J Mater Sci: Mater Med (2011) 22:1357–1364
DOI 10.1007/s10856-011-4299-2

# In vivo tests of a novel wound dressing based on biomaterials with tissue adhesion controlled through external stimuli

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Received: 20 October 2010 / Accepted: 16 March 2011 / Published online: 25 March 2011


**Abstract** The high incidence of wounds by second intention and the high costs associated with their treatment give rise to the need for the development of wound dressings that protect not only the wounds themselves but that are also able to promote cell proliferation and skin regeneration. Moreover, it is also very important that no damage to the new regenerated tissue is generated while removing the dressing. In this work, a novel wound dressing, which would be able to favor tissue repair and be removed at an appropriate scheduled moment by means of an external stimulus without promoting extensive damage to the new tissue, was produced and tested. Polyurethane membranes were modified by grafting polymers based on poly(*n*-isopropylacrylamide) (P-N-IPAAm). P-N-IPAAm undergoes a phase transition at approximately 32°C, which changes its behavior from hydrophilic (below 32°C) to hydrophobic. It was hypothesized that, by reducing the temperature near the wound dressing to values lower than 32°C, the detachment of the dressing would become more effective. The wound dressings containing P-N-IPAAm grafts were tested in vivo by covering excisional wounds produced in mice. The produced dressings were placed in direct contact with the lesions for 3 days. Results showed that the hypothermia due to anesthesia required to remove the dressings from mice lowered the local temperature to 28°C and favored the detachment of the wound dressings containing P-N-IPAAm grafts. Histological analyses showed that lesions covered by dressings presented less intense inflammatory events and denser connective tissue than did the wounds without dressings. The wounds covered by polyurethane membranes with P-N-IPAAm grafts showed signs of more intense re-epithelization and angiogenesis than did the lesions covered by polyurethane without grafts.

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## 1 Introduction

In Latin America, there are approximately 1.1 million new cases of chronic wounds annually. Approximately 4% of the individuals with diabetes present skin ulcers, while 1% of the people over 65 years of age present pressure ulcers [1]. Medical and social services associated with these types of wounds are estimated in thousands of US dollars per treatment [2]. Severe burns, diabetes, pressure ulcers (decubitus), and veined ulcers are commonly associated with chronic wounds [3, 4] that require longer to heal or in which healing may not be completely observed [5]. Wounds can also be classified depending on the severity of tissue damage. Wounds that heal by secondary intention are most commonly those in which the borders are distant from each other and in which granulation tissue is visible. Chronic wounds usually heal through second intention.

The number, cost, and impact on both individuals and countries have motivated the development of a series of wound dressings that can protect wounds as well as enhance the healing rate [6–11]. These wound dressings generally consist of a porous support (typically made of polyurethane or silicone) and a hydrogel that can store water and exudates [12, 13]. Although these devices can be considered successful in protecting the wounds and allowing healing to occur, their application very often leads to high levels of adhesion between the recently-formed tissue [14] and the wound dressing itself, which can ultimately result in tissue damage when removing the dressing.

The present work aimed to produce and test in vivo novel wound dressings that would not only provide the appropriate environment for healing to occur, but would also substantially reduce adhesion to the new tissue by applying external stimuli, such as temperature. Wound dressings were produced by grafting poly(*n*-isopropylacrylamide) (P-N-IPAAm) from a polyurethane membrane. P-N-IPAAm undergoes a phase transition at approximately 32°C, which shifts the behavior of the polymer from hydrophilic (below 32°C) to hydrophobic [15–20]. This change in behavior of the polymer can then be potentially used to switch the surface of the wound dressing from non-adherent (below 32°C) to adherent (above 32°C) in relation to the recently-formed tissue. This on–off type of switch can then be used to aid in the detachment of the wound dressing when required without provoking extensive tissue loss. In this work, the on–off mechanism, as well as the healing process of wound dressing containing P-N-IPAAm, was investigated in vivo.

