



## Structure–process–property relationship of the polar graphene oxide-mediated cellular response and stimulated growth of osteoblasts on hybrid chitosan network structure nanocomposite scaffolds

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### ABSTRACT

We here describe the structure–process–property relationship of graphene oxide-mediated proliferation and growth of osteoblasts in conjunction with the physico-chemical, mechanical, and structural properties. Chitosan–graphene network structure scaffolds were synthesized by covalent linkage of the carboxyl groups of graphene oxide with the amine groups of chitosan. The negatively charged graphene oxide in chitosan scaffolds was an important physico-chemical factor influencing cell–scaffold interactions. Furthermore, it was advantageous in enhancing the biocompatibility of the scaffolds and the degradation products of the scaffolds. The high water retention ability, hydrophilic nature, and high degree of interconnectivity of the porous structure of chitosan–graphene oxide scaffolds facilitated cell attachment and proliferation and improved the stability against enzymatic degradation. The cells infiltrated and colonized the pores of the scaffolds and established cell–cell interactions. The interconnectivity of the porous structure of the scaffolds helps the flow of medium throughout the scaffold for even cell adhesion. Moreover, the seeded cells were able to infiltrate inside the pores of chitosan–graphene oxide scaffolds, suggesting that the incorporation of polar graphene oxide in scaffolds is promising for bone tissue engineering.

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### 1. Introduction

Chitosan (CS) is the partially deacetylated form (poly- $\beta$ (1,4)-2-amino-2-deoxy-D-glucose) of chitin and is a potential biomaterial for tissue engineering from the viewpoint of repair of osseous and chondral defects [1]. There is currently a need to enhance the mechanical properties and promote the biological response of CS for bone tissue engineering applications [2,3]. Chemical approaches involving the synthesis of CS derivatives with diverse chemical and molecular structures have been attempted in recent years to enhance the physico-chemical properties and cellular response [4–7]. PEGylation was observed to enhance the water solubility of CS, while incorporation of hyaluronic acid improved the biological response of pure CS [8,9].

The ability of CS to support cell attachment and proliferation is related to the structural and chemical properties of the polysaccharide backbone of CS, which is structurally similar to glycosaminoglycans, the major component of the extracellular matrix of bone and cartilage. Other advantages of CS from the view point of tissue

engineering include the highly porous and interconnected pore structure, osteoconductivity, and ability to enhance bone formation [10,11]. The pore interconnectivity enhances the diffusion of nutrients, while providing room for neovascularization.

While CS is biocompatible and is a potential candidate to repair osseous and chondral defects, the mechanical properties and biological response of CS scaffolds are inadequate to qualify them for bone tissue engineering [12] or facilitate transfer of the applied load at the implant site and to participate in matrix mineralization. Furthermore, tissue engineering scaffolds should possess an interconnected porosity (preferably >90%) to promote cell adhesion, in-growth and reorganization, in vitro. A number of studies have been directed at enhancing the mechanical strength of CS by reinforcement, with such materials as hydroxyapatite (HA) [13].

Carbon nanotubes (CNT), nano-clay, and hydroxyapatite are being increasingly considered as reinforcement materials to enhance the modulus and mechanical strength of polymers. CNT have attracted significant interest in tissue engineering, in diagnostics, for drug delivery, and for their anti-bacterial activity [14–17]. However, in spite of the promise of CNT-based scaffolds for stimulated cell growth [18], the application of CNT as a biomaterial is not considered viable because of the toxic nature of the impurities

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present, whose removal is expensive [19]. Furthermore, they tend to aggregate because of their hydrophobicity and poor dispersibility in biological media, leading to heterogeneous interaction with the cell components [20]. Thus it is preferred to reinforce biopolymers with metal-free nanofillers that enhance the mechanical properties and favorably modulate the biological properties of polymer scaffolds.

Graphene, a single layer of  $sp^2$  bonded carbon atoms in a two-dimensional hexagonal lattice, has attracted considerable attention as a potential biomaterial because of its physico-chemical properties, including a large surface area, high dispersibility and hydrophilicity [21,22]. If we consider graphene oxide (GO), functional side groups (hydroxyl and carboxyl) bound to the surface of GO are expected to promote interfacial interactions between GO and a polymer matrix in a manner similar to functionalized CNT [23]. The interfacial interaction is particularly important for strong interfacial strength, enabling efficient transfer of stress from the polymer matrix to GO. Interfacial interaction can be successfully achieved by establishing a chemical bond between GO and a polymer [24,25].

In this regard we have investigated the biological response of chitosan–GO scaffolds, where the carboxyl groups of GO chemically react with the amine group of chitosan (CS) with the consequent formation of a chemical bond between GO and the biopolymer (chitosan), as shown in Scheme 1.

Here we describe the physico-chemical properties and biological response of osteoblasts cells seeded on CS–GO scaffolds, where

GO is covalently linked to the biodegradable CS and not just randomly dispersed in the matrix.

