Stem cells, growth factors and scaffolds in craniofacial regenerative medicine

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Introduction

- Large craniofacial defects:
  - Congenital defect
  - Trauma
  - Cancer resection

https://www.3ders.org/articles/20150616-surgeons-use-3d-printing-to-help-fix-serious-skull-defects-for-young-south-african-girls.html

http://metro.co.uk/2017/07/07/man-who-lost-half-his-face-to-cancer-successfully-has-it-rebuilt-from-parts-of-his-legs-6761660/

Repair of extensive defects

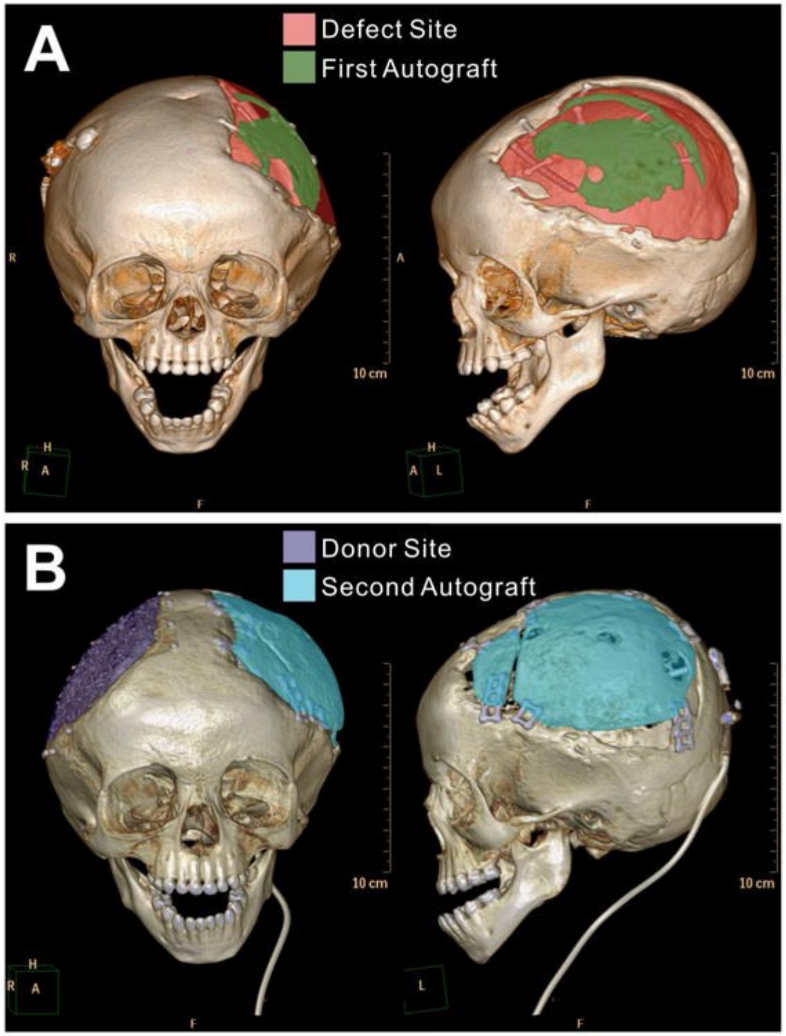


Figure 1

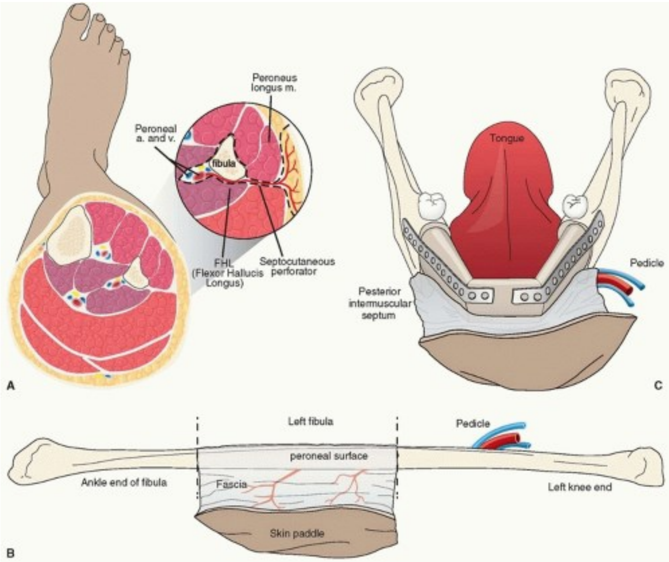
- Extensive defects prevent spontanous re-ossification
- Autologous bone grafts:
  - Cranium
  - Tibia
  - Rib
  - Iliac crest