## 2 Experimental

### 2.1 Preparation of the polyurethane membrane

Thermoplastic polyurethane Elastollan ELA585A10 (Basf), kindly provided by Polymeric Petropol, was dissolved in pyridine (20% w/v), and films were obtained by solution casting, followed by drying. Porous membranes were obtained by dispersing LiCl (10% w/w and an average particle size of 5 μm) in the polyurethane solution and washing the membrane several times with deionized (D.I.) water to remove the salt particles and any remaining solvent.

### 2.2 UV treatment of polyurethane membranes

Ultraviolet radiation was used to oxidize the original surface of the polyurethane (PU) membranes to yield surfaces containing polar species and free radicals. PU membranes were placed at a 50 mm distance from a UV lamp (30 W; wavelength 256–370 nm) for four hours.

### 2.3 Grafting of *n*-isopropylacrylamide (N-IPAAm) from the polyurethane film

Grafting of N-IPPAm was accomplished by immersing UV-treated PU membranes in a an aqueous solution containing 5% w/v of a N-IPAAm monomer, 0.04 N of nitric acid and 0.1% ceric ammonium nitrate. PU samples not exposed to the UV treatment were also used in the grafting process as a reference. Grafting of N-IPAAm was performed within an $N_2$ atmosphere at room temperature (20°C) for 12 h. Polyurethane membranes submitted to the grafting procedure were washed extensively with pure water for 4 h to remove any non-reacted and non-grafted species.

### 2.4 Preparation of the wound dressings

Discs of 10 mm in diameter and 0.5 mm in thickness were obtained by cutting both polyurethane membranes, with or without P-N-IPAAm grafts. The obtained discs were sterilized using ultraviolet light and were then inserted between two cellulose films (one of the films with an adhesive) which had been sterilized beforehand. Cellulose films were used to protect the wound dressing and to allow for the overall device to attach to the tissue around the wound.

### 2.5 In vivo tests

A proposal reporting the protocol associated with the in vivo tests were submitted to and approved by the Committee of Ethics in Animal Experimentation from the Federal University of Minas Gerais (UFMG-CETEA protocol 069/09).

In vivo tests were performed to monitor the healing process of excisional wounds that had received PU membranes as well as to check the hypothesis that the novel wound dressing would display a tailorable behavior in terms of adhesion to the recently-formed tissue.

The in vivo tests were carried out using Swiss mice, which were anesthetized, with 80 mg/kg of cetamine and 10 mg/kg of xylazine. After, the mice's backs were shaved. Next, two circular excisional wounds, with an area of 19.6 $mm^2$, were produced on each mouse by using a surgical tool commonly used for procedures associated with biopsies. The animals were divided into two groups: Group A—five mice with no wound dressings and Group B—five mice with PU wound dressings (ten animals with two wounds each). Animals in Group B had one of the wounds covered by PU wound dressings with no P-N-IPAAm grafts, while the other wound was covered by wound dressings with P-N-IPAAm grafts (Fig. 1).

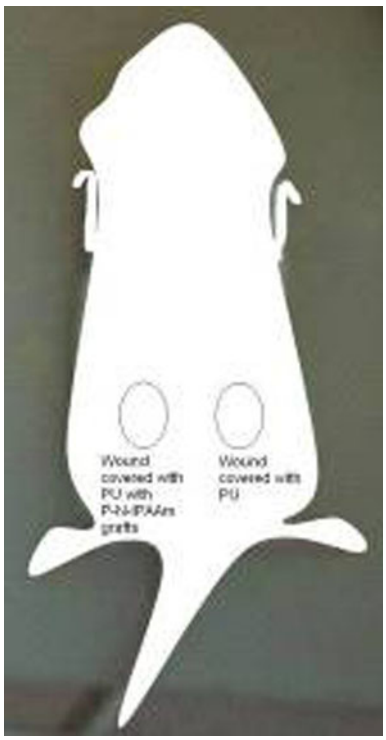


**Fig. 1** Location of wounds and wound dressings in mice

The level of adhesion between the wound dressings and the newly-formed tissue was measured by removing the wound dressings after 3 days and analyzing the amount of tissue which had adhered to the dressing and could be viewed by the naked eye (or by using an optical microscope, if necessary). Before removing the wound dressings, the animals were anesthetized by using the same protocol employed during the production of the excisional wounds and the temperature at the wound dressing was measured using an infrared thermometer. After removing the wound dressings, the wounds were photographed, and their larger (R) and smaller (r) dimensions were measured by a digital caliper. The area (A) was then calculated by the Eq. 1 [7]:

