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EXPERIMENTAL STUDY

Nicotine-induced chondrogenic differentiation of human bone marrow stromal cells in vitro

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Abstract
Purpose Nicotine has been reported that it has a dose-dependent effect on matrix mineralization by human bone marrow cells. However, there is no relevant research concerning on chondrogenic differentiation potential of bone marrow stromal stem cells (BMSCs) treated with nicotine in vitro. The aims of the study were to examine the effects of nicotine (0, 10^{-7}, 10^{-6} and 10^{-5} M) on the proliferation and chondrogenic differentiation of BMSCs from three healthy donors in vitro.
Methods BMSCs proliferation was analyzed by CCK8 assay and real-time polymerase chain reaction was used to assay the expression of type II collagen, aggrecan, type I collagen and type X collagen. The proteoglycan content was stained by Alcian blue, and the sulfated glycosaminoglycan (sGAG) content of BMSCs was quantified spectrofluorometrically using dimethylmethylene blue.
Results The cell viability was not significantly impaired until up to a concentration of 10^{-5} M nicotine. Nicotine promoted the proliferation and enhanced the expression of type II collagen at the level up to 10^{-6} M ($P < 0.05$). The expression of aggrecan was reduced at the concentration of 10^{-5} M nicotine at day 14 ($P < 0.05$), and there was no significant difference in aggrecan gene expression at 10^{-7} and 10^{-6} M nicotine levels compared to control group (n.s.). Also the fibroblastic and hypertrophic gene expressions were down-regulated in the chondrogenic medium with 10^{-7}–10^{-5} M nicotine ($P < 0.05$).
Conclusion It was implied that local application of nicotine at an appropriate concentration may be a promising approach for enhancing chondrogenic differentiation capacity of BMSCs in cell-based cartilage tissue engineering. Also these results indicate that nicotine maybe a potentially useful drug for the treatment of Osteoarthritis.

Keywords Bone marrow stromal stem cells (BMSCs) · Chondrogenic · Nicotine · Type II collagen · Aggrecan · Cartilage tissue engineering

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Introduction

Articular cartilage has limited capacity to repair damage caused by trauma or disease because of its avascularity and low cellular mitotic activity [13]. The chondral lesions often result in progressive deterioration and eventual osteoarthritis [19]. Surgical procedures are quite unsatisfactory in long-term evaluation and often lead to endoprothetic joint replacement. Other strategies for cartilage repair include transplantation of autograft cartilage or culture-expanded autologous chondrocytes [3]. However, the in vitro expanded chondrocyte experiment results in a progressive loss of specific markers that lead to fibrocartilage formation, devoid of the specific properties of the

articular cartilage, as well as dedifferentiation. Schulze et al. [27] found that dedifferentiation makes chondrocytes which were expanded in vitro no longer capable of redifferentiation when they were re-implanted. In recent years, the use of bone marrow stromal stem cells (BMSCs) represents a good alternative to all of these techniques. BMSCs are characterized by a multilineage differentiation potential and a high proliferative capacity without losing their genetic stability [1]. Therefore, BMSCs have considerable potential for use in the therapeutic regeneration of tissues due to their ability to differentiate into various cell lineages, including osteoblasts and chondrocytes [1, 29].

Cigarette smoking has been associated with chronic musculoskeletal conditions, such as low-back pain and degenerative disc disease [6, 15]. Jaiswal et al. [14] using a case-controlled study to look at whether smoking has a deleterious effect in the outcome of autologous chondrocyte implantation for the treatment of full thickness chondral defects of the knee, and they found that patients who smoke have worse pre-operative function and obtain less benefit from this procedure than non-smokers. Recently, Meidinger et al. [20] found that smoking was one of the risk factors with a statistically significant influence on the development of a non-union after medial open-wedge high tibial osteotomy (HTO). Of the more than 400 agents found in cigarette smoke, nicotine is one of the most physiologically active components. Gullahorn et al. [8] reported that nicotine could upregulate collagen synthesis of human articular chondrocytes in vitro. Also we previously showed that nicotine can promote the proliferation of articular chondrocytes isolated from normal human, and osteoarthritis patients enhance the expression of cartilage-specific type II collagen [34]. Recently, autologous culture-expanded BMSCs have been applied in patients with osteoarthritis [32], and its therapeutic potential for osteoarthritis or the repair of cartilage defect holds out new hope to reverse a painful and debilitating condition suffered by millions of people globally. To our knowledge, there was no relevant research concerning on chondrogenic differentiation potential of BMSCs treated with nicotine. The aims of the study were to examine the effects of various concentrations of nicotine (0, 10^{-7}, 10^{-6} and 10^{-5} M) on the proliferation and chondrogenic differentiation of human BMSCs in vitro. We hypothesized that nicotine had a significant positive effect on cell proliferation and chondrogenic differentiation of BMSCs.

