EFFECT OF GLUTERALDEHYDE CROSS-LINKED COLLAGEN ON PC-12 CELL VIABILITY

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Abstract: In vitro cell culturing has been rapidly changing to accommodate the growing field of tissue engineering. The use of dynamic cell culturing in vitro now has applications in biomedical, environmental and other related fields. In order to improve on the applications of dynamic cell culturing to tissue engineering many key issues must be addressed. Tissue engineering scaffolds are 3D materials that provide temporary support for cell culturing. To achieve the goal of tissue engineering, these scaffolds must meet some specific requirements. A few of the requirements are: (1) The surface should permit cell adhesion, promote cell growth, and allow the retention of differentiated cell functions; (2) they should be biocompatible, neither the polymer nor its degradation by-products should provoke significant inflammation or toxicity in vivo; (3) they should be biodegradable and eventually be eliminated. To accomplish goals (1-3) from above the following experiments have been initiated. First, collagen was cross-liked with gluteraldehyde at increasing concentrations from 0.1% to 25% and placed in 35 mm polystyrene cell culture dishes in order to form stable collagen gelatin scaffolding. Plates containing cross-linked collagen were then either exposed to Millipore filtered water for 1 week or had pheochromocytoma (PC-12) cells plated, 1x10⁶ cells/plate, and maintained on them for a week. We demonstrated that scaffold integrity decreased over time in higher amounts at lower cross-linker concentrations. Furthermore, we revealed that increasing doses of cross-linker, although dose-dependently stabilizing the collagen gelatin, became increasingly toxic to the cells. Thusly it became pertinent to optimize collagen cross-linked stability with cell viability. A comparison of PC-12 cell attachment, growth, and confluence using F-12K media without scaffolding vs. the use of same media with the cross-linked scaffolding was conducted. Cell viability was measured with a fluorescent assay using propidium iodide (staining cells reddish orange) to identify dead cells and calcien-AM (staining cells green) to identify living cells. Cell counts were used to determine total number of cells and % viability based on (calcien-AM positive cells/total cells)*100. We found that our highest cell viability (75% survival) occurred at lowest concentrations of cross-linker (0.1% gluteraldehyde) and determined that our maximum cross-linker stability associated with a minimum of %50 survival could be no more than (12.5% gluteraldehyde).

Keywords: PC-12 Cells, Tissue Scaffolding, Crosslinker

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