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Journal Name

ARTICLE

Methylsulfonylmethane-loaded electrospun poly(lactide-co-glycolide) mats for cartilage tissue engineering

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Methylsulfonylmethane (MSM) is popularly used for the therapy of arthritic and rheumatic diseases but seldom in tissue engineering application for cartilage regeneration. In this study, biodegradable poly(lactide-co-glycolide) (PLGA) fibrous mats containing MSM with different doping levels were fabricated by electrospinning. The MSM-loaded mats were interconnected with smooth, uniform micro- and nano-fibers. *In vitro* drug release of MSM-loaded mats and the biological activity for chondrocytes were investigated. The results showed that the total MSM release of 0.01 wt%, 0.1 wt%, 1 wt% and 10 wt% MSM/PLGA mats within 48 hours were 83.5%, 80.7%, 72.2%, and 51.5%, respectively. Because of the excellent bioactivity of MSM, the MSM-loaded mats showed much better cell proliferation and ECM formation ability than that of PLGA mat. Among them, the 0.1 wt% MSM/PLGA mat showed the best cell proliferation. More importantly, the MSM/PLGA mats, especially for the 10 wt% group, also promoted extracellular matrix (ECM) formation, the cartilage related gene expression of collagen type II, aggrecan, and collagen type I, and cartilage specific protein expression of collagen type II. Together, findings from this study have revealed that the electrospun MSM-loaded PLGA mat is a promising candidate for cartilage regeneration.

Introduction

Tissue engineering is a promising approach for cartilage tissue regeneration using cells, scaffolds, and growth factors, alone or in combination.¹ Although growth factors are necessary for cartilage regeneration in adjusting growth, differentiation and extracellular matrix (ECM) synthesis of chondrocytes, they are still limited in clinical applications because of high expense, short half-life, and the potential risk of inducing cancer cell development.^{2,3} Another distinctive problem is that the growth factors are usually unstable and hard to be manipulated when they are processed to be combined with polymer scaffolds for local delivery.⁴ Thus, searching for some molecules with bioactivity, stability and easy processability with polymers is necessary for polymeric tissue engineering scaffolds.

Methylsulfonylmethane (MSM, (CH₃)₂SO₂), also known as dimethyl sulfone or methyl sulfone, is a naturally occurring organosulfur molecule. Because of its sulfur content, MSM is used in body to maintain normal connective tissues. The commercial MSM is synthesized by reacting dimethyl sulfoxide (DMSO) and hydrogen peroxide, and is popularly used for arthritic and rheumatic pain.⁵ MSM has anti-inflammatory activities, chemopreventive properties, prostacyclin (PGI₂) synthesis inhibition, anti-atherosclerotic action, salutary effect on eicosanoid metabolism, and free radical scavenging activity.⁶ Oshima et al.⁷ investigated that MSM incubation of

chondrocytes derived from humans with moderate severity osteoarthritis reduced the activation of genes coding for the manufacture of pro-inflammatory cytokines. It indicated that MSM might have an ability to protect articular cartilage in osteoarthritis. However, there are few reports of MSM application in biomedical fields, such as cartilage tissue engineering. The extensive solubility of MSM in acetone helps in its easy incorporation into polymer scaffolds by the electrospinning approach for tissue engineering application.

Drug delivery in tissue engineering is often associated with the delivery of therapeutic agents for the treatment of some diseases such as osteoarthritis, it can also be applied to the delivery of bioactive agents for tissue regeneration.⁸ Recently, many studies have demonstrated the application of electrospinning as a platform technology to generate fibrous scaffolds for drug delivery in tissue engineering applications.⁹⁻¹¹ Electrospinning process was firstly developed to produce ultra-fine polymer fibers and afterwards used as a novel tool to generate nano-scale fibers that mimic ECM.¹² Ma et al.⁹ reviewed that fibers with diameters ranging from nano-meter to micro-meter scales could be prepared by electrospinning. The electrospun fibers have been generated from numerous biodegradable natural and synthetic biopolymers, such as chitosan,¹³ cellulose,¹⁴ collagen,¹⁵ and poly(lactide) (PLA),¹⁰ to mimic the ECM in structure. These biodegradable polymeric micro- and nano-fibers have been investigated for a multitude of biomedical applications such as tissue engineering scaffolds, wound dressing, drug delivery, and vascular grafts.^{16,17} Among them, PLGA is a frequently used biomaterial for tissue engineering application, such as cartilage tissue engineering.^{18,19}

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In this study, MSM-loaded micro- and nano-fibrous PLGA mat was successfully fabricated by electrospinning. The morphology and chemical properties of electrospun mats were characterized by an environmental scanning electron microscope (ESEM, XL30 FEG, Philips) and ^1H Nuclear Magnetic Resonance (^1H NMR). The *in vitro* drug release of MSM-loaded mats was also investigated. Meanwhile, the effects of electrospun mats on chondrocytes proliferation, ECM formation, cartilage related gene expression, and cartilage specific protein expression were further studied.

