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Tissue Engineering Material KLD-12 Polypeptide /TGF-β1 the Protective Effect and Mechanism of Nanofiber Gel on Early Intervertebral Disc Degeneration

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ABSTRACT

Intervertebral disc degeneration (IDD) is a common clinical symptom of multifactorial disease. The treatment and expenditure of IDD cause huge economic and psychological harm to patients, and there is no root treatment in the clinic. However, the appearance of tissue engineering materials provides a new idea for the treatment of early IDD. KLD-12 polypeptide material is a new kind of polypeptide scaffold material, which can be used to repair early IDD and TGF-β1Transforming growth factor-1 plays an important role in the proliferation of Intervertebral disc cells and inhibition of inflammatory response. In order to further understand the tissue engineering material kld-12 polypeptide / TGF-β1 the biomechanical properties of nanofiber gel, and to clarify tissue engineering material KLD-12 polypeptide TGF-β1nanofiber gel provides an experimental basis for the protection and mechanism of early IDD. In this paper, tissue engineering material KLD-12 polypeptide /TGF-β1 is mainly studied as the protective effect and mechanism of nanofiber gel on early IDD. In this paper, through the study of the tissue structure of the intervertebral disc, the composition of the nucleus pulposus, annulus fibrosus and cartilage endplate was studied. The objective was to study the relationship between transforming growth factor TGF-β1 and IDD and to understand its important role in the proliferation of intervertebral disc cells and inhibition of inflammatory response. In this paper, we studied the molecular basis of IDD, the main reason is the imbalance of extracellular matrix synthesis and degradation of Intervertebral disc cells, to understand the structural characteristics of cartilage endplate and the composition of Intervertebral disc fibroblasts. In this study, we studied the cell proliferation activity, the ratio of surviving dead cells, the content of glucosaminoglycans, the content of polyproteoglycan and type II collagen in the gel, and studied the protective effect and mechanism of tissue engineering material KLD-12 polypeptide /TGF-β1 nanofiber gel on early IDD. The results showed that kld-12 polypeptide / TGF-β1 was more effective in the proliferation activity of annulus fibrosus cells of nanofiber gel is higher than that of KLD-12 polypeptide/annulus fibroin nanofiber gel. On 2d, the difference in cell proliferation activity was not obvious, KLD-12 polypeptide / TGF- β 1 the fibrous annulus cell proliferation activity of nanofiber gel was 0.796, and the proliferation activity of KLD-12 polypeptide/annulus fibroin nanofiber gel was 0.786. On the 14d, KLD-12 polypeptide / TGF- β 1 the fibrous annulus cell proliferation activity of nanofiber gel was 1.204, and the proliferation activity of KLD-12 polypeptide/annulus fibroin nanofiber gel was 1.034.

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Introduction

Lumbar disc degeneration is one of the most common causes of low back pain. The current clinical treatment methods can not fundamentally solve the problem (1-2). Intervertebral disc degeneration (IDD) is a process of chronic fatigue. It is difficult to study IDD (3-4). First of all, the experimental subjects are not easy to obtain. When many symptomatic patients feel the symptoms, IDD has developed to a very serious degree (5-6). Secondly, IDD is a process of slow progress, and the experimental period that needs observation and research will be very long, which is not conducive to the achievement of transformation (7-8). Moreover, this kind of clinical research will be affected by the subjective and objective factors of the observation object, such as gender, age, living habits, genetic variables, amount of activity, different drugs used and environmental factors (9-10). These non-experimental factors will seriously interfere with the accuracy of the experimental results and the reliability of the data, resulting in a bias in the research results, and the reliability of the research results can not be guaranteed (11-12). The main pathological changes of early IDD are the apotheosis of nucleus pulposus

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cells, decrease of water content and protestant content in the nucleus pulposus, and there is no effective treatment in the clinic (13-14). With the rapid development of cartilage tissue engineering in recent years, there is a new research direction for repairing degenerative Intervertebral disc that is, using tissue engineering materials to construct cartilage, and then transplanting it to degenerative lumbar Intervertebral disc to complete the treatment purpose (15-16).

The research mechanism of tissue process materials on early lumbar disc degeneration is the research focus of scholars at home and abroad in recent years. Based on the research on nanomaterials particularly nanofiber gel, they have made some progress (17-19). Nakazawa K R and Duran S research indicate that the normal nucleus pulposus tissue itself is a highly hydrated gelatin viscoelastic tissue with a water content of 70%-85%. Therefore, the material used to construct the tissue engineering material for repairing the nucleus pulposus should also have biomechanical properties similar to the normal nucleus pulposus tissue. At the same time, the biocompatibility of implant Table biopeptide materials must be tested before in vivo experiments, so as to ensure the safety of in vivo application (20, 21). The loss of proteoglycan and type II collagen in the intervertebral disc matrix leads to the decrease of water content in the intervertebral disc matrix, which changes the microenvironment of Intervertebral disc cells in vivo, affects the normal morphology and biological activity of Intervertebral disc cells, and reduces the secretion of proteoglycan and type II collagen, which maintain the biological stability of Intervertebral disc, Apoptosis of Intervertebral disc cells leads to IDD (22-23).

