Study on in vitro biocompatibility of PHBV/Wollastonite composites

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Statement of Purpose:

As bone is a mineral–biopolymer composite material, the polymer matrix composite containing a particulate bioactive inorganic component appears a natural choice for substituting cortical bone. Recently, we have reported the incorporation of wollastonite into PHBV to prepare bioactive and degradable composite scaffolds [1-2], and demonstrated that the incorporation of wollastonite with PHBV could not only enhance bioactivity and tailored mechanical properties but also alter the degradation behavior of PHBV. In the present study, the in vitro biocompatibility of degradable and bioactive composites consisting of polyhydroxybutyrate-co-hydroxyvalerate (PHBV) and wollastonite (W) was studied by culturing osteoblasts on the PHBV/W substrates, and the cell adhesion, morphology, proliferation, and alkaline phosphatase (ALP) activity were evaluated.

Methods:

The PHBV/wollastonite composites were prepared by polymer coagulation and hot compression moulding. First, the composite powders of PHBV and wollastonite were prepared by co-precipitation in methanol. Then, the composite powders were hot-compression moulded into discs at a pressure of 10 MPa at 200℃ for 30 min. The surface of the as obtained composites were coated with gold and analyzed by SEM. Osteoblasts isolated from rat calvaria were incubated on the different substrates for different time periods. The cell adhesion and proliferation was assessed at the different time points using the MTT method. Alkaline phosphatase (ALP) activity was assayed by the method of Lowry (1954) [10]. Ionic concentrations of released silicon (Si) and calcium (Ca) from composites in culture medium were determined using inductively coupled plasma optical emission spectroscopy (ICP-OES).

Results / Discussion:

The results showed that an increase of the cell adhesion percentage was observed with the increase of the wollastonite content in the PHBV substrates. Only 60% of cell adhesion was obtained on the pure PHBV substrates, while 70% and 82% were obtained on the composites with 10 and 20wt% wollastonite content, respectively. The cells on the PHBV/W composite attached well and began to spread while those on the pure PHBV substrates were round and weakly attached. After culturing for 1 day, the cell number on the pure PHBV substrates was similar to that of the PHBV/10wt%W composite substrates, which were significantly lower than that on the PHBV/20wt%W composite substrates. After culturing for 3 days, the cell number on all of the substrates increased quickly and the osteoblasts cultured on the PHBV/20wt%W composite substrates showed the highest proliferation rate. In addition, fifteen-fold increase of the osteoblasts number on the PHBV/10wt%W composite was observed, which was significantly higher than that on the pure PHBV substrates. After culturing for 7 days, the osteoblasts continued to proliferate and the cell numbers on the PHBV/10wt%W and PHBV/20wt%W composite were still significantly higher than that on the pure PHBV substrates. The ALP activities of osteoblasts on all substrates increased over time. However, the ALP activities of the osteoblasts on composite substrates were significantly higher than that of the osteoblasts on the pure PHBV substrates, and an increase of osteoblasts ALP activity was also observed with the increased of the wollastonite content in the composites. The ICP-OES analysis showed that Si release was found to be 31.42 μg/ml for PHBV/10wt%W composites and 49.39 μg/ml for PHBV/20wt%W composites, respectively. The Ca concentration was 46.08 μg/ml for PHBV/10wt%W composites and 60.8 μg/ml for PHBV/10wt%W composites, respectively. However, the Ca concentration in the cell culture medium containing the pure PHBV was only 4.87 ug/ml and no Si ions could be detected, which were the same to those in the pure cell culture medium. This result suggests that the Si and Ca containing ionic products of wollastonite in the composites might be the reason for the stimulatory effect on cell proliferation and differenciation.

Conclusions:

The results of this study suggest that incorporation of wollastonite into PHBV could enhance the biocompatibility of the composite materials by enhancing cell adhesion and stimulating cell proliferation and differentiation. This stimulatory effect is dependent on the amount of wollastonite incorporated, and the Si and Ca containing ionic products of wollastonite dissolution from the composites might be the reason for this stimulatory effect. It is concluded that the PHBV/W composites are biocompatible and might be suitable for preparation of bone implant and tissue engineering scaffolds.

References:

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