Polycaprolactone/calcium sulfate whisker/barium titanate piezoelectric ternary composites for tissue reconstruction

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Abstract
The piezoelectric materials with excellent bioactivity have attracted more attentions recently and have broad potential applications in tissue engineering. In this article, the barium titanate (BT) particles were filled into the polycaprolactone (PCL)/calcium sulfate whisker (CSW) (15 wt%) composites to prepare the PCL/CSW/BT ternary composites. Due to the reinforcement synergy between the CSWs and the BT particles, the mechanical properties of the ternary composites were increased by 50% compared with the PCL/BT binary composites. The piezoelectric coefficient of the ternary composites is still in the range of natural bone. The ternary composite can promote the adhesion and proliferation of cells. The composites in this study have potential applications in tissue engineering.

Keywords
polymers, smart materials, mechanical property, piezoelectric property

Introduction
In living organisms, piezoelectricity is shown to be effective in natural pathways, because the generated charge produces electrical stimulation on cells. The electrical stimulation can produce β-TGF, a potential key factor in cell growth, differentiation, extracellular matrix synthesis, inflammation, and tissue reconstruction.¹,² The most feasible method for obtaining piezoelectric scaffold is to select a suitable piezoelectric material, such as piezoelectric polymers, piezoelectric ceramics, or polymer/ceramic piezoelectric composites as a biological scaffold matrix. At present, the piezoelectric polymers for cartilage and bone tissue engineering mainly contain polyvinylidene fluoride (PVDF),³,⁴ poly(vinylidene fluoride-three fluoroethylene) (P(VDF-TrFE)),⁵ poly(3-hydroxybutyric acid-3-hydroxyvalerate),⁶ poly(l-lactic acid),⁷,⁸ and natural biopolymers (cellulose, collagen, and chitin).⁹–¹¹ PVDF, P(VDF-TrFE), and polyamide are thermoplastic polymers with good biocompatibility and high chemical and physical properties. For non-piezoelectric biodegradable materials, piezoelectric property could be obtained by adding piezoelectric ceramics particles. Piezoelectric ceramics has higher piezoelectric coefficient compared with piezoelectric polymers. In lead-free
piezoceramics, barium titanate (BT) with perovskite structure has a high piezoelectric coefficient ($d_{33}$) is 191 pC N$^{-1}$) and biocompatibility. The phase symmetry depends on the size of BT particle. Cubic phase is for ultrafine particles and tetragonal phase is for larger particles size.$^{12}$ For composites respectively with perovskite ceramic nanoparticle, including calcium titanate, strontium titanate, and BT, BT composites have weak enhancement effect of osteogenic genes expression.$^{13}$ This may be caused by the nonpiezoelectric property for the BT nanoparticle. For the cytocompatibility of BT, it is reported that BT particles high concentration still show cytocompatibility.$^{14}$ Some study has shown that the osteoblast adhesion and cell proliferation of PLGA composite were improved by the addition of BT nanoparticles.$^{15}$ We also found that for PCL/BT composite, the adhesion and proliferation of cell were enhanced by the addition of BT.$^{16}$ In addition, the mechanical properties of the composite scaffolds could be reinforced by BT nanoparticles.$^{17}$ Therefore, piezoelectric BT is a suitable ceramic for scaffold and has broad application prospects in tissue engineering.

It is noteworthy that the piezoelectric ceramic has high piezoelectric coefficient with large brittleness and difficult degradation. The piezoelectric biodegradable polymer is restricted by the low piezoelectric property and mechanical strength. For the composite of the piezoelectric polymer and piezoelectric ceramic, the excellent property of them could be combined. The mechanical strength could be improved by the addition of piezoelectric ceramic particles. The piezoelectric characteristic could be like that of natural bone. Polycaprolactone (PCL) is a non-negligible biodegradable biopolymer with easy processing and biocompatibility. In previous work, we found that the strength of PCL was reinforced by 50% by adding 15 wt% calcium sulfate whisker (CSW).$^{18}$ In this article, the PCL/CSW/BT ternary composites were finally prepared using PCL/CSW (15 wt%) as matrix. The synergistic effect of CSW and BT on the mechanical improvement was discussed. The piezoelectric characteristic and the cytocompatibility of the ternary composites were also studied.

PCL ($W_g = 50000$) was purchased from Dow Chemical Co., Ltd. (Shanghai, China) Tetrahydrofuran and ethanol (analytically pure) were purchased from Guangzhou and Jiangsu chemical reagents Co., Ltd. (Guangzhou, Nanjing, China) BT (particle size smaller than 2 μm) was purchased from Sigma–Aldrich Co., Ltd. (St. Louis, Missouri, USA). CSW was from Jian-kun Co., Ltd. (Hefei, Anhui, China) Chemical Dulbecco’s modified Eagle’s medium powder medium and newborn calf serum were purchased from Gibco Co., Ltd. (Grand Island, NY, USA) The mixture of trypsin and ethylenediamine tetra acetic acid (EDTA) (0.25% trypsin and 0.1% EDTA), penicillin-streptomycin (100 mL$^{-1}$ penicillin and streptomycin), and phosphate buffer solution (phosphate buffer solution) were self-made in our laboratory. Fibroblast L929 is provided by Guangdong stemmatological hospital.

The sample was prepared using solution blending method. Firstly, PCL particles were dissolved in tetrahydrofuran at 50°C to prepare PCL solution, and the BT particles were dispersed in tetrahydrofuran and stirred. After 2 h, the PCL solution was blended with the BT suspension and stirred for 2 h, and then the blend liquid was poured into the ethanol to precipitate the PCL/BT blends, afterward, the flocs of PCL/BT blends were obtained after filtration. Finally, the flocs of the blends were molded on the flat vulcanizing machine for 5 min with the condition of 15 MPa and 70°C to obtain the composite samples. The sample was polarized in the flat plate with the following polarization process: after hot polarization for 10 min, the polarization temperature of sample was quickly dropped to room temperature, and then followed by cold polarization for 1.5 h.