The purpose of this study was to study the protective effect and mechanism of tissue engineering material KLD-12 polypeptide /TGF-β1 nanofiber gel on early IDD. In this paper, through the study of the tissue structure of the Intervertebral disc, the composition of the nucleus pulposus, annulus fibrosus and cartilage endplate was studied. The objective was to study the relationship between TGF-β1and IDD and understand its important role in the proliferation of Intervertebral disc cells and inhibition of inflammatory response. In this paper, we studied the molecular basis of IDD; the main reason is the imbalance of extracellular matrix synthesis and degradation of Intervertebral disc cells, to understand the structural characteristics of cartilage endplate and the composition of Intervertebral disc fibroblasts. In this study, we studied the cell proliferation activity, the ratio of surviving dead cells, the content of glucosaminoglycans, the content of polyproteoglycan and type II collagen in the gel, and studied the protective effect and mechanism of tissue engineering material KLD-12 polypeptide /TGF-β1 nanofiber gel on early IDD.

Tissue Engineering Material KLD-12 Polypeptide/TGF-β1Nanofiber Gel and Early IDD

Structure of Intervertebral Disc

The human spine consists of 23 Intervertebral discs and their connected vertebrae. Anatomically, the Intervertebral disc consists of the nucleus pulposus, annulus fibrosus and cartilage endplate. The nucleus pulposus is a gelatinous structure located at the center of the Intervertebral disc. It directly bears the stress conduction of the spine and transforms the axial stress into the force scattered around it. Annulus fibrosus is a circular structure around the nucleus pulposus, which is composed of 15-25 layers of fibrous tissue rich in type I collagen. Its main function is to receive the radial pressure from the nucleus pulposus and play a role in maintaining the shape and stability of the spine. The cartilage endplate is composed of a layer of hyaline cartilage, which plays an important role in nutrient diffusion and metabolic waste excretion (24).

Nucleus pulposus

The nucleus pulposus lies in the gelatinous structure at the center of the Intervertebral disc, consisting of a large amount of proteoglycan and collagen fibers scattered between them. Proteoglycan consists of a protein core and at least one mucopolysaccharide chain. Due to the hydrophilicity of mucopolysaccharide, the water content in the nucleus pulposus is higher, which makes it have higher expansibility and play an important role in resisting vertebral pressure. The decrease of proteoglycan can reduce the water content of the nucleus pulposus and the height of the intervertebral disc, which is one of the important characteristics of IDD. At the same time, the nucleus pulposus contains more collagen, the highest content of which is type II collagen. Its distribution characteristics are high in the

center, and the content gradually decreases near the annulus fibrosus (25).

Nucleus pulposus originates from notochord tissue. At the early stage of development, there are more cells in the nucleus pulposus. After birth, the number of cells gradually decreases. There are two kinds of cells in the nucleus pulposus in the early stage: notochord cells with large volume and lots of vacuoles and chondroid cells with small volume. In adult nucleus pulposus, chordate cells disappeared, and nucleus pulposus cells were round and scattered in the extracellular matrix, with a density of about 4 × 106 cells/ml. Although the density of nucleus pulposus cells in the extracellular matrix is small, they play an important role in maintaining the normal physiological function of the nucleus pulposus: nucleus pulposus cells are the source of extracellular matrix synthesis and secretion, and they can express and secrete a variety of molecules to regulate and maintain the homeostasis of nucleus pulposus (26).

Annulus fibrosus

The main function of the annulus fibrosus is to resist the stress caused by the deformation of the nucleus pulposus. The main component is collagen fiber, but the content of proteoglycan is low. In the collagen fibers, the content of type I collagen was the highest, and its expression gradually decreased from the outer layer to the inner layer. In the outer layer of the annulus fibrosus, the collagen fibers in the same layer were arranged in a dense parallel arrangement, which was 60 ° to the longitudinal axis of the spine, these fibers connect with vertebrae; in the inner layer of the annulus fibrosus, the arrangement of collagen fibers was relatively loose, and the arrangement angle gradually changed to 45 °, more than cartilage endplate fixation. The annulus fibrosus cells are long fusiform, and their functions are similar to fibroblasts. They are arranged in the same direction as the surrounding collagen fibers. The density of annulus fibrosus cells is about 9 × 106 cells/ml. Its main function is to secrete related functional proteins and maintain the normal physiological function of the annulus fibrosus (27).

