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THE CHARACTERIZATION OF WOUND DRESSING POLY (VINYL PYRROLIDONE) HYDROGELS USING GAMMA RADIATION

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ABSTRACT

The first hydrogel for wound dressing processed by radiation was developed in Poland in 1986 by the inventor Janusz M. Rosiak and reached the local market in 1992. Laboratories of developing countries, which face all kinds of restrictions, were seduced by the simplicity of the process and low cost of its raw materials. It was a technological breakthrough due to its painless product characteristics and having improved healing properties such as absorbing a high water capacity, attached to healthy skin, and being easy to remove, plus its intelligent production process combining sterilization and crosslinking in a simultaneous operation. The use of hydrogels as biomaterials has increased recently. Hydrogel wound dressings were prepared using the gamma ray irradiation technique. Radiation was applied as a tool for crosslinking and sterilization of these materials. The hydrogels are composed of poly (N-vinyl-2-pyrrolidone) (PVP), poly (ethylene glycol) (PEG) and agar at radiation doses of 15, 20, 25, 30 kGy. The influence of some process parameters on their properties was investigated by: sterilization, gel fraction, swelling measures and mechanical properties. Hydrogels with less than 20 kGy of radiation were not properly sterilized. The gel fraction and swelling increased with increasing radiation dose due to increased crosslinking density, and at 25kGy, obtained optimum swelling. No significant differences were found for the test of mechanical properties but hydrogel matrices of different doses of gamma radiation.

1. INTRODUCTION

Hydrogels have received considerable attention in the last 50 years due to their promise in a wide range of biomedical applications. Such as wound care products [1- 2], dental materials [3] and ophthalmic [4-5], drug delivery systems [6] and implant elements [7]. They have a

degree of flexibility very similar to natural tissue due to their great water content [8]. They exhibit softness, elasticity and capacity to store a fluid preserving its integrity, making the hydrogel a unique material [9-10].

Hydrogels are defined as two- or multi-component systems consisting of a three-dimensional network of polymer chains and water that fills the space between macromolecules capable of absorbing large amounts of water and biological fluids in their structure without dissolving [9-11]. The ability to swell and the extent of swelling of the hydrogels are mainly governed by two factors, namely the hydrophilicity of the polymer chains and the crosslink density [12].

Synthetic hydrogels have aroused interest with the establishment of the former by Wichterle and Lim in 1954. But especially during the last two decades, natural hydrogels were gradually replaced by synthetic hydrogels, as these have a long shelf life, high water absorption capacity, high gel resistance and stable at temperature fluctuations. [8].

The poly (N-vinyl-2-pyrrolidone), also known as polyvinylpyrrolidone (PVP), is classified as a synthetic polymer, was obtained at the beginning of War 2 from the polymerization of the cyclic amide N-vinyl-2-pyrrolidone [13]. This polymer is characterized by its biocompatibility, its amphipathic character, which allows the formation of hydrogels, as well as the inclusion of molecules of interest [14] and exhibits properties similar to those of a protein because of its pyrrolidone structure [15].

Polyvinylpyrrolidone (PVP) has been widely used for the production of hydrogels, because they have good biocompatibility, are composed of about 90% of water [16], permeability to oxygen and can also retain solubilized substances. With specific properties it has been successfully used as a basic material for the manufacture of hydrogel dressings being membranes composed of crosslinking (or interlaced) polymer systems [1-17]. The crosslinking of PVP in aqueous solution for hydrogel formation was done for the first time by Charlesby and Alexander in 1995 [18].

Hydrogels based on polyvinylpyrrolidone (PVP), poly (ethylene glycol) (PEG) and agar, were first developed as dressings for burn wound by Dr. Rosiak, of Polish origin [19]. This has met with great success with requesting medical treatment for other types of injuries and diseases since its introduction in 1992 [20]. They can be produced at a relatively low cost, a process being simple, safe and efficient [21]. This work utilized a mixture of PVP-PEG-agar-water in the preparation of hydrogel [22-23] with different doses of gamma radiation.

Since the first biomaterials of polymer origin, scientists have encountered a problem arising from the use of chemical initiators in their crosslinking reactions and the consequent toxicity thereof. This problem was the starting point for the search for new forms of reaction considered "clean", that is, to manufacture a pure product, uncontaminated with ballast materials or the residues of toxic initiators, but at the same time produce materials comparatively similar to those achieved by methods previously tested [9-24-25].

