent murine ESCs1,2 and we are now translating this knowledge to human ESCs. We have studied several passaging techniques in order to achieve a robust single cell suspension to inoculate the suspension bioreactors. Enzymatic, mechanical and chemical protocols were compared based on cell-fold expansion over a four day culture period following dissociation. We are currently investigating the effects of inoculation density and agitation rate on growth kinetics and overall cell yield culturing the CA-1T hESC line (UBC) in suspension using 125mL spinner flask bioreactors. Results will be presented for dissociation protocols as well as suspension culture kinetics. Future studies will investigate whether these protocols may be transferred directly to other hESC lines.

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