Research Article

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Antibacterial polymer nanofiber-coated and high elastin protein-expressing BMSCs incorporated polypropylene mesh for accelerating healing of female pelvic floor dysfunction

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Abstract: To solve the bio-inertness of widely used polypropylene (PP) mesh for treating pelvic floor dysfunction (PFD), a novel strategy of incorporation with elastin gene-transfected bone marrow stem cells (BMSCs) and antibacteria drug-loaded polylactic acid (PLA) nanofibrous mat covering layer was proposed to overcome the limitation of the pristine PP mesh. Then, a series of physicochemical and *in vitro* experiments were applied to investigate the improvement of the as-prepared material. The elastin protein expression was proved to be upregulated without obvious cytotoxicity influence after the gene transfection and also improved the cell migration rate. In addition, the antibacteria drug-loaded PLA nanofibrous mat on the PP mesh could efficiently inhibit bacteria and showed no significant impact on cell adhesion and proliferation. Thus, we believe that the incorporation of the elastin gene-transfected BMSCs and nanofiber-coated PP mesh would be a potential candidate in the application of female PFD.

Keywords: antibacteria nanofiber-coated polypropylene mesh, pelvic floor reconstruction, elastin gene transfection

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1 Introduction

Nanotechnology was applied in several research directions, and materials with different components and nanostructures were also developed to fulfill different requirements [1–5]. As a kind of nanomaterials, nanofibers prepared by organic or inorganic materials through the method of electrospinning were widely researched due to their advantages such as high porosity, specific surface area, and lightweight [6–8]. In the biomedical field, polymer nanofibers, such as polylactic acid (PLA), chitosan, and silk fibroin [9–11], can be applied as tissue regeneration scaffold and drug delivery system [12]. Moreover, the nanofibrous structure could well mimic the natural extracellular matrix (ECM) and help cell proliferation [13].

As a kind of human tissue problem, pelvic floor dysfunction (PFD) including stress urinary incontinence (SUI) and pelvic organ prolapse (POP) is a common clinical symptom for parous or aged women [14–16]. For POP and SUI treatment, the traditional methods are hysterectomy and vaginal wall folding repairing, which were gradually replaced by the alternative method of mesh reconstruction due to the high recurrence [17,18]. Based on the advantages of short surgery period, high recovery ratio, and small trauma, mesh reconstruction technique was greatly developed and various materials were investigated to screen out suitable candidates, such as synthetic polymers and biological source materials [19–22].

Polypropylene (PP) mesh, which is fabricated via initial melt spinning to monofilament and subsequent weaving to mesh, was found to be the eligible material due to the nontoxicity, low weight, mechanical durability, and well processability, thus widely applied in this field and a large amount of corresponding products have come into the market [23–26]. On the other hand, as a chemically inert material, PP is nearly ungradable in human body, showing good resistance to immunological rejection and recurrence rate. However, the complications such as mesh exposure and low healing rate limit

its application to some degree [27–29]. The major factors of this phenomenon include the tissue compatibility to the mesh and the fiber un-degradation caused by inflammation around the mesh; therefore, a second operation should be undertaken to address this problem [25]. To avoid this after mesh implanting, the materials have to be modified and improved.

Recent research revealed that some biocompatible macromolecules, including collagen, could significantly improve the biocompatibility and healing response after coating on the mesh surface [30,31]. In addition, growth factor like bFGF was also incorporated to the mesh via certain methods [32]. Based on the fundamental PP raw materials, some growth factors were incorporated to prepare composite fiber and further weave to mesh, such as nano-sized pearl powder; the hydrophilicity and biocompatibility were both raised which made it more appropriate as PFD reconstruction material [33].

