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Research paper

The use of fucosphere in the treatment of dermal burns in rabbits ☆

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Abstract

The aim of this study was to prepare a new microsphere (fucosphere) system based on polyion complexation of fucoidan with chitosan, and to evaluate its treatment efficiency on dermal burns.

The physicochemical properties such as mean particle size and distribution, zeta potential and bioadhesive properties of the microspheres were investigated. The formulation which had the high surface charge, narrow size distribution and the highest bioadhesive property was selected and applied on seven male New Zealand white rabbits with dermal burns. Biopsy samples were taken on day 7, 14 and 21. Each burn site was evaluated macroscopically and histopathologically and the findings were compared with controls of fucoidan solution and chitosan microspheres.

The microspheres between the size ranges of 367 and 1017 nm were obtained. The work of bioadhesion of microspheres, with the surface charges +6.1 to +26.3 mV, changed between 0.081 and 0.191 mJ cm^{-2}. Macroscopically and histopathological observations indicated that the fastest healing of the burns was obtained in group treated with fucosphere after 21 days of treatment ($P < 0.05$). Rete peg formation values and nuclear organize regions (NORs) were higher with treated fucospheres than the other groups on day 14.

In conclusion, *in vitro* and *in vivo* evaluation of fucospheres indicated that the new microsphere system shortened the treatment period of burns and provided fast and effective healing by improving regeneration and re-epithelization. Hence fucosphere may find application in the treatment of dermal burns.

Keywords: Fucoidan; Chitosan; Biopolymer; Fucosphere; Microsphere; Burn healing; Animal experiment

1. Introduction

Third-degree burn is the most serious injury because it destroys all the layers of the skin and the healing begins with the formation of granulation tissue, often associated with hypertrophic scars [1,2]. Recently, dermal substitution and burn healing have become one of the most exciting research areas in biomaterial sciences. Although there have been many recent advances in this field, commercially available products and the biological materials currently described in experimental studies are still incapable of fully substituting for natural living skin [2,3]. On the other hand, healing of dermal wounds with macromolecular agents such as natural polysaccharides is preferred as a skin substitute as they possess useful properties such as high biocompatibility and non-toxicity [4].

There are two stages of burn healing. The first stage involves the inflammatory phase and the second stage is the new tissue formation phase [5]. During the inflammatory phase, infiltrating neutrophils aid in the removal of foreign agents in the burn area. It was found that

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polysaccharides such as chitin and chitosan could accelerate the infiltration of inflammatory cells, consequently accelerating wound cleaning [6]. When the new tissue formation phase occurs, fibroplasia begins by the formation of granulation tissue within the wound space.

Chitosan is a mucopolysaccharide with structural characteristics similar to glycosamines. This biopolymer, produced by deacetylation of chitin, is derived from the exoskeleton of crustaceans. Chitosan has been used as a wound dressing in burn healing for proliferation and activation of inflammatory cells in granulation tissue [7]. Fucoidan is a sulphated polysaccharide extracted from brown seaweeds (e.g., *Fucus vesiculosus*, *Ascophyllum nodosum*). Fucoidan is made up of α-L-fucose units linked by (1 → 4) and (1 → 3) glycosidic bonds and sulphated at positions 2 and/or 3 and/or 4 depending on the algal species. Fucoidan is endowed with significant gel contraction-promoting ability and integrin expression-enhancing heparinic activity [8]. A great number of studies on pharmacological properties of fucoidan have been carried out but there is limited research on the use of fucoidan for the treatment of burn healing [9–11].

The aim of this study was to prepare a new microsphere (fucosphere) system based on polyion complexation of negatively charged fucoidan with positively charged chitosan, and to evaluate its treatment efficiency on dermal burns.

