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Natural collagenic skeleton of marine sponges in pharmaceutics: Innovative biomaterial for topical drug delivery

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ABSTRACT

The growing interest in the use of recyclable and biodegradable natural materials has become a relevant topic in pharmaceutics. In this work, we suggest the use and valorization of natural horny skeleton of marine sponges (Porifera, Dictyoceratida) as bio-based dressing for topical drug delivery. Biomaterial characterization focusing on morpho-functional traits, swelling behavior, fluid uptake performances, glycosaminoglycans content and composition and microbiological quality assessment was carried out to investigate the collagenic skeleton properties. After grinding and sieving processes, L-cysteine hydrochloride-loaded formulations were designed in form of powder or polymeric film by testing various drug concentrations and different drying parameters. Drug content, SEM analyses and *in vitro* permeation studies were performed to test the suitability of skeleton-based formulations. To this respect, drying time and temperature are key parameters for skeleton-mediated drug crystallization. Consequently, this behavior seems to influence drug loading and permeation profiles of formulations. The high percentages of drug are found after absorption into sponge powder and *in vitro* permeation studies demonstrate that cysteine is released more slowly than the pure drug within 1 h. Such a system is attractive because it combines the known healing properties of cysteine with the advantageous potentials of the collagen/proteoglycan network, which can act as biocompatible carrier able to absorb the excess of the wound exudate while releasing the drug. Furthermore, due to its glycosaminoglycans content, natural sponge skeletal scaffold might act as bioactive-biomimetic carrier regulating the wound healing processes.

1. Introduction

A chronic wound is described as a break in the skin, which lasts at least 6 weeks and has a frequent recurrence. It might affect mainly epidermis and dermal inner layers. Several factors are involved in wound healing; it is indeed a complex and dynamic process of renovating cellular components and tissue layers [1]. A wide range of topical wound dressings are now available such as films, foams, hydrogels containing antiseptics, antibiotics and/or factors involved in the healing process [2–6]. The treatment of wounds will be dependent on the wound type, healing phases, condition of patient and it will include the concept of moisture [7,8]. Optimal conditions comprise a proper circulation of oxygen to support restoring cells and tissues, effective moisture around the wound and minimum microbial load [9,10]. Despite moisture is fundamental for proper healing, excessive wetness on the wound might become problematic. A wet-wrap dressing applied to highly exudative wounds can lead to tissue maceration. Moreover, chronic wound fluid may actively interfere with the healing process, since it contains proteases that damage extracellular matrix (ECM) components, while inhibiting fibroblast proliferation. Fibroblasts produce indeed different kinds of substances important for wound repair, *i.e.* glycosaminoglycans (GAGs) and collagen [11]. An ideal wound dressing should remove the excess exudate without totally absorbing it, creating an excessively dried wound as a consequence. The absence of amino acids and proteins may also compromise the wound healing process [12]. In particular, effectiveness in promoting epithelial regeneration by sulfur amino acids is well known: they influence the cell proliferation and new cell formation [13]. For example, cysteine is an essential component in re-establishing wound inflammation and increasing the concentration of

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wound fibroblasts. Furthermore, cysteine is necessary for disulfide bonds that play an important role in the formation of the collagen triple helical structure, strengthening its components [14]. In antiquity, the collagenic skeletons of some horny sponge species (phylum Porifera, class Demospongiae, subclass Keratosa, order Dictyoceratida) now commonly known as bath sponges were recommended for wound cleaning and drying before applying a bandage, in order to absorb fluids and promote wound healing [15,16]. Recently the pharmaceutical interest in sponge-derived molecules/biomaterials and their sustainable production focus on a wide array of applied research [17,18]. Sponges are basal Metazoa and their *bauplan* has proved successful in evolution since Cambrian in a wide array of aquatic environments [19]. The body architecture of Demospongiae is structured as ectosome with its skeleton as a selective and protective barrier at the outer surface, and the choanosome with its skeleton in the inner region containing flagellated chambers, choanocytes, and canals of the aquiferous system. The jelly ECM of these ancient multicellular animals resembles that of higher taxa, being composed of collagen, sulfated polysaccharides, proteoglycans (PGs), and minor amounts of structural proteins and glycosaminoglycans (GAGs) [20–26]. In the sponge ECM collagen fibrils are arranged to form thin fibrils which tend to be more densely packed to form the fibrous skeleton [23]. In horny sponges skeleton a high number of fibers are spatially arranged in a hierarchical network of primary and secondary fibers. From chemical studies on sponge collagen, glycosylated hydroxylysine together with a high content of aspartate and glutamate were found to represent the main components of fibrils [23]. In order to reproduce the interesting structure of these animals, during recent years spongy like matrices obtained by crosslinking of different polymers have been developed as new drug delivery systems targeted to wound healing [1,27,28].

The aim of this study is to suggest a new bio-based dressing in form of powder or polymeric film for cutaneous wound healing. This biomaterial consists of marine sponge natural collagenic skeleton of a target species loaded with L-cysteine hydrochloride. The main objective of the work is to evaluate the morpho-functional performances of this bioactive-biomimetic material, easily available in the wild and/or on the market, for pharmaceutical application as natural alternative to the development of polymeric spongy-like matrices. Due to its collagenic network of fibers, it can adsorb the excess of wound exudate and modulate the release of the entrapped drug, thus contributing to a suitable environment for the re-epithelialization process. In view of that, this biomaterial used as carrier for the drug delivery can be a new promising approach to develop environmentally sustainable formulations aimed at wounds treatment.