Cartilage endplate

The cartilage endplate is composed of hyaline cartilage, which is responsible for connecting the Intervertebral disc and vertebral body. Its cells are chondrocytes, mainly composed of proteoglycan and type II collagen. The thickness of the adult cartilage endplate is about 0.5-1 mm. In the early stage of Intervertebral disc development, the cartilage endplate is rich in vascular tissue; after birth, the number of blood vessels is reduced, and finally, there is no vascular tissue in adults (28).

TGF- β the Relationship between the Degeneration of Disc

TGF-β1 is one of the most widely studied cytokines in the treatment of IDD. TGF-β1 plays an important role in the proliferation of intervertebral disc cells and the inhibition of inflammatory responses (29).

Immunohistochemical study of normal and degenerative disc tissues revealed that TGF was expressed in both groups- β 1 and its receptor, but in degenerated TGF- β expression was significantly lower than that of the normal disc. TGF transfection of human disc tissue- β The content of proteoglycan and type II collagen was significantly increased after 1. TGF- β can also inhibit the expression of matrix degradation enzymes such as MMPs and ADAMTS, and TGF in the nucleus pulposus cells- β 1 by inhibiting NF- κ B plays an anti-inflammatory role, in addition, TGF- β 1. It has the function of regulating stem cell differentiation. In short, the degeneration process of the disc is complex and diverse, and there are many factors. Although the recently popular biological treatment methods such as gene and stem cell transplantation can promote the regeneration of disc, their clinical application, safety and long-term effect need further research and conformation. Based on the research foundation of the present research on disc degeneration, the key to preventing the development of degenerative diseases of the disc lies in fully understanding the risk factors and the mechanism to promote disc degeneration. Through the inhibition or release of the role of these risk factors, the situation of disc degeneration is changed and the regeneration of disc cells can be promoted (29).

Molecular Basis of IDD

Human IDD is a complex and incompletely understood multifactorial process, which is affected by many factors, including genes, mechanical stress, cell aging and lack of vascular nutrients. With regard

to the mechanical loading of Intervertebral discs, there is a delicate balance between the normal mechanical loading required to maintain the optimal phenotype of Intervertebral disc cells and the damage caused by excessive mechanical loading. Overload may be caused by overweight or trauma, and produce many degenerative manifestations that can be seen by histological and radiological methods. A comparative study between the degenerative disc and the non-degenerative disc shows that the proportion of aging cells in the degenerative disc is increased, and these cells lose the ability of division, which may lead to the further reduction of disc cells in degenerative disc disease. Moreover, when the function of aging cells decreases, the extracellular matrix produced by them will decrease, which will further aggravate the degeneration of the intervertebral disc (30).

Nucleus pulposus cells depend on the diffusion of nutrients from the cartilage endplate to meet their metabolic needs. Therefore, nucleus pulposus cells are damaged by the limited blood supply, nutrient supply and accumulation of metabolites. The imbalance between the overproduction rate of metabolites and the decomposition of extracellular matrix components leads to a series of changes, including the decrease of matrix synthesis, the decrease of proteoglycan synthesis and the transformation of type II collagen to type I collagen, the decrease of cell viability and activity and the change of cytokine distribution accelerate proteoglycan decomposition, leading to disc dehydration and loss of mechanical integrity. Dehydration of the disc reduces the mechanical support provided by the swelling pressure of previously hydrated nucleus pulposus cells. This changes the mechanical load on the exposed outer annulus fibrosus, thereby changing the tension of collagen in the annulus fibrosus (31). This resulted in the subsequent progressive minimally invasive injury of this annulus fibrosus. In this kind of annulus fibrosus degeneration and minimally invasive injury structure, patients are prone to disc herniation caused by IDD, in which disc tissue fragments protrude through this annular defect, leading to nerve compression and nerve root pain. With the gradual deterioration of the mechanical and structural integrity of the Intervertebral disc, neurovascular infiltration may occur through the annular tear. In clinical studies, it has been confirmed that neurovascular infiltration expands to nucleus pulposus cells through annular vessels in painful Intervertebral discs. On the contrary, there was vascular infiltration in the control disc. This new innervation process of degenerative Intervertebral disc is considered to be an important reason for the development of back pain (32).

