

## Research Paper

# Non-cytotoxic hybrid bioscaffolds of chitosan-silica: Sol-gel synthesis, characterization and proposed application



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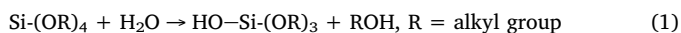
## ABSTRACT

Biohybrid chitosan-silica scaffolds were synthesized through the sol-gel and the freeze drying processes. Hydrolysis and condensation of chitosan with tetraethylorthosilicate (TEOS) in the presence of 3-isocyanatopropyl triethoxysilane (ICPTES) were successfully carried out. Results obtained from FTIR, swelling test and pyrolysis confirmed that the hybrid scaffolds containing covalent coupling between the organic and inorganic networks were formed with high crosslink density of Si–O–Si bridging and could be classified as the class II material. The hybridization also resulted in improvements on mechanical strength and stability comparing to the pure chitosan. *In vitro* investigations on the guided bone regeneration and the cytotoxicity were also performed. SEM-EDS was used to examine the proliferation of calcium phosphate mineral at the scaffold surface after an immersion in simulated body fluid. The results revealed that the hybrid scaffolds exhibited a rapid induction of calcium phosphate mineral without cytotoxicity effect, reflecting an excellent *in vitro* bone bioactivity which was superior to the pure chitosan scaffold.

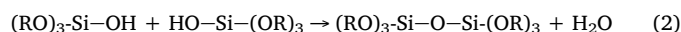
## 1. Introduction

Recently, the organic-inorganic hybrid polymeric composites have been of great interest and widely explored due to their ability to combine the advantages of polymeric matrix with those of inorganic components and their superior properties compared with the pure counterparts. Various techniques for the preparation of hybrid materials based on organic polymeric materials for broad application prospects have been developed and reported (Pandey & Mishra, 2011). Dai, Zhang, and Zhao (2017) fabricated a crosslinked hybrid polymer sensor for the application of humidity sensing by the thiol-ene click reaction. Bandaru, Ahmad and Bhatnagar (2017) prepared hybrid thermoplastic composite armors using the vacuum assisted compression molding technique. In the case that both organic and inorganic components are physically bonded, the obtained material is classified as the class I hybrid. Another alternative route for the preparation of organic-inorganic polymeric composites is the sol-gel process. Two reactions used to describe the sol-gel process are hydrolysis (Eq. (1)) and condensation (Eqs. (2) and (3)).

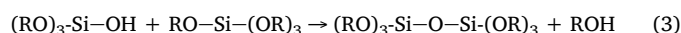
## Hydrolysis:



## Polycondensation:



and/or



In general, the starting materials are polymeric molecules and metal-organic alkoxides, especially tetramethoxysilane (TMOS) and tetraethoxysilane (TEOS). The final product of a three-dimensional network can be fabricated containing siloxane linkage. In this case, covalent coupling is obtained between the organic and inorganic networks and the fabricated material is classified as the class II hybrid (Owens et al., 2016).

Advantages of the sol-gel hybridization are its mild condition, simplicity and possibility of organic-inorganic ratio and nature of starting material variation. Several studies reported that the structure and property of hybrid materials prepared by the sol-gel process could be altered and controlled by different parameters, such as reaction pH, catalyst, type and content of alkoxide precursor, temperature and reaction time (Rashti et al., 2016; Seraji, Sameri, Davarpanah, & Bahramian, 2017; Wu, Gaharwar, Schexnailder, & Schmidt, 2010). Different synthetic techniques have been used in the sol-gel process to generate polymer-silica hybrid materials with a variety of applications. Budnyak et al. (2016) described an *in situ* formation of siloxane inorganic network through hydrolysis of TEOS in the

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presence of chitosan solution. The obtained chitosan-silica composites were tested for the application of heavy metal adsorption. Ryan and Delezuk (2016) developed silica-based photonic crystals embedded in chitosan-TEOS interpenetrating polymer networks. They reported that the TEOS crosslinking enhanced mechanical property of the material and the obtained composite was suitable for the application in terms of strength and stability.

Natural polysaccharide chitosan is a deacetylated derivative of chitin. Chitosan possesses an excellent forming ability and can be readily fabricated into different forms such as spherical bead, film and porous scaffold. The high content of reactive functional groups along chitosan molecule provides possibilities of structure modification and network formation which can be easily accomplished. Chitosan has been intensively investigated and developed for several applications because of its antifungal activity, hydrophilicity, good biocompatibility, biodegradability and non-toxicity (Ma, Yang, Yan, Kennedy, & Meng, 2013). *In vitro* and *in vivo*, chitosan can be degraded by enzymatic mechanism and its biodegradation product, glucosamine, can be metabolized by the action of human enzymes (Cunha-Reis et al., 2007; Kean & Thanou, 2010). Several works have reported the potential applications of chitosan including drug delivery systems, wound dressing materials and bone tissue regeneration (LogithKumar et al., 2016; Ma et al., 2010; Serra et al., 2015; Tang et al., 2010). To the best of our knowledge, there has been no report on the sol-gel synthesis of chitosan-silica hybrid scaffold using a coupling reaction of an isocyanate silane compound for the biomedical application.