Experimental

Materials

Poly(lactide-co-glycolide) (PLGA, $M_w = 70,000 \text{ g mol}^{-1}$) with a lactic acid-glycolic acid ratio of 80:20 was synthesized in our laboratory according to the reference.²⁰ D,L-Lactide (LA) and glycolide (GA) were purchased from Purac (Netherlands). MSM (analytical grade) was presented by Jilin Herun Chemical Co. (Jilin, China). 3-(4,5-Dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was purchased from Sigma-Aldrich (USA). The reagents for cell experiments were purchased from Gibco (USA). Animals were purchased from the Institute of Experimental Animal of Jilin University, China.

Fabrication and Characterization of MSM-loaded Mats

MSM powder was dissolved completely in acetone to prepare MSM/acetone solution. The solution was dispersed into 10% (w/v) PLGA/chloroform solution to prepare the composite solution of MSM/PLGA. The volume ratio of acetone and chloroform was the same and the contents of MSM in the composites were 0.01 wt%, 0.1 wt%, 1 wt% and 10 wt%, respectively. The solutions were gently stirred overnight at room temperature in order to obtain a homogenous suspension. PLGA without addition of MSM was served as a control group.

The electrospinning set-up employed in this study has been depicted previously.^{21,22} Briefly, the electrospinning parameters were as follows: applied voltage: 40 kV; air gap distance: 20 cm; inner diameter of spinneret: 0.4 mm; and flow rate of the solution: 0.07-0.10 mL min^{-1} . The electrospinning experiment was carried out under ambient condition. In order to remove the residual solvent, the collected mats were dried in vacuum at room temperature for about 48 hours.

The surface morphology and fiber size of the electrospun mats were determined by ESEM and statistically analyzed with ImageJ software. The accelerating voltage for ESEM was 15 kV. The elemental composition of the mats was analyzed with an energy dispersive X-ray spectrometry (EDS) (Philips, XL-30W/TMP, Japan). ^1H NMR spectra was recorded on a Bruker AV 400M in CDCl_3 .

In Vitro MSM Release

20 mg of 0.01 wt%, 0.1 wt%, 1 wt% and 10 wt% MSM-loaded mats were accurately weighed and soaked in 12 mL Phosphate buffered saline (PBS) (pH=7.2) at 37 °C and shaking at 100 rpm,

respectively.²³ The release liquid was taken from 0.5 h to 48 h, and refreshed with 12 mL PBS for each sample. The quantitative of sulphur (W_s) in the release liquid was determined using the inductively coupled plasma atomic emission spectroscopy (ICP-OES, Leeman Prodigy High Dispersion ICP, USA). Then the release amount of MSM (W_{MSM}) in each period was obtained from the following equation:

$$W_{\text{MSM}} = W_s \times M_{\text{MSM}} / M_s$$

M_{MSM} and M_s indicate the Molar mass of MSM and sulphur with 94 g mol^{-1} and 32 g mol^{-1} , respectively.

The productivity of the electrospun mats were also measured via ICP-OES.

Cell Isolation and Culture

Chondrocytes were isolated and cultured according to our previous method.²⁴ The use of animals was in accordance with the institutional guidelines for care and use of laboratory animals. Briefly, full-thickness articular cartilage was obtained from newborn New Zealand White Rabbit, and chipped into small pieces. Chondrocytes were isolated by trypsin (2.5 g L^{-1}) treatment of cartilage pieces for 30 min, followed by type-II collagenase (2 g L^{-1}) digestion for 3-4 h, and filtered through a nylon sieve with the pore size of 75 μm . After rinsed for 3 times with 0.1 M PBS and centrifuged at 1500 rpm for 5 min, the obtained cells were re-suspended with Dulbecco's modified Eagle's medium (DMEM) supplemented by 10 vol% FBS, 50 mg L^{-1} L-ascorbic acid, 10 mM HEPES, $1.0 \times 10^5 \text{ U L}^{-1}$ penicillin and 100 mg L^{-1} streptomycin, and incubated at 37 °C in a humidified atmosphere with 5% CO_2 . The medium was changed every 2 days. After 7-10 days of culture, the monolayer chondrocytes were harvested and re-suspended in the medium for the following assessment.