Due to the influence of human occupation and the external environment, the intervertebral disc is prone to degenerative changes under the action of a variety of physiological or pathological factors. The main reason is related to the imbalance of extracellular matrix synthesis and degradation. Under the long-term continuous action of various internal and external pressure stress loads, the collagen fibers in the outer layer of the annulus fibrosus can be denatured and broken, the nucleus pulposus loses a lot of water, solidifies and hardens, and the viscoelasticity decreases. Under the stimulation of long-term axial compressive stress load, the nucleus pulposus can protrude to the weak link, most of which are backward or left or right backward, resulting in a series of clinical symptoms, such as low back pain, lower limb radiation pain, etc. Therefore, to find the etiology of low back pain and other related diseases, and to understand the specific pathogenesis, we can prevent the occurrence of IDD in advance, and achieve the three early prevention strategies of early detection, early diagnosis and early treatment. Early application in clinical practice can greatly reduce the pain of patients (33).

Structural Characteristics of Cartilage Endplate

The chemical composition of the cartilage endplate contains water and proteoglycan molecules, which are enhanced by the network of collagen fibers. There are many small pores on the surface of the cartilage endplate, and the function of these pores is closely related to the Intervertebral disc, mainly for the transportation of various nutrients and metabolic waste. Proteoglycans, which also form the extracellular matrix of the cartilage endplate, can make some macromolecular substances in and out because of their special structure and polarity. Although the cartilage endplate is one of the components of the intervertebral disc, there is no nerve distribution in it. Therefore, under the external force, stimulation, inflammatory mediators and other damage factors, the cartilage endplate is not only

unable to appear pain symptoms but also unable to self-repair (34).

The special position of the cartilage endplate in the vertebral body determines the importance of its function. The nucleus pulposus and annulus fibrosus are connected between the upper and lower cartilage endplates. The cartilage endplate itself has plasticity, when it is under pressure, it will change its shape to buffer the stress, so as to prevent vertebral compression due to external force. In addition, a large amount of nutrients and metabolic wastes required by the Intervertebral disc mainly depend on the permeability of the cartilage endplate to exchange these substances. Usually, the nutrition supply of the intervertebral disc not only comes from the cartilage endplate pathway, that is, the material is transported through the medullary cavity to the microvessels, then from the microvessels to the cartilage endplate surface, and finally to the nucleus pulposus and annulus fibrosus according to the osmotic pressure difference; there are also a few sites from the annulus fibrosus pathway, which is often "self-sufficient" (35).

When the cartilage endplate calcification, thickness thinning and elasticity decline, it will cause the decline of its ability to transfer nutrients, and the metabolites of the Intervertebral disc accumulate around the micropores of the cartilage endplate, resulting in insufficient nutrition supply of the Intervertebral disc, reduced excretion capacity, resulting in the decrease of water content in the nucleus pulposus and annulus fibrosus, and gradually become dry, or even cracks, Finally, the degeneration of Intervertebral disc occurred. After the degeneration of the intervertebral disc, the stability of the spine is weakened, the stress of the intervertebral disc is uneven, and the stress of different parts is uneven, which leads to the degeneration of the cartilage endplate. It can be said that the degeneration of cartilage endplate and the degeneration of the intervertebral disc interact with each other, and they are closely related. When the cartilage endplate function is normal, it has strong plasticity and can play a buffer role on the pressure of the spine. However, when the cartilage endplate is damaged or degenerated, its shaping ability and the role of buffering stress will also decline. Once it exceeds its range, the cartilage endplate will produce irreversible damage. When the cartilage endplate structure is seriously damaged, because it can not buffer the stress from all directions of the spine, resulting in the redistribution of stress in the Intervertebral disc, the peripheral annulus fibrosus, therefore, bears several times higher pressure than before and eventually leads to the acceleration of IDD (36).

Composition of Intervertebral disc Fibroblasts

Annulus fibrosus cell

Theoretically, autologous annulus fibrosus cells are the most ideal source of seed cells for annulus fibrosus tissue engineering. They not only have the same function but also have no immune rejection and need not be induced to differentiate. When cultured in monolayer in vitro, annulus fibrosus cells are spindle-shaped and prone to dedifferentiation after multiple passages; in three-dimensional culture, the cells were nearly round chondrocyte-like, high-level expression of type II collagen and secretion of proteoglycan, which was easier to maintain the stability of its phenotype. To improve the proliferation of annulus fibrosus cells, obtaining as many seed cells as possible in a short time and maintaining their normal phenotype can also be achieved by coculturing annulus fibrosus cells with other cells. It was found that the co-cultured cells showed more vigorous proliferation activity than the two cultured cells alone, and also showed a significant increase in the secretion of polysaccharides. Under physiological conditions, the annulus fibrosus of the intervertebral disc is constantly stimulated by tensile force, pressure and shear force. Therefore, the living environment of annulus fibrosus cells does not conform to their natural living conditions in vivo. The introduction of a pressure device in cell culture can effectively simulate the real pressure environment in vivo, which provides a good research strategy for annulus fibrosus tissue reconstruction. It has been shown that constant or dynamic pressure can enhance the ability of type II collagen production, and also affect the secretion and expression of polysaccharides. In animal experiments, people can easily obtain annulus fibrosus cells with physiological functions for research. However, it is difficult to use human annulus fibrosus cells for tissue engineering, because it is difficult to obtain normal human annulus fibrosus cells. At the same time, it is also faced with the problem of gradual aging of cells with the growth of culture time (37,38).