It was in this scenario that the use of high energy radiation in the preparation of biomaterials appeared. High energy radiations such as gamma radiation are an example of the types of radiation currently used for this purpose [9].

The formation of hydrogels via ionizing radiation can occur through gamma or UV-C radiation. The use of radiation in the formation of hydrogels can be defined as the result of a mutual combination of radical macros. Gamma radiation, in particular, has become more efficient and viable, since it enables crosslinking and sterilization simultaneously, as well as being a technology that simplifies the production process [23-26].

The crosslinking reactions caused by gamma (ionizing) radiation occur through the free radicals formed during the transfer of energy from the radiation to the electron system of the atom or molecule, for this reason it is also called ionizing radiation. The interaction of ionizing radiation with matter promotes physical-chemical events at the atomic level. Figure 1 shows the two paths for the formation of a polymeric radical: the direct and the indirect [27].

Direct crosslinking:

$P \xrightarrow{\gamma\ e^-} P^*$ (Radical formation)

$P^* + P^* \longrightarrow P - P$ (Recombination of polymer radicals)

Where P is the polymer

Indirect crosslinking:

$H_2O \xrightarrow{\gamma\ e^-} {}^*OH + H^*$ (Radical formation)

$^*OH + P \longrightarrow P^* + H_2O$ (Hydrogen abstraction)

$P^* + P^* \longrightarrow P - P$ (Recombination of polymer radicals)

Figure 1. Formation of the polymeric radical [28]

In the swollen state these spaces contain water or, generally, solvent molecules, and may also obtain active additions to the hydrogel shown in Figure 2. The characteristic dimensions in this region depend on the degree of crosslinking. The higher the degree of crosslinking, the less water can be absorbed [9].

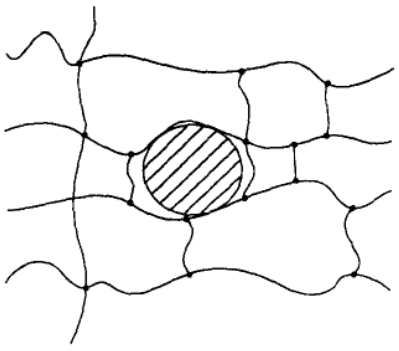

Figure 2. Scheme of hydrogel network. Circle denotes available space for diffusion [9]

The process of formation of gamma-ray hydrogels has some advantages while the reaction occurs as the easy control in the process, crosslinking at low temperatures, sterilization in a single technological stage, relatively low costs of operation and absence of contamination by chemical initiators, That this type of reactions is increasingly being used in the synthesis of materials for biomedical application [9-23-26].

2. EXPERIMENTAL

2.1. Material

Polymer matrix membrane was obtained from Poly (N-vinyl-2-pyrrolidone) (PVP) K90 (powder; MM = 360,000; Sigma-Aldrich) using poly (ethylene glycol) (PEG) 300 (liquid; MM = 300; Sigma-Aldrich) as plasticizer and agar (powder; pure; Isofar) as gelling agent. The solvent used was deionized water. All these materials were used without further purification.

2.2. Preparation of Hydrogel Solutions

To prepare the hydrogel, PVP K90 (6%), PEG 300 (1.5%) was added in a glass vessel and filled with deionized water at room temperature for complete dissolution of the components. After standing for 24 h, the mixture was warmed to the boil and agar (0.5%) was added, keeping it under heating until complete dissolution of the components.

The prepared solution was briefly cooled and added in circular molds up to 3 mm thick, which, after complete cooling, were sealed with plastic film, properly packed and sent for crosslinking.

2.3. Gamma Cross-linking

Crosslinking the solution was done using gamma radiation to obtain the hydrogels, a total radiation of 15, 20, 25 and 30 kGy was used. The irradiation was performed in a 60Co source, manufactured by MDS Nordion in Canada, model / serial number IR-214 and type GB-127, of circular geometry, located in the Gamma-LIG Irradiation Laboratory of CDTN / UFMG, Belo Horizonte, Brazil.

2.4. Characterization

2.4.1. Sterilization

For this test a portion of gamma-radiation-crosslinked hydrogel was added in containers with nutrient agar, all aseptically and incubated at 35 ° C. After four days in the incubator, the samples were taken and photographed for growth analysis of microorganisms.

