J Mater Sci: Mater Med (2010) 21:2937–2945
DOI 10.1007/s10856-010-4124-3

Thermoresponsive poly(*N*-vinylcaprolactam) cryogels: synthesis and its biophysical evaluation for tissue engineering applications

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Received: 16 April 2010 / Accepted: 29 June 2010 / Published online: 13 July 2010

Abstract The thermoresponsive poly(*N*-vinylcaprolactam) (PVCl) based cryogel network were synthesized and characterized with respect to physical and biological properties. The PVCl cryogel crosslinked with polyethylene glycol-diacrylate (PEGda) was synthesized in 1% dimethyl sulfoxide containing aqueous medium at −12°C for 12–14 h. The cryogel synthesized in this manner were highly spongy in nature and can absorb water in its porous network. These polymeric cryogel networks have good physical morphology as confirmed by scanning electron microscopy. The estimated porosity of these cryogels was 90% as demonstrated by various methods based on absorption of water and cyclohexane. The median pore diameter and surface area was 30 μm and 2.0253 m^2/g, respectively as confirmed by analysis on mercury porosimeter. These materials can interact with biological system without any cytotoxic effects. Change in temperature influenced the adsorption of fetal bovine serum (FBS) on PVCl scaffold which showed maximum protein adsorption at 37°C, as compared to that at 25°C. Furthermore, the fibroblast cell adhesion studies showed the potential of these PVCl based cryogels as tissue engineering scaffolds.

Abbreviations

BSA	Bovine serum albumin
DMSO	Dimethyl sulfoxide
FBS	Fetal bovine serum
MBAAm	Methylene bis-acrylamide
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide
PBS	Phosphate buffer saline
PVCl	Poly(*N*-vinylcaprolactam)
PVC1-PEGda	Poly(*N*-vinylcaprolactam)–polyethleneglycol diacrylate
SEM	Scanning electron microscopy
VCl	*N*-vinylcaprolactam

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1 Introduction

Development of new biomaterials with novel properties is the current requirement for biotechnological and medical applications such as drug delivery, tissue engineering, implantable devices, and as in situ gelling materials. Biocompatible and degradable networks of biomaterials are advantageous in tissue engineering, where a temporary scaffold design is needed for structural support, cell attachment, and growth [1]. These scaffolds mainly involved polymeric materials and the majority of synthetic polymeric biomaterials fall in the class of polyesters. Generally, the synthesis of these polymers utilizes organic solvent, sometimes even after washing traces of organic solvent get trapped in polymeric scaffold which can cause toxicity towards biological system. Therefore, there has been a continuous need of new polymers with aqueous based synthesis for biomedical application. Recently lactam polymers were extensively studied for the development of biomaterial scaffolds due to its biocompatibility towards biological systems [2]. Poly(*N*-vinylcaprolactam) (PVCl) is thermo-responsive polymer with the lower critical solution temperature (LCST) or cloud point of about 35°C. Above and below the LCST, the PVCl can switch its property from hydrophobic to hydrophilic (insoluble to

soluble state), respectively and this transition is reversible in nature [3]. PVCl has been reported as biocompatible polymer [2, 4] but not much research has been focused on its potential applications which perhaps were limited by its complex synthesis process [5]. The lactam based polymeric scaffolds were prepared in different format from macroporous gels to micro/nanoparticles. But hydrogels are becoming more and more appealing scaffold material because they structurally resemble the extracellular matrix of many tissues and provide three dimensional matrices for cell migration and proliferation. Additionally, they can often be processed under relatively mild conditions, and may be implanted in a minimally invasive manner [6]. However, the limitations associated with these hydrogels are their low porosity, sometimes not a proper three-dimensional (3-D) architecture, and can retain bound organic compounds, if synthesized in organic solvents which can be toxic towards cell growth [7].

Recently specifically designed supermacroporous cryogel materials have shown great promises for various bioengineering and biotechnological applications [8, 9]. Aqueous based synthesis of these materials at sub-zero temperatures provides the cryogels with large pores (up to 200 μm), spongy and elastic morphology. Briefly, the monomer or polymer precursors was dissolved in water as solvent and polymerized at sub-zero temperature for desired time period. At such low temperature water forms ice crystals which can 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/or 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, cell culture and cell-biomaterial applications [10–12]. The cryogel scaffold has shown interesting applications like capture and release of particular cell type [13] by squeezing method which is possible due to highly spongy and mechanically strong cryogels [14] and for culturing the cells for therapeutic protein production such as urokinase [8] and monoclonal antibodies [15]. In our previous works it was shown that the cryogel may provide suitable 3-D architecture for tissue engineering applications [16, 17] and cell culture bioreactor [18].