$$A = \pi Rr \tag{1}$$

The healing of the wound was monitored by comparing the initial area ($A_0$) to the area after 3 days ($A_l$) by using Eq. 2 [7]:

$$M \pm SD = \frac{(A_0 - A_l)}{A_0} \tag{2}$$

After 3 days of producing excisional wounds and applying wound dressings (as well as after the detachment of the wound dressings under anesthesia), the animals were sacrificed in a gas chamber. The entire region of each wound, including its borders, was then removed using a surgical scissor. The collected tissues were cross-sectioned and processed using traditional histotechniques, that is, the tissues were set in formalin and embedded in paraffin (Fig. 2). The embedded tissues were then sectioned with a microtome and stained using hematoxylin and eosin (H&E).

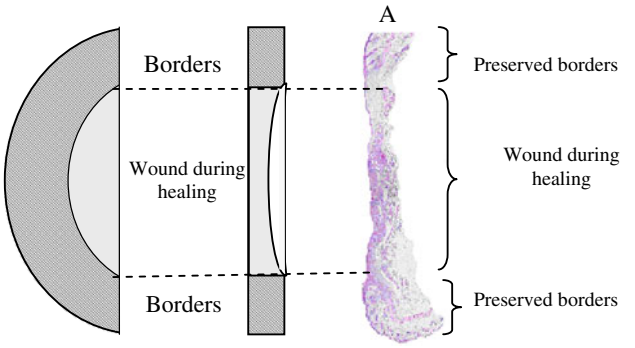


**Fig. 2** Representative outline of the collected tissue after in vivo test, showing that both the original tissue (borders of the wounds) and the area of the wounds were analyzed

## 3 Results

After 3 days of application, the wound dressings, based on PU with P-N-IPAAm grafts, were easily removed from the wounds (with no visual damage to the tissue below the dressing). A temperature close to 28°C was measured in the areas around the wounds, indicating that mice had undergone hypothermia due to the anesthesia. The measured temperature was lower than the transition temperature (LCST = lower critical solution temperature), usually defined as 32°C for P-N-IPAAm [17–20]. At this temperature, the hydrophilic type of surface due to P-N-IPAAm would favor tissue detachment.

Figure 3 shows that only an average of 20% of the original size of the wound was healed after 3 days of the application of the wound dressings. The inflammatory