2. Materials and methods

2.1. Collection and sampling of sponge skeletons

The collagenic skeletons of wild marine sponges (Porifera) used in this study were collected as stranded skeletons from Sardinian beaches (Western Mediterranean Sea) and belong to four species *i.e. Spongia lamella* (Schulze, 1879), *Spongia officinalis* (Linnaeus, 1759), *Hippospongia communis* (Lamarck, 1814) (Demospongiae: Dictyoceratida: Spongiidae) and *Sarcotragus spinosulus* (Schmidt, 1862) (Demospongiae: Dictyoceratida: Irciniidae). Before to be processed each skeleton was accurately rinsed with freshwater in order to remove any contamination in form of salt, sand, shell and rock fragments entrapped in the fibrous skeletal network. Finally, they were air dried at room temperature. Samples isolated from skeletons comprised three groups selected on the base of preliminary tests: one designed for morphological characterization and swelling performances comprising all the four species; a second group for the determination of water uptake and for powder preparation (*S. lamella*, *S. officinalis*, and *S. spinosulus*); a third group for the determination of GAGs (*S. lamella*, *S. officinalis*, and *H. communis*). On the basis of the results, selected subsamples of *S. lamella* were considered as experimental target material for further studies, such as determination of viable aerobic count, sterilization, preparation and SEM characterization of both drug loaded powder and alginate-based dressing, and *in vitro* permeation assays.

2.2. Materials

L-cysteine hydrochloride (CysHCl) (>98%) ammonium acetate (99.9%) neocuproine (>98%) and glycerol (99.5% Eur. Ph.) were purchased from Sigma Aldrich (Italy). Copper (III) chloride dehydrate (99% ACS) was obtained from Aldrich Chemical Company Ltd. (Gillingham, England). (USA). Tryptone soya agar (oxide), Sabouraud dextrose agar (oxide), tryptone soya broth and thioglycolate U.S.P. were purchased from Oxoid LTD (Basingstoke, Hampshire, England). Sodium alginate (Protanal LF 120L, batch 907788) was purchased from NovaMatrix/FMC Biopolymer (Sandvika, Norway). All other chemicals and reagents were of analytical grade. Alcian Blue 8GX (Cod. A9186), papain, hyaluronidase from *Streptomyces hyaluroniticus*, Chondroitinase-AC from *Arthrobacter aurescens*, (Cod. C8618), Chondroitinase ABC from *Proteus vulgaris* (Cod. C3667), chondroitin sulfate A from bovine trachea (Cod. C9819), chondroitin sulfate B from porcine intestinal mucosa (Cod. C-3788), chondroitin sulfate C from shark cartilage (Cod. C-4384), heparan sulfate from bovine kidney (Cod. H7640), were Sigma Aldrich reagents. Titan III-H cellulose acetate plates (6.0 × 7.5 cm) were from Helena BioSciences. DEAE Sephacel was from GE Healthcare (Cod. 17-0500-01).

2.3. Characterization of sponge skeletons

2.3.1. Sponge skeleton morphology

Each skeleton was observed by light microscopy (LM, Leitz DMRB) and scanning electron microscopy (SEM Vega3 Tescan, Czech Republic) to characterize the diagnostic traits of skeletal architecture, namely texture of reticulate network, spatial arrangement of conules and of primary-secondary spongin fibers, abundance and morphometries of collagenic filaments, surface and morphometries of fibers, and presence of foreign material in fibers core. Identification of each specimen at the species level was carried out following standard techniques [29]. The taxonomic status of species was validated following Pronzato and Manconi [30].

2.3.2. Extraction, purification and characterization of glycosaminoglycans (GAGs)

GAG analyses were performed in triplicate on dry skeletons (see Section 2.1). Horny skeletons were re-hydrated for 24 h at 4 °C with 0.1 M sodium acetate buffer (pH 6.0) containing 5 mM ethylenediaminetetraacetic acid (EDTA)and 5 mM cysteine (pH 6.0), and exhaustively digested with papain at 56 °C for 48 h (0.3 U/mg of dry tissue). Free GAGs were purified by anion-exchange chromatography on (DEAE)-Sephacel, equilibrated with 50 mM sodium acetate buffer (pH 6.0) and eluted with 2.0 M LiCl, followed by GAGs precipitation by incubating with 4 volumes of cold absolute ethanol at −20 °C overnight. Sulfated polysaccharides were recovered by centrifugation and dried for quali-quantitative analyses. Total hexuronic acid content was estimated by carbazole reaction, by using glucurono-lactone as a standard. Purified GAGs were submitted to discontinuous cellulose acetate electrophoresis [31]. Following Alcian Blue staining, images were acquired by means of GS-800 calibrated densitometer (Bio-Rad) and analyzed by using Quantity One v4.6.3 software (Bio-Rad). Aliquots of GAGs underwent enzymatic and chemical depolymerization with standard methods for vertebrate GAGs characterization. The treatment with hyaluronidase (0.5 U/100 μg of hexuronic acid) was conducted in 0.15 M NaCl, 0.02 M Na acetate buffer, pH 6 for 3.5 h at 60 °C. The treatment with chondroitin-AC-or-ABC lyase (0.1 U/100 μg of hexuronic acid) was performed in 50mMTris-HCl, 50 mM Na acetate buffer, pH 7.5 for 5 h at 37 °C. To establish the presence of glycosaminoglycan containing