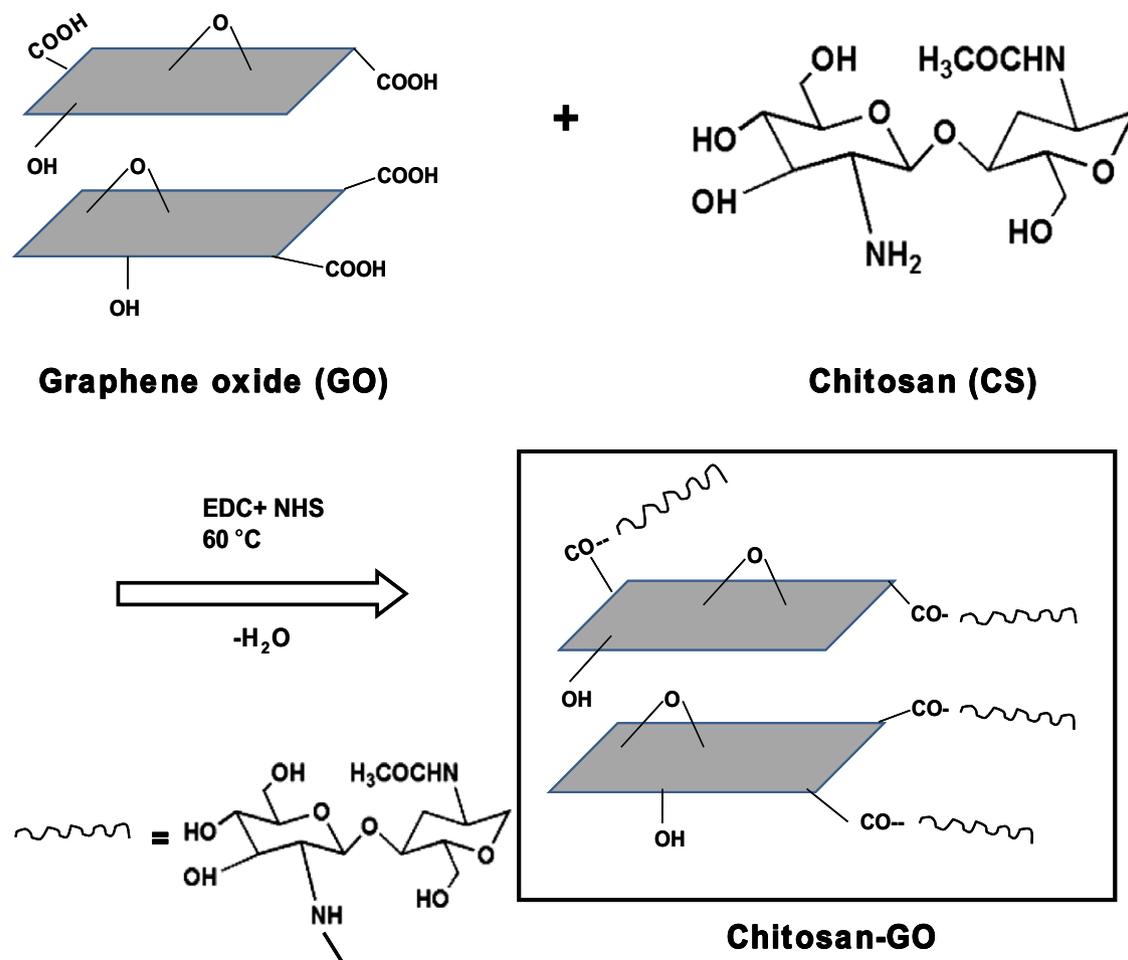
## 2. Experimental

### 2.1. Materials

Low molecular weight CS (50 kDa, 80% degree of deacetylation, as provided by the supplier) was obtained from Aldrich (St. Louis, USA). The reagents 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), N-hydroxyl succinimide (NHS), sodium hydroxide, and acetic acid were also obtained from Aldrich. Alpha minimum essential medium ( $\alpha$ -MEM) and fetal bovine serum (FBS) were purchased from Gibco®, Invitrogen Corp. Penicillin (10,000 IU) and streptomycin ( $10,000 \mu\text{g ml}^{-1}$ ), trypsin–EDTA (0.25% trypsin, 0.53 mM EDTA) in Hank's buffered salt solution, and phosphate-buffered saline (PBS) without calcium and magnesium were obtained from American Type Cell Culture Collection (Manassas, VA).

### 2.2. Preparation of a chitosan–graphene oxide network structure scaffold

The scaffolds were synthesized by covalent linking of the carboxyl ( $-\text{COOH}$ ) groups of GO with the amino ( $-\text{NH}_2$ ) groups of CS. First, the carboxyl groups of GO were activated using EDC



**Scheme 1.** Schematic illustration of the reaction for the synthesis of chitosan (CS)–graphene oxide (GO) scaffolds in the presence of EDC and NHS. The reaction scheme shows covalent linking of carboxyl groups of GO with amine groups of CS to form a CS–GO network structure scaffold.

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