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Induced apoptosis of osteoblasts proliferating on polyhydroxyalkanoates

Yang Wang [a,b,1], Xian-Li Jiang [b,1], Si-Wu Peng [c,d,1], Xiao-Yong Guo [d,e], Guan-Guan Shang [d], Jin-Chun Chen [b], Qiong Wu [b], Guo-Qiang Chen [b,f,*]

[a] State Key Laboratory of Plant Physiology and Biochemistry, National Plant Gene Research Center, College of Biological Sciences, China Agricultural University, Beijing 100193, China
[b] MOE Key Lab of Bioinformatics, School of Life Sciences, Tsinghua University – Peking University Joint Center for Life Sciences, Tsinghua University, Beijing 100084, China
[c] Center for Vascular Biology, University of Connecticut Health Center, Farmington, 06030 CT, USA
[d] Multidisciplinary Research Center, Shantou University, Shantou, 515063 Guangdong, China
[e] Shenzhen Institutes of Advance Technology, Chinese Academy of Science, Shenzhen, 518055 Guangdong, China
[f] Center for Nano and Micro Mechanics, Tsinghua University, Beijing 100084, China

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ABSTRACT

The mechanism study on behaviors of cells influenced by biomaterial surface properties can provide profound guidances for functional tissue engineering scaffolds design. In this study, regulation of integrin-mediated cell–substrate interactions using rat osteoblasts incubated on PHA films was investigated. Compared with tissue culture plate (TCP), poly-3-hydroxybutyrate (PHB), copolymer of 3-hydroxybutyrate and 3-hydroxyvalerate (PHBV) and copolymer of 3-hydroxybutyrate and 3-hydroxyhexanoate (PHBHHx), osteoblasts inoculated on a terpolymer of 3-hydroxybutyrate, 3-hydroxyvalerate and 3-hydroxyhexanoate (PHBVHHx) were found to have higher apoptosis rates. Several integrin subunits in osteoblasts grown on PHBVHHx showed altered expressions. Simultaneously, extracellular matrics (ECM) were also remodeled on the material surface. Osteoblasts showed a higher expression of integrin subunit β3 and αv on PHBVHHx films compared with that on TCP. On the other hand, less vitronectin, osteopontin and fibronectin, the main ligands for integrin β3 were expressed and deposited in ECM. The unligated integrin β3 could recruit caspase-8 to the membrane and activate its downstream signaling which was proven by the caspase-8 activation assay. It was therefore concluded that the induced apoptosis of osteoblasts on PHBVHHx was regulated by recruitment of caspase-8 to the unligated integrin β3.

1. Introduction

Polyhydroxyalkanoates (PHA) are non-cytotoxic, biodegradable microbial polyesters, which process flexible physical and chemical properties due to their rich monomer compositions [1–3]. PHA have attracted increasing interests in the biomedical fields, because of their biodegradability, and ability to support cell adhesion and proliferation both *in vitro* and *in vivo* [4–7], and no risk of carcinogenicity [8]. As a new member of PHA, terpolyester poly(3-hydroxybutyrate-co-3-hydroxyvalerate-co-3-hydroxy-hexanoate) (PHBVHHx) was produced by recombinant *Aeromonas hydrophila* 4AK4 [9]. Compared with common copolymers PHBV and PHBHHx, terpolymer PHBVHHx has higher surface roughness, lower crystallinity and more hydrophobicity [10]. Human keratinocyte cell line HaCaT [11], mouse osteoblasts cell line MC3T3-E1 [10], human umbilical cord Wharton's Jelly-derived MSCs [12] and rat primary bone marrow-derived mesenchymal stem cells (MSCs) [13,14] could adhere, proliferate and differentiate on PHBVHHx films and scaffolds, indicating its potential as a tissue engineering material.

