Poly(*N*-vinylcaprolactam) Based Cryogel Scaffold for Tissue Engineering Applications: Synthesis & Biophysical Characterization

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Statement of Purpose: Recently specifically designed macroporous cryogel material has shown great promise for various bioengineering and biotechnological applications [1]. Synthesis of these materials at subzero temperatures provide cryogels with large pores (upto 200μm), with a spongy and elastic morphology. Briefly, the monomer or polymer precursor is dissolved in water containing media and polymerized at subzero temperature for the desired time period. At such low temperature water forms ice crystals which grow and connect to each other. After complete polymerization and thawing of gels at room temperature the ice crystal melts, leaving behind large interconnected pores. The cryogel materials can be produced from both hydrophilic and hydrophobic monomers and polymeric precursors in different sizes and formats (monoliths, sheets, discs, micro-titer plate formats, etc.), depending upon the application and scale of operation. These characteristics in combination with osmotic, chemical and mechanical stability and convective flow properties can be well utilized in cell separations[2] and tissue engineering [3]. The aim of this work is to produce three-dimensional scaffold for soft tissue engineering applications. The polymer selected for scaffolds preparation is poly(vinylcaprolactam)(PVCl) from lactam family. The polymer is also known for its biocompatibility and temperature responsiveness.

Methods: The PVCl cryogel was synthesized in 5% dimethyl sulfoxide (DMSO) containing aqueous medium and in water as solvent. The crosslinker were used i.e. MBAAm and PEG-da and the synthesized cryogel were termed as PVCl-Bis cryogel and PVCl-Peg cryogel respectively. The gels were then thawed and washed at room temperature. These cryogels were then dried under vacuum and stored for further analysis. The physical characterization of cryogel scaffolds included water holding capacity and determination of interconnected porosity, flow rate and permeability. The porosity of these scaffolds was further characterized by mercury porosimeter and solvent based swelling capacity. The scanning electron microscopy was done to observe the internal porous morphology of cryogel scaffolds. Further for tissue engineering application the PVCl cryogel scaffolds were checked for its biocompatibility and biodegradability. The BSA and FBS adsorption test was carried out to demonstrate the adsorption of protein at 37°C under hydrophobic condition of polymer. The blood compatibility of these cryogels was also carried out to demonstrate its biocompatibility. The direct contact test was done to check the toxicity of PVCl scaffold and finally fibroblast cells were grown on the PVCl cryogel scaffold and analyzed by SEM and proliferation of cells was checked by MTT for 15 days.

Results: A poly(*N*-vinylcaprolactam) (PVCl) cryogel were synthesized and characterized with respect to physical and biological properties. The cryogel network has good physical morphology as confirmed by scanning electron microscopy. The porosity of PVCl-Bis and PVCl-Peg cryogels was 96% and 98%, respectively as determined by solvent absorption method and by mercury porosimeter analysis. The permeability of the two types of cryogels was 1.01×10^{-12} m4/Ns and 1.66×10^{-12} m4/Ns, respectively. The effective diffusion coefficients (*D*eff) of bovine serum albumin (BSA) in PVCl cryogel cryogel were 3.5×10^{-7}cm2/s to 3.4×10^{-7}cm2/s. The PVCl-peg cryogel were spongier and mechanically stronger than PVCl-Bis cryogel. These materials were further characterized to demonstrate its interaction with biological system. The blood biocompatibility studies showed minimal hemolysis (4–6%) caused by these materials and a very low adsorption of BSA (0.001–0.002 mg/g dry scaffold). However, the fetal bovine serum (FBS) adsorption studies demonstrate the protein binding at 37◦C. these scaffold has shown more than 15% degradation after 1month. Furthermore, cytotoxicity test and the fibroblast cell adhesion studies showed the potential of these PVCl-based cryogels for suitable biomaterial applications. The *in vivo* testing of PVCl material was done on C57BL/6J mice model. The histological studies were performed to demonstrate the localized biocompatibility these developed cryogel materials. Additionally systemic toxicity of these materials was also tested by screening of TNF-α marker in mice blood serum at different time intervals.

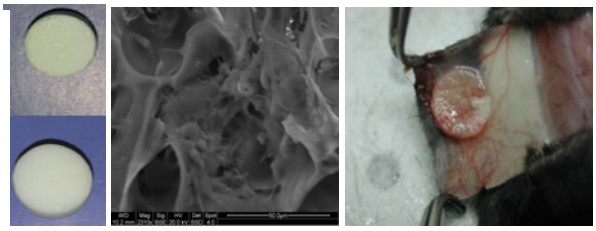

Figure 1. (a) PVCl cryogel (i) Dry (ii) Swollen. (b) SEM images shown Cell grown on PVCl-peg and *in vivo* implantation of PVCl based cryogel in rat model.

Conclusions: The biocompatible PVCl cryogel can fulfill the pre-requirement of cell culture scaffold for its biomedical application. The PVCl-peg provides better physical morphology than PVCl-Bis cryogel. The porosity of these cryogels is large enough to accommodate the animal cell and provide them better 3-D environment to grow and proliferate. The blood compatibility, direct contact assay and cell culture experiments demonstrate no cytotoxicity of these materials and further proves the biocompatibility of PVCl based cryogel.

References: (1) Lozinsky VI. *TRENDS* Biotechnol. 2003;21:445.
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