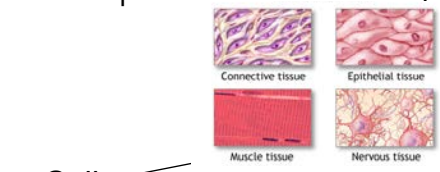
Tissue Engineering

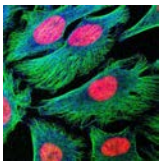
– A technology where artificial organs and tissues are constructed in vitro and transplanted in vivo for the recovery of lost or malfunctioned organs or tissues.

– The use of a combination of cells, engineering methods and materials, and suitable biochemical factors to improve or replace biological functions.

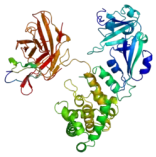
Tissue engineering starts from components of biological tissues



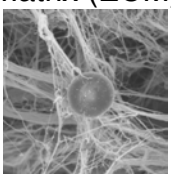
Cells



Growth factors



Extracellular matrix (ECM)



Cell Sources

Autologous: Come from the person that needs the new cells.

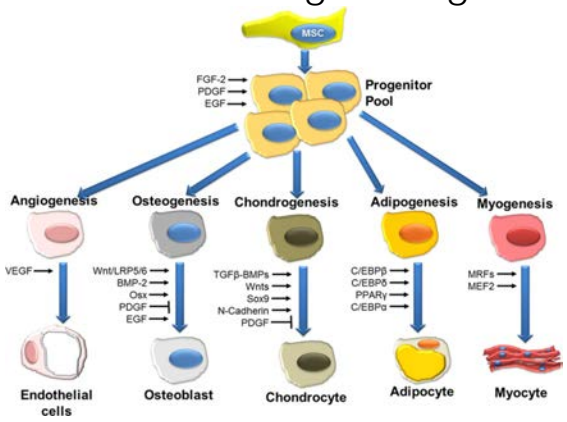
Allogeneic: Come from a body from the same species.

Xenogenic: Come from a different species then the organism they're going into.

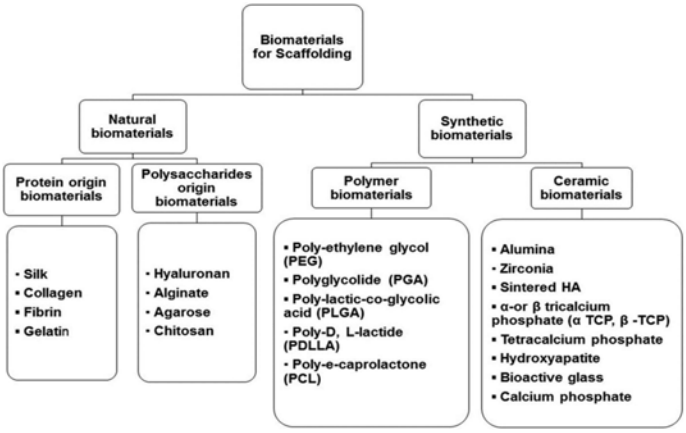
Isogenic (Syngenic): Come from identical twins.

Stem cells: Undifferentiated cells with the ability to divide in culture and give rise to different forms of specialized cells

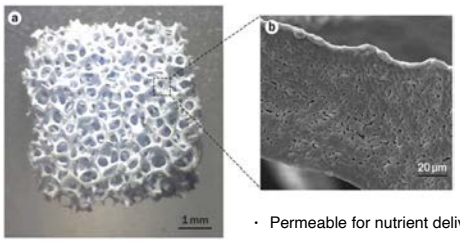
MSCs are the most common cells in tissue engineering



Scaffolds

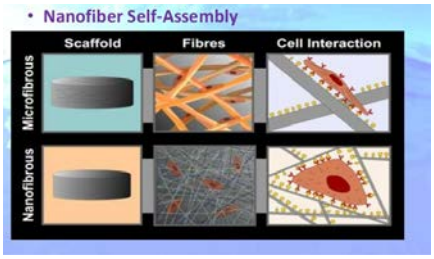


Scaffold Requirements



- Biocompatible
- Bioabsorbable
- Degrade with healing
- Highly porous for cell penetration
- Permeable for nutrient delivery and gas exchange
- Provide appropriate stress environment
- Surface conducive to cell attachment
- Promote extracellular matrix production and deposition
- Carry and transmit biomolecular signals