Materials and methods

BMSC isolation and culture

Bone marrow was aseptically aspirated from the iliac crest of three human donors (aged 35–56 years, 1 man and 2 women) who were undergoing elective orthopedic procedures. All of the three patients were non-smokers. Full ethical consent was obtained from all patients, and the study was granted ethical approval by the Medical Ethical Committee of the Second Affiliated Hospital, Wenzhou Medical College. Upon collection, bone marrow was washed with growth culture medium (DMEM, Gibco) supplemented with 10% (V/V) fetal bovine serum (FBS, Gibco), 1% (V/V) penicillin and streptomycin (Gibco). The mixture of bone marrow and medium was gently added to the 50% Percoll solution (Sigma) and centrifuged at 3,000 rpm for 30 min. The cell suspension was obtained between the layer of Percoll and the supernatant liquid layer. Cells were plated and then incubated in a humidified atmosphere of 5% CO_2 at 37°C. The BMSCs were passaged every 3–4 days using 0.25% (w/v) trypsin–EDTA solution (Gibco), and the third or fourth passage was used in our experiments. To induce chondrogenic differentiation, the BMSCs were cultured in 96-well culture plates with chondrogenic medium (growth culture medium supplemented with 50 μg/ml ascorbate-2-phosphate (Fluka), Premix ITSþ (BD Biosciences), 10 ng/ml TGF-β3 (Sigma) and 100 nM dexamethasone (Sigma)) at an initial density of 1×10^4 cells/cm^2. After that they were exposed to varying dosages of nicotine (0, 10^{-7}, 10^{-6} and 10^{-5} M) (Sigma) which bracketed the average 10^{-8}–10^{-7} M seen in chronic smokers [12].

Cytotoxicity studies

Cytotoxicity of nicotine was evaluated by the neutral red assay [2, 30]. Cells that cultured in 96-well plates at 1×10^4 cells per well were treated with increasing concentrations of nicotine (0, 10^{-7}, 10^{-6} and 10^{-5} M). After 24-h incubation, the medium was replaced by medium containing neutral red (3-amino-m-dimethylamino-2-methylphenazine hydrochloride) (Sigma) and cells were incubated for 3 h. The neutral red dye was extracted by a bleaching solution containing 50% ethanol, 1% acetic acid and 49% H_2O. Absorbance was read at 540 nm (ELX 800, Bio-Tek), and cell viability was calculated as percentage of medium treated control cells.

Cell proliferation by cell counting kit-8 (CCK8) assay

For the cell proliferation assays, BMSCs were cultured in 96-well plates at 1×10^4 cells per well with growth culture medium. Twenty-four hours later, cells were switched to the nicotine containing media. The proliferation of BMSCs was determined by the cell counting kit-8 (Kumamoto, Japan) and measured by microplate reader scanning at 450 nm as previously described elsewhere [35].

Alcian blue staining of BMSCs

Alcian blue staining was used to detect proteoglycan synthesis as an indicator of cartilage matrix production. Four groups of cells were stained: control group (BMSC cultured only with chondrogenic medium), chondrogenic medium with 10^{-7} M nicotine, chondrogenic medium with 10^{-6} M nicotine and chondrogenic medium with 10^{-5} M nicotine. The cells at an initial density of 1×10^4 cells were washed with phosphate-buffered saline (PBS) after 7 and 14 days, fixed for 10 min with 4% paraformaldehyde, stained with 1% alcian blue (Fluka, Ronkonkoma, NY) for 30 min and rinsed with distilled water. The cells were assessed by an inverted microscope.