→ Second surgical site

→ donor site morbidity:

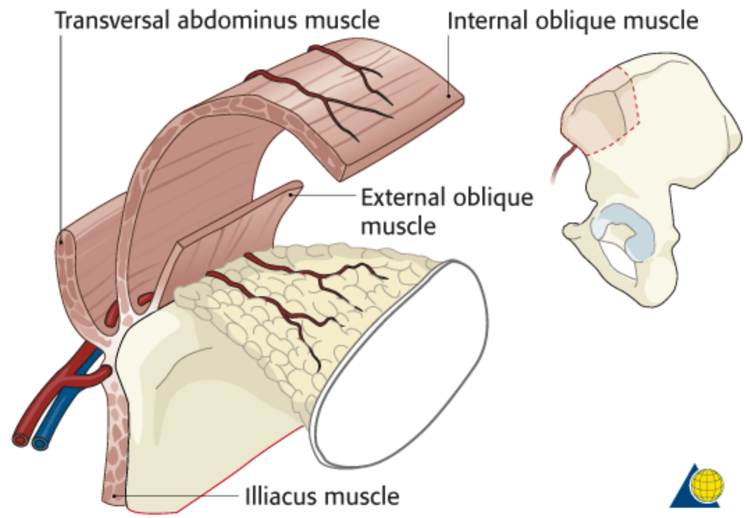
→ Infection, pain bleeding, fraction etc.