Besides the implanted mesh, the attention should also be paid to the healing ability of the injured tissue itself. Some research studies reveal that the elastin protein amount of muscular and connective tissues decreased for such patients; thus, stimulating elastin protein secretion of cells to accelerate the tissue regeneration will be the another method to PFD [34,35]. It is well known that bone marrow stem cells (BMSCs) could differentiate to epithelial cells and smooth muscle cells, which are frequently regarded as seed cells for tissue engineering [36–38]. However, pure BMSCs show weak elastin protein secreting ability; thus, proper stimulus could be given to solve this problem. Gene transfection technique is well performed in cell science through transfecting target gene fragment to recipient cells; therefore, the BMSCs with high elastin expression capacity would be obtained [39–42]. However, due to the chemical inert surface and large holes of PP mesh, the gene-transfected cells could not be loaded and implanted together to the injured tissue; thus, certain method should be developed to deal with this problem.

In this study, after measuring the expression ability of elastin gene-transfected BMSCs, nanofibrous mat of biocompatible polymer was initially coated on PP mesh as a support of cell adhering (Figure 1). Due to multiparameters that would affect the properties of nanofibers, such as morphology, mechanical properties, biocompatibility, and degradability, PLA was applied as the main component of the nanofibrous mat [4,43]. In addition, to prevent probable inflammation during implantation, antibacteria component was also loaded in the nanofibrous mat [44], then the antibacterial efficiency and biocompatibility were investigated to estimate the viability of the applied materials [45,46].

2 Methods/experimental

2.1 Materials

PLA and ciprofloxacin hydrochloride (CIP) were purchased from Aladdin Chemistry, Co., Ltd (Shanghai, China). PLA was further purified via dissolving and alcohol precipitating for three times. PP filament was melt spun and woven in laboratory. Other agents were all obtained from Sigma-Aldrich Co., Ltd, without a specific description.

2.2 Primary rat BMSCs culture

The primary rat BMSCs, isolated from 6 month old female Sprague-Dawley rats, were obtained from the Chinese Academy of Science (Shanghai, China) and cultured in low glucose Dulbecco's modified Eagle's medium containing 10% of fetal bovine serum, 100 U/mL of penicillin, and 100 µg/mL of streptomycin at 37°C in a humidified atmosphere of 5% CO₂.

2.3 Reverse transcription polymerase chain reaction (RT-PCR) and target DNA preparation

RT-PCR was performed using the following primers: elastin, forward 5'-TAG AGC TAG CGA ATT CAT GGC GGG TCT GAC GGC G-3', reverse 5'-CTT TGT AGT CGG ATC CTT TTC TCT TCC GGC CAC AA-3'.

Plasmid DNA was first mixed with BamH I and Xba I systems for 6 h at 37°C to carry out the restriction enzyme digestion; then, the product was evaluated by agarose gel electrophoresis to obtain the target band. Afterward, the target band was blended with target DNA fragment in the T4 DNA ligase solution at 16°C overnight to achieve the joining of target and carrier DNA fragments.

2.4 Plasmid extraction and lentivirus package

DH5α *Escherichia coli* (*E. coli*) were used as competent cells to receive the joined DNA product. After mixing with the joined NDA product and incubating on ice for 30 min, the competent cells were heat shocked at 42°C for 90 s and

Figure 1: Schematic strategy of composite PP mesh incorporated with elastin gene-transfected BMSCs and CIP-loaded PLA nanofibrous mat coating for the PFD therapy.

immediately transferred to incubation on ice for 2 min. Afterward, the certain volume of Luria–Bertani (LB) fluid medium was added and further incubated in a 37°C shaker for 1 h. The suspension was casted on ampicillin-resistant LB plate to incubate at 37°C overnight; then, colony PCR was directly undertaken and positive clones were screened. Subsequently, recombinant plasmid digestion identification was applied and correctly digested plasmid DNA was sequenced, the sequencing primers were as mentioned above.

