

# Effects of hydrolysis on dodecyl alcohol-modified bioactive glasses and PDLA/modified bioactive glass composite films

YanLing Zhou · Yuan Gao · Jiang Chang

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**Abstract** In this article, mesoporous 58S and 58S bioactive glasses (BGs) were surface modified by dodecyl alcohol through esterification reaction and PDLA/modified BGs composite films were prepared. The purpose of this study was to investigate the properties of the modified BGs particles and the PDLA/modified BGs composite films before and after hydrolytic treatment. The modified BGs powders and composite films were treated in boiling water for 20 min to remove the dodecyl chains. After hydrolytic treatment, the modified BGs powders showed increased hydrophilicity and the FTIR analysis revealed that most dodecyl chains were removed. Furthermore, the hydrophilicity of the PDLA/modified BGs composite films was also greatly improved. The tensile strength of the composite films after hydrolysis decreased slightly, but was still much higher than that of pure PDLA film. In addition, bone marrow mesenchymal stem cells from dogs on the composite films after hydrolytic treatment showed the highest proliferation rate. The results suggest that hydrolytic treatment is an effective and practicable method to remove alcohol chains from surface-modified BGs and polymers/modified BG composites, which may be used for preparation of bioactive scaffolds for tissue engineering applications.

## Introduction

During the last decades, the studies on bioactive glasses (BGs) have allowed important advances in the development of preparation techniques and controlling the composition and structure [1–5]. In 2004, Yan et al. [6] firstly synthesized mesoporous BGs and these new biomaterials possessed superior bioactivity *in vitro* compared to normal sol–gel-derived BGs. Although BGs show favorable bioactivity, lack of *in situ* moldability and the relative brittleness limit their applications [7]. Therefore, many scientists have started focusing on preparing polymers/BGs composites in order to obtain biomaterials with improved properties [8–11]. Poly(lactic acid) (PLA) is one of the most popular synthetic polymers considered for tissue engineering [12, 13]. Previous studies have demonstrated that combining PLA and BGs together could obtain materials with optimized properties [14–16].

Among the factors affecting the properties of the polymers/inorganic composites, the interface adhesion of inorganic particles to polymer matrix plays a very important role [17, 18]. It was found that some polymers/inorganic composites lost their strength rapidly in physiological environment and the failures occurred mainly at the interface between inorganic particles and the polymer matrix [19]. The main reason was the tendency for inorganic particles to agglomerate in the polymer matrix due to their small dimensions and incompatible polarity with polymers [20–22]. Therefore, improving poor dispersion of inorganic particles in polymeric matrix was critical for preparing composite materials with improved performance.

Surface modification of inorganic particles using organic molecular was an effective method to solve this problem [21]. In our previous work [23], BGs particles were surface modified by esterification reaction with

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Y. Zhou · Y. Gao · J. Chang (✉)  
State Key Laboratory of High Performance Ceramics and Superfine Microstructure, Shanghai Institute of Ceramics, Chinese Academy of Sciences, 1295 Dingxi Road, Shanghai 200050, People's Republic of China  
e-mail: jchang@mail.sic.ac.cn

Y. Zhou · Y. Gao  
Graduate School of the Chinese Academy of Sciences, 319 Yueyang Road, Shanghai 200050, People's Republic of China

dodecyl alcohol and poly-(D,L-lactic acid) (PDLLA)/modified BGs composite films showed more uniform particle distribution and enhanced mechanical strength compared with unmodified samples, nevertheless, the organic dodecyl chains remarkably decreased the hydrophilicity of the composites films which was closely associated with the biocompatibility of the biomaterials. Previous research showed that appropriate hydrophilicity could enhance protein adsorption and cell adhesion onto the surface of the biomaterials [24]. Therefore, it would be ideal if the dodecyl chains could be removed from composite films after achievement of uniform distribution of inorganic particles in polymer matrix.

During the modification, the hydroxyl groups of dodecyl alcohol condensed with the Si–OH groups on the surface of BGs particles, and the ester bonds could be broken through hydrolysis reaction under certain conditions [21, 25]. Obviously, the properties of the composite films after the removal of dodecyl chains were the most concerned issues subsequently.

In this study, mesoporous 58S (m58S) and 58S BGs were surface modified by esterification with dodecyl alcohol, and PDLLA/modified BGs composite films with homogeneous dispersion of modified BGs particles in PDLLA matrix were prepared, then the modified composite films were treated in boiling water to remove the dodecyl chains. The aim of this article was to investigate the properties of modified BGs particles and the PDLLA/modified BGs composite films before and after hydrolytic treatment, and the interaction between cells and PDLLA/BGs composites films was studied.