The cryogel technology was used to synthesize PVCl based cryogel as cell culture scaffold [19], which we anticipate as the introduction of a new scaffold material in the field of biomedical engineering. Here, the PVCl cryogel crosslinked with polyethylene glycol-diacrylate (PEGda) was synthesized in presence of minimum organic solvents and further characterized with respect to porosity, interconnectivity, and biocompatibility which provide in-depth material property suitable for desired biomaterial applications.

2 Materials and methods

2.1 Materials

N-vinylcaprolactam (VCl), polyethylene glycol-diacrylate (PEGda) and Dulbecco's Modified Eagle's Medium (DMEM) was purchased from Sigma–Aldrich Inc. (Steinheim, Germany), *N,N,N′,N′*-tetramethylethylenediamine (TEMED) was purchased from Sisco research laboratory (Mumbai, India), cyclohexane was purchased from SD fine-chemical limited (Chennai, India). Ammonium persulfate and bovine serum albumin were purchased from Merck chemicals (Mumbai, India). Fetal bovine serum (FBS) and antibiotic penicillin/streptomycin (P/S) solution were obtained from Hyclone (Utah, US). All other chemicals used were of analytical grade.

2.2 Methods

2.2.1 Synthesis of cross-linked poly(N-vinylcaprolactam)–polyethylene glycol-diacrylate (PVCl–PEGda) cryogel

The PVCl–PEGda cryogels were synthesized in 1–10% DMSO containing water as solvent. The cryogels were prepared by mixing 6% (w/v) *N*-vinylcaprolactam (VCl) monomer, 4% (w/v) PEGda as cross-linker in degassed and deionized 1–10% DMSO containing water as solvent. The mixture was then pre-cooled in ice bath and 0.1% initiator and activator (TEMED/APS) were then added into reaction mixture. The reaction mixture was then poured into 2.5 ml (3 cm × 0.9 cm) syringe and was immediately frozen at −12°C for 12–14 h. After complete polymerization the gels were thawed immediately with distilled water at room temperature and the resulting gels were subjected to purification by repeated washing with deionized water and water/alcohol mixture. The gels were then air dried followed by vacuum drying and then stored at room temperature.

2.2.2 Microscopy

PVCl–PEGda cryogels were analyzed by scanning electron microscopy (SEM). All the samples were ethanol dried. The samples were put consecutively in increasing concentration of ethanol that is 20, 40, 60, 80 and finally in

100% (v/v) ethanol [10]. The samples were then vacuum dried overnight before gold coating. The SEM analyses were carried out using FEI Quanta 200 and the pore diameters of cryogels were measured arbitrarily. The PVCl–PEGda sections (500 μm) were also visualized on florescent microscope. The 3-D and 2-D images were taken to demonstrate the pore morphology of PVCl–PEGda cryogels.

2.2.3 Determination of swelling kinetics

The kinetics of swelling was carried out by conventional gravimetric procedure [20]. Briefly, it involved measurement of water uptake by samples placed in deionized water at room temperature. The PVCl based cryogels were dried under vaccum. The xerogels were swollen at room temperature in deionized water and removed from aqueous swelling medium at regular time intervals. The excess water on surface was whipped off by filter paper and the weight of all the gels was taken after regular time intervals until the equilibrium was reached. The samples were of 1 cm in length and 0.9 cm in diameter. At least five samples with similar dimension of each concentration of the gel were used for the study. The weight-swelling ratio (q_w) can be calculated as:

$$q_w = \text{weight of swollen gels}(W_s)/\text{weight of dry gels}(W_d)$$

The rate of swelling was demonstrated as increase in swelling ratio over a period of time till the swelling equilibrium was achieved.

2.2.4 Flow rate determination

The flow resistance of the PVCl–PEGda cryogel columns (2.5 ml; 2 cm × 0.9 cm) were evaluated up to flow rates of 1–5 ml/min using peristaltic pump, registering the flow rate at given pump settings. In a separate experiment, the pump settings were calibrated against flow rate with no column connected according to Adrados et al. [21].

