International Journal of Advances in Medical Biotechnology

Journal homepage: http: www.journalamb.com/index.php/jamb

Intelligent copolymers based on poly (N-isopropylacrylamide) PNIPAm with potential use in biomedical applications. Part i: PNIPAm functionalization with 3-butenoic acid and piperazine

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ARTICLE INFO

Keywords:
Poly (N-Isopropylacrylamide)
3-Butenoic Acid, Piperazine
Thermosensitive copolymers
LCST Temperature

ABSTRACT

The synthesis and characterization of the thermosensitive copolymers based on Poly (N-Isopropylacrylamide) (PNIPAm) and 3-butenoic acid and functionalized with piperazine was carried out. The free radical polymerization of the PNIPA copolymer with 3-butenoic acid was performed under microwave radiation. After obtaining this copolymer, the carboxyl groups present in the copolymer chain were activated with 1-ethyl- (3-3-dimethylaminopropyl) carbodiimide in the presence of N-hydroxysuccinimide, improving its reactivity to incorporate the piperazine through its amino group. The characterization consisted: differential scanning calorimetric and ultraviolet-visible spectrophotometry to determine the LCST phase transition temperature, ranging from (30-35)°C. Structurally it was analyzed by infrared spectroscopy. A morphological analysis was performed using scanning electron microscopy, after simulating an injectable process, with the objective to observe internally the porosity and interconnectivity. The biocompatibility was evaluated through hemocompatibility tests and it was observed that the copolymers obtained were not cytotoxic. In base of the results, the chemical structure of these new copolymers confers a functionality that allows them to serve as nuclei to graft other molecules, such as polysaccharides. Then, the results obtained on the LCST temperature, porosity, interconnected pore network morphology, the ability to be injectable and the biocompatible nature of these copolymers are indicative that these new synthetic biomaterials have the potential to be used in biomedical, pharmacological and for tissue engineering. Also, once their biocompatibility was demonstrate, they may serve to generate interesting compounds having chemical anchor points for the possible addition of polysaccharides using insertion reactions, thereby generating graft copolymers with potential use in biomedical applications.

1. Introduction

Over the years, metals, ceramics and polymers had been the basic materials for the design of biomaterials due their reproducibility and properties[1], but are the polymers (like polyesters and biopolymers) who have presented the broadest and most versatile class of biomaterials that are widely used for biomedical applications, greatly impacting the advancement of modern medicine [2,3]. One of the most important characteristics of a biomaterial that can be used in biomedicine to interact with a biological system is that it be biocompatible [4]. Among the biomaterials, based on polymers, we can find hydrogels, which are hydrophilic polymers that due to their macromolecular chain and at their disposal in the form of a three-dimensional network can absorb water without being soluble under conditions of temperature and physiological pH [5].

Currently, the development of systems called intelligent hydrogels has taken great interest in the area of bioengineering and biomedicine. These novel materials can drastically change their volume upon application of a specific stimulus, such as a change in temperature, pH, electric fields or light, and such changes are reversed once the stimulus is removed [6-8].

In the case of hydrogels sensitive to changes in temperature are characterized by having a lower critical solution temperature (LCST) [9,10]. Among the smart polymers we can mention PNIPAm, which is a non-biodegradable polymer having an LCST of approximately 32-34°C (close to human body temperature 37.4°C) [7,11]. However, being a synthetic polymer, the PNIPAm could present problems

ARTICLE HISTORY:
Received 20 October 2017; Received in revised form 12 November 2017;Accepted 10 December 2017
Available online 04 January 2018

of biocompatibility and biodegradability, so it is of great interest, the incorporation of a phase that can improve this condition, especially if it is intended to be used in some biomedical applications [12]. In addition, it has been reported in the literature that copolymerizing PNIPAm with other monomers provides a way to incorporate a wide range of functionality while retaining thermal response [10]. Considering the above, it is proposed to obtain a copolymer based on PNIPAm modified and functionalized with 3-butenoic acid and piperazine. This research, presented in this first part, may serve to generate interesting compounds having chemical anchor points for the possible addition of polysaccharides using insertion reactions (how it will be done in the continuation of this project and will be presented in a second part), thereby generating graft copolymers with potential use in biomedical applications