Cell Proliferation

The cell proliferation of chondrocytes on electrospun mats was determined using the MTT assay. Each surface of the mats was sterilized by ultraviolet rays (UV) for 1 h. Round samples of the mats with diameter of 15 mm and thickness of 0.06 mm were placed in 24-well tissue culture plates avoiding them floating. The chondrocytes of 2nd to 3rd passage in a density of 5.0×10^4 cells/500 μL /well were seeded onto the mats and left undisturbed in the incubator for 4 h to allow cells attachment. Then each well was supplemented with additional medium. The plates were returned to the humidified incubator and cultured for 1, 4, 7 and 10 days. The medium was renewed every 2 days. Four hours before each culture time interval, 100 μL of MTT (5 g L^{-1} in PBS) was added to each well and the cells were incubated for an additional 4 h. Then the medium was removed and 800 μL of acidified isopropanol (0.2 mL of 0.04 N hydrochloric acid (HCl) in 10 mL of isopropanol) was added to each well to solubilize the converted dye. The plates were returned to the humidified incubator and cultured for 30 min. Then 200 μL of the solution in each well was transferred to a 96-well plate, and optical density was measured at 540 nm wavelength on a Full wavelength Microplate Reader (Infinite M200, TECAN). The

mean value of three parallel samples for each material was used as the final result.

Meanwhile, the mats seeded with chondrocytes were collected after incubation for 7 days and fixed with 25 g L⁻¹ glutaraldehyde for 2 h at room temperature. The samples were washed with distilled water for 3 times, dehydrated through a graded series of ethanol and freeze-dried for 48 h before ESEM observation.

Quantitative Real-time PCR analysis

The chondrocytes cultured on the mats for 7 days were also collected for evaluation of cartilage related gene expression.

Total RNA was extracted using a Qiagen RNeasy micro kit according to the manufacturer's instructions. Quantitative real-time PCR was carried out and analyzed to assess the gene expression of collagen type II, aggrecan, and collagen type I. RNA samples were reverse transcribed using the Quantitect reverse transcription kit (Qiagen). Real-time PCR was carried out using Mx3005P (Stratagene, USA) with the following primers: collagen-II (F-CTCAAGTCCCTCAACAACC, R-AGTAGTCACCGCTCTTCC), aggrecan (F-CAAGGACAAGGAGGTGGTG, R-GTAGTTGGGCAGCGAGAC), collagen-I (F-CTCGTCCACCACCTTCTC, R-TAACCACTGCTCCACTCTG), (Shengong Inc, Shanghai, China) and GAPDH (F-GATGGTGAAGGTCGGAGTG, R-TGTAGTGGAGGTCAATGAATG) was served as the housekeeping gene to normalize expression for the genes. Primer sequences for the genes were validated by dissociation curve/melt curve analysis.

Immunofluorescence Staining

Chondrocytes grown on the mats for 7 days were also fixed in 40 g L⁻¹ paraformaldehyde in PBS for 30 min, followed with PBS washing for 3 times. The samples were incubated with 10 vol% normal blocking serum in PBS for 20 min to suppress non-specific binding of IgG, followed with collagen II antibody (Abcam) and then appropriate secondary antibody conjugated to FITC. The nucleus were stained with DAPI. The cells were observed with an inverted fluorescence microscope (TE 2000U, NIKON) and the pictures were taken by a digital camera (DXM 1200F, NIKON). Meanwhile, the samples treated with collagen II antibody/FITC-conjugated secondary antibody were further stained with DAPI and observed under a confocal laser scanning microscopy (CLSM, Leica DMIRE2, Germany).

Statistical Analysis

All quantitative data were analyzed with OriginPro 8.0 (Origin Lab Corporation, USA) and expressed with the mean \pm standard deviation. Statistical comparisons were carried out by analysis of

variance (ANOVA, OriginPro 8.0). A value of $p < 0.05$ was considered to be statistically significant.

Results and Discussion

Morphology of Electrospun Mats

Tissue engineering requires a scaffold to provide a three dimensional (3D) structure mimicking natural extracellular matrix (ECM) to support cells and guide cell behavior.²⁵ Electrospun mats have a great potential to mimic the important features of natural ECM in terms of structure and can be further functionalized via incorporation with bioactive species (e.g. drugs, enzymes, DNAs and growth factors) to better control the proliferation and differentiation of cells.^{26, 27} Some therapeutic drugs, such as metronidazole and nitrofurazone, have been incorporated into electrospun fibers.^{28, 29} In this study, we prepared MSM-loaded mats via electrospinning intending to develop its application in cartilage tissue engineering.