In this study, chitosan was hybridized with a silica precursor, TEOS, in the presence of a silane coupling agent, 3-isocyanatopropyl triethoxysilane (ICPTES). Porous hybrid bio-composite scaffolds containing different organic-inorganic composition ratios were then fabricated by the sol-gel process and the freeze drying method. The hybrid scaffolds were extensively studied in terms of thermal and physico-chemical properties and water absorption capacity. The influences of inorganic incorporation on the morphology, guided bone-regeneration in simulated body fluid and cell viability were also assessed and discussed in detail.

## 2. Experimental

### 2.1. Materials

A medium molecular weight chitosan (CH), No.448877: 75–85% deacetylated degree, was purchased from Sigma-Aldrich. 3-isocyanatopropyl triethoxysilane (ICPTES) was obtained from TCI Chemical. Tetraethylorthosilicate (TEOS) and acetic acid were supplied from Merck. Distillation water was used in all preparation procedures.

### 2.2. Synthesis of chitosan-silica hybrid scaffold

The component ratios for scaffold preparation in this work are listed in Table 1. A solution of chitosan was prepared by dissolving chitosan in 2% v/v acetic acid using a magnetic stirrer. ICPTES was then added into the obtained solution. After 3 h stirring, a desired amount of TEOS was

**Table 1**  
Notation and composition of different chitosan-silica biohybrid scaffolds.

Sample code	Organic:Inorganic component weight ratio	%Weight ratio of chitosan	
		ICPTES	TEOS
CH	100:0	–	–
CH-10Te	100:10	–	10
CH-Cp	100:10	10	–
CH-Cp-5Te	100:15	10	5
CH-Cp-10Te	100:20	10	10
CH-Cp-25Te	100:35	10	25

incorporated and the solution was left under stirring at 35 °C for 21 h. The mixture was poured into molds, frozen at –18 °C for 24 h, and then freeze-dried at –100 °C for 6 h. The obtained scaffolds were removed from the molds and kept in a desiccator at room temperature.

### 2.3. Characterization of the hybrid scaffolds

#### 2.3.1. ATR-FTIR analysis

Functionality of the prepared scaffolds was analyzed using an attenuated total reflectance Fourier transform (ATR-FTIR) spectrometer (PerkinElmer-Frontier) by accumulation of 250 scans at a wave number range of 4000–400 cm<sup>-1</sup> and a resolution of 4 cm<sup>-1</sup>.

#### 2.3.2. Porosity

Porosity of the scaffolds was examined by a gravimetric method using heptane as an inert solvent to minimize the effect of network swelling and keep the scaffolds in their initial morphology. The porous samples were dried, weighed and fully immersed in heptane. The experiments were performed three times, and the pore volume occupied by the solvent was calculated using mass and density (0.6795 g/cm<sup>3</sup>) of heptane as

$$V_{pore} = \frac{M_{wet} - M_{dry}}{\rho_{heptane}}$$

where  $M_{wet}$  represents the wet weight (g) of sample after immersion in heptane and  $M_{dry}$  is the weight (g) of dry scaffold.

The porosity (%) was calculated as a function of the pore volume ( $V_{pore}$ ) and the total volume of the scaffold ( $V_{pore} + V_{CHI}$ ), where the volume of chitosan was  $V_{CHI}$  and density of the polymeric chitosan was 1.25 g/cm<sup>3</sup>.

$$Porosity(\%) = \frac{V_{pore}}{V_{pore} + V_{CHI}}$$

#### 2.3.3. Water absorption capacity

To measure the water absorption behavior of the porous scaffold, dry samples were immersed in distilled water at room temperature for 6 h. At each time interval, the wet weight of sample was determined and the absorption capacity was calculated by:

$$Waterabsorption = \left( \frac{W_{wet} - W_{dry}}{W_{dry}} \right)$$

where  $W_{dry}$  and  $W_{wet}$  are the weights of scaffold before and after immersion in water, respectively.

#### 2.3.4. Pyrolysis of the hybrid scaffolds

The hybrid scaffolds were oven dried at 40 °C and weighed prior to this study ( $W_{dry}$ ). The samples were then tested by pyrolysis at 850 °C for 2 h using a muffle furnace under air atmosphere and the residue weight was recorded ( $W_{pyrolysis}$ ). All experiments were repeated at least three times. Ash content (%) of the scaffolds was obtained according to the following equation:

$$Ash(\%) = \frac{W_{pyrolysis}}{W_{dry}} \times 100$$

#### 2.3.5. Morphology of the hybrid scaffolds

The surface morphology and microstructure of the hybrid scaffolds were examined using a scanning electron microscope (Model LEO 1450VP). The samples were mounted on stubs and sputter-coated with gold under high vacuum condition before an SEM investigation.

#### 2.3.6. Mechanical property of the hybrid scaffolds

The mechanical strength of the hybrid scaffolds was investigated by compression test at ambient temperature. The samples were produced

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