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Evaluation of healing activity of PVA/chitosan hydrogels on deep second degree burn: Pharmacological and toxicological tests

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ABSTRACT

Hydrogel based on poly(vinyl alcohol) containing 0.25% of chitosan was synthesized by gamma irradiation and evaluated as wound dressing material in a burn rat model. Histological analyses, Primary Irritation Index (P.I.I.) and Ocular Irritation Index (O.I.I.) were investigated. The comparative study showed that the wounds treated with PVA/chitosan hydrogel healed on the 9th day, while those treated with paraffin gauze dressing and cotton gauze healed on the 16th day. Histological analysis showed that new granulation tissue and epithelialization progressed better in wound treated with hydrogel PVA/chitosan. The determined values of P.I.I. and O.I.I. of the PVA/chitosan hydrogel were, respectively 0.5 and zero. These values indicate that the PVA/chitosan hydrogel can be considered as non-irritating to the skin.

1. Introduction

Every year, hundred thousand patients suffer from burns due to domestic and industrial accidents [1], which along with enormous cost of treatment, cause mortality and considerable morbidity. Depending on their severity, burn wounds are classified into two categories: wounds with tissue loss and without tissue loss. The common challenge encountered in plastic and reconstructive surgery is how to enhance the healing of such wounds.

Various formulations have been developed such as passive products, interactive and bioactive products [2]. An ideal wound dressing should protect the wound from bacterial infection, provide a moist and healing environment and be biocompatible [3].

Hydrogels have been considered to be advantageous in their application as a wound dressing material [4–6]. During the last decade, very promising materials for wound dressing have been synthesized with poly(vinyl pyrrolidone) [7–9], poly(vinyl alcohol) [10], poly(ethylene oxide) [11] and polysaccharides such as chitosan, alginate, collagen, and cellulose [12,13].

The application of radiation for the formation of hydrogels for medical use offers a unique possibility to combine the formation and sterilization of the product in a single technological step.

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Chitosan is a polysaccharide derived from chitin which is natural polysaccharide present in crustacean exoskeletons (crab, shrimps), squid pens, fungi, insects, and some algae. Chitosan possess many properties that are advantageous for wound healing like biocompatibility, biodegradability [14], hemostatic activity [15], healing acceleration, non-toxicity, adsorption properties and anti-infection properties [16–18]. The most important characteristic of chitosan is its degree of deacetylation (DD) and molecular weight which influence its physicochemical behavior [19]. Chitosan extracted from squid pen chitin is inherently purer than crustacean chitosan; this purity makes it particularly suitable for medical and cosmetic application [20,21].

The main aim of this study was to synthesize by gamma irradiation poly(vinyl alcohol) (PVA) hydrogels containing chitosan, and to investigate in vivo the influence of the presence of chitosan in their composition on the wound healing process.

2. Materials and methods

2.1. Materials

Chitosan with degree of deacetylation (DD) of 70% and M_w = 471 kDa was produced locally from squid (*Loligo* sp.) pens chitin. Poly(vinyl alcohol) with a degree of polymerization of 4200, degree of hydrolysis of 86%, and M_w of 205 kDa, polyethylene glycol (PEG 400) (Fluka) and agar (Bacto) were used without any purification. All the reagents used were of medical grade. Deionized water was used for solutions preparation.

2.2. Preparation of hydrogel

Hydrogel was prepared by dissolving chitosan powder at concentration 0.25% in an aqueous solution of 0.25% acetic acid. PVA powder added to the chitosan solution to a total polymer concentration of 5%; PEG and agar were added to the mixture at 1.5% and 1%, respectively. The mixture was irradiated with gamma rays to a total dose of 25 kGy which represents the dose of cross-linking and sterilization.

2.3. Pharmacological test

2.3.1. Animals

Male Albino Wistar rats were obtained from the Pharmaceutical Products Control Laboratory. Rats weighting between 260 and 320 g were used in this study. They were randomly separated into three groups of seven animals each. In the first group, which served as control, the wound was covered with cotton gauze only, whereas the second and third groups were treated with paraffin gauze dressing and PVA/chitosan hydrogel, respectively.

2.3.2. Creation of a burn wound model

Rats were anesthetized with sodium thiopental with a dose of 40 mg/kg of body, administered by an intra-peritoneal injection; the hair on their upper back was shaved. A copper punch (1.5 cm × 1.5 cm) heated to 100 °C was applied for 5 s on the shaved dorsal skin of the rat to cause the burn. An analgesic was administered to the animals for 7 days. After 48 h, the resultant dead area was removed surgically under general anesthesia to obtain a severe burn wound model. Such a wound was used as a burn wound model for the pharmacological study. After wounding, each rat was housed individually in a sterilized cage with a 12 h light/dark cycle and at a constant temperature (25 ± 1 °C) and humidity (60 ± 5%).

According to the various stages of healing, the dressings were renewed every 2 days in the inflammatory phase (fibrin and necrosis), every 3–4 days in the proliferative phase and every 4 to 7 days in the maturation phase. During the change of the dressing on the 1st, 2nd, 6th, 9th, 12th, and 16th days, the wounds were photographed with a digital camera in order to calculate the wound surface areas (WSA) with the software AUTOCAD. The change in wound surface area in a given day (WSA_{day-x}) was expressed in a percentage of the wound area on the second day (WSA_{day-2}) using the following equation:

$$WSA = \frac{(WSA_{day-2} - WSA_{day-x}) \times 100}{WSA_{day-2}}$$

2.4. Toxicological test

2.4.1. Test of skin irritation

A primary skin irritation test was performed on a rabbit [22]. Hydrogel was applied on two sites of six albino rabbits.

Each rabbit received four parallel epidermal abrasions to remove the stratum corneum with a sterile needle on one test site while the skin at the opposite site remained intact. The dose level of hydrogel administered to the skin was 0.5 g and the treated skin areas were covered with surgical gauze. Erythema and edema were evaluated after 24 and 72 h in view to calculate the Primary Irritation Index (P.I.I.) and compare with reference index (Table 1) [22].

2.4.2. Test of mucosal membranes irritation

The Draize rabbit eye test is the only widely used test for the effect of substances on the eye. This study was conducted according to the requirement [22]. Weight of 0.1 mg of the PVA/chitosan hydrogel was inoculated into the conjunctival sack of six rabbits. The other eye was left untreated. The eye lid was kept closed by using two fingers for a few seconds to assure close contact with the cornea. The ocular lesions in the eye media, cornea, iris, and conjunctiva were evaluated for 1, 24, 48, 72 and 144 h, in order to calculate the maximum Ocular Irritation Index (O.I.I.).

Table 1 – Primary irritation index (P.I.I.).

Irritating effects	Primary Irritation Index (P.I.I.)
No irritating	P.I.I. < 0.5
Mildly irritating	0.5 ≤ P.I.I. < 2
Moderately irritating	2 ≤ P.I.I. < 5
Severely irritating	5 ≤ P.I.I. < 8