Glycosaminoglycan quantification

For glycosaminoglycan quantification assays, cells were seeded in 24-well plates at 1×10^4 per well. At indicated time intervals (at 7 and 14 day), cells were collected and digested for 18 h at 60°C using 300 mg/ml papain solution (1 ml/sample papain solution). Sulfated glycosaminoglycan (sGAG) accumulation was quantified spectrofluorometrically using dimethylmethylene blue (DMMB) [5]. Standard curves for DMMB assay was generated using an aqueous solution of chondroitin sulfate C (Sigma), with concentrations ranging from 0 to 25 mg/ml. The DNA content in each sample was quantified using a DNA Quant-iT kit (Molecular Probes, Eugene, OR) according to the manufacturer's instructions. All absolute sGAG quantities were normalized by their DNA content.

RNA isolation and real-time polymerase chain reaction (real-time PCR)

Real-time PCR was used to detect the expression of several chondrogenic differentiation related marker genes (COL II, aggrecan, COL I, COL X) at 7, 14 and 21 days respectively. Total RNA was extracted using TriZol (Invitrogen) according to the manufacturer's instructions and quantified. Its concentration was determined spectrophotometrically at 260 nm (HP 8452A Diode Array Spectrophotometer). First strand complementary DNAs (cDNAs) were synthesised from 0.3 mg of the isolated RNA by oligo(deoxythymidine) (oligo(dT)) using DyNamoTM cDNA Synthesis Kit (Fermentas) and used as templates for real-time PCR. The expression of mRNAs was determined quantitatively using DyNamo SYBR1 Green qPCR kit (Takara, Japan).The PCR was performed on a final volume of 25 ml containing 2 ml cDNA, 7.5 pmol of each primer, 1 ml ROX reference dye and 12.5 ml of SYBR Green Master mix (TIANGEN), with ABI Prism 7300 (Applied Biosystems, Foster City, CA, USA). The samples underwent 40 cycles consisting of the following steps: initial denaturation at 95°C for 5 min, followed by a set cycle of denaturation at 94°C for 10 s, different annealing temperatures for each pair of primers (ranging between 53 and 62°C) for 10 s, extension at 72°C for 27 s and a final elongation at 72°C for 5 min. Fold increment of any assayed gene was calculated by normalizing its expression level to that of the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene, which was used as an internal control. Each gene analysis was performed in triplicate. Primer's sequences of the targeted genes were listed in Table 1.

Measuring type II collagen secretion by ELISA

BMSCs were plated at 1×10^4 cells/well in a 96-well plate in chondrogenic medium with different concentrations of nicotine. Four days later, the medium was removed and the cells were washed twice with PBS and lysed in 0.5 ml PBS by three freeze–thaw cycles. Finally, 20–200 μl of cell lysate was analyzed for type II collagen with the use of an ELISA kit (United States ADL's). The results are indicated as μg/l of protein in each sample. The content of type II collagen was measured at 450 nm as OD values using a spectrophotometer after cooling. The amount of type II collagen secreted by the BMSCs under the chondrogenic medium with different concentrations of nicotine was calculated and compared to control group. The results were rounded off to one decimal place.

Statistical analysis

All experiments reported in this study were performed independently at least three times, and data (expressed as mean ± SD) from a representative experiment are shown. Results were expressed as mean ± SD. Analysis of variance (ANOVA) was used to determine statistical significance; values of $P < 0.05$ were considered significant.

Table 1 Sequences of primers used in real-time polymerase chain reaction (real-time PCR)

Gene	Primer sequences (5′–3′)
COL II	Forward GGC AAT AGC AGG TTC ACG TAC A
	Reverse CGA TAA CAG TCT TGC CCC ACT T
Aggrecan	Forward ACT TCC GCT GGT CAG ATG GA
	Reverse GGT GAT GAT CTG GCA CGA GA
COL I	Forward GGC TCCTGC TCC TCT TAG
	Reverse CAG TTCTTG GTC TCG TCA C
COL X	Forward CAG ATTTGA GCT ATC AGA CCA ACA A
	Reverse AAA TTCAAG AGA GGC TTC ACA TAC G
GAPDH	Forward TCT CCTCTG ACT TCA ACA GCG AC
	Reverse CCC TGT TGC TGT AGC CAA ATT C