Studies on cell–matrix interaction mechanism have become one of the focuses in the field of biomaterial research [15–17]. In this regard, biomaterials including PHA could be considered as one type of extracellular matrix (ECM) which has important influences on cell behaviors [18]. Recently, PHBHHx films was reported to have some chondrogenic induction effects on mouse bone marrow-derived MSCs, and the mechanism underlying was associated with complex microRNAs regulation [19]. Various surface properties of PHA were found to cause different interfacial behaviors of attached cells [20]. It was interesting to observe that the proliferation rate of osteoblasts on the surface of PHBVHHx films was decreasing over time [8]. Therefore, the present study intended to understand the mechanism of slowing growth on the terpolyester. To do that, cell growth status including adhesion, proliferation, cell cycle and apoptosis of osteoblasts on films of PHB, PHBV, PHBHHx

* Corresponding author. Center for Nano and Micro Mechanics, Tsinghua University, Beijing 100084, China. Tel.: +86 10 62783844; fax: +86 10 62794217.
E-mail address: chengq@mail.tsinghua.edu.cn (G.-Q. Chen).
[1] These authors contributed equally to this work.

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Table 1
Sequence information of primers for real-time PCR.

Gene	Accession	Primer sequence (sence/antisence)
GAPDH	NM_017008	GGCACAGTCAAGGCTGAGAATG ATGGTGGTGAAGACGCCAGTA
Integrin α2	XM_345156.3	TCGGTGCAGCAGCTTACG TGTCAGGGAAGCCACTCCAT
Integrin α5	NM_00110811.1	CCTGTATCCTGCATCAACCTTAGC TCTGCCAGTCCAGTTGGAGTT
Integrin αv	NM_001106549	TAGCCACACGGACTGCACAAG AATGCCGTCACCATTGAAGTCTC
Integrin β1	NM_017022.2	TGCACAGATCCCAAGTTCCAAG TGAAGGCTCTGCACTGAACACA
Integrin β3	NM_153720.1	TTCAATGCCACCTGCCTCAA TGAAGCTCACCGTGTCTCCAA
Vitronectin	NM_019156.2	CCTTCACCGACCTCAAGAAC GAA GCC GTC AGA GAT ATT TCG GAAGCCGTCAGAGATATTTCG
Osteopontin	NM_012881.2	GACGGCCGAGGTGATAGCTT GTGGCCTTGGGATCGATGT
Fibronectin1	NM_019143.2	GCGACTCTGACTGGCCTTAC CCGTGTAAGGGTCAAAGCAT

and PHBVHHx were investigated down to integrin receptor related signaling pathway.

2. Materials and methods

2.1. Preparation of PHA films

PHB (poly-(R)-3-hydroxybutyrate) was obtained from our own lab. PHBV [poly(R-3-hydroxybutyrate-co-5.7 mol% R-3-hydroxyvalerate)], and PHBHHx [poly(R-3-hydroxybutyrate-co-12 mol% R-3-hydroxyhexanoate)] were kindly donated by Zhejiang Ningbo TianAn Biomaterials Co. Ltd., Shandong Lukang, respectively. All of these PHAs had weight average molecular weights (M_w) around 300 kDa. Terpolyester PHBVHHx [poly(R-3-hydroxybutyrate-co-5 mol% R-3-hydroxyvalerate-co-12 mol% R-3-hydroxyhexanoate)] with M_w of 900 kDa was produced in-house using *A. hydrophila* 4AK4. The 2%(*w*/*v*) PHB, PHBV, PHBHHx and PHBVHHx films were prepared by solution casting method [21]. Briefly, each polymer material was completely dissolved in chloroform at 60 °C. Each solution was then cast into a Petri dish of equal diameter and the solvent was evaporated at room temperature in a fume cupboard. All films were sterilized by immersion in 75% (*v*/*v*) ethanol overnight, then rinsed in phosphate buffered saline (PBS) three times.