2 Materials and Methods

2.1 Materials

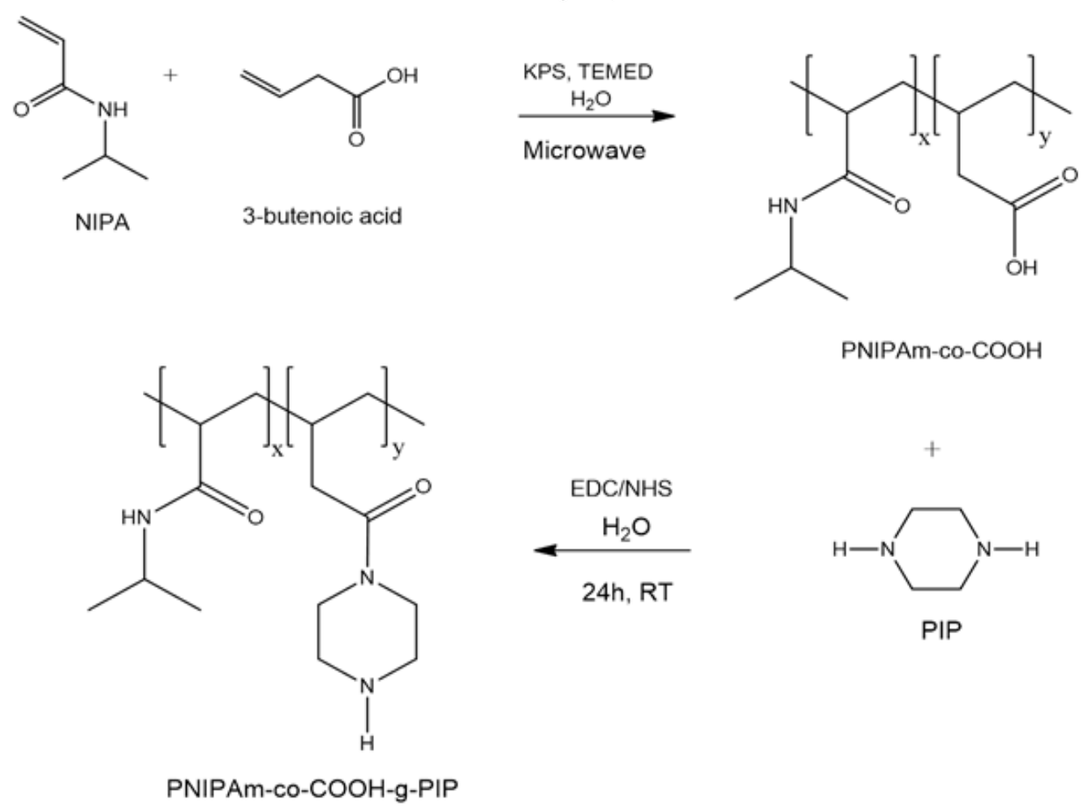
N-Isopropylacrylamide (NIPA), 3-butenoic acid, piperazine (PIP), 1-ethyl-(3-3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS) were purchased from Sigma-Aldrich. Potassium peroxydisulphate (KPS, Riedel - de Haën). All reagents were used analytical grade.

2.2 Methods

2.2.1 Synthesis of copolymer PNIPAm-co-3-butenoic acid (PNIPAm-co-COOH) via a microwave

PNIPAm-co-COOH was synthesized by free radical polymerization via a microwave. First, the NIPA monomer and 3-butenoic acid as the co-monomer, were placed in a teflon reactor with 25ml of deionized water (19:1 and 18:2 molar ratios, respectively) (Fig. 1). Dried nitrogen ($N_{2(g)}$) was bubbled in to the solution for 5min prior to polymerization to avoid formation of oxidation products. KPS initiator was added and the reactor was closed. The mixture was immediately placed into the microwave. The microwave-assisted polymerization reactions was performed in a microwave mark MAR model CEM and power at 300 W (at 70%) for 5min. The use of microwave radiation allowed to reduce the reaction times to only 5min. By using conventional reaction methods reaction times can be extended up to 24 hours. The system recorded that it reached temperatures between 60-70 ºC and a pressure around 10psi. After the reaction, the purification consisted of the following: the solutions were dialyzed for

Figure 1 - Scheme of synthesis of the copolymer PNIPAm-co-COOH and functionalization with piperazine (PNIPAm-co-COOH-g-PIP).



48h at room temperature, followed by successive washes with deionized water and centrifugation. To complete the purification process, and taking advantage of the phase change undergoing the PNIPAm, the copolymer synthesized were dissolved in 5mL of water, stirred vigorously for a few minutes and heated (40°C) to promote phase separation and forming the gel. The reactants that did not react would remain in the aqueous phase, which was carefully removed. This procedure was performed three times. Finally the samples were frozen and lyophilized for 24h at -50ºC and 500psi.

2.2.2 Functionalization of PNIPAm-co-COOH with piperazine (PNIPAm-co-COOH-g-PIP)

Once the PNIPAm functionalized with 3-butenoic acid has been obtained and characterized, the PNIPAm backbone has -COOH groups available for reaction with an amino group. Therefore, the functionalization of PNIPAm-co-COOH with piperazine will be performed. Carbonyl group of the copolymer PNIPAm-co-COOH was activated with the condensing agents EDC/NHS (2:1 ratio) [13,14]. Improving its reactivity for subsequent incorporation of the amino group present in the piperazine, obtained the copolymer PNIPAm-co-COOH-g-PIP. The synthesis pathway is summarized in Figure 1. The PNIPAm-co-COOH: PIP ratio was 20:1 and 20:1.2. The reaction was carried out for 24h under constant stirring at room temperature. The purification process was performed in a similar manner as when the PNIPAm-co-COOH copolymer was obtained.

2.3 Characterization

2.3.1 FTIR-ATR spectroscopy of the copolymers PNIPAm-co-COOH and PNIPAm-co-COOH-g-PIP

The identification of functional groups and changes in bonding environments during reaction were monitored using a Thermo Scientific – Nicolet IS5 Fourier transform infrared (FTIR) spectrometer equipped with a ZnSe Attenuated Total Reflectance (ATR). The experiments run with air as the background. Scans were accumulated 32 sweeps with a resolution of 4cm^{-1} for each spectrum. FTIR spectra were taken in the wavelength region 4000-400cm^{-1} at ambient temperature.