As shown in Fig. 1, the surface morphology of electrospun PLGA and MSM/PLGA mats containing different amounts of MSM observed by ESEM. The fibers were interconnected and uniform. The diameters of fibers in PLGA, 0.01 wt%, 0.1 wt%, 1 wt% and 10 wt% MSM/PLGA mats were $1.04 \pm 0.27 \mu\text{m}$, $0.83 \pm 0.29 \mu\text{m}$, $0.56 \pm 0.19 \mu\text{m}$, $0.65 \pm 0.23 \mu\text{m}$ and $0.84 \pm 0.22 \mu\text{m}$, respectively. Incorporation of different amounts of MSM did not seem to apparently affect the diameter of electrospun fibers. MSM is well soluble in acetone so it can be uniformly mixed into electrospun system. Consequently, the incorporation of MSM does not affect the morphology of fibers and the electrospun mats have the ECM-like microstructure, which are expected to provide space gradually for supporting cell ingrowth and migration.

¹H NMR and EDS Analysis

The surface chemistry of fibrous mats was characterized via ¹H NMR and EDS analysis. As shown in Fig. 2a, according to the ¹H NMR results, the hydrogen peaks of PLGA fibers were located at 1.5, 5.0, 5.1, and 7.2 ppm, respectively. The hydrogen peak of MSM was at 2.9 ppm and its characteristic peak appeared in the MSM-loaded fibers due to the addition of MSM (Fig. 2b). The EDS element analysis showed that there was existence of sulfur (S) on the surface of the MSM/PLGA fibers (Fig. 2b) compared to that of PLGA fibers (Fig. 2a). The results indicated that MSM was doped into the fibers successfully and the detection of sulfur might be a simple way to prove the addition of MSM into the MSM-loaded mats.

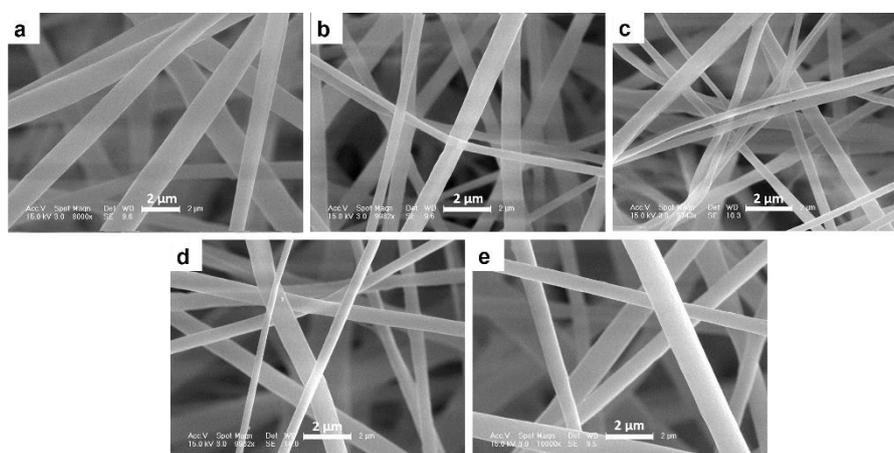


Fig.1. ESEM micrographs of the electrospun mats of PLGA (a) and MSM/PLGA with the MSM content of 0.01 wt% (b), 0.1 wt% (c), 1 wt% (d) and 10 wt% (e). Bar lengths are 2 μm .

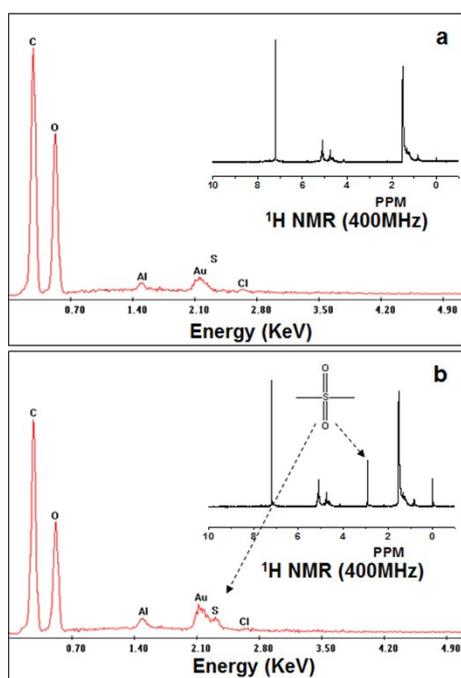


Fig.2. ^1H NMR spectra (400MHz) (inside) and EDS analysis of PLGA (a) and 10 wt% MSM/PLGA (b) mats.