2.2.5 Porosity determination

The pore volume was measured by the uptake of cyclohexane. This was done by immersing the dried samples of the PVCl–PEGda cryogel into the cyclohexane for 1 h followed by measurement of the weight of the swollen sample. The pore volume was calculated as follows:

$$\left(m_{\text{swollen gel}} - m_{\text{dried gel}}\right)/m_{\text{swollen gel}}$$

All measurements were done in triplicate and average values were taken. Porosity was then calculated as percentage of pore volume to total volume of cryogel [21].

Porosity of the cryogels was also measured using the Archimedes's principle [22]. After determining its dry mass, each cryogels was pre-wet by placing it in ethanol under negative pressure. It was then saturated with water and weighed to determine its wet mass. Next, it was completely immersed in water and its submerged mass was measured. Porosity was then calculated as follows.

$$\text{Porosity}(\%) = \left(M_{\text{wet}} - M_{\text{dry}}\right)/\left(M_{\text{wet}} - M_{\text{submerged}}\right)$$

Five samples were tested for each cryogel.

The pore distribution of PVCl based cryogels was characterized by mercury porosimetry Poresizer 9310 (Micromeritics Instrument Corporation, Norcross, GA). The sample of 1.5 cm length and 0.8 cm diameter (0.06 g dry wt) was taken and maximum pressure applied was 100 psi. Analysis was done on mixed mode of pressure and volume. The pore diameter, D was calculated using the Wasburn equation [23, 24].

$$D = (4g\cos\theta)/P$$

where P is the applied pressure; 'θ' the contact angle of mercury on the surface was determined by mercury intrusion porosimetry. The critical pressure P_c, i.e. the minimum pressure required to intrude the largest pore was determined by plotting the following equation [25].

$$\ln[V(\infty) - V(P)] = \ln[V(\infty) - V(P_c)] - m[\ln P - \ln P_c]$$

where $V(\infty)$ is the volume of intruded mercury at the maximum intrusion pressure, $V(P)$ the volume of intruded mercury at pressure P and m is the slope of the graph.

2.2.6 In vitro degradation

Dry cryogel samples of PVCl–PEGda were weighed and transferred to 15 ml tubes filled with sterile 0.1 M PBS (pH 7.4). The tubes were incubated in a water bath at 37°C for 4 weeks under sterile conditions. After every 3 days the scaffolds were removed and washed with de-ionized water. The samples were dried overnight under vaccum. The degree of degradation was determined by dry weight change.

$$D.D(\%) = W_1 - W_2/W_1 \times 100$$

where, $D.D$ is degradation percentage, W_1 is initial dry weight of sample before incubation and W_2 is final dry weight of sample after incubation.

2.2.7 Fetal bovine serum (FBS) adsorption test

Protein adsorption was performed by incubating the scaffolds in phosphate buffered saline (0.1 M PBS, pH 7.4) containing 5% FBS [26]. The total protein concentration in this solution was 0.57 mg/ml. The disc type cryogel specimens (dry wt 0.25 mg) with dimensions of 9 mm in

diameter and 5 mm in thickness were used. Before incubation in the medium containing proteins, the specimens were pretreated with ethanol for 30 min and then washed by PBS three times overnight under gentle shaking. Specimens were then put into 24-well culture plates (one specimen for each well) and 1 ml of FBS (5%) in PBS (0.1 M, pH 7.2) solution was added into each well (total protein concentration 0.57 mg/ml). The scaffolds were incubated at 37°C for a fixed incubation time. The concentration of the protein in the FBS solution was then quantified using bicinchoninic acid reagent according to smith et al. [27], using bovine serum albumin (BSA) as standard. The amount of absorbed proteins was determined by subtracting the amount of proteins left in the FBS solution after adsorption from the amount of proteins in control FBS solution (without specimen) under the same incubation conditions.

2.2.8 Direct contact test

A near confluent monolayer of fibroblast (COS-7) was grown in 24-well culture plates. The culture medium was removed and replaced with 1 ml of fresh medium. Cryogel specimens were then carefully placed in individually prepared cultures and incubated for 24 h at 37°C in a humidified CO_2 incubator. The culture medium and specimens were then removed and cell toxicity was tested by MTT assay. MTT assay is a quantitative colorimetric assay for determining the mammalian cell survival and cell proliferation. The serum free culture medium containing thiazolyl blue (MTT) (0.5 mg/ml) were added in each well. Cells were cultured for 4 h and then the medium containing MTT was removed. After that, DMSO was added into each well to mix the formazan crystals formed by the living cells in each well, and incubated for 15 min. The solution from each sample well was removed and the absorbance of the solution was measured at 490 nm.