2.4.2. Determination of gel fraction

Samples of gamma cross-linked hydrogel membranes were oven dried at a temperature of 50 °C to constant weight. After drying, in triplicate, they were packaged in nonwoven tissue bags and placed for removal of the soluble fraction for 36 hours in the Soxhlet extractor, using deionized water as the solvent. After this time the samples were again dried in an oven at a temperature of 50 °C until reaching constant weight. It is assumed that all soluble material has been extracted by water and the insoluble residual material (cross-linked part) is the gel fraction, which was calculated in percentage according to ASTM D 2765 [29] in equation 1.

$$\text{Gel fraction (\%)} = (Mf/Mo) \times 100 \quad (1)$$

Being the: Mf is the mass of the dry sample after extraction of the soluble fraction and Mo is the mass of the dried sample before extraction.
The tests were done on crosslinking hydrogels in a 100mm diameter and 60mm container both 3mm high, to verify if the crosslinking occurred in a similar way.

2.4.3. Swelling measurements

This assay was performed with phosphate buffered saline (PBS) pH 7.4, biological fluid simulator solution. 3 samples of each prepared matrix, weighing about 0.5 g each, were weighed. Samples were immersed in phosphate buffered saline (PBS) pH 7.2, biological fluid simulator solution, and mass measurements were performed every 10 minutes for the first 30 minutes and every 1 hour for a period of 8 hours and finally after 24 hours from the start of the test. The tests were performed at room temperature and the degree of swelling was calculated by equation 2.

$$\text{Swelling (\%)} = [(m_s - m_d)/m_d] \times 100 \quad (2)$$

Where m_s is the mass of the swollen polymer and m_d is the mass of the dried polymer. The final result of the degree of swelling for each sample was taken as the numerical mean of the results of the corresponding specimens tested.

2.4.4. Mechanical properties

The mechanical properties of the hydrogels with different radiation doses (15, 20, 25, 30 kGy) were evaluated using a Brookfield texturometer (model CT3). The analysis was performed by the study of the mechanical properties of the films by the principle of perforation. Among the known mechanical resistance tests, the test of resistance to perforation and deformation at the rupture of the films was studied using hydrogel films free of physical imperfections and with 30 mm of diameter and 2 mm of thickness, and the tests were performed in triplicate.

The specimens were fixed in a support, and a stainless steel "probe" with a cylindrical tip of 4 mm diameter was used at a penetration rate of 1 mm / s. The system was calibrated with a maximum force of 10 kg for all tests. The software used was the *TexturePro CT V1.4 Build 17*.

3. RESULTS AND DISCUSSION

3.1. Sterilization

After the irradiation process of the hydrogel membranes, they remained sealed and stored in closed styrofoam boxes in a controlled laboratory for approximately 180 days. It was noticed after this date that the hydrogel irradiated at the dose of 15 kGy had a distinct staining of the other membranes, as illustrated in Figure 3a.

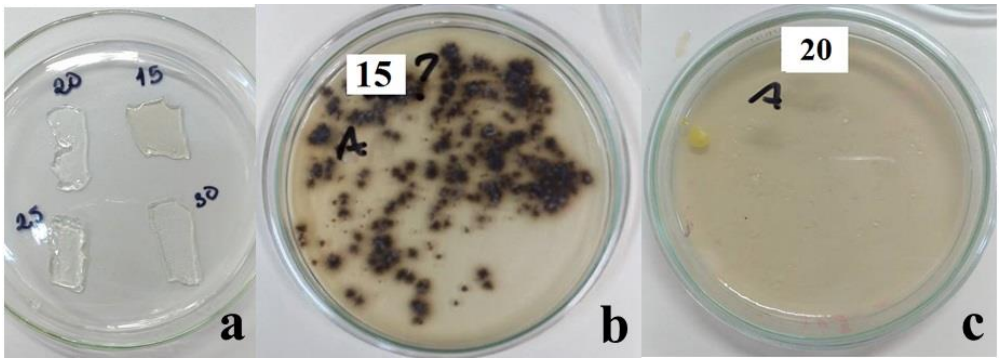

Figure 3: Hydrogel sterilization. (4a) hydrogel irradiated at different doses of radiation without culture of microorganisms; (4b) hydrogel irradiated at 15 kGy with culture of microorganisms; (4c) hydrogel irradiated at 20 kGy with culture of microorganisms.