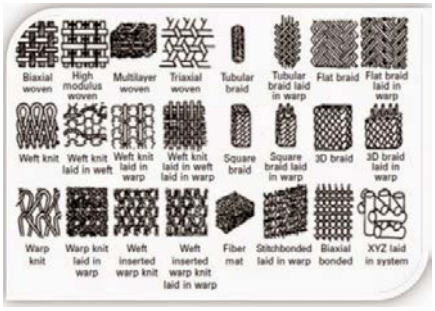
Scaffolds Synthesis - Nanofiber self-assembly



- Usually hydrogel scaffolds
- Low toxicity
- High biocompatibility

Scaffolds Synthesis - Textile technologies

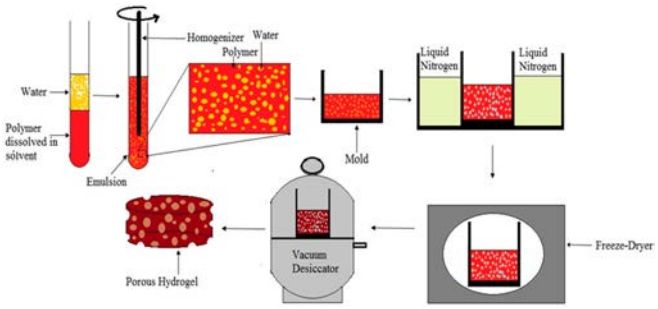
Major limitation: difficulties in obtaining high porosity and regular pore size



Technical Textiles
↓
Medical Textiles
↓
Tissue Engineering
↓
Textile Scaffolds

Scaffolds Synthesis - Freeze-drying

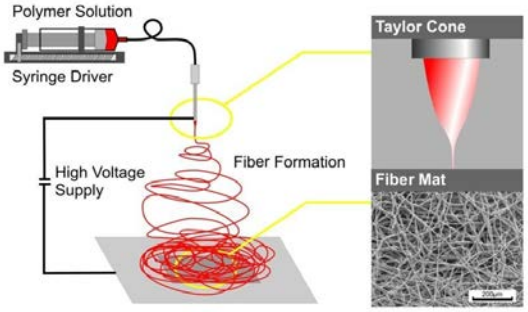
1)A synthetic polymer is dissolved into a suitable solvent
2) Water is added and the two liquids are mixed in order to obtain an **emulsion**.
3) Before the two phases separate, the emulsion is cast into a mold and quickly frozen by immersion into liquid nitrogen.
4) The frozen emulsion is freeze-dried to remove dispersed water and the solvent, thus leaving a solidified, porous polymeric structure



Scaffolds Synthesis - Electrospinning

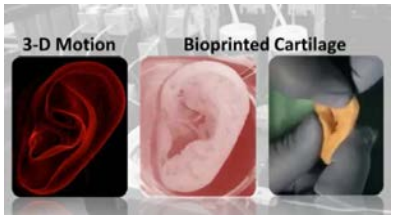
1) A solution is fed through a spinneret
2) A high voltage is applied to the tip
3) Electrostatic repulsion within the charged solution, causes it to eject a thin fibrous stream
4) A collector plate with an opposite charge draws in continuous fibers, which form a porous network.

Advantages: simplicity and ease of variation (by modifying distance to collector, magnitude of applied voltage, or solution flow rate, the scaffold architecture changes dramatically).

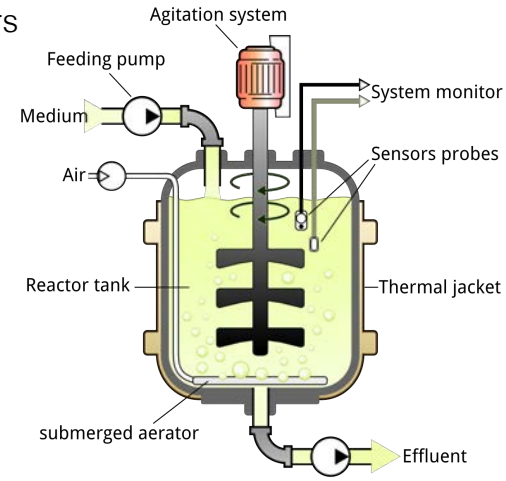


Scaffolds Synthesis - 3D bio printing

Layer-by-layer method to deposit bioinks (cells, matrix and nutrients) to create tissue-like structures that are later used in medical and tissue engineering fields.