Nucleus pulposus cell

The main pathological mechanism of IDD is that the decrease of the intervertebral disc matrix leads to the loss of water in nucleus pulposus tissue, which leads to IDD. Therefore, autologous nucleus pulposus cells are also optional seed cells for Intervertebral disc tissue engineering. The nucleus pulposus cells cultured in vitro were injected into the degenerative annulus fibrosus. It was found that chondrocyte-like colonies were formed in the inner layer of annulus fibrosus and type II collagen was synthesized, which slowed down the degree of degeneration compared with the control group (39).

Mesenchymal stem cells

The development of stem cell technology provides a powerful source of seed cells for the construction of tissue engineering annulus fibrosus. Mesenchymal stem cells have sufficient sources and low immunogenicity, which can avoid the limitations of some annulus fibrosus cells, such as cell aging and lack of autologous homologous cell donors. Adipose-derived mesenchymal stem cells and bone marrow-derived mesenchymal stem cells can be expanded and differentiated in a specific direction in vitro. Fibroblast-like cells were isolated and cultured from human adipose tissue, and they could differentiate into chondrocytes, adipocytes, osteoblasts and muscle cells under specific conditions. Although great progress has been made in the experimental and clinical application of bone marrow mesenchymal stem cells, there are still some problems, such as the determination of cell markers in different stages of differentiation, the precise control of proliferation and differentiation conditions. At the same time, the low proliferation rate of bone marrow mesenchymal stem cells, the ability of induced differentiation will decline with age, which is also a constraint for the application of elderly patients (40).

Disc Correlation Algorithm

Hough transform algorithm

Hough transform can calculate the overall description according to the local measurement and has good fault tolerance and robustness. It can tolerate some discontinuous parts of the feature boundary and is less affected by noise. Considering the detection of straight lines in the Intervertebral disc image, the parameter expression formula is as follows (41):

$$\rho = x\cos\theta + y\sin\theta \quad [1]$$

The steps of the algorithm of extracting the spinal cord by Hough transform are as follows: Hough transform is applied to the image of the spinal cord after the region is reduced:

$$H = hough(C_s, Dtheta) \quad [2]$$

Where h is the Hough transformed matrix and dtheta is the angular interval of Hough transform along the θ-axis. Then, 12 peak points of Hough transform are found, and the coordinate index of a non-zero pixel in image H is calculated:

$$[r, c] = houghpeaks(H, 12) \quad [3]$$

The distance function is used to estimate the degenerative disc,the position between the discs was calculated:

$$\text{distance}(p_{j+1} - p_j) \in [1.7dis\tan ce(p_j - p_{j-1}), 2.3dis\tan ce(p_j - p_{j-1})] \quad [4]$$

Among them, p_j refers to the position of the current disc, p_{j-1} refers to the position of the previous disc, p_{j+1} refers to the next possible disc position, when the distance between the next possible position and the previous position is 1.7-2.3 times of the current distance from the previous disc, we estimate that there is a disc degeneration in it. If the 23rd disc is not identified and stops at the 22nd, the estimation function can be used to estimate the position of the 23rd disc:

$$Esimate(x_j, y_j) = (x_{j-1}, y_{j-1}) + h_a(x_{j-1}, y_{j-1}) * \sum_{i=1}^{j-1} \frac{h_s(x_i, y_i)}{h_a(x_i, y_i)} \quad [5]$$

Where $Esimate(x_j, y_j)$ is the estimated position of the j-th disc, (x_{j-1}, y_{j-1}) is the position of the j-1st disc, $h_a(x_i, y_i)$ is the average height of the i-th vertebrae in the training set, and $h_s(x_i, y_i)$ is the height of the i-th vertebrae in the currently processed image.

