

Design of Intelligent Polymer of Gelatin- Poly N-isopropylacrylamide under Gamma radiation for Cellular Applications

Saeed Heidari-Keshel^{a, b*}, Bita Soleimani^c, Maryam Ebrahimi^b, Asghar Ashrafi Hafez^d, Razieh Fallahakbarpour^e, Mohammad Khaledian^e, Fatemeh Yousefi^e, Maryam Rostampour Kakroudi^e, Forough Fathi Arateh^e

^a Proteomics Research Center, Shahid Beheshti University of Medical sciences, Tehran, Iran.

^b Tissue Engineering Department, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran.

^c Department of Chemistry, Tonekabon Branch, Islamic Azad University, Tonekabon, Iran.

^d Medical Education Research Center, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran.

^e Department of Biomaterials Engineering, Tonekabon Branch, Islamic Azad University, Tonekabon, Iran.

ARTICLE INFO

Article Type:

Research Article

Article History:

Received: 2013-09-13

Revised: 2013-10-02

Accepted: 2013-10-09

ePublished: 2013-10-15

Keywords:

Thermo- Sensitive Polymer

Gelatin

N-Isopropylacrylamide

Gamma Radiation

Epithelial Cell Sheet

ABSTRACT

Thermo-sensitive polymers were prepared by graft copolymerization of gelatin with N-Isopropylacrylamide via gamma radiation. Characterization of polymers such as DSC analysis, swelling in different ratios and cell assays were investigated. DSC and solubility analysis showed gelatin and N-Isopropylacrylamide monomers were grafted via gamma radiation successfully. Results show swelling of samples increased as gelatin increased. Swelling ratio and curves results administrated hydrophilicity / hydrophobicity of hydrogel that this property is due to presence of N-Isopropylacrylamide in different temperatures. The polymer was tested for harvesting epithelial cells after carrying out cell culture at 37 °C and incubating the confluent cells at 10°C for spontaneous detachment of cell sheet from polymer surface without enzyme treatment. These unique properties of the hydrogel would make it a promising support for drug delivery systems and tissue regeneration.

*Corresponding Author: Saeed Heidari-keshel, E-mail: saeedhey@yahoo.com

Introduction

During recent decades several materials and medical devices have been produced for medical purposes. For tissue engineering, it is desirable to recover the monolayer cells in a cell sheet structure at the end of the culture stage without using a biochemical or chemical reagent [1, 2, 3]. Such a cell sheet constructed in vitro could be useful in various clinical situations to regenerate tissues (especially epithelial tissues) such as artificial skin and artificial cornea [4, 5]. Cell sheet engineering has been developed to avoid tissue reconstruction limitations using biodegradable scaffolds or single cell suspension injection [6, 7, 8].

Cell sheets are developed by thermo-responsive culture dishes [9, 10, 11]. Thermo-responsive polymers are grafted to dishes covalently, which allows different cell types to attach and proliferate at 37°C [12, 13, 14]. Cells detach spontaneously without using enzymes when the temperature decreases below 32°C; this is due to the natural specification of the intelligent polymers, and also to the detachment of the cell metabolic changes made by the polymer resulting from decreasing temperature [15, 16, 17].

PNIPAAm and polyacrylic acid are successfully grafted into different substrates such as polytetrafluoroethylene (PTFE) and polyvinylidene fluoride (PVDF) or polyethyleneterephthalate (PET) using gamma radiation [18, 19, 20]. The hydrogels such as chitosan and other natural polymers can be used for grafting with PNIPAAm by different methods. These intelligent polymers increased cell attachment [21-30]. In this work, hydrogels based on gelatin (hydrophilic material) grafted N-isopropylacrylamide were prepared by ⁶⁰Co gamma radiation, the thermo

sensitivity and swelling & cellular properties of the polymers were also investigated.

Materials and Methods

Gelatin was purchased from Fluka Company (Gelatin from porcine skin, Type A. Sigma-aldrich). N-Isopropylacrylamide (NIPAAm, Aldrich) were recrystallized from nhexane and methanol freshly before use.

