Applied Surface Science 347 (2015) 307–314

Contents lists available at ScienceDirect

Applied Surface Science

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

# A dense and strong bonding collagen film for carbon/carbon composites

Sheng Cao, Hejun Li*, Kezhi Li, Jinhua Lu, Leilei Zhang

*State Key Laboratory of Solidification Processing, Northwestern Polytechnical University, Xi'an, Shaanxi, China*

ARTICLE INFO

*Article history:*
Received 17 November 2014
Received in revised form 11 April 2015
Accepted 13 April 2015
Available online 19 April 2015

*Keywords:*
Osteoblast
Carbon/carbon composites
Collagen
Surface modification

ABSTRACT

A strong bonding collagen film was successfully prepared on carbon/carbon (C/C) composites. The surface conditions of the modified C/C composites were detected by contact angle measurements, scanning electron microscope (SEM), X-ray photoelectron spectroscopy (XPS) and Raman spectra. The roughness, optical morphology, bonding strength and biocompatibility of collagen films at different pH values were detected by confocal laser scanning microscope (CLSM), universal test machine and cytology tests in vitro. After a 4-h modification in 30% $H_2O_2$ solution at 100 °C, the contact angle on the surface of C/C composites was decreased from 92.3° to 65.3°. Large quantities of hydroxyl, carboxyl and carbonyl functional groups were formed on the surface of the modified C/C composites. Then a dense and continuous collagen film was prepared on the modified C/C substrate. Bonding strength between collagen film and C/C substrate was reached to 8 MPa level when the pH value of this collagen film was 2.5 after the preparing process. With 2-day dehydrathermal treatment (DHT) crosslinking at 105 °C, the bonding strength was increased to 12 MPa level. At last, the results of in vitro cytological test showed that this collagen film made a great improvement on the biocompatibility on C/C composites.

© 2015 Elsevier B.V. All rights reserved.

## 1. Introduction

Carbon/carbon (C/C) composites are high-temperature structural materials for aircraft and aerospace applications. They are also considered to be potential orthopaedics materials due to their favorable mechanical properties and good biocompatibility with blood and tissues [1,2]. On the basis of these advantages, C/C composites have been studied as artificial bone replacement candidates for many years. However, this kind of material may release carbon debris to surrounding tissues during and/or after a bone replacement surgery [3]. To eliminate this problem, several kinds of films and coatings have been applied on the surface of C/C composites, such as pyrolytic carbon coatings, titanium films and calcium phosphate coatings [4–9]. Though the release of carbon debris from C/C composites would be minimized by these coatings, the bioinert surface of C/C composites is still a big issue. The lack of biological activity or weak bonding strength of coatings still limited the further biomedical application of C/C composites [10–12]. Therefore, it is a need to address these problems by surface modification of C/C composites.

In most studies of surface modification, oxidation is a main strategy for grafting active functional groups on carbon materials. For this reason several kinds of oxidation methods were applied on C/C composites to endow their surface activities. Li et al. reported an acidic mixture oxidization method with nitric acid ($HNO_3$) and sulfuric acid ($H_2SO_4$), whose volume ratio was 1:3 [13]. This method is an effective way to graft oxygen-containing functional groups on C/C composites, but it leads to the matrix damages, which would decrease the mechanical properties of C/C composites. Xiong et al. used hydrogen peroxide ($H_2O_2$) and ammonium persulfate ($(NH_4)_2S_2O_8$) to modify C/C composites. These two methods successfully grafted carboxyl functional groups onto the surface of C/C composites [11,12]. In their studies, carboxyl functional groups played role of bridges in enhancing the bonding strength between C/C composites and hydroxyapatite (HA) coating by chemical bonding. These studies concluded that oxygen-containing functional groups formed on C/C composites could increase their surface hydrophilia and enhance their surface activity.

Type I collagen is the most abundant and common protein, which is the major constituent of skins, tendons and bones [14,15]. Type I collagen molecule, which has a triple-helix structure, has the length of about 300 nm and the diameter of about 1.5 nm. This molecule is soluble in an aqueous solution, but its solubility would be variable when the pH value, temperature, or ionic concentration of the aqueous solution change [16]. By controlling these factors, a self-assembly process would be processed between collagen molecules, and the molecular aggregates would be formed. The sizes of these aggregates are several times or even hundred

* Corresponding author. Tel.: +86 029 8849 5004; fax: +86 029 8849 5764.
*E-mail address:* lihejun@nwpu.edu.cn (H. Li).

http://dx.doi.org/10.1016/j.apsusc.2015.04.081
0169-4332/© 2015 Elsevier B.V. All rights reserved.

times larger than that of single collagen molecule. Gobeaux systematically studied the physicochemistry and formation of type I collagen molecules, when they were self-assembled into fibrils in vitro. In a high concentration range, which was close to that of living tissues (40–300 mg $mL^{-1}$), collagen solutions yielded strong gels over wide pH and ionic strength ranges [17]. In addition, collagen membranes in different molecule aggregation structures would also be obtained by controlling these self-assembly factors. Gabriele et al. cultured retinal pigment epithelial (RPE) cells on ultrathin collagen membranes [18]. His research indicated that the collagen membranes were non-toxic and did not elicit any rejection or inflammatory response when it was implanted into subconjunctivally or subretinally in rabbits. Ma reported collagen/chitosan porous scaffolds with improved biostability for skin tissue engineering [19]. Tillman et al. had researched the stability of electrospun polycaprolactone–collagen scaffolds for vascular reconstruction in vivo [20]. For bioactive film on artificial bone, collagen molecules were mixed with HA powder and deposited on the surface of titanium by electrochemical deposition [21]. The Collagen/HA film shown great bioactive and this method was also could be used on C/C composites [22]. However, surface heterogeneity of C/C composites would limit their using. We consider that if the collagen skin could be used as a bioactive film on C/C composite, the continuous and homogeneous features could address this issue. The continuous collagen film could be a buffer between C/C composites and bone to reduce the corrosion of C/C composites and carbon wear debris accumulation.