Bioreactors



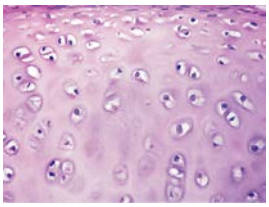
Engineering cartilage

Objectives
Immediate functionality (mechanical, metabolic); capacity for further development and integration

Culture requirements
High initial cell density
Nutrient and gas exchange
Growth factors (TGFbeta, IGF… sequential application)
Hydrodynamically active environment

Engineering cartilage

Cartilage is avascular, aneural, alymphatic and contains only a sparse population of a single cell type (chondrocyte):
- no spontaneous regeneration
- suitable for tissue engineering



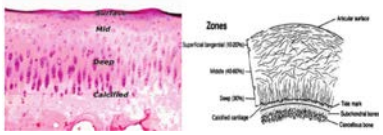
1. Orthopaedic applications: defects in articular joint or meniscus

2. Head and neck applications: reconstruction of an auricle, trachea, nose, larynx, or eyelid for aesthetic or functional purpose

Engineering cartilage

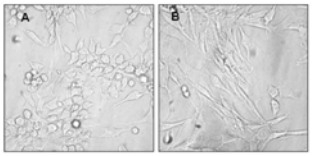
No consensus on the optimal cell source for orthopedic cartilage engineering: chondrocytes or MSCs?

The most important function of orthopedic cartilage is to bear weight.
Engineered neo-cartilage should:
1. integrate with the subchondral bone and adjacent cartilage for stable load distribution and mechanotransduction;
2. match the mechanical properties of the adjacent native cartilage in order to avoid tissue degradation caused by strain disparity;
3. be resistant to load under large deformations and motions;
4. recapitulate the distinct zonal architecture in order.



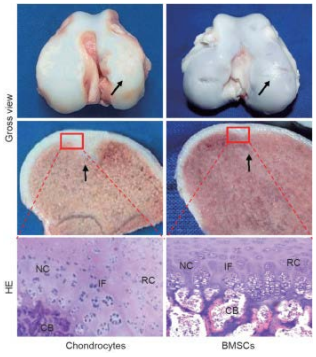
Chondrocytes for cartilage engineering

- logical choice of seed cells
- isolating chondrocytes from joint surface is difficult and causes secondary injury leading
- non-articular "heterotopic" chondrocytes are easier to harvest, associated with lower donor-site morbidity, and possess a higher proliferation rate. However, it remains unclear whether heterotopic chondrocytes would produce cartilage with a desired type (such as hyaline cartilage) and function during defect healing
- chondrocytes tend to de-differentiate in culture



MSCs for cartilage engineering

MSCs
- can be harvested from a number of sources that do not affect cartilage activity,
- maintain multipotency after numerous expansions,
- can be differentiated to generate both cartilage and bone, making the tissue-specific repair of osteochondral defects possible



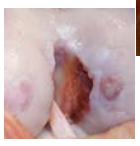
Repair of autologous osteochondral defects by polyglycolic acid (PGA) scaffold loaded with chondrocytes or bone marrow stromal cells (BMSCs), respectively. Both cells realized cartilage repair with a smooth surface. Chondrocytes failed to realize tissue-specific repair in the subchondral region. HE: haemotoxylin and eosin; NC: native cartilage; IF: interface; RC: regenerated cartilage; CB: subchondral bone.

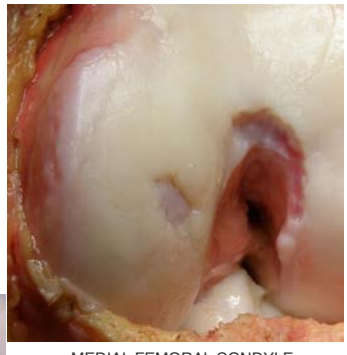
Engineering cartilage: products on the market

maci
autologous cultured chondrocytes on porcine collagen membrane

MACI® (autologous cultured chondrocytes on porcine collagen membrane) is an autologous cellularized scaffold product that is indicated for the repair of single or multiple symptomatic, full-thickness cartilage defects of the adult knee, with or without bone involvement.

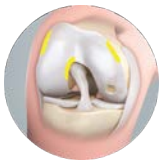
DEFECT WITH BONE INVOLVEMENT
DEFECT:
2.5cm x 1.5cm = 3.75cm2 (0.8cm depth)
PATIENT:
22 years old, gymnast, sports injury at 15 years old





MEDIAL FEMORAL CONDYLE
DEFECT:
2.7cm x 1.3cm = 3.51cm2
PATIENT:
28 years old, occupational therapist, runner

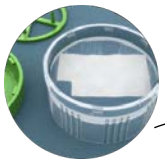
MACI PROCEDURE



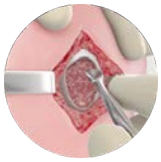
STEP 1: BIOPSY TAKEN
A small biopsy of healthy cartilage is taken arthroscopically from a non weight-bearing area of the patient's knee.
Typical harvest sites include the intercondylar notch and the proximal aspect of the medial and/or lateral femoral condyle.