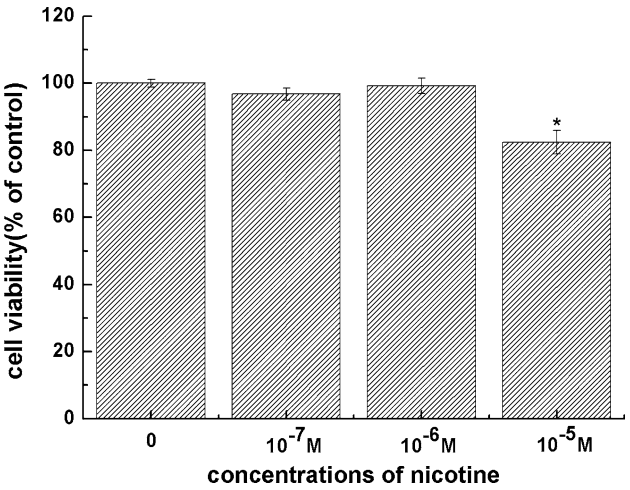

Fig. 1 Effect of nicotine on BMSCs viability was determined by the neutral red assay, and cell viability is expressed as percentage of control (untreated cells). Cells were treated with increasing concentrations of nicotine for 24 h. Nicotine did not impair cell viability until at the concentration of 10^{-5} M. *$P < 0.05$

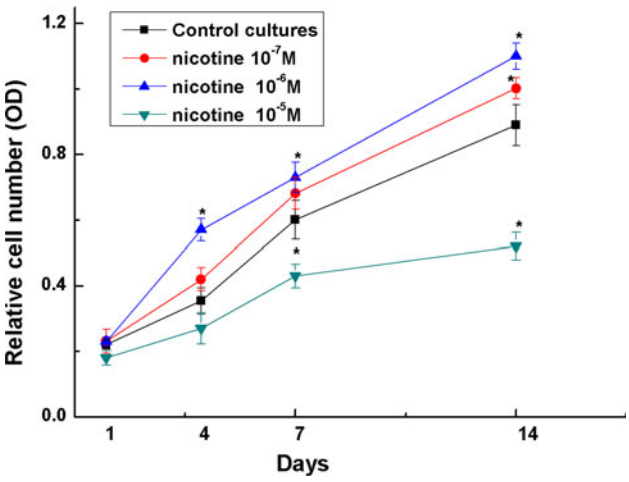

Fig. 2 Effect of nicotine on cell proliferation was analyzed at 1, 4, 7 and 14 days. The relative cell number (value of OD) was significantly increased at the concentration of 10^{-6} M compared to the control in a time-dependent manner, and it was inhibited significantly in the culture treated with 10^{-5} M compared to the control. *$P < 0.05$

Results

Potential cytotoxic effects of nicotine

To study the effect of nicotine on cell viability, Cells were treated with increasing concentrations of nicotine (0, 10^{-7}, 10^{-6} and 10^{-5} M). As shown in Fig 1, cell viability of BMSCs was not significantly impaired until up to a concentration of 10^{-5} M nicotine.

Cell proliferation

Cell proliferation of BMSCs was analyzed at 1, 4, 7 and 14 days respectively. By the CCK8 assay (Fig. 2), the relative cell number (value of OD) was significantly increased at the concentration of 10^{-6} M compared to the control in a time-dependent manner ($P < 0.05$). We also found that the cell proliferation was inhibited significantly in the cultures treated with 10^{-5} M nicotine compared to the control ($P < 0.05$).

Alcian blue staining

After 7 and 14 days of chondrogenic differentiation, the proteoglycan content was stained by Alcian blue. The BMSCs treated with nicotine (10^{-5} M) showed slightly reduced alcian blue staining compared to the control group at day 14 (Fig. 3).

Quantification of sulfated glycosaminoglycans

Because differentiation in Alcian blue staining with or without nicotine treatment could be subtle and non-quantitative, we evaluated total sGAG synthesis, normalized to total DNA content at 7 (Fig. 4a) and 14 (Fig. 4b) days. The sGAG content of the BMSCs that cultured under the chondrogenic medium with 10^{-7} and 10^{-6} M nicotine showed a little increased compared with the control group, but no significant differences were observed (n.s).

The mRNA expression of chondrogenic differentiation related marker genes

Real-time PCR was used to detect the expression of several chondrogenic differentiation related marker genes (COL II, aggrecan, COL I, COL X) at 7,14 and 21 days respectively (Fig. 5). Collagen type II was up-regulated in chondrogenic medium with 10^{-7} and 10^{-6} M nicotine respectively (Fig. 5a). The expression of aggrecan was reduced at the concentration of 10^{-5} M nicotine at 14 days ($P < 0.05$), and there was no significant difference in aggrecan gene expression with other nicotine concentrations (n.s) (Fig. 5b). Both the fibroblastic and hypertrophic gene expressions were down-regulated in the chondrogenic medium with 10^{-7} to 10^{-5} M nicotine, as compared to the control group (Fig. 5c, d).