In actual applications, a collagen membrane cannot be directly used as biomaterials, because of their water-swellable and readily biodegradable properties in vivo [23]. For this reason, a crosslinking modification must be applied on collagen membranes to improve their mechanical properties, reduce their water-swellable property and enhance its resistance ability of enzymatic degradation [24,25]. Formaldehyde, carbodiimide (EDC), glutaraldehyde (GA) and other chemical reagents could crosslink the residues of amino acid side chains and enhance the practicability of collagen membrane, but most of the crosslinking agents still have some residual cytotoxicity [26]. Dehydrathermal treatment (DHT) is one of the most common physical crosslinking methods and always is used to improve the stability of collagen films without introducing cytotoxicity. This method could reduce the molecule distance and lead to direct crosslink between collagen molecules [23,27]. After this crosslink the deformation temperature and mechanical strength could be improved and free amino acid content of collagen film could be reduced. Scotchford used collagen/polyvinyl alcohol (COL/PVA) as substrates for the culture of osteoblasts to observe the attachment, adhesion and proliferative responses of these cells [28]. The studies mentioned above show the DHT is an effective physical modification to enhance the biostability of collagen films with good biocompatibility.

In this study, type I collagen film was applied onto $H_2O_2$ modified C/C composites. Collagen films were prepared in different pH values and crosslinked using different DHT parameters. The effects of $H_2O_2$ surface modification on C/C composites were investigated. Bonding strengths and biological activities of the collagen films were also tested to find out a suitable coating preparation progress and parameters for C/C composites.

## 2. Materials and methods

### 2.1. C/C composites and collagen

C/C composites were prepared by chemical vapor infiltration (CVI) process in our laboratory. Carbon perform used was carbon fiber needled felts and the carbon source used for infiltration was natural gas [29]. Samples were cut into $10 \times 10 \times 3\ mm^3$ and their densities were between 1.70 and 1.75 g $cm^{-3}$. Prior to surface modification and film preparation, each sample was polished to $10 \times 10 \times 2\ mm^3$ with SiC sandpapers in the number sequence of 400, 800, 1200 and 1500. After that, they were rinsed with distilled water, cleaned ultrasonically in acetone and dried in a vacuum environment [22].

Non-soluble and bovine achilles tendon type I collagen (LS001654, Worthington Biochemical Corporation, U.S.A.) was purchased from LSBIO company (China). One gram of non-soluble collagen was mixed with 0.1 g pepsin in 0.5 M acetic acid liquid (200 mL), and then stirred for 24 h to get a soluble collagen solution at 25 °C. At last, the collagen solution was filtrated by a stainless steel sieve cloth with 2 μm pore size and a 0.2 μm syringe filter (PALL Life Science, U.S.A.), respectively.

### 2.2. Surface modifications of C/C composites

Each C/C sample was immersed in 50 mL 30% $H_2O_2$ solution, which had been maintained at 100 °C in a thermostat water bath. The oxidation times were 1, 2, 4 and 8 h, respectively. These specimens were washed by deionized water for at least three times and dried in a vacuum environment, after they were taken out from $H_2O_2$ solution.

### 2.3. Collagen film preparations

For film preparation, the 4-h modified C/C specimens were used as the substrates and marked as H—C/C substrates.

The pH value of the soluble collagen solution was adjusted by adding a few drops of 30% ammonia water ($NH_3 \cdot H_2O$) to increase the value from 2.5 to 7.0. Then each of these collagen solutions was pumped for half an hour after the H—C/C substrates were immersed. These H—C/C substrates covered with a small amount of collagen solution were placed in a 6-well cell culture plate and the remaining collagen solutions were pumped again and centrifugalized to remove air. After that, the pumped collagen solutions were poured into the wells to cover the H—C/C substrates and had 8 mm height above their surfaces. At last, the cell culture plates with samples and collagen solutions were dried by air in a clean bench for three days.

The same collagen films were also prepared on slides as a control team for roughness and optical morphology detections.

### 2.4. DHT corsslinking and neutral adjustment

DHT crosslinking was carried out in a vacuum environment [23]. The treatment was set at 105 °C for 1 day. To research how the DHT temperature and duration affect the bonding strength and bioactive of collagen films, three groups were added in. The treatments used different temperature and duration: at 105 °C for 2 days, at 120 °C for 1 day and at 120 °C for 2 days, respectively.

The crosslinked samples were immersed in 1 M phosphate buffer solution (PBS) for 3 times, and then were washed with deionized water. Finally, samples were immersed in 75%, 95% and absolute ethyl alcohol respectively for gradient dehydration.

### 2.5. Surface characterizations

The wettability of $H_2O_2$ modified and unmodified C/C specimens was investigated by an optical contact angle meter (DataPhysics OCA20, Germany), and the contact medium is deionized water. The surface morphology of H—C/C substrate was observed by a TESCAN VEGA3 (CZECH) scanning electron microscope (SEM). The chemical states of the atoms in the surface of untreated and treated C/C specimens were investigated by a Thermo Scientific K-Alpha

Download English Version:

https://daneshyari.com/en/article/5358255

Download Persian Version:

https://daneshyari.com/article/5358255

Daneshyari.com