Advanced Manufacturing Research for Healthcare

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Invited Special Issue

06th August 2019

24 key papers by key leading researchers covering every part of the world
Microbubble size reduction

(A): **Triple (3) T-junction**
- Capillary size: 65 μm
- Channel gap size: 65 μm
- Gas Pressure: 80 kPa
- BSA solution: 10 wt %
- Flow Rate: 650 μL / min

The mean MB size is: **12.4 μm**

(B): **Triple (3) T-junction**
- Capillary size: 25 μm
- Channel gap size: 25 μm
- Gas Pressure: 80 kPa
- BSA solution: 5 wt %
- Flow Rate: 400 μL / min

The mean MB size is: **12.8 μm**
**C: Triple (3) T-junction**
- Capillary size: \(25 \, \mu m\) (gas inlet) and \(65 \, \mu m\) (rest)
- Channel gap size: \(65 \, \mu m\)
- Gas Pressure: \(80 \, kPa\)
- BSA solution: \(10 \, wt \%\)
- Flow Rate: \(700 \, \mu L / \text{min}\)

The mean MB size is: \(9.9 \, \mu m\)

**D: Quadruple (4) T-junction**
- Capillary size: \(100 \, \mu m\)
- Channel gap size: \(100 \, \mu m\)
- Gas Pressure: \(80 \, kPa\)
- BSA solution: \(10 \, wt \%\)
- Flow Rate: \(700 \, \mu L / \text{min}\)

The mean MB size is: \(8.7 \, \mu m\)
Microbubble size reduction with fission processes:

**Quadruple (4) T-junction**
- Capillary size: 65 μm
- Channel gap size: 100 μm
- Gas Pressure: 80 kPa
- BSA solution: 10 wt %
- Flow Rate: 600 μL / min

The mean MB size is: **6.6 μm**

**Quadruple (4) T-junction**
- Capillary size: 25 μm (gas inlet) and 100 μm (rest)
- Channel gap size: 150 μm
- Gas Pressure: 80 kPa
- BSA solution: 15 wt %
- Flow Rate: 700 μL / min

The mean MB size is: **6.30 μm**

**Quadruple (4) T-junction**
- Capillary size: 25 μm (gas inlet) and 65 μm (rest)
- Channel gap size: 100 μm
- Gas Pressure: 80 kPa
- BSA solution: 10 wt %
- Flow Rate: 650 μL / min

The mean MB size is: **2.80 μm**
Fission processes:

- MB size can be further reduced by fission process. (But lose the monodispersity of MBs)

- When the channel gap size is greater than the diameter of capillary, fission process occurred

**Quintuple (5) T-junction**
- Capillary size: **100 μm**
- Channel gap size: **150 μm**
- Gas Pressure: **80 kPa**
- BSA solution: **15 wt %**
- Flow Rate: **500 μL / min**
## Comparison study:

