Int J Clin Exp Pathol 2015;8(2):1093-1103
www.ijcep.com /ISSN:1936-2625/IJCEP0004439

Original Article

Development of a KLD-12 polypeptide/TGF-β1-tissue scaffold promoting the differentiation of mesenchymal stem cell into nucleus pulposus-like cells for treatment of intervertebral disc degeneration

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Received December 4, 2014; Accepted February 3, 2015; Epub February 1, 2015; Published February 15, 2015

Abstract: Objective: To develop tissue engineering scaffolds consisting of self-assembling KLD-12 polypeptide/TGF-β1 nanofiber gel, for the induction of mesenchymal stem cell (MSCs) differentiation into nucleus pulposus (NP)-like cells. Methods: The release of TGF-β1 from KLD-12 polypeptide gels containing varying TGF-β1 concentrations was detected by ELISA. MSCs were isolated with a density gradient method and their differentiation into NP-like cells was analyzed in KLD-12 polypeptide/TGF-β1- or KLD-12 polypeptide control nanofiber-gel 3D-cultures. The Alcianblue method, Real-time quantitative PCR (RT-qPCR), and immunocytochemistry were used to measure the expression of extracellular matrix (ECM) molecules, such as aggrecan, glycosaminoglycans (GAGs), and type II collagen. Results: ELISA results documented favorable time-dependent release characteristics of TGF-β1 in the KLD-12 polypeptide/TGF-β1 gel scaffolds. The results of CCK-8 cell proliferation assay showed the TGF-β1 containing scaffolds induced higher growth rate in MSCs compared to the control group. Calcein-AM/PI fluorescent staining showed: the cells in the gel grew well, maintaining the circular shape of cells, and the spindle and fusiform shape of cells on the gel edges. The cell viability displayed a survival rate of 89.14% ± 2.468 for the TGF-β1 group with no significant difference between the two groups at 14 d of culture. The production of ECM was monitored showing higher expression of GAGs in the TGF-β1 group ($P < 0.01$) with highest amounts at 10 d and 14 d compared to 4 d and 7 d ($P < 0.05$). Real-time PCR results revealed that the expression levels of collagen II and aggrecan mRNA were higher in the TGF-β1 group ($P < 0.05$). Finally, immunocytochemical staining of collagen II confirmed the higher expression levels. Conclusion: A scaffold containing a KLD-12 polypeptide/TGF-β1-nanofiber gel and MSCs differentiated into NP-like cells is able to produce ECM and has the potential to serve as a three-dimensional (3-D) support scaffold for the filling of early postoperative residual cavities and the treatment of intervertebral disc degeneration.

Keywords: KLD-12 polypeptide, transforming growth factor-β1, mesenchymal stem cells, nucleus pulposus cells, intervertebral disc tissue engineering

Introduction

Degenerative disc disease, affecting one or more intervertebral discs, is often accompanied my severe neck (cervical) or lower back (lumbar) pain or sciatica. The degenerative disease has a high prevalence and represents a major public health problem with high socioeconomic impact. Once degeneration starts, it is difficult to reverse, and there is no effective treatment for early disc degeneration. The intervertebral disc is a cartilaginous structure, with the nucleus pulposus (NP) as the core structure containing a gel-like, shock absorbing extracellular matrix (ECM). The degeneration of the integrity of this gel-like ECM and dehydration in the nucleus pulposus causes pressure on the spinal nerves, which leads to pain and possibly affects nerve function. Stem cell transplantation therapy shows good treatment prospects. It promotes the formation of new ECM in the NP, repairs the degenerated disc structures, and rebuilds its function by supplementing the reduced numbers of NP cells caused by the internal disc degeneration [1-3]. Mesenchymal stem cells (MSCs) are a class of somatic stem

cells with a high degree of self-renewal and pluripotency [4]. They can be directionally differentiated into osteoblasts, adipocytes, NP-like cells, muscle and other cells under the appropriate induction conditions [5-7]. TGF-β1 is a well-studied cytokine that inhibits the proliferation of most cells, while stimulating the proliferation of bone marrow mesenchymal cells and the production of ECM [8]. KLD-12 polypeptides belong to a class of molecules with self-assembling properties that are biocompatible and may serve as scaffold material for tissue engineering [9-11]. The present study was designed to induce differentiation of purified MSCs into NP-like cells using a 3D-culture consisting of a KLD-12 polypeptide/TGF-β1 nanofiber gel scaffold. These studies are the foundation for the tissue engineering of KLD-12 polypeptide/TGF-β1/MSCs scaffolds to be implanted in intervertebrate discs for the treatment of intervertebral disc degeneration and for the filling of residual cavities after early surgical procedures.

Materials and methods

Materials

New Zealand white rabbits were obtained from the Laboratory Animal Center of the School of Medicine, Shihezi University, TGF-β1 96t Elisa Kit was purchased from Cloud-Clone Corp (Houston, TX, USA), recombinant human TGF-β1 from PeproTech (Rocky Hill, NJ, USA), collagen II antibody from ThermoFisher (Waltham, MA, USA), and calcein-AM/PI from Japan Institute Colleagues Chemistry. Alcian blue and sodium chondroitin sulfate were from Sigma-Aldrich (St. Louis, MO, USA), CCK-8 cell counting kit from Beyotime Biotechnology (Haimen, Jiangsu, China), and Human Peripheral Blood Lymphocyte Separation Solution from Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China). KLD-12 polypeptide (ACN-KLDLKLDLKLDL-CNH2) was generated using a peptide synthesizer, and further purified and analyzed using HPLC and mass spectroscopy (Sangon Biotech Co. Ltd, Shanghai, China).

