

Summary & Highlights from Tissue Engineering: State of the Art and Future Perspectives

UNSW Biomedical Engineering
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As told by Cameron Wilson,
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Introduction to Tissue Engineering

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Introduction to Tissue Engineering

- Clinical and production issues...
 - Inert implants in dynamic environment.
 - Lack of viable materials & litigation risk.
 - Longer patient life - wear of implants.
 - Diseases limit ability to heal.
 - Accidents & sports injuries.
- Availability of cells and blood supply.
 - Apoptosis
 - Stimulate angiogenesis or seed endothelial cells.

Scaffolds

- Mimic ECM signals / structural cues.
- Options: natural, synthetic, composite, permanent, degradable, preformed, formed *in situ*.
 - CSIRO developing 2-part injectable polymer for bone repair.
 - Beads (eg demineralized bone) avoid contraction of fibrous/gel scaffolds...
 - Bioreactors to suit cell type.

Cells & Models

- Mesenchymal / embryonic stem cells / transfected cells.
- Can grow MSCs in 3-d matrix - eg collagen sponge / demineralized bone matrix.
- Animal model considerations...

Design & Fabrication of Scaffolds for Tissue Engineering Applications

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National University of Singapore

- Working on bone/cartilage TE &, more recently, skin.
- Rapid prototyping with polytechnics.
- Milestones set:
 1. Scaffold design & fabrication.
 2. Cell culturing & seeding in static env't.
 3. Tissue growth in dynamic environment.
 4. Transplantation.

Types / Classifications

- Biodegradable vs bioresorbable.
- Guided tissue regeneration.
 - Membrane prevents soft tissue invasion.
- Synthetic organic / inorganic materials.
- Natural organic / inorganic materials.

Material Developments

- Important to search related fields!
- Some materials well established.
- Recently approved polymers (eg):
 - Polyorthoesters
 - Polyanhydrides
 - Polyhydroxyalkanoates (PHA)
 - Hyaluronic acid derivatives.
- Emerging materials for specific cell types
 - eg poly(lactic acid-co-lysine).

Scaffold Characteristics

- "Ideal" characteristics...
- Loss of mech. properties in degradation.
- "Bioerodable" polymers.
- Degradation by-products.
- Range of hydrophilicity acceptable to cells (depends on cell type)
 - Increase with surface modifications
 - NaOH, glow discharge, etc.

Scaffold Characteristics (2)

- Pore size & % porosity.
- Body stops replacing scaffold in time - problem with large defects.

Problems & Opportunities

- Sinus lift - bone tissue engineering.
 - Currently use graft + patient-derived PDGF
- Can't presently grow blood vessels in scaffold *in vitro*.
- Tissue engineering not so much cells & ECM on surface as filling 3-d space.

Developments at NUS

- Forming 3-d structure by combining cell / fibrinogen mixture with thrombin in culture medium.
- Seeding cells onto Ti (with fibrin glue) to form bone prior to implantation.
- Shape memory alloy micro-gripper for precision positioning.

Gene Technology in Tissue Engineering

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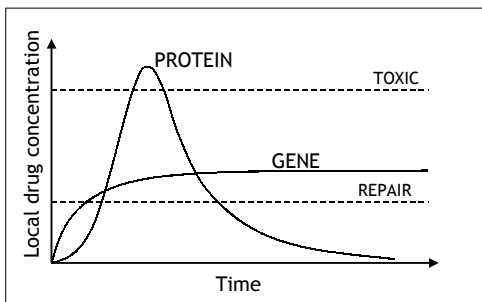
Transgenes in Tissue Engineering

- Introduce transgenes, via vectors, to achieve *higher* levels of expression.
- Stable:
 - Correction of genetic abnormalities.
 - Restricted to life-threatening diseases.
- Transient:
 - Tissue engineering "mainstay".
 - "Kick-start" or boost healing.

Applications

- Therapeutic gene expression compromised.
- Correction of underlying condition.
- Accelerate repair / rehabilitation.
- Alternatives (eg protein delivery) may be ineffective.
 - Dosage, degradation, duration...

