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Short communication

Effect of discarded keratin-based biocomposite hydrogels on the wound healing process in vivo

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ABSTRACT

Biocompatible keratin-based hydrogels prepared by electron beam irradiation (EBI) were examined in wound healing. As the EBI dose increased to 60 kGy, the tensile strength of the hydrogels increased, while the percentage of elongation of the hydrogels decreased. After 7 days, the dehydrated wool-based hydrogels show the highest mechanical properties (the % elongation of 1341 and the tensile strength of 6030 g/cm^2 at an EBI dose of 30 kGy). Excision wound models were used to evaluate the effects of human hair-based hydrogels and wool-based hydrogels on various phases of healing. On post-wounding days 7 and 14, wounds treated with either human hair-based or wool-based hydrogels were greatly reduced in size compared to wounds that received other treatments, although the hydrocolloid wound dressing-treated wound also showed a pronounced reduction in size compared to an open wound as measured by a histological assay. On the 14th postoperative day, the cellular appearances were similar in the hydrocolloid wound dressing and wool-based hydrogel-treated wounds, and collagen fibers were substituted with fibroblasts and mixed with fibroblasts in the dermis. Furthermore, the wound treated with a human hair-based hydrogel showed almost complete epithelial regeneration, with the maturation of immature connective tissue and hair follicles and formation of a sebaceous gland.

1. Introduction

Biomaterials have been developed rapidly in the medical field and adapted broadly to many innovative medical applications [1]. One of the most commonly used and environmentally–friendly biomaterials is keratin. Keratin proteins have three-dimensional mesh structures associated with structural fibrous proteins that are the key structural component of hair, wool, feathers, horns, and nails [2]. Keratins are composed of cysteine-rich structural proteins, which have a large number of disulfide bonds and have significant mechanical properties due to their hard and fibrous structures. More than 300,000 t of protein-rich hair waste is produced worldwide each year. These discarded keratins can be used for generating water-soluble compounds by reducing the disulfide bonds [3,4]. A significant amount of research has focused on the function and composition of biomaterials produced by keratins [5,6].

Hydrogels are three-dimensional polymer networks formed by hydrophilic polymer chains via either physical or chemical bonds. Hydrogels have widespread applications [7–11] in multiple fields, particularly in the medical and pharmaceutical sectors, because of their biocompatibility and high water content. Hydrogels are a soft and wet material that can be used in applications such as tissue engineering, drug delivery devices, artificial skin, and materials for contact lenses.

Ionizing radiation, including gamma radiation and electron beams (EB), has been widely used in cross-linking to produce hydrogels rendering the use of cross-linkers and toxic reagents unnecessary. Thus, radiation-treated hydrogels can be used in wound dressings without further post-processing due to their simultaneous cross-linking and sterilizing effects [12–14]. Keratin hydrogels can be used as wound dressings because they are abundant and bioactive, and they are a realistic source of autologous proteins [15].

The primary function of the skin is to serve as a barrier to the environment. Due to this essential function, skin wounds need to be efficiently repaired within a very short time frame [16]. The wound repair process comprises three orderly overlaid stages: inflammation, cell proliferation, and tissue regeneration [17]. Injured tissue goes through four phases to repair the wound: hemostasis, inflammation, proliferation (granulation), and remodeling. Hemostasis occurs immediately after injury. When the tissue is disrupted and the platelets adhere to the exposed collagen and to each other. Generally, inflammation begins 24 hours after injury. Neutrophils are the predominant cell marker in the inflammation phase. Along with macrophages, neutrophils remove

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the cell debris resulting from the wound by phagocytosis. The proliferation phase is initiated approximately 4 days after injury. This phase is characterized by angiogenesis and epithelialization, after which granulation tissue begins to invade the wound space, and numerous new capillaries are generated in the tissue. The predominant cells of the proliferative phase in the wound site are fibroblasts which are responsible for producing the new matrix necessary to restore structure and function to the injured tissue. The remodeling phase begins approximately 3–4 weeks after injury but only after the inflammation and proliferation phases have been successfully completed. In this phase, remodeling of the granulation tissue occurs, and immature connective tissues change into mature connective tissues through extracellular collagen formation.

Considering the cellular attachment property and theories regarding the effect of hydrogels composed of human and animal hair in wound healing, the present study was conducted using two experimental hydrogels, one extracted from human hair and another from sheep hair, that were compared with the most commonly used Comfeel Transparent Dressing (Transparent Wundverband, Coloplast, Denmark) and the natural wound healing process. Additionally, complimentary histological studies were carried out to determine the mechanism of stimulatory action in the healing process.

In our previous work, we successfully prepared biocompatible hydrogels made by natural polymer wastes (such as human hair and wool) for the first time using EBI and investigated the corresponding mechanical properties, gel fraction, and swelling behavior [18]. The objectives of this study were to reduce environmental contamination and produce environmentally friendly biocompatible hydrogels for wound healing.