In vitro MSM release

Drug encapsulation efficiency is highly influenced by the characteristic of the drugs, such as sublimability and

compatibility between the drugs and polymer matrix. The productivity of MSM-loaded electrospun mats, prepared under

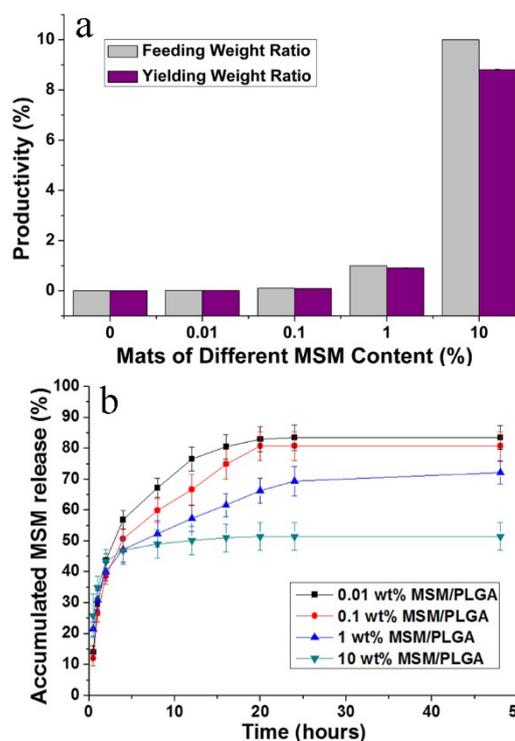


Fig.3. The release curves of MSM/PLGA mats immersed in PBS buffer at 37 $^{\circ}\text{C}$ for 0.5 h to 48 h.

different feed weight percentages of MSM, were summarized in Fig.3a. The content of MSM in the mats increased with the increase of feed weight fraction. Due to the sublimation of MSM at room temperature, the MSM content is corresponding lower than the theoretical value and with the increase of MSM loading, the MSM encapsulation efficiency decreases. The similar phenomenon was also observed in the incorporation of metronidazole into PCL-gelatin electrospun fibers and the drug cannot completely encapsulated into the fibers.²⁸

Fig. 3b showed the release curves of the MSM-loaded mats in PBS buffer. The release amount of MSM in all MSM/PLGA mats increased with time extension and up to about 50% of the total amount during the first 4 h with an initial burst release. Then, the release of 10 wt% MSM/PLGA mat turned into plateau after 8 h. The release amount of 0.01 wt%, 0.1 wt% and 1 wt% MSM/PLGA mats continuously increased up to 83%, 81% and 66% at 20 h, respectively, and then reach to a plateau. Total amount of MSM released from 0.01 wt%, 0.1 wt%, 1 wt% and 10 wt% MSM/PLGA mats within 48 h were 83.5%, 81.7%, 72.2%, and 51.5%, respectively. MSM is a good water-soluble drug, so a fast initial drug release was obtained with the dissolution from the surface of fibers. Then with the drug dissolution and degradation of PLGA, a stable and sustained release was observed. The burst release of MSM could have a preliminary effect for the cartilage defect and the gradual dissolved MSM could accelerate cell proliferation and ECM formation. As the results showed, we can obtain satisfactory sustained MSM releasing mats with adjusting the amount of drug in MSM-loaded mats to get preliminary cure and substantial effect to the cartilage defect.

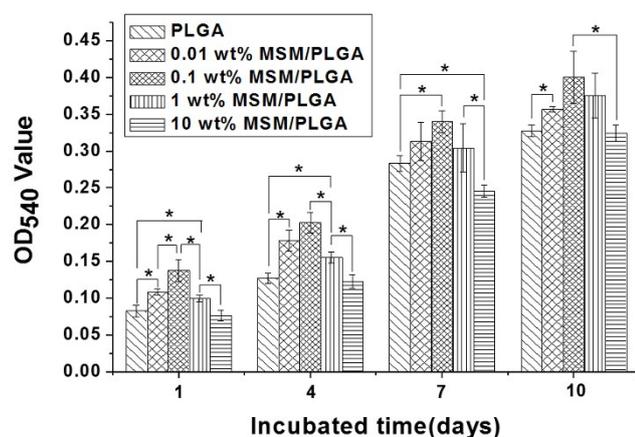


Fig.4. Proliferation of chondrocytes cultured on the electrospun mats for 1 to 10 days *in vitro*. * $p < 0.05$ compared to the other group, $n = 3$.

The drug release study indicated that the controlled release of MSM could be accomplished by the electrospun MSM/PLGA mats. And the controlled release of MSM by the electrospun polymer fibers may provide a novel approach for prolonging its *in vivo* process for the therapy of osteoarthritis or tissue engineering application. Furthermore, it is necessary to adjust and improve the electrospinning strategies such as preparing multilayered mats to obtain more substantial release for cartilage tissue repair.²⁹