Autologous bone transplants



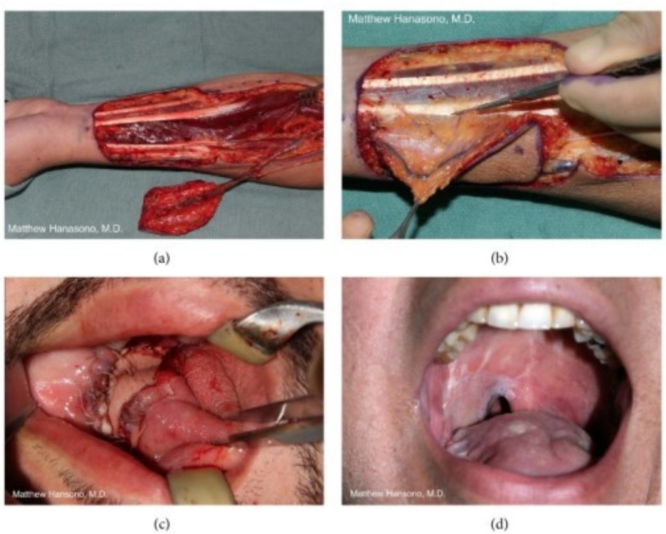
Fibula flap

https://plasticsurgerykey.com/mandible-reconstruction-with-free-fibula-flap/



Iliac crest flap

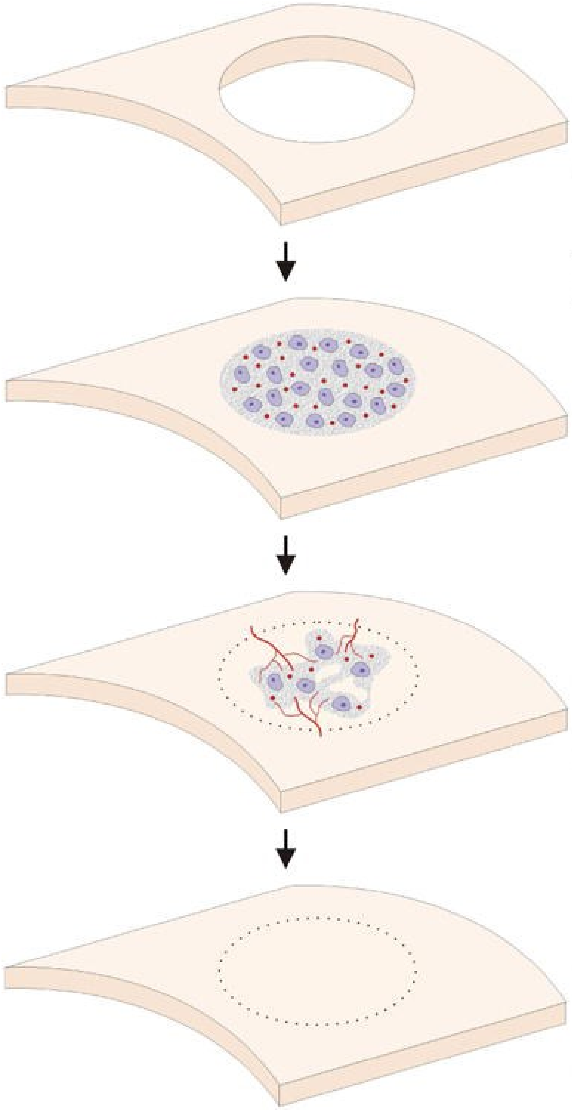
https://www2.aofoundation.org/wps/portal/surgerymobile?contentUrl=/srg/96/05-RedFix/Midface/B3/P520_03A-IliacCrestInternalObliqueFreeFlap



Radial forearm flap

https://openi.nlm.nih.gov/detailedresult.php?img=PMC4590972_AMED2014-795483.010&req=4

Biocompatible implants



- Drawbacks of alloplasts:
  - Rigid fixation → problem in children
  - Great risk of infection
- Biocompatible implants:
  - Osteoinductive scaffolding
  - stem cells
  - growth factors

→ osteoblastic + endothelial progenitor cell differentiation

→ bone formation,

→ integration into surrounding bone

Figure 2

Stem cells in bone regeneration

- BMSCs
  - Promising when seeded on Tricalcium-phosphate scaffold
  - limited supply, donor site morbidity
- ADSCs
  - Easier to harvest, easily expandable, similar osteogenicity
  - Still invasive procedure to harvest
- UCMSCs
  - Limited supply
- USCs
  - Easy excess, non-invasive
  - Similar to ADSCs
  - Still poorly studied