2. Materials and methods

2.1. Materials

Urea, sodium disulfite ($Na_2S_2O_5$), sodium dodecyl sulfate (SDS), acetone (CH_3COCH_3), ethanol (C_2H_5OH), poly(ethylene imine) (PEI, branched, average M_n~10,000 by GPC, average M_w ~ 25,000 g/mol by light scattering) and poly(vinyl alcohol) (PVA) (Mw = 85,000–124,000 g/mol, 87–89% hydrolyzed) were purchased from Sigma Aldrich, Co. (St. Louis, USA). Human hair was obtained from local hair salons free of charge, and discarded wool (Merino) was provided by local bedding factories. Double-distilled water was used for preparing aqueous solutions. Disposable sterilized polystyrene square dishes (125 × 125 × 20 mm) were purchased from SPL Life Sciences Co. (Republic of Korea).

2.2. Hydrogel preparation

Wool (Merino) and human hair were thoroughly washed with water containing 0.5% SDS, rinsed with fresh water and then air-dried. Keratin was extracted by sulfitolysis [19–21]. The cleaned hair and wool were separately extracted with ethanol and acetone for 12 hours using a Soxhlet apparatus to remove external lipids and impurities. The dried hair and wool samples (150 g each) were cut into fragments of several millimeters and placed into 1.5 l of an aqueous solution containing 8 M urea, 75 g SDS and 150 g $Na_2S_2O_5$. The mixture was heated to 100 °C at a rate of 2 °C/min, gently stirred for 30 min and then cooled in a water bath at 30 °C. The resulting mixture was filtered through a stainless-steel mesh, and the filtrate was dialyzed against 15 l of water containing 0.1% (w/v) $Na_2S_2O_5$ using cellulose tubing (molecular-weight cutoff of 12,000 Da) for 3 days. The dialysis solution was changed twice per day.

Hydrogels that contained keratin protein were produced in the form of a sheet (125 mm × 125 mm × 5 mm, width × length × thickness). Briefly, a S-sulfo keratin solution containing various weight fractions of S-sulfo keratin was blended with the PVA solution to improve the gelation of the S-sulfo keratin aqueous solution. The optimal concentrations of the S-sulfo keratin and PVA solutions were 5.0% (w/v) and 5.0% (w/v) respectively, 0.01% (w/v) PEI included in the PVA solution. Subsequently, the S-sulfo keratin/PVA blended solution was poured into the square dishes, and irradiated with an electron-beam accelerator (beam energy of 2.5 MeV, beam current of 8.5 mA, irradiation width of 110 cm, conveyor velocity of 10 m/min, dose rate of 6.67 kGy/s, roller type handling system, EBTech Co., Ltd., Korea) at a dose of 10 kGy–100 kGy at room temperature under normal atmospheric conditions. After gelation, the hydrogels were cut into the required sizes for conducting various analyses.

2.3. Characterizations

A standard procedure was used to create hydrogel samples for scanning electron microscopy (SEM) measurements. Freeze-dried hydrogel samples were coated with platinum (ion-sputter, Hitachi E-1010, Japan) under vacuum, and were used to investigate the morphology of keratin protein hydrogels. SEM images of the surface and fractured morphologies of the hydrogels were recorded at 15 kV with different magnifications using a Jeol JSM-5900 SEM machine. Mechanical properties of discarded keratin-based hydrogels were performed using hydrogel samples after 0, 3, 7, and 14 days fixed with adhesive bandage in the same way as in vivo test. The tensile strength at rupture and the elongation at break were measured using a Universal Test Machine (UTM) (Lloyd., US/LRIOK) according to the ASTM D882 standard method. Strips with dimensions of 50 mm × 15 mm × 5 mm were prepared by cutting a portion from the hydrogel. A cross-head speed of 50 mm/min was used. The ambient temperature and relative humidity were maintained at 21 °C to 22 °C and 50% to 55%, respectively. The data were transferred to a computer for evaluating the stress–strain curve.

A keratin biomaterial was tested (n = 6) and compared to negative control of no treatment (n = 7) and a positive control of GelFoam® (n = 6).

Three injuries inflicted on each rat (n = 11) were dressed with ultrashort peptide hydrogels Ac-ILVAGK-NH2 and Ac-LIVAGK-NH2, and silicone-coated polyamide net Mepitel®.

Dermal irritation tests were performed using Sprague–Dawley rats (n = 4 per group).

2.4. Animals, wounding and histology

Male Sprague Dawley rats (8 weeks old) were used for all of the experiments. The animals were anesthetized with sodium pentobarbitone by intra-peritoneal injection (40 mg/kg). Each rat was then placed in the prone position, and the hair was removed from the dorsal region using a razor blade. Four full thickness 1 cm^2 wounds were made through the panniculus carnosus on the dorsum of each animal at the same level. Two injuries inflicted on each rat were randomly chosen as treatment group dressed with human hair-based hydrogel (HH) and wool based-hydrogel (WH) (n = 4, respectively), and the third wound was compared as a blank control (BC, negative control) of no treatment (n = 4). The forth wound was covered with a commonly used hydrocolloid wound dressing (Transparent Wundverband T.N. Comfeel, COLOPLAST, Denmark) as a positive control (WD) (n = 4). The wounding day was considered day 0. Healing wounds were observed on days 3, 7 and 14 after wounding [22]. All animal handling followed the guidelines of and was approved by the Institutional Animal Care and Use Committees (IACUC) at Chonbuk National University, South Korea. At the 3rd, 7th and 14th postoperative days, the wounds were excised and fixed in 10% neutralized buffer formalin. After fixation, the specimens were embedded in paraffin and sectioned at 5 μm with a normal rotor microtome. The sections were stained with hematoxylin–eosin (H–E stain).