Cell Proliferation and ECM Formation

The cell proliferation of chondrocytes grown on MSM-loaded electrospun mats was determined using MTT assay. As shown in Fig. 4, the number of chondrocytes in all groups increased obviously with incubation time. The proliferation of chondrocytes was similar on 0.01 wt%, 0.1 wt% and 1 wt% MSM/PLGA mats, better than that of PLGA and 10 wt% MSM/PLGA mats during 10 days of culture, albeit with some slight differences. Among them, 0.1 wt% MSM/PLGA mat presented the highest density of chondrocytes at all the time intervals, indicating the best effect on chondrocyte proliferation. When the MSM content increasing from 1 wt% to 10 wt%, the cell density decreased significantly. The cell density on 10 wt% MSM/PLGA mat was even lower compared to those of PLGA mat during all time intervals, especially for 7 days. The results indicated that MSM was a bioactive molecule promoting the proliferation of chondrocytes with appropriate dosage. The adequate content of MSM in PLGA mats for cell proliferation is 0.1 wt%, but overdose of MSM in the mats might decrease their bioactivity or even inhibit cell growth. Kim et al.⁶ reported that MSM was not toxic to murine macrophages when the cells were exposed to 10 mg/mL MSM for 24 h. *In vivo* study also showed that oral administration of 1.5g/kg/day MSM in rats for 90 days did not cause any adverse effects or mortality.³⁰

Fig. 5 showed ESEM micrographs of chondrocytes grown on different mats. After 7 days of culture, chondrocytes attached and grew well on all mats. The cell morphology and ECM formation of 0.01 wt% MSM/PLGA mat (Fig. 5b and g) was similar to those of PLGA mat (Fig. 5a and f). However, much ECM formation was observed on 0.1 wt%-10 wt% MSM/PLGA mats (Fig. 5c-e and h-j). The ECM was confluent on the surfaces of mats and the cells could not be distinguished clearly in these groups. The results indicated that the incorporation of MSM could improve the ECM formation of chondrocytes on MSM/PLGA mats as the MSM content up to 10 wt%. Especially, 10 wt% MSM/PLGA mat also presented an increased ECM formation similarly to 0.1-1 wt% MSM/PLGA mats even if its cell proliferation was lower than the others.

The constituents of cartilage matrix are proteoglycans, containing considerable chondroitin sulfate and keratan sulfate, and the collagen

based network. Therefore, it may be considered that some sulfur compounds or collagen supplementation can have a beneficial effect on joint diseases. However, cartilage formation was not observed in rats with intake of MSM at a series of dose.³¹ Richmond et al. found that MSM could provide a source of sulfur amino acid in the guinea pig suggesting that MSM acts as a sulfur resource to any compounds that were involved with protection and development of cartilage and prevented knee joint damage.³² In this study, cartilage matrix was obviously observed with incorporation of different amount of MSM into the fibers.

Combining with the results of cell proliferation, we might conclude that the incorporation of MSM could improve the ECM formation of chondrocytes with the content of 0.1-10 wt%, especially for 10 wt% MSM-loaded mats.

Gene and Protein Expression

According to Joung et al.'s study,³³ MSM can promote osteogenic differentiation of mesenchymal stem cells (MSCs) and intensify the growth of bone. However, it remains unclear that how MSM affect the gene expression of chondrocytes even if it has been widely used as an effective drug for arthritic and rheumatic.

The cartilage related gene expression of chondrocytes cultured on the electrospun mats for 7 days was analyzed using quantitative real-time PCR. As shown in Fig. 6, the gene expression of collagen type II, aggrecan and collagen type I was promoted obviously with the incorporation of MSM. The expression of collagen type II was significantly higher for 0.01-1 wt% MSM/PLGA groups than PLGA group ($p < 0.05$) and 10 wt% group was higher than all the other groups ($p < 0.05$). There was no significant difference among the 0.01-1 wt% MSM/PLGA groups. It indicated that all the MSM-loaded mats could promote collagen type II synthesis of chondrocytes, especially for the 10 wt% group. The gene expression of aggrecan and collagen type I was significantly promoted by 10 wt% MSM/PLGA mat compared with the other groups ($p < 0.05$). Whereas, no obviously difference was found among PLGA and 0.01-1 wt% groups. As collagen type I is not the main component of cartilage, its gene expression was a little lower than that of aggrecan and collagen type II for all the groups.

The results of quantitative real-time PCR analysis indicated that the incorporation of MSM in PLGA mat enhanced the chondrogenic differentiation of chondrocytes, especially for the 10 wt% MSM/PLGA mat even if its lower cell proliferation. It could also well explain that the difference of ECM formation among all mats according to the ESEM observation. It was different from the results of the literature.⁷ They found that MSM did not show an increase in proteoglycan synthesis in cultured chondrocytes or an increase of cartilage matrix production in normal and osteoarthritic chondrocytes at the mRNA level.