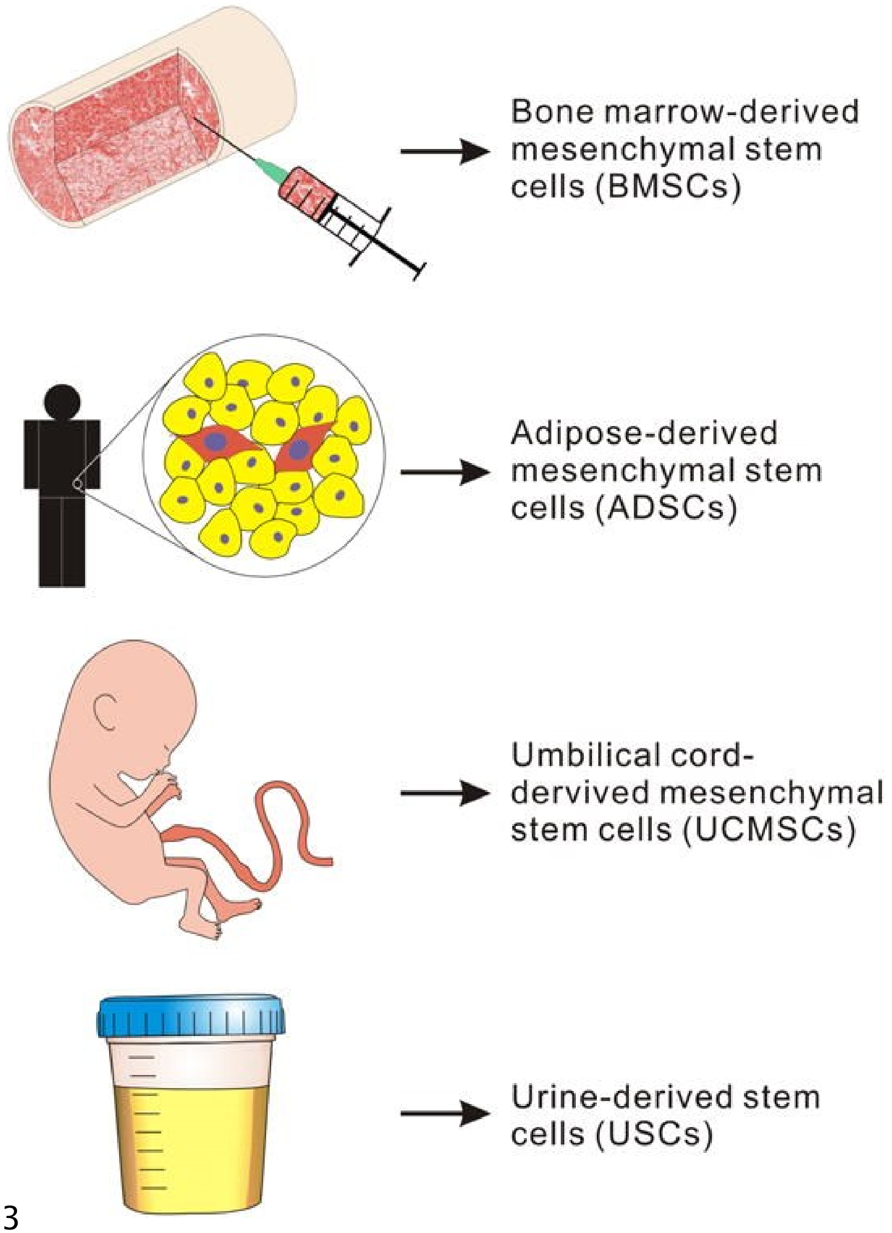