STEP 2: BIOPSY PROCESSED
The biopsy is sent to the Vericel cell-processing facility in Cambridge Massachusetts.
A state-of-the-art cell-processing facility provides optimal product quality and safety.

STEP 3: CHONDROCYTES EXTRACTED AND LOADED
Chondrocytes are extracted from the biopsy, expanded, and, using proprietary methods are uniformly seeded onto a resorbable Type I/III collagen membrane.
MACI delivers a controlled, uniform dose of cells with a density of at least 500,000 cm² on a Type I/III collagen membrane.



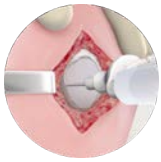
STEP 4: MACI DELIVERED
MACI is delivered via courier to the treatment facility for the procedure.



STEP 5: DEFECT DEBRIDED
The defect area is debrided back to healthy, stable cartilage.

STEP 6: TEMPLATE CREATED
To ensure precise sizing, use sterile materials to create a template of the defect.

STEP 7: MACI IMPLANTED
The MACI implant is secured in place using fibrin sealant. Suture fixation is not required.
The MACI implant can be easily cut and shaped to the appropriate size.

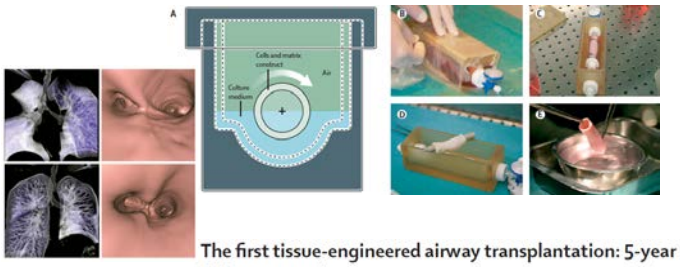


Engineering cartilage for head and neck defects

Clinical transplantation of a tissue-engineered airway

Paolo Macchiarini, Philipp Jungebluth, Tetsuhiko Go, M Adelaide Asnaghi, Louisa E Rees, Tristan A Cogan, Amanda Dodson, Jaume Martorell, Silvia Bellini, Pier Paolo Parnigotto, Sally C Dickinson, Anthony P Hollander, Sara Mantero, Maria Teresa Conconi, Martin A Birchall

Lancet 2008; 372: 2023–30



The first tissue-engineered airway transplantation: 5-year follow-up results

Alessandro Gonfiotti, Massimo O Jaus, Daniel Barale, Silvia Baiguera, Camilla Comin, Federico Lavorini, Giovanni Fontana, Oriol Sibila, Giovanni Romboli, Philipp Jungebluth, Paolo Macchiarini

Lancet 2014; 383: 238–44

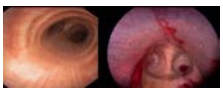
Paolo Macchiarini: A surgeon's downfall

BBC World Service, September 2016

A few questions have dogged Paolo Macchiarini

1. **Decision-making around operations.** Had the risk of each operation been properly assessed? Were the patients ill enough to require such drastic intervention? Did the patients understand the risks involved?
2. **Academic publications.** Footage from surgical cameras conflicted with the descriptions of the patient in published articles. Was the success of the operations misrepresented, omitting or even fabricating data in his published articles?
3. **Absence of pre-clinical large animal studies**

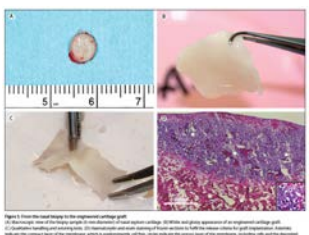
Patient	Location	When operated	Outcome
Andemariam Beyene	Stockholm	June 2011	Deceased Jan 2014
Keziah Shorten	London	Sept 2011	Deceased Jan 2012
Christopher Lyles	Stockholm	Nov 2011	Deceased March 2012
Julia Tuulik	Krasnodar	June 2012, Aug 2013	Deceased Sept 2014
Alexander Zozulya	Krasnodar	June 2012, Nov 2013	Deceased Feb 2014
Yasim Cetir	Stockholm	Aug 2012, July 2013	Survives (remains hospitalised)
Hannah Warren	Peoria, US	April 2013	Deceased July 2013
Sadiq Kanaan	Krasnodar	Aug 2013	Deceased (date unknown)
Dmitri Onogda	Krasnodar	June 2014	Survives (synthetic trachea removed)

