Silk Biomaterials for Regenerative Medicine

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Driven by the ever-increasing demand of donor tissues and organs from patients suffering from end-stage tissue and organ loss.

Registrations for Organ Waiting List (Total number: 117,398)

<table>
<thead>
<tr>
<th>Organ Type</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td>92,584</td>
</tr>
<tr>
<td>Liver</td>
<td>16,750</td>
</tr>
<tr>
<td>Pancreas</td>
<td>1,451</td>
</tr>
<tr>
<td>Kidney/Pancreas</td>
<td>2,268</td>
</tr>
<tr>
<td>Heart</td>
<td>3,181</td>
</tr>
<tr>
<td>Lung</td>
<td>1,836</td>
</tr>
<tr>
<td>Heart/Lung</td>
<td>77</td>
</tr>
<tr>
<td>Intestine</td>
<td>251</td>
</tr>
</tbody>
</table>

Sep 06, 2010 data on size of waiting list in United States for waiting list candidates for vital organ transplants.

Source: Organ Procurement and Transplantation Network (OPTN)
The Rise of Regenerative Medicine

Factors that pushed for clinical need of engineered tissues:

• Lack of suitable donor organs
• Need for immuno-suppressive drugs for allogeneic tissues/organs
• Donor site morbidity for autograft options
• Possibility of cross-species genetic virus infections in xenotransplantation
• Long-term application issues for mechanical organ assist/ replacement devices

Consequently, it was highlighted that regenerative medicine would be the “vanguard of 21st century health care”, with National Institutes of Health and National Academies acknowledging regenerative medicine as a significant core component of modern medical practice.

Current Treatment Methodologies for Conditions Requiring Musculoskeletal Tissue Replacement

- **Autografts**
  - eg. Hamstring Tendon/ Patellar Tendon/ Achilles Tendon

- **Allografts**

- **Xenografts**

- **Synthetic Graft Replacements**

**Limiting Factors**
- × Donor Site Morbidity
- × Immunological Foreign Body Response
- × Disease Transmission
- × Donor Shortages
- × Donor-Recipient Compatibility
- × Infections
- × Implant Fatigue Failure

Source: [http://www.scoi.com](http://www.scoi.com)
Tissue Engineering and Regenerative Medicine

An interdisciplinary field that applies the principles of engineering and the life sciences towards the development of biological substitutes that restore, maintain, or improve tissue function.

Stimuli:
- Biochemical cues
- Mechanical cues
Scaffolds

Scaffold Materials:

• Biocompatible
• Biodegradable
• Chemical Properties
• Mechanical Properties
• Proper architecture
New opportunities for an ancient material

Silk Fibroin Material

➢ **Strength and toughness**
  ➢ [Perez-Rigueiro et al., 2000]
  • UTS: $566 \pm 71.6$ MPa
  • Modulus: $15.9 \pm 2.63$ GPa
  • % Strain at break: 4–16%

➢ **Gradual degradation over long duration**
  • Proteolytic degradation mediated by foreign body response [Horan et al., 2004]

➢ **Biocompatible upon removal of sericin**
  • Sericin is main allergen of silk leading to Type I allergic response [Altman et al., 2003]

➢ **Advantageous side chemistries**
  • Silk consists of diverse amino acid side chemistries for facile coupling of selected growth or cell adhesion factors [Sofia et al. 2001]

Biocompatibility Assessment

- Silk sheath induce discrete intracytoplasmic granules with no cell lysis or reduction in cell growth in the tested mouse connective tissue cell line (NCTC clone 929), indicating non-cytotoxicity.

- Sensitization tests indicated limited erythema and oedema at challenged skin site over 48 hours.

- No significant biological reactivity findings compared to the respective negative control groups in the acute systemic and sub-chronic repeated dose toxicity assessments.

- Genotoxicity (Ames test) showed that the sheath was non mutagenic in the tested bacterial strains of Salmonella typhimurium and Escherichia coli.

Silk sheath was cut length-wise to form a rectangular mat (A), which was rolled into a cylinder (L5 × Ø2 mm) (B) for filling into a 2 mm circular bone defect made by ring drill along the diaphysis of the femur in a rabbit model (C, D). Histological sectional images at filled defect 12 weeks post implantation (E: 200×, F: 400× magnifications).