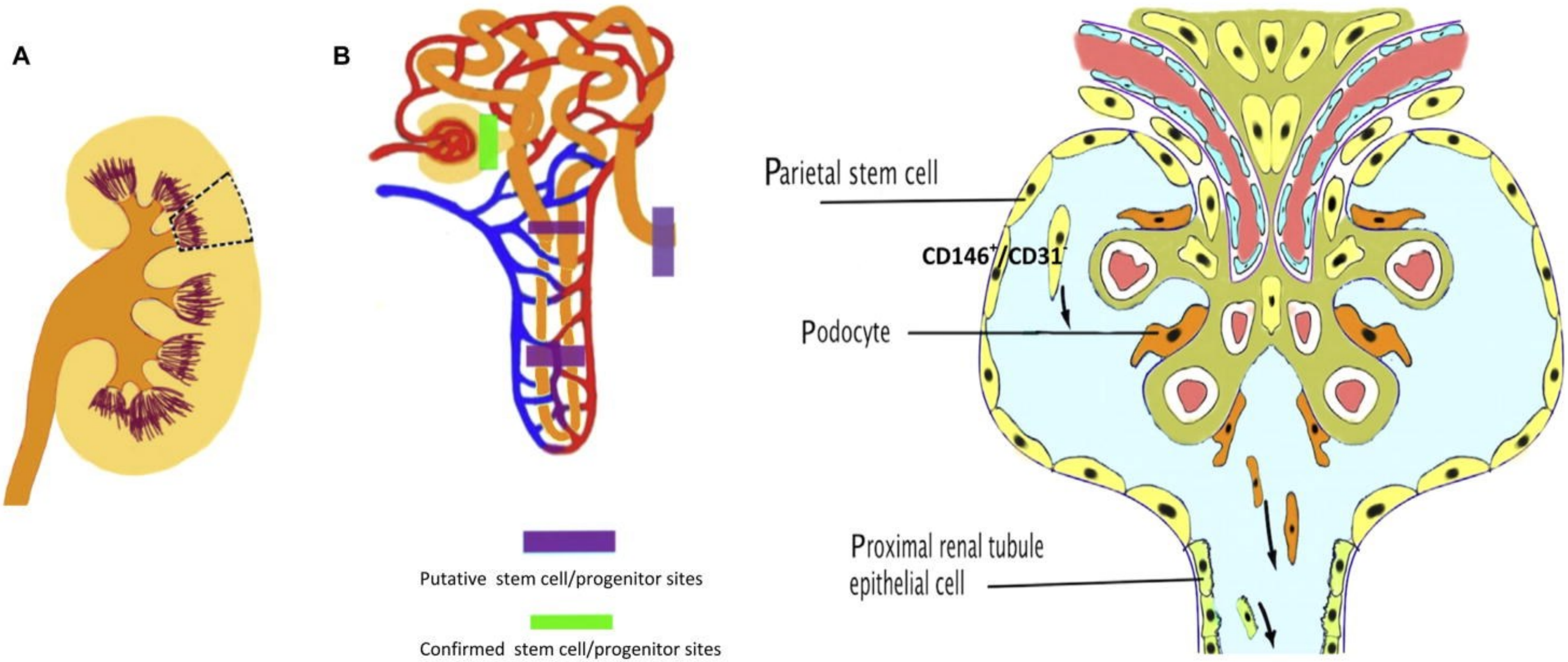