Screening for appropriate complex concentration and ratio of KLD-12 polypeptide/TGF-β1

TGF-β1 stock solution (10 ng/μl) was diluted with distilled water to various working concentrations and added into an EP tube containing lyophilized KLD-12 polypeptide. The tube was vortexed for 30 min. PBS buffer was added drop wise to the tube, and the tube was placed into a 37°C incubator for 20 min as a trigger for KLD-12 polypeptide self-assembly, forming a transparent gel. The final concentration for TGF-β1 was 100 ng/ml or 300 ng/ml, 0.05% KLD-12 and the PBS supernatant was collected daily and 200 μl new PBS was added to the gel, which was stored in a 37°C incubator for a total of 10 d. The TGF-β1 concentration was determined by ELISA.

Isolation and cultivation of rabbit MSCs

New Zealand white rabbits were anesthetized by injecting chloral hydrate (10%) intravenously in the ear vein. Approximately 4 ml of bone marrow was extracted under sterile conditions through the femoral bone and the bone marrow mixed with an equal volume DMEM/LG medium was added to 3 ml lymphocyte separation solution for centrifugation (15 min, 2000 rpm), and the mist-like cells in the middle layer were isolated using a pipette. The cell suspension was mixed with DMEM/LG medium and centrifuged again for 5 min, 1000 rpm). The supernatant was discarded, and the procedure was repeated twice. Culture medium containing 10% FBS was added, and cells were seeded in two separate culture flasks and cultivated at 37°C, in 5% CO_2. Half the medium was changed after 24 h, and again after 48 h, and the whole medium was changed every 3 d. When cells reached 80%-90% confluency, 0.25% trypsin was used for cell splitting at a ratio of 1:2.

Immunocytological characterization of bone marrow MSCs

The MSC cultures (P3) were digested by 0.25% trypsin, and the resulting cell suspension was counted and cell density was adjusted to 10×10^6/ml. The cell suspension was washed twice with PBS, fixed with 4 ml dehydrated alcohol at -20°C for 24 h. After washing twice in blocking solution (1% bovine serum albumin in PBS) was added and after another washing step, 100 μl isothiocyanate (FITC) labeled CD34, CD44, CD45, CD90 and a Lamine monoclonal antibodies were incubated in the dark for 30 min, and the samples were analyzed by flow cytometry.

Table 1. Related gene sequences of primers

Genes		Sequences of primers	bp
β-actin	Forward	TGCTTCTAGGCGGACTGTTA	314
	Reverse	CGTCACATGGCATCTCACGA	
Collagen II	Forward	CTGTCCTGTGCGACGACATA	140
	Reverse	TCCTTTCTGCCCCTTTGGTC	
Aggrecan	Forward	GCTACGGAGACAAGGATGAGTTC	114
	Reverse	CGTAAAAGACCTCACCCTCCAT	

Generation of KLD-12 polypeptide/TGF-β1/MSC 3D-cultures

KLD-12 polypeptide (10 mg) freeze-dried powder was dissolved in 10% sucrose solution containing 5 μl of TGF-β1 (0.01 μg/μl) to prepare a 0.56% polypeptide (w/v) solution. The solution was mixed for 30 min in a scroll-type oscillator. MSCs (P3) were digested with 0.25% trypsin to obtain a cell suspension, which was counted, and its density was adjusted to 10×10^6/ml. The cell suspension was mixed with the KLD-12 polypeptide/TGF-β1 solution to form the KLD-12 polypeptide/TGF-β1/MSC suspension containing a final concentration of 0.5% KLD-12 polypeptide and 100 ng/ml TGF-β1. The self-assembly and 3D-gel formation of the KLD-12 polypeptide/TGF-β1/MSC suspension was induced by incubating with PBS solution at 37°C for 20 min. The upper layer of PBS was carefully removed, and the culture medium (L-DMEM, 10% FBS, 10 ng/ml TGF-β1, 100 nmol/l dexamethasone, 50 μg/ml ascorbic acid 2-phosphate, 100 μg/ml sodium pyruvate, 40 μg/ml L1 proline, ITS-premix) was added, and the culture placed in a humidified incubator in a 5% CO_2, 95% air atmosphere at 37°C. The medium was replaced regularly and the distribution and morphology of the cells in the gel was observed under an inverted microscope. The control group was prepared without TGF-β1.

Cell proliferation assay

KLD-12 polypeptide/TGF-β1/MSC- and KLD-12 polypeptide/MSC 3D-cultures as controls were cultivated in 96-well culture plates, and the experiment was performed 6 times (n = 6) for each group. Medium (200 μl) was replaced every 24 h. After 1 d, 4 d, 7 d, and 14 d a 10% CCK-8 cell counting solution in complete medium was added, and incubated at 37°C for 4 h. The absorbance (A value) was detected by the Eliasa at an excitation wavelength of 450 nm.