A series of gelatin-g-NIPAAm hydrogels were prepared in the following procedures: pure gelatin dissolved in 5% aqueous acetic acid (25 ml) in a glass reaction bottle, the monomer was added to the gelatin solution (W% gelatin/monomer =1/1 and 3/1). Mohr's salt (ammonium ferrous sulphate) was added to the mixture to minimize homopolymerization during irradiation. The solution was deoxygenated by purging with nitrogen for 30 min. The sealed reaction bottles were irradiated at doses 10 and 20 kGy. After irradiation, the product was extracted with methanol in a Soxhlet extractor for 48 h, in order to remove the unreacted monomer, homopolymer and other impurities. The hydrogel was dried at 40°C in a vacuum oven overnight (Figure 1).

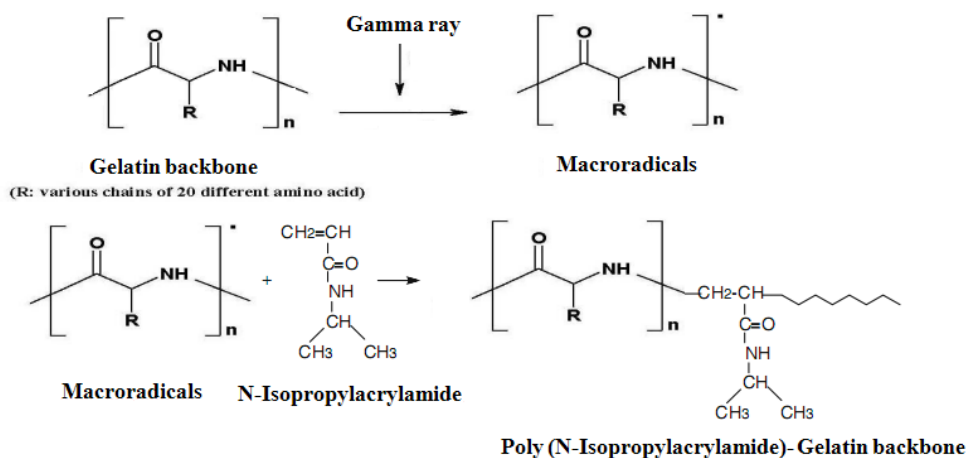


Fig. 1. Mechanism of graft polymerization of poly-NIPAAm with gelatin by gamma radiation.

For cell culture; fibroblast (1929) cells (obtained from National Cell Bank of Iran, NCBI) were cultured in RPMI 1640 supplemented with 10% fetal calf serum, 100 U/ml penicillin and 100 µg/ml streptomycin. They were incubated at 37°C in a humidified CO₂-incubator with 5% CO₂ and 95% air. For Cytotoxicity assay, The effect of diethylenetriaminepentacetic acid (DTPA) on these cell lines, 3-(4,5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide (MTT), colorimetric assay was applied. Briefly, growing cells (1.5 × 10⁴ cells/ml) were transferred into 96-well culture plates containing 200 µl of medium and incubated for 24 h. Various concentrations of DTPA (0-100 µM) were added and incubated for different time intervals followed by MTT assay. The percent of cell viability was calculated as the (mean OD of treated cells/mean OD of control cells).

Swelling ratio of the hydrogels in different doses (10 and 20 KGy) and temperatures (10-40°C) in the distilled water was investigated. The samples were investigated by thermal analysis using the DSC device (NETZSCHDSC200F3), with the heating rate of 5 degree per minute from 0°C to 60°C in a nitrogen gas atmosphere.

Results and Discussion

‘Table 1’ indicated swelling ratio of the hydrogels in different doses. Swelling ratio for 10 and 20 KGy were calculated which demonstrated hydrophilicity of samples for 10 KGy and NIPAAm increasing was about 14 which showed hydrophilicity of the samples. ‘Figure 2’ showed swelling / temperature

ratio of hydrogels. The curve slope related to critical temperature of the gels at 30°C and this demonstrated presence of PNIPAAm in the hydrogels and no significant change in polymer LCST during radiation and graft process.