## Materials and methods

### Materials

58S BGs with size around 1  $\mu\text{m}$  were prepared using sol-gel techniques previously described by Zhong and Greenspan [26]. In brief, tetraethyl orthosilicate (TEOS), triethyl phosphate (TEP), and  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$  (Si/Ca/P = 58:23:9, weight ratio) were added into HCl solution, respectively. After hydrolysis for 1 h, the solution was aged in 60  $^\circ\text{C}$  for 55 h and then the getting sample was calcined at 700  $^\circ\text{C}$  to obtain 58S powder. Fibrous m58S BGs with length of 1  $\mu\text{m}$  were prepared using sol-gel and hydrothermal processing according to the procedure reported in the literature [27]. In a typical synthesis of m58S, TEOS, TEP, and  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$  (Si/Ca/P = 58:23:9, weight ratio) and P123 ( $\text{EO}_{20}\text{-PO}_{70}\text{-EO}_{20}$ ) as structure-directing agent were dissolved into HCl solution and stirred at 35  $^\circ\text{C}$  for 24 h. The suspension was hydrothermalized at 100  $^\circ\text{C}$  for 24 h and then was dried at 100  $^\circ\text{C}$  for 24 h. The as-synthesized

sample was calcined at 650  $^\circ\text{C}$  in air for 6 h to get m58S powder.

PDLLA with an average molecular weight ( $M_w$ ) of 45 kDa was obtained from Chengdu Institute of Organic Chemistry (Sichuan, China). Dodecyl alcohol was purchased from A.R. Shanghai Chemical Reagent Co., Ltd. (Shanghai, China).

### Surface modification of BGs powders

m58S and 58S BGs powders were modified by esterification reaction with dodecyl alcohol according to our previous work [23]. In brief, 5 g BGs powders was dispersed into 20 mL 95% ethyl alcohol by ultrasonic treatment for 15 min. After evaporated the ethyl alcohol, 100 mL dodecyl alcohol and 0.1 g para-toluenesulfonate as catalyst were added to the BGs powders. The esterification reaction was carried out at 260  $^\circ\text{C}$  for 4 h. The modified BGs powders (abbreviated to modified-m58S and modified-58S BGs) were obtained through centrifugation and washed with ethyl alcohol for three times.

### Preparation of the PDLLA/modified-BGs composite films

PDLLA/modified-BGs composite films were synthesized in accordance with the literature [23]. PDLLA was dissolved in chloroform to form 10% (m/v) solution, and then modified-BGs powders (the weight proportion of PDLLA and the BGs powders was 85:15) were put into the PDLLA solution to form suspension. The suspension was stirred for 2 h to ensure a homogeneous dispersion of the modified-BGs powders, and then poured into a glass plate to evaporate the chloroform in air and in vacuum for 12 h, respectively. PDLLA/BGs composite films were prepared by the same way as control.

### Hydrolytic treatment of modified-58S powders and PDLLA/modified-BGs composite films

The results in our previous study [23] showed that the modification was more effective on 58S BGs compared to m58S BGs, so 58S BGs particles were chosen for the hydrolytic experiments. Modified-58S BGs powders were soaked in boiling deionized water for 20 min, then filtrated and dried in vacuum at room temperature (named as modified-58S-20B). PDLLA/modified-BGs composite films were hydrolyzed under the same conditions as the treatment of the modified-58S BGs (named as PDLLA/modified-58S-20B and PDLLA/modified-m58S-20B, respectively).

## Characterization of modified-58S powders

### *Fourier transform infrared spectroscopy*

Fourier transform infrared (FTIR) spectroscopy (Thermo Nicolet nexus-IR spectrometer) was employed to identify the specific functional groups on the surface of modified and unmodified-58S BGs. Samples were prepared by mixing BGs particles with KBr powders. The spectra were collected after 64 scans in 4500–400  $\text{cm}^{-1}$  region at a resolution of 4  $\text{cm}^{-1}$ .

### *Dispersion stability of modified-58S powders in different solvents*

Dispersion stability of modified-58S-20B powders in water and hexane was determined by sedimentation experiments according to previous report [21]. 0.1 g of sample was ultrasonically dispersed in 10 mL of water or hexane. The sedimentation behavior was measured by the time required for all particles precipitating out of the solution, which became completely transparent and clear. 58S and modified-58S BGs were also observed as controls.