Calcein-AM/PI fluorescence staining and determination of the ratio of living/dead cells in 3D-cultures

KLD-12 polypeptide/TGF-β1/MSC- and KLD-12 polypeptide/MSC 3D-cultures as controls were cultivated in 48-well culture plates, and the experiment was performed 4 times (n = 6) for each group. The media were replaced every 2 d. At 7 d and 14 d the cultures were washed twice with PBS, and a solution of Calcein-AM (2 μmol/l) and PI (4.5 μmol/l) was added to the culture wells, and incubated at 37°C in the dark for 20 min. Living cells (green cytoplasmic fluorescence) and dead cells (red nucleus) were observed in an inverted fluorescence microscope: using the blue and red excitation channel.

For the determination of the specific ratio of living cells calcein-AM/PI fluorescence staining was conducted for KLD-12 polypeptide/TGF-β1/MSC or KLD-12 polypeptide/MSC 3D-cultures as controls. Five different well areas were selected and the numbers of living vs. dead cells was counted with 40 × magnification using an inverted fluorescence microscope. The specific ratio of living cells was calculated as the number of living cells/(number of living cells + number of dead cells) × 100%.

Real-time quantitative PCR (RT-qPCR) for collagen II and aggrecan

KLD-12 polypeptide/TGF-β1/MSC- or KLD-12 polypeptide/MSC 3D-cultures as controls were incubated in 6-well culture plates. The experiment was performed 4 times (n = 5) for each group. After 14 d in culture, the MSCs were collected, lysed with Trizol reagent, and the total RNA was extracted. Using a First Strand cDNA Synthesis Kit cDNA reverse transcription was conducted to obtain cDNA as a template for RT-qPCR reactions. The PCR primers for collagen II and aggrecan were synthesized by Sangon Biotech Co. Ltd. (Shanghai, China) (**Table 1**), with β-actin as an internal control RT-qPCR was performed according to standard protocols and each sample was repeated five times. Ct (average value for 5 wells) represents the number of cycles needed to reach the threshold for the objective amplified products.

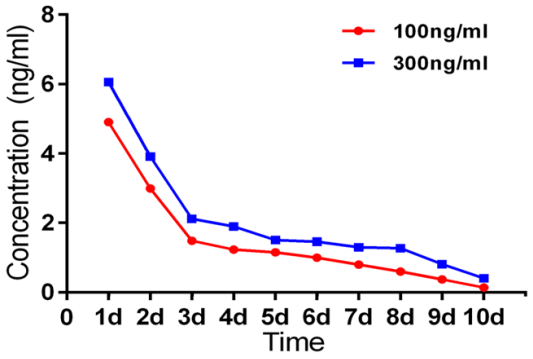

Figure 1. Release curve of different concentrations of TGF-β1. TGF-β1 can be slowly released from KLD-12 polypeptide/TGF-β1 gel, and showed a tendency from rapid to slow. The release rates for the gel scaffold with concentration of 300 ng/ml TGF-β1 were higher than with a concentration of 100 ng/ml.

The relative expression of genes (N) in the experimental group relative to the control group can be calculated by the formula: $N = 2^{-\Delta\Delta Ct}$.

Immunocytochemical staining for collagen II in 3D-cultures

KLD-12 polypeptide/TGF-β1/MSC and KLD-12 polypeptide/MSC 3D-cultures as controls were incubated in 6-well culture plates with a cover slip inserted. The experiment was performed 4 times (n = 4) for each group. The medium was replaced every 3 d. After 14 d of incubation, the culture was fixed with 1% paraformaldehyde solution and immunocytochemical staining using the collagen II antibody (dilution 1:1000) was performed according to standard protocol provided by the manufacturer.

Glycosaminoglycan (GAG) assay of 3D-culture supernatants

KLD-12 polypeptide/TGF-β1/MSC and KLD-12 polypeptide/MSC 3D-cultures as controls were incubated in 6-well culture plates. and the experiment was performed 5 times (n = 5) for each group. The media were replaced every 3 d, and a sample of the medium was collected at 1 d, 4 d, 7 d, 10 d and 14 d for the determination of the GAG content using the Alcian blue assay.

Statistical analysis

Statistical analysis of variance and Student's t test was performed using the SPSS 19.0 software (SPSS, Inc., Chicago, IL, USA). Data were expressed as mean values ± standard deviation. $P < 0.05$ was considered statistically significant.

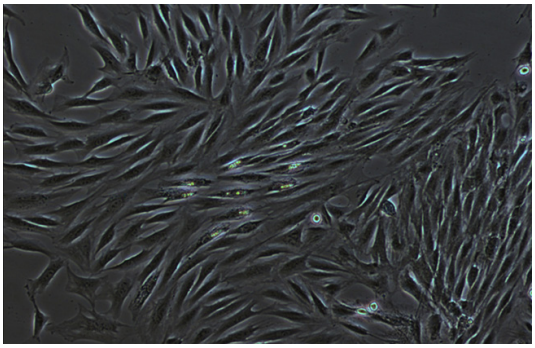

Figure 2. The growth of MSCs at the 10th days. The MSCs in the colonies were constantly amplified, radically extended to the surrounding, and gradually integrated with the neighboring colonies.