Shape change statistical model algorithm

The feature points of the object can be described by the point distribution model, the shape vector is as follows (42):

$$x = (x_1, y_1, ..., x_n, y_n)^T \quad [6]$$

For each training set, the shape x can be approximated by the following formula:

$$x = \bar{x} + p_s b_s \quad [7]$$

$$b_s = p_s (x - \bar{x}) \quad [8]$$

$$g = \bar{g} + p_s b_s \quad [9]$$

Where $\bar{x}$ is the average shape vector in the training set, p_s is an orthogonal matrix describing the shape change, b_s is a parameter vector of gray level, and $\bar{g}$ is the average normalized gray level vector. There is a certain relationship between the shape and the gray level, in the following form:

$$\begin{pmatrix} W_s b_s \\ b_g \end{pmatrix} = b = \begin{pmatrix} Q_s \\ Q_g \end{pmatrix} c = Qc \quad [10]$$

Where W_s is a diagonal matrix, q is an orthogonal matrix, and C is an appearance parameter vector controlled by shape and gray level.

Materials and methods

Subjects

The subjects of this study were two groups of lumbar annulus fibrosus cells from patients with early IDD and confirmed by clinical manifestations and imaging. Two groups of lumbar annulus fibroblast cells were cultured in vitro for third generations, and the experimental group was treated with lumbar KLD-12 and /TGF-β1nanofiber gel. KLD-12 polypeptide / TGF-β1was prepared in nanofiber gel, the control group was KLD-12 peptide / fibrous annulus cell nanofiber gel, after the experimental preparation, two groups of fibroblast cells were cultured in vitro for 14d. By observing the cell proliferation activity, the ratio of surviving dead cells, the content of Glucosaminoglycan and the content of polyproteoglycan and type II collagen in the gel, we studied the KLD-12 polypeptide /TGF-β1of tissue engineering material. The protective effect and mechanism of nanofiber gel on early IDD.

Experimental Process Steps

The two groups of lumbar annulus fibrosus cells were immersed in a povidone-iodine solution and ethanol solution for 15 minutes respectively. The annulus fibrosus cells were cut in the ultra-clean working environment, and 1 / 3 of the annulus fibrosus tissue was removed for cultivation. 4 ml 0.25% trypsin was added into the culture medium. After 25 minutes of digestion, the cells were centrifuged at a radius of 13 cm and 1000 r / min for 5 minutes, and trypsin was discarded, add 5 ml of 0.25% type II collagenase, digest for three and a half hours until the large tissue mass disappears, and gently blow the digestive juice. The digestive juice was collected and centrifuged at a radius of 13 cm and 1000 r / min for 5 minutes; 3 ml l-dmem medium was added, centrifuged at a radius of 13 cm and 1 000 r / min for 5 min, and the supernatant was discarded; Add 2 ml of l-dmen medium containing 10% FBS, blow and mix well, press 1 × 105 cells/ml were inoculated in 6-well plates for culture. Repeat this step and prepare for the experiment after three generations in vitro.

Results and discussion

Tissue Engineering Material KLD-12 Polypeptide / TGF- β 1 Nanofiber Gel and Experimental Study of Early IDD

Proliferation Activity of Annulus Fibrosus Cells in Nanofiber Gel

In order to prove that kld-12 polypeptide / TGF is a tissue engineering material- β 1 the protective effect and mechanism of nanofiber gel on early IDD, understand TGF- β 1 in the role of Intervertebral disc cell proliferation, we experimentally studied the proliferative activity of annulus fibrosus cells in nanofiber gel, and the results are shown in Figure 1.

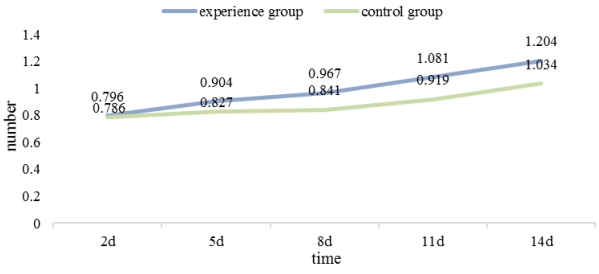

Figure 1. Proliferation activity of annulus fibrosus cells in nanofiber gel

As can be seen from Figure 1, KLD-12 polypeptide / TGF- β1 plays an important role in the proliferation activity of annulus fibrosus cells of nanofiber gel is higher than that of KLD-12 polypeptide/annulus

fibroin nanofiber gel. On 2d, the difference in cell proliferation activity was not obvious, KLD-12 polypeptide / TGF- β1 the fibrous annulus cell proliferation activity of nanofiber gel was 0.796, and the proliferation activity of KLD-12 polypeptide/annulus fibroin nanofiber gel was 0.786. On the 14d, KLD-12 polypeptide / TGF- β 1 the fibrous annulus cell proliferation activity of nanofiber gel was 1.204, and the proliferation activity of KLD-12 polypeptide/annulus fibroin nanofiber gel was 1.034.

