

# Physical, chemical and in vitro biological profile of chitosan hybrid membrane as a function of organosiloxane concentration <sup>☆</sup>

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## Abstract

We attempted to prepare chitosan–silicate hybrid for use in a medical application and evaluated the physico-chemical properties and osteocompatibility of the hybrids as a function of  $\gamma$ -glycidoxypolytrimethoxysilane (GPTMS) concentration. Chitosan–silicate hybrids were synthesized using GPTMS as the reagent for cross-linking of the chitosan chains. Fourier transform infrared spectroscopy, <sup>29</sup>Si CP-MAS NMR spectroscopy and the ninhydrin assay were used to analyze the structures of the hybrids, and stress–strain curves were recorded to estimate their Young's modulus. The swelling ability, contact angle and cytocompatibility of the hybrids were investigated as a function of the GPTMS concentration. A certain fraction of GPTMS in each hybrid was linked at the epoxy group to the amino group of chitosan, which was associated with the change in the methoxysilane group of GPTMS due to hybridization. The cross-linking density was around 80% regardless of the volume of GPTMS. As the content of GPTMS increased, the water uptake decreased and the hydrophilicity of the hybrids increased except when the content exceeded amolar ratio of 1.5, when it caused a decrease. The values of the mechanical parameters assessed indicated that significant stiffening of the hybrids was obtained by the addition of GPTMS. The adhesion and proliferation of the MG63 osteoblast cells cultured on the chitosan–GPTMS hybrid surface were improved compared to those on the chitosan membrane, regardless of the GPTMS concentration. Moreover, human bone marrow osteoblast cells proliferated on the chitosan–GPTMS hybrid surface and formed a fibrillar extracellular matrix with numerous calcium phosphate globular structures, both in the presence and in the absence of dexamethasone. Therefore, the chitosan–GPTMS hybrids are promising candidates for basic materials that can promote bone regeneration because of their controllable composition (chitosan/GPTMS ratio).

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**Keywords:** Chitosan;  $\gamma$ -Glycidoxypolytrimethoxysilane (GPTMS); Organic–inorganic hybrid; Osteocompatibility; Cell viability

## 1. Introduction

Tissue engineering has been regarded as an ultimate medical treatment for the reconstruction of defective tissues in biomedical engineering fields in recent years. Guided tissue regeneration (GTR) or guided bone regeneration (GBR) is such a treatment that reconstructs new tissue by using a barrier membrane to guard the defected area

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from invasion of other tissues, especially fibrous connective tissues. GTR/GBR is a comparatively simple treatment compared with other tissue construction. Therefore, GTR/GBR treatment and relevant biomaterials have been paid special attention by many researchers recently [1–3]. Because it uses barrier membranes, the GTR/GBR technique has been suggested as a potential method for periodontal therapy [4–8]. In periodontal therapy the barrier membranes prevent apical migration of gingival epithelial cells into the bony defect site and promote the growth of progenitor bone and periodontal ligament cells. Membranes for this purpose include GORE-TEX<sup>®</sup>, collagen membrane; Bio-Gide<sup>®</sup>, Vicryl Periodontal Mesh, polylactic acid sheet; and Guidor, which has demonstrated successful periodontal regeneration [9–10]. General requirements for the barrier membranes with excellent biocompatibility in the GTR/GBR process are suitable mechanical strength, mechanical stability, optimal porosity and biodegradability. A porous structure both at the surface and in the sub-layer of the membranes is essential for cellular adaptation and sufficient nutrient permeation [9]. Non-biodegradable membranes, such as GORE-TEX, required a secondary surgical procedure for retrieval and this remains a significant drawback [11]. In order to avoid the second-stage operation of removing the non-absorbable membrane and to guarantee the continuous healing of tissue, the GTR/GBR membrane should be able to be completely bio-absorbable after it has performed its function. Bioabsorbable membranes, such as synthetic polyesters and collagen, do not require secondary surgery for membrane removal. However, the degradation products of the synthetic polymers reduce the local pH, potentially accelerating the polymer degradation rate and inducing an inflammatory response [12]. On the other hand, collagen is potentially immunogenic and can be expensive [13]. Furthermore, there can be great variations between the batches of collagen produced.