Results

Analysis of the release of TGF-β1 from KLD-12 polypeptide/TGF-β1 gel scaffolds

We first tested identity and quality of the synthesized KLD-12 polypeptide by mass spectroscopy and HPLC analysis. The relative molecular weight of 1467.81 and a purity of >95.36% was determined for the peptide. To evaluate the slow-release properties of TGF-β1 from KLD-12 polypeptide/TGF-β1 gel scaffolds containing 100 ng/ml or 300 ng/ml TGF-β1 the gel supernatants collected daily for 10 d were analyzed by a TGF-β1 ELISA. TGF-β1 release was rapid in the beginning and gradually decreased (**Figure 1**). As expected, the release rates for the gel scaffold with concentration of 300 ng/ml TGF-β1 were higher than with a concentration of 100 ng/ml.

Characterization of cultured MSCs isolated from rabbit bone marrow

The MSCs isolated by a density gradient method appeared rounded with good refraction under an inverted microscope. They were mixed together with red blood cells and other cells. After 24 h a small amount of adherent cells, mostly with short spindle shapes were observed. After incubation for 7 d significant proliferation and colony formation were observed. Cells had long spindle shapes, large

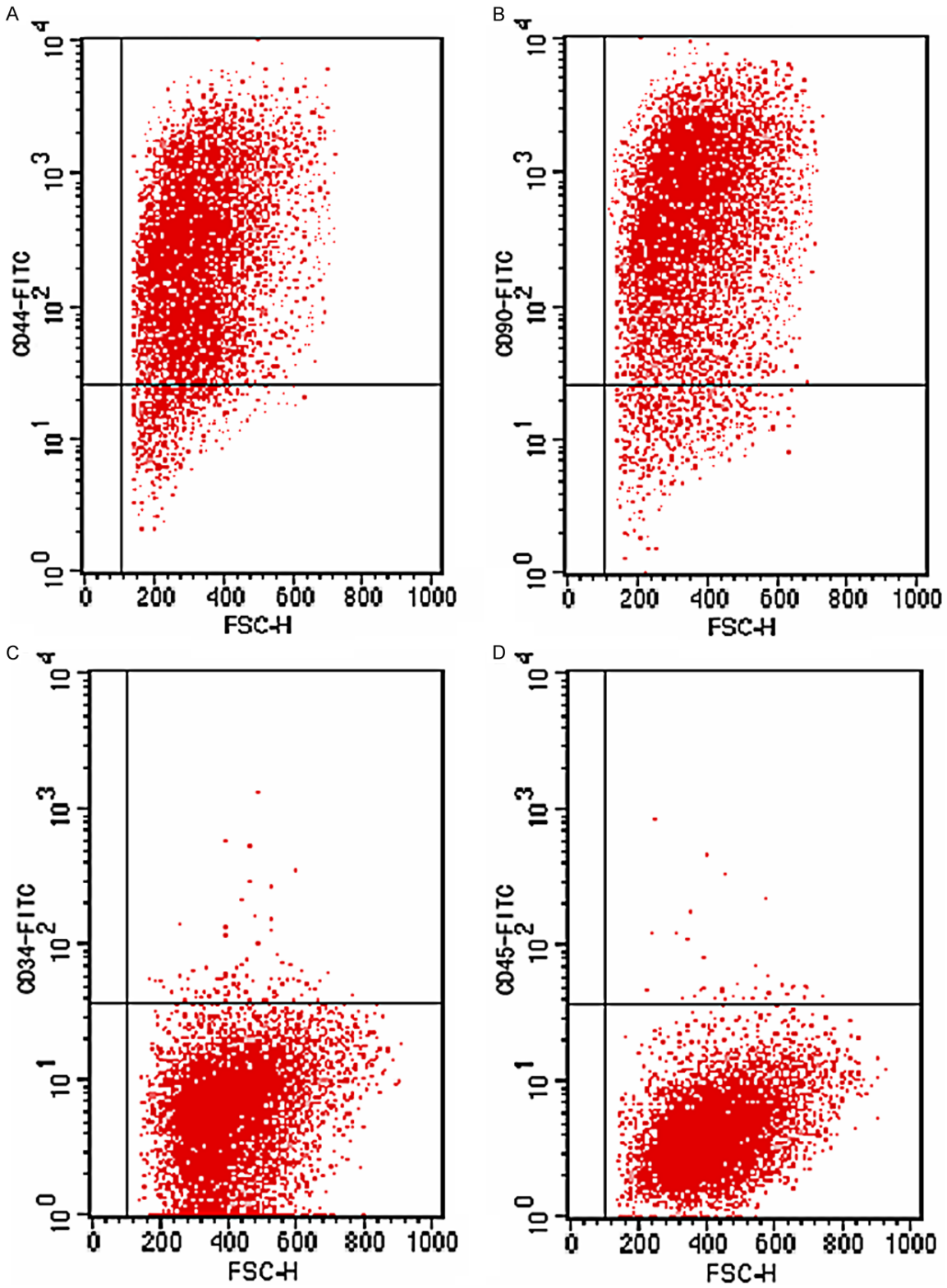

Figure 3. Appraisal results of MSCs. A. CD44: 92.03%; B. CD90: 93.71%; C. CD34: 1.12%; D. CD45: 2.18%.