Potential Benefit



Problems

- Public perception re GMO's.
- Risk vs benefit.
- Regulatory path will be difficult!

Solutions (?)

- Can T.E. be treated as special case?
- Genetic modification prior to seeding
→ vector not delivered to patient.
- Cell-specific promoters for targeting.
 - Doesn't seem to be happening at present.

Mesenchymal Stem Cells

Bruce Milthorpe
Head of
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Mesenchymal Stem Cells

- Act as reservoir for replacing cells.
- Found in connective tissue & muscle.
 - Usually harvested from marrow.
- Risks of use:
 - May produce undesirable phenotype.
 - Cancer.
 - Loss of control on implantation.

Desirable Characteristics

- Capacity for "infinite" expansion.
- Can send along any differentiation path.
- Able to genetically manipulate?
- Non-immunogenic when implanted:
 - Prefer autologous.

Growing in Culture

- Low cytokine / hormone conditions.
- Low O₂ conditions beneficial.
- Differentiate with:
 - Bone: 1,25dh-D₃, dex, β-glycerophosphate.
 - Cartilage: BMP-2, suspension culture.
 - Adipose: bFGF.
 - Muscle / tendon / ligament: ?

Tissue Sealing, Haemostasis & Wound Healing

Rick Tocchetti
State Red Cell Serology Reference Laboratory
IMVS Haematology

Fibrin-Based Tissue Sealants

- Well established - many applications.
- Avoid problems with sutures & synthetic adhesives.
- [Fibrinogen] → strength & adhesion.
- [Thrombin] → setting time.
- Coagulation risk in intravascular use.

Sources

- IMVS concentrates & precipitates fibrinogen from donor blood (~1½ days).
 - Ideally autologous.
- Bovine thrombin - some risks.

Future Developments @ IMVS

- Improvements to service & production.
- New clients.
- Validity testing of autologous thrombin.
- Develop as delivery vehicle for therapeutics.

Autologous Platelet Gel

- Source of growth factors.
 - Synergy between degranulation factors in bone healing?
- Autologous platelet gel (Gronthos *et al*):
 - Patient's own GFs → less costly.
 - Can mix with morselized autograft.
 - Mostly for elective surgery.
 - Often used with fibrin glue.

Case Study: Skin Tissue Engineering

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The Market

- Range of commercial products avail.
- Skin grafting / chronic wounds: large market.
- Dressings / grafts / tissue-eng'd skin.
 - Ultimate aim is "true skin".
 - Epidermal sheets fragile.
 - Deep wounds: dermal graft followed by epidermal sheet - expensive.
 - Neonatal foreskin fibroblasts:
 - Low allergenicity (?) Cell survival? Expensive.

Tissue Engineered Skin

- To simulate true skin, fibroblasts submerged; keratinocytes exposed to air.
- Success with mixing keratinocytes in fibrin glue & spraying/injecting.
 - Higher up-front cost, but no on-going treatment.
 - Fibrinogen etc stimulate angiogenesis.

Tissue Engineered Skin (2)

- NUS: biaxially-stretched PCL films.
 - Keratinocytes on poly(ϵ -caprolactone);
 - Fibroblasts in foam with growth factors.
- Parenteau "Skin: The First Tissue Engineered Products" *Scientific American* 1999 April, 83-85.

Caution!

- Fibroblasts contract substrates.
- Rat models - itching starts with epithelialization!
- Some treatments slow repair but reduce contraction & scarring.

Case Study: Cartilage Tissue Engineering

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Cartilage Defect Treatments

- Conduction / Embedding / Scaffolding
- Cell Seeding / Transplants:
 - Autogenic - culture from biopsy.
 - Brittberg *et al.*: inject, cover with periosteum & seal with fibrin glue.
 - Seed in hydrogel for 3-D construct.
 - Tissue develops better in dynamic environment - eg perfusion chambers (Sittinger *et al.*)

Cell Seeding / Transplants

- Tissue mechanics depend on cell source - articular superior "hardness".
- Periosteum often used as MSC source.
 - Many treatments fail w/out periosteum.
- MSC's achieve better results than chondrocytes.

