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In vitro evaluation of novel chitosan derivatives sheet and paste cytocompatibility on human dermal fibroblasts

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ABSTRACT

The cytocompatibility of novel chitosan derivative sheets and pastes were evaluated *in vitro* for possible utilization in wound dressing applications for wound healing. In this study, the cytotoxicity of oligo-chitosan (O-C), *N,O*-carboxymethyl-chitosan (NO-CMC) and *N*- carboxymethyl-chitosan (N-CMC) derivatives in sheet like and paste forms were evaluated using primary normal human dermal fibroblast cultures and hypertrophic scars; a fibrotic conditions representing a model of altered wound healing with overproduction of extracellular matrix and fibroblast hyperproliferative activity. Cytotoxicities of these chitosan derivatives were assessed using 3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyl tetrazolium bromide (MTT) assay. The results indicate that both chitosan derivative sheets and pastes have appropriate cytocompatibility and appear promising as safe biomaterials with potential wound healing applications. The SH120 (NO-CMC) derivatives sheet exhibited highest cytocompatibility property and may be regulated by MMP-13 in controlling the cell growth and its expression level.

1. Introduction

Chitosan, a de-*N*-acetylated analog of chitin, is a heteropolysaccharide consisting of linear β-1,4-linked GlcN and GlcNAc units and is widely distributed in nature as the principal component of crustaceans and insects exoskeletons as well as the cell walls of some bacteria and fungi (Jang, Kong, Jeong, Lee, & Nah, 2004). Because chitosan is relatively insoluble, its potential applications are also limited. To exploit the unique properties of these versatile polysaccharides, attempts are being made to derivatize them. Chitosan and modified derivatives display excellent biological properties including biodegradability in the human body (Sashiwa, Saimoto, Shigemasa, Ogawa, & Tokura, 1990; Shigemasa, Saito, Sashiwa, & Saimoto, 1994), as well as immunological (Mori et al., 1997; Nishimura et al., 1984), antibacterial (Tanigawa, Tanaka, Sashiwa, Saimoto, & Shigemasa, 1992; Tokura, Ueno, Miyazaki, & Nishi, 1997), and wound-healing activities (Khnor & Lim, 2003; Kweon, Song, & Park, 2003; Okamoto et al., 1993), and thus, these compounds possess unlimited application potential for use in a wide range of fields (Harish Prashanth & Tharanathan, 2007). The biodegradability, biocompatibility and non-toxicity of chitosan and its derivatives allow for widespread applications in wound healing (Muhammad, Lim, & Khor, 2001). Chitosan has been shown to posses both material and bioactive properties that may enhance wound repair (Mattioli-Belmonte et al., 1997) and the ability of chitosan and chitin to form gels, films, fibers, and sponges demonstrate their inherent versatility (Majeti & Kumar, 2000; Nakade et al., 2000; Yoshikawa, Otsuki, Midorikawa, & Terashi, 1997).

Biocompatibility has been defined as "the ability of a material to perform with an appropriate host response in a specific application" (Williams, 1987) and is one of the important prerequisites for the usefulness of biomaterials. Several procedures have been described using cell culture techniques for preliminary biocompatibility evaluation of materials intended for medical application (Pizzoferrato et al., 1994). Because much of the work describing the effects of chitosan was performed on specific cell lines or animal primary fibroblasts, fibroblasts primarily isolated from human skin cells were used in this study (Chung, Schmidt, Hamfyn, & Sagar, 1994; Mori et al., 1997; Schmidt et al., 1993). Fibroblasts

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are crucial to various organism processes that range from synthesizing ECM to mediate ECM remodeling by cytokine and metalloproteinase activity in the wound healing process (Thomas, O'Neil, Harding, & Shepperd, 1995). Fibroblast hypertrophic scars are a form of excessive dermal fibrosis and cutaneous scarring (Amadeu, Braune, & Porto, 2004) caused by deregulation of cellularity increases and decreases during the wound healing process (Sahl & Clever, 1994; Szulgit, Rudolph, & Wandel, 2002; Tanaka, Hatoko, & Tada, 2004).

The determination of cell viability is a mean of observing the *in vitro* cytotoxicity of biomaterials through detrimental intracellular effects on mitochondria and metabolic activity. The colorimetric MTT test, based on the selective ability of viable cells to reduce 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide into purple formazan has been used to screen the *in vitro* cytotoxicity of biomaterials (Mosmann, 1983).

Currently, studies that investigate the effect of chitosan and its derivatives on primarily cultured human skin cells are limited. Furthermore, very few studies have assessed the compatibility of chitosan with human hypertrophic scars or investigated the final product of chitosan derivatives in sheet and paste forms. Thus, the purpose of this study is to evaluate the cytocompatibility of novel chitosan derivative (O-C, NO-CMC and C-MC) sheets and pastes on primary human dermal fibroblasts and hypertrophic scars.