2.2.9 Cell culture

The monkey fibroblast cell line COS-7 was grown over PVCl–PEGda to check the adherence and growth of cells. Initially, the PVCl cryogels samples were sterilized by ethanol and then autoclaved. The scaffolds of 0.8 cm diameter and 5 mm thickness were placed in 24-well polystyrene tissue culture dish. The dried scaffolds were equilibrated with DMEM supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (P/S). The medium were then removed from the scaffolds and 2×10^5 cells (COS-7) were seeded. The cells were allowed to culture in 5% CO_2 in humidified environment at 37°C. The medium was changed after every 2 days and the cell attachment was observed on SEM after 1 week of culture.

3 Results and discussion

The non-crosslinked PVCl were synthesized in 10% or more DMSO containing water as solvent [5]. In our initial work [19] it was shown that PVCl based cryogel can be prepared in 1, 2, 5 and 10% DMSO concentration but the PVCl cryogel synthesis in 2% DMSO concentration in water provide all cryogel like features. The cryogels so developed were not completely biocompatible and expected to be degradable because *N,N,N′,N′* methylene bis-acrylamide was used as cross-linker. Hence the synthesis of completely biocompatible and degradable PVCl based cryogels with all desired properties, was the major challenge to provide it as tissue engineering scaffolds. The PEGda was chosen as cross-linker which is biocompatible and biodegradable [28]. The synthesis of PVCl–PEGda cryogel were optimized (Table 1) with varying DMSO and PEGda concentration and it was found that in the presence of 5–10% DMSO-water solvent, the PVCl–PEGda cryogels can be prepared at −12°C for 12–14 h. The cryogel synthesized in water were physically loose and weak and on the other hand cryogel synthesized in 1% DMSO containing water showed good physical morphology. Contradictorily, if DMSO concentration increased above 5% in water then synthesized cryogel were loose with low mechanical strength which does not retain shape upon drying. This may be because the polymerized PVCl chains can also get dissolved in DMSO containing water as solvent during thawing which can cause collapse of porous network in cryogel. It was also observed that upon air drying, the cryogel synthesized in water as solvent does not retain its physical shape while cryogel synthesized in 1% DMSO containing water can retain the physical shape.

The physical morphology of PVCl–PEGda cryogel are shown in Fig. 1a, it demonstrates that scaffold shape was retained even after drying the cryogel at 60°C and further dried in vacuum and can be re-swelled in water. The 2-D autofluorescent images of PVCl–PEGda cryogels demonstrated porous morphology with thicker side walls and 3-D images showed long interconnected channels of pores and pore distribution (Fig. 1b). The pores were also visualized in fluorescent microscope and it was found that PVCl–PEGda cryogel have thicker sidewalls and pore size up to 50 μm. The developed cryogel posses macroporous morphology and high porosity as demonstrated by SEM images (Fig. 1c). It showed smooth walled porous morphology in PVCl–PEGda cryogels. The PVCl–PEGda cryogels have shown highly porous system with the majority of pores having pore size in the range of 20–50 μm in diameter. It was also shown that PVCl–PEGda cryogels have dense and rigid pore walls in comparison to PVCl cryogel prepared with *N,N,N′,N′* methylene bis-acrylamide in our previous work [19]. The SEM pictures depicts the presence of

Table 1 Optimization of DMSO and monomer concentration for the synthesis of PVCl–PEGda cryogels and their physical appearance

PVCl (%)	PEGda (%)	DMSO (%)	APS/TEMED (%)	Physical appearance
6	6	5	0.12/0.1	Less spongy
6	4	0	0.12/0.1	Spongy
6	4	1	0.12/0.1	Spongy
6	4	5	0.12/0.1	Very spongy
6	4	10	0.12/0.1	Spongy
5	3.33	0	0.12/0.1	Less spongy