Tissue Engineering - Horse Model

- Poly lactic-co-glycolic acid / agarose successful in regeneration
 - Close to native composition in 12 months.
 - Some structural abnormality - alignment.
- Recently Ca-PO₄ matrix.

Problems

- Scaffolds are often contracted by cells.
- Neovascularization.
- Fibrotic cell immigration.
- Separation between new cartilage, membrane & host tissue.
- Current treatments of "full-flesh osteochondral defects" unsuccessful.
 - Need scaffold to support large repair (?)

Cartilage Scaffolds

- NUS:
 - Bi-compartment chamber for bone / cartilage growth.
 - PCL → cartilage in islets but partial encapsulation; » collagen I & IX c.f. native.
 - TGF-β1 in fibrin glue to stimulate MSC's.
 - Non-seeded scaffolds → non-contact between implant & cartilage.

Case Study: Bone Tissue Engineering

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Challenges

- Presently no resorbable scaffolds for diaphyseal defects/fractures.
- Will be difficult to tissue engineer compact bone due to complex structure.
- Not presently able to recreate endochondral ossification.
- Photosensitive resorbable polymers for stereolithography?
 - Some in development.

Current Methods & Research

- Davies - coating to block osteoclast activation (Desirable?).
- Calvarial models - intramembranous.
- GTR - membrane to prevent soft tissue invasion.
- TE'd bone graft - "unlimited" availability.
- Ma & Zhang (1999) - attempt to copy collagen structure.

Current Methods & Research (2)

- Marrow-impregnated foam (*Plast Reconstr Surg* (1999) **104**:705-12).
 - Inhomogeneous cell population, but aim to stimulate bone growth.
 - Marrow-coated PCL scaffold most successful, but slow regeneration.
 - No regeneration w/out scaffold.
 - Rohner et al (2001) - 12 month results; more coming.
- NUS: CT scanning + rapid prototyping.

An Opportunity

- Large skull defects → cell explant culture:
 - Bone cement not ideal.
 - Ti mesh "gold standard" but expensive.
 - Scaffolds large & difficult to handle → cells seeded at implantation.
 - Vasculature from dura & surrounding tissue → expect slow regeneration.

Regulation & Testing of Tissue Engineered Medical Devices

Arthur Brandwood
Medical technology consultant;
UNSW Graduate School of Biomedical Engineering;
ISO Technical Committee 150.

Key Points for All Devices

- Risk analysis - early & often.
 - ISO 14971 - very good!
- Standards.
- Quality systems (good lab, clinical & manufacturing practices).
- Documentation.

Regulation

- Pre-/post-market
- Classifications.
 - Determine assessment required.
- FDA & EU systems virtually "international standard".
- *Essential to consider requirements from early stages of development!*

Tissue-Engineered Devices

- Not new issues, but greater biological focus.
 - Biocompatibility.
 - Biological safety.
- Conventional issue still apply.
- Regulation increases with manipulation.
- Presently outside Australian Medical Device Directive.

Australia

- Messy state/federal regulatory system.
 - Federal government likes deregulation.
- Public perceptions/concerns.
- Technical & funding pressures.
- *Current regulatory status...*
 - Transplants, extracts, tissues...
- *Proposed classifications according to level of manipulation.*

Regulations Evolving...

- EU working on classification system.
- FDA started afresh in 1997.
- Most regulating as medical devices, but adding requirement for minimization of disease transmission.
- Range of related standards; some being devised for TE'd devices - ASTM, ISO - early stages yet.
- Global Harmonization Task Force.

Discussions & Interesting Asides...

Discussions & Interesting Asides

Dietmar Hutmacher:

- Hutmacher *et al* - PLLA nails that swell slightly @ 37°C.
- Plastic/reconstructive surgery - important to close periosteal flap.
- No evidence of cortical bone formation in tissue engineering - studies too short?
 - Hutmacher & Brandwood commencing 2-year goat skull defect study.
- May be problems getting vascularization into large scaffolds fast enough.

Discussions & Interesting Asides (2)

- Rick Tocchetti:
 - RAH: lymph angiogenesis - shown to be critical in lizard tail regeneration (although less so than blood supply).
 - IMVS: experience in precipitation of proteins from plasma.