The eligibly sequenced bacteria suspension was incubated with 10 mL antibiotic containing LB fluid medium at 37°C overnight, and the process of plasmid extraction was carried out following the description of endotoxin removed plasmid medium extraction kit (Tiangen Biotech Co., Ltd, Beijing, China). For lentivirus package, the recombinant plasmid and viral packaged protein vector were cotransfected into 293T cells, and the supernatant after incubating for 24 and 72 h was collected for further filtrating through 0.22 µm PVDF filter membrane and concentrated.

2.5 Cell proliferation and western blotting

The investigation of cell proliferation was carried out to evaluate the influence of pure vector and DNA-loaded vector. Herein, normal BMSCs and pure vector transfected BMSCs were used as negative control groups.

Different BMSCs were first seeded in a 96-well plate with a concentration of 1,000 per well and cultured in an incubator at 37°C. After culturing for the certain time point, 10 µL solution of Cell Counting Kit-8 (CCK-8; Beyotime, Biotech, Shanghai, China) was added in each well and kept culturing for another 4 h; then, the plate was placed in a microplate reader and scanned under the wavenumber of 450 nm. The obtained data were analyzed, and cell proliferation curves were drawn.

For western blotting study, the transfected BMSCs were cultured to fully cover the plate and then extracted with RIPA buffer, the supernatant was centrifuged at 12,000 rpm for 15 min, and the total protein content of which was evaluated by Coomassie Brilliant blue solution. Afterward, denaturing SDS-PAGE was applied to resolve the proteins followed by transferring onto the PVDF membrane. The used primary and second antibodies against elastin were purchased from Abcam Ltd (England). The immunoblot signal was detected via Immobilon Western Chemiluminescent HRP Substrate (WBKLS0050; Millipore, USA) according to the specification.

2.6 Cell cycle and apoptosis assays

Transfected BMSCs were dissociated and dispersed in PBS to take cell cycle and apoptosis assays. For this part, Cell Cycle and Apoptosis Analysis Kit-8 (Beyotime;

Biotech, Shanghai, China) was used, the cells were fixed and stained following the specification, and then the flow cytometry was applied to analyze the ratio of cells in different stages of the cell cycles.

2.7 Cell migration and invasion

For the cell migration test, the cells were initially dissociated and dispersed in non-FBS cultural medium with a density of $1 \times 10^5/\text{mL}$, then 100 μL of cell dispersion was added in the upper transwell chamber, and 500 μL of cultural medium containing 20% FBS was added in the lower chamber. Afterward, the cultural plate was cultured for 12 h, and the cells on the undersurface of the transwell chamber membrane were stained with crystal violet for the observation under a microscope. In addition, the cells adhered on the membrane undersurface and lower chamber were both dissociated and experienced CCK-8 assay to quantitatively analyze the cell number.

For cell invasion test, 100 μL of Matrigel/non-FBS cultural medium solution with a concentration of 200 $\mu\text{g}/\text{mL}$ was added in the upper transwell chamber and placed in the incubator till the fluid was dried; then, the similar procedure of cell migration test was undertaken.

2.8 Wound healing experiment

Parallels were premarked at the back of six-well cultural plate ensuring that the parallel was across each well; then, different cells were seeded at a concentration of 1×10^5 per well and cultured for 24 h. Then, scratch was made by using pipette nozzle, which was compared with the scratch marked parallel at the back, and the cells were washed with PBS three times. The plate was placed in the incubator and cultured for certain time periods, the plate was observed under microscope, and the images of the scratch were recorded for further analysis via Image J software.