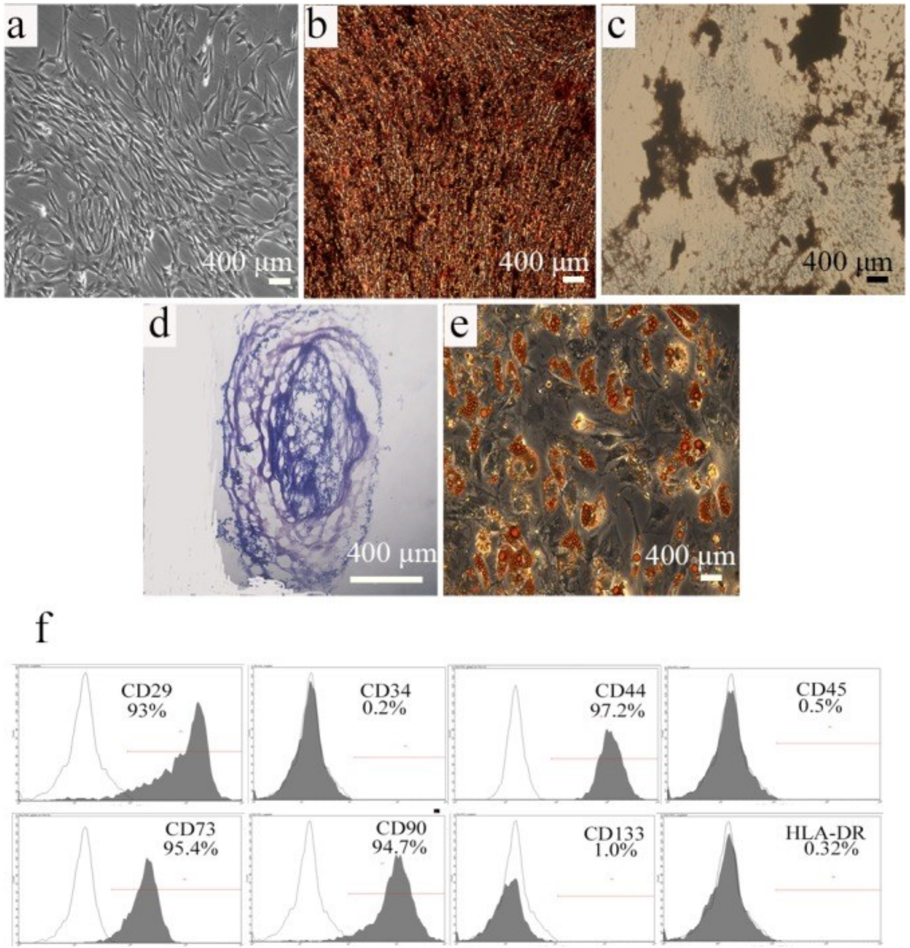
Figure 3

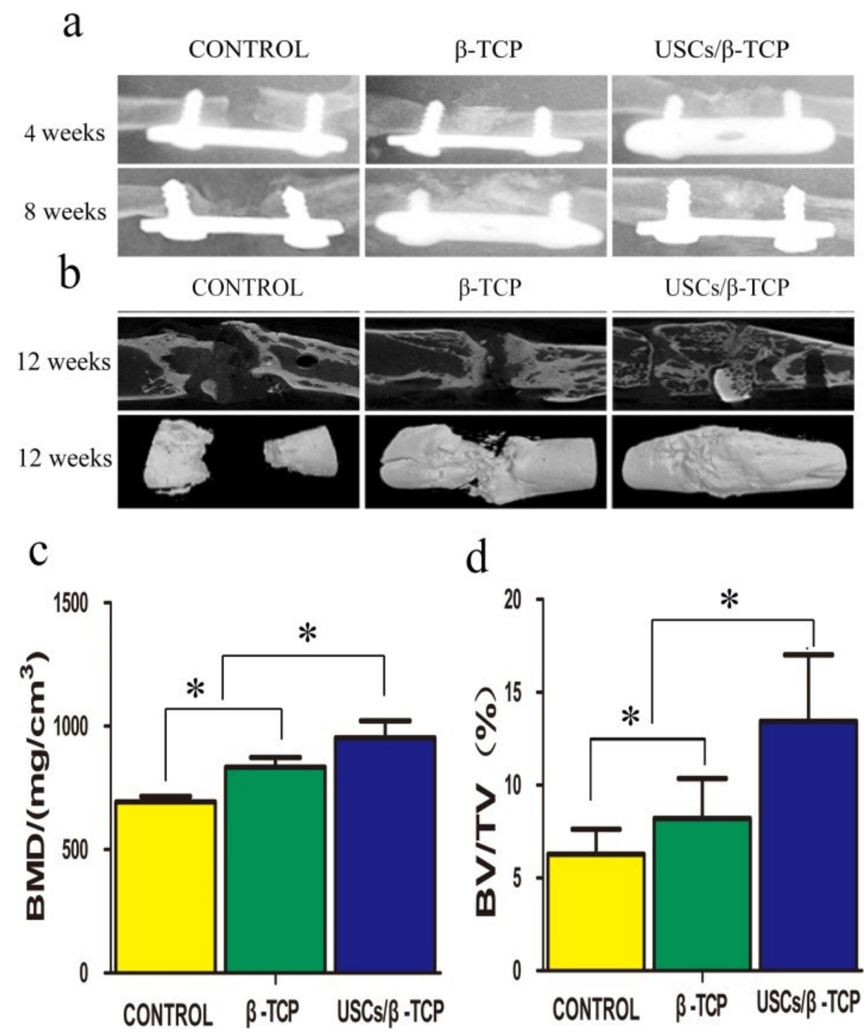
Urine-derived stem cells



Genes Dis. 2014 Sep 1;1(1):8-17.Urine-derived stem cells: A novel and versatile progenitor source for cell-based therapy and regenerative medicine. Zhang D1, Wei G2, Li P3, Zhou X4, Zhang Y5.

Urine-derived stem cells





PLoS One. 2015 May 13;10(5):e0125253. doi: 10.1371/journal.pone.0125253. eCollection 2015.Human Urine Derived Stem Cells in Combination with β-TCP Can Be Applied for Bone Regeneration.Guan J1, Zhang J1, Li H2, Zhu Z1, Guo S3, Niu X3, Wang Y4, Zhang C4.

