Colloids and Surfaces B: Biointerfaces 138 (2016) 41–49

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

# Boron nitride nanotubes included thermally cross-linked gelatin–glucose scaffolds show improved properties

Özlem Şen, Mustafa Culha*

*Department of Genetics and Bioengineering, Yeditepe University, Atasehir, Istanbul 34755, Turkey*

ARTICLE INFO

*Article history:*
Received 11 August 2015
Received in revised form
13 November 2015
Accepted 19 November 2015
Available online 23 November 2015

*Keywords:*
Boron nitride nanotubes
BNNT-OH
Scaffold
Gelatin
Mechanical strength
Cell proliferation

ABSTRACT

Boron nitride nanotubes (BNNTs) are increasingly investigated for their medical and biomedical applications due to their unique properties such as resistance to oxidation, thermal and electrical insulation, and biocompatibility. BNNTs can be used to enhance mechanical strength of biomedical structures such as scaffolds in tissue engineering applications. In this study, we report the use of BNNTs and hydroxylated BNNTs (BNNT-OH) to improve the properties of gelatin–glucose scaffolds prepared with electrospinning technique. Human dermal fibroblast (HDF) cells are used for the toxicity assessment and cell seeding studies. It is found that the addition of BNNTs into the scaffold does not influence cell viability, decreases the scaffold degradation rate, and improves cell attachment and proliferation compared to only-gelatin scaffold.



## 1. Introduction

Nanomaterials are increasingly applied in tissue engineering to strengthen the 3-D scaffold structure, and control the degradation and swelling ratio. In addition, the presence of a nanomaterial may influence scaffold chemical structure, and increase the cell adhesion, spread and differentiation [1]. Therefore, several nanomaterials including gold nanoparticles (AuNPs) [2], silver nanoparticles (AgNPs) [3], titanium dioxide ($TiO_2$) nanoparticles [4,5], carbon nanotubes (CNTs) [6,7], and boron nitride nanotubes (BNNTs) [8] were used to enhance the scaffold properties.

Chou et al. showed that the addition of AuNPs or AgNPs into poly(ether) urethane scaffolds improved the biocompatibility and biostability [9]. In addition, AuNPs were used to create a biodegradable, biocompatible and conductive scaffold for skeletal muscle repair [2]. In that study, despite low cell proliferation, the composite scaffold showed high elastic modulus and yield stress, and no toxic effect. In another study, the AuNPs were added into the collagen scaffolds to improve mechanical properties and resistance to degradation [10]. The results of the study demonstrated that the degradation rate was slower and the cell proliferation was improved in the long term compared to the collagen scaffolds without AuNPs. In a different study, gelatin-based scaffolds containing AgNPs showed antibacterial effect and good cytocompatibility on adult human mesenchymal stem cells [3]. In titania-based studies, $TiO_2$ nanoparticles were used to improve cell proliferation and attachment on the composite surfaces and it was found that $TiO_2$ helps to reduce the swelling ratio [4,5].

Carbon nanotubes (CNTs) are studied to strengthen 3-D scaffold structure, provide mechanical and electrical support. Polyaniline–carbon nanotube/poly(*N*-isopropyl acrylamide-*co*-methacrylic acid) composite nanofibres were fabricated as a conducting scaffold using electrospinning. The high cell viability and growth were observed when the CNTs were added into the scaffold composite [6,7]. The BNNTs were used for the first time to reinforce glass composites [8]. The results showed that the BNNTs enhanced the flexure strength and fracture toughness of the glass. Lahiri et al. used the BNNTs to reinforce a composite and found that the mechanical strength was improved. In addition, a good biocompatibility on osteoblast and macrophages was observed [11,12]. The BNNTs and hydroxylated BNNTs were used to fabricate polycarbonate (PC) and polyvinyl butyral (PVB) composites. It was found that the addition of the hydroxylated BNNTs improved mechanical performance of the composite [13]. Furthermore, 2-D boron nitride nanosheets (BNNSs) were used to produce polymethyl methacrylate (PMMA)/BNNSs composites and

* Corresponding author at: Department of Genetics and Bioengineering, Faculty of Engineering, Yeditepe University, Ataşehir, Istanbul 34755, Turkey. Fax: +90 216 578 0829.
*E-mail addresses:* mculha@yeditepe.edu.tr, mculha2@gmail.com (M. Culha).

http://dx.doi.org/10.1016/j.colsurfb.2015.11.036
0927-7765/

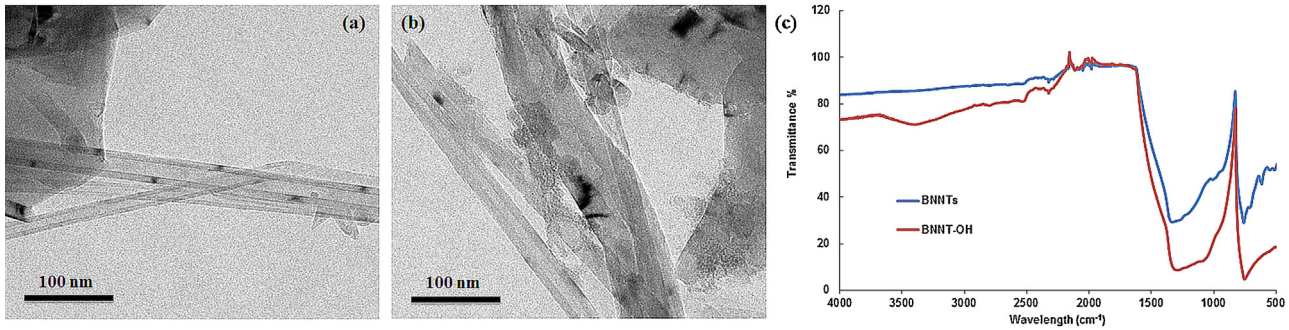


**Fig. 1.** TEM images of BNNTs (a) and BNNT-OH (b). (c) FT-IR spectra of BNNTs and BNNT-OH.

the composites showed enhanced elastic modulus and strength [14]. Although the BNNTs are structural analogues of the CNTs, their physical and chemical properties are rather different from the CNTs. For instance, the BNNTs have higher resistance to oxidation due to a wide band gap of approximately 5.5 eV. The BNNTs are more stable up to about 1000 °C compared to the CNTs. They have also many other advantageous properties such as higher mechanical strength, chemical stability and electrical insulating ability [15]. Zhi et al. also reviewed theoretical investigation, fabrication, structure, physical properties, modification and applications of BNNTs [16].