2. Materials and methods

2.1. Materials

Chitosan (MW 250 kDa, deacetylation degree ⩾90%, Pronova A/S, Norway; MW 400 kDa, deacetylation degree ⩾60%, Fluka, Germany; MW 750 kDa, deacetylation degree ⩾75%, Sigma, USA), fucoidan (MW 80 kDa, from *Fucus vesiculosus*), sodium sulphate (Merck, Germany) and lactic acid (85% m/v) were purchased from Sigma, USA. All other reagents used were of analytical grade.

2.2. Preparation of the microspheres

Fucospheres were prepared by mixing positively charged chitosan and negatively charged fucoidan using a polyion complexation method [12,13]. Briefly, 10 ml aqueous solution of fucoidan was added dropwise into 10 ml chitosan solution in 1% m/v lactic acid using high shear homogenisation (Ika, Euroturrax T20, Germany) at 20,000 rpm. Formed microspheres were separated by centrifugation at 15,000 rpm and then freeze-dried (Lyovac GT 2E, Steris, Germany). For comparison, chitosan microspheres were prepared by precipitation method as previously described [14]. To prepare chitosan particles, 10 mL of sodium sulphate solution (20% m/v) was dropped into 10 mL of acidic solution of chitosan (2% m/v) and stirred (Ika-Werke GmbH & Co, Germany) for 1 h at 500 rpm. Chitosan microspheres were washed, separated by centrifugation at 12,000 rpm and then freeze-dried. A number of variables were investigated for the purpose of optimization of the microsphere formulations (Table 1).

2.3. Scanning electron microscopy (SEM)

Particles were mounted on the metal grids using double-sided adhesive tape and coated with gold about 500×10^{-8} cm in thickness using SC7640 Sputter Coater (Quorum technologies, Newhaven, UK) under high vacuum, 0.1 Torr, 1.2 kV and 50 mA at 25 ± 1 °C. The surface morphology of microspheres was investigated with scanning electron microscopy (SEM) (Joel, JSM-5200, Japan) at 20 kV.

2.4. Determination of particle size

Measurements were performed at 25 °C, using a Climet submicron particle size analyzer (Climet CI-1000 liquid counter system, USA) with a 180 Series Laser Diode illuminated light scatter submicron sensor (Measurement range: 0.2–50 μm). The particle suspensions were sonicated in an ultrasonic bath for 5 min prior to analysis and bidistilled water was used as a dilution medium. Analyses were performed in three different batches and the results were expressed as a mean of three measurements.

2.5. Zeta potential

The zeta potential values of microspheres were determined in a 0.2 M KCl solution after measurement of the electrophoretic mobility for 20 s at 25 °C using a Zetasizer

Table 1
Mean particle diameter, zeta potential and bioadhesion values of the microspheres

Formulations	Fucoidan concentration (%)	Chitosan concentration (%)	Chitosan origin	Mean particle size (nm ± SD)	Polydispersity index ± SD	Zeta potential (mV ± SD)	Work of bioadhesion (mJ cm^{-2} ± SD)
A1	1.50	0.50	Sigma	367 ± 34	0.268 ± 0.032	23.1 ± 0.9	0.104 ± 0.003
A2	2.00	0.50	Sigma	575 ± 43	0.146 ± 0.021	13.3 ± 0.4	0.164 ± 0.006
A3	2.50	0.50	Sigma	901 ± 61	0.234 ± 0.041	8.2 ± 0.5	0.187 ± 0.005
A4	–	0.50	Sigma	616 ± 23	0.318 ± 0.074	26.3 ± 0.4	0.082 ± 0.004
B1	2.00	0.25	Sigma	416 ± 31	0.213 ± 0.045	9.7 ± 0.5	0.081 ± 0.005
B2	2.00	0.75	Sigma	768 ± 22	0.098 ± 0.021	16.7 ± 0.4	0.191 ± 0.005
C1	2.00	0.50	Protan 243	801 ± 40	0.314 ± 0.054	6.1 ± 0.3	0.134 ± 0.005
C2	2.00	0.50	Fluka (M.W)	1017 ± 73	0.358 ± 0.081	10.5 ± 0.6	0.141 ± 0.006