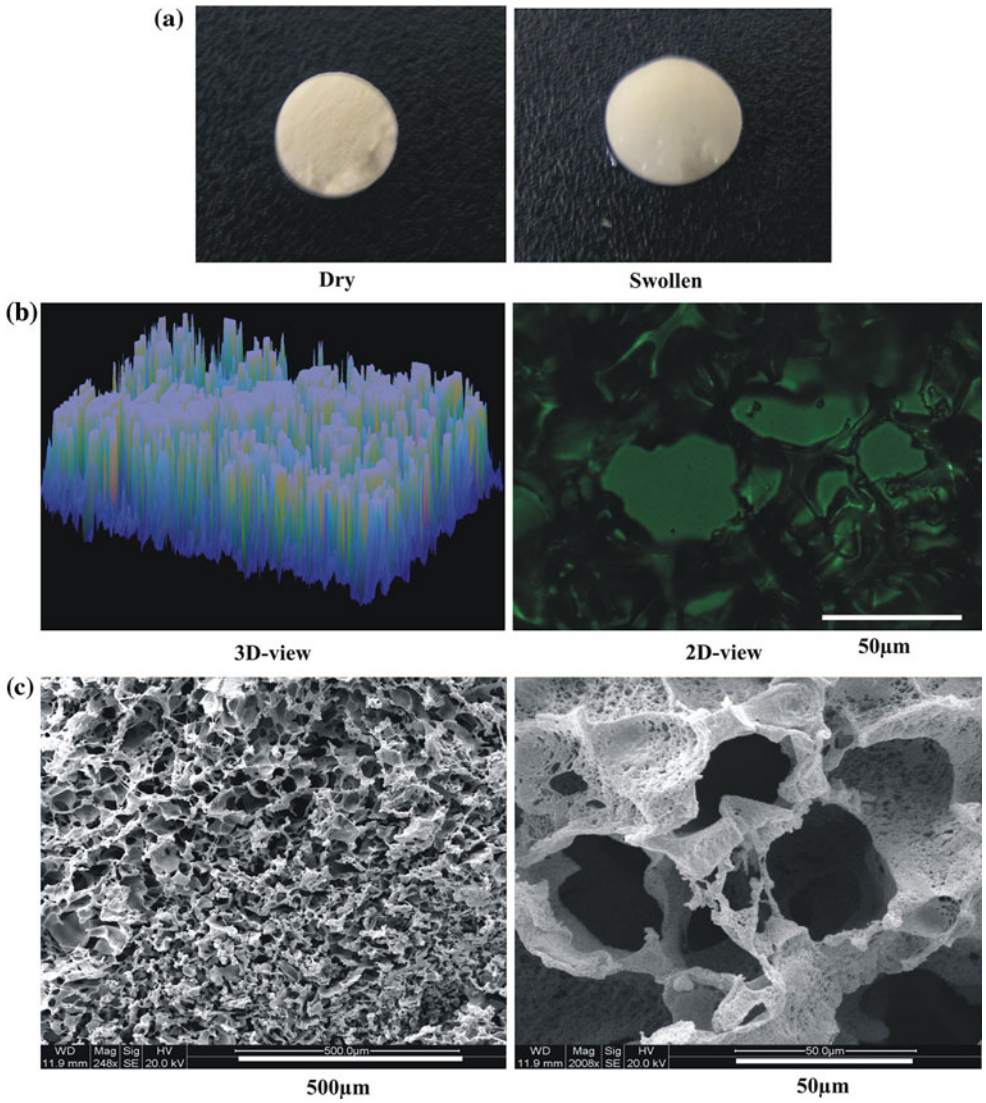

Fig. 1 (**a**) Dry and swollen form of PVCl–PEGda cryogels. (**b**) Autofluorescent 3-D and 2-D image of PVCl–PEGda cryogel sections (500 µm). (**c**) Scanning electron images of PVCl–PEGda cryogels at different magnification (×250 and ×2000)

widely distributed pore size which results in the convective flow of liquid as confirmed by flow rate experiments.

