Novel Use of Biomaterials in a Clinical Setting: One Surgeon’s Journey from the Clinic to the Laboratory and Back Again

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Resorbable keratin-based biopolymer as a bone substitute material

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In Vivo Sheep Trial 1

• ... and 38.5 million sheep

• 2007 Agricultural Production Census, Statistics New Zealand
• NZ is the 3rd largest (17%) global wool producer (after Australia and China), and 2nd largest exporter (after Australia)
• Intact keratin proteins

From: Strelkov SV, et. al. 2000
Keratin protein fractions

- Matrix protein
- Intermediate filament protein
Reversible protection of cystine crosslinks

\[ W\text{--S--S--R} + \text{SO}_3^{2-} \rightarrow W\text{--S--SO}_3^- + R\text{--S}^- \]
Electrophoresis

SDS 1D

SDS 2D
Biopolymer materials

Bone graft
Bone fixation
Incorporation of other materials, eg: HA
Biopolymer materials

Membranes, coatings, fibrous assemblies
Biopolymer materials

Soluble powder
Gel forming liquid
Hydrogels
Keratin hydrogel
Control of porous structure

Leaching of porogens
Control of porous structure
<table>
<thead>
<tr>
<th></th>
<th>KERATIN HA</th>
<th>CANCELLOUS BONE*</th>
</tr>
</thead>
<tbody>
<tr>
<td>% density</td>
<td>29</td>
<td>20-25</td>
</tr>
<tr>
<td>Cell size / intertrabecular spaces (µm)</td>
<td>100-500</td>
<td>200-500</td>
</tr>
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* Schenk, Biology of Fracture Repair
In Vivo Histomorphological Results
Results - Biological response

Collagen implant 3 weeks
Results - Biological response

Porous keratin-4%HA implant 3 weeks
Results - Biological response

Porous keratin-4%HA implant 8 weeks
Results - Biological response

Porous keratin-4%HA implant 12 weeks
Results - Biological response

Dense keratin implant 12 weeks
Keratin HA/ Week 4/ Micro-CT
Osteoinduction

- KP/HA/BM 21 days sub-muscularly in Lewis rats
- Some cells show tendency towards osteoblastic differentiation & widespread osteoid may have formed with increased implantation times.
Osteoinduction

- KP/HA/BM 21 days sub-muscularly in Lewis rats
Week 1 (VG)

Sham

Keratin/HA
Week 2 (VG)

Collagen

Keratin-40%HA
Trial 3: Keratin/HA Week 4  VG (x4)
Trial 3: Keratin/HA Week 4 VG (x40)
Trial 3: Keratin/HA Week 4  VG (x40)
Trial 3: Keratin/HA Week 8  VG (x10)
Unique Properties of Keratin/HA

• Scaffold for cells and protein binding
• Calcifiable, biodegradable matrix
• Biocompatible
• Non-antigenic
• Stimulates osteoconduction, osteogenesis, and possibly osteoinduction

Patents


Novel hybrid resorbable sutures

- A novel processing method was established to produce dairy protein based bio-absorbable hybrid medical suture, which retain positive biological properties of dairy proteins.

- The methods involved in processing are simple and similar to melt extrusion fibre manufacturing.

- This processing methodology uses moderate mixing temperature without any chemical agents, hence will not affect the physiological or biological benefits of proteins.

- The hybrid suture demonstrated unique characteristics of surface and matrix morphologies.
Suture diameters

- The manufacturing process can produce variable suture diameters.

- Approximately 30-50 μm diameter of mono-filament suture was produced for this investigation.

- Processing methodology demonstrated that this melt-extrusion process can also be applied to produce multi-filament sutures.
Mechanical performance of sutures

- Hybrid sutures (DP01 & DP02) comprising desirable proportions of dairy proteins exhibited good mechanical properties similar to the control (PCL based biodegradable suture) in dry and wet conditions.

- Tensile strength (in dry & wet conditions) results demonstrated that the hybrid suture having suitable mechanical characteristics that is desirable in a medical suture.
In vitro/in vivo results

- The tensile strength (TS) and knot strength (KS) of hybrid sutures in particular DP02 suture remained almost unchanged after in vivo subcutaneous implantation (in rats) for 14 days. In contrast, these properties of the control suture (PCL based commercial) decreased with time.

- Significant drop in KS of PCL suture which may be of concern in clinical applications.

- Hybrid sutures constantly retained TS and KS (DP02 suture) during subcutaneous in vivo implantation at the different time-points. These results indicate that hybrid sutures possibly integrating with skin tissues leading to enhancement of TS & KS in vivo at 14 days.
In vitro/in vivo results

- The hybrid sutures demonstrated biocompatibility and satisfactory keratinocyte (skin cell) proliferation, comparable to PCL.

- The following skin closure *in vivo* investigation was based on these results.
SKIN/WOUND CLOSING STUDY IN VIVO
SKIN/WOUND CLOSING STUDY \textit{IN VIVO}

- Skin/wound closing study was conducted in the Lewis rat.
- The wound strengths were approximately 30\% greater at the 2 experimental suture wound closing sites compared to the PCL control at 7 days.
- This important finding illustrates that the hybrid suture comprising dairy protein are fully bioabsorbable and enhances wound healing leading to significant increase in wound strength compared to PCL based suture.
- The skin healing/closing results of hybrid sutures \textit{in-vitro} and \textit{in-vivo} (animal model) studies reveal that sutures comprising dairy protein demonstrating remarkable skin wound healing/closing performance.
## SKIN/WOUND CLOSING STUDY IN VIVO

<table>
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<tr>
<th></th>
<th>Control</th>
<th>Material A</th>
<th>Material B</th>
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<tbody>
<tr>
<td><strong>Day 7</strong></td>
<td>549.95 µm²/µm</td>
<td>578.29 µm²/µm</td>
<td>661.68 µm²/µm</td>
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<tr>
<td><strong>Day 14</strong></td>
<td>1249.04 µm²/µm</td>
<td>722.26 µm²/µm</td>
<td>422.47 µm²/µm</td>
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<tr>
<td><strong>Day 28</strong></td>
<td>665.97 µm²/µm</td>
<td>510.76 µm²/µm</td>
<td>496.92 µm²/µm</td>
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Competitive Advantages with our technology

- The current medical sutures (both resorbable and non-resorbable) in the market provide mechanical support to lesions in tissues. These do not claim any physiological and biological benefits.

- The hybrid sutures produced by incorporating dairy protein based biomaterials into biocompatible polymer demonstrate significantly improved healing performance and tissue-integration, with desirable mechanical properties. These balanced attributes demonstrates significant potential for creating new generation of medical sutures.

- However, suture production process may require further improvement to achieve higher mechanical properties, and to fuse needle onto the suture (eye-less needle).

- Further small and large animal trials are also required prior to pre-clinical trials.

- While exact manufacturing cost is unknown at this stage; it is envisaged that the cost would be approximately 10% higher than the current PCL based sutures.
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