Osteoinductive factors

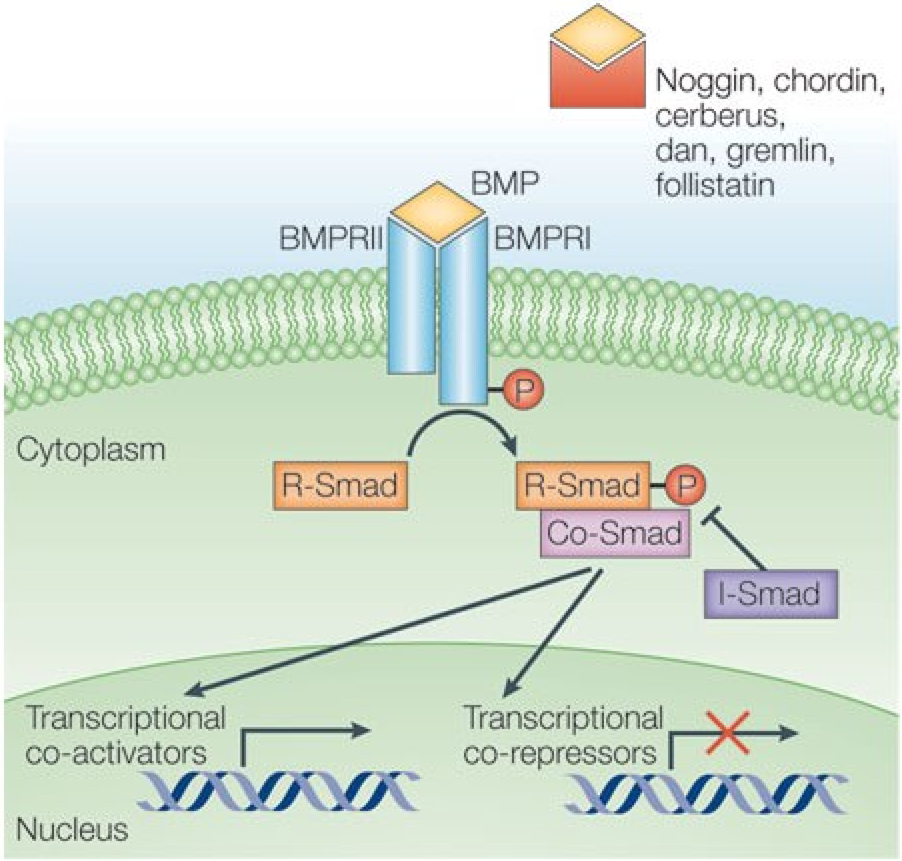
Growth Factor	Osteoblastic Differentiation	Osteoblast Proliferation	Neovasculogenesis
TGF-B	promoting	promoting	
FGF		promoting	
VEGF			promoting/inducing
PDGF	promoting *	promoting	promoting
BMP-2	inducing	promoting early; inhibiting late	
BMP-4	inducing	promoting early; inhibiting late	
BMP-6	inducing	promoting early; inhibiting late	
BMP-7	inducing	promoting early; inhibiting late	
BMP-9	inducing	promoting early; inhibiting late	

* Only PDGF-AA has been shown to promote osteoblastic differentiation in MSCs

Table 1: Osteoinductive growth factors

Bone morphogenic proteins (BMPs)

- TGFbeta family
- Bind to multiple stem cell types
- Osteoblastic differentiation through Smad signaling pathway
- FDA-approved scaffolds containig BMP 2/7



Nature Reviews | Neuroscience

Osteoinductive factors in scaffolds

- Soaking scaffold in growth-factor solution → fast release
- Incoroporated/linked to scaffold → slow release
- Cells modified to express/secrete osteoinductive factors → constant relase, but gene transfer (virally) necessary
- Osteoiunductive small molecules: statins, immunosuppressants, Phenamil

- High dose requirements
- Ectopic bone formation
- Paradoxal increase of bone resorption
- Mismatch growth factor release – bone regeneration

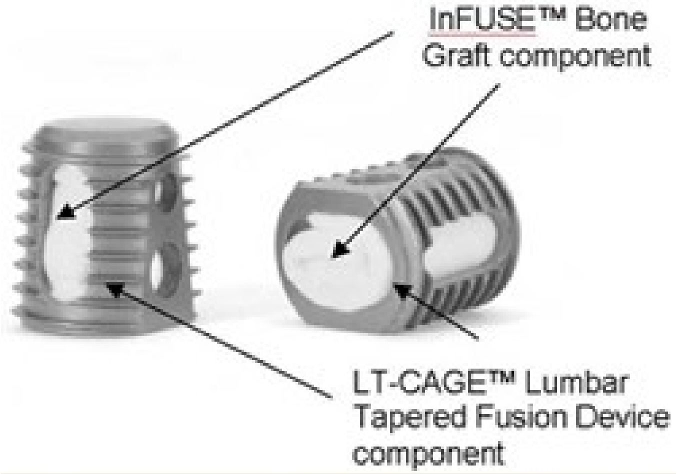


Photo showing the 3 parts of InFUSE™ Bone Graft/LT-CAGE™ Lumbar Tapered Fusion Device - P000058"