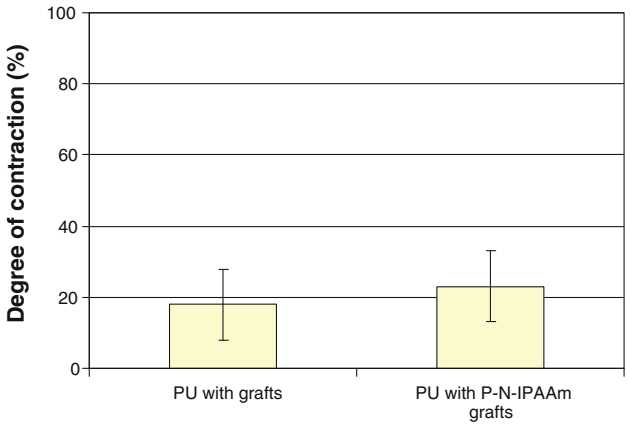


**Fig. 3** Degree of contraction of the wounds calculated by Eq. 2

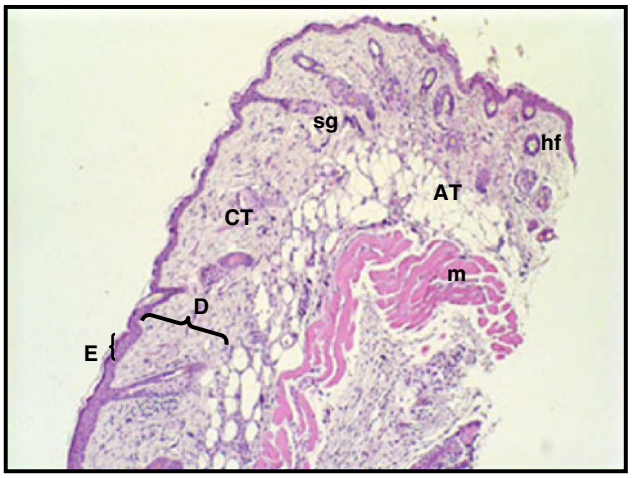


**Fig. 4** Histology of normal skin showing epidermis (*E*) with keratin, dermis (*D*) consisting by connective tissue (CT), with hair follicles (*hf*), sebaceus gland (*sg*), and adipose tissues (*AT*). Muscles (*m*) can be seen below the dermis. (H&E, magnification of 4×)

response to skin wounds, which involves damage to both the epidermis and the dermis, frequently requires several days for the repair process to become substantial [21]. No statistical difference between wound dressings with or without P-N-IPAAm grafts could be identified.

The healing process of the wounds was investigated by performing histological analyses on the tissues derived from the in vivo tests. Figure 4 shows the structure of normal skin removed from the mice at areas close to the wounds. In Fig. 4, the epidermis containing epithelial cells, which forms a stratified epithelium rich in keratin, can be observed. Beneath the epidermis, one can observe the dermis, which consists of a connective tissue with blood and lymphatic vessels, nerves, hair follicles, and sebaceous glands. Below the dermis, fatty and muscular tissue are present.

Figure 5 shows a histological section of wound not covered by a wound dressing after 3 days of healing. An extensive necrosis and granulation tissue can be observed close to the surface of the wound as compared to the undamaged tissue at the borders of the wound. A granulation tissue, containing blood vessels and inflammatory cells (neutrophils, macrophages, and lymphocytes), together with a connective tissue made up of lightly packed fibers, can be seen under the necrotic area.

In the wounds covered by PU membranes that were not modified by P-N-IPAAm grafts (Fig. 6), the superficial necrosis was less extensive than that observed in wounds that were kept for 3 days without wound dressings (Fig. 5). As in Fig. 5, a granulation tissue can also be observed but with a smaller number of inflammatory cells and a connective tissue comprised of much thicker and more densely packed fibers.

Figure 7 illustrates the histological images of wounds after 3 days of healing that had been covered by PU membranes with P-N-IPAAm grafts. No necrosis and few inflammatory cells could be observed. Moreover, signs of reepithelization and new blood vessels can also be noted when Fig. 7 is compared to Fig. 4, which indicates that the healing process is well-established.

## 4 Discussion

Temperatures lower than the LCST of P-N-IPAAm were observed in areas close to the wounds while they were being removed. At temperatures lower than LCST, P-N-IPAAm grafts should clearly expose the hydrophilic groups, thus leading to an increase in the overall hydrophilic behavior of the surface of the wound dressing. This type of surface can be fully hydrated when exposed to an aqueous environment, which can then lead to the desorption of proteins and the detachment of cells, as often occurs in common hydrogels [22].

However, Fig. 3 shows that the level of healing was low for all samples. Therefore it is reasonable to assume that the cell population at the wound sites remained limited even after 3 days, which may well be partially responsible for the low levels of adhesion observed between wound dressings based on PU with P-N-IPAAm grafts and the wounds themselves.

The comparison between Figs. 5, 6 indicates that, after 3 days, the repair process associated with wounds covered by PU membranes is much more well-established than in wounds without dressings. Although the histological analyses were performed only 3 days after the creation of the wounds, the results indicate that covering wounds with PU membranes is enough to improve the healing rate and resolve the inflammatory response, as has been well-documented for many wound dressings [1].