## Characterization of the PDLLA/modified-BGs composite films

### *Hydrophilicity of PDLLA/modified-BGs composite films*

The hydrophilicity of the PDLLA/modified-BGs composite films before and after hydrolytic treatment was measured by testing water contact angles of the films using the sessile drop method. The composite films were cut into square pieces with the size of 10 × 10  $\text{mm}^2$ , purified in ethyl alcohol through ultrasound, and dried in vacuum at room temperature. One droplet (about 0.5  $\mu\text{L}$ ) of distilled water was added to the surface of the samples. The photograph of the sample with water droplet on it was taken under an optical microscope and the water contact angles were measured by Photoshop Software (Adobe, USA). The water contact angles of composite films with unmodified-BGs were also measured under the same conditions as control. All data were presented as mean  $\pm$  standard deviation. At minimum, five samples were represented for each data point.

### *Mechanical properties of PDLLA/modified-BGs composite films*

Mechanical properties of PDLLA/modified-BGs composite films before and after hydrolytic treatment were measured by tensile test on an AG-1 Shimadzu mechanical machine (Shimadzu Co., Japan) at a crosshead speed of 2 mm/min.

Dumbbell-shaped specimens (with a size of 30 × 8  $\text{mm}^2$  for the effective area) were cut from the prepared composite films for testing. Pure PDLLA and the composite films with unmodified-BGs were used as controls. Ten samples of each kind of films were tested.

## Cell experiments

### *Cell culture*

In this cell culture experiment, the fifth passage of bone marrow mesenchymal stem cells from dogs (dMSC) was employed. dMSCs were isolated and cultured according to the procedure proposed by Ringe et al. [28]. Circular-shaped specimens with the diameter of 1 cm were cut from the prepared composite films for cell culture experiments. After sterilization with ethanol and ultraviolet light, samples were placed into sterile 48-well tissue-culture plates, and dMSCs suspensions were added into each well at a density of  $1.5 \times 10^4$  cells/sample.

### *Cell proliferation*

The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) test [29] was used for the evaluation of cell proliferation. After culturing cells on the composite films for 7 days, the samples were moved to new 48-well tissue-culture plastic dishes. Then MTT was added (100  $\mu\text{L}$  per well of a 0.5 mg/mL solution in PBS) and cultured for 4 h at 37 °C. After washed with D-Hanks solution, 100  $\mu\text{L}$  DMSO was added to each well, and the plate was shaken for 5 min. Optical density (OD) at 590 nm was measured with a microplate reader (ELX800, Bio-TEK, USA).

### Statistical analysis

Statistical analysis for determination of differences in the measured properties between groups was accomplished using one-tailed analysis of variance, performed with a computer statistical program (Student's *t*-test), and *p*-values < 0.05 were considered statistically significant.

## Results

### Characterization of modified-58S powders after hydrolytic treatment

#### *FTIR spectra*

The FTIR spectra were used to identify the functional groups of 58S BGs particles before and after hydrolytic

treatment. Intensive absorption bands attributed to the C–H stretching vibrations in modified-58S BGs were observed at 2930 and 2860  $\text{cm}^{-1}$  for surface-modified dodecyl chains (Fig. 1). After 58S BGs were treated in boiling water for 20 min, the intensity of the bands related to C–H stretching vibrations decreased significantly and even became indistinguishable, indicating that most long alkyl chains had been removed.

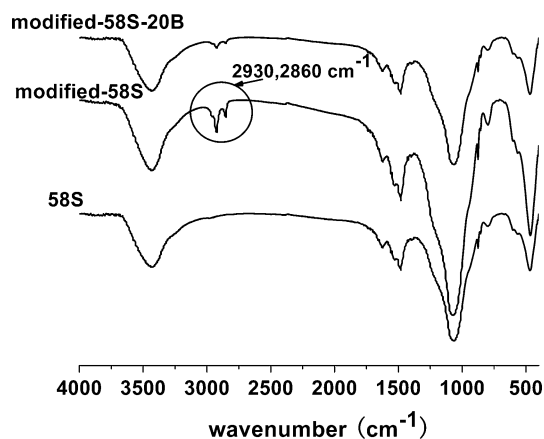
#### Dispersion stability of 58S BGs powders in different solvents

The time required for the sedimentation of modified-58S BGs powders and modified-58S-20B BGs powders out of different solvents was shown in Table 1. After hydrolytic treatment, the sedimentation time of modified-58S BGs powders in solvents changed dramatically. Compared to modified-58S BGs, the complete sedimentation time of modified-58S-20B BGs powders in polar solvent (water) was much longer, but shorter than that of the unmodified-58S BGs. Otherwise, in non-polar solvent (hexane), the three types of 58S BGs powders completely precipitated within 1 h, and unmodified-58S BGs powders were the fastest.