<table>
<thead>
<tr>
<th>Capillary size:</th>
<th>100 μm</th>
<th>65 μm</th>
<th>25 μm</th>
<th>25 μm for gas inlet</th>
<th>65 μm for rest capillaries</th>
</tr>
</thead>
<tbody>
<tr>
<td>Channel gap distance:</td>
<td>100 μm</td>
<td>65 μm</td>
<td>25 μm</td>
<td>65 μm</td>
<td></td>
</tr>
<tr>
<td>Fission Process</td>
<td>Not occurred</td>
<td>Not occurred</td>
<td>Not occurred</td>
<td>Not occurred</td>
<td></td>
</tr>
<tr>
<td>The smallest MB size:</td>
<td>22.8 ± 1.4 μm</td>
<td>12.4 ± 1.2 μm</td>
<td>12.8 ± 1.7 μm</td>
<td>9.9 ± 1.3 μm</td>
<td></td>
</tr>
<tr>
<td>The optimal conditions:</td>
<td>Triple (3) T-junction Gas Pressure: 80 kPa BSA solution: 15 wt % Flow Rate: 800 μL min⁻¹</td>
<td>Triple (3) T-junction Gas Pressure: 80 kPa BSA solution: 10 wt % Flow Rate: 650 μL min⁻¹</td>
<td>Triple (3) T-junction Gas Pressure: 80 kPa BSA solution: 5 wt % Flow Rate: 400 μL min⁻¹</td>
<td>Triple (3) T-junction Gas Pressure: 80 kPa BSA solution: 10 wt % Flow Rate: 700 μL min⁻¹</td>
<td></td>
</tr>
<tr>
<td>lifetime of MBs (without adding SiQD):</td>
<td>40.0 ± 2.0 mins</td>
<td>25.0 ± 1.5 mins</td>
<td>5.0 ± 0.5 mins</td>
<td>25.0 ± 2.5 mins</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Capillary size:</th>
<th>100 μm</th>
<th>65 μm</th>
<th>25 μm</th>
<th>25 μm for gas inlet</th>
<th>65 μm for gas inlet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Channel gap distance:</td>
<td>100 μm</td>
<td>100 μm</td>
<td>50 μm</td>
<td>150 μm</td>
<td>100 μm</td>
</tr>
<tr>
<td>Fission Process</td>
<td>Not occurred</td>
<td>Occurred</td>
<td>Occurred</td>
<td>Occurred</td>
<td>Occurred</td>
</tr>
<tr>
<td>The smallest MB size:</td>
<td>8.7 ± 0.8 μm</td>
<td>6.6 ± 2.1 μm</td>
<td>6.3 ± 1.4 μm</td>
<td>3.3 ± 1.4 μm</td>
<td>2.8 ± 1.5 μm</td>
</tr>
<tr>
<td>The optimal conditions:</td>
<td>Quadruple (4) T-junction Gas Pressure: 80 kPa BSA solution: 15 wt % Flow Rate: 800 μL min⁻¹</td>
<td>Quadruple (4) T-junction Gas Pressure: 80 kPa BSA solution: 10 wt % Flow Rate: 600 μL min⁻¹</td>
<td>Quadruple (4) T-junction Gas Pressure: 80 kPa BSA solution: 5 wt % Flow Rate: 300 μL min⁻¹</td>
<td>Quadruple (4) T-junction Gas Pressure: 80 kPa BSA solution: 15 wt % Flow Rate: 700 μL min⁻¹</td>
<td>Quadruple (4) T-junction Gas Pressure: 80 kPa BSA solution: 10 wt % Flow Rate: 650 μL min⁻¹</td>
</tr>
<tr>
<td>lifetime of MBs (without adding SiQD):</td>
<td>35.0 ± 4.0 mins</td>
<td>20.0 ± 2.0 mins</td>
<td>5.0 ± 1.0 mins</td>
<td>20.0 ± 1.0 mins</td>
<td>15.0 ± 4.5 mins</td>
</tr>
</tbody>
</table>
Multi-Layer Capsules
(prepared using electrohydrodynamics)

Many coaxial Needles

Generation 1


Generation 2


Generation 3

Conventional equipment

Precision syringe pumps
User control board
High voltage
Power converter

Generation 4

EPSRC GRANT: EP/P022677/1
Collaboration School of Pharmacy (Prof Duncan Craig) AND MECHANICAL ENGINEERING
**Pressurised Gyration & SISTER PROCESSES**

**EPSRC grant EP/L023059/1:** Exploitation of Pressurised Gyration as an Innovative Manufacturing Route for Nanofibrous Structures. Industrial Support: BASF, Astra Zeneca (Collab: Professor Duncan Craig, UCL School of Pharmacy)

*Invited Feature: 2018*

*Featured in Advanced Science News July 23rd 2018*

*BEST OF MACROS 2018*

*BEST OF MACROS 2019*
Creation and Exploitation of Pressurised Gyration to Manufacture Core-Sheath Structures


Polymers 2020, 12, 1709; doi:10.3390/polym12081709
Mahalingam, S; Huo, S; Homer-Vanniasinkam, S; Edirisinghe, M

Layered hybrid fibers could be used to build anti-viral masks, researchers say

Current methodologies and approaches for the formation of core–sheath polymer fibers for biomedical applications
S. Mahalingam, R. Matharu, S. Homer-Vanniasinkam and M. Edirisinghe
Applied Physics Reviews 7, 041302 (2020); https://doi.org/10.1063/5.0008310
EPSRC Grants: EP/N034228/1 & EP/N034368/1
Antimicrobial filters for hospital air and water system
(Collab: Dr Lena Ciric UCL Eng Civil Eng. (microbiologist) & UoH Eng.)

Mark-II of device (2015-16)
Nanocomposite filters were developed using pressurised gyration, during which poly(methylmethacrylate) (PMMA) solutions were loaded with **antimicrobial nanoparticles** and processed into nanofiber meshes.
Filters were tested using phosphate buffered saline solution spiked with high concentrations of *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The bacterial suspensions were passed through a peristaltic pump at 500 mL min\(^{-1}\) to simulate standard UK tap pressure.

The viability of bacterial populations were quantified and analysed using flow cytometry to determine live and dead cells after filtration.