The interconnected porosity of the cryogels was demonstrated by calculating swelling kinetics of PVCl–PEGda cryogels in water (Fig. 2). The high swelling rate and swelling ratio of PVCl–PEGda cryogels demonstrate that these gels were swelled immediately within few minutes in aqueous solvent. The PVCl cryogel reached to swelling equilibrium within 2 min due to presence of highly interconnected large pores. The cryogels demonstrate high

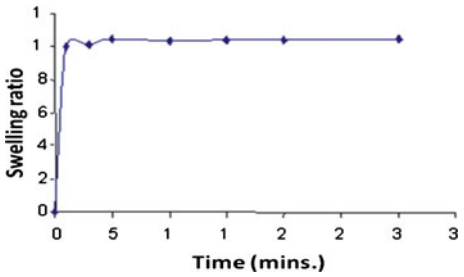

Fig. 2 Swelling kinetics of PVCl–PEGda cryogel at room temperature. The data is presented as an average of five sample analyses with $P < 0.05$

swelling rate which supports the fact that in PVCl cryogels the pore walls are also porous as demonstrated by SEM images and cause quick swelling.

The flow rate studies were carried out to demonstrate the permeability of the matrix. The PVCl–PEGda cryogels showed flow rates in the range of 3–4 ml/min without generating back pressure (Table 2). There was unhindered movement of solvent confirmed by studying flow properties of PVCl–PEGda cryogels. The high flow rate of these cryogels also confirms the presence of interconnectivity between large pores in the scaffolds.

Pore volume of the PVCl based cryogels were calculated by incubation of dried cryogels in water as well as in hydrophobic solvent like cyclohexane to provide free volume of cryogel without the contribution of polymer bound solvent and the results are presented in Table 2. The pore volume (%) of PVCl–PEGda by incubation in cyclohexane and water were 82.17 and 89.2%, respectively. The estimated porosity of PVCl based cryogels by using Archimedes principle was 96.93%. The study further demonstrates highly porous characteristics of these cryogels. The high porosity of these gels enhanced the potential of these cryogels for different biomedical applications.

Table 2 Physical characteristics of PVCl–PEGda cryogel synthesized in 1% DMSO containing aqueous solvent

Physical characteristics	PVCl–PEGda cryogel
Pore volume[a]	
A (%)	82.17
B (%)	89.2
Porosity[b]	96.93
Flow rate (ml/min)	3–4 ml/min
Thermal shrinkage (at 37°C)	1 mm (change in diameter) (10–11% change in diameter)
Swelling ratio	12
Appearance	Spongy and smooth surface

[a] Pore volume A—cyclohexane absorption and Pore volume B—water absorption

[b] Porosity—archemdies principle

The average porosity of 90% was estimated from all the above mentioned method.

The pore size distribution of PVCl–PEGda cryogels was further characterized by mercury porosimeter (Fig. 3). The results demonstrate that the median and average pore diameter was 30 and 11.7672 μm, respectively. The pore size distribution of PVCl cryogel demonstrate that the majority of pore size were in the range of 20–50 μm in a broader range (0.3–100 μm) and the median pore size was around 30 μm. The total intrusion volume was 5.958 cc/g, the large volume of mercury intruded at low pressure was due to presence of large diameter pores, small pores and mesopores. The calculated surface area of developed cryogel was 2.0253 m^2/g. The surface area is large enough to accommodate cells for growth and proliferation. It may also be possible that many large pores are interconnected with each other via narrow pores which cause the mercury intrusion at high pressure which results into the measurement of the pore sizes of those narrow connecting pores. Due to presence of mesopores in the sidewalls in PVCl cryogel, the matrix adopt large surface area which provide better mass transfer and support for cell growth. Such material surface characteristics clearly demonstrate the PVCl–PEGda cryogel have proper 3-D architecture which can be useful for animal cell growth and proliferation for its application as tissue engineering implants. The large pore size and high surface area accommodate the cells and interconnected porosity provides better nutrient transfer for cell growth and proliferation.

The degradation of PVCl–PEGda was checked by measuring the change in weight over the period of one month and it was found that the overall weight loss occurs in physiological condition. A degradation capacity of PVCl–PEGda cryogel was found to be 35 ± 1.01% (Fig. 4) with almost linear degradation rate. This may be possible due to crosslinking with degradable PEGda crosslinker which can undergoes hydrophilic degradation