#### Characterization of the composite films after hydrolytic treatment

##### Hydrophilicity determination

Table 2 showed the water contact angles of the PDLLA/modified-BGs composite films before and after hydrolytic treatment. The water contact angle of pure PDLLA was 61.5°, whereas that of the PDLLA/unmodified-BGs composite films was around 40°, which was significantly lower than that of the pure PDLLA. In contrast, PDLLA/



**Fig. 1** FTIR spectra of modified 58S BGs powders before and after hydrolytic treatment

**Table 1** The sedimentation time of the modified 58S powders before and after hydrolytic treatment in the solvent of water and hexane

Complete sedimentation time	58S	Modified-58S	Modified-58S-20B (min)
Water	2 h	/	50
Hexane	20 s	1 h	8

“/” means the powders sedimented quickly so that the time could not be recorded

**Table 2** Water contact angles of PDLLA/BGs composite films before and after hydrolytic treatment

Samples	Water contact angle (°)		
	Unmodified	Modified	Hydrolyzed
PDLLA/58S	41.2 ± 2.03	57.3 ± 1.91	48.9 ± 1.86
PDLLA/m58S	46.2 ± 1.75	56.6 ± 1.28	52.5 ± 1.81
PDLLA	61.5 ± 1.64		

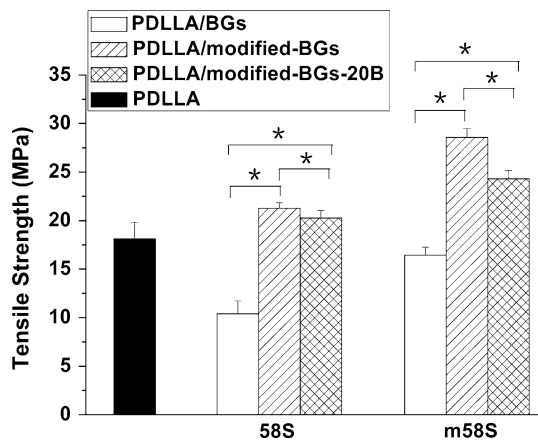
modified-BGs, which showed much higher water contact angles, almost were the same as that of pure PDLLA film. However, after the hydrolytic treatment of PDLLA/modified-BGs films in boiling water, the water contact angles were decreased remarkably. The water contact angles of the PDLLA/modified-58S-20B and PDLLA/modified-m58S-20B fell sharply to 52.5° and 48.9°, respectively, after hydrolytic treatment.

##### Tensile strength

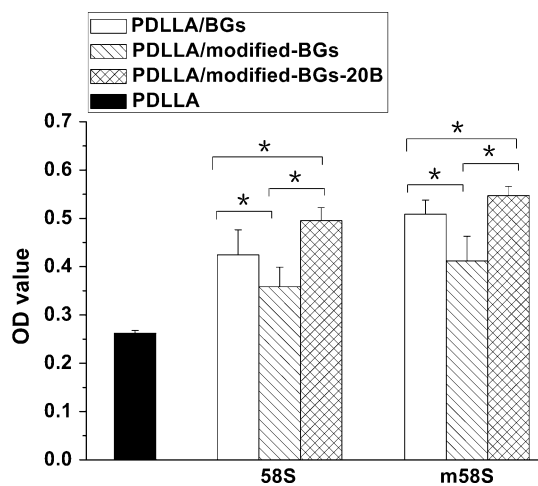
As shown in Fig. 2, significant differences in tensile strength were observed between PDLLA/BGs and PDLLA/modified-BGs composite films before and after hydrolysis, and PDLLA/modified-m58S and PDLLA/modified-58S composite films showed notably higher tensile strength than that of pure PDLLA films. After treated in boiling water for 20 min, as shown in Fig. 2, the tensile strength of PDLLA/modified-BGs decreased slightly, but the tensile strength of PDLLA/modified-58S-20B and PDLLA/modified-m58S-20B were still much higher than that of pure PDLLA films.

##### Proliferation of dMSC

Figure 3 showed the results of the MTT assay of dMSC on different composite films indicating cell proliferation. After cultured for 7 days, the OD values of all composite films were significantly higher than that of pure PDLLA films. The proliferation rate of dMSC on PDLLA/BGs composite films was higher than that on PDLLA/modified-BGs composite films and cells on PDLLA/modified-BGs-20B composite films showed the highest proliferation rate.