nuclei, and prominent nucleoli. After incubation for 10 d colonies radically extended to the periphery and gradually integrated with neighboring colonies (**Figure 2**). After cell passaging

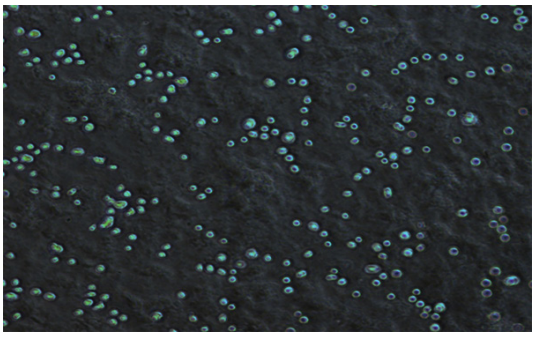

Figure 4. MSCs in the KLD-12 polypeptide/TGF-β1 nanofibers gel. Cells were rounded and evenly distributed, with the presence of some cell clusters, indicating that cell grew well.

growth was rapid and cell morphology appeared uniform showing a larger volume compared with the previous, long spindle shapes. After the third passage the cells reached about 80% confluency within 3-4 d. To confirm the identity of MSCs in culture, flow cytometry was performed (**Figure 3A-D**). The analyzed cells displayed homogeneity expressing high percentages of interstitial cell markers CD44 (92.03%), CD90 (93.71%), but no expression for the blood cells markers CD34 (1.12%) and CD45 (2.18%). Thus, the cultured cells isolated by a density gradient method were confirmed as MSCs.

Characterization of MSC morphology, proliferation and survival rate in KLD-12 polypeptide gel scaffolds

KLD-12 polypeptide/TGF-β1/MSC 3D-cultures were maintained for 7 d and MSCs were observed under an inverted microscope (**Figure 4**). Cells were rounded and evenly distributed, with the presence of some cell clusters, indicating cell growth. To quantify cell proliferation in KLD-12 polypeptide/TGF-β1/MSC versus control cultures a CCK-8 cell proliferation assay was performed. The increase in culture time (1, 4, 7 and 14 d) led to significantly increased cell numbers (**Table 2**). There was no significant difference in cell proliferation at 1 d and 4 d between the experimental and the control group. At 7 d and 14 d growth rates in the experimental group were higher than in the control group (**Figure 5**, $P < 0.01$).

To evaluate the MSC survival rate Calcein-AM/PI fluorescent staining of KLD-12 polypeptide/TGF-β1/MSC gels was employed. After 7 d in culture the 3D-gels displayed a large number of living cells maintaining a round cell morphology, but fusiform and spindle shapes for cells on gel edges were observed. Proliferating cells presented with "dumbbell" shapes. After 14 d culture the number of proliferating, dumbbell-shaped cells increased (**Figure 6A**). Compared with the experimental group, cell proliferation in the control group was not obviously (**Figure 6B**). The cell viability was determined by calculating the specific ratio of living cells. At 14 d of culture the cell viability in the experimental group was 89.14% ± 2.468, and for the control group it was 87.53% ± 4.201 with no significant difference between the two groups (**Figure 7**).

Production of ECM in differentiated MSCs in KLD-12 polypeptide/MSC 3D-cultures

RT-qPCR analysis of KLD-12 polypeptide/TGF-β1/MSC- and control cultures determined that mRNA expression of the ECM molecules collagen II and aggrecan was found in both groups. The expression levels of collagen II in the experimental group and the control group were 1.258 ± 0.114 and 1.007 ± 0.131, respectively, showing a statistically significant difference (**Figure 8**, $P < 0.05$). The expression levels of aggrecan were 1.555 ± 0.368 and 1.008 ± 0.138 in the experimental and control group, respectively. The difference between the two groups was statistically significant (**Figure 9**, $P < 0.05$). These data indicate that the expression levels of the ECM molecules collagen II and aggrecan were higher in the experimental group compared to the control group.

To confirm the results for collagen II on the protein level, immunocytochemical staining was performed. The collagen II antibody detected strongly stained particles in the cytoplasm of MSCs with more intense staining around the nucleus in the experimental group after 14 d in culture (**Figure 10**). The expression level of collagen II was lower in the control group compared to the experimental group (**Figure 11**).

Finally, GAG expression levels were analyzed in KLD-12 polypeptide/TGF-β1/MSC- and control cultures. The GAG content in the cell supernatants significantly increased with time in the experimental group and in the control group. The increase rate of GAG content in the cell supernatants of the experimental group was

Table 2. Comparison of cell proliferation of different days detected by cck-8 between experimental group and Control group

Group	1 d	4 d	7 d	14 d
Experimental group	0.16 ± 0.041	0.26 ± 0.063	0.48 ± 0.098	0.74 ± 0.077
Control group	0.15 ± 0.034	0.21 ± 0.021	0.23 ± 0.01	0.39 ± 0.132
t	0.297	1.906	6.280	5.584
P	> 0.05	> 0.05	< 0.01	< 0.01

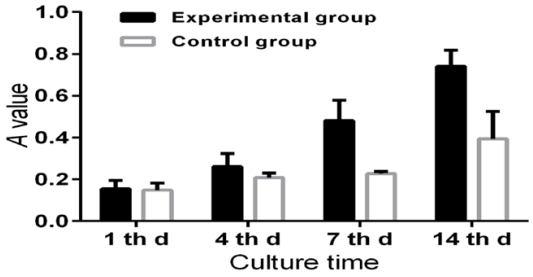

Figure 5. Cell proliferation of different days between experimental group and control group.

higher than in the control group between 1 d and 14 d (**Table 3**). Moreover, no significant difference in GAG expression levels was observed between the two groups at the 1 d. At 4 d and 7 d the GAG expression level in the experimental group was significantly higher than in the control group ($P < 0.05$); At 10 d and 14 d the GAG expression level in the experimental group showed a statistically significant difference of $P < 0.01$ (**Figure 12**).