Finally, protein expression of collagen type II by the chondrocytes grown on the electrospun mats was evaluated by immunofluorescence staining. Fig. 7a-c showed the images of collagen type II observed by inverted fluorescence microscope. After 7 days of culture, expression of collagen type II on the 0.1 wt% and 10 wt% MSM/PLGA mats (Fig. 7b-c) was much more than that on PLGA mat (Fig. 7a). The cell density of chondrocytes grown on 0.1 wt% MSM/PLGA mat (Fig. 7b) was greatly higher than those on PLGA and 10 wt% MSM/PLGA mats (Fig. 7a and c). Although the cell density of 10 wt% MSM/PLGA mat decreased and was similar to that of PLGA mat, its protein expression of collagen type II was enhanced. It was in well accordance with the results of MTT assay, ESEM observation and gene expression.

The results of collagen type II protein expression were further verified by the confocal microscopy as shown in Fig. 7d-f. The area of collagen type II staining (green) outside the nuclei (blue) for a single cell was larger on 0.1 wt% (Fig. 7e) and 10 wt% MSM/PLGA mats (Fig. 7f) than that on PLGA mat (Fig. 7d). It was deduced that the protein expression of collagen type II on MSM/PLGA mats can be up-regulated as MSM content was up to 10 wt% even if the cell proliferation of chondrocytes was decreased at the same amount of MSM. The protein expression results were well accordance with the enhanced gene expression and ECM formation with incorporation of MSM in the fibers. Even if the results were different from the literature,^{7, 31} MSM-loaded electrospun mats were also expected to play a critical role in promoting the regeneration of defected cartilage according to our work.

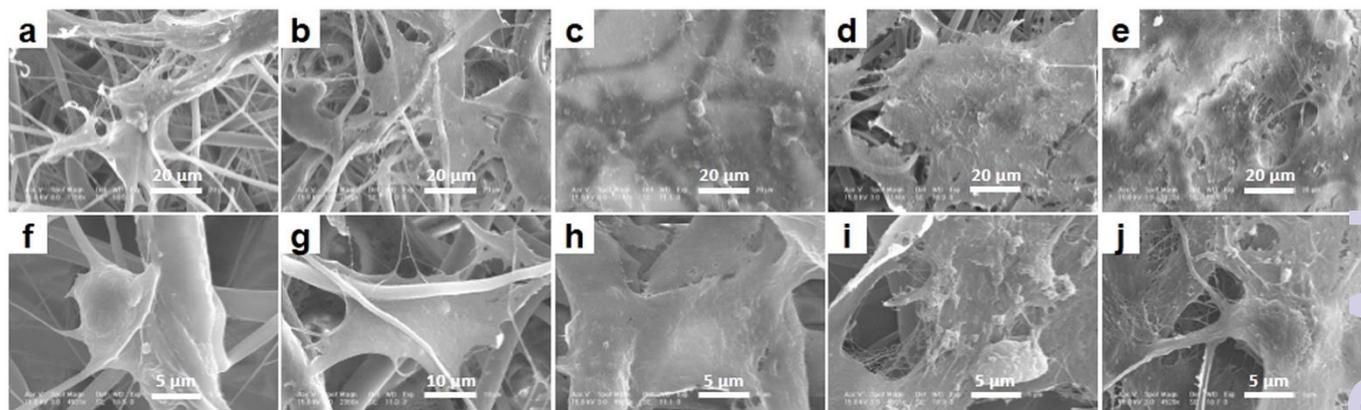


Fig.5. ESEM micrographs of chondrocytes grown on the mats of PLGA (a and f), 0.01 wt% MSM/PLGA (b and g), 0.1 wt% MSM/PLGA (c and h), 1 wt% MSM/PLGA (d and i) and 10 wt% MSM/PLGA (e and j) for 7 days. Scale bars are 20 μm (a, b, c, d and e), 10 μm (g) and 5 μm (f, h, i and j).

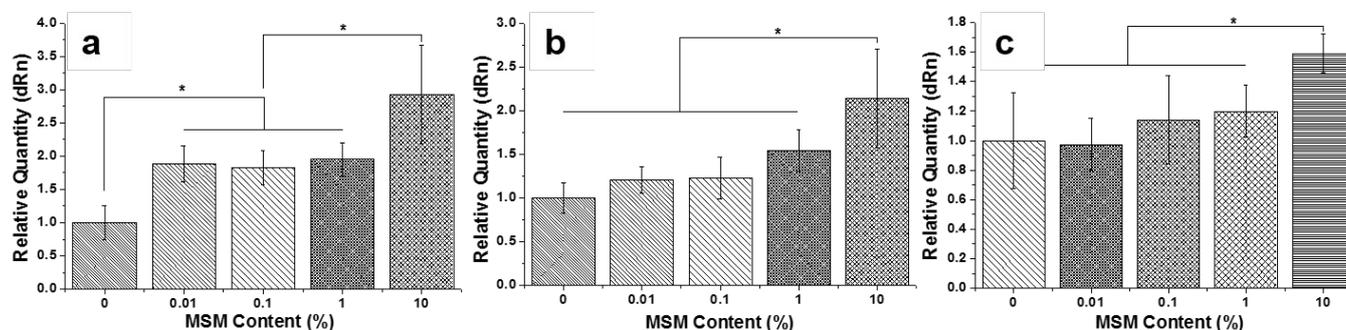


Fig.6. Quantitative real-time PCR analysis showed that cartilage related gene expression of collagen type II (a), aggrecan (b), and collagen type I (c) by rabbit chondrocytes cultured on the electrospun mats for 7 days. The expression level was normalized to that of the respective expression of GAPDH, which was used as a reference standard. * $p < 0.05$.