Detecting type II collagen by ELISA

The content of type II collagen produced by BMSCs which were cultured under the chondrogenic medium with 10^{-7} and 10^{-6} M nicotine was significantly increased compared with control group ($P < 0.05$). Also we discovered that nicotine had shown to keep type II collagen expression positive and increasing from 0 to 10^{-6} M in a

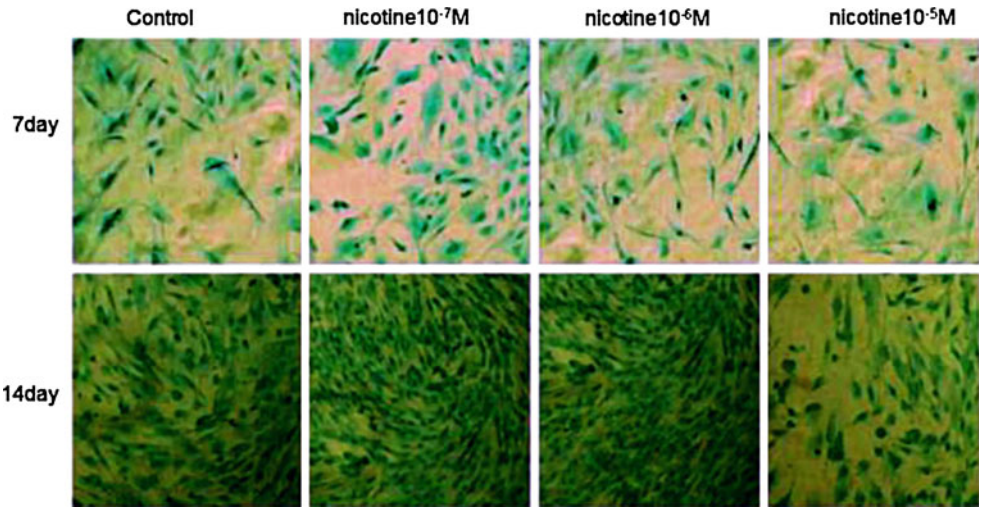

Fig. 3 Alcian blue staining for proteoglycan accumulation in BMSCs treated with chondrogenic media and nicotine. The BMSCs treated with nicotine (10^{-5} M) showed slightly reduced alcian blue staining compared to the control group at day 14. All images were taken at 100× magnification

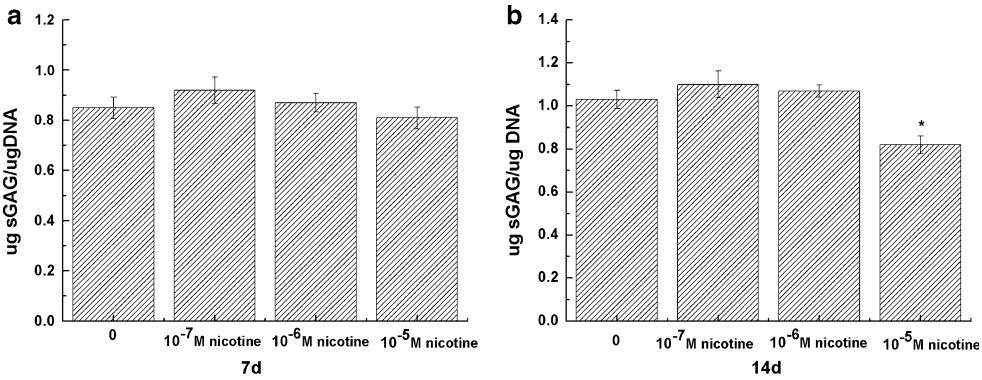

Fig. 4 Quantification of sulfated glycosaminoglycans normalized to total protein content at 7 (**a**) and 14 days (**b**) respectively. The sGAG content was reduced in the BMSCs that cultured under the chondrogenic medium with 10^{-5} M nicotine and were increased at 14 days when compared to the day 7 in all groups except the 10^{-5} M nicotine group. Values are mean ± SD, $*P < 0.05$

time-dependent manner from day 7 to 21 ($P < 0.05$) (Table 2).