Table 1. Swelling ratio of copolymers with to different doses for 1 hour and 1 week at 25°C.

Sample	NIPAAm/gelatin(3/1) at 10 KGy	NIPAAm/gelatin(3/1) at 20 KGy	NIPAAm/gelatin(1/1) at 10 KGy	NIPAAm/gelatin(1/1) at 20 KGy
1hours	5.5±0.3	5.4±0.1	7.6±0.4	7.1±0.1
1 weeks	11.1±0.1	10.8±0.2	14.3±0.4	14.1±0.1

(The data are presented as the mean values S.D, P < 0.05).

The grafted samples’ DSC analysis review showed a critical temperature of the grafted PNIPAAm. ‘Figure 3’ shows the DSC thermo-gram in which the curve

slope in 30°C is obtained. This shows no significant change in the smart polymer critical temperature during radiation and graft process.

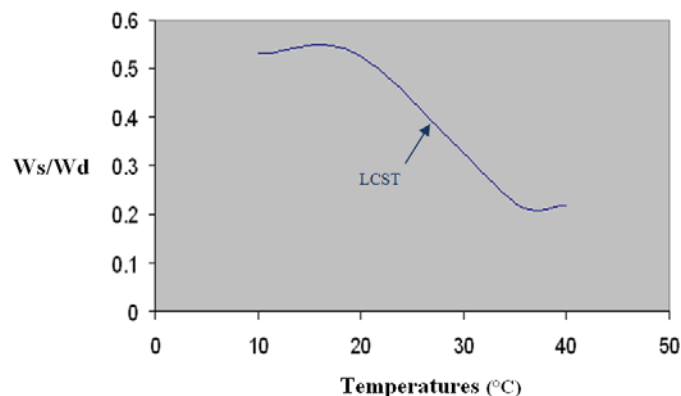


Fig. 2. Ws/Wd ratios to T°C, the curve slop in 30°C showed presence of PNIPAAm in hydrogel (dose :10KGy , NIPAAm/gelatin:1/1).

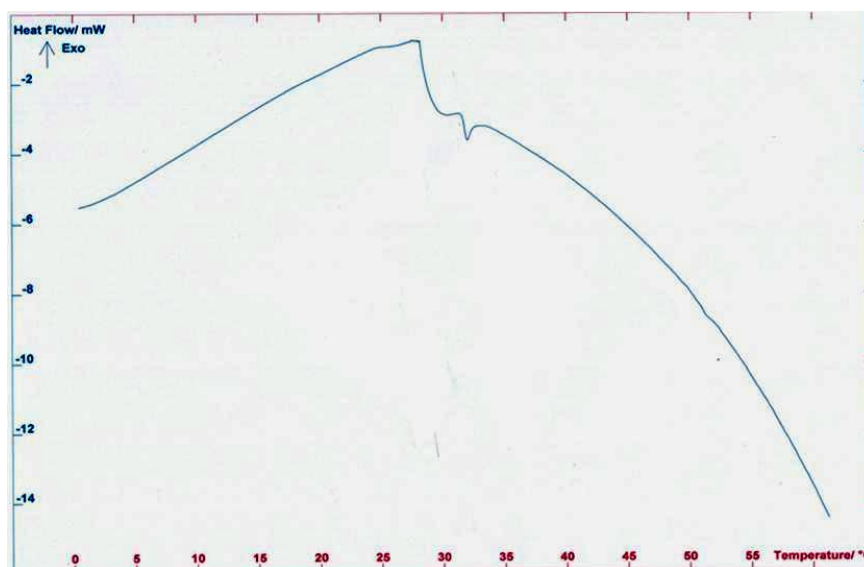


Fig. 3. DSC spectra of the hydrogel grafted by gamma radiation (radiation dose : 10 KGy)

