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Cryotopic Macroporous Polymeric Matrices for Regenerative Medicine and Tissue Engineering Applications

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Abstract : Three-dimensional matrices were fabricated from blend of natural-natural polymers like carrageenan-gelatin and synthetic -natural polymers such as PEG- gelatin (PEG of different molecular weights (2,000 and 6,000) using two different crosslinkers; glutaraldehyde and EDC-NHS by cryogelation technique. Blends represented a feasible approach to design 3-D scaffolds with controllable mechanical, physical and biochemical properties without compromising biocompatibility and biodegradability. These matrices possessed interconnected porous structure, good mechanical strength, biodegradable nature, constant swelling kinetics, ability to withstand high temperature and visco-elastic behavior. Hemocompatibility of cryogel matrices was determined by coagulation assays and hemolytic activity assay which demonstrated that these cryogels have negligible effects on coagulation time and have excellent blood compatibility. In vitro biocompatibility (cell-matrix interaction) inferred good cell adhesion, proliferation, and secretion of ECM on matrices. These matrices provide a microenvironment for the growth, proliferation, differentiation and secretion of ECM of different cell types such as IMR-32, C2C12, Cos-7, rat bone marrow derived MSCs and human bone marrow MSCs. Hoechst 33342 and PI staining also confirmed that the cells were uniformly distributed, adhered and proliferated properly on the cryogel matrix. An ideal scaffold used for tissue engineering application should allow the cells to adhere, proliferate and maintain their functionality. Neurotransmitter analysis has been done which indicated that IMR-32 cells adhered, proliferated and secreted neurotransmitters when they interacted with these matrices which showed restoration of their functionality. The cell-matrix interaction up to molecular level was also evaluated so to check genotoxicity and protein expression profile which indicated that these cryogel matrices are non-genotoxic and maintained biofunctionality of cells growing on these matrices. All these cryogels, when implanted subcutaneously in balb/c mice, showed no adverse systemic or local toxicity effects at implantation site. There was no significant increase in inflammatory cell count has otherwise been observed after scaffold implantation. These cryogels are supermacroporous and this porous structure allows cell infiltration and proliferation of host cells. This showed the integration and presence of infiltrated cells into the cryogel implants. Histological analysis confirmed that the implanted cryogels do not have any adverse effect in spite of host immune system recognition at the site of implantation, on its surrounding tissues and other vital host organs. In vivo biocompatibility study after in vitro biocompatibility analysis has also concluded that these synthesized cryogels act as important biological substitutes, more adaptable and appropriate for transplantation. Thus, these cryogels showed their potential for soft tissue engineering applications.

Keywords : cryogelation, hemocompatibility, in vitro biocompatibility, in vivo biocompatibility, soft tissue engineering applications
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