Biodegradable scaffolds are designed to offer a flexible structural template for cell attachment and tissue organization by mimicking 3-D environment of native tissue [17]. Gelatin is a natural polymer and derived by hydrolysis from collagen. It shows non-toxicity, non-immunogenicity, good biocompatibility and biodegradability. It has also some disadvantages such as heterogeneous composition and inconsistent physicochemical properties. Although gelatin shows good water solubility, a scaffold should be insoluble in water during the cell culture to provide mechanical support to the cells until tissue formation is completed. Thus, a cross-linking procedure is necessary to decrease the water solubility and enhance its mechanical properties. Adding reducing sugar to the gelatin scaffolds enhance fibre properties due to Maillard reaction, which occurs between a non-protonized amine group and the electrophilic carbonyl group of a reducing sugar [18–20].

In this study, the influence of inclusion of BNNTs and BNNT-OH into the gelatin fibre-based scaffolds was investigated. The BNNTs including gelatin fibres prepared with electro-spinning were thermally cross-linked with glucose. The water-resisting properties of the scaffolds were evaluated with contact angle measurements. The biological stability was tested using collagenase and trypsin enzymes. HDF cells were used for the biocompatibility and cell proliferation studies. The cell attachment and proliferation on the scaffolds were monitored for 1, 3 and 7 days using SEM and fluorescence microscopy.

## 2. Materials and methods

### 2.1. BNNTs synthesis

In BNNT synthesis, a boron compound as boron source and nitrogen compound as nitrogen source mixed with a catalyst, mostly a metal oxide, heated to high temperatures. The experimental conditions and precursor compounds are critical for the quality of the BNNTs. The toxicity of catalyst and precursor compounds can be an important factor for medical or biomedical applications of BNNTs. Low-toxic metal oxides such as FeO and MgO were used to obtain highly pure BNNTs in wide temperature range of 1100–1700 °C [21]. In our study, the BNNTs were synthesized by using a chemical vapor deposition (CVD) method as described previously [22]. Briefly, a 2 g colemanite and 160 mg $Fe_2O_3$ was dispersed in 2 mL dd$H_2O$ at 100 °C. Then, this colemanite-$Fe_2O_3$ mixture was placed onto an alumina boat and heated until water evaporated to dry. Next, the mixture was placed into a tubular furnace (Protherm, Furnaces PTF 14/50/450) and the BNNTs synthesis were performed under $NH_3$ atmosphere for 3 h at 1280 °C. The BNNTs that were formed at the top of alumina boat was collected for purification process. The BNNTs were stirred in a 4 M HCl solution at 90 °C for 4 h. After centrifugation (14,000 rpm, 30 min), the precipitate was added to a 1 M $HNO_3$ solution at 30 °C and stirred for 6 h. Then they were centrifuged at 14,000 rpm for 30 min. In order to remove all acid residues, the precipitate was washed with dd$H_2O$ and the pure BNNTs were obtained.

### 2.2. Modification of BNNTs with hydroxyl groups

The hydroxylation of BNNTs was performed using a method previously reported by us [23]. Briefly, a 100 mg of pure BNNTs was added into 10 mL 30% $H_2O_2$ solution and sonicated for 1 h at 25 °C. The mixture was refluxed by stirring for 48 h at 110 °C. Then, the BNNT-OH was centrifuged for 15 min at 10,000 rpm and washed with dd$H_2O$ water for five times and dried at 60 °C. The BNNTs and BNNT-OH were characterized with TEM and FT-IR spectroscopy.

### 2.3. Preparation of fibrous scaffolds

Gelatin from porcine skin was dissolved in a 10 M acetic acid solution at about 40 °C by stirring to obtain solutions containing 25% gelatin [19]. In order to prepare the gelatin/BNNTs and gelatin/BNNT-OH scaffolds, 0.5% (w/v) from each BNNTs and BNNT-OH were added to the gelatin solutions at about 40 °C while stirring. For the gelatin–glucose scaffolds, D(+)-glucose monohydrate was added into stirring 25% gelatin solution. When prepared, the D(+)-glucose monohydrate concentration was 10% in the final scaffold mixture. To prepare the gelatin–glucose/BNNTs and gelatin–glucose/BNNT-OH scaffolds, the BNNTs and BNNT-OH were added into the stirring gelatin–glucose solution at 40 °C to make the final concentration of the BNNTs 0.5% (w/v). These solutions were filled into a syringe and a voltage of 18 kV was applied at a speed of 1 mL/h to obtain scaffolds by electrospinning. The scaffolds were cross-linked thermally in an oven for 3 h at 175 °C and the SEM images were obtained with a Carl Zeiss Evo-40 instrument under high vacuum before and after the cross-linking.

### 2.4. Contact angle measurements

For contact angle measurements, a droplet of water was placed onto the scaffolds using a Hamilton syringe. A camera that was at the opposite side of the droplets recorded the droplets and the contact angles were measured from the droplet images.