2.2. Surface topography study using atomic force microscopy (AFM)

The surface topography of PHB, PHBV, PHBHHx and PHBVHHx films was investigated with AFM (MultiMode NanoscopIIIa, Digital Instrument, USA) by

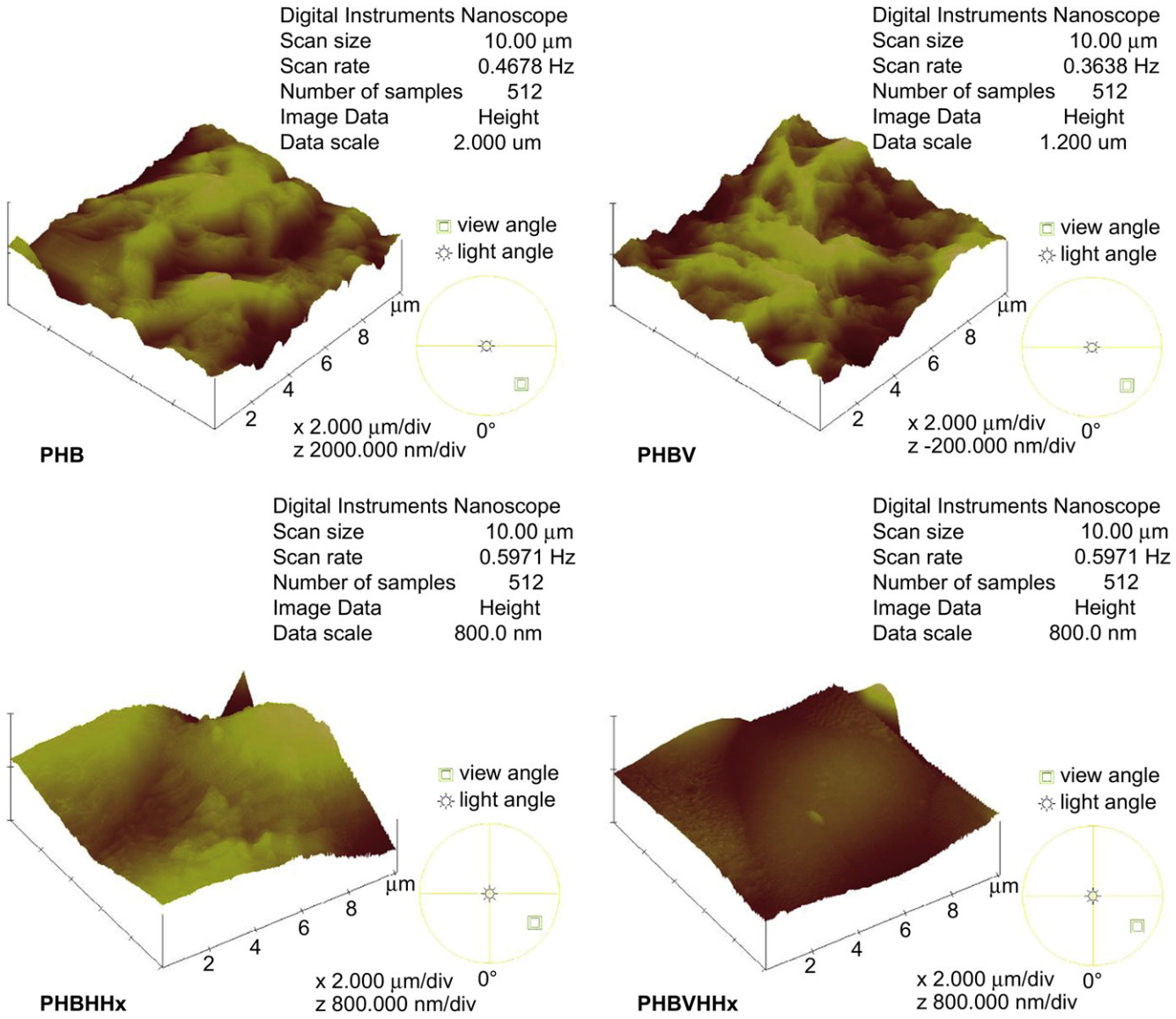

Fig. 1. AFM study of surface topography of PHA films. The surface roughness was represented by three-dimensional images.