published result  footage from surgical cameras

Engineering cartilage for nose reconstruction

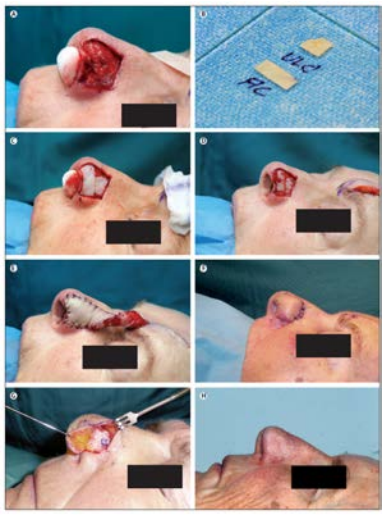
Engineered autologous cartilage tissue for nasal reconstruction after tumour resection: an observational first-in-human trial

Ilario Fulco*, Sylvie Miot*, Martin D Haug, Andrea Barbero, Anke Wixmerten, Sandra Feliciano, Francine Wolf, Gernot Jundt, Anna Marsano, Jian Farhadi, Michael Heberer, Marcel Jakob, Dirk J Schaefer, Ivan Martin



Lancet 2014; 384: 337–46
Published Online
April 11, 2014
http://dx.doi.org/10.1016/S0140-6736(14)60544-4
See Comment page 288
*Contributed equally
Department of Surgery and Department of Biomedicine (I Fulco MD, S Miot PhD, M D Haug MD, A Barbero PhD, A Wixmerten MSc, S Feliciano BSc, F Wolf BSc, A Marsano PhD, Prof M Heberer MD, Prof M Jakob MD, Prof D J Schaefer MD, Prof I Martin PhD), and Institute of Pathology (Prof G Jundt MD), University Hospital Basel, University of Basel, Basel,

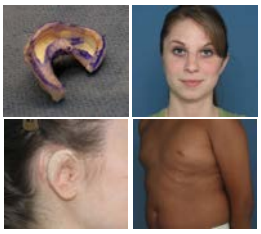
Figure 3: Surgical procedure in one patient
(A) Two-layer defect after wide local excision of the skin cancer on the alar lobule. (B) Tissue engineered cartilage cut to the right shape and ready for implantation; this patient needed cartilage support to achieve stability in the alar lobule (labelled AC) and at the upper lateral site (labelled ULC). (C,D) Tissue engineered cartilage was inserted to replace the structural support and secured by absorbable sutures. (E) Reconstruction of the outer layer with a paramedian forehead flap. (F) Division of the flap pedicle 2 weeks after reconstruction. (G) Intra-operative appearance of the implanted engineered tissue during refinements 6 months after reconstruction. (H) Follow-up 1 year after reconstruction.



Engineering cartilage for ear reconstruction

MEDPOR: the patient's own skin is grafted over a polyethylene framework

Rib Cartilage Ear Construction



Since the early 1990s, tissue engineering has become increasingly popular in the field of reconstructive surgery. In particular, when an in-vitro-manufactured auricular-shaped cartilage implant was implanted on the back of a nude mouse, reconstructive surgeons were intrigued and patients' expectations were raised.

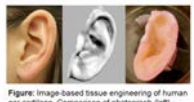
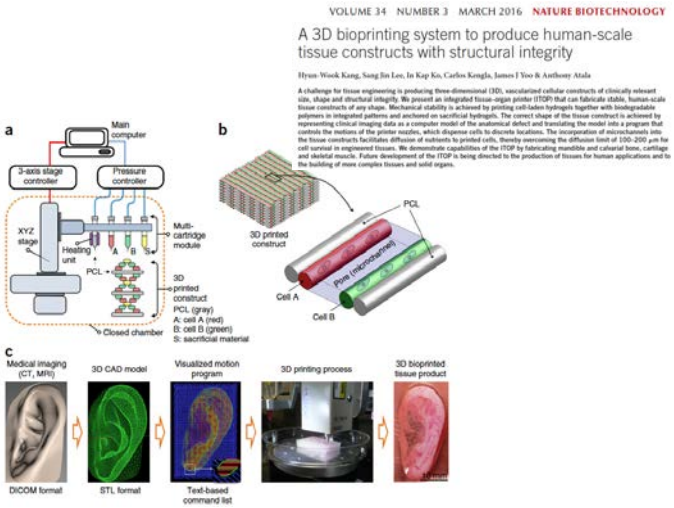


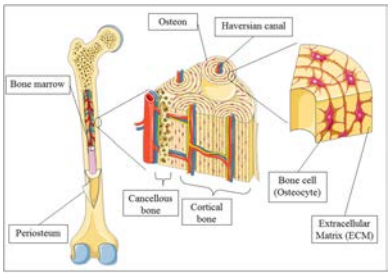
Figure: Image-based tissue engineering of human ear cartilage. Comparison of photograph (left), digitized image (middle), and tissue engineered ear cartilage after two weeks in culture.