Discussion

The most important finding of the present study was that nicotine could promote the proliferation of human BMSCs from 0 to 10^{-6} M and enhance the expression of type II collagen at the level up to 10^{-6} M. Also the study demonstrated that 10^{-7} and 10^{-6} M nicotine could decrease the expression of fibrocartilage marker genes (collagen type I) and hypertrophic marker (collagen type X) in human BMSCs.

The concentrations (10^{-7}, 10^{-6} and 10^{-5} M) of nicotine that we used in the study were in the range of those (1 μM–10 mM) in previously reported studies [4]. Hukkanen et al. [12] reported that blood concentrations of nicotine obtained from chronic cigarette smokers were in the range of 10^{-8}–10^{-7} M. However, the concentrations of nicotine in the saliva of chronic snuff users reached from 0.6 to 9.6 mM [10]. Therefore, the effects of high but physiological concentrations of nicotine on the proliferation and chondrogenic differentiation of cultured BMSCs were shown in the present study.

The results of cell proliferation in the study were consistent with the research from Pereira et al. [24], and they found that exposure of human bone marrow (HBM) cells to nicotine resulted in increased cell proliferation at levels up to 0.2 mg/ml, an initial inhibitory effect in cell growth in the presence of 0.3 mg/ml and a dose-dependent deleterious effect at higher levels. The present study also proved that 10^{-5} M nicotine could decrease the cell viability, and this marked decrease at the concentration of 10^{-5} M was most likely a result of cytotoxic effects. Schraufstatter et al. [26] also reported that high concentrations of nicotine (10^{-5} M)-induced hMSC apoptosis, physiological concentrations (10^{-7}–10^{-6} M) did not interfere with cell survival.

Cartilage extracellular matrix is the place where chondrocytes play the physical effect in vivo. It is the place chondrocytes absorption nutrition and as a carrier to deliver the signal. Metabolic balance of the extracellular matrix is an important part to maintain the normal function of cartilage. Type II collagen and aggrecan were the most widely recognized marker of chondrogenic differentiation [28, 31, 33].