Chitosan, a mucopolysaccharide with structural characteristics similar to glycosamines, is derived from the alkalideacetylation of chitin obtained from the exoskeleton of crustaceans [14,15]. It is biodegradable, biocompatible, non-antigenic and non-toxic [15]. Chitosan and some of its complexes have been studied for a number of biomedical applications, including wound dressings, drug delivery systems and space-filling implants [15–17].

In a previous study [18], a hybrid of chitosan and  $\gamma$ -glycidoxypropyltrimethoxysilane (GPTMS) with a defined composition was prepared; the hybrid presented excellent cytocompatibility with respect to MG63 osteoblastic cells compared to chitosan. That is, the Si—OH and —Si—O—Si— groups derived from the GPTMS favored cell attachment and proliferation, suggesting the importance of silicate ions in the promotion of cell differentiation. Therefore, this hybrid has potential for use as a barrier membrane in dental GTR/GBR. In this study, with the importance of silicate ions in promoting cell differentiation in mind, chitosan–GPTMS hybrids with a wide range of

compositions were prepared to further clarify the effects of GPTMS on the physical, chemical and in vitro biological aspects of the proliferation and differentiation of human osteoblastic cells.

## 2. Materials and methods

### 2.1. Preparation of the hybrids

Chitosan (Aldrich<sup>®</sup>, high molecular weight) was dissolved in 0.25 M acetic acid aqueous solution to attain a concentration of 2% (w/v). Then GPTMS (Chisso, Tokyo, Japan) was added to the chitosan solution. Table 1 indicates the composition of the hybrids, together with sample codes. The resultant chitosan–GPTMS solutions were poured into polypropylene containers and kept at 60 °C for 2 days to yield hybrid membranes. The obtained membranes were cut into disks of  $\phi = 12$  mm in size. All specimens were soaked in 0.25 N sodium hydroxide to neutralize the remaining acetic acid, washed well with distilled water and dried at 60 °C for 2 days.

### 2.2. Structural characterization of the hybrids

Fourier transform infrared (FTIR) spectra were measured with an FTIR spectrometer (Nexus470, Thermo Nicolet Corp., WI, USA) using the KBr method, by which signals from 128 scans were accumulated with a resolution of 4 cm<sup>-1</sup>. Then <sup>29</sup>Si CP-MAS NMR spectra were recorded at 7.05 T and 59.6 MHz, with 10.0  $\mu$ s ( $\pi/2$ ) pulses, 10 s recycle delays and 1500  $\mu$ s contact time on a Varian UNITY INOVA300 FT-NMR spectrometer, equipped with a CP-MAS probe. The samples were placed in a zirconia sample tube. The sample spinning speed at the magic angle to the external field was 5.0 kHz. The signals for about 3400 pulses were accumulated. The chemical shift is conventionally represented by  $\delta$  (ppm). Polydimethylsilane (PDMS;  $\delta = -34$  ppm with respect to tetramethylsilane:  $\delta = 0$  ppm) was used as the secondary external reference. The degree of cross-linking was evaluated by ninhydrin assay, which was defined as the percentage of free amino groups in the hybrids. Pulverized hybrids were suspended in 0.25 M acetic acid aqueous solution for 1 h at room temperature. Ninhydrin solution (ninhydrin reagent L-8500 Set, Wako Pure Chemical Industries, Ltd., Japan) was added to the suspension and the resultant ninhydrin-containing suspension was kept at 80 °C for 20 min. Then the optical absorbance of the supernatant solution was recorded at 570 nm with an ultraviolet–visible spectrophotometer (UV-2550, Shimadzu Corp., Kyoto, Japan), from which absorbance percentage of free amino groups in the

Table 1  
Starting composition of chitosan–silicate hybrids (molar ratio)

	Ch	ChG01	ChG05	ChG10	ChG15	ChG20
Chitosan	1.0	1.0	1.0	1.0	1.0	1.0
GPTMS	0	0.1	0.5	1.0	1.5	2.0

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