At physiological temperature (37°C), the hydrogel turns into a rigid gel within 10 min and the phase transition is reversible. Biocompatibility data demonstrated that the hydrogel supported cell adhesion and proliferation and the cells also maintained high viability 'Figure 4'. After cultured for 7 days on gel, all cells are alive, suggesting that hydrogel is suitable for cell attachment and proliferation 'Figure 4a' and viability is 85% . When cells were placed outside the incubator and the medium cooled from 37 to 10°C, almost all cells are alive 'Figure 4b' and viability is up 80% . 'Figure 5a' shown good cells grown on gel surface (85%) at physiological temperature (37°C), 'Figure 5b' shown cells grown detached from the hydrogel surface spontaneously, in the absence of enzymes (trypsin/EDTA). Cell detachment efficiency from the hydrogel was high. After a longer period of cell cultivation for 7 days, confluent cells formed a continuous monolayer cell sheet on the surface of the hydrogel. The cell sheet spontaneously detached from the surface of the thermo-reversible hydrogel when cooled to 10°C without treating with any enzymes. As shown from 'Figure 5b' detachment of the cell monolayer started from the edge of the cell

monolayer. After 60 min incubation at 10°C, a monolayer cell sheet could be lifted up from the edge upon mild perturbation of the medium. A living cell sheet completely detached from the culture surface could be obtained within 60 min 'Figure 5b'. Although the cell sheet was folded into an irregular shape by contractile forces between cells, cell-cell connections were well preserved in the rolled-up sheet. These results demonstrate that cold treatment effectively released the cell sheet from the plate without considerable damage of the cell-cell connections.

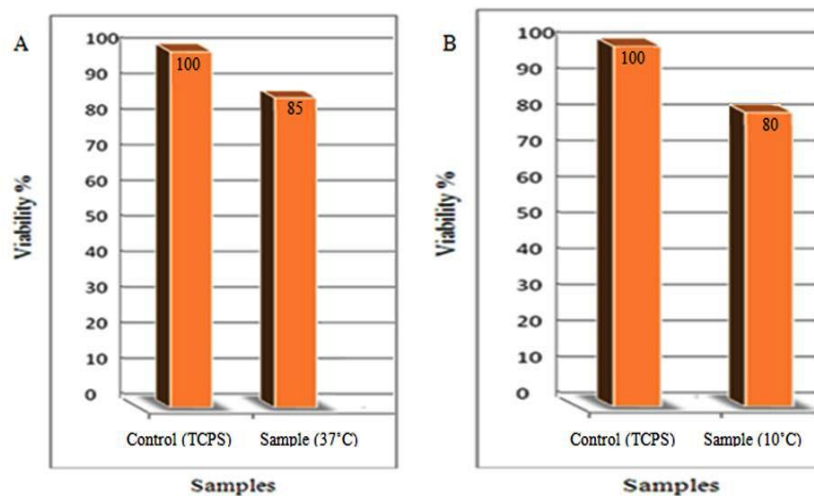


Fig. 4. Cells viability on gel surface at physiological temperature (37°C) (A), and after 60 min incubation at 10 °C (B) and control surface (TCPS).

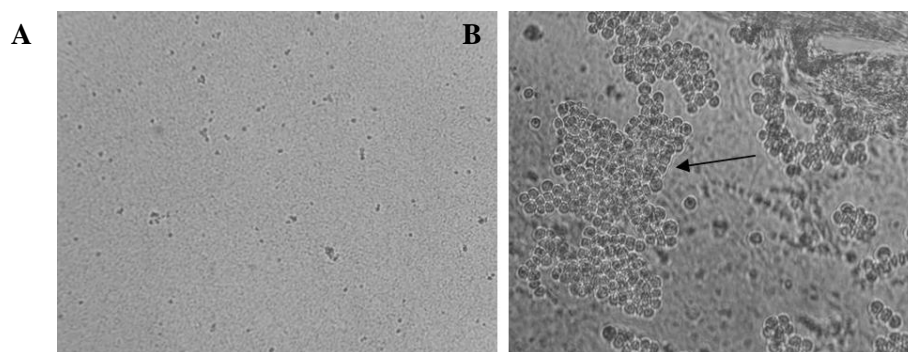


Fig. 5. Cells growth on hydrogel. A) Good cells grown on gel surface at physiological temperature (37°C) , B) Detachment of the cell monolayer after 60 min incubation at 10 °C .