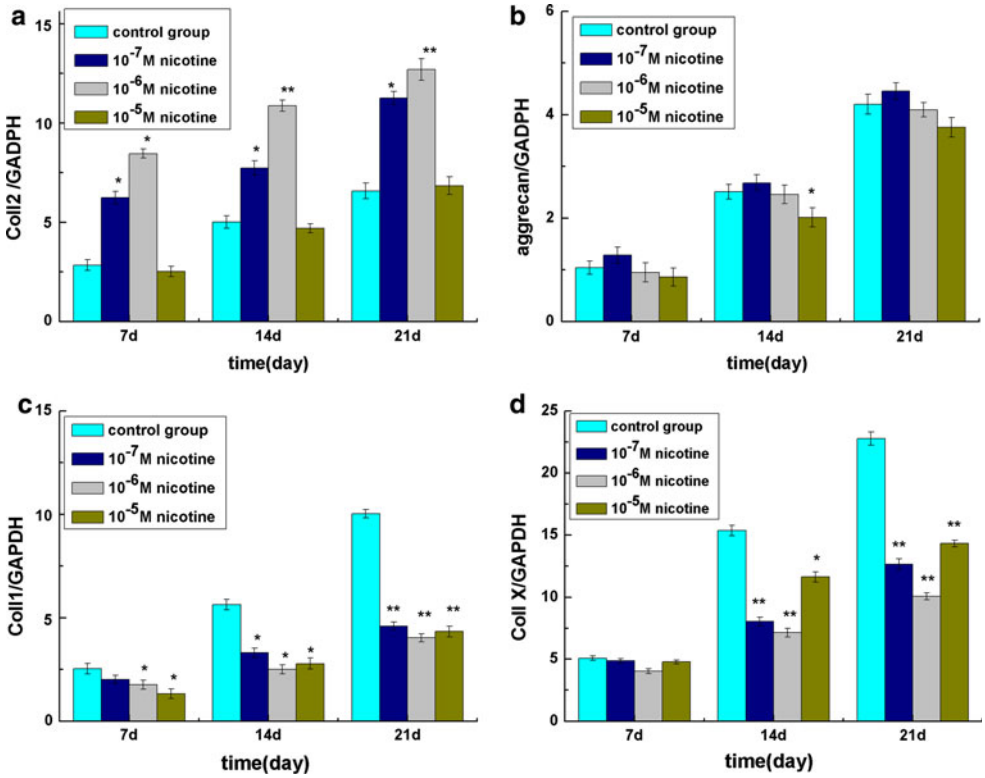

Fig. 5 Real-time polymerase chain reaction analysis indicated more chondrogenic markers but less fibroblastic and hypertrophic markers of BMSCs cultured in chondrogenic medium with different concentrations of nicotine than control group (chondrogenic medium without nicotine): **a** COL II: 10^{-7} M nicotine (collagen type II: day 7–day 21, $P < 0.05$) and 10^{-6} M nicotine (collagen type II: day 7, $P < 0.05$; day 14–day 21, $P < 0.01$); **b** aggrecan; **c** COL I: from day 7 to 21 (day 7–day 14, $P < 0.05$; day 21, $P < 0.01$); **d** COL X: from day 7 to 21 (day 7, $P > 0.05$; day 14–21, $P < 0.01$). Values are mean ± SD, $n = 8$, $^{*}P < 0.05$, $^{**}P < 0.01$

Table 2 The content of type II collagen produced by BMSCs in chondrogenic medium with different concentrations of nicotine ($n = 8$)

Grouping	n	7d	14d	21d
Control group	8	72.6 ± 10.7	95.8 ± 12.4	111.6 ± 14.9
10^{-7} M nicotine	8	103.2 ± 14.6*	123.8 ± 15.5*	147.5 ± 16.3*
10^{-6} M nicotine	8	118.7 ± 12.5*	144.8 ± 13.8*	171.2 ± 18.4*
10^{-5} M nicotine	8	68.5 ± 9.8	91.2 ± 16.2	110.4 ± 17.1

* Compared with control group, $P < 0.05$

The changes of type II collagen and aggrecan in the quantity, and quality are the direct cause of the loss of the normal articular cartilage biomechanical properties [9]. In this study, we concluded that nicotine has shown to keep type collagen expression positive and increasing from 0 to 10^{-6} M in a time-dependent manner, and it was reduced at the higher nicotine level of 10^{-5} M. RNA level for aggrecan was not influenced at the concentration of 10^{-7} to 10^{-5} M at 7 days when compared to control group; however, it was decreased at 10^{-5} M nicotine at 14 days. Additionally, we detected the proteoglycan content by Alcian blue stain after 7 and 14 days. The BMSCs treated with nicotine (10^{-5} M) showed slightly reduced alcian blue staining compared to the control group at 14 days. Because the differences in Alcian blue staining with or without nicotine treatment could be subtle and non-quantitative, we evaluated total sGAG synthesis, normalized to total DNA quantities. We found that the sGAG content was a little increased at 10^{-7} and 10^{-6} M nicotine levels compared with the control group, although there were no significant differences and it was reduced in the BMSCs that cultured under the chondrogenic medium with 10^{-5} M nicotine. In the literature, there was no study published, related to the interaction of nicotine with chondrogenic differentiation capacity of BMSCs. However, the studies related to the effect of nicotine on chondrocytes had been reported previously. Gullahorn et al. [8] reported that nicotine (2.5, 12.5 and 25 ng/ml) can up-regulate collagen II synthesis of human articular chondrocytes in vitro. Also, it was consistent with what we have reported previously that nicotine can promote the proliferation of articular chondrocytes isolated from normal human and osteoarthritis patients enhance the expression of cartilage specific type II collagen [34]. In contrast, Kawakita et al. [17] discovered that nicotine, from cigarette smoking, acted directly on growth plate chondrocytes to decrease matrix synthesis, suppressed hypertrophic differentiation via alpha7 nAChR, leading to delay skeletal growth. The great variability of results regarding chondrogenic response to nicotine reflected differences in the experimental conditions maybe relate to the differences of cell sources and culture conditions. In Kawakita study, the chondrocytes were harvested from growth plate of human, a major component of endochondral ossification, and

cultured in agarose gel culture and alginate bead culture. But in Gullahorn study, the chondrocytes were harvested from human articular and cultured in monolayer in vitro.