A simple sterilization test was performed to better understand the efficiency of radiation doses in sterilization, where the total elimination of the microbiological life of these materials should occur, because when the microorganisms are found, the energy breaks the DNA chain of these beings, leading to death or inability to reproduce. As the electromagnetic waves have great penetration power, organisms can be reached wherever they are, in sealed packages as well as in products in various ways, which guarantees the total efficiency of the process. After the test, a large number of microorganisms were found on the plate related to the hydrogel irradiated at 15 kGy (Fig. 3b), different from that found on the hydrogel plate irradiated at 20 kGy (Fig 3c), which presented a similar result as the Irradiated hydrogel membranes at the dose of 25 and 30 kGy, where a single point of contamination was found, and presumed to have occurred during manipulation to perform the test. We can say that at the radiation doses of 20, 25 and 30 kGy the sterilization of the hydrogel membranes was efficient.

3.2. Determination of gel fraction

In this test, the hydrogels crosslinked at different doses of radiation and placed in containers of 60 mm diameter had high gel content, indicating a high crosslinking between the polymer molecules. However, the hydrogels in 100 mm diameter containers obtained showed low crosslinking results. The results are shown in Fig. 4.

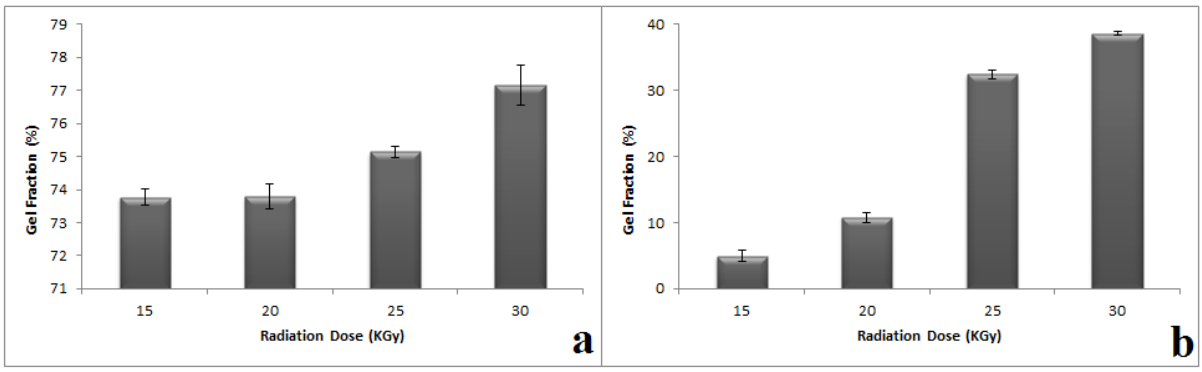

Figure 4: Gel fraction obtained by the hydrogel at different doses of radiation. (4a) 60 mm containers; (4b) 100 mm containers.

The gel fraction is the mass fraction of the material resulting from a three-dimensional network formation, showing the crosslinking behavior of the hydrogel. The hydrogel macromolecules formed by radiation are linked together by covalent bonds. [9]. Only the PVP molecules undergo crosslinking in the radiation process. Other materials do not participate in the structural network, PEG acts as a plasticizer and can improve the physical properties of the hydrogel membranes and the agar acting as a gelling agent. [23-30].

The results obtained from gel fraction tests (Figure 4a) showed a good level of crosslinking at all proposed radiation doses. By setting the 25 KGy dose as a reference for comparison to the study, a 75% crosslinking percentage, i.e. a high crosslinking network, was obtained. The result presented in a similar study by Ajji et al (2005), with 6% of PVP irradiated at 25 kGy was about 81%, which is in agreement with our data. An increase in the gel fraction is observed each time the radiation dose is increased, a fact also observed by Benamer *et al*. (2006) and by Foroutan *et al*. (2007).

The gel fraction tests shown in Figure 4b do not present satisfactory results, since the percentage of cross-linking was below expected. By setting the 25 KGy dose as a comparison reference for the study, a percent cross-linking of 32%, i.e., a network with little cross-linking was obtained. It is assumed that due to the size of the container the cross-linking may have been incomplete or some problem occurred in the allocation during the radiation. Although they did not show the expected result, an increase in the gel fraction was observed each time the radiation dose was increased, as expected.