Discussion

The prevalence of degenerative intervertebral disc disorder is high, and it is the main cause for neck and lower back pain in the population. The main clinical treatment options are conservative treatment, such as physical therapy or anti-inflammatory drugs, or surgical treatment, including vertebral fusion. These methods can alleviate the symptoms, but do not aim directly at the underlying mechanism causing disc degeneration, such as reduced numbers of NP cells and secondary reduction in ECM consisting of proteoglycans, collagen II and other molecules [12]. In recent years, the development of tissue engineering techniques to repair degenerated discs has provided a new research avenue and potential for the treatment of intervertebral disc degeneration. The tissue engineering approaches for regenerating intervertebral discs include three components: seed cells, growth factors and cytoskeleton. MSCs isolated from bone marrow present with a high degree of self-renewal and pluripotency, and can be differentiated into cell types, including intervertebral disc chondrocyte-like cells and NP-like cells. Therefore, MSCs have become a research focus for the seed cells of intervertebral disc tissue engineering [13]. The key issue when using MSCs is how to differentiate these pluripotent cells into NP-like cells, which can be used for the treatment of intervertebral disc degeneration.

TGFs or tumor growth factors are a family of growth factors with an important regulatory role in cell growth, differentiation and immune function. Among them, TGF-β1 is the preferred growth factor for disc tissue engineering research, because it can stimulate cell proliferation, division and differentiation of stem cells of mesenchymal origin. Risbud et al. [14] found that bone marrow-derived rat MSCs cultured in a 3D-alginate scaffold under hypoxic conditions can be differentiated with TGF-β1 towards the NP-like phenotype. The studies of Steck et al., [15] showed that TGF-β1-induced bone marrow-derived MSCs increase synthesis capacity for proteoglycans and collagen II. In summary, TGF-β1 can stimulate MSCs proliferation and promote the generation of ECM, and therefore plays an important role in the process of the differentiation of MSCs into NP-like cells.

Self-assembling polypeptides are ideal scaffolds for tissue engineering. They not only solve issues of poor compatibility between materials and cells, but also maintain favorable 3D-characteristics compared to other scaffold materials which promote cell activity, and imitate ECM structures [16]. The KLD-12 polypeptide (ACN-KLDLKLDLKLDL-CNH2) is a novel self-assembling polypeptide, which forms a hydrogel consisting of a nanofiber web-like structure. The KLD-12 polypeptide and its degradation products neither induce host immune or hemolytic reactions, nor affect the biological activity of seed cells, such as MSCs, and thus show good biocompatibility [10, 11]. The porous nano to microfiber environment formed by the KLD-12 polypeptide provides a good microenvi-

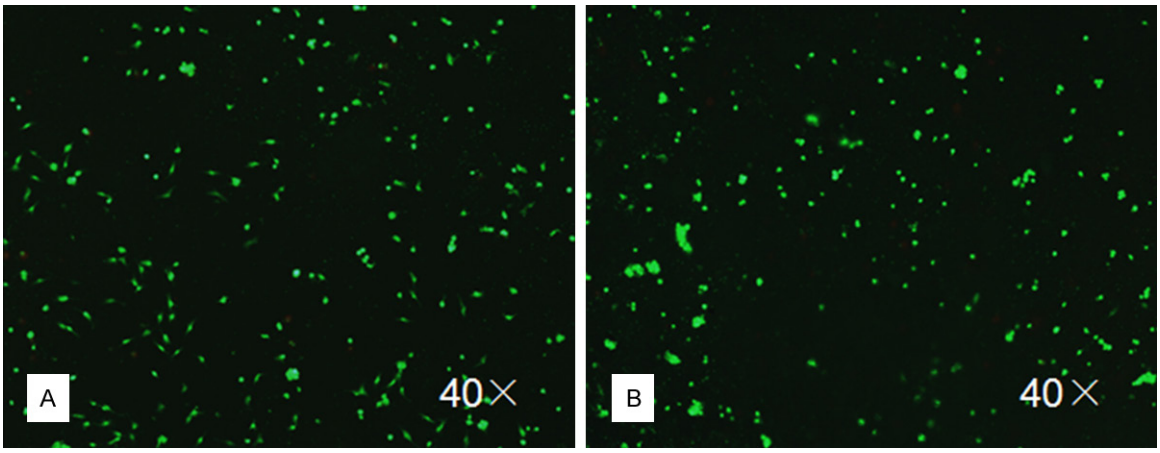

Figure 6. The morphology of MSCs by Calcein-AM/PI fluorescein staining at the 14th days. A. The MSCs in KLD-12 peptide/TGF-β1 gel maintained round shape. The number of proliferating, dumbbell-shaped cells increased. Few of dead MSCs was detected (red color); B. Compared with the experimental group, cell proliferation in the control group was not obviously.