- Histopathological assessments of the extracted femurs in a 12 weeks implantation study in the rabbit model indicated moderate foreign body response with presence of neovascularization and bone ingrowth.
Various Forms of Silk for Regenerative Medicine

- Knitted silk
- Silk sponge
- Electrospun silk
- Sheets/films
- Submicron-micron fibers
- Fibers
- Highly dense blocks
- Gels-knit hybrids
- Silk putty
Cell Guidance using Electrospun Silk and Topography

- Consistent and significantly **more viable cells in the AL groups** (both static and dynamic) compared to other respective groups from day 7 onwards

- **AL (dynamic)** have **more viable** cells than **AL (static)** on day 14

- Significant proliferation was observed in AL (dynamic), AL (static) and RD (dynamic) through the 14-day culture


Confocal micrograph illustrating actin fibers (red) and nuclei (blue) of fluorescent stained MSCs seeded on RD (A,C,E) and AL (B,D,F) scaffolds and cultured for 3 days (A,B), 7 days (C,D) and 14 days (E,F).
Anterior Cruciate Ligament Damage

ACL injuries remain one of the common sports-related injuries

Treatment

Autografts: Hamstring graft
Silk Scaffolds for ACL Replacement

Loaded BMSCs
6 Months after Surgery

A

Lateral femoral condyle
Medial femoral condyle
Native ACL

C

Lateral femoral condyle
Medial femoral condyle
Regenerated ACL
Mechanical properties of native ACL and regenerated ligaments of experimental and control groups. The femur–ACL–tibia (A) and femur–regenerated ligament–tibia complexes (B) and control group (C) were firmly fixed on Instron machine to perform mechanical test.

The load–deformation curves of native ACL (D), regenerated ligament (E) and control group (F) were recorded at 24 weeks postoperatively.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Maximum load (N)</th>
<th>Stiffness (N/mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native ACL</td>
<td>770.1±105.2</td>
<td>93.9±15.9</td>
</tr>
<tr>
<td>Scaffold</td>
<td>398.8±69.5</td>
<td>58.5±16.8</td>
</tr>
<tr>
<td><strong>Experiment group</strong></td>
<td><strong>404.9±49.9</strong></td>
<td><strong>57.5±9.4</strong></td>
</tr>
<tr>
<td>Control group</td>
<td>120.3±14.2</td>
<td>10.9±1.8</td>
</tr>
</tbody>
</table>
Interface TE

Scaffold modifications

Tunnel bone calcification

Animal studies

Ligament/Tendon TE

Bioreactor

Topography

Intra Vertebral Disc (IVD) TE

Ligament/tendon – bone

Nano-HA

Growth factor delivery

PEMF

3D Tumor model

Silk-based Technology

Cardiac Patch

Interface TE

Ligament/tendon – bone

Cartilage – bone
Acknowledgement
Members of the Tissue Repair Group

Current Team Members
Dr Thomas Teh
Dr Neo Puay Yong
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Ms Png Si Ning
Ms Maria Christine Asuncion
Mr Ernest Tan Xuan Hao
Ms Liang Yeshi

Past Team members
Prof Ouyang Hongwei
Prof Ge Zigang
Prof Liu Haifeng
Prof Fan Hongbin
Dr Sambit Sahoo
Dr Shi Pujiang

Dr He Pengfei
Dr Chen KeLei
Dr Ng Kian Siang
Dr Pamela Tan
Dr Eugene Sim
Dr Yu Jiangwei

Special thanks: Ms Saikwan, Chul Thai Silk Company
Up Coming IFMBE-Sponsored Conferences

NBC 2017 and EMBEC 2017 on 11-15 June 2017 in Tampere, Finland

ASIA-PACIFIC CONFERENCE ON MEDICAL AND BIOLOGICAL ENGINEERING 2017
November 2017, Sydney, Australia

World Congress on Medical Physics & Biomedical Engineering
June 3–8, 2018, Prague, Czech Republic
Thank you