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Designing an Antimicrobial Knitted Spacer Fabric for Vacuum Assisted Wound Closure

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OUTLINE

- BACKGROUND
  - Skin wound care
  - Requirement for wound dressings
  - Vacuum assisted wound therapy
  - Spacer fabric
  - Antimicrobial treatment
  - Surface modification
- EXPERIMENT
- RESULTS
- DISCUSSION
- FUTURE RECOMMENDATION
- SUMMARY
- CONCLUSION
- MOTIVATION

Start → Ends

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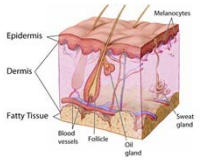
BACKGROUND

➢ Skin Components



Skin and its layers

- Serve as Protective barrier
- Consisting of three layers
  - Epidermis
  - Dermis
    - Associated glands and vessels (fatty tissue, blood vessels, follicle, oil gland, sweat gland)

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BACKGROUND

- Two major skin injuries

Diabetic foot ulcers

- One of the most common complications of poorly controlled diabetes
- Two major reasons: peripheral neuropathy and ischemia from peripheral vascular disease
- The incidence ranges from 15% to as high as 25%
- Adding around $9 to $13 billion to the direct annual costs for treatment

Burn injuries

- Dynamic injuries affecting the local and systemic tissue, characterized by an intense inflammatory and hyperdynamic response
- Affecting 1.25 million people in the US
- Over 480,000 burns cases receive medical treatment

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BACKGROUND

Wound Bed Preparation

- Debridement: Sharp surgical / Autolytic / Enzymatic / Mechanical / Biological
- Infection Control: Bacterial in wound management
  - Wound contamination
  - Wound critical colonization
  - Wound infection

- Moisture balance:
  - Intense inflammatory phase
  - Proliferation, migration, and differentiation of keratinocytes
    - Earlier restoration of the epidermal barrier
  - Fibroblast proliferation and collagen synthesis, earlier angiogenesis
  - Earlier wound contraction
- Edge Effect: Clinical observation of the edge of the wound

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BACKGROUND

Requirement for wound dressings

- ❑ Adjustable shape of wound dressings
- ❑ Moisture control
  - Absorb liquid to avoid maceration
  - Moist environment to promote wound healing
- ❑ Seal the wound from the outside environment
  - Reduce pain and infection
- ❑ The freedom of movement
- ❑ Control the growth of microorganisms
- ❑ Gaseous exchange
- ❑ Thermally insulates the wound

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BACKGROUND

Vacuum-assisted closure (VAC) system

Mechanism

- Contraction of the wound (macro-deformation at the foam-wound interface)
- Removal of extracellular fluid
- Stabilization of the wound environment

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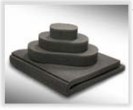
BACKGROUND

Current materials for vacuum-assisted closure (VAC) system



VAC® GranuFoam™ (KCI™)

- Polyurethane foam dressing
- Open
- 600
- unifo
- nega
- wou

PICO N
Nephe

- Redu
on r
- Manage
exudate

VAC® GRANUFOAM SILVER™ Dressing (KCI™)

- Polyurethane foam dressing
- ontinuous delivery of silver directly
o the wound bed

ALYST™ and ALLY™ NPWT
essories
Open cell, reticulated,
ydrophobic, high tensile strength

Limitation

- Retention of foam pieces in wounds
- In-growth of granulation tissue
- Rigidity of foam
- High costs

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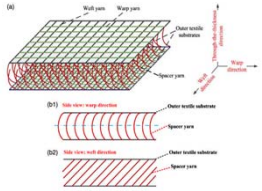
BACKGROUND

Spacer Fabric



- ❑ Properties of spacer fabric
  - Open-cell structures with high porosity
    - ➢ Liquid absorption capacity
    - ➢ Transfer moisture
  - Thermal insulating environment
  - Softness
  - Good resilience
    - ➢ Provide a good cushioning effect
  - Breathable performance
  - Flexibility
  - Tensile strength

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BACKGROUND

Antimicrobial treatment – Chitosan

- Natural polysaccharide obtained from the chitin extracted from the shell of crustacean shells
- A wide inhibition spectrum for not only Gram-positive and Gram-negative bacteria but also yeasts and molds
  - Mechanisms
    - Disrupted bacterial cell membranes with the effect of -NH_3^+
      - Electrostatic interaction
- Biodegradability
  - Depolymerizes to release N- acetyl-β-d-glucosamine
    - Enhance fibroblast proliferation
- Enhance in ordered deposition of collagen and stimulate increased level of natural hyaluronic acid synthesis
- Stopping bleeding