2.3.2 Lower critical solution temperature (LCST) of the copolymers obtained by differential scanning calorimetric (DSC) and UV-spectrophotometry (UV)

Copolymers synthesized were tested in a Perkin-Elmer DSC 7 to determine their lower critical solution temperature (LCST). Samples were prepared as follows: after having been freeze-dried, 5mg of each material was weighed with 5 µL of distilled water in hermetically sealed aluminum pans. After calibrating with indium and obtaining the corresponding baseline under inert $N_{2(g)}$, each sample was submitted to a heating scan: 0°C → 50 °C at 20°C/min.

Additionally, the phase transition of PNIPAm and copolymers solutions was measured using an UV/VIS spectrophotometer HP AGILENT 8452 with diode arrangement for temperature control. The absorbance of visible light at 450nm was measured while the cells were subjected to heating with a water bath to promote the phase change by thermal effect. The temperature range studied was 20-45ºC. LCST correspond to the temperature at which the transmittance is 50% [15,16].

2.3.3 Morphologic study of the copolymers obtained by scanning electron microscopy (SEM)

A JEOL JSM6390 scanning electron microscope was used for the morphological analysis of copolymers synthesized porosity. First, simulating an injection process, given their gel character, these copolymers were dissolved in deionized water and loaded into an injector by passing the thin needle. Then, the solutions obtained were subjected to a bath which reached a temperature equal to or higher than the LCST of these materials (~ 40°C). Once the phase change occurred, the samples were immediately frozen and lyophilized to be observed by SEM. The samples studied were cryogenically fractured in liquid N_2 to ensure the morphological observation of the inside of each sample. Then, they were coated with a thin layer of gold in a Balzers-SCD 030 sputter coater. The voltage of the SEM equipment was set to 20 kV.

2.3.4 Cytotoxicity test by cell hemolysis on blood agar of the copolymers synthesized

Blood compatibility was evaluated with hemolysis assay. The blood was mixed with agar in a totally sterile medium and was gelled for 2h in an oven at 37 °C. Each lyophilized sample was subjected to 1h of irradiation UV, necessary to sterilization. Once the gels were sterile, they were brought into contact with blood/agar system and were transferred under sterile conditions to the surface of the blood/agar gel and then placed at 37 °C and a 5% CO_2 flow in the oven for cell culture. Protocol performed according to ISO-10993-4: 2002. The surface around each gel sample was observed for a total time of 48h and was compared to the controls (negative control = a blood agar gel which had not been in contact with any gel; another or positive control = blood agar gel which had been in contact with a toxic gel using of NIPA monomer).

3 Results and Discussion

3.1 Characterization of PNIPAm and PNIPAm-co-COOH and PNIPAm-co-COOH-g-PIP

The signals corresponding to the groups present in the FTIR-ATR spectra of PNIPAm and the copolymers PNIPAm-co-COOH and PNIPAm-co-COOH-g-PIP were analyzed by the Fourier Transform Infrared Spectroscopy (Fig 2-A). The PNIPAm, being the majority component, was the starting point and reference for the study of the other infrared spectra. The broad band representative of the amide NH stretching is presented at 3408cm^{-1} and two