In vitro culture of BMSCs eventually leads to hypertrophic chondrocytes, which differentiates toward osteogenic lineages [23]. Gene expression of collagen type X has been reported to be the marker of hypertrophic chondrocytes [22]. In this study, the expression of collagen type I, dominantly expressed in fibrocartilage, was down-regulated in hBMSCs cultured in the chondrogenic medium with 10^{-7}–10^{-5} M nicotine, as compared to the control group. Moreover, the gene expression level of the hypertrophic marker, collagen type X, was also lower in hBMSCs cultured in the chondrogenic medium with 10^{-7}–10^{-5} M nicotine at day 7, 14 and 21. Our results indicated that the chondrogenic medium with different concentrations of nicotine inhibited fibrocartilage formation and hypertrophy in hBMSCs.

Ma et al. [18] showed recently that nicotine at low dose had no significant effect on the expression of bone morphogenetic protein-2 and on the radiodensity of bone regeneration. However, the delayed bone healing was detected in the nicotine group by histologic examination, subsequently, there is a potential risk of compromised bone healing in patients taking nicotine medication and maybe also then smoking as well. Nelson et al. [21] discovered that smoking not only reduced body length but also brought ossification retardation in a rat-smoking model. From these results, we speculated that nicotine may suppress the mesenchymal stem cells on osteogenic pathway and guide them instead into a chondrogeneic route. Many studies had proved that elements of the cholinergic system including acetyltransferase, acetylcholinesterase and acetylcholine receptors (AChRs) were expressed in a large array of non-neural cells including epithelial and endothelial cells, mature blood cells, hematopoietic progenitors, osteoblasts and mesenchymal stem cells [7, 11, 25]. And nicotine, as an agonist of the nicotinic acetylcholine receptors (nAChR), maybe play an important part in the process of the proliferation and chondrogenic differentiation of BMSCs. Kalamida et al. [16] reported that a nAChR consisting of a homopentamer of α7 atypically provides gating to calcium ions. Schraufstatter et al. [26] discovered that nicotine evoked transient calcium fluxes and increased intracellular calcium. From these, we speculated that changes in the intracellular levels of Ca^{2+} ions, following binding of nicotine to its receptor and subsequent activation of voltage-dependent Ca^{2+} channels, may modulate, at least in part, the effects of nicotine on the proliferation and chondrogenic differentiation of BMSCs.

Articular cartilage has limited capacity to repair damage caused by trauma or disease because of its avascularity and low cellular mitotic activity [13]. The chondral lesions often result in progressive deterioration and eventual osteoarthritis [19]. Articular hyaline cartilage injuries still pose a big challenge to orthopedic surgeons, because these defects have poor capacity for intrinsic repair. In recent years, cell-based articular cartilage tissue engineering studies were focused on using either differentiated chondrocytes or BMSCs for transplantation. However, limited proliferative capacity of BMSCs possesses a major challenge in providing adequate cell numbers for viable transplantations and cartilage repair. Construction of a 3D biomaterial with autologous BMSCs to regenerate defected articular cartilage may become a viable clinical option. In our study, we found nicotine could promote the proliferation of human BMSCs and enhance the expression of type II collagen, these results implied that local application of nicotine at an appropriate concentration with 3D biomaterial such as Poly-lactic-co-gly-colic acid (PLGA) may be a promising approach for enhancing chondrogenic differentiation capacity of BMSCs in cartilage tissue engineering.

The limitation of this study was that we did not test the chondrogenic differentiation capacity of BMSCs with nicotine influence ceases, so further studies upon the influence of nicotine ceases and the molecular mechanism would be performed to bring to light how nicotine affects the process of chondrogenic differentiation of BMSCs. As the results of nicotine on chondrogenic differentiation of BMSCs in the present study were cultured in monolayer and in vitro, a three-dimensional culture needs further validation in vitro and in vivo.

Conclusion

This is the first study to demonstrate that nicotine could promote the proliferation of human BMSCs from 0 to 10^{-6} M and enhance the expression of type II collagen at the level up to 10^{-6} M. However, both the cell proliferation and the express of collagen type II were inhibited when treated with 10^{-5} M nicotine. The expression of aggrecan was reduced at the concentration of 10^{-5} M nicotine at 14 day, and there was no significant difference in aggrecan gene expression at 10^{-7} M and 10^{-6} M nicotine levels compared to control group. Also the reduced expressions of the fibroblastic and hypertrophic gene in the chondrogenic medium with 10^{-7} to 10^{-5} M nicotine were demonstrated in the study. These results implied that local application of nicotine at an appropriate concentration may be a promising approach for enhancing chondrogenic differentiation capacity of BMSCs in cartilage tissue engineering.

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