2.9 Preparation of nanofiber-coated PP (NF-PP) mesh

The PP mesh with tricot warp-knitting structure was used as the main support, which was then adhered on a collector. PLA was dissolved in a blend solvent of dichloromethane and *N,N*-dimethylformamide (volume ratio of 7:3) at a

concentration of 10 wt%, and various amounts of CIP (weight ratio to PLA was 0.4%, 0.8%, or 1.6%) were also added. Then, the solution was electrospun to PP mesh collector under the condition including voltage of 16 kV, extrusion rate of 15 $\mu\text{L}/\text{min}$, and distance between the collector and syringe needle (0.4 mm in diameter) of 15 cm. Ambient temperature and relative humidity were kept at 25°C and 30%, respectively. After electrospinning for certain period, the PP mesh was flipped over and kept electrospinning for same time. Finally, the nanofibers were observed under scanning electron microscopy (SEM), and the samples were marked as NF, NFC-1, NFC-2, and NFC-3. Additionally, the NF-PP mesh would be marked as NFC-PP, for instance, NFC-3-PP means that the CIP concentration was 1.6%.

2.10 Antibacteria test of NF-PP mesh

In this study, *E. coli*, a Gram-negative bacterium, was selected to investigate the antibacteria property of the NF-PP mesh via the inhibition zone observation. Generally, *E. coli* was activated in a liquid medium of trypticase soy broth at 37°C in a shaker for 24 h, which was subsequently spread onto the agar plates. Then, NF-PP mesh with or without CIP with a shape of 1.5 mm round in diameter was placed onto the independent agar plate. After incubating at 37°C for 24 h, the images of each plate were taken to measure the difference of inhibition zone among all samples.

2.11 Biocompatibility of NF-PP mesh

Elastin gene-transfected BMSCs were used to investigate the biocompatibility of NF-PP mesh via CCK assay. The mesh with or without antibacteria drug was cut into appropriate size and placed in 24-well cultural plates; then, the plates were transferred to disinfection ethanol atmosphere overnight. Afterward, 2×10^4 cells per well were seeded and kept culturing for 1, 4, and 7 days to perform the CCK-8 assay. For cell morphology observation, the cells seeded 3 days were washed with PBS and fixed with 4% glutaraldehyde for 30 min at 4°C, then soaked in 0.1% Triton X-100 for 5 min and washed again. For cytoplasm, the cells were stained with Alexa Fluor@488 phalloidin solution (165 nM) for 10 min, and for nucleus, the cells were stained with DAPI solution (100 nM) for 10 min. Afterward, the cells were observed under confocal laser scanning microscopy (CLSM).

Figure 2: Green fluorescence pictures of BMSCs treated with (a) pure and (b) gene-loaded vectors, respectively; (c) CCK-8 assay of BMSCs with different treatments; and (d) photograph of elastin measurement with western blot experiment ($\times 400$).

Figure 3: Flow cytometry identification of G1, S, and G2 ratio of BMSCs with different treatments.

Table 1: Calculated ratio of G1, S, and G2 phases of BMSCs with different treatments

	Control %	Vector %	Elastin %	<i>p</i>	
				(1 vs 2)	(2 vs 3)
G1	81.7 ± 1.7	82.5 ± 1.6	74.9 ± 1.8	0.644	0.005
S	9.9 ± 0.9	11.6 ± 0.7	15.2 ± 0.9	0.052	0.006
G2	8.4 ± 0.8	5.9 ± 0.9	9.9 ± 1.0	0.098	0.006

2.12 Statistical analysis

The values are all presented as mean $\pm$ standard deviation. Statistical analysis was carried out through a two-way analysis of variance and Scheffe's *post hoc* test.

Figure 4: Calculated result of (a) cell migration, (b) staining images of control, (c) empty vector, and (d) elastin gene-loaded vector groups ($\times 100$).

The criteria for statistical significance were $*p < 0.05$ and $**p < 0.01$.

3 Results and discussions

3.1 Cytotoxicity and elastin gene expression in BMSCs

CCK-8 assay was used to evaluate the cytotoxicity of empty or elastin gene-loaded vector transfected BMSCs. Due to the green fluorescence from the vector, transfected cells were observed under fluorescence microscopy to study the success of transfection. As shown in Figure 2b and c, the fluorescence images of cells transfected with empty and gene-loaded vector indicated that both groups were well transfected and displayed

strong green fluorescence. In addition, the curves of O.D. value to time point are displayed in Figure 2a; during 6 days culture, there was no obvious difference comparing each group, indicating that the empty and gene-loaded vector showed good biocompatibility to BMSCs and would not affect cell proliferation. Moreover, western blot experiment was used to illustrate the elastin gene expression of transfected BMSCs, the result is displayed in Figure 2d, and elastin protein was only detected in the target gene-transfected group, which further demonstrated the successful expression of the transfected gene.