3.3. Swelling measurements

Swelling is defined as the amount of fluid absorbed by the polymeric material at equilibrium when it is submerged in fluid for a period of time sufficient for the system to attain constant volume [31].

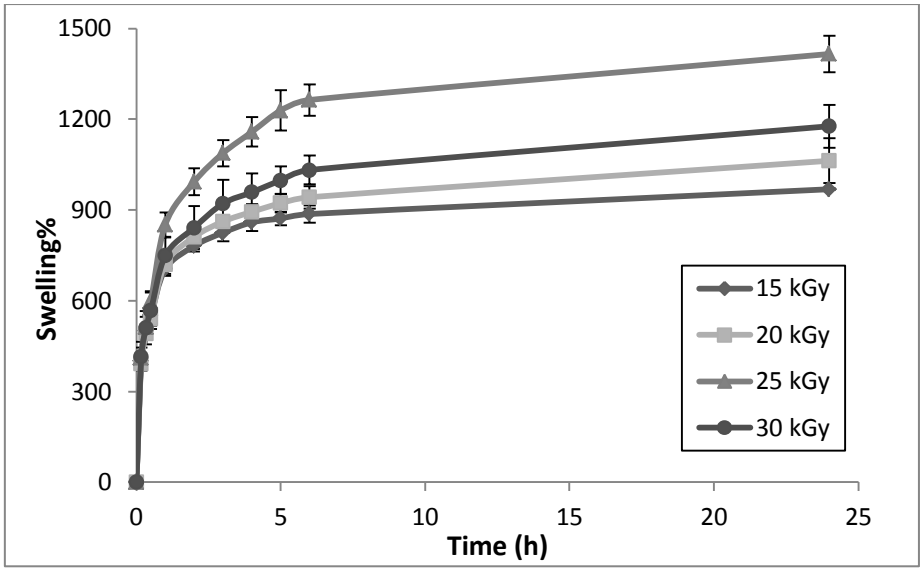

Figure 5: Swelling curve of crosslinked hydrogels at different radiation doses, ambient temperature and common atmosphere.

At all radiation doses, the swelling capacity for the PVP hydrogel equilibrium was reached 7 h as shown in Fig. 5. The hydrogels cross-linked at a dose of 25 kGy, was close to 1400% after 24 h. These results are similar to those obtained by Ajji et al, 2005 and to this dose of radiation presenting the best swelling curve as well as Benamer et al. (2006). In the study by Ajji et al, 2005 with hydrogel matrices composed of PVP, PEG and agar at different doses of radiation the matrix with 5% PVP and irradiated at a dose of 25 kGy showed a degree of expansion of about 1500% after 24 h. These results are very similar to ours, 1400%, considering that we use 6% of PVP. At a given dose of irradiation, the degree of equilibrium swelling depends on the concentration of polymer [12].

With the results shown in Figure 5, it is apparent that the ratio of crosslinking is one of the most important factors affecting the swelling of the hydrogels. Polymer matrices irradiated with 30 kGy (ca. 1200%) obtained a higher percentage of swelling compared to irradiated 25 and 15 kGy (ca. 950% and 1050% respectively). The higher the ratio of crosslinking the greater amount of crosslinking of the hydrogel making the structure more rigid. Consequently, swelling will be greater when compared to the same hydrogel when it has a lower ratio of crosslinking [11-32].

The gel fraction (Fig. 4a) and the degree of swelling (Fig. 5) demonstrate that this is a highly cross-linked system and shows a high level of swelling in the PBS solution, this system is suitable for use as a polymer matrix [22].

3.4. Mechanical properties

The mechanical properties of the hydrogels are associated with the ability of the material to withstand mechanical forces, especially with the ability of the polymer to form bonds in the polymer chains, making it difficult or not to separate these chains when subjected to mechanical forces.