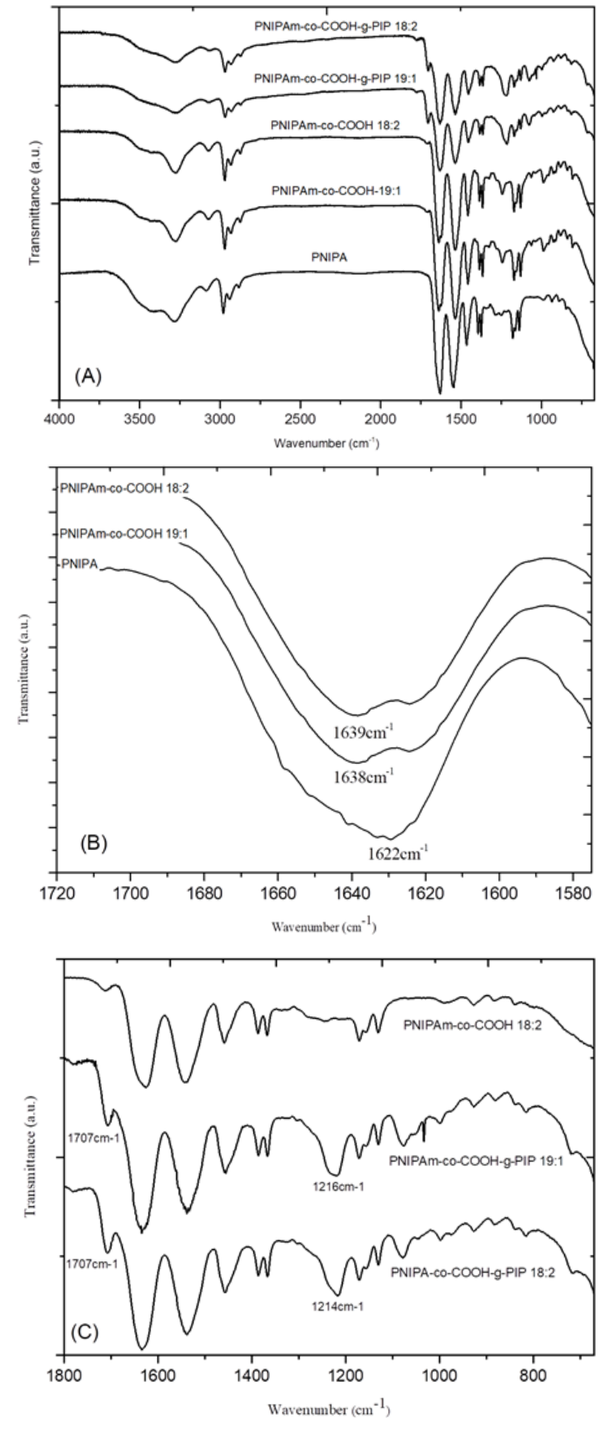


Figure 2 - FTIR spectra of:
(A) PNIPAm, PNIPAm-co-COOH and PNIPAm-co-COOH-g-PIP in its 19:1 and 18:2 formulations (region 4000-500cm^{-1}).
(B) PNIPAm, PNIPAm-co-COOH 19: 1 and 18: 2 (region 1700-1500cm^{-1}).
(C) PNIPAm-co-COOH 18: 2 and PNIPAm-co-COOH-g-PIP 19: 1 and 18: 2 (region 1800-700cm^{-1}).

peaks close to 1300cm^{-1} are also observed, representing the flexion vibration of the isopropyl group [17,18]. Once the copolymerization of the PNIPAm with 3-butenoic acid (PNIPAm-co-COOH) is carried out, the carbonyl signal of the synthesized product is displaced at a higher wavelength with respect to the PNIPAm homopolymer and the signal is unfolded, indicating the presence of the 2 types of carbonyl groups present in these news copolymers (See Figure 2-B). However, this shift is not as noticeable because of the high proportion of NIPA relative to the incorporated 3-butenoic acid.

Rejinold (2011) chemically modified the PNIPAm with mercaptopropionic acid by free radical polymerization. In this work the characterization of the copolymers by infrared spectroscopy was made, considering it valid evidence indicating the modification and obtaining of the desired product as a function of the displacement observed for the carbonyl group [13]. Then when the PIP was subsequently incorporated, it was possible to evidence the presence of a new band near the 1700cm^{-1} characteristic of a tertiary amide (-CO-NR2), corroborating the formation of the same between PNIPAm-co-COOH and PIP. Additionally it is possible to identify a band around the 1200cm^{-1} corresponding to the secondary amine (R-NH-R) present in the piperazine (Fig 2-C). When the piperazine is incorporated into the PNIPAm-co-COOH (PNIPAm-co-COOH-g-PIP) by forming an amide bond, another amino group is provided by the piperazine for subsequent modification of the backbone by graft-like reactions.

3.2 Study of the LCST of the synthesized copolymers

In Figures 3A and 3B, it is seen how the phase change occurs in each of the samples analyzed by the selected analytical techniques. When the DSC curves (figure 3B) were used it is observed that all the PNIPAm-co-COOH copolymers decrease the phase transition temperature LCST with respect to the PNIPAm homopolymer. This

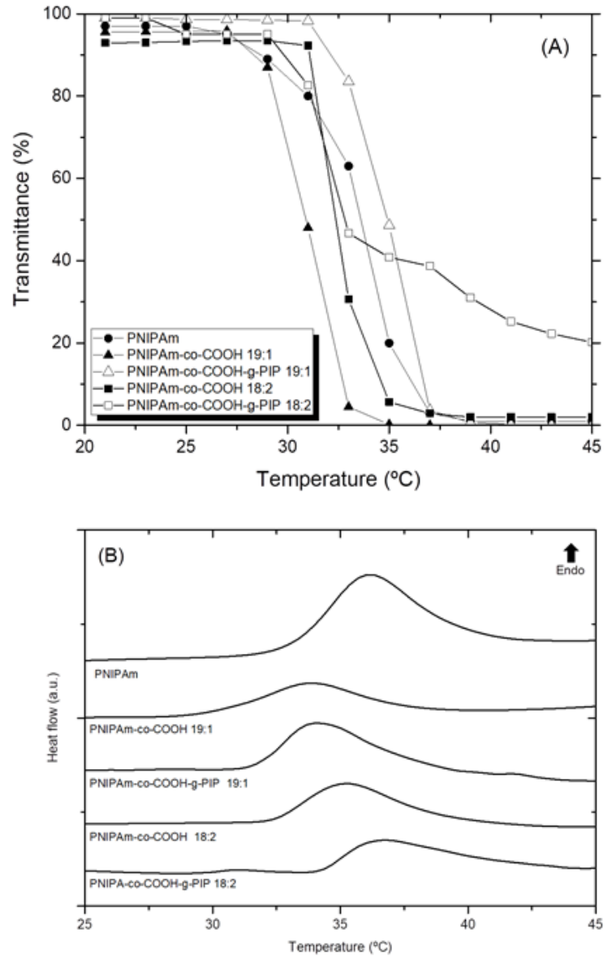


Figure 3 - Determination of LCST transition for PNIPAm, PNIPAm-co-COOH and PNIPAm-co-COOH-g-PIP formulations by techniques: (A) UV-vis spectrophotometry, and (B) DSC.