Results in Fig. 7, in which no necrosis and many signs of reepithelization and vascularization were noted, suggest that P-N-IPAAm grafts displayed low levels of toxicity since no extensive inflammatory reaction could be noticed. The normal body temperature of mice varies from 35.2 to 37.9°C; this means that, during the healing process, P-N-IPAAm chains should have been exposed to temperatures that were higher than their LCST. In this light, P-N-IPAAm grafts should display a hydrophobic behavior that commonly favors the adhesion of proteins and cells [9, 10].

Many changes have been observed in terms of wound dressings used to protect and help the healing process in the last two decades [23]. New materials have been proposed, tested and used that would improve tissue healing by contributing for the formation of a moist environment close to the wounds and restricting the presence of bacteria

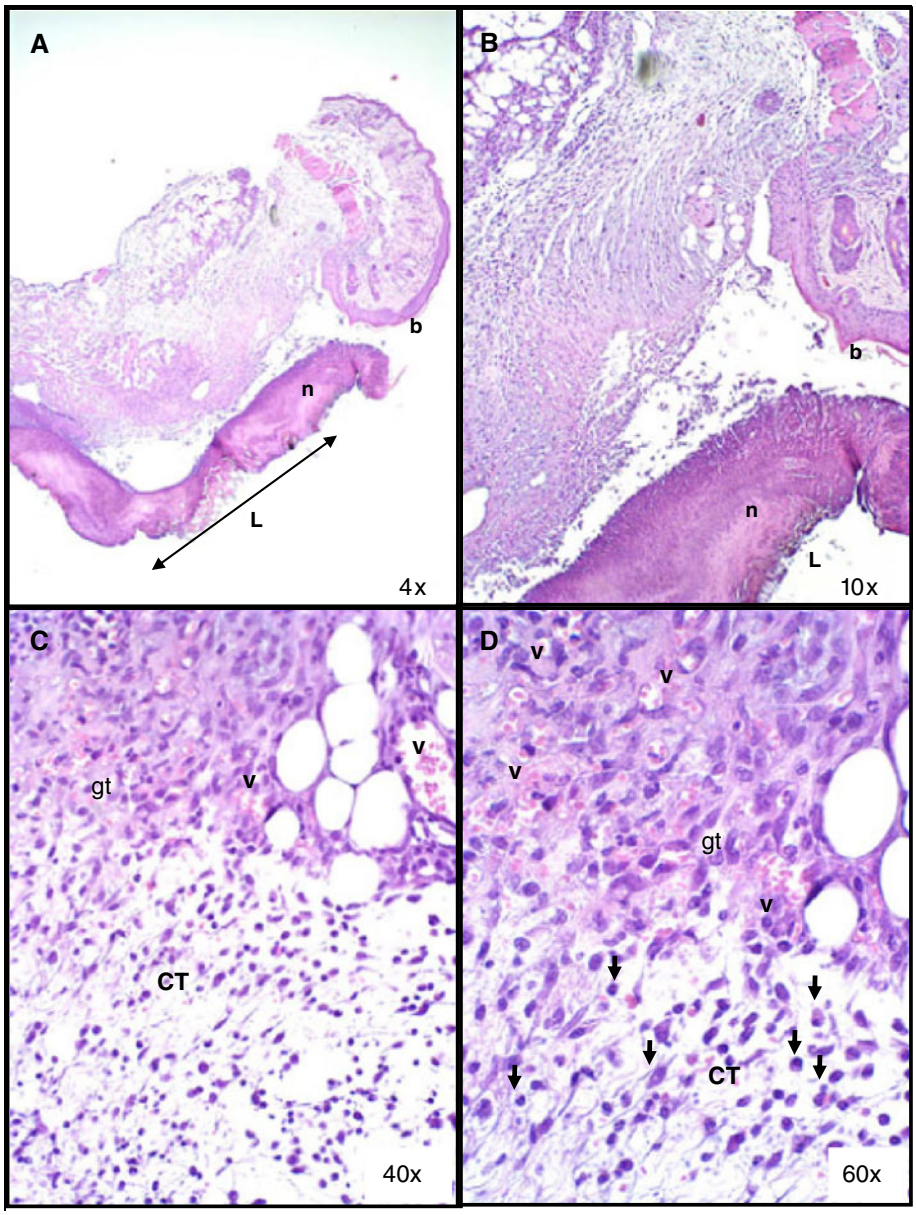


**Fig. 5** Wounds not covered by wound dressings after 3 days of healing: **A** Damaged area (*L*) and the preserved borders (*b*); **B** necrosis (*n*) at the surface of the wound; **C**, **D** presence of connective tissue (*CT*), blood vessels (*v*) and a thick inflammatory infiltrate (*arrows*) that corresponds to a granulation tissue (gt). **B**–**D** are different magnifications of (**A**). H&E staining