Bacteria	MIC* (ppm)
Agrobacterium tumefaciens	100
Bacillus cereus	1000
Corinebacterium michiganence	10
Erwinia sp.	500
Erwinia carotacora subsp.	200
Escherichia coli	20
Klebsiella pneumoniae	700
Micrococcus luteus	20
Pseudomonas fluorescens	500
Staphylococcus aureus	20
Xanthomonas campestris	500
Fungi	
Botrytis cinerea	10
Fusarium oxysporum	100
Drechatera sorokiana	10
Mirmactrilla nivalis	10
Piricularia oryzae	5000
Rhizoctonia solani	1000
Trichophyton equinum	2500

* MIC: minimum growth inhibitory concentration.

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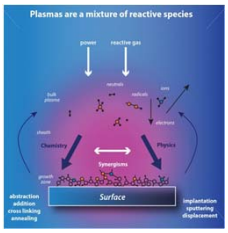
BACKGROUND

Surface modification by Plasma Treatment



Plasma surface treatment is a process that raises the surface energy of many materials so as to easily improve the bonding, gluing and painting characteristics

(1) Action of atomic oxygen:

$RH + 2O \rightarrow R\cdot + H\cdot + O_2$

$R1 + R2 + O \rightarrow R1\cdot + R2\,O\cdot$

$RH + O\cdot \rightarrow R\cdot + OH\cdot$

(2) React with oxygen molecules:

$R\cdot + O_2 \rightarrow ROO\cdot$

(3) Reaction of superoxide free radical:

$R1OO\cdot + R2H \rightarrow R1OOH + R2$

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OBJECTIVE

To successfully fabricate a 3D spacer fabric with the appropriate structure and achieve antimicrobial activity at surface by applying a chitosan coating to the plasma treated fabric

To determine whether or not the structural parameters and physical properties of the selected spacer fabric material are equivalent or superior to the existing foam wound dressing product

To determine whether or not the mechanical and moisture management properties of the selected spacer fabric material are equivalent or superior to the existing foam wound dressing product.

To determine by chemical characterization and antibacterial testing whether or not the plasma treatment has been effective in grafting the chitosan to the textile surface

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Material Selection

Acelity - V.A.C.® GRANUFOAM™ Dressing

- Polyurethane
- Open-cell structure
- Hydrophobicity
- 400-600μm pore diameter
- Foam size: 26 X 15 X 3.2 cm^3

VS

3D Warp knitted Spacer fabric

- Nylon 66 yarn
  - 100 denier, 34 filament
  - Unifi Inc., Greensboro, NC, USA.
- Antimicrobial agent
  - Chitosan
    - 95.3% deacetylate and a number average molecular weight of 740,000 (740 kDal).
    - Dr. Sam Hudson's lab , NCSU, Raleigh, NC.Z

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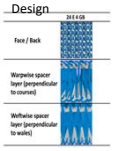
FABRICATION 3D WARP KNITTED SPACER FABRIC

Structure Design

Warping yarn

Warp knitting



- 3D Spacer Fabric
  - Same technique face and back
    - Tricot stitch to enhance tensile strength in both warp and weft direction
  - Spacer layer
    - GB 2 and GB 3 were used for spacer yarns
    - "Z" direction
  - Ruis double needle bed warp knitting machine
  - Using 24 Gauge, 4 guide bar

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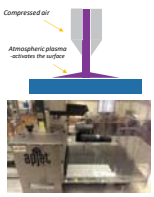
SURFACE MODIFICATION

Atmospheric plasma generator and stage



Atmosphere	Helium (99%)	Oxygen (1%)
Flow rate	39.6 L/min	0.9 L/min

In-line localized plasma treatment

- The velocity of the sample platform through the plasma was 6mm/min.
- Immediately after plasma activation, the samples were placed DZ1 in the 3% chitosan solution at room temperature for 6 mins
- After removal from the solution, they were placed in an oven at 95 ℃ for 10 mins to allow the grafting reaction to continue.
- The nylon spacer fabrics grafted with chitosan were then washed by immersion in deionized water 5 times. Then they were dried at 65 ℃ for 1 hour.