3.2 Cell cycle and apoptosis

To determine the influence of elastin gene transfection on cell division activity, cell cycle and apoptosis tests were carried out via flow cytometry. The results are

shown in Figure 3, and the calculated ratio to analyze the distribution at each phase is listed in Table 1. Obviously, after elastin gene transfecting, the G1 phase was 74.9 ± 1.8 , which is shorter than control and empty vector groups (81.7 ± 1.7 and 82.5 ± 1.6). Moreover, the S and G2 phases were 15.2 ± 0.9 and 9.9 ± 1.0 , respectively, which were significantly longer than the other two groups: 9.9 ± 0.9 and 8.4 ± 0.8 for the control group and 11.6 ± 0.7 and 5.9 ± 0.9 for the empty vector group. These might indicate that the cell division activity was enhanced through elastin gene transfecting.

3.3 Cell migration and invasion

In addition to the good biocompatibility and proliferation capacity, another vital aspect depends on the ability

of migration and invasion when stimulating wound healing; thus, corresponding investigations were carried out in this part. According to the migration results of cell number measurement and staining images (Figure 4), empty vector transfected BMSCs showed similar cell count to normal cells, indicating that the vector would not raise migration to BMSCs. However, elastin gene-loaded vector presented obvious improvement on migrated cell count, which suggested that the elastin gene transfection could enhance the migration ability to BMSCs.

Moreover, the cell invasion property could also reveal the cell movement ability, and the results (Figure 5) showed similar tendency to migration measurement; the cell count was significantly raised through elastin gene transfecting, which further proved that the cells could obtain considerable moving capacity after elastin gene transfection.

Figure 5: Calculated result of (a) cell invasion, (b) staining images of control, (c) empty vector, and (d) elastin gene-loaded vector groups ($\times 100$).

Figure 6: Optical images of BMSC migration with different treatments after incubating for various time periods ($\times 200$).

Figure 7: Migration distance calculation of BMSCs with different treatments after incubating for 6 and 12 h.

After acquiring the above conclusion, the elastin protein expression ability was improved after the gene transfecting. Actually, as a main structural protein of the body tissue, elastin protein could provide elasticity and strength and play a key role of growth factor in the cell

proliferation process. Thus, elastin protein will further affect the rate of wound healing and tissue repair.

Then, the wound healing experiment was applied to further verify the cell migration. As shown in Figures 6 and 7, the healing images were presented and cell migration distance of each group was also calculated. The wound was initially created on fully cell-covered cultural plate with certain width; then, the cells were continuously incubated for 6 and 12 h to estimate the wound healing ability. According to the optical observation, with incubating, the distance between the two sides became reduced in different extent.

After 6 h of incubation, the migration distances of control, empty vector, and gene-loaded vector groups showed no obvious difference about 100 μm , while which was enlarged after 12 h of incubation. The migration distance of control and empty vector groups was under 250 μm , which was over 300 μm for the gene-loaded vector group with great statistical significance to the other two groups. Summarizing with the above results, the elastin gene-transfected BMSCs indeed showed great movement abilities, which revealed that

Figure 8: SEM pictures of NF and NFC nanofibrous mats.

the method of elastin gene transfection was a promising way to accelerating wound healing in the PFD application.