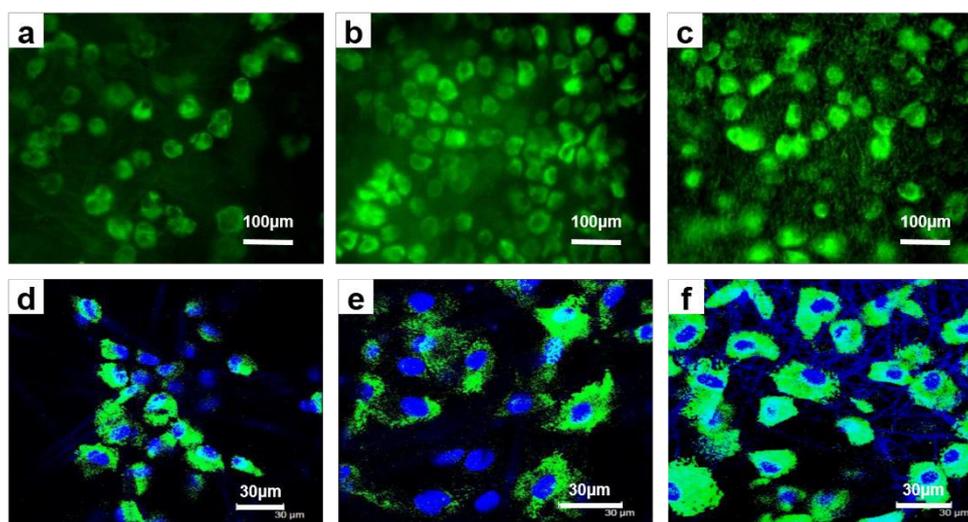


Fig.7. Immunofluorescent images of collagen type II protein for PLGA (a and d), 0.1 wt% MSM/PLGA (b and e), and 10 wt% MSM/PLGA (c and f) mats. (a-c) were observed by an inverted fluorescence microscope and (d-f) were observed by a confocal laser scanning microscope. DAPI staining for nuclei (blue) and FITC-conjugated secondary antibody for collagen type II (green). Scale bar lengths are 100 μm (a-c) and 30 μm (d-f).

The results of this study showed that MSM-loaded PLGA mat was biocompatible supporting cell attachment, migration, and proliferation, as well as the ECM formation of chondrocytes. Especially, the gene and protein expression of collagen type II by chondrocytes could be obviously promoted by the addition of MSM in PLGA mat. It was regarded that the interaction between cells and engineered ECM was crucial for modulating or redirecting cell functions *in vitro*. Biocompatibility of tissue-engineered scaffold was of primary concern since it affected cell attachment, proliferation, and further growth.³⁴ Besides, collagen type II was the main extracellular component of cartilage which provided the tissue with structural support and sufficient mechanical properties. Furthermore, as a naturally occurring

organosulfur molecule, MSM was cheap and easily manipulated for delivery. Thus, we can speculate that the MSM-loaded electrospun mats may be one of bioactive polymeric scaffold for cartilage defect repair. In our future study, a systematical investigation on articular cartilage regeneration with polymeric scaffold containing MSM will be undertaken *in vivo*.

Conclusions

In this study, we have successfully prepared MSM-load PLGA mats by electrospinning. The obtained fibers were uniform and interconnected with incorporation of MSM. Incorporation of MSM did not apparently affect the diameter of electrospun fibers.

The controlled release of MSM has been accomplished by the MSM-loaded mats, indicating that it will be helpful to maintain the bioactivity of MSM for longer time. The MSM-loaded electrospun mats were cytocompatible and bioactive because the cell proliferation and ECM formation of chondrocytes were promoted significantly. Moreover, the promoted cartilage related expression of collagen type II, aggrecan and collagen type I verified by quantitative real-time PCR and protein expression by immunofluorescence staining for collagen type II indicated that the MSM-loaded electrospun mats were beneficial for cartilage tissue regeneration. It will be available for tissue engineering application and drug delivery. Thus, the next step of our research will focus on the application of MSM-loaded mats in cartilage tissue engineering and in the therapy of cartilage defects *in vivo*.

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