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DZ1 Should we include all these details?
Daxian Zha, 8/28/2019

RESULTS

Physical Properties of Spacer Fabric

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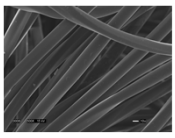
PHYSICAL PROPERTIS OF YARN

- The physical properties of the yarn used to warp all four beams and to knit the spacer fabrics are listed

100 denier, 34 filament Nylon 66 yarn

	Linear density (denier)	Tenacity (gf/den)	Elongation (%)	Shrinkage (%)
Mean	105.9	4.33	29.9%	4.25%
Std. Dev.	0.6	0.12	1.4%	0.00%



SEM photomicrograph (500X) of the nylon 66 indicates circular cross-sectional shape

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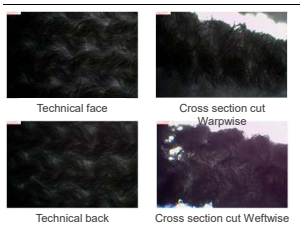
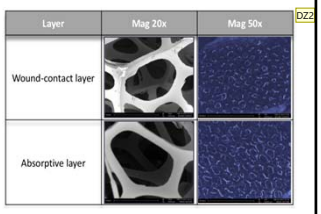
SURFACE MORPHOLOGY



Surface morphology by optical microscopy (20x)



Photomicrograph of GranuFoam™ by SEM

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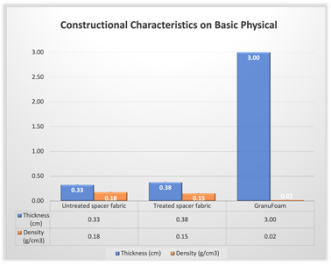
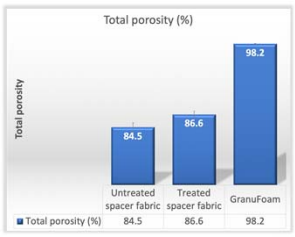
DZ2 confused by the MAG 50
Daxian Zha, 8/28/2019

CONSTRUCTIONAL CHARACTERISTICS





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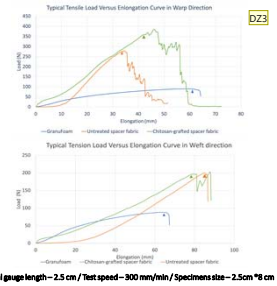
MACHNICAL PROPERTIES

Load(N)-displacement(mm) curve



DZ3

Comparison of tensile force at break (N)

Initial gauge length – 2.5 cm / Test speed – 300 mm/min / Specimens size – 2.5cm *8 cm

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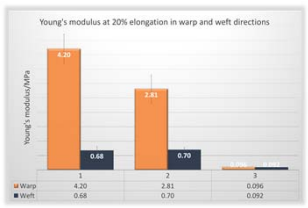
MACHNICAL PROPERTIES

Young's modulus



DZ4

- Tearing of the foam at the time of dressing removal and causing remnants of the foam dressing remaining in the wound
- Superior tensile properties of the spacer fabric dressing
  - Remain intact at the time of removal from wound.

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DZ3 make sure normalization
Daxian Zha, 8/28/2019

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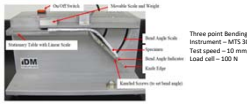
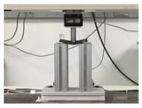
DZ4 change the figure
Daxian Zha, 8/28/2019

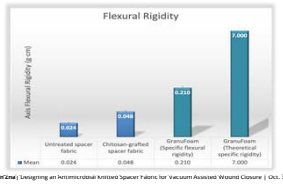
FLEXURAL RIGIDITY



Three point Bending Test
Instrument – MTS 30/G
Test speed – 10 mm/min
Load cell – 100 N



Cantilever Bending Test
Instrument – Model SD15
Cantilever Bendign Stiffness Tester



- The higher flexural rigidity, the harder it was for the materials to bend
- Superior flexibility of the spacer fabric compared to the GranuFoam™
- The chitosan surface coating has a positive impact on the flexural rigidity
  - Promoting surface adhesion between the filaments and the yarns

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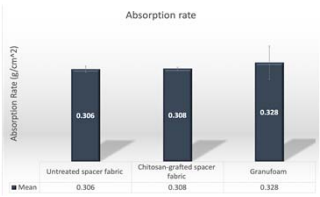
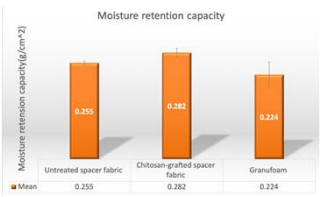
MOISTURE MANAGEMENT





Error bar = ± standard deviation

- The chitosan-grafted spacer fabric shows its comparability to the foam dressing in terms of its rate of moisture absorption and its superior moisture retention capacity.
  - Lower the maceration of the skin around the wound edges and promote exudate drainage when connected to VAC devices.