Table 1 - Transition temperature LCST determined by UV and DSC, of the synthesized copolymers.

Sample	UV	DSC	
	T_{LCST} (±1) ºC	$T_{(onset)LCST}$ (±0.1) ºC	ΔH_{LCST} (±0.1) J/g
PNIPAm	34	33.1	17.8
PNIPAm-co-COOH 19:1	31	30.5	14.5
PNIPAm-co-COOH$_{-g-PIP}$ 19:1	32	31.5	22.9
PNIPAm-co-COOH 18:2	32	32.3	6.0
PNIPAm-co-COOH$_{-g-PIP}$ 18:2	34	34.6	7.1

response may be an indication that with the presence of the 3-butenoic acid fraction the hydrophilic character of the copolymer is increased, but in turn the polymer-polymer interactions are favored, which causes it to displace smaller temperatures in relation to the homopolymer. The same effect is observed in the curve obtained by the analysis performed using UV spectroscopy (fig. 3A). In the case of the curve obtained by UV, initially a transparent solution was obtained, for which they had a maximum transmittance. As the temperature in the cells increased, the solutions became opaque giving rise to the phase change (gelation) and decreasing the transmittance due to that turbidity. This change is what allows to determine the average value of the temperature LCST.

These results show how two techniques that are fundamentally different, allow to identify the temperature in which the phase change occurs and therefore the intelligent nature of these materials. However, the advantage of the DSC analysis is the possibility of knowing the value of the energy cost (enthalpy) associated with this transition (see Table 1). It is seen, in Table 1, that as more of the 3-butenoic acid co-monomer is incorporated, the copending capacity of the copolymer is reduced by reducing its enthalpy. But after functionalizing with the piperazine, it appears structurally that the polymer chain is able to order or extend its intramolecular interactions in its gel form, which in turn can translate into a slight increase in its phase transition temperature LCST.

The intelligent character of the copolymers obtained is based on the phase transition LCST that presented around a temperature range T~30-35°C, values close to the body temperature (37.4°C), giving the material potentiality for its use in biomedical applications. In the literature it has been reported that by adding a new component to PNIPAm (covalently bound or by physical mixtures) it is possible to change its LCST transition temperature to higher or lower values [11,12,19]. What has been mentioned is that when some hydrophilic phase is added to the PNIPAm, the LCST is displaced at higher temperatures[5], while in the presence of compounds with greater hydrophobic character, the opposite effect occurs [2,20]. All the formulations of PNIPAm-co-COOH decrease the transition temperature with respect to the PNIPAm homopolymer, despite the hydrophilic character of 3-butenoic acid, which seems to contradict the above; but in this case, it is possible that, if the polymer-polymer interactions prevail, a lower temperature is require to initiate the phase transition.

In order to support this, another effect can be measured, and for that reason an additional measure was to determine the pH of the solution of these copolymers in aqueous medium. By showing that the 18:2 copolymers have a lower pH value than in the case of 19:1, which is to be expected. For polymers such as PNIPAm, it has been reported in the literature that as the pH increases, there is a decrease in the LCST transition. According to this statement, when the pH is high the protonation is weak and the polymer-polymer interactions are favored, which leads to phase separation occurring at a lower temperature [21]. This makes it possible to justify these slight differences in TLCST between formulations 19:1 and 18:2. On the other hand, by incorporating the piperazine as a grafted functional group, an electrostatic repulsion is generated which makes the polymer more soluble and produces an increase in LCST due to an easier protonation of the amino group [21].

3.3 Study of copolymer morphology observed by SEM

The microphotographs of the copolymers allow to appreciate homogeneous porous three-dimensional structures, especially for the 18:2 PNIPAm-co-COOH formulation (Figure 4 (a, b, c, d)); Which has a honeycomb-like morphology formed by interconnected pores. The morphology of the obtained biomaterials was studied after simulating its injectability process, where it was possible to observe scaffold structures with interconnected pores, whose homogeneity varies depending on the formulation. The aim of simulated the injectability process, ie., the passage of the aqueous formulation by an injector, is what would be the morphology once the phase change (gel or solid) inside the organism.