[23, 24]. Among the new materials used in wound dressings, polyurethane membranes have been studied and showed that they are very successful in absorbing exudates and keeping a humid environment that favors wound closure [23, 25]. The same type of result was also observed in this study. Figure 6 clearly shows that covering wounds with polyurethane membranes helped in promoting a higher healing rate. To improve healing, it is also important that wound dressings could provide support for cell adhesion and proliferation [26]. However, wound dressings with high adherence to tissues may not only be painful and difficult to remove but also cause tissue damage during the detachment procedure [23, 27, 28]. A combination of high levels of cell and tissue adhesion during the initial process of healing together with a progressive reduction in adherence would probably be an ideal situation [23]. Many studies have been performed, pursuing this idea. The use of biodegradable natural polymers and synthetic polymers has been investigated as biodegradable wound dressings, since they could be progressively degraded by the body environment, while tissue healing was being processed [29–32]. This type of wound dressing would not need to be removed and preservation of the healed tissue would be assured. Wound dressings based on collagen [29], chitosan [30], poly(lactic acid) [31], alginates [32], among others have been extensively tested. Although biodegradable wound dressings usually show good results in terms of healing, it is typically difficult to match the time required for biodegradation to occur and the time needed for a specific wound to heal. Therefore, in this work, results that showed