Survival Ratio of Annulus Fibrosus Cells in Nanofiber Gel

In order to understand the survival ratio of fibrous annulus cells in nanofiber gel, the experimental group and the control group were cultured and stained, and the number of surviving and dead cells was observed under a fluorescence microscope. The proportion of living cells was calculated according to the number of living cells/living cells + dead cell number *100%. The result is shown in Figure 2.

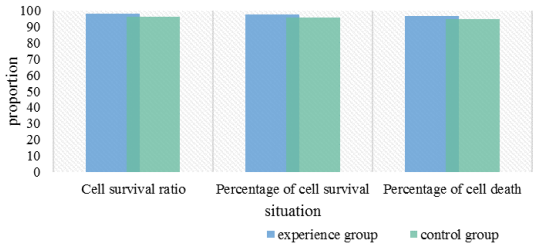

Figure 2. Analysis of survival ratio of annulus fibrosus cells in nanofiber gel

From Figure 2, it can be seen that in the analysis of the survival rate of fibrous annulus cells in nanofiber gel, there was no significant difference in the ratio of cell survival and death between the experimental group and the control group, and the difference between the final cell survival rates was not statistically significant and had no reference value.

Absorbance Standard of Glucosaminoglycan

In order to study Glucosaminoglycan in nanofiber gel, the content of Glucosaminoglycan in the culture medium was detected by experimental culture. The detection method used in the experiment was the chondroitin sulfate new alcian blue method. When a certain amount of chondroitin sulfate is prepared into a standard solution with the same concentration for culture, the absorbance of the chondroitin sulfate standard solution shall be considered for the detection standard, and the value of absorbance standard is shown in Table 1.

Table 1. Absorbance standard of Glucosaminoglycan

Chondroitin sulfate concentration	absorbance
100ug/mL	2.01275
80ug/mL	2.00575
60ug/mL	1.993
40ug/mL	1.07275

It can be seen from Table 1 that the corresponding absorbance value measured according to the concentration of chondroitin sulfate is the corresponding culture environment to maintain the Glucosaminoglycan content in the culture medium. When the concentration of chondroitin sulfate was 100ug / ml, the absorbance was 2.01275; when the concentration of chondroitin sulfate was 80ug / ml, the absorbance was 2.00575; when the concentration of chondroitin sulfate was 60ug / ml, the absorbance was 1.993; when the concentration of chondroitin sulfate was 40ug / ml, the absorbance was 1.07275.

Content Analysis of Glucosaminoglycan in Fibrous Annulus Cells of Nanofiber Gel

In order to understand the content of Glucosaminoglycan in nanofiber gel fibrous annulus cells, the experimental group and the control group were cultured in accordance with certain culture medium standards and absorbance standards. The alcian blue staining solution was added into the culture plate. The absorbance of the medium was detected by enzyme labelling instrument. The content of Glucosaminoglycan in the medium was calculated by the standard curve equation. The results are shown in Figure 3.

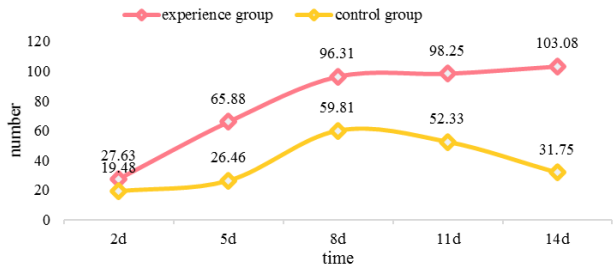

Figure 3. Content analysis of Glucosaminoglycan in fibrous annulus cells of nanofiber gel

It can be seen from Figure 3 that in the statistical analysis of the Glucosaminoglycan content secreted by the fibrocytes, the Glucosaminoglycan content in the control group did not rise with the increase of time, but reached the peak at 8 days, and then decreased with the increase of time. The content of Glucosaminoglycan in the experimental group can be seen that it is rising rapidly before 8 days. After 8 days, the growth rate begins to slow down, and the content of Glucosaminoglycan begins to stabilize.

Relative Expression of Proteoglycan and Type II Collagen in Nanofiber Gel

In order to study the relative expression of proteoglycan and type II collagen in nanofiber gel, the experimental group and the control group were cultured in accordance with certain culture steps. 14d was cultured in vitro, and the ratio of II and collagen protein extracted from annulus fibrosus was compared. The results are shown in Figure 4.