For the case of the PNIPAm-co-COOH-g-PIP samples (Figure 4 (e-h)), a significant variation in morphology is

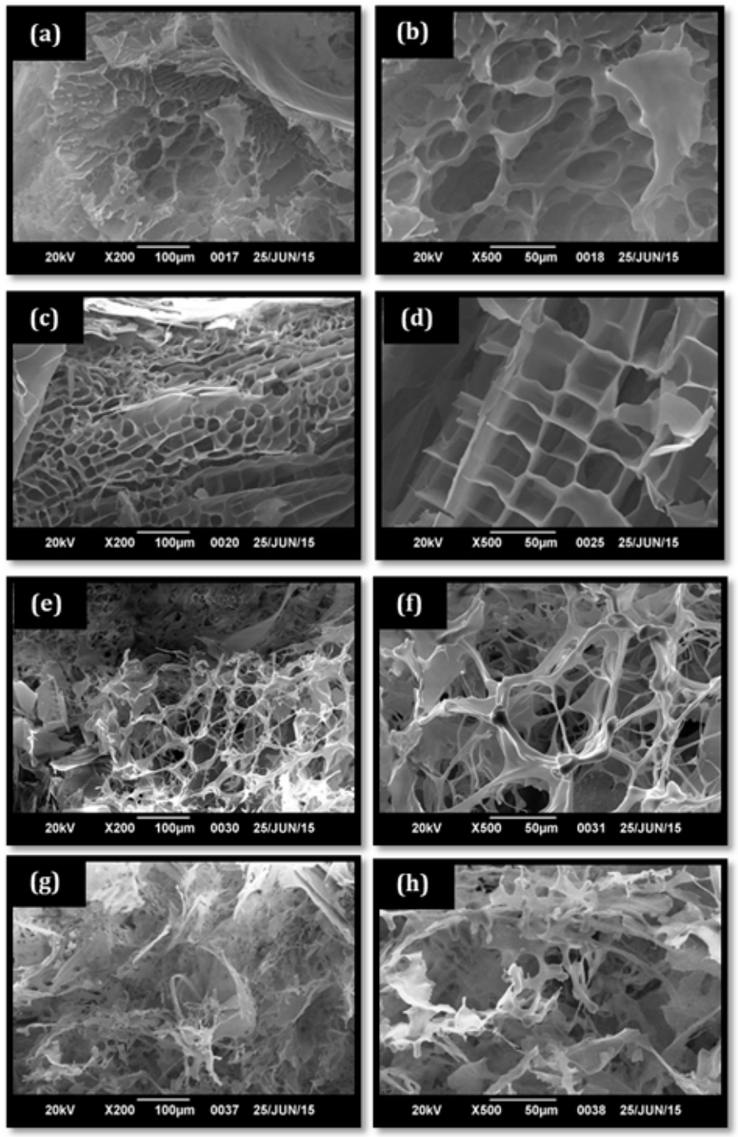


Figure 4 - Micrographs SEM of PNIPAm-co-COOH 19: 1 (a, b), PNIPAm-co-COOH 18: 2 (c, d), PNIPAm-co-COOH-g-PIP (e,f) and PNIPAm-co-COOH-g-PIP (g, h).

observed compared to the starting material. However, it continues to present as a scaffold-like structure, having pore walls much thinner than those obtained in its precursors, ie, porosity of the open and interconnected type.

At present, the study and design of functional biological systems has been demanding the development of interconnected porous and three-dimensional structures (scaffolds) that serve as vehicles for transporting drugs or other biomolecules [22-24], as well as for cell culture and tissue regeneration [12,25]. The purpose of scaffolds in the field of tissue engineering is to support the cells to proliferate and differentiate, given their interconnectivity between the pores necessary to allow vascularization, the passage of fluids that transport cells, nutrients, Oxygen and to remove waste [23,26]. This type of morphology observed for these new copolymers are important requirements to be able to estimate the biomedical or tissue engineering applications that these novel materials can have, where their cytotoxic response plays an extremely primordial role.

3.4 Cytotoxic studies

The hemocompatibility test is very common and

important during the development of biomaterials, as it measures their tolerance when subjected to contact with an active biological system such as blood [27,28]. Hemolysis testing, despite their simplicity, are of great relevance for evaluating medical devices or implants that will be in contact with blood flow [29]. Even more so if think of devices type scaffold [30].

Figure 5 shows the results of the hemocompatibility test of the synthesized samples and their respective controls. As control it is presented to the NIPA monomer (which has been reported to be cytotoxic) [31], and a whitish halo is clearly seen around the sample (hemolysis) deposited in the center of the agar / blood system, due to cell death around the sample. However, for the synthesized copolymers, which were subjected to a thorough purification process, this trend was reversed obtaining copolymers that according to the results and under the conditions evaluated are presented as non-cytotoxic. Based on this, it can be affirmed that the obtained compounds were not toxic, since, none generated evidence of cellular lysis. Therefore,

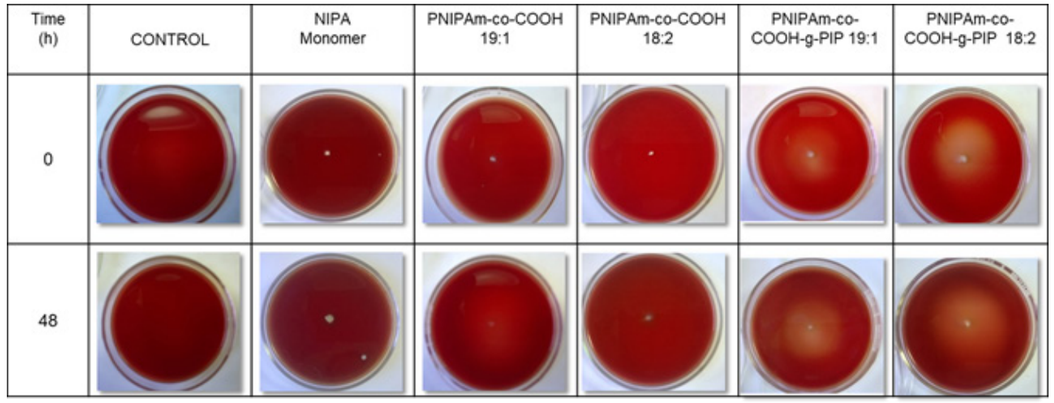


Figure 5 - Non-cytotoxic evidence of PNIPAm-co-COOH and PNIPAm-co-COOH-g-PIP in contact with agar/blood at time 0 and after 48h.