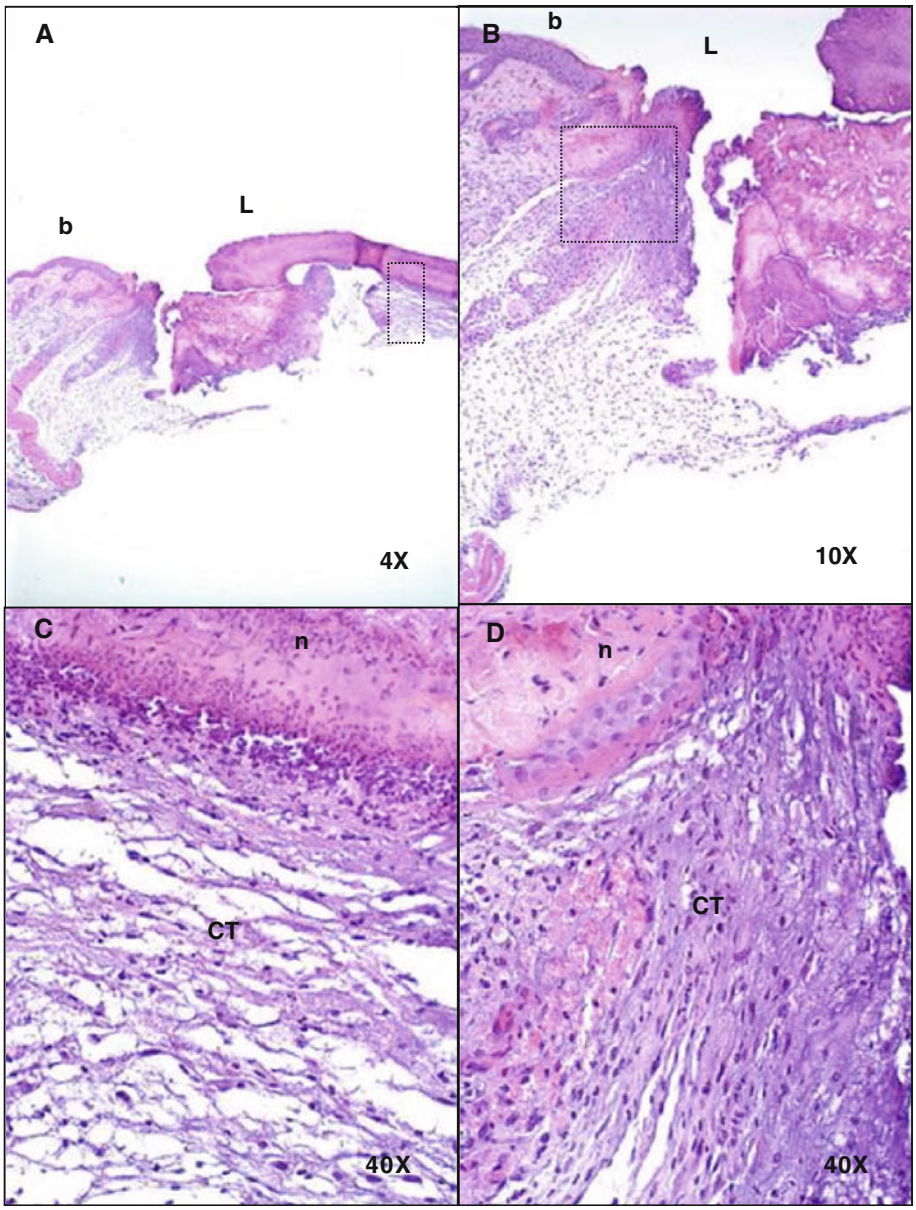


**Fig. 6** Wounds covered by PU wound dressings without P-N-IPAAm grafts after 3 days of healing: **A, B** damaged area (*L*) and the preserved borders (**B**); **C**, **D** necrosis (*n*) at the surface of the wound and the presence of a dense connective tissue (*CT*). **C**, **D** are amplified views of the *dotted square* in (**B**). H&E staining

evidences during in vivo tests that polyurethane membranes containing P-N-IPAAm grafts not only would be able to help wound healing but also could be switched from tissue adherent to non-adherence by reducing the temperature close to the wound site were obtained. This type of behavior had been used by others to detach cells from substrates in cell cultures [33]. The application of this strategy to wound dressings, as demonstrated in this work, would result in significant reduction in both pain and tissue damage during wound dressing removal. Moreover, the wound dressings could be removed anytime by applying an external stimulus (such as a reduction in the temperature at the wound site).

## 5 Conclusions

New wound dressings were created by grafting P-N-IPAAm from a porous polyurethane membrane surface. The obtained wound dressings were tested in vivo by applying them to cover excisional wounds produced in mice. These wound dressings would possibly switch from an adherent to a non-adherent tissue by reducing the temperature near the wound to below 32°C (usual LCST reported for P-N-IPAAm). This behavior would then be appropriate in removing the wound dressing without causing extensive tissue damage. To test this hypothesis, the wound dressings were removed from the wounds, and

