Acta Biomaterialia 42 (2016) 329–340

Contents lists available at ScienceDirect

Acta Biomaterialia

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

Full length article

The effects of lactate and acid on articular chondrocytes function: Implications for polymeric cartilage scaffold design

Xiaolei Zhang [a,b,c], Yan Wu [a,c], Zongyou Pan [a,b,c], Heng Sun [a,c], Junjuan Wang [a,c], Dongsheng Yu [a,c], Shouan Zhu [a,c], Jun Dai [a,c], Yishan Chen [a,c], Naifeng Tian [b], Boon Chin Heng [f], Noelle D. Coen [g], Huazi Xu [b,*], Hongwei Ouyang [a,c,d,e,*]

[a] Dr. Li Dak Sum & Yip Yio Chin Center for Stem Cells and Regenerative Medicine, School of Medicine, Zhejiang University, China
[b] Department of Orthopaedics, The Second Affiliated Hospital, Wenzhou Medical University, China
[c] Key Laboratory of Tissue Engineering and Regenerative Medicine of Zhejiang Province, School of Medicine, Zhejiang University, China
[d] Department of Sports Medicine, School of Medicine, Zhejiang University, China
[e] State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, The First Affiliated Hospital, School of Medicine, Zhejiang University, 310003 Hangzhou, China
[f] Faculty of Dentistry, The University of Hong Kong, Pokfulam, Hong Kong
[g] Point Loma Nazarene University, United States

ARTICLE INFO

Article history:
Received 12 January 2016
Received in revised form 8 June 2016
Accepted 22 June 2016
Available online 23 June 2016

Keywords:
Polymeric cartilage scaffold
Lactate
Chondrocyte

ABSTRACT

Poly (lactic-co-glycolic acid) (PLGA) and poly-L-lactate acid (PLLA) are biodegradable polymers widely utilized as scaffold materials for cartilage tissue engineering. Their acid degradation products have been widely recognized as being detrimental to cell function. However, the biological effects of lactate, rather than lactic acid, on chondrocytes have never been investigated. This is the major focus of this study. The amounts of lactate and the pH value (acid) of the PLGA and PLLA degradation medium were measured. The effects of PLGA and PLLA degradation medium, as well as different lactate concentrations and timing of exposure on chondrocytes proliferation and cartilage-specific matrix synthesis were investigated by various techniques including global gene expression profiling and gene knockdown experiments. It was shown that PLGA and PLLA degradation medium differentially regulated chondrocyte proliferation and matrix synthesis. Acidic pH caused by lactate inhibited chondrocyte proliferation and matrix synthesis. The effect of lactate on chondrocyte matrix synthesis was both time and dose dependent. A lactate concentration of 100 mM and exposure duration of 8 h significantly enhanced matrix synthesis. Lactate could also inhibit expression of cartilage matrix degradation genes in osteoarthritic chondrocytes, such as the major aggrecanase ADAMTS5, whilst promoting matrix synthesis simultaneously. Pulsed addition of lactate was shown to be more efficient in promoting COL2A1 expression. Global gene expression data and gene knock down experiments demonstrated that lactate promote matrix synthesis through up-regulation of HIF1A. These observed differential biological effects of lactate on chondrocytes would have implications for the future design of polymeric cartilage scaffolds.

Statement of Significance

Lactic acid is a widely used substrate for polymers synthesis, PLGA and PLLA in particular. Although physical and biological modifications have been made on these polymers to make them be better cartilage scaffolds, little concern has been given on the biological effect of lactic acid, the main degradation product of these polymers, on chondrocytes. Our finding illustrates the differential biological function of lactate and acid on chondrocytes matrix synthesis. These results can facilitate future design of lactate polymers-based cartilage scaffolds

1. Introduction

Self-healing and repair of human articular cartilage injuries is clinically challenging due to the limited self-regenerative capacity

* Corresponding authors at: Dr. Li Dak Sum & Yip Yio Chin Center for Stem Cells and Regenerative Medicine, School of Medicine, Zhejiang University, 310003 Hangzhou, China (H. Ouyang).
E-mail addresses: spinexu@163.com (H. Xu), hwoy@zju.edu.cn (H. Ouyang).

http://dx.doi.org/10.1016/j.actbio.2016.06.029
1742-7061/

of cartilage tissue [1]. Therefore, much clinical research effort has been focused on restoring cartilage defects. Tissue engineering is a promising strategy for cartilage defect repair. The three major elements of tissue engineering are the seed cells, scaffold to support cells and growth factors to modulate cell biological function. The choice of a suitable scaffold is the first step in tissue engineering.

A diverse array of biomaterials, in particular polymers, have been developed as scaffolds for cartilage tissue engineering. Poly (lactic-co-glycolic acid) (PLGA) [2–4] and poly-L-lactate acid (PLLA) [5–7], also known as poly-lactic acid (PLA), are two polymers widely utilized as scaffolds in cartilage tissue engineering. L-Lactic acid (hereafter referred to as lactic acid) is a common substrate for synthesis of these polymers [8], and is also the principal degradation product of these polymers [9]. Acid products have been widely recognized as being detrimental to cell function, however the biological effects of lactate, rather than lactic acid, on chondrocytes has never been investigated.