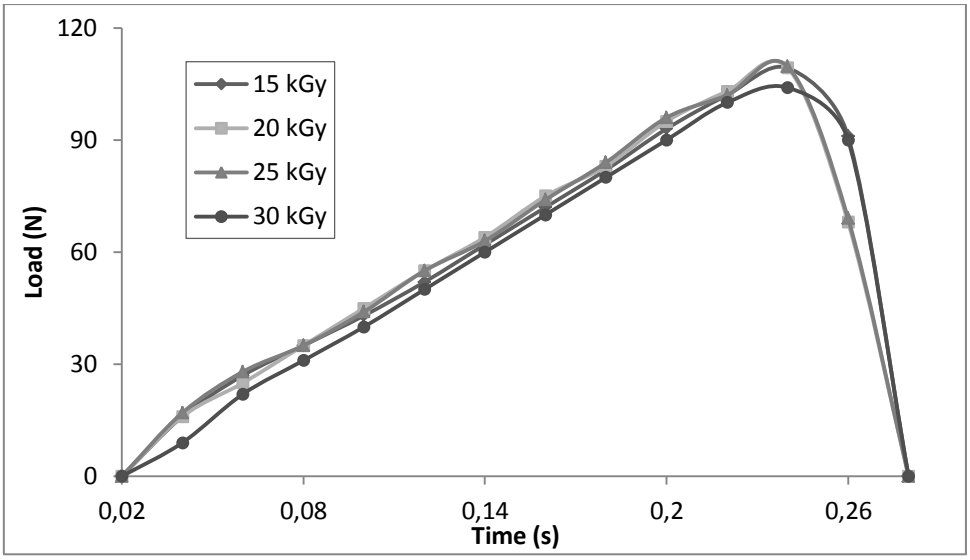

Figure 6: Graphical representation of the results of the tests of mechanical resistance of the hydrogels in relation to the perforation, obtained by texturometer.

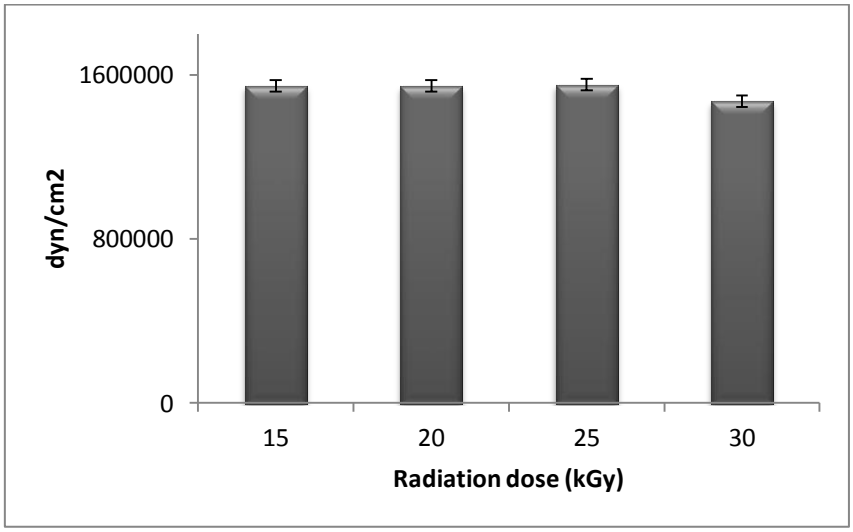

Figure 7: Strain peak of the irradiated hydrogels with different doses of radiation.

The drilling test measures the resistance to understanding, in N, as a function of the time to which the film is subjected. The results shown in Figure 6 show an insignificant difference related to the perforation of the irradiated material at different doses, so there is no difference between them. The strain peak voltage, Fig. 7, confirms these results showing similar results among the comparison parameters.

4. CONCLUSIONS

The results reported in this study are consistent with the expectations, demonstrating in the sterilization test that dose irradiation of 15 kGy was not sufficient to eliminate the microorganisms, requiring a dose of 20 kGy or greater to make the hydrogel sterile and safe for use as a wound dressing. The gel fraction tests presented the crosslinking hydrogels

arranged in 60 mm diameter containers with high gel content, indicating a high crosslinking between the polymer molecules, thus obtaining better results. The hydrogels presented good results for the swelling test demonstrating that this is a highly crosslinking system, and obtaining a better result for the matrices irradiated at 25 kGy. The mechanical properties of the hydrogels presented similar results for all matrices. The methodology used in the study is suitable for use as a polymer matrix wound dressing, suggesting new investigations for future applications as matrix for wound treatment.

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