**Project Partners:**
GAMA Healthcare Ltd
Intrinsiq Materials Limited
Pall Corporation
Pathogen Solutions
Professor John Oxford
AMNP8.1 filters showed reproducible results upon reuse every 30 minutes for 6 hours.

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The Effect of Graphene-Poly(methyl methacrylate) Fibres on Microbial Growth.

Graphene nanoplatelets (GNPs) are the most recently discovered carbon-based nanomaterial. They are the two-dimensional counterpart of carbon nanotubes and are composed of a single layer of sp2 hybridized carbon atoms arranged in a regular hexagonal lattice.

The microbial properties of a novel class of ultra-thin PMMA fibres containing 2, 4 and 8 wt% of (GNPs) were studied. A series of polymer solutions loaded with GNPs were prepared and processed into continuous, tubular and smooth nanofiber meshes using pressurised gyration. As GNP concentration increased, the average fibre diameter increased from 0.75 μm to 2.71 μm.

Gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa* were used to investigate the bacterial properties of the composite fibres. Pure PMMA fibres were used as the control. The presence of 2 and 4 wt% GNP loaded fibres promoted microbial growth, whilst 8 wt% GNP loaded fibres showed antimicrobial activity.

New project: Making a new generation of wound healing bandages

- Air Flow!!!
- Bigger Collector (from 150 to 300 mm in diameter)

<table>
<thead>
<tr>
<th>Sample Series</th>
<th>Relative Humidity (%)</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 PLA to 100 BC</td>
<td>41.4 - 45.3</td>
<td>23.1 – 24.8</td>
</tr>
<tr>
<td>100 PCL to 100 BC</td>
<td>42.6 - 45.7</td>
<td>22.2 - 23.7</td>
</tr>
<tr>
<td>PLA:PCL</td>
<td>51.3 – 54.5</td>
<td>21.3 – 25.8</td>
</tr>
<tr>
<td>PLA+PCL : BC (70:30 wt ratio)</td>
<td>48.5 - 51.9</td>
<td>20.1 – 23.6</td>
</tr>
</tbody>
</table>
New project: Making a new generation of wound healing bandages

PLA:PCL samples from collector to bandage form
PEO 15 wt.% + HA 30 wt.% at 36000 rpm and 0.3 MPa
PEO 15 wt.% + HA 30 wt.% at 36000 rpm and 0.3 MPa

- EDS line scan along a fibre

![EDS line scan](image)
**Electrospinning**

- Electric field overcomes surface tension of polymer solution which allows for a highly tuneable fibre production (voltage increase very incremental)
- Can create very fine fibres with wide range of biomedical applications
- Due to electric field, polymer solution must be conductive, which limits usage of various polymer systems

**Pressurised Gyration**

- Centrifugal force and applied gas pressure overcome surface tension of solution which allow for a large range of materials to be used, in solution form or otherwise
- Very rapid fibre production rate with great potential for scale-up
- The full potential of this technology has not yet reached
Differences in Polymer Preparation

**Electrospinning**
- Polymer solution must be prepared by dissolving a polymer within a solvent
- Polymer solution must be conductive in order to form fibres
- Solution must be homogenous otherwise it can effect the fibre formation
- Non-dissolved solutions can cause needle and pipe clogging and potentially damage setup

**Pressurised Gyration**
- Polymer solution can be prepared just like with electrospinning
- Non-homogenous solution can be spun as vessel is large and creates mixing during spinning
- Polymer melts can also be spun using melt gyration
- Materials in vessel can be in different phases, but can still form fibres
- Thick and viscous solutions can be spun which can be a problem with EHD

- Voltage can be adjusted very incrementally to give great control over final fibre morphology
- Flow rate is carefully governed to suit desired application and fibre size
- Collection distance can be altered to optimise fibre production by controlling fibre drying times
- Needle size can also have an effect on fibre production and morphology

- Rotation speed influences centrifugal force and can be adjusted to control fibre morphology
- Applied gas pressure can also be increased to reduce fibre diameter, and control bead production
- Collection distances and setups do influence fibre structure
- Relative humidity can be used to regulate drying times and thus fibre morphology
- Melt temperature and infusion rate can further used to regulate fibre production
Differences in Fibre Production Rate

Electrospinning

- Typical flow rates are 50-150 μL/min in a single needle setup
- Typical fibre production in 1 hour is approximately 0.6 grams of fibre for normal single-needle nanofibre setups
- Can be scaled up with multiple needle set-ups and advanced needle and collector devices which minimise solution-related problems

