Biodegradable Nanofibers For The Immobilization Of Bioactive Molecules

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Requirements for wound dressing

- Mechanical integrity
- Appropriate adherence to the wound
- Allow gas exchange
- Absorb exudates

For serious wounds functionalized wound dressing is required
FUNCTIONALIZED WOUND DRESSING

- Prohibit bacterial growth
  - Wound beds provide an ideal condition for the growth of microbial which will cause inflammation

- Key Attributes of Antimicrobial Dressing
  1. Moist environment
  2. Broad-spectrum antimicrobial activity

- Accelerate wound healing

- Not available commercially up to date
ELECTROSPUN NANOFIBERS FOR WOUND DRESSING

- Excellent mechanical strengths
- Ultra fine, soft
- High porosity
- Extremely large specific surface-area ratio
- Antibiotics loaded to protect wound from bacteria and infection
- Growth factors loaded to accelerate wound healing
- Mechanical integrity
- Appropriate adherence to wound
- Allow gas exchange
- Absorb exudates
- Prohibit bacterial growth
- Accelerate wound healing
Develop functionalized electrospun fibrous dressings that will provide a locally-controlled release of drugs as well as accelerated wound healing

1. A novel “emulsion-electrospinning” process to incorporate a hydrophilic antibiotic drug into the core of electrospun fibers without losing drug bioactivity
2. Demonstrate the sustained release behavior of the drug from electrospun fibrous dressing
3. Test the ability to inhibit bacterial growth
4. Immobilization of growth factor (a protein) onto nanofibers
ELECTROSPINNING APPARATUS

Electrospinning setup in our textile lab
MATERIALS FOR ELECTROSPINNING

- Poly (Ethylene Glycol)-Poly (Lactide) (PEG-PLA)
  - PEG: hydrophilic
  - PLA: hydrophobic
  - PEG (MW<5000) and PLA approved by FDA for internal uses in human body
IMMOBILIZATION OF DRUGS

reservoir-type structure to enclose drug in the polymer matrix

Core sheath structure
1. Preparation of water-in-oil emulsions containing Tetracycline hydrochloride (TC)
2. Preparation of TC-loaded electrospun fibrous mats
3. In vitro drug release studies
4. Antibiotic activities of TC-loaded fibrous dressings
5. Immobilization of Bovine Serum Albumin (BSA)
polymer solution: PEG-PLA /chloroform

surfactant: Triethyl benzyl ammonium chloride (TEBAC)

drug: Tetracycline hydrochloride (TC) (broad-spectrum antibiotics)
RESULTS AND CONCLUSION

Fig1. SEM images of 3.0 wt% TC-loaded PEG-PLA nanofibers

average diameter: 650nm

uniform, smooth, no drug crystals
TC release rate as TC% decreases with increased concentration of TC.

The hydrophilic drug was incorporated into PEG-PLA fibers forming core-sheath structure, greater amount of TC in the electrospinning emulsion resulted in a thinner core and a thicker sheath of fibers.

So, drug diffused more slowly through the relatively thicker PEG-PLA sheath.

Fig 2. Release profiles of TC from 1.0 and 3.0 wt% TC-loaded nanofibers.
DISK DIFFUSION

Day 0

Day 1

Day 2

Day 3

Day 4
clear inhibitory zones were observed around TC-loaded fiber mats, fiber mats loaded with TC showed longest inhibitory effects to S. aureus due to more sustained release of TC
3% TC-loaded fiber mat showed stronger and longer inhibitory effects to S. aureus, the inhibition zone difference of 3% TC-loaded fiber mat was not as big as that of 1% TC-loaded fiber mat.
IMMOBILIZATION OF PROTEIN ONTO NANOFIBERS

- Fiber Materials: PLA
- Protein: BSA, a model protein
- Approach:
  - Surface functionalization (expose –COOH)
  - Cross-lined with FITC-labeled BSA
PRELIMINARY RESULTS

confocal image of FITC-BSA immobilized PLA nanofibers
1. Emulsion electrospinning can efficiently immobilize hydrophilic antibiotic drugs into polymer nanofibers

2. The result TC-loaded nanofibrous mats can provide a controlled-release of antibiotic drugs and remain its antimicrobial ability during a certain period

3. Surface functionalization of nanofibers renders them better substrates for immobilization of bioactive molecules
Both antibiotics and growth factors can be loaded onto nanofibrous mat

The functionalized nanofibrous mat can be developed into wound dressing for wounds healing process, like foot ulcers which need a suitable dressings combining debridement and antimicrobial activity with moisture control.
REFERENCES


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THANK YOU
Appendix
In vitro drug release studies

incubated at 37 °C in 20 ml of phosphate buffered saline (PBS, pH 7.4).

sample was transferred to 20 ml of fresh buffer solution and the released TC in the original buffer solution was determined by UV spectrometer at 366nm
Antibiotic activities of TC-loaded fibrous dressing

- **Disk Diffusion**

- Disk with 0.6 cm diameter cut from nanofibrous mats are placed on agar plates seeded with S. aureus incubated in a CO2 incubator at 37 ºC for 24 h. Every 24 h, the tested disks were transferred to the new agar plates.

- Inhibitory effects were determined by disk diffusion method which is to measure the diameters of the inhibition rings.
Crosslinking with EDC and NHS and immobilization of BSA

EDC

Carboxylate molecule

Unstable reactive o-acylisourea ester

Sulfo-NHS

Stable amide bond

Regenerated carboxyl group

Stable amide bond
1) add EDC and NHS to PLA nanofibrous mats in PBS buffer
2) react 15min at room temperature
3) add 2-mercaptoethanol to quench EDC
4) add BSA solution and react at room temperature for 2h