3.4 Morphology observation of nanofibers

PLA is a widely used biocompatible and biodegradable polymer in biomedical field, which is also easy to prepare and nontoxic to human body. In PFD application, the present pure PP mesh might have the problem including adhesion between tissues due to the direct contacting; thus, a layer was introduced to interdict this direct contacting, nanofiber mat might be a superior candidate, which could well mimic ECM and support cell growth at the two sides of PP mesh. In addition, PLA was reported having good mechanical properties due to relatively high crystallization degree, which would make it much more durable to stand the friction from the tissue movement. Therefore, PLA nanofiber mat was chosen

as a covering layer on the PP mesh to overcome the current problem on the PFD surgical operation.

As shown in SEM images in Figure 8, PLA nanofibers with or without CIP were successfully prepared through the electrospinning technique, and the morphology of all samples was beadless and smooth with similar diameter distribution (the average diameter is 394, 405, 417, and 402 nm, respectively, measured by the software of Image Pro Plus), revealing that blending with CIP would not influence the nanofiber preparation.

3.5 Antibacteria ability and biocompatibility of NF-PP mesh

CIP loaded on the NF-PP mesh was expected to present an efficient antibacteria ability; thus, the corresponding test of the inhibition zone was performed. Herein, gauze was chosen as the control group instead of pure PP mesh

Figure 9: Antibacteria test of NF-PP and NFC-PP with different CIP concentrations.

for the reason of simulating the fully covered surface of NF-PP, whose surface was covered with nanofibrous mats at the two sides.

As shown in Figure 9, after incubating for 24 h, no visible inhibition zone was found on NF-PP mesh without CIP loading; the plate was fully covered with bacteria. However, the CIP loading samples presented great bacteria inhibition ability, and most area of plate was clean except seldom bacteria at the edge. In addition, the CIP concentration seemed no obvious influence to the antibacteria activity. The results indicated that the CIP could successfully release from PLA nanofibers and displayed a strong antibacteria activity in a relative low concentration.

To evaluate the biocompatibility of CIP-loaded NF-PP mesh, BMSCs were seeded and CCK assay was applied to study the cell proliferation. During the *in vitro* investigation, NF-PP without CIP was chosen as the control group instead of pure PP mesh due to which the much bigger pores of PP mesh would allow the cells to fall to the bottom of the cultural plate and could not support the adhesion of sufficient cells.

As seen in Figure 10, after incubating for 1, 4, and 7 days, the O.D. value kept increasing with time prolonging, while which was not significantly changed between NF-PP mesh with or without CIP. This might reveal that the mesh was well biocompatible and the incorporation with CIP would not influence the cell proliferation. Moreover, BMSCs were stained to observe the cell morphology under

CLSM. As shown in Figure 11, the cytoplasm and nucleus were stained to red and blue, respectively, and cells seeded on all samples grew well with the shape along the axial direction of nanofibers, further suggesting the biocompatibility of newly prepared NF-PP mesh which would not adversely affect the cell viability and morphology. Considering the aforementioned results, this novel high elastin expressing BMSCs incorporated NF-PP mesh obtained great potential for female PFD.

Figure 10: Cell proliferation assay for BMSC seeding on NF-PP and NFC-PP with different CIP concentrations for different periods.

Figure 11: CLSM images of BMSCs seeded on NF-PP and NFC-PP with different CIP concentrations (×200).

4 Conclusion

In this study, a novel PFD therapy method was proposed by the incorporation of antibacteria polymer nanofibers and elastin gene on the PP mesh. As expected, the gene-transfected BMSCs showed better elastin protein expression and migration ability than the original cells, which

would accelerate the healing rate. PLA nanofibrous mat was covered on the PP mesh to support cell adhesion due to the ECM-mimicked structure and potentially avoided the direct contact between the organs. Additionally, the antibacteria drug CIP loaded in PLA nanofibers could efficiently inhibit bacteria without obvious effects on cell growth and morphology.

Therefore, the strategy of incorporation with elastin gene-transfected BMSCs and CIP-loaded PLA nanofibrous mat coating on PP mesh showed great potential in the PFD therapy.

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