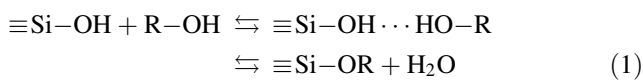
**Fig. 2** Tensile strength of the composite films. Asterisk indicates that the difference between the two data was significant ( $p < 0.05$ )



**Fig. 3** The dMSC proliferation on different composite films. OD value on Y-axis represented the number of living cells. Asterisk indicates that the difference between the two data was significant ( $p < 0.05$ )

**Discussion**

There were plenty of silanol groups ( $\equiv\text{Si-OH}$ ) on the surface of BGs, which can be modified with alcohols through esterification reaction [30–32]. Esterification reaction was an equilibrium reaction and the reverse reaction occurs in boiling water [30], as shown in Eq. 1.



Ossenkamp et al. [30] found that the hydrophobic silica surface modified with 1-octanol first floated on the water, as hydrolysis progressed more and more silanol groups became exposed and the water contact angle was reduced until the material eventually sank. In the present hydrolytic experiment, most surface modified-58S BGs powders,

which floated firstly on the water, sank to the bottom of the beaker after treatment in boiling water for 20 min. The measurement of the sedimentation time confirmed that the modified-58S-20B were much more stable in water than modified-58S particles, which meant hydrophobic dodecyl chains were removed from the surface of the modified-58S particles. The FTIR spectra further verified the result that hydrolytic treatment was a useful method to remove the dodecyl alcohol linked on the surface of modified-58S BGs powders.

Regarding PDLLA/BGs composite films, the results of the water contact angle tests showed that hydrolysis in boiling water also resulted in an increase in the hydrophilicity of the composite materials obviously, which was consistent with that of 58S BGs powders, and suggested that most dodecyl chains were removed from the PDLLA/modified-BGs composite films through hydrolytic treatment.

Uniform dispersion of inorganic particles in composite films was critical to improve the mechanical properties of the materials. The tensile strength of the composite films with dodecyl alcohol-modified BGs increased obviously, since the surface-modified BGs could be homogeneously dispersed in PDLLA matrix. After treatment in boiling water for 20 min, the tensile strength of PDLLA/modified-BGs decreased slightly, but still much higher than that of PDLLA/unmodified-BGs composite films and pure PDLLA films. The results suggested that the treatment in boiling water did not change the distribution of inorganic particles in PDLLA matrix.

The hydrophilicity of surface characteristics played an essential role in cells adhesion on biomaterials [33, 34]. According to our experimental results, pure PDLLA films was high hydrophobic and resulted in the lowest cell proliferation rate. The hydrophilicity of the PDLLA/BGs composite films increased greatly because of the added hydrophilic BGs particles, which resulted in a higher proliferation rate of dMSC cells as compared with pure PDLLA films. After BGs modified with dodecyl chains, the hydrophobicity of the composite films increased remarkably and caused lower cell proliferation on PDLLA/modified-BGs composite films. However, when treated in boiling water for 20 min, the composite films regained hydrophilicity and the BGs particles maintained the uniform dispersion in the PDLLA films, which resulted in the highest proliferation rate of dMSC cells. Hydrophilicity contributed to the physicochemical interaction between cells and materials surfaces [35]. Webb et al. [36] found that hydrophilic surfaces supported cell attachment significantly as compared to hydrophobic surfaces. Our results suggested that the hydrolytic treatment resulted in the recovery of the hydrophilicity due to the removal of the alkyl chains, which enhanced the biocompatibility of the PDLLA/modified-BGs composite films.

## Conclusion

In this study, two types of BGs (m58S and 58S) particles were surface modified with dodecyl alcohol, and the modified BG particles were homogeneously dispersed in PDLA matrix to obtain composite films. Modified-58S particles and the obtained composite films were treated in boiling water for 20 min to remove the dodecyl chains. After hydrolytic treatment, hydrophobic modified-58S powders and PDLA/modified-BGs composite films regained hydrophobicity. PDLA/modified-BGs-20B composite films retained high mechanical properties compared to pure PDLA films. Attributed to improved hydrophilicity and well dispersion of BGs particles, cells on PDLA/modified-BGs-20B composite films had the highest proliferation rate compared with those on PDLA/BGs and PDLA/modified-BGs composite films. In conclusion, hydrolysis treatment was an effective method to prepare hydrophilic polymers/inorganic bio-composite films with improved properties.

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