Conclusion

In this work, hydrogels based on gelatin grafted N-isopropylacrylamide was prepared by 60°C gamma radiation, the thermo sensitivity and swelling and cellular properties of the polymers were also investigated. The gelatin-g-NIPAAm hydrogels showed good thermo-sensitivity and swelling property. Results show swelling of samples decreased with gelatin increasing also swelling ratio of samples decreased with radiation dose increasing due to more cross linking between polymeric chains. Swelling ratio and curves results administrated hydrophilicity / hydrophobicity of hydrogel that this property is due to presence of PNIPAAm in different temperatures. Fibroblast cells grew well on the hydrogel surface at 37°C and showed high viability that this administrated biocompatibility and non toxicity of our hydrogel. MTT analysis showed good viability of hydrogel at 37°C. Cells also (cell sheet) detached spontaneously when temperature decreased at 10°C, without using enzymes. MTT analysis showed good viability of the hydrogel at 10°C that this administrated no significant change in cell viability. These unique properties of the hydrogel would make it a promising support for drug delivery systems and tissue regeneration.

Conflict of interest

Authors certify that no actual or potential conflict of interest in relation to this article exists.

References

- [1] Park KD, Suzuki K, Lee WK, Lee JE, Kim YH, Sakurai Y, Okano T. Platelet adhesion and activation on polyethylene glycol modified polyurethane surfaces. *ASAIO J.* 1996;42:876–881.
- [2] Kikuchi A, Okano T. Nanostructured designs of biomedical materials applications of cell sheet engineering to functional regenerative tissues and organs. *J Control Release.* 2005;101:69–84.
- [3] Ricardo MP, da SilvaJoao F, Manoand Rui L. Smart thermoresponsive coatings and surfaces for tissue engineering: switching cell-material boundaries. *TRENDS in Biotechnology.* 2007;25:577–583.
- [4] Akiyama Y, Kikuchi A, Yamato M, Okano T. Ultra thin poly (N-isopropylacrylamide) grafted layer on

poly(styrene) surfaces for cell adhesion/detachment control. *Langmuir.* 2004;20:5506–5511.

- [5] Ozyürek Z. Thermoresponsive Glycopolymers via Controlled Radical Polymerization (RAFT) for Biomolecular Recognition. *Macromolecular Chemistry and Physics.* 2007;208:1035–1049.
- [6] Yamato C, Kohno A, Nishimoto H, Sekine F, Okano T. Cell sheet engineering: recreating tissues without biodegradable scaffolds. *Biomaterials.* 2005;26:6415–6422.
- [7] Lim YM, Jeun JP, Lee JH, Lee YM, Nho YC. Cell Sheet Detachment from Poly(Nvinylcaprolactam- co-N-isopropylacrylamide) Grafted onto Tissue Culture Polystyrene Dishes. *J.Ind. Eng. Chem.* 2007;13:21–26.
- [8] Priscilla S, Curtia M, de Mouraa R. Characterization of PNIPAAm photografted on PET and PS surfaces. *App.Sur. Sci. J.* 2005;245:223–233.
- [9] Yamada N, Okano T, Sakai H, Karikusa F, Sawasaki Y, Sakurai Y. Thermo- Responsive Polymeric Surfaces; Control of Attachment and Detachment of Cultured Cells. *Macromol. Chem. Rapid Commun.* 1990;11:571–576.
- [10] Yaping H, Andrew R, Ashley M, Smitherman M, Allen S. Thermoresponsive nanocomposite hydrogels with cellreleasing behavior. *Biomaterials.* 2008;29:3175–3184.
- [11] Gil ES, Hudson SM. Stimuli-responsive polymers and their bioconjugates. *Prog. Polym.Sci.* 2004;29:1173–1222.
- [12] Yamato M, Okano T. Cell Sheet Engineering. *Materials Today.* 2004;7:42–47.
- [13] Caner H, Hasipoglu H, Yilmaz O, Yilmaz E. Graft copolymerization of 4-vinylpyridine on to Chitosan-1. By ceric ion initiation. *Eur. Polym. J.* 1998;34:493–497.
- [14] Singh DK, Ray AR. Radiation-induced grafting of N,N0-dimethylaminoethyl methacrylate onto chitosan films. *J. Appl.Polym. Sci.* 1997;66:869–877.
- [15] Mima S, Miya M, Iwamoto R, Yoshikawa S. Highly deacetylated chitosan and its properties. *J. Appl. Polym.Sci.* 1983;28:1909–1917.
- [16] Kumber SG, Aminabhavi TM. Synthesis and characterization of modified chitosan microspheres:effect of the grafting ratio on the controlled release of nifedipine through microspheres. *J. Appl. Polym. Sci.* 2003;89:2940–2949.
- [17] Shantha KL, Harding DRK. Synthesis and characterization of chemically modified chitosan microspheres. *Carbohyd. Polym.* 2002;48:247–53.
- [18] Wang MZ, Qiang JC, Fang Y, J.Polym Sci, Part A. Preparation and properties of chitosan-poly(Nisopropylacrylamide) semi-IPN hydrogels. *Polym. Chem.* 2000;38:474–481.
- [19] Kim SY, Cho SM, Lee YM, Kim SJ. Thermo and pH-responsive behaviors of graft copolymer and