Pressurised Gyration

- 5mL of polymer solution can be produced into fibres in 15 seconds
- Typical fibre production rate in 1 hour is approximately 120 grams
- Infusion gyration can further increase production rate to exceed 6 kg/hour
- Just like electrospinning, multiple gyration pot setups can be created to increase production rate
Differences in Fibre Morphology

**Electrospinning**
- Without a modified collector electrospun fibres form mesh-like fibres useful in mimicking the ECM
- Increased collection times allow for stacking up of layers to create 3D structures
- Very low fibre diameters can be achieved as low concentration polymers can be used

**Pressurised Gyration**
- PG fibres can easily form highly aligned structures suitable for use in neural tissue engineering
- The fibres can be collected into mats, bandages and other shapely materials and used directly as bandages without an additional step
- High production capable of fulfilling demand in medical scenarios
Pressurised Gyration Vs. Electrospinning for Drug Delivery

Electrospinning
PVP-Itraconazole
Average Fibre Diameter: (0.94 μm ± 0.34)

Gyration
PVP-Itraconazole
Average Fibre Diameter: (1.60 μm ± 0.87)

Differences in physical applications

Electrospinning

- Can be used to create filters with small mesh sizes that are capable of trapping microbes
- Portable EHD gun can be used to coat a layer of protective fibres directly onto the wound site in an emergency state

Pressurised Gyration

- Rapid spinning process allow for macro-scaled materials to be produced fast allowing for use in more physical applications such as wound dressings
- As the spinning material does not have to be in a solution, a wide range of antimicrobial and pro wound-healing additives can be incorporated into the bandages

Combination of Gyration and Electro-spinning/spraying

- PCL high-yield fibres produced by gyration which can be collected as bandage-like sheets
- Fibrous ring is cut into pieces and robot transfers bandage to the electrospinning site
- Electrospinning of active nano-fibrous patches made of materials such as collagen and chitosan can be accurately printed onto the bandage
Production of Bioactive Bandages & Patches

- PCL fibres produced via pressurised gyration which act as the bandage
- Collagen and Chitosan solutions are electrospun onto the bandages to provide a bioactive patch which both protects the wound site from bacterial infections and accelerates the wound healing process.

Bandage with patch on 90 mm Petri dish

**Gyration** (1.71 ± 0.47 μm)  **Electrospinning** (142 ± 31 nm)
Next generation wound dressing made of nanofibers incorporated with antimicrobial peptides (AMPs).

- Materials including hydrogels, decellularized dermal porcine dermal matrix and freezing dried or gas foaming scaffolds have been studied for wound healing applications, however, lack the ability to recapitulate the architecture of the skin’s extracellular matrix (ECM).
- A new generation of wound dressing materials is anticipated to have a higher moisture level, and thereby provide sustained release results, which will enhance the healing of wounds due to the distinctive biological and non-sterile wound environment and to the difficult process of wound healing.
- Pressurised gyration is selected in this research because of its ability to mass produce bandage like meshes with simple separation and enhanced process control.
- A wound dressing material is created by loading peptides into water-soluble polymers that are proficient of efficient release. Thus, the growth of microorganisms is regulated by the antibacterial agents embedded in the structure of the fiber.
- A series of PEO integrated AMP nanofibers are prepared using GH12-COOH-M2 and AMP2 antimicrobial peptides, which are synthesized by solid phase peptide synthesis (SPPS) using an AAPPTec Focus XC peptide synthesizer, supplied by Kansas University (USA).

Schematic illustration of the antibacterial assessment of AMP integrated PEO nanofibers

Preliminary testing

Average fiber diameters using M2 peptides at 35 µg/ mL at 0.1 – 0.3 MPa

- Average fiber diameter at 0.1 MPa – 0.3 MPa: 506 nm ± 155, 395 nm ± 101 nm and 191 nm ± 55 nm respectively.

Scanning electron microscopy (SEM) images and size distribution graphs at 15% PEO/water using M2 35 µg/ mL, a) 0.1 MPa, b) 0.2 MPa, c) 0.3 MPa.
AMP characterisation using fluorescence microscopy

- Fluorescence microscopy was used to produce brightfield and polarisation contrast images
- Brightfield image A, and polarisation image B both serve as PEO control fibers. Images display fibers without attachment, this confirms samples are pure PEO fibers only
- Both polarisation images C and D display attachment of peptides (M2 and AMPs at 105 µg/mL) dispersed evenly inside and along fiber surface, illustrated as dots
- AMPs are embedded in the polymer matrix and fiber surface.

