Electrospun Polyvinyl Alcohol/Cyclodextrin/Tobramycin Nanofibrous Matrix for Drug Delivery

[2]Bhullar, P; [1]Song, W; [1]Jin, X; [1]Shi, T; [3]Markel, D; [1, 3]Ren, WP*

[1]Department of Biomedical Engineering, Wayne State University, Detroit, MI

[2]School of Medicine, Wayne State University, Detroit, MI

[3]Detroit Medical Center/Providence Hospital Orthopedic Surgery Residency Program, Detroit, MI

Statement of Purpose:

Prosthesis infection remains a potential major course for implant failure and loosening. Development of periprosthetic antibiotics delivery devices is highly desired. The burst drug release represents one of the main limitations of periprosthetic drug delivery approaches. Electrospinning is one of the most promising methods to create 3-D fibrous scaffolds with enormous surface area. We propose that the embedding of antibiotics in these 3-D nanofibrous matrix will extend the drug release time. Tobramycin (TB) was an aminoglycoside antibiotic that is widely used in orthopedic infections. Cyclodextrins (CD) are cyclic oligosaccharides that are frequently used as drug carriers. Polyvinyl alcohol (PVA) is biocompatible and has good fiber forming capability. The purpose of this study is to develop and characterize the profile of electrospun PVA/CD/TB nanofibrous matrix for extended and controllable TB release.

Methods:

Preparation of PVA-CD-TB solution

Firstly, the TB aqueous solution (8 mg/mL) was mixed with the equal volume of CD aqueous solution (20 mg/mL) (1/1, v/v). This mixture was freeze-thawed for three cycles. Each cycle contains 1h freezing at -20ºC and 30min thawing at room temperature to yield TB-embedded CD solution. Secondly, the PVA aqueous solution (13%, w/v) was prepared through homogenizing at 90ºC. The TB-CD solution was then added to PVA solution yielding PVA-CD-TB mixture with a ratio of 1/2 (v/v). The mixture was homogenized at 60ºC and cooled down to room temperature. The pure PVA solution and PVA-CD solution were included as control.

Preparation of electrospun nanofibers

A set of electrospining device was equipped to fabricate nanofibers, which was composed of a high voltage supplier (Gamma high voltage research, 40kV, FL), a rotating collector (Arrow Eng., PA) and a syringe pump (Razel Sci., VT). The running parameters were set as: voltage—20kV, the rotation speed of collector—250 rpm and the rate of syringe pump—3 mL/h. Glass-made cover slips and Ca-P ceramics substrates were attached onto the collector to collect spun fibers. After 1h spinning, cover slips were detached and treated with UV light for 24h.

Morphology of electrospun nanofibers

Morphologies of electrospun nanofibers on cover slips were visualized by utilizing light microscope (Zeiss, US), while nanofibers on Ca-P substrates were analyzed by scanning electron microscope (SEM) (Hitachi, JP)

Anti-bacterial activity assay

A Mueller–Hinton broth inoculated with *S. aureus spores* was cultured at 37°C until an optical density (OD) at 625 nm reached to 0.08–0.1. This OD value is equal to roughly 1–2 $\times 10^8$ CFU/ml of *S. aureus spores*. The cover slips with nanofibers were covered with sterilized PBS for 22h. Then 100 µL of PBS was collected and added into 800 µL of the bacterial broth, and incubated at 37 °C for 2, 4, 6 and 8h, respectively. The OD value at 625 nm was measured for the culture medium collected at different time points.

Cytotoxicity of nanofibers co-culturing with pre-osteoblasts:

Osteoblast precursor cells from mouse cell line MC3T3, were seeded onto the surface of cover-slips with nanofibers in a density of 2,000 cells/plate. Cells were incubated for 1 week at 37ºC. MEM Alpha medium (invitrogen) was replaced every 3 days. Live/Dead staining was performed according to the instruction of manufacture (Invitrogen, US) to determine cell viability.