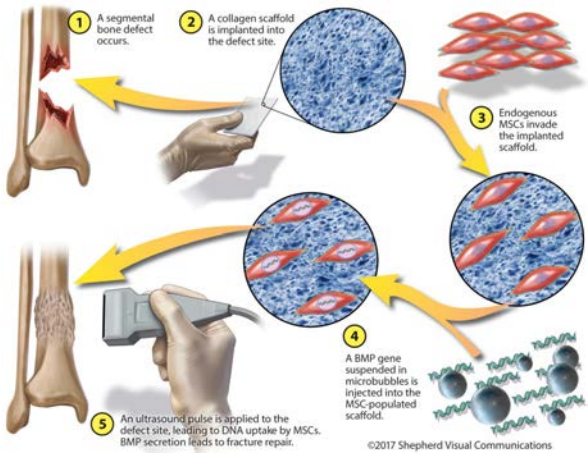


Engineering bone

Objectives
Immediate functionality (mechanical, metabolic)
Capacity for further development and integration
Functional hierarchy

Culture requirements
Nutrient and gas exchange
Regulatory molecules (dex, BMP-2, etc)
Hydrodynamically active environment (interstitial flow)



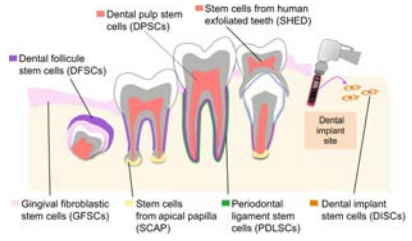


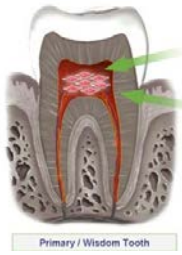
"In situ bone tissue engineering via ultrasound-mediated gene delivery to endogenous progenitor cells in mini-pigs," *Science Translational Medicine* (2017).

MSCs of oral origin

Table 1: Mesenchymal Stem Cells from dental tissues

Name	Site	Date of discover	Authors	Country	Institution
DPSCs	Dental Pulp	2000	S. Gronthos, M. Mankani, J. Brahim, P.G. Robey, S. Shi	USA, Bethesda, Maryland	National Institute on Dental Research, National Institutes of Health
SHED	human Exfoliated Deciduous Teeth	2003	M. Miura, S. Gronthos, M. Zhao, B. Lu, L.W. Fisher, P. G. Robey, S. Shi	USA, Bethesda, Maryland	National Institute on Dental Research, National Institutes of Health
PDLSCs	Periodontal Ligament	2004	B. M. Seo, M. Miura, S. Gronthos, P.M. Bartold, S. Batouli, J. Brahim, M. Young, P.G. Robey, C.Y. Wang, S. Shi	USA, Bethesda, Maryland	National Institute on Dental Research, National Institutes of Health
SCAP	Apical Papilla	2006	W. Sonoyama, Y. Liu, D. Fang, T. Yamaza, B.M. Seo, C. Zhang, H. Liu, S. Gronthos, C.Y. Wang, S. Wang, S. Shi	USA, Los Angeles, California JAPAN: Okayama	University of Southern California School of Dentistry; Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences
DFSCs	Dental Follicle	2005	C. Morsczeck, W. Götz, J. Schierholz, F. Zeilhofer, U. Kühn, C. Möhl, C. Sippel, K.H. Hoffmann	GERMANY. Bonn	Stiftung Caesar, Center of Advanced European Studies and Research
hPCy-MSCs	human Periapical Cyst	2013	M. Marrelli, F. Paduano, M. Tatullo	ITALY. Crotone	Calabreodental, Unit of Maxillofacial Surgery; Tecnologica Research Institute, Biomedical Section



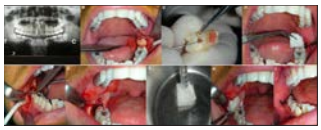


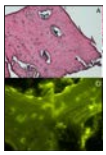
Dental pulp stem cells for bone regeneration

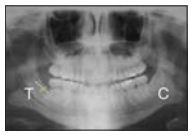
HUMAN MANDIBLE BONE DEFECT REPAIR BY THE GRAFTING OF DENTAL PULP STEM/PROGENITOR CELLS AND COLLAGEN SPONGE BIOCOMPLEXES

Riccardo d'Aquino[1,2], Alfredo De Rosa[1], Vladimiro Lanza[1], Virginia Tirino[1], Luigi Laino[1], Antonio Graziano[1], Vincenzo Desiderio[1], Gregorio Laino[1] and Gianpaolo Papaccio[1]*

[1]Dipartimento di Discipline Odontostomatologiche, Ortodontiche e Chirurgiche, [2]Dipartimento di Medicina Sperimentale, Sezione di Istologia ed Embriologia, Tissue Engineering and Regenerative Medicine (TERM) Division, Secondo Ateneo di Napoli, Naples, Italy







European Cells and Materials Vol. 18 2009 (pages 75-83) DOI: 10.22203/eCM.v018a07

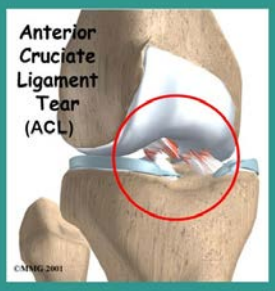
Engineering ligament

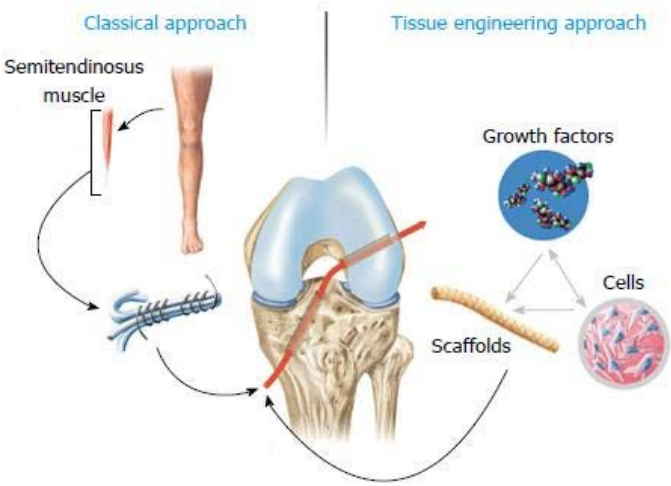
Objectives
Immediate functionality (mechanical, metabolic)
Capacity for bonding with adjacent bones

Culture requirements
High initial cell density
Nutrient and gas exchange
Physical signals
Perfusion
Mechanical stimulation (ligament-like)

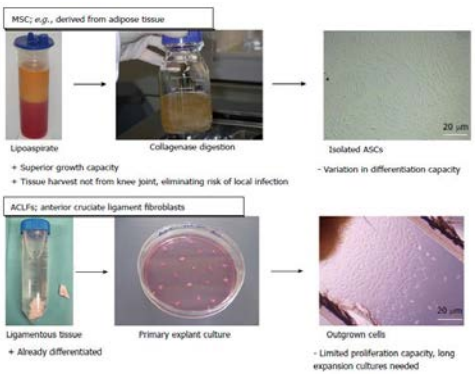
Need for Ligament Tissue Engineering

- Knee ligaments cannot self repair
- High injury rate, especially the anterior cruciate ligament (ACL)
  - > 200,000 ACL surgeries/year
  - > 5 billion dollars
- Surgery options
  - Disease transfer
  - Tissue rejection
  - Poor mechanical strength (current synthetic grafts)





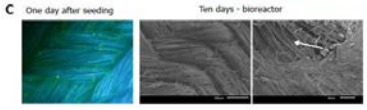
Primary choice of cells for ACL regeneration:
1. mesenchymal stem cells (MSC)
2. ACL fibroblasts



Mechanical stimulation of silk grafts with a bioreactor system

A: The silk-based anterior cruciate ligament (ACL) scaffold is produced of *Bombyx mori* silk fibers in a wire-rope design

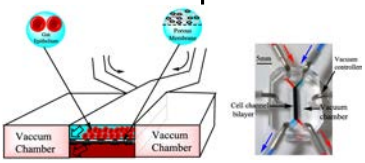
B: The scaffold is seeded with ASCs for 24 h and then transferred into bioreactor and cultured under linear and rotational displacement for 10 days.

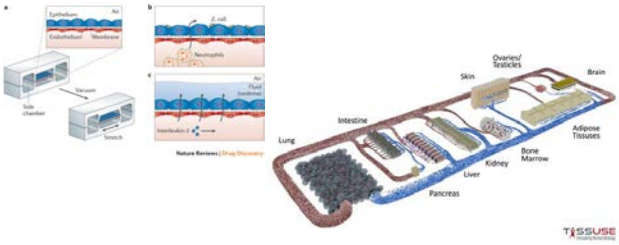


The mechanically stimulated ACL scaffolds show sheets of extracellular matrix. The arrow in the bottom panel indicates an artefact of scanning electron microscopy preparation. In this area, the covering extracellular matrix sheet has been flushed away due to too intense flushing, allowing the view to the underlying silk fibers.

Adipose-derived stem cells cultured on silk-based ligament grafts produce sheets of extracellular matrix proteins under mechanical stimulation *via* a bioreactor system

Organs on chip

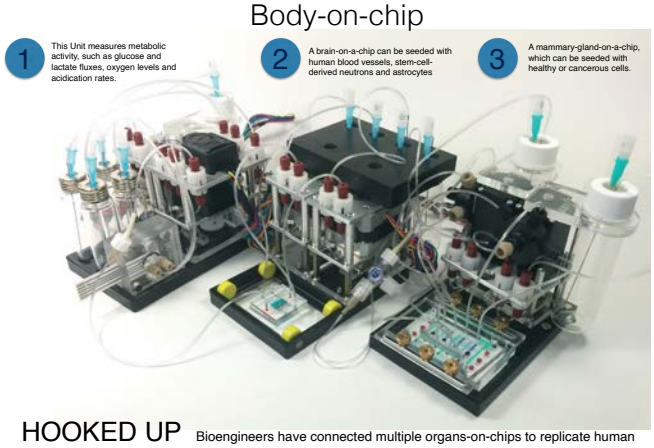




German spin-off TissUse is producing chips with two or four organs on each.

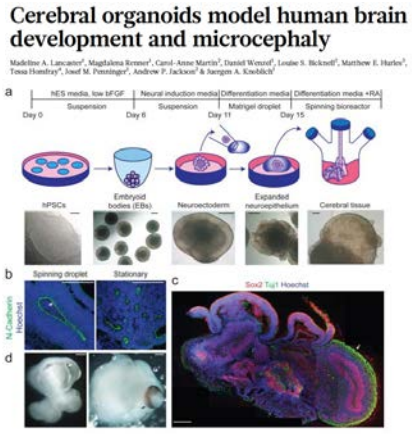
Tissue Engineering for Precision Medicine in Cancer

Body-on-chip



HOOKED UP Bioengineers have connected multiple organs-on-chips to replicate human physiology. They hope to use the set-up to study the spread of metastatic breast cancer to the brain.

Tissue Engineering, Organoids and Precision Medicine



Cerebral organoids model human brain development and microcephaly

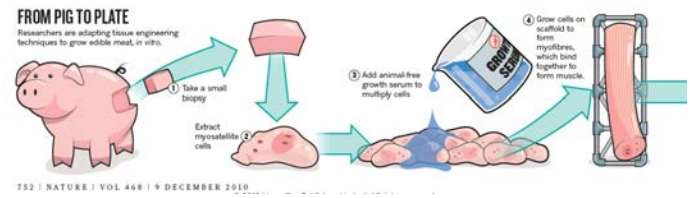
Madeline A. Lancaster[1], Magdalena Renner[1], Carol-Anne Martin[2], Daniel Wenzel[3], Louise S. Bicknell[4], Matthew E. Hurles[5], Tessa Homfray[6], Josef M. Penninger[3], Andrew P. Jackson[4] & Juergen A. Knoblich[1]

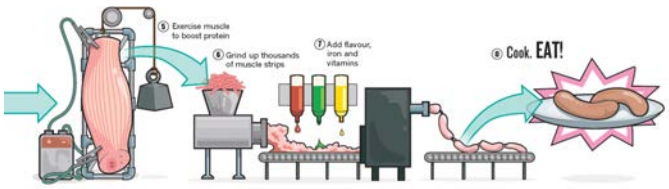
In vitro or cultured meat

Cultured meat, also called clean meat or in vitro meat, is meat grown in cell culture, using many of the same tissue engineering techniques traditionally used in regenerative medicine, instead of inside animals.

- First peer-reviewed journal article published in 2005 in Tissue Engineering.
- In 2008, PETA (People for the Ethical Treatment of Animals) offered a $1 million prize to the first company to bring lab-grown chicken meat to consumers by 2012

In vitro or cultured meat





Start-ups producing cultured meat

1. Memphis meat (San Francisco, Silicon Valley)

- beef metballs
- chicken tenders
- duck à l'orange

2. Supermeat (Israel)

https://thechicken.kitchen