As a degradation product of polymers, lactate released by PLA film was reported to maintain neuronal progenitor cell phenotype [10]. Within the nervous system, lactate may serve as an alternative energy source for neural cells under particular circumstances [11]. A previous study found that lactate could also induce hypoxia signaling to promote cell growth and angiogenesis [12]. Lactate was also reported to facilitate cell survival in an unfavorable environment [13].

On the other hand, lactate is considered to be an end waste product of polymeric scaffold degradation that can potentially exert adverse biological effects. Patients who have received PLA-based implants have been reported to display foreign-body granulomatous inflammatory reaction at the injection site [14]. Additionally, PLGA that undergoes degradation *in vivo* within about 2–4 weeks, is known to cause acute inflammatory reaction as degradation progressed [15], which would imply the pathological effects of lactate on tissues and cells. Moreover, lactate is known to accumulate in muscles after physical exercise and is thought to be responsible for exhaustion after exercise [16]. There was reported to be dramatically increased concentration of lactate in septic arthritis [17]. These studies thus suggested that lactate is harmful to cells.

Currently, the effects of lactate exposure on chondrocyte proliferation and function are unclear. This would warrant further investigation on the biological effects of lactate on chondrocytes and the underlying mechanisms involved, which may facilitate future design of polymeric scaffolds for cartilage tissue engineering.

2. Materials and methods

2.1. Human cartilage sample collection and processing

The patient's informed consent was obtained for all samples, and approval of the Zhejiang University ethics committee was obtained prior to harvesting of any human tissue samples. Normal articular cartilage samples were obtained from three different trauma patients who were undergoing arthroscopic knee surgery. The cartilage samples were obtained from either the lateral femoral condyles or medial femoral condyles of the distal femur. None of the three patients had a history of joint disease, and none of the samples from these three patients exhibited any macroscopically obvious progressed OA changes. OA articular cartilage samples were obtained from three different osteoarthritis patients undergoing total knee replacement surgery. The cartilage samples were obtained from the distal part of both the lateral femoral condyles and medial femoral condyles of the knee joint.

2.2. Human chondrocyte culture

The methods for isolation and treatment of primary chondrocytes were carried out in accordance with the approved guidelines. Chondrocytes were isolated by enzymatic digestion as described previously [18]. Briefly, cartilage tissues were washed with sterile phosphate buffered saline (PBS) and minced into 1 mm^3 tissue slices, which were digested in Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM/F-12, Gibco, USA) medium with 0.2% (w/v) collagenase type II for 10 h at 37 °C, followed by seeding in tissue culture flasks in DMEM/F-12 supplemented with 10% (v/v) fetal bovine serum (FBS, Gibco, USA) within a 5% CO_2 incubator set at 37 °C. After reaching 70–90% confluence after about 7 days, chondrocytes were passaged and cultured as a monolayer. Every 3–5 days, chondrocytes reached 70–90% confluence and were passaged. Passage 1–2 chondrocytes were used in western blot and immunofluorescence studies, and passage 2–3 chondrocytes were used in the rest studies.

Normal chondrocytes were used to evaluate the effects of polymer degradation media as well as lactate and pH on proliferation and cartilage-specific matrix synthesis on chondrocytes; osteoarthritic patients' chondrocytes were used to evaluate the effects of polymer degradation media as well as lactate and pH on cartilage matrix degradation

2.3. Degradation study and L-lactic acid quantification

Poly (lactic-co-glycolic acid) (PLGA) (85:15, Mw = 10,000 g/mol, 100,000 g/mol and 700,000 g/mol) and poly-L-lactate acid (PLLA) (Mw = 10,000 g/mol, 100,000 g/mol and 1000,000 g/mol) were purchased from Jinan Daigang Biomaterial Co., Ltd. 1 g of PLGA and PLLA with different molecular weight were immersed in 3 ml PBS and vibrated for 7 days, the degradation media was collected and subjected to pH value measurement and lactic acid quantification. Lactic acid was quantified with lactate assay kit (Sigma Aldrich, MAK064) followed the manufacturer's protocol.

2.4. Cell proliferation assay

Cell proliferation was assessed by the Cell Counting KIT-8 (CCK-8, Dojindo Laboratories, Kumamoto, Japan). Chondrocytes treated with either PGLA and PLLA degradation media or L-sodium lactate (sigma, 71718) with indicated concentration for 2 days were incubated with culture medium supplemented with 10% (v/v) CCK-8 solution in a 5% CO_2 incubator at 37 °C for 2 h. The absorbance of the culture medium was measured at 450 nm.

2.5. Semi-quantitative real-time polymerase chain reaction (Q-PCR)

Chondrocytes were lysed in TRIzol (Invitrogen Inc., Carlsbad, CA, USA) and total RNA was extracted according to the manufacturer's instructions. Q-PCR was performed using SYBR Green QPCR Master Mix (Takara) with a Light Cycler apparatus (Bio-Rad, CFX-96). The relative expression level of each target gene was calculated using the $2^{-\Delta\Delta}$Ct method. Q-PCR was performed on at least 3 different experimental samples and the representative results are displayed as target gene expression normalized to the reference gene *β-actin*. The primer sequences utilized for Q-PCR are listed in Table 1.

2.6. RNA interference

Chondrocytes at 30–40% confluency were transfected with 100 nM of siRNA utilizing Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol. Small inhibitory RNAs (siRNAs) targeting HIF1A and HIF2A were obtained from Ruibo