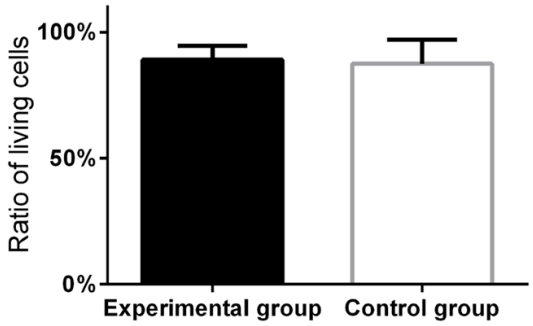

Figure 7. Ratio of living cells at the 14th days between experimental group and control group. The cell viability in the experimental group was 89.14% ± 2.468, and for the control group it was 87.53% ± 4.201. There is no difference in the survival rate between day between experimental group and control group.

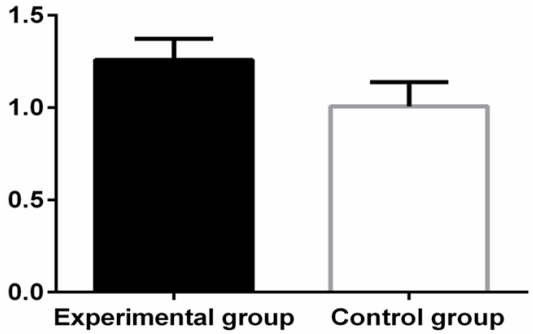

Figure 8. The relative expression of collagen type II.

ronment for NP cell adhesion, growth and proliferation, and the functions of NP cells seeded in

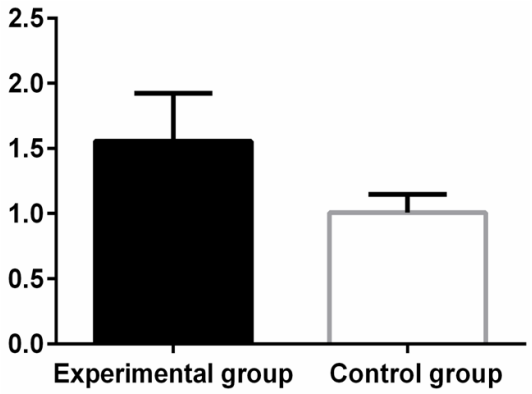

Figure 9. The relative expression of aggrecan.

a KLD-12 polypeptide 3-D gel are normal at the gene and molecular level [12].

The use of KLD-12 polypeptide/TGF-β1 nanofiber gel as scaffold material with biological activity to induce MSC differentiation into NP-like cells for purposed of intervertebral disc tissue engineering has not been described yet in the literature. In this study, we have shown that MSCs can be successfully isolated from rabbit bone marrow with high purity by a density gradient method. After several passages the cells grew rapidly, were evenly distributed, and showed a typical, long spindle-like shape. MSCs seeded in a KLD-12 polypeptide/TGF-β1 nanofiber gel grew well showing uniform distribution and round shapes, but with spindle and fusiform shapes for some cells at the edges of the gel. Using the sustained release characteristics

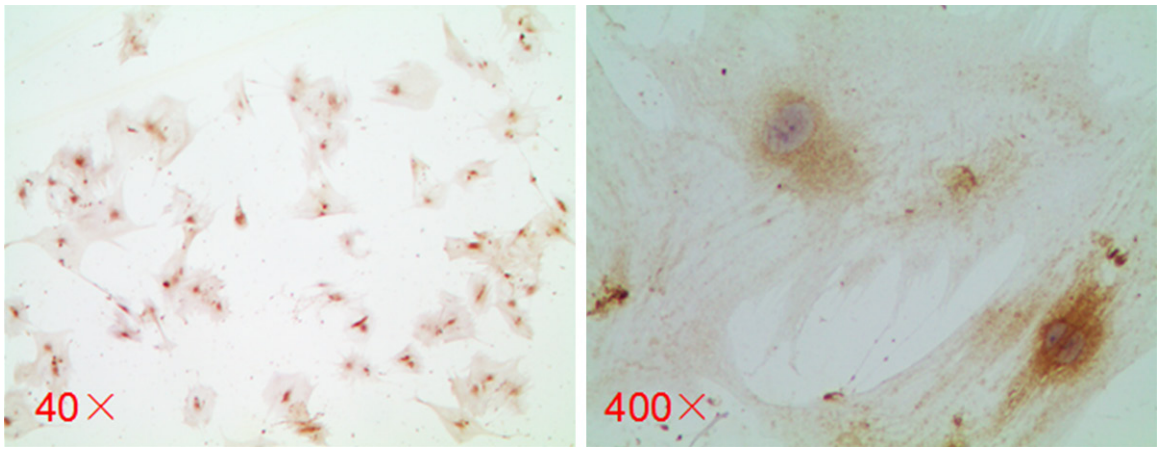

Figure 10. Expression of collagen type II detected by immunohistochemistry in experimental group.