these could be used in more advanced or exhaustive in vitro tests, in order to put these materials in direct contact with primary cell cultures or cell lines; Or in vivo experiments with experimental animals, as these results give indications of their potential biocompatibility [25,32,33]. This obtained biocompatibility is a relevant result, since for this research, these copolymers will serve as an anchor point for the insertion of polysaccharides such as chitosan, hyaluronic acid and alginate, thus leaving evidence that new biocompatible compounds derived from these Which are being studied in this research.

4 Conclusions

A copolymer based on NIPA and 3-butenoic acid (PNIPAm-co-COOH) was synthesized in order to generate a highly reactive block copolymer. From this acid group it was then possible to perform a chemical modification by the incorporation of piperazine (PNIPAm-co-COOH-g-PIP). The synthesized copolymers exhibited characteristics such as: phase change temperature (LCST close to body temperature), porosity, interconnected pore morphology, water solubility, ability to be injectable and biocompatible, indicative that these new biomaterials have the potential to be used in biomedical applications and for tissue engineering. In addition, the structure of the modified PNIPAm has anchor points for future insertion reactions that allow graft-like copolymers to be obtained between this and biopolymers, such as polysaccharides.

Acknowledgment

The authors would like to express their thanks to the following laboratories within the USB: research group B5IDA, Polymer research group GPUSB-1, Laboratory of Surfaces for SEM (Laboratory E), Laboratory of Microbiology of the Department of Cell Biology, Laboratory of instrumental analysis of Chemistry Department. Likewise, the deans of professional studies and dean of research and development (DID).

Reference

1. Wahab IF, Razak SIA. Polysaccharides as Composite Biomaterials. In: Composites from Renewable and Sustainable Materials [Internet]. 2016. Available from: http://www.intechopen.com/books/composites-from-renewable-and-sustainable-materials/polysaccharides-as-composite-biomaterials
2. Kulshrestha AS, Mahapatro A. Polymers for biomedical applications. In: ACS Symposium Series. 2008. p. 1–7.
3. Bret UD, Lakshmi NS, Laurencin CT. Biomedical Applications of Biodegradable Polymers. J Polym Sci Part B Polym Phys. 2011;3(49):832–64.
4. Chen H, Yuan L, Song W, Wu Z, Li D. Biocompatible polymer materials: Role of protein-surface interactions. Vol. 33, Progress in Polymer Science (Oxford). 2008. p. 1059–87.
5. Coronado R, Pekerar S, Lorenzo AT, Sabino MA. Characterization of thermo-sensitive hydrogels based on poly(N-isopropylacrylamide)/hyaluronic acid. Polym Bull. 2011;67(1):101–24.
6. Drury JL, Mooney DJ. Hydrogels for tissue engineering: Scaffold

design variables and applications. Vol. 24, Biomaterials. 2003. p. 4337–51.

7. Coronado R, Pekerar S, Lorenzo AT, Müller AJ, Sabino MA. Obtención y caracterización de hidrogeles inteligentes del tipo red interpenetrada basados en poli (N-Isopropil Acrilamida). *Rev Latinoam Metal y Mater*. 2009;2(1):65–6.

8. Pollock JF, Healy KE. Mechanical and swelling characterization of poly(N-isopropyl acrylamide -co- methoxy poly(ethylene glycol) methacrylate) sol-gels. *Acta Biomater*. 2010;6(4):1307–18.

9. Katime IA, Katime O, Katime D. Materiales inteligentes: Hidrogeles macromoleculares. Algunas aplicaciones biomédicas. Vol. 53, *Journal of Chemical Information and Modeling*. 2013. 1689-1699 p.

10. Haq MA, Su Y, Wang D. Mechanical properties of PNIPAM based hydrogels: A review. Vol. 70, *Materials Science and Engineering C.* 2017. p. 842–55.

11. Chang B, Ahuja N, Ma C, Liu X. Injectable scaffolds: Preparation and application in dental and craniofacial regeneration. *Mater Sci Eng R Reports* [Internet]. 2017;111:1–26. Available from: http://dx.doi.org/10.1016/j.mser.2016.11.001

12. Vieira JN, Posada JJ, Rezende RA, Sabino MA. Starch and chitosan oligosaccharides as interpenetrating phases in poly(N-isopropylacrylamide) injectable gels. Mater Sci Eng C. 2014;37(1):20–7.