The mechanism involved in exhibiting cytocompatibility effects by selected chitosan derivatives that exhibited highest cytocompatibility property need to be elucidated. At present, very little information is available on the mechanisms controlling matrix metalloproteinase-13 (MMP-13) expression in both normal and pathological conditions. The MMP-13 has broad substrate specificity for collagen and other ECM macromolecules and has a pivotal role in wound healing and the pathogenesis of invasive cancers and arthritis (Ala-aho & Kahari, 2005; Leeman, Curran, & Murray, 2002).

2. Materials and methods

2.1. Materials

The sterile (irradiated) sheet-like and paste-like (Fig. 1) of novel chitosan derivatives oligo-chitosan (O-C), *N*,*O*-carboxymethyl-chitosan (NO-CMC) and *N*-carboxymethyl-chitosan (N-CMC) were tested in this study. The samples were prepared and derivatized using new formulation and/or method of preparation to form sheet-like and paste-like final product (Section 2.1.1). The O-C was prepared using enzymatic reactions to achieve the desired viscosity (which is related to the molecular weight) of the oligo-chitosan solution to obtain a structurally suitable film which has not been reported before. The novelties of NO-CMC and N-CMC are the crosslinking of both polymers with PVP and PEG through gamma ray. In addition, the source of chitosan was from local shrimp exoskeleton. General information regarding sample composition is shown in Table 1. Each chitosan derivative sheet was cut into 0.5 cm × 0.5 cm square pieces.

2.1.1. Preparation of sheets and pastes

Chitosan sheet was prepared by dispersing 5 g chitosan powder in 150 ml isopropanol, while stirring with 13 ml of 20% (w/v) sodium hydroxide solution for another 1 h at room temperature. Then 6 g sodium monochloroacetate was added and heated about 1–2 h at 55 °C. It was washed with 100 ml aqueous ethanol for three times and dried in oven at 60 °C. On the other hand, chitosan paste was prepared by dispersing O-C (MW 45,660 Da), NO-CMC and N-CMC in distilled water. PVP was dissolved (Average MW 30 kDa, K 90, B.P.) into chitosan solution, then PEG was added (Average MW 200–500, Lutrol E400 BASF) at equal percentage to PVP in the range of 11%:11% to 14%:14% by weight/volume. The solution was stirred well until smooth. The pH was adjusted to about 5–6 before pouring into container. Final concentration of the water soluble chitosan/chitosan derivatives was in the range of 1–5%. The mixture was irradiated using gamma rays at 25 kGy to crosslink as well as to sterilize it in its final package.

Table 1
Properties of chitosan derivative sheets and pastes. O-C, oligo-chitosan; NO-CMC, *N*,*O*-carboxymethyl-chitosan; N-CMC, *N*-carboxymethyl-chitosan; PVP, polyvinyl pyrrolidone; PEO, polyethylene oxide; SH, sheet; P, paste.

No.	Sample code	Properties
1	SH120	NO-CMC, 3 phr PVP
2	SH121	NO-CMC, 5 phr PVP
3	SH123	O-C (3 h), PEO, PVP
4	SH124	O-C (6 h), PEO, PVP
5	P133	1% O-C, 11% PVP
6	P134	2% NO-CMC, 11% PVP
7	P136	1% NO-CMC, 11% PVP
8	P137	1% N-CMC, 11% PVP
9	P138	1% O-C, 14% PVP
10	P139	2% NO-CMC, 14% PVP
11	P141	1% NO-CMC, 14% PVP
12	P142	1% N-CMC, 14% PVP

phr, par per hundred = specific weight of material in 100 ml solution; h, hour of degradation process using chitanese enzyme.

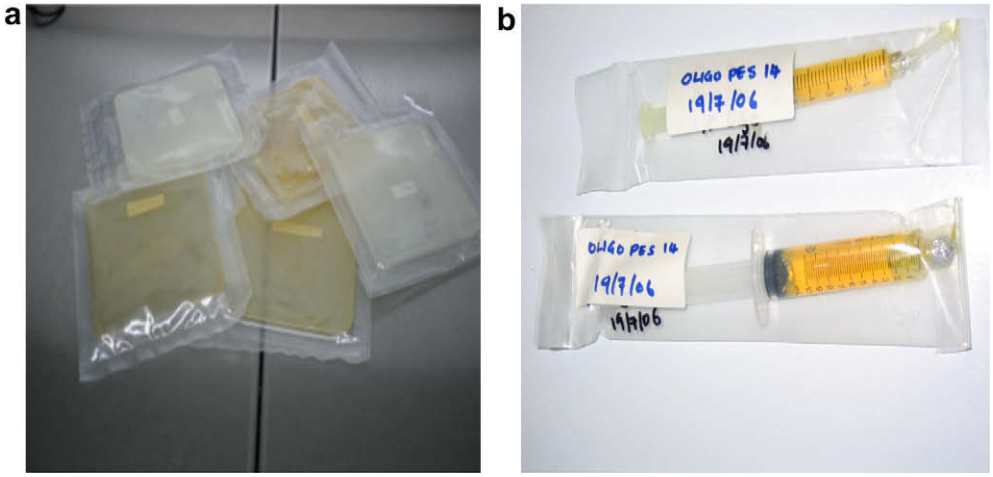

Fig. 1. Samples of chitosan derivatives (a) sheets and (b) pastes.