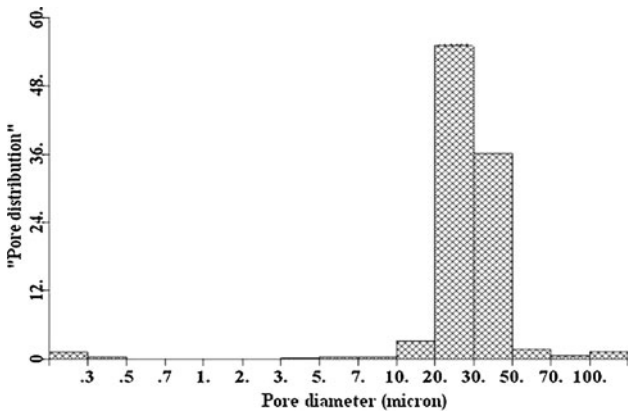

Fig. 3 Pore size distribution of PVCl–PEGda cryogels at 100 psi intrusion pressure using mercury porosimetry

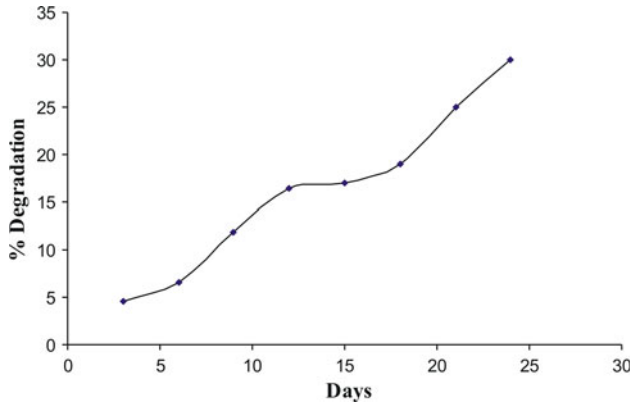

Fig. 4 The percentage degradation of PVCl–PEGda cryogel over a period of 1 month. There was 30% change in weight after 25 days

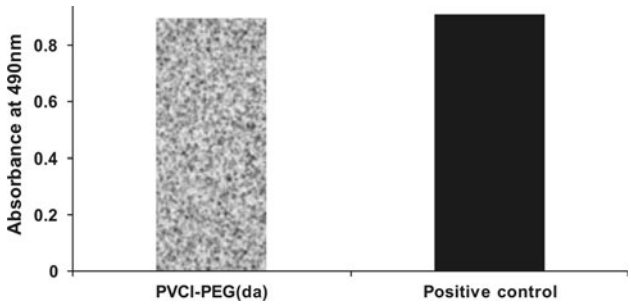

Fig. 5 MTT assay for direct contact test of PVCl–PEGda cryogel on 1 day culture of fibroblast cells, compared with positive control (cells without scaffolds). PVCl–PEGda cryogels demonstrated high cell viability which was comparable with positive control (monolayer of cells devoid of cryogel specimen). The data is the average values from five sample analyses with $P < 0.05$

through the hydrolysis of ester bond when expose to aqueous environment. Degradation potential may be high when used in in vivo or with biological systems, where enzymes and other biomolecules may act on these polymeric scaffolds [29].

Biocompatibility is the foremost requirement for all the developing biomaterials. In the present study, the assessment of biocompatibility has been made on the basis of following in vitro tests, viz. FBS adsorption test and cell cytotoxicity test. PVCl–PEGda cryogel scaffolds were incubated in FBS solution to investigate protein adsorption at two different temperatures i.e., 37 and 25°C, respectively. At 37°C in all PVCl based scaffolds, the protein adsorption reached equilibrium in 72 h and there was no significant increase of protein adsorption when incubated further. The PVCl–PEGda cryogel did adsorb significant amount of proteins, i.e., 0.52 mg/g dry scaffold from an initial serum protein concentration of 0.57 mg/ml. The adsorption of serum protein in PVCl cryogel is due to its hydrophobic nature at 37°C. The surface roughness and large surface area of PVCl cryogel could also enhance the protein adsorption. This study can further explain that large amount of protein adsorption in the PVCl based scaffold is required for cell adherence and growth. Such large amount of FBS adsorption on PVCl–PEGda cryogels can be explained as the globular protein, bovine serum albumin (BSA), is a major component of fetal bovine serum and can largely adsorb on PVCl–PEGda cryogels. One can also assume the complete adsorption of other cell adhesion globular proteins, like fibronectin, vitronectin or other proteins present in the FBS. It is also likely possible that after the adsorption of these globular proteins it further causes protein–protein interaction and more amount of BSA present in FBS can bind to the scaffold.