Comparison of antimicrobial effect of PEO fibers loaded with M2 and AMP2 at 105 µg/mL against S. epidermidis

Bacterial viability

- Bacterial viability was assessed at various concentrations using only M2 integrated PEO nanofibers
- An increase in the M2 content has produced greater antimicrobial activity.

Antimicrobial effect of PEO fibers loaded with M2 peptides (105 µg/mL – 175 µg/mL) against S. epidermidis evaluated with AlamarBlue cell viability assay

Comparison of bacterial viability at 105 µg/mL using M2 and AMP2 suggest that antimicrobial activity was significantly higher using AMP2, indicating a greater release rate when tested against S. epidermidis.
Fourier-transform infrared spectroscopy

FTIR spectra of the nanofiber samples

- FTIR depicts that characteristic peaks of PEO observed at 2900 cm\(^{-1}\) (methylene group CH\(_2\) molecular stretching), and at 1100 cm\(^{-1}\) and 960 cm\(^{-1}\) (C O C group stretching).
- FTIR results showed that there was no change in chemical structure as a result of M2 integration to fibers.
- FTIR of M2 samples integrated with PEO in two different concentrations did not reveal new chemical bonds.
- Therefore, FTIR peaks of PEO fiber and fiber samples containing M2 are similar.

Conclusions

- The novel PEO nanofibers incorporated with M2 and AMP2 peptides generated an effective antibacterial activity against \textit{S. epidermidis} to enhance wound healing applications.
- The nanofibers created a high efficiency against \textit{S. epidermidis} which is a common bacteria associated with wounds.
- AlamarBlue assay confirms that AMP2 (105 \(\mu g/\) mL) performed the greatest antibacterial activity.
- Therefore, loading antibacterial peptides with water soluble polymers such as PEO is an effective delivery method for the easy release of antimicrobrial agents into a wound site.
- Using water-based systems also allows to be more environmentally friendly whilst also ensuring maximum biocompatibility in an open wound scenario.

Antimicrobial peptide structures

- Secondary structure models of the peptides were obtained from the amino acid sequences using PEP-FOLD 3.5.8.
- Chimeric peptides with AMP2 incorporated demonstrated effective antibacterial function against \textit{S. epidermidis} bacteria.
New Portable Gyrator Device - Very High Speed/Pressure

- Portable
- Computer control
- Rotation speed up to 50000 RPM
- Pressure up to 0.7 MPa
- Flow Control
- Excellent Morphology
- Micro/Nano scale
- Different products
- Safe

Mass Production
Experimental and theoretical investigation of the fluid behavior during polymeric fiber formation with and without pressure

**C.S at 7000, 8500, and 10000 rpm no pressure VS P.G at 10000 rpm at 0.1, 0.2, and 0.3 MPa**

**Graphs and diagrams showing the fluid behavior and fiber formation results.**

- C.S at different speeds with no pressure.
- P.G at 10000 rpm at various pressures.

**Fiber diameter distribution**

- Frequency (%)
- Fiber diameter (nm)

**Mean and Standard Deviation**

- C.S: Mean=1636.67, Std. Dev=714.83, N=100
- P.G: Mean=620.75, Std. Dev=178.89, N=100
Groundbreaking Research on Polymeric Fibers

Creating Miracles with Polymeric Fibers

Amer Inst Phys News 15th October 2019

https://publishing.aip.org/publications/latest-content/creating-miracles-with-polymeric-fibers/

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https://www.sciedaily.com/releases/2019/10/191015131616.htm

Experimental and theoretical investigation of the fluid behavior during polymeric fiber formation with and without pressure

Applied Physics Reviews 6, 041401 (2019); https://doi.org/10.1063/1.5110965

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Key Sponsors
– The Royal Society, The Wolfson Foundation
  – Leverhulme Trust
  – Unilever
– Worshipful Co. Armourers & Brasiers
– Islamic Development Bank
– Orthopaedic Research UK (Furlong Foundation)
  – JRI Orthopaedics Ltd
– Danish Research Council, Veloxis Pharmaceuticals

Key Academic/Other Collaborations
– University of Oxford
  – UCL Div. of Medicine, UCLH, Moorfields
– UCL School of Pharmacy, UCL Business
  – University of Cambridge
  – University of Hertfordshire
  – University of Sheffield
    --Marmara University
  – University of Padua
  – Hacettepe University
– China University of Geosciences, Beijing, China
  --University of Kansas
  --University of Sichuan, Chengdu, China
  --North Dakota University
  --Helsinki University
  --National Institute for Materials Science, Ibaraki, Japan

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