13. Sanoj Rejinold N, Sreerekha PR, Chennazhi KP, Nair S V., Jayakumar R. Biocompatible, biodegradable and thermo-sensitive chitosan-g-poly (N-isopropylacrylamide) nanocarrier for curcumin drug delivery. *Int J Biol Macromol.* 2011;49(2):161–72.

14. Peng J, Su Y, Chen W, Zhao X, Jiang Z, Dong Y, et al. Polyamide nanofiltration membrane with high separation performance prepared by EDC/NHS mediated interfacial polymerization. *J Memb Sci.* 2013;427:92–100.

15. Liu XM, Wang LS, Wang L, Huang J, He C. The effect of salt and pH on the phase-transition behaviors of temperature-sensitive copolymers based on N-isopropylacrylamide. *Biomaterials.* 2004;25(25):5659–66.

16. Yang J, Van Lith R, Baler K, Hoshi RA, Ameer GA. A thermoresponsive biodegradable polymer with intrinsic antioxidant properties. *Biomacromolecules.* 2014;15(11):3942–52.

17. do Nascimento Marques N, Curti PS, da Silva Maia AM, Balaban R de C. Temperature and pH effects on the stability and rheological behavior of the aqueous suspensions of smart polymers based on *N* -isopropylacrylamide, chitosan, and acrylic acid. J *Appl Polym Sci* [Internet]. 2013;129(1):334–45. Available from: http://doi.wiley.com/10.1002/app.38750

18. Han J, Wang K, Yang D, Nie J. Photopolymerization of methacrylated chitosan/PNIPAAm hybrid dual-sensitive hydrogels as carrier for drug delivery. *Int J Biol Macromol.* 2009;44(3):229–35.

19. Dimitrov I, Trzebicka B, Müller AHE, Dworak A, Tsvetanov CB. Thermosensitive water-soluble copolymers with doubly responsive reversibly interacting entities. Vol. 32, Progress in Polymer Science (Oxford). 2007. p. 1275–343.

20. Gui R, Wang Y, Sun J. Encapsulating magnetic and fluorescent mesoporous silica into thermosensitive chitosan microspheres for cell imaging and controlled drug release in vitro. Colloids Surfaces B Biointerfaces [Internet]. 2014;113:1–9. Available from: http://dx.doi.org/10.1016/j.colsurfb.2013.08.015

21. Gan LH, Gan YY, Deen GR. Poly(N -acryloyl- N '-propylpiperazine): A New Stimuli-Responsive Polymer. *Macromolecules*. 2000;33(21):7893–7.

22. Lannutti J, Reneker D, Ma T, Tomasko D, Farson D. Electrospinning for tissue engineering scaffolds. Mater Sci Eng C. 2007;27(3):504–9.

23. Sandino C, Ginebra MP, Planell JA, Lacroix D. Reconstrucción computacional de andamios para ingeniería de tejidos. *Biomecánica*. 2011;19(1):25–34.

24. Hidalgo IA, Sojot F, Arvelo F, Sabino MA. Functional electrospun poly (lactic acid) scaffolds for biomedical applications: experimental conditions, degradation and biocompatibility study. *Mol Cell Biomech [*Internet]. 2013;10(2):85–105. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24015478

25. Chaim IA, Sabino MA, Mendt M, M??ller AJ, Ajami D. Evaluation of the potential of novel PCL-PPDX biodegradable scaffolds as support materials for cartilage tissue engineering. J Tissue Eng *Regen Med.* 2012;6(4):272–9.

26. Ye M, Mohanty P, Ghosh G. Morphology and properties of poly vinyl alcohol (PVA) scaffolds: Impact of process variables. *Mater Sci Eng C Mater Biol Appl* [Internet]. 2014;42:289–94. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25063121

27. Balan V, Verestiuc L. Strategies to improve chitosan hemocompatibility: A review. Vol. 53*, European Polymer Journal*. 2014. p. 171–88.

28. Fazley Elahi M, Guan G, Wang L. Hemocompatibility of surface modified silk fibroin materials: A review. Vol. 38, *Reviews on Advanced Materials Science*. 2014. p. 148–59.

29. Henkelman S, Rakhorst G, Blanton J, van Oeveren W. Standardization of incubation conditions for hemolysis testing of biomaterials. *Mater Sci Eng C.* 2009;29(5):1650–4.

30. Liu Y, Cai D, Yang J, Wang Y, Zhang X, Yin S. In vitro hemocompatibility evaluation of poly (4-hydroxybutyrate) scaffold. I*nt J Clin Exp Med*. 2014;7(5):1233–43.

31. Conzatti G, Cavalie S, Combes C, Torrisani J, Carrere N, Tourrette A. PNIPAM grafted surfaces through ATRP and RAFT polymerization: Chemistry and bioadhesion. Vol. 151, Colloids and Surfaces B: *Biointerfaces.* 2017. p. 143–55.

32. Sabino MA, Feijoo JL, Nuñez O, Ajami D. Interaction of fibroblast with poly(p-dioxanone) and its degradation products. *J Mater Sci*. 2002;37(1):35–40.

33. Porto ICC de M. Polymer Biocompatibility. In: Gomes ADS, editor. *Polymerization* [Internet]. Rijeka: InTech; 2012. Available from: http://dx.doi.org/10.5772/47786