The flexural property of the composite was tested by the universal electronic test machine (LR5K Plus, Lloyd instruments Ltd., London, UK), with the test rate of 2 mm min$^{-1}$ according to the American Standard ASTM D790. The microstructure of the composite was observed by scanning electron microscopy (SEM) (XL30, Philips) after the impact section was vacuum plated Aurum. The piezoelectric coefficient $d_{33}$ was tested by quasi-static test method with ZJ-3A quasi-static piezoelectric constant measuring instrument developed by Acoustics Institute of Chinese Academy of Sciences.

PCL/CSW (85/15) was chosen as the matrix to prepare PCL/CSW/BT ternary composites. The micromorphology

![Figure 1](image-url)
Table 1. The effect of BT content on the mechanical properties of ternary and binary composites.

| Volume fraction of BT (v%) | Flexural strength (MPa) | Flexural modulus (GPa) |
|---------------------------|------------------------|-----------------------|
| 15                        | 50.5                   | 5.133                 |
| 20                        | 50.6                   | 6.810                 |
| 25                        | 42.1                   | 5.650                 |

Table 2. The effect of BT content on the piezoelectric coefficient $d_{33}$ of the composites.

| Volume fraction of BT (v%) | Piezoelectric coefficient $d_{33}$ (pC N$^{-1}$) |
|---------------------------|-----------------------------------------------|
| 15                        | Bone (Ternary composites)                     |
| 20                        | 20                                           |
| 25                        | 25                                           |
|                           | 0.22                                         |
|                           | 0.1                                          |
|                           | 0.2                                          |
|                           | 0.8                                          |

|                           | 0.1                                          |
|                           | 0.8                                          |
|                           | 1.0                                          |

BT: barium titanate.

of the ternary composites observed by SEM is shown in Figure 1. It can be seen from photographs that CSW and BT particles disperse uniformly in PCL matrix with no obvious agglomeration. As the white arrow shown in Figure 1(b), there is a smooth trace left by the CSW. It indicates that the CSW absorbs a part of the impact energy during the impact process. It also indicates that the adhesion strength between the CSW and the PCL matrix needs to be improved. Due to its large specific surface area, the volume of the composite material is occupied by the BT particle, reducing the indirect contact probability of the CSW and the PCL matrix. This fact reduces the adhesion strength between the CSW and the PCL matrix. Therefore, there is a smooth trace left by the CSW.

The data in Table 1 shows the mechanical properties of PCL/CSW/BT composites. It is shown that the flexural strength of the composite is not obviously changed, when the content of BT is 15 and 20 v%, respectively. When the content of BT is 25 v%, the bending strength of the composite is reduced. The agglomeration of BT particles is the possible reason of this phenomenon. It is worth to note that, with the same content of BT, the flexural strength of PCL/CSW/BT composite is higher than that of PCL/BT composite. This phenomenon indicates that CSW and BT particles have synergistic effect on the reinforcement of PCL. CSW is a kind of anisotropic inorganic short fiber with high longitudinal strength and low transverse strength. BT particles can be regarded as the isotropic inorganic particles. The BT particles around the whiskers can share a part of the stress loaded in the transverse direction of CSW, achieving a synergy of reinforcement. Therefore, the flexural strength of PCL/CSW/BT composites is higher than that of PCL/BT composites.

Results show that the flexural modulus of PCL/CSW/BT composite is higher than that of the PCL/BT composites, and it increases first and then decreases with the increase of BT content. The modulus of CSW is as high as 70 GPa owing to the few defects of crystallization on the surface. It is shown that the flexural modulus of PCL/CSW/BT composite is still in the range of the piezoelectric coefficient of bone. Marino and Gross reported that the piezoelectric of bone is about 0.22 pC N$^{-1}$.

To observe the growth of the cells on the surface of the material, the cell morphology and spreading on the surface of the pure PCL and PCL/CSW/BT composite with 15 v% BT after cultured for 1 week were observed by SEM, and the results were shown in Figure 3. It is observed that, after cultured for 1 week, the cells on the surface of the pure PCL have not spread massively. The cells on the surface of the PCL/CSW/BT composite containing 15 v% BT spread massively and flatly, with mitosis and reproduction. The results are similar to the reported research work.

To confirm the cell viability, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) experiments of PCL and PCL/CSW (15%)/BT composites were done through fibroblasts. The results were shown as Figure 4. The results show that the best cell viability is the ternary composites with 15% BT. This advantage is obvious with...
the increase of BT dosage. These results agree with the observation of SEM.

The PCL/CSW (15 wt%)/BT ternary composites are prepared by solution method with the PCL/CSW (15 wt%) as matrix. Results show that the mechanical properties are improved by 50%. The enhanced synergy between CSW and BT contributes to the reinforcement of the ternary composites. The cytocompatibility of the ternary composites is studied by MTT and SEM. The ternary composites with piezoelectric characteristic are benefit to the mitoschisis and reproduction of the cells. In general, the strength and piezoelectric properties of PCL/CSW/BT ternary composites are improved compared with that of pure PCL. The PCL/CSW/BT ternary composites with piezoelectric characteristic are benefit to mitoschisis and reproduction of the cells having potential applications in the field of tissue reconstruction.
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Figure 4. Cell viability of PCL and PCL/CSW (15%)/BT composites tested by MTT. PCL: polycaprolactone; CSW: calcium sulfate whisker; BT: barium titanate; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.