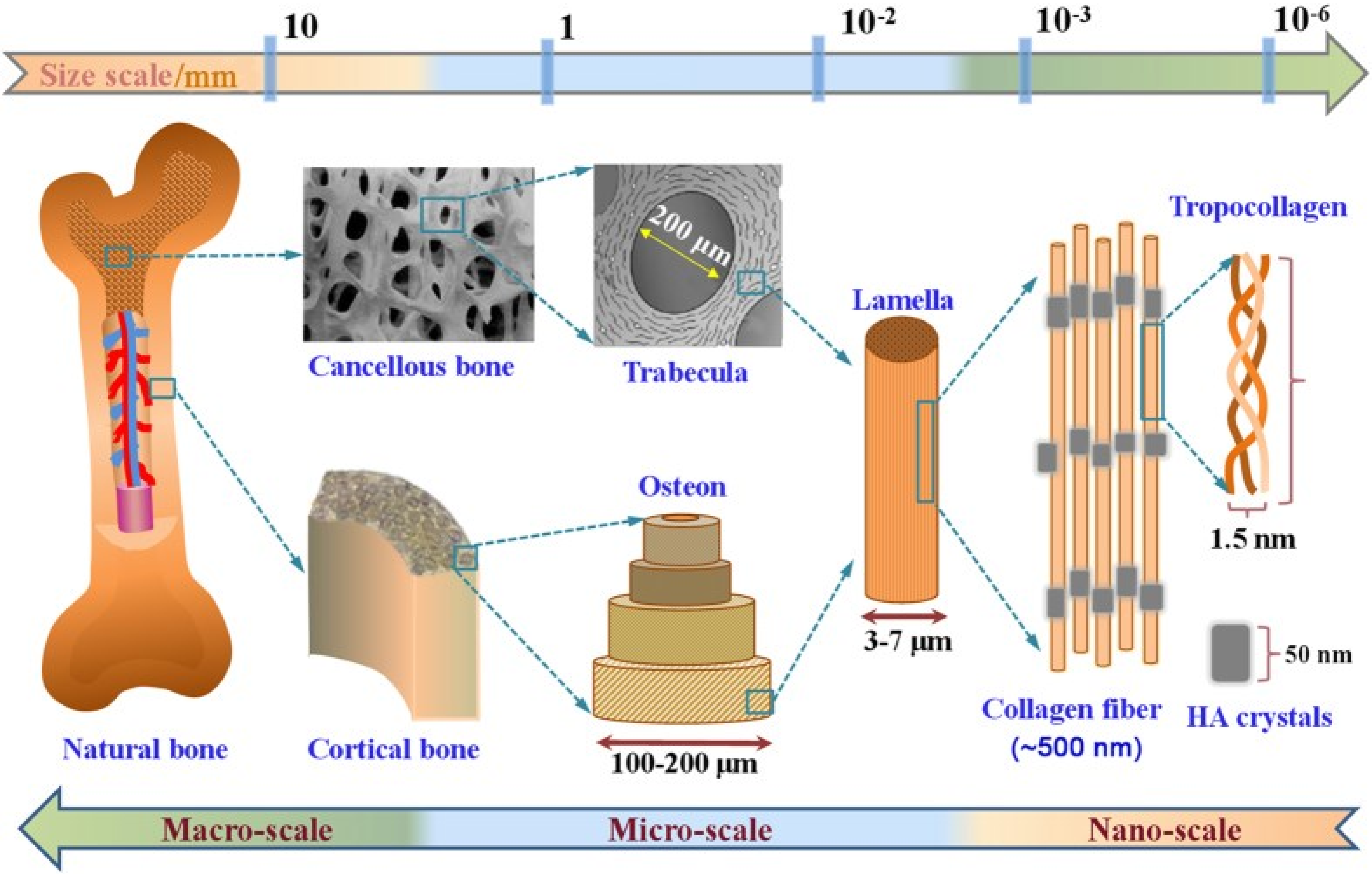
Scaffolds

Osteoconductive biomaterials for scaffold construction	
Allogenic bone derivative	Demineralized bone matrix (DBM)
Ceramics	Hydroxyapatite (HA)
	Tricalcium phosphate (TCP) biphasic calcium phosphate
	Calcium carbonate
Polymers	poly(lactic acid) (PLA)
	poly(glycolic acid) (PGA)
	poly(lactic-co-glycolic acid) (PLGA)
	Poly(propylene fumarate) (PPF)
	Polycaprolactone (PCL)
	Polyamide (PA)
	Chitosan
Metals	Titