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Nano medicines, a essential component of tissue engineering strategies

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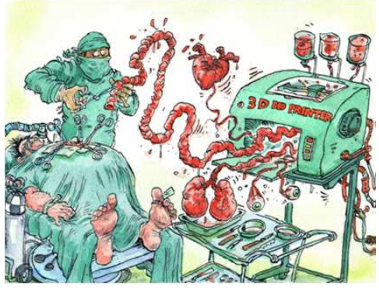
1. Introduction
2. Cells
3. Scaffolds
4. Signaling molecules
5. Application: bone healing

Tissue regeneration

- In certain tissues, cells capable of initiating regeneration or repair after injury.
  - Constant renewing: skin, bone marrow, intestinal epithelium and mucosa, …
  - Liver, bones, …
- Depend of
  - Cell type
  - Nature/extend of injury
  - Age
- Not for neurons, heart muscles, …
- Tissue engineering is one strategy.

Regenerative medicine/Tissue Engineering

?



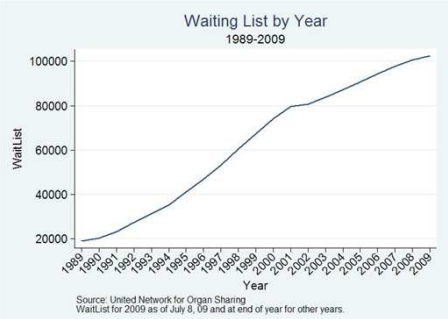
http://www.economist.com/node/15543683?story_id=15543683

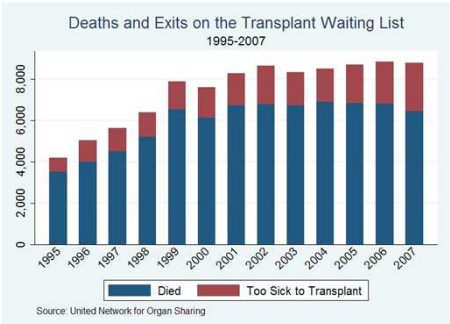
Regenerative medicine

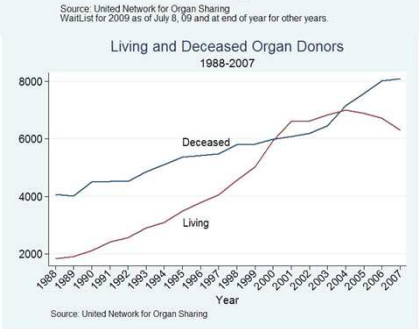
- Restore structure and function of damaged tissues and organs.
- Create solutions for organs that become permanently damaged.
- Cure previously untreatable injuries and diseases.

http://www.regenerativemedicine.net/What.html

A few numbers…







http://www.econlib.org/library/Columns/y2009/Tabarroklifesaving.html

Current therapies

- Autografting: tissue from one location in the patient's body transplanted to an other location in the same patient.

examples: coronary bypass

+ : best clinical results, no rejection

- : lack of suitable harvest sites, pain, infection, blood loss from harvesting procedure

Current therapies

- Allografting: tissue from one donor transplanted to an other donor.

examples: heart, lung, … transplant

+ : live saving by total replacement of failing/non functional organs

- : rejection (life-long medication), donor/organ shortage

Current therapies

- Xenografting: tissue from animal sources transplanted to humans.

examples: heart, lung, … transplant

+ : readily available potential supply, possibility to standardize (transgenic animals recognized as humans)

- : rejection (life-long medication), disease transmission

Current therapies

- Man-Made, biomimetic devices to replicate functions performed by biological systems.

examples: artificial hearts, valves, orthopedic prosthesis, …

+ : solve many problems, delay the need for transplantation

- : fatigue, fracture, toxicity of the devices, no remodeling (growth), no physiological behavior

Regenerative medicine

- Four concentrations in the field of regenerative medicine:
  - Medical devices and artificial organs
  - Tissue engineering and biomaterials
  - Cellular therapies
  - Clinical translation

http://www.regenerativemedicine.net/What.html

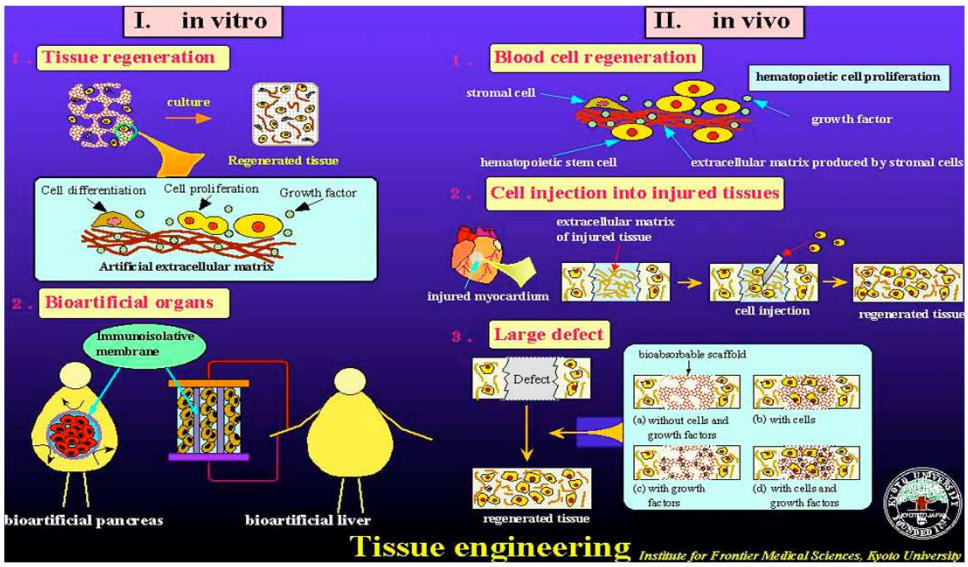
Tissue engineering

- The first definition from Drs. Langer and Vacanti:

"An interdisciplinary field that applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function or a whole organ."

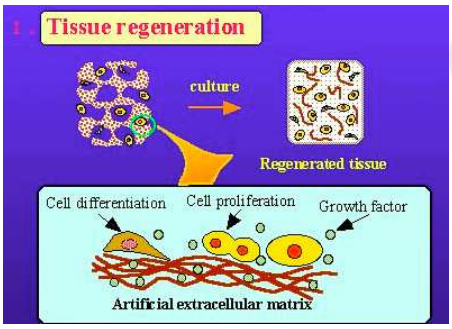
Tissue engineering

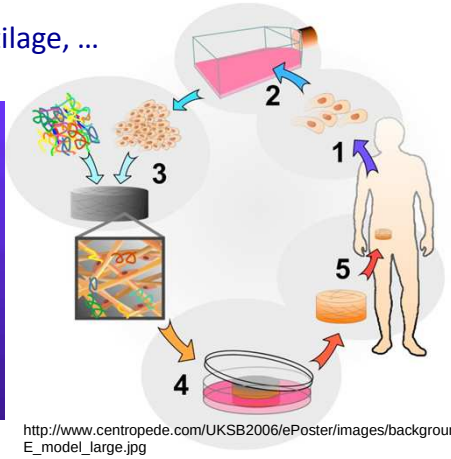
- 2 general approaches:



Design and grow human tissues outside the body for later implantation

- Reduce tissue harvest, surgical and post operative costs and pain
- ex: skin graft, bone, cartilage, …

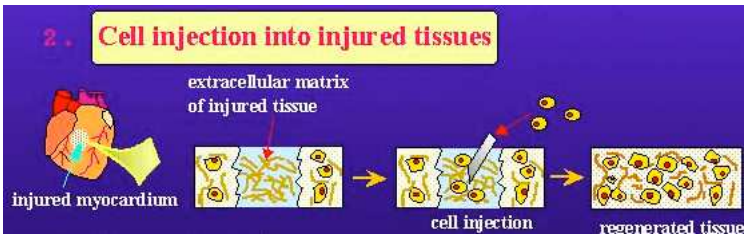




http://www.centropede.com/UKSB2006/ePoster/images/background/TE_model_large.jpg

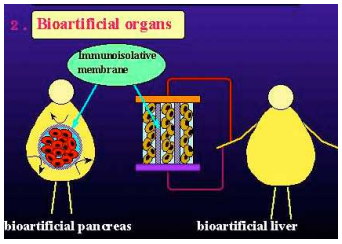
Direct injection of bolus cells into the tissue of interest

- Myocardium regeneration



External/internal devices containing human tissues to replace the function of diseased tissues

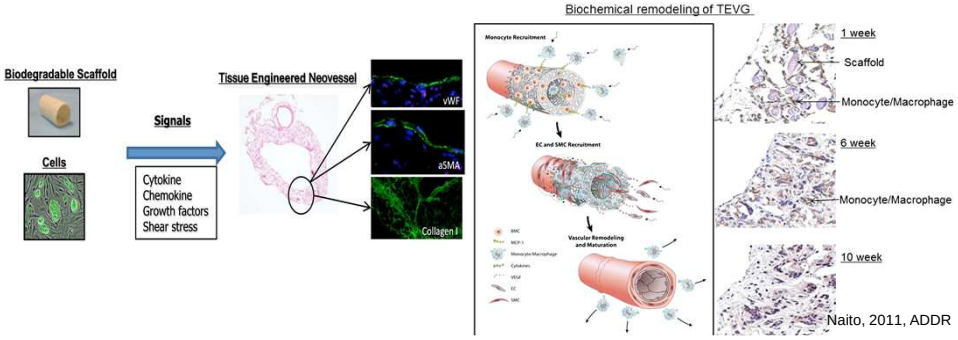
- Perform organ function without transplantation/improve biocompatibility of implanted artificial device
- Artificial liver, pancreas, cell-lined vascular grafts



http://www.shubhanmedical.com

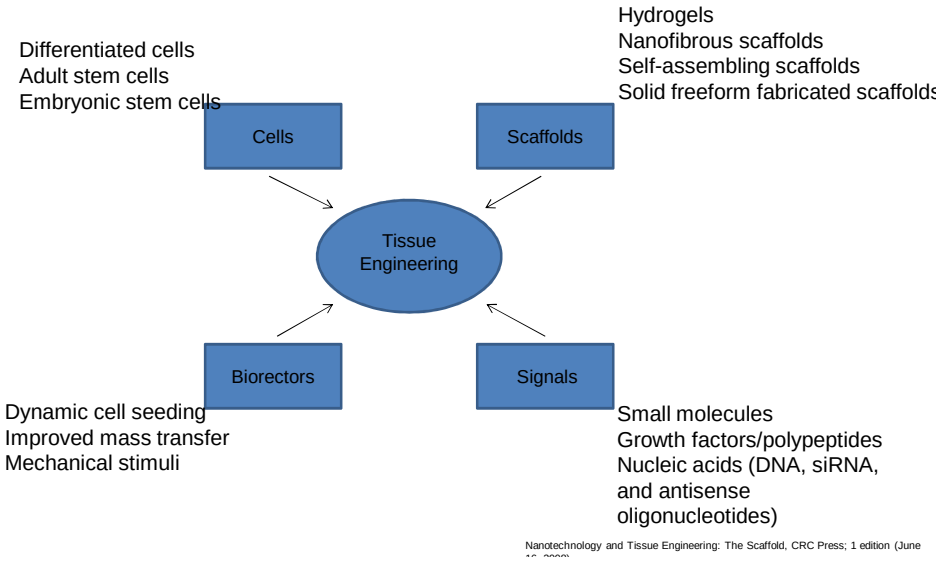
Implantation of scaffold-based delivery systems to induce the regeneration of functional tissues

- Scaffolds + bioactive molecules + cells
- Remodeling/disappearance of implant
- Bone, cartilage, skin, vascular grafts, …



Naito, 2011, ADDR

Tissue engineering



Nanotechnology and Tissue Engineering: The Scaffold, CRC Press; 1 edition (June

Steps in Tissue engineering

- Appropriate cell source must be identified, isolated and produced in sufficient numbers. Cells seeded onto or into material, maintaining function, morphology
- Appropriate biocompatible material that can be used as a cell substrate or cell encapsulation material
- Appropriate signals for cell infiltration, survival, proliferation, differentiation, …

CELLS

Cells sources

- Primary cells
- Stem cells
  - Embryonic stem cells
  - Adult stem cells

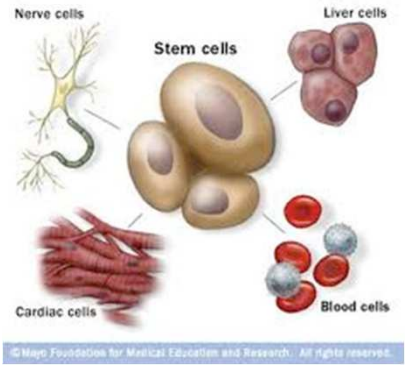
Primary cells

- Mature cells from a specific tissue type *(ex: osteoblastes from femoral heads during hip replacement)*
- Harvested by surgery
- Advantages
  - Immunilogical biocompatibility
- Disadvantages
  - Differentiated: no proliferation, de-differentiation

Primary cells

- Example:
  - Langerhans cells encapsulated in alginate beads to treat type I diabetes
  - Porcine islets implanted in humans (Prof. Dufrane's work, UCL, Belgium)

Stem cells



"Undifferentiated cells that can proliferate and have the capacity of both self-renew and differentiate to one or more types of specialized cells"

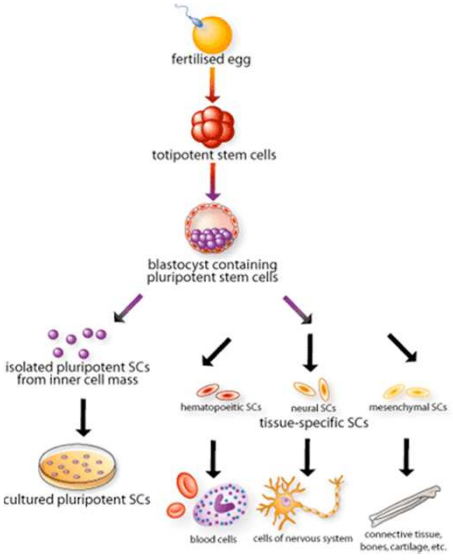
Umbilical cord blood

- Placenta and umbilical cord: source of hematopoietic stem cells.
- A higher chance of matching family members than stem cells from bone marrow.
- late 1980s, used to treat a number of blood and immune-system related genetic diseases, cancers, and disorders (+/- 75 diseases).
- In 1993, the first two successful unrelated donor cord blood tr… …cured acute lymphoblastic leukemia.

http://www.thefastertimes.com/pediatrics/2010/08/26/umbilical-cord-blood-banking-is-it-worth-it/

Embryonic stem cells

- The most plastic cell source available
  → totipotent
- Isolated from the inner cell mass of developing blastocytes *(1998)*
  → ethical concerns



http://www.scq.ubc.ca/stem-cell-

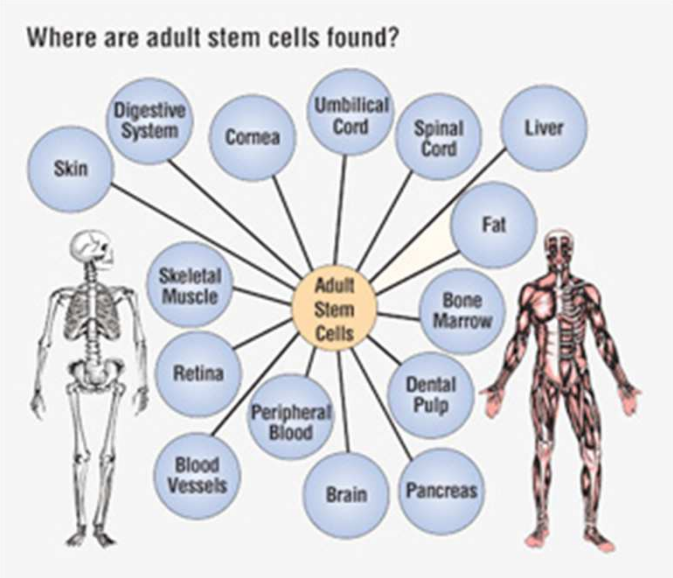
Embryonic stem cells

- Advantages:
  - Proliferate indefinitely in the undifferenciated state
  - Retain the capacity to differentiate to all mature somatic phenotypes under the appropriate signals
- Disadvantages:
  - Ethical concerns
  - Tricky culture conditions
  - Potential risk of genetic mutations, teratocarcinoma
  - Lack of hindsight

Adult stem cells

- Advantages:
  - Multipotency (mesenchymal and non mesenchymal origin)
  - Long-term self-renewing (life time)
  - Immunomodulatory properties
  - Autograft/allograft
  - Isolation/expansion doable
- Disadvantages:
  - Source/access: morbidity at the site of sampling
  - Low occurrence

Adult stem cells

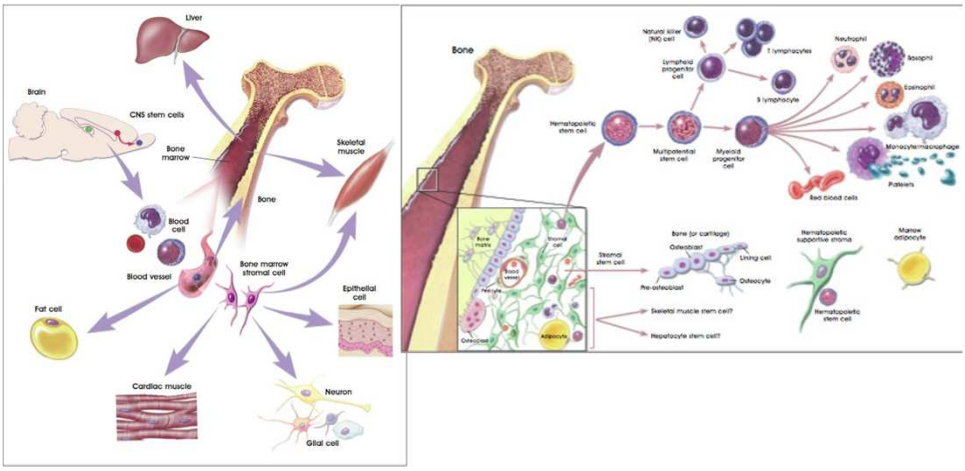


Mesenchymal stem cells

- Bone marrow stem cells
- Umbilical stem cells
- Fat stem cells

- Easy access, autograft, acceptable morbidity.
- The most studied/used.

MSC differentiation



http://stemcells.nih.gov/info/scireport/chapter4.asp

Cell therapy Clinical trials

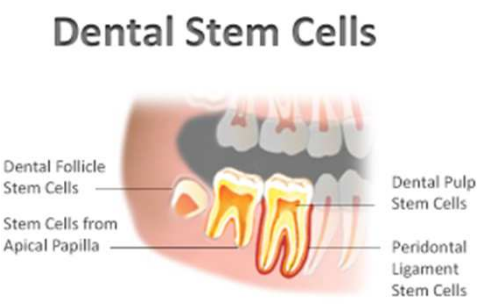
- 265 studies on MSC worldwide

http://www.clinicaltrials.gov/

Cell therapy Clinical trials

Conditions	Interventions	Phases	Start Date
Ischemia|Stroke	Genetic: Autologous mesenchymal stem cells	Phase 2	August 2010
Pened Chest Surgery for Programmes Coronary Bypass	Procedure: harvest of a small bone marrow sample		November 2008
Chronic Myocardial Ischemia|Left Ventricular Dysfunction	Genetic: Mesenchymal stem cells	Phase 1|Phase 2	October 2009
Osteoarthritis|Knee Arthrosis|Osteochondral Defects|Osteonecrosis	Procedure: Transplantation of Bone Marrow Stem Cells Activated in Knee Arthrosis	Phase 0	July 2010
Prostate Cancer|Erectile Dysfunction	Biological: injection of bone marrow mononucleated cells	Phase 1|Phase 2	April 2010
Tibial Fractures|Fractures, Open|Bone Marrow Transplantation	Procedure: Osteosynthesis		September 2007
Osteoarthritis	Biological: Autologous adipose derived stem cells administrated for intra-articular use	Phase 1	April 2012
Ovarian Cancer|Sarcoma|Small	Biological: dactinomycin|Biological: filgrastim|Drug: carboplatin|Drug: cyclophosphamide|Drug: doxorubicin hydrochloride|Procedure: peripheral blood stem cell transplantation|Radiation:		http://www.clinicaltrials.gov/

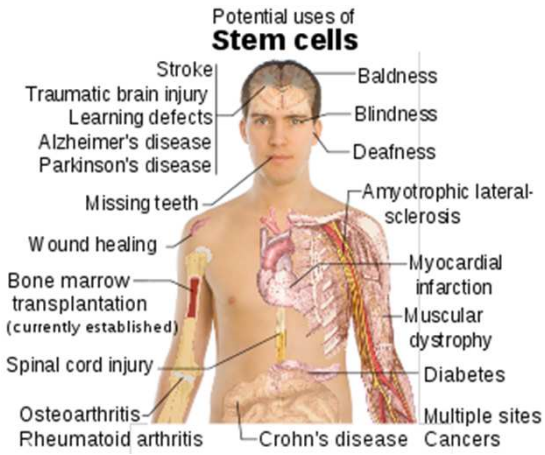
Dental stem cells



- Easy access
- Autograft
- High proliferation rate
- From neural crest: good candidate for CNS regeneration

Stem cells

- Stem cells can provide a virtually inexhaustible cell source for a lot of applications.
- Stability, potential risks have yet to be fully evaluated.

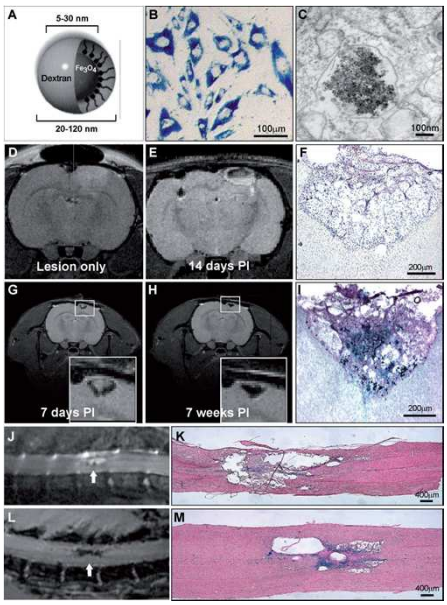


Stem cells for tissue engineering

- Actions
  - Cell replacement
  - Bioactive molecule secretion
- Challenges
  - Survival
  - Delivery
  - In vivo tracking post implantation

In vivo tracking

- Magnetic nanoparticles for labeling stem cells
  - Superparamagnetic iron oxyde nanoparticles (SPIO)-MRI
  - Gadolinium-rhodamine-dextran conjugates
  - Manganese oxide nanoparticles



Kubinova et al. 2010

SCAFFOLDS

Scaffolds

- Central components of tissue engineering strategies
  - Architectural context in which extracellular matrix, cell-cell and growth factors interactions combine

- Challenges
  - Design and manufacture of scaffolds
    - Highly porous structure
    - Controlled release kinetic of growth factors

Sokolsky et al., 2007

Materials forming scaffolds

- Selection criteria
  - Appropriate mechanical properties matching targeted tissue.
  - Acceptable biocompatibility
  - Mimic native extracellular matrix
  - Interface adherence: cell adhesion and proliferation
  - Adapted degradation rate

Sokolsky et al., 2007

Drug releasing scaffolds

- High loading capacity (therapeutic levels)
- Homogenous drug distribution
- Appropriate binding affinity to allow slow release
- Controlled release kinetic
- Maintain drug stability

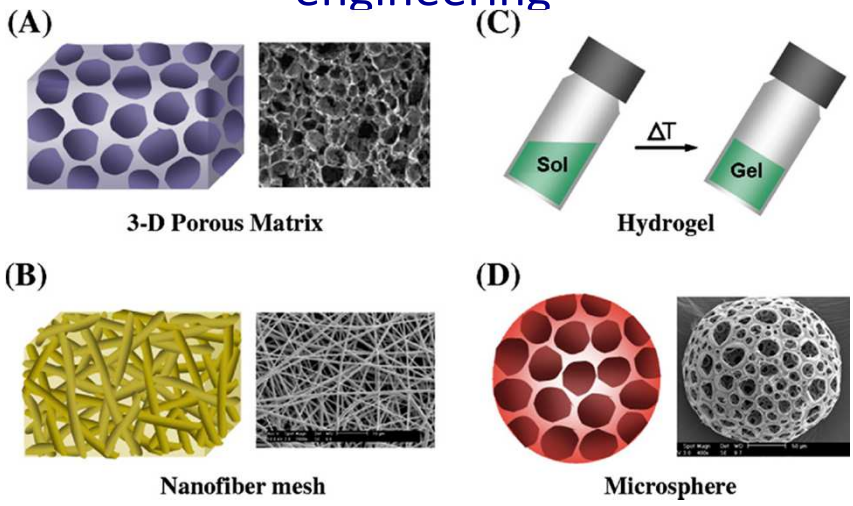
Natural materials

- Minimized chronic inflammation
- Intrinsic biological activity
- Often soluble in water: mild fabrication conditions
- Examples: silk, collagen, gelatin, chitosan, alginate, agarose, hyaluronic acids, fibrin, …

Synthetic materials

- Biocompatible, biodegradable
- Tunable
- More controlled structure and properties
- Longer degradation rates
- Examples: poly(a-hydroxyester)s, polyanhydrides, polyorthoesters,…

Forms of scaffolds for tissue engineering



Chung et al., 2007

SIGNALING MOLECULES

Signaling factors

- Precise control over the signaling of factors in a local area may potentially allow control over a regenerative process.
- Growth factors: proteins which affect cell migration, proliferation and differentiation.

Table 2
Manifestations of growth factors on cellular effects are multifaceted.

Action of growth factors on cells
– An onset of vectorial migration (chemotaxis effect)
– An onset of random migration (chemokinesis effect)
– A stimulation of cell division (mitogenic effect)
– An induction of cell differentiation (control cell fate)
– Patterning of cells (morphogenesis)
– An initiation of programmed cell death (apoptotic effect)
– An inhibition of cellular activity
– A modulation of metabolic activity
– A combination of above

Lee et al., 2011, Metha et al., 2012

Popular growth factors

abbreviation	tissues treated	representative function
Ang-1	blood vessel, heart, muscle	blood vessel maturation and stability
Ang-2	blood vessel	destabilize, regress and disassociate endothelial cells from surrounding tissues
FGF-2	blood vessel, bone, skin, nerve, spine, muscle	migration, proliferation and survival of endothelial cells, inhibition of differentiation of embryonic stem cells
BMP-2	bone, cartilage	differentiation and migration of osteoblasts
BMP-7	bone, cartilage, kidney	differentiation and migration of osteoblasts, renal development
EGF	skin, nerve	regulation of epithelial cell growth, proliferation and differentiation
EPO	nerve, spine, wound healing	promoting the survival of red blood cells and development of precursors to red blood cells.
HGF	bone, liver, muscle	proliferation, migration and differentiation of mesenchymal stem cells
IGF-1	muscle, bone, cartilage, bone liver, lung, kidney, nerve, skin	cell proliferation and inhibition of cell apoptosis
NGF	nerve, spine, brain	survival and proliferation of neural cells
PDGF-AB (or -BB)	blood vessel, muscle, bone, cartilage, skin	embryonic development, proliferation, migration, growth of endothelial cells
TGF-α	brain, skin	proliferation of basal cells or neural cells
TGF-β	bone, cartilage	proliferation and differentiation of bone-forming cells, anti-proliferative factor for epithelial cells
VEGF	blood vessel	migration, proliferation and survival of endothelial cells.

Lee et al., 2011

Commercially available growth factors

Table II. Commercially available growth factor delivery systems approved for clinical use

Growth factor	Commercial name	Administration	Clinical condition	Company
BMP-2	INFUSE® Bone Graft/LT-Cage	BMP-2 absorbed in collagen sponge	Degenerative disc disease	Medtronic (http://www.medtronic.com/for-healthcare-professionals/products-therapies/spinal-orthopedics/bone-graft-options/infuse-bone-graft/index.htm)
BMP-7	OP-1™ Implant/ Putty[a]	BMP-7 in collagen-based carrier	Fractures of long bones, lumbar fusions	Olympus Biotech Corporation (http://www.olympusbiotech.com/us/index.html)
KGF	Kepivance® (palifermin)	Injected intravenously	Oral mucositis	Amgen/Biovitrum (http://www.kepivance.com/)
PDGF	REGRANEX® (becaplermin)	PDGF impregnated in a hydrogel	Lower extremity neuropathic ulcers	OMJ Pharmaceuticals (http://www.ncbi.nlm.nih.gov/pubmedhealth/PMH0001057/)
PDGF-BB	Augment™[b]	PDGF in tricalcium phosphate matrix	Open orthopedic surgical procedures	Biomimetic (http://biomimetics.com/products.htm)
PDGF-BB	GEM 21S®	Synthetic bone matrix (β-TCP)	Periodontal bone defects and associated gingival recession	Osteohealth (http://www.osteohealth.com/GEM21S.aspx)

a Approved under Humanitarian Device Exemptions (HDE).

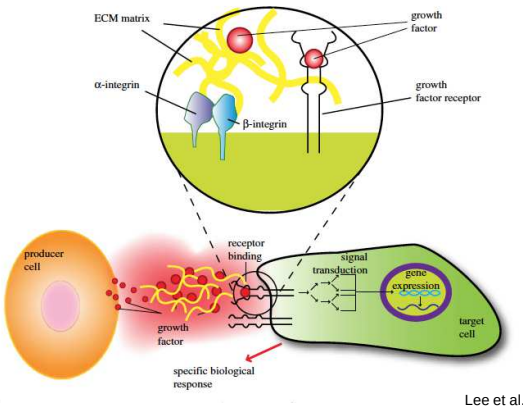
b Approved for use in Canada and Australia.

β-TCP = beta-tricalcium phosphate; **BMP** = bone morphogenetic protein; **KGF** = keratinocyte growth factor; **PDGF** = platelet-derived growth factor.

Koria et al., 2012

Mechanism of action

- Growth factors instructs cell behavior through binding to specific trans-membrane receptors on targeted cells.

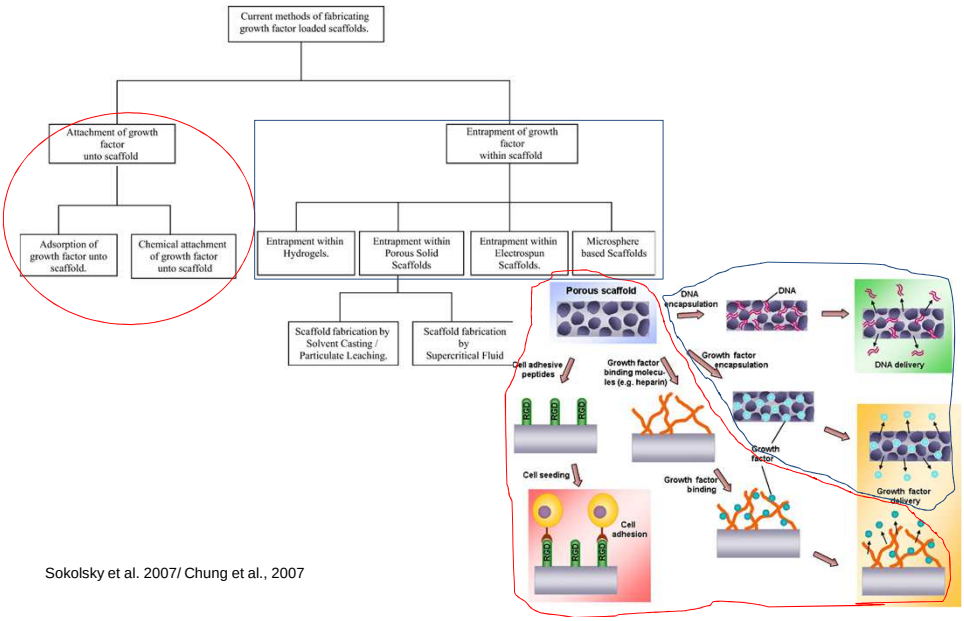


Lee et al., 2011

Clinical studies

- Growth factors tested in clinical trials (GF injected locally).
  - Promising Phase I
  - Not the expected results for Phase II
- Causes: formulation, dose, route of administration.
- Often neglected: mode of delivery
- Administration of supraphysiological concentrations may lead to severe side effects.

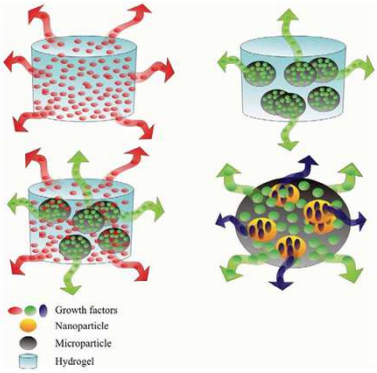
Delivery strategies for tissue engineering



Sokolsky et al. 2007/ Chung et al., 2007

Incorporation in polymeric matrices

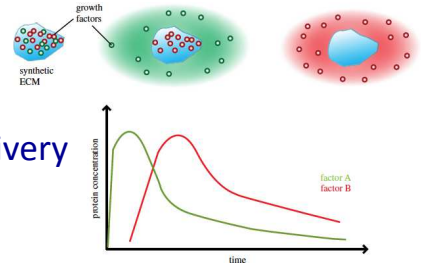
- Growth factor directly incorporated into the matrix
- Growth factor encapsulated in nano/microparticles before incorporation in implants
- Combination of both



Stevens, 2008

Delivery strategies for tissue engineering

- Integrative approach: nanoparticulate systems with hydrogels or scaffolds.
  - Delivery of multiple growth factors
  - Better control of release (decrease of burst release and diffusion)
  - Spatio/temporal delivery of distinct factors
  - Results in controlled sequential waves of GF delivery



Lee et al., 2011

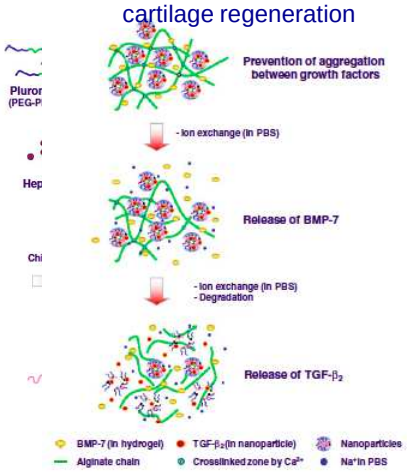
Combinatorial approaches

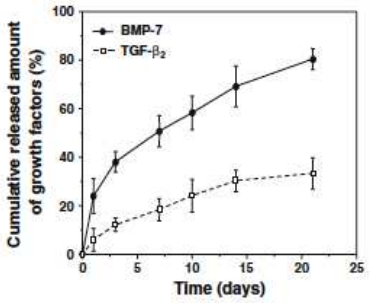
- Improved efficacy of growth factors in tissue regeneration.

- Sequential release critical for tissue regeneration: improper sequence=little effect and undesirable effects

Combinatorial approaches

- TGFb2-loaded poly-ion complex nanoparticles in a BMP-7 containing hydrogel for cartilage tissue engineering *(Lim et al., 2010).*
  - A faster release of BMP-7 and a slower release of TGF2b, desirable for cartilage regeneration





APPLICATIONS

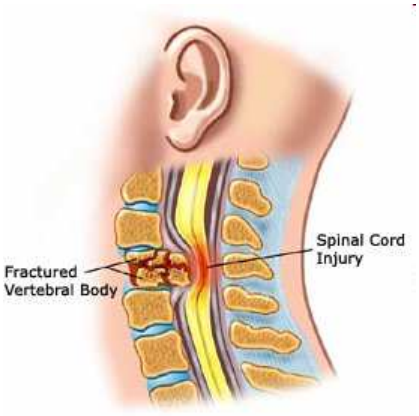
INFLUENCE OF GDNF DELIVERY FROM HYDROGELS ON SPINAL CORD REGENERATION

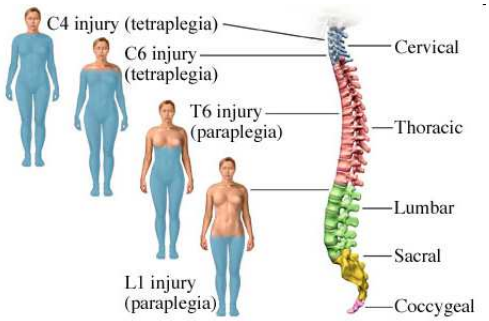
Spinal cord injury

- 1.2 million individuals worldwide
- Young adults (82%=males 16-30 years old)
- Incomplete/complete injury
- Loss of function

Spinal cord injury resource center
http://www.spinalinjury.net/

Spinal cord injury



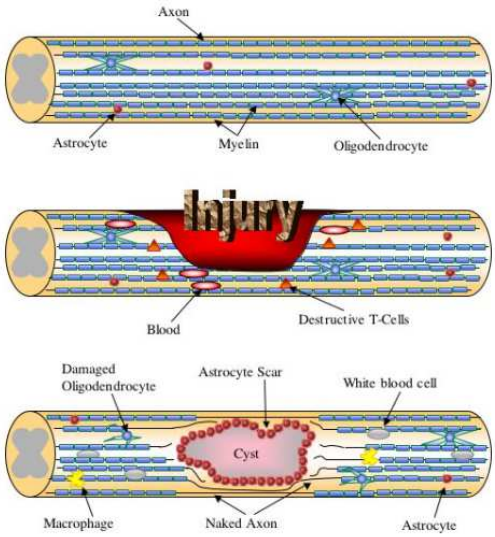


http://www.topnews.in/health/scientists-develop-promising-new-nano-engineered-gel-spinal-cord-injury-21748

http://www.articleslounge.com/medical/spinal-cord-injury-sci/

Spinal cord injury

- Acute phase: spinal shock
- Sub-acute phase
- Chronic phase



Spinal Cord Injury:Progress, Promise, and Priorities, C. T. Liverman et al., 2005

Hypothesis

Precise control over the signaling of growth factors in a local area may potentially allows control over a regenerative process.

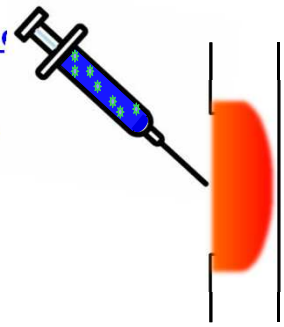
Spinal cord regeneration

Requirements:

Injectable hydrog
Biocompatible
Biodegradable
Stimulate regener

Growth factors
Free
Encapsulated

Cells
Stem/primary cells



To do:

Injectable hydrogel
Select
In vitro/In vivo compatibility

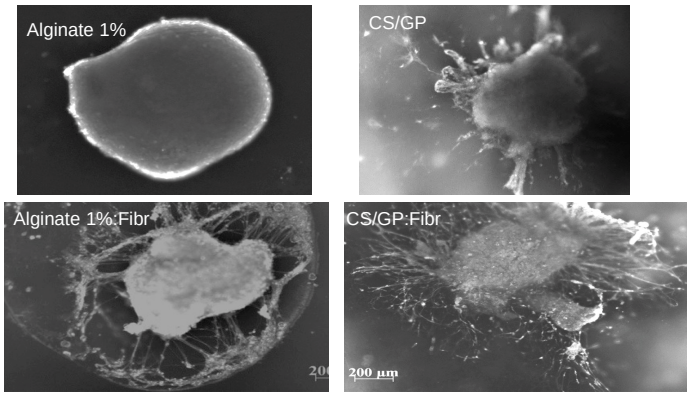
Growth factors
Develop formulations
Study release from hydrogels

Cells
Incorporation in hydrogels
Influence in vivo

Injectable Hydrogels

- Composition
  - 0,5 % MVG Alginate (Novamatrix™)
  - 1.5%Chitosan / β-Glycerophosphate disodium salt hydrate (Crabe shell, Sigma)
  - +/- Fibrinogen (Tisseel™ fibrin sealant kit, Baxter International Inc.)
- Characterization
  - Mechanical properties (Rheology): Alginate 0.5% close to spinal cord modulus (+/- 200 Pa.s) and CS/GP modulus very low (31 Pa.s).
  - Biocompatibility (MTS): No influence of fibrinogen addition to alginate but higher proliferation on CS/GP + fibrinogen.

Ex vivo neurite growth



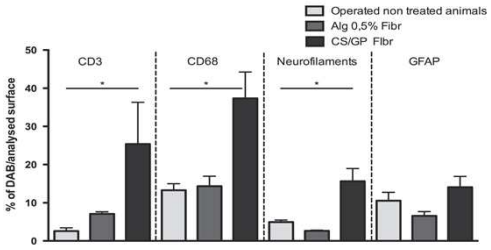
➡ CS/GP>Alg

Fibrinogen ↗ neurite growth

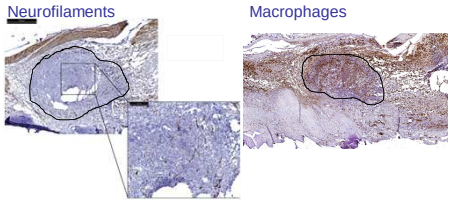
➡ Selection of Hydrogels + Fibrinogen for in vivo experiments

Ansorena et al., submitted

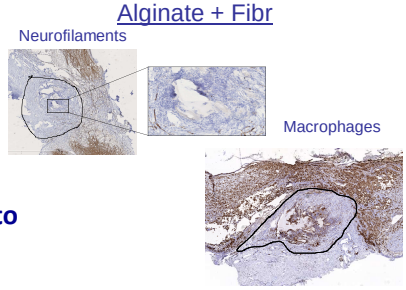
Influence on spinal cord injury in vivo



CS/GP + Fibr



Alginate + Fibr



- CS/GP+ fibrinogen induced the highest neurite growth but also the more severe inflammatory reaction.
- **0,5% Alginate+ fibrinogen was selected to study the influence of GDNF release on spinal cord lesion.**

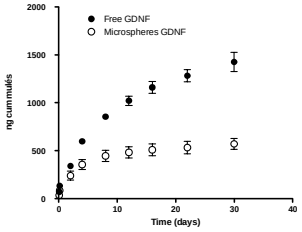
Ansorena et al., submitted

GDNF delivery from hydrogels

- Formulation
  - Microspheres or free GDNF (solution) incorporated in alginate hydrogels
- Incorporation in hydrogels
  - In vitro release
  - Influence on spinal cord injury (3 months implantation)- functional test and IF

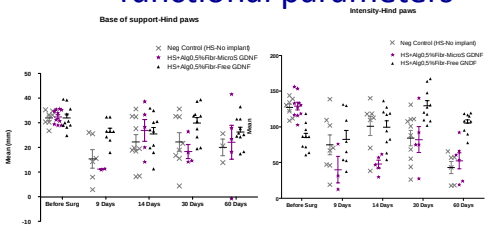
GDNF release from hydrogel

- Release profiles



- Sustained release of GDNF for the 2 formulations.
- Very slow release of GDNF from microspheres-loaded hydrogel.

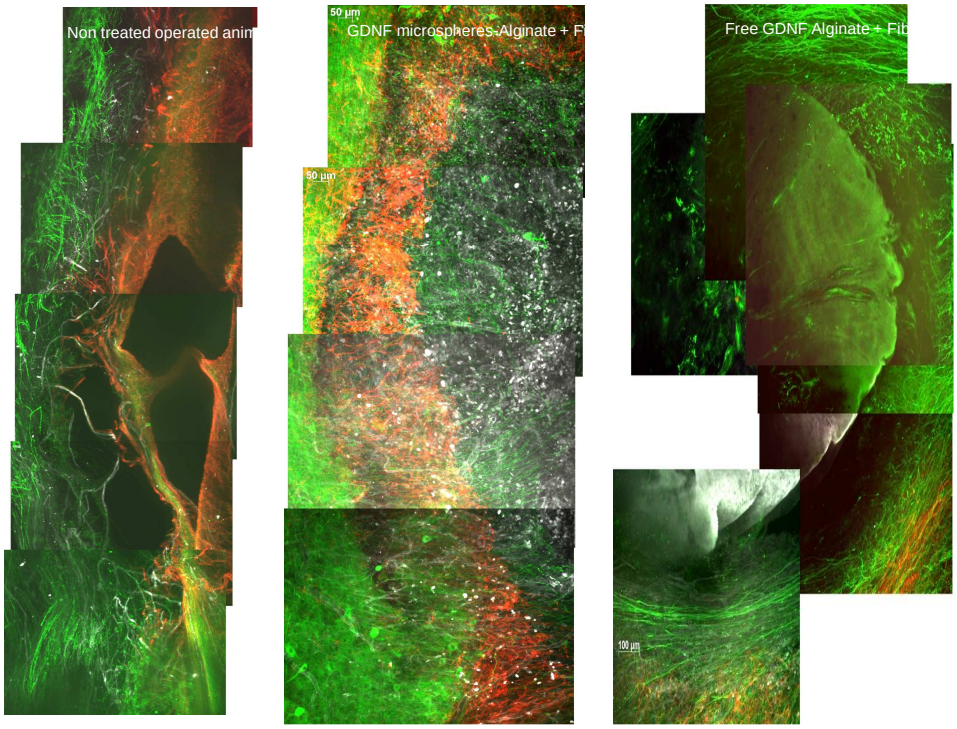
- Influence on functional parameters



- Improvement of certain CatwalkTM parameters over 1 month for rats injected with free GNDF-alginate.

GDNF release from hydrogel

- Influence on spinal cord lesion



Conclusion

- Promising results but not efficient enough.
- Test other hydrogels that will stimulate neurite growth without triggering inflammation.
- Reinforce GDNF action by other growth factors.
- Introduce stem cells.

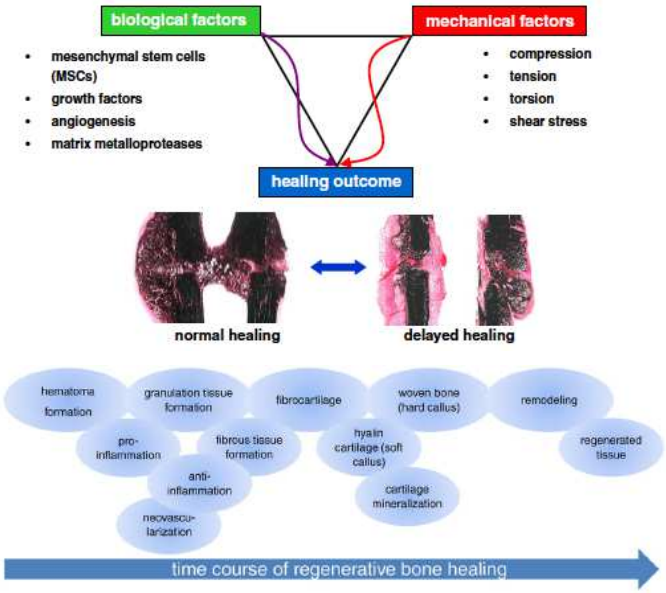
Large bone defect treatment

1. Bone healing
2. Current tissue engineering strategies for bone
3. Growth factor and delivery methods in treatment of bone defect
1. Future outlook

Bone healing

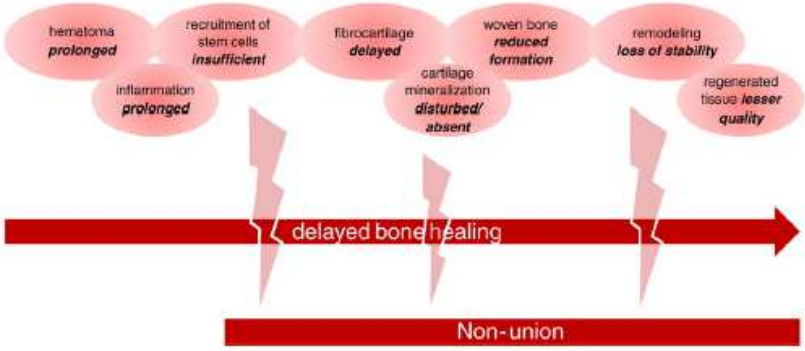
- Bone transplantation one of the most common clinical procedure.
- Bone loss from trauma, tissue resection, necrosis, spinal deformities, infections … leading to poor healing.
- Major clinical and socioeconomic problem.

Bone healing



Metha et al., 2012

Failure in bone healing



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Classical treatments

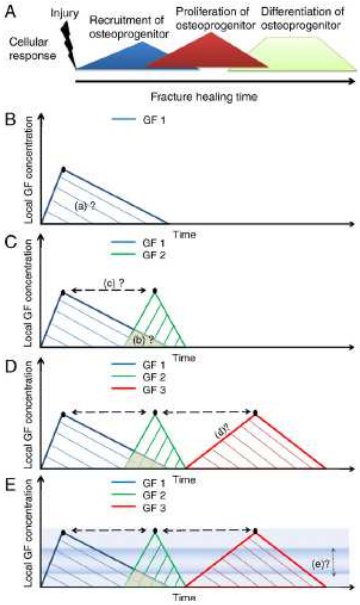
- Historically, amputation.
- In the past decades, bone autografts + metal implants but delayed union, prolonged treatment time and revision surgeries.
- The gold standard: autogenous bone grafting.
  But: donor site morbidity, pain, paresthesia, prolonged hospitalisation, risks of deep infection, inflammation, restricted availability.
- Other options
  - Allografts and xenograft. But: risk of infection and immune response.
  - Synthetic bone graft substitutes. But not reached yet clinical efficacy

Metha et al., 2012

New strategies

- Bone tissue engineering based on delivery of cells, matrix and bioactive molecules.
- Still inferior to the gold standard, mainly due to poor control over the delivery of growth factors, rapid degradation, and insufficient local concentration.
- Need to orchestrate spatiotemporal delivery of cues.

Delivery of multiple signals



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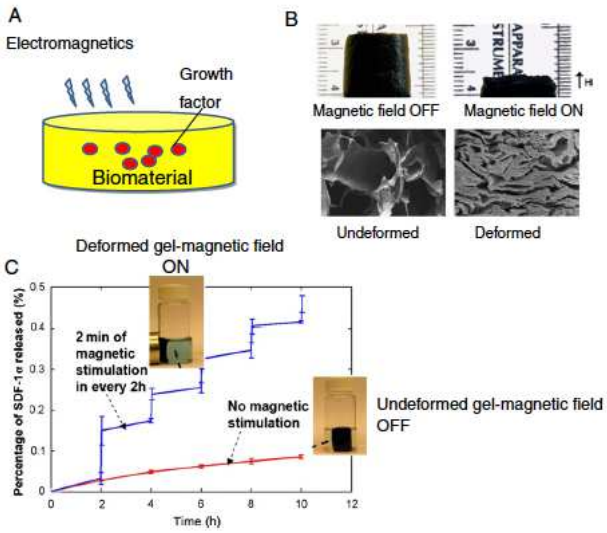
Delivery of multiple signals

Multiple GF release systems in bone regeneration.

Multiple growth factor system	Carrier	Species	Model	Testing temporal regulation	Ref.
PDGF/IGF-1	Titanium implant	Dog	Jaw bone	No	[107]
IGF-1/TGF-β1	Poly(D,L-lactide)-coated titanium K-wires	Rat	Tibia	No	[178]
BMP-2/FGF	Collagen sponge	Rat	Femur	No	[179]
BMP-2/TGF-β3	Alginate scaffold	Rat	Femoral defect	No	[255]
BMP-2/TGF-β3	Composite scaffold	Mice	Muscle	No	[256]
BMP-4/VEGF	PLGA scaffold	Mice	Subcutaneous	No	[170]
BMP-7/TGF-β1	Collagen	Baboon	Extraskeletal sites	No	[257]
Bone Protein/PDGF/IGF	Collagen/PLG	Rat	Skull caps	No	[177]
BMP-2/VEGF	Composite scaffold	Rat	Cranial	Yes	[258]
BMP-2/VEGF	Composite scaffold	Rat	Subcutaneous and femoral defect	Yes	[259]

Metha et al., 2012

On-demand release



Metha et al., 2012

TAKE AWAY MESSAGE

- Importance of the extracellular environment in determining cell behaviour: need for regenerative materials to provide cells with biological cues.

- Outcome of growth factor administration can be improved enormously with the use of technically simple slow-release schemes.

- Over-engineering devices difficult to translate to clinical use.