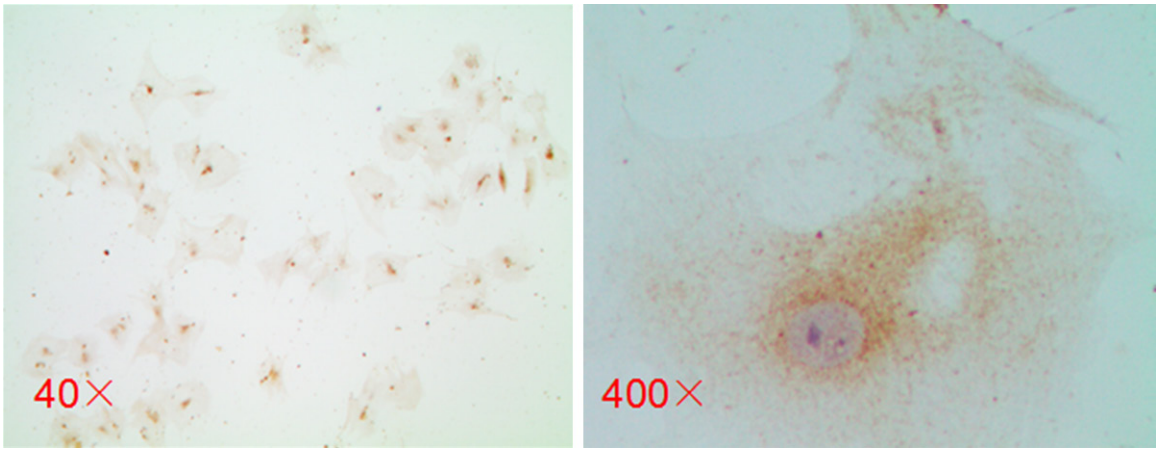

Figure 11. Expression of collagen type II detected by immunohistochemistry in control group. The deeply stained darker brown particles can be seen in the MSCs cytoplasm after induction for 14 d in the experimental group. The closer to the nucleus, the deeper for the staining. The expression levels of the control group were lower than the experimental group.

Table 3. Comparison of the expression of GAG content between experimental group and Control group

Group	1 d	4 d	7 d	10 d	14 d
Experimental group	2.39 ± 1.455	12.52 ± 4.399	31 ± 7.864	97.81 ± 15.958	135.62 ± 21.737
control group	2.29 ± 0.97	5.32 ± 2.143	19.6 ± 4.653	52.62 ± 15.279	72.63 ± 15.857
t	0.127	3.290	2.790	4.574	5.234
P	> 0.05	< 0.05	< 0.05	< 0.01	< 0.01

of KLD-12 polypeptide, the different concentrations of TGF-β1 were combined with the KLD-12 polypeptide, and the slow-release amounts were measured. The optimal concentration for TGF-β1-induced MSCs differentiation to the nucleus pulposus -like cells was 10 ng/mL [17]. Elisa results indicated that the KLD-12 polypeptide/TGF-β1 gel releases TGF-β1 from the gel which gradually decreased over time, and the slow-release amount of TGF-β1 with the gel concentration of 100 ng/mL was closer to the optimal concentration of MSCs induction differentiation. The TGF-β1 released from the gel with time was gradually decreased, and the slow-release amount of TGF-β1 with the gel concentration of 100 ng/mL was closer to the

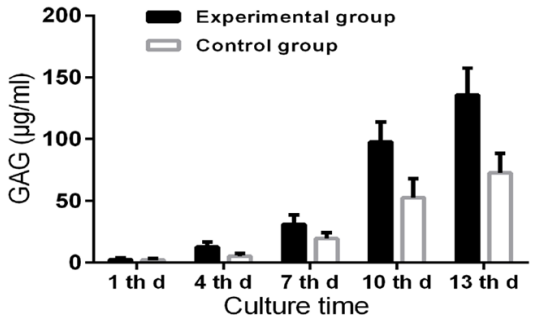

Figure 12. The content of GAG between experimental group and control group.

optimal concentration of MSCs induction differentiation. When MSCs were grown in KLD-12 polypeptide/TGF-β1 3-D nanofiber gels for 14 days, RT-qPCR results showed increased expression of the ECM components collagen II and aggrecan. Moreover, collagen II immunoreactivity was higher in the KLD-12 polypeptide/TGF-β1 group compared to controls. The Alcian Blue test for GAGs revealed an increase in this component of proteoglycans in KLD-12 polypeptide/TGF-β1/MSC 3D-nanofiber gels, indicating that the KLD-12 polypeptide/TGF-β1 scaffold significantly promoted the differentiation of MSC into NP-like cells to produce cell-specific ECM components, such as GAGs, collagen II and the proteoglycan aggrecan.

In summary, in this tissue engineering approach we successfully developed a 3D-gel scaffold consisting of a KLD-12 polypeptide/TGF-β1 nanofiber gel and NP-like cells derived from rabbit MSCs which may be implanted in intervertebrate discs to delay disc degeneration and for the filling of residual cavities after surgical procedures.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (project 81160224) and the Crops Doctor Foundation of China (project 2011BB017).

Disclosure of conflict of interest

None.

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