- blendbased on chitosan and N-isopropylacrylamide. *J. Appl.Polym.Sci.* 2000;78:1381–1391.
- [20] Li Y, Yu H, Liang B, Fang Y. Study of radiationinduced graft copolymerization of butyl acrylate onto chitosan in acetic acid aqueous solution. *J. Appl. Polym.Sci.* 2003;90:2855–2860.
- [21] Zhai ML, Liu N, Li J, Yi M, Li JQ, Ha HF. Radiation preparation of PVA-g-NIPAAm in a homogeneous system and its application in controlled release. *Radiat. Phys. Chem J.* 2000;57:481–484.
- [22] Liu PF, Zhai ML, Wu JL. Study on radiationinduced grafting of styrene onto chitin and chitosan. *Radiat. Phys. Chem J.* 2001;61:149–153.
- [23] Yoksan R, Akashi M, Biramontri S, Chirachanchai S. Hydrophobic chain conjugation at hydroxyl group onto g-ray irradiated chitosan. *Biomacromolecules.* 2001;2:1038–1044.
- [24] Biazar E, Zeinal R, Montazeri N, et al. The Cell engineering: nanometric grafting of poly-N-isopropylacrylamide onto polystyrene film by different doses of gamma radiation. *Int.J.Nanomed.* 2010;5:549–56.
- [25] Biazar E, Khorasani MT, Daliri M. Cell sheet engineering: solvent effect on nanometric grafting of poly-N- isopropylacrylamide onto polystyrene substrate under ultraviolet radiation. *Int.J.Nanomed.* 2011;6:295–302.
- [26] Biazar E, Khorasani MT, Daliri M. Cell adhesion and surface properties of poly styrene surfaces grafted with Poly(N-Isopropylacrylamide). *Chinese journal of polymer sciences.* 2013;31:1509–1518.
- [27] Rezaei Tavirani M, Heidari Keshel S, Biazar E. Effect of plasma radiation on intelligent surface grafted to NIPAAm with chemical initiator under UV radiation. *Oriental journal of chemistry.* 2011;27:1457–1464.
- [28] Biazar E, Poursamsian Kh. Synthesis and properties of thermo- sensitive hydrogels based on PVA/Chitosan/PNIPAAm. *Oriental journal of chemistry.* 2011;27:1443–1449.
- [29] Biazar E, Mohammadi H, Asefnejad A, et al. Design of smart surface by nanometric grafting of NIPAAm with benzophenone initiator under UV radiation. *Oriental journal of chemistry.* 2011;27:997–1003.
- [30] Biazar E, Montazeri N, Pourshamsian K, et al. Platelet adhesion and activation on polyethylene glycol modified polyurethane surfaces. *ASAIO J.* 1996;42:876–881.