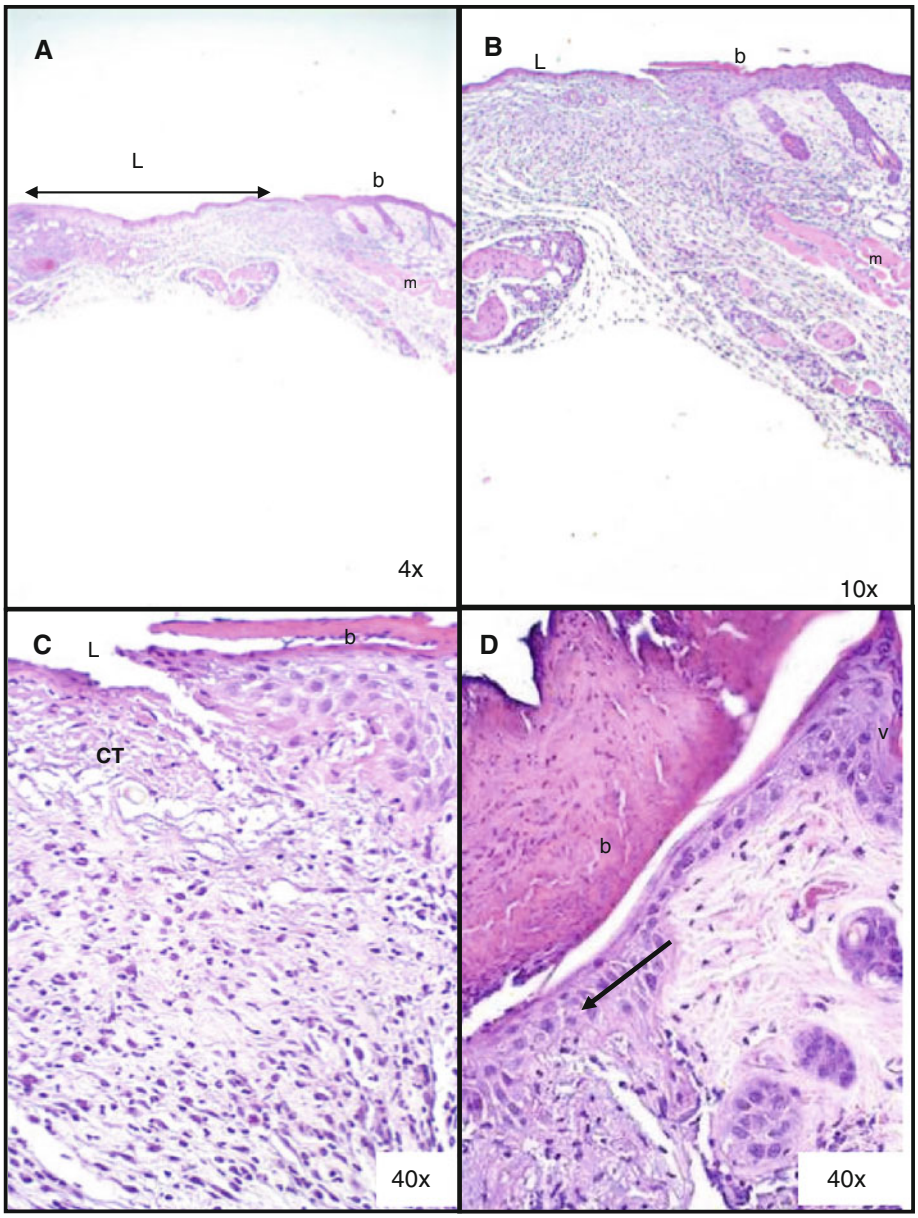


**Fig. 7** Wounds covered by PU wound dressings with P-N-IPAAm grafts after 3 days of healing: **A**, **B** damaged area (*L*), the preserved borders (*b*) and muscles (*m*); **C** presence of a dense connective tissue (*CT*); **D** signs of reepithelization (*arrow*). H&E staining

any tissue damage promoted by this procedure was checked. However, while removing the wound dressings, after 3 days of application, it was noted that the mice had undergone hypothermia due to the anesthesia. This hypothermia lowered their body temperatures to values close to 28°C, and the wound dressings were removed easily with no signs of tissue damage. Within this temperature range, P-N-IPAAm grafts should display a hydrophilic behavior that can favor the desorption of biological molecules, such as adhesive proteins, and the detachment of cells. Histological analyses performed on the wounds after 3 days of healing showed that covering the wounds with polyurethane membranes was able to improve the healing rate. When polyurethane membranes containing P-N-IPAAm grafts were used, histological sections of the wounds, after 3 days, revealed the presence of a new tissue with a limited number of inflammatory cells, connective tissue with highly packed fibers, and signs of reepithelization and angiogenesis. The results of this work suggest that novel wound dressings consisting of a porous polymer membranes and P-N-IPAAm grafts are non-toxic, can support skin growth, and may be detached from wounds by applying an external stimulus, such as the lowering the temperature at the wound site.

**Acknowledgments** The authors wish to acknowledge the financial support received from the National Council for Scientific and Technological Development (CNPq), a foundation linked to the Ministry of Science and Technology (MCT) of the Brazilian Government; the State of Minas Gerais Research Foundation (FAPEMIG); and Unileste.

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