The protein adsorption study at 25°C showed that there was negligible amount of protein adsorption on PVCl cryogel. The PVCl behaves as hydrophilic polymer below 35°C and does not cause any protein adsorption. The serum protein adsorption occurs at high temperature i.e., 37°C which in turn cause cell attachment and proliferation but at low temperature (below 35°C) there is not any significant protein adsorption and may not cause cell attachment.

3.1 Direct contact test

The result of cytotoxicity test performed on PVCl–PEGda cryogel showed that the developed scaffold had biocompatible nature when using fibroblast cells (Fig. 5). The cell viability in the fibroblast grown in wells containing PVCl–PEGda cryogel specimens was checked by MTT test which revealed that the absorbance value of colored formazan in cryogel incubated fibroblast monolayer correspond to fibroblast monolayer in tissue culture well plates. As in negative control only scaffolds were incubated for same time period in media and in MTT assays it did not show the formation of formazan. The study further proves that the biocompatibility of these PVCl–PEGda based scaffolds is significant and has potential as cell scaffold for tissue engineering applications.

3.2 Cell adherence

The cell attachment and growth was also observed on PVCl–PEGda cryogel. It is clearly demonstrated that mouse fibroblasts COS-7 cells are well adhered over PVCl–PEGda cryogel. The PVCl–PEGda is thermoresponsive polymer and provide hydrophobic conditions above 35°C. This may be the possible reason for fibroblast cell attachment over temperature responsive PVCl–PEGda cryogel. The cells have shown flattened cell shape and secreted extra-cellular matrix (ECM) as the whole scaffold was covered with the ECM as shown by SEM images (Fig. 6). This demonstrates the potential of thermoresponsive PVCl–PEGda cryogel as

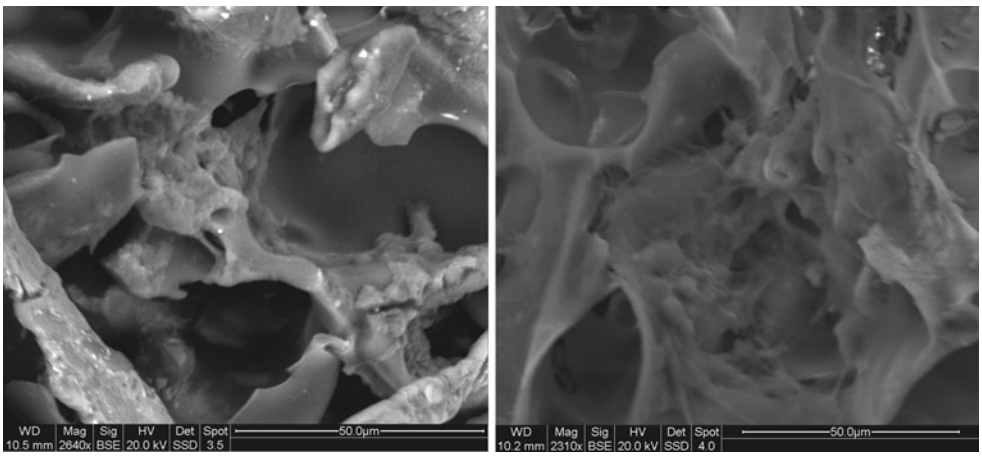

Fig. 6 Scanning electron microscopic image of fibroblast cells (COS-7) grown on PVCl–PEGda cryogel for 1 week

cell scaffold which may alter the adherence of cells by switching external temperature.

In conclusion the developed supermacroporous PVCl based cryogel have provided useful properties of the material for its applications as cell culture scaffold. The cryogel developed in this manner has demonstrated its broader range of pore size with a diameter ranging from 30 to 100 μm (average pore size in the range of 20–50 μm). In addition the materials have 3-D properties, sponginess, biocompatibility and degradation which can interact with biological system without showing any toxicity. PVCl based cryogels are thus introduced as a new material which can show potential for tissue engineering applications such as neo-vascularisation, fibroblast ingrowth, skin tissue engineering and as scaffold for stem cell transplantation. Further work is planned in this direction and also in vivo cytotoxicity analysis in mouse model will be carried out.

Acknowledgements The work was financially supported by Department of Biotechnology (DBT) and Department of Science and Technology (DST), Ministry of Science and Technology, Government of India Organizations. AS gratefully acknowledges the fellowship received from University grants commission, India.

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