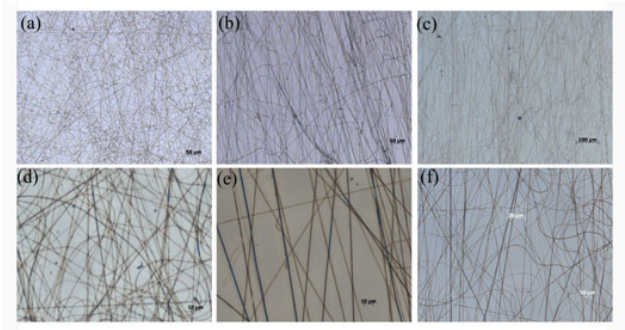

Fig.1 Light microscopic morphology of electrospun nanofibers on cover slips: (a, d) PVA, (b, e) PVA-CD, (c, f) PVA-CD-TB. Magnification: 100× (a-c); 400×: (d-f)

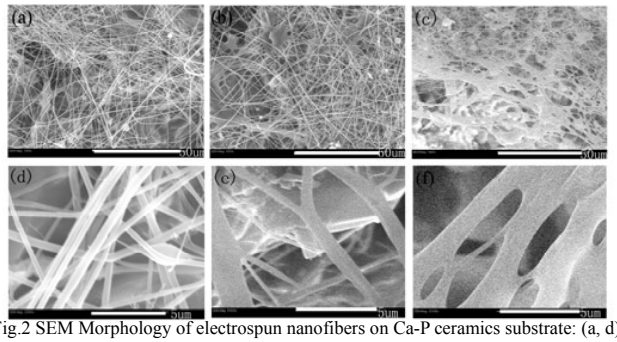

Fig.2 SEM Morphology of electrospun nanofibers on Ca-P ceramics substrate: (a, d) PVA, (b, e) PVA-CD, (c, f) PVA-CD-TB. Magnification: 1000× (a-c); 10000×: (d-f)

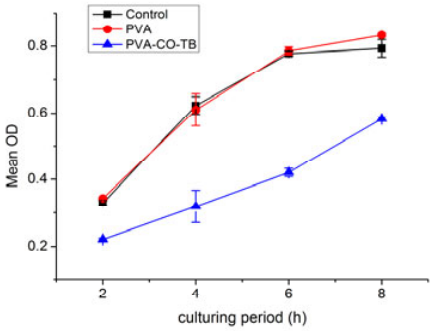

Fig.3 The profile of anti-bacterial activity assay. The OD was measured at 625 nm, n=3

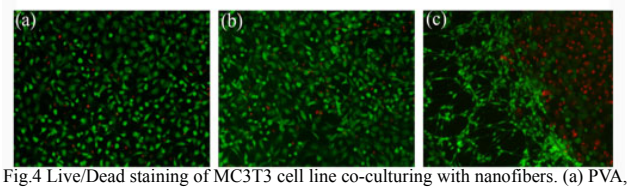

Fig.4 Live/Dead staining of MC3T3 cell line co-culturing with nanofibers. (a) PVA, (b) PVA-CD, (c) PVA-CD-TB. Magnification: 100×.

Results:

The electrospun nanofibers were randomized onto both cover slips (Fig.1) and Ca-P substrate (Fig.2)

The densities of PVA, PVA-CD and PVA-CD-TB fibers were equal. The diameter scale of fibers approximated to less than 1 µm. PVA-CD-TB nanofibers showed certain conglutination.

The bacteria proliferation was inhibited by TB in eluant (Fig.3)

The eluant of PVA and PVA-CD has no inhibitory effect on bacterial growth. But the eluant of PVA-CD-TB inhibited the bacterial growth by about 2-folds within 8h.

The PVA-CD-TB showed certain cytotoxicity (Fig.4)

The PVA and PVA-CD nanofibers showed excellent cytocompatibility, while PVA-CD-TB nanofibers demonstrated cytotoxic effects on MC3T3 cell line at given concentration.

Conclusion:

The feasibility of preparation of drug-loaded nanofibers via electrospining was proven in this study. The antibiotics released from fibers effectively inhibit the bacterial growth, which might provide a possible solution to orthopedic infection. However, the dosage of drug incorporated in nanofibers needs to be optimized since it can also influence the cytocompatibility. Electrospun nanofibers with excellent biocompatibility, biodegradability and delivery of functional antibiotics would be a new and promising approach to implant surface fabrication strategy.

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