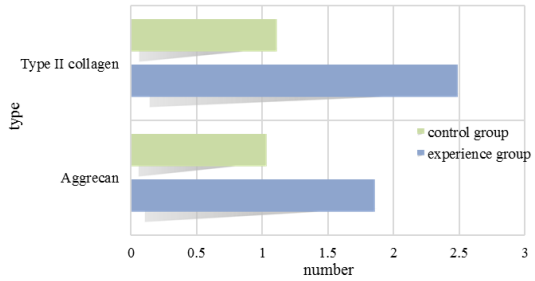

Figure 4. Analysis of relative expression of polyproteoglycan and type II collagen in nanofiber gel

From Figure 4, we can see that in the nanofiber gel, the relative expression level of PPG and type II collagen in the experimental group was significantly higher than that in the control group, and the difference was statistically significant. The relative expression level of GPC in nanofiber gel was 1.87, while that in control group was 1.04. The relative expression of type II collagen in nanofiber gel showed that the relative expression level of the experimental group was 2.51, while that of the control group was 1.12.

Detection and Analysis of Cell Proliferation in Experimental Group and Control Group

KLD-12 polypeptide /TGF-β1was studied in the protective effect and mechanism of nanofiber gel on early IDD. To understand TGF-β1 in the role of Intervertebral disc cell proliferation, the experimental group divided lumbar annulus fibrosus cells with KLD-12 polypeptide nanofiber gel and /TGF-β1 nanofiber gel, the control group was KLD-12 peptide / fibrous annulus cell nanofiber gel, after the experimental preparation, two groups of fibroblast cells were cultured in vitro for 14d. The cell proliferation activity in the two groups of gel was detected by observation, and the results are shown in Table 2.

Table 2. Analysis of cell proliferation in experimental group and control group

	2d	5d	8d	11d	14d
experience group	0.793± 0.056	0.896± 0.045	0.963± 0.01	1.07± 0.041	1.184± 0.024
control group	0.746± 0.057	0.82± 0.014	0.837± 0.021	0.881± 0.059	0.981± 0.043
p	>0.05	<0.05	<0.01	<0.01	<0.01

The experimental results were written in the format of X ± S, at the same time, according to the results of the data, it is of reference significance to choose whether the cell proliferation activity in the gel can be detected by variance analysis in different detection time. When $p<0.05$, the data result is of reference significance. It can be seen from Table 2 that the data of cell proliferation activity on day 2 is not of reference significance among the observation, extraction and detection time points.

Detection and Analysis of Glucosaminoglycan Content in Experimental Group and Control Group

The content of Glucosaminoglycan in nanofiber gel fibrous annulus cells was studied in this study. In the experimental group, the lumbar fibroblast cells and KLD-12 polypeptide nanofiber gel and /TGF-β1 were used. KLD-12 polypeptide / TGF-β1was prepared in nanofiber gel, while the control group was KLD-12 polypeptide/annulus fibroin nanofiber gel. The two groups were cultured, and alcian blue staining solution was added to the culture plate. The absorbance of the culture medium was detected by an enzyme reader, and the content of Glucosaminoglycan in the culture medium was calculated by a standard curve equation. The results are shown in Table 3.

Table 3. Detection and analysis of Glucosaminoglycan content in experimental group and control group

	2d	5d	8d	11d	14d
experience group	27.14± 27.77	68.16± 20.57	97.81± 27.34	98.65± 30.24	101.88± 33.67
control group	15.19± 19.48	34.44± 17.06	64.81± 23.05	50.82± 30.33	35.75± 19.3
p	>0.05	<0.05	<0.01	<0.01	<0.01

The experimental results were written in the format of X ± s. At the same time, according to the data results, the analysis of variance was used to determine whether the detection value of glucosamine content in different detection times has reference significance. When $p < 0.05$, the data results have reference significance. It can be seen from Table 3 that the data of cell proliferation activity on day 2 is not of reference significance at several time points of observation, extraction and detection.

Conclusions

With the development of transgenic technology and soft tissue engineering, there is a new way to repair degenerative discs. In the aspect of cartilage formation, TGF- β 1, plays a very important role. Using TGF- β1The seed cells were induced to transform into nucleus pulposus cells to repair degenerative disc, and a certain number of chondrocytes could be cultured in vitro and then transplanted to degenerated disc tissue to achieve the purpose of repair. But it is still in the basic research stage and is restricted by many experimental conditions and factors. The mechanism of the induction of the transforming factor has not been clarified, which signal pathway plays a role, how to regulate the target gene expression in vivo can not only be sustained, but also avoid overexpression, and how to select the best gene. With the development of gene engineering technology and the in-depth study of transforming factors, the application of exogenous genes to repair degenerative disc tissue will be from experiment to clinical, which will provide a powerful weapon for the human to overcome the disease and pain.

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Interest conflict

The authors declare that they have no conflict of interest.

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