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BACKGROUND

Characterization of Chitosan Grafting

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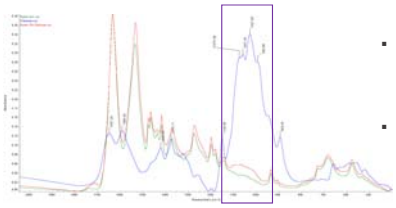
FOURIER-TRANSFORM INFRARED SPECTROSCOPY (FTIR)



FTIR Spectrum for spacer fabric(green),spacer fabric with chitosan(red) and chitosan(blue)

- The major characteristic absorption peaks of chitosan at 1057 cm^{-1} and 1027 cm^{-1} due to C-O stretching vibration
- RF plasma modification enabled the chitosan to be grafted to the surface of the nylon 66 spacer fabric

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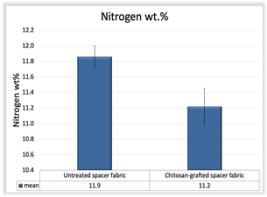
ELEMENTAL ANALYSIS - CHN



- A combustion analysis approach
  - Three elements presents: carbon, hydrogen and nitrogen
- Nylon 66 polymer
  - Nitrogen wt. %: 12.5% (theoretical composition)
  - Oxidation of the surface: marginally lower at 11.9%
- Chitosan
  - Nitrogen wt.%: 10.7% (theoretical composition)
- Chitosan/nylon 66 coated fabric
  - Nitrogen wt.%: 11.22% (experimental value)

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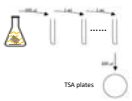
DETERMINING ANTIMICROBIAL ACTIVITY UNDER DYNAMIC CONTACT CONDITIONS

Procedure to determining antimicrobial activity

- Test organism
  - *Escherichia coli,* ATCC™ 25922™, a Gram negative bacteria
  - *Staphylococcus aureus,* ATCC™ 29213™, a Gram positive bacteria

50ml bacterial inoculum
Fabric: 1.0 ± 0.1g weight

Incubator shaker
36 °C T at a speed of 200 rmp



At each time point(0h, 1h, 6h, 12h,24h)
100 µl liquid from flasks was pipetted out and serially diluted.

Place plates in incubator
36 ± 2°C for 24 h

Count the # of colonies

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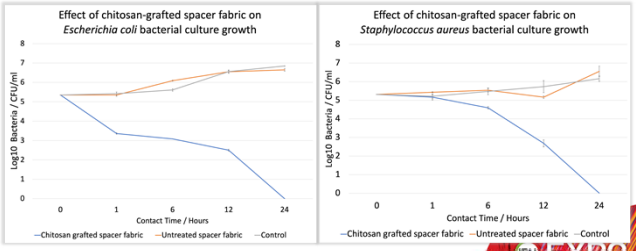
DETERMINING ANTIMICROBIAL ACTIVITY UNDER DYNAMIC CONTACT CONDITIONS

- The chitosan grafted sample gave 100% reduction in 24 hours, and displayed excellent antibacterial activity by controlling both strains.



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SUMMARY AND CONCLUSION

- The selected structure of the prototype spacer fabrics has successfully shown superior performance properties compared to the existing foam product: GranuFoam™.
- The 3D knitted spacer fabric prototypes showed superior mechanical properties such as tensile strength as well as flexural rigidity and equivalent moisture management performance compared to the GranuFoam™ product. The chitosan-coated spacer fabric further optimized the above performance.
- The plasma treatment was effective in grafting the chitosan to the surface of nylon 66 spacer fabric, and a positive antimicrobial activity was generated by the chitosan-coated samples.

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FUTURE RECOMMENDATIONS

- Evaluation of porosity properties
  - Design and develop a porosity gradient with an improved structure
- Evaluate the uniformity of the chitosan coating on the surface of the spacer fabric.
- The durability of the antimicrobial agent needs to be evaluated to ensure that the contact "killing" time survives for at least a week.
- *In vitro* cell culture studies with fibroblasts and keratinocytes
- Evaluate the properties of spacer fabric under constant vacuum.
- *In vivo* animal studies and clinical trials

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ACKNOWLEDGEMENT

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  - Dr. Martin W. King
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  - Brain Davis
  - Teressa White
  - Jeffrey Krauss
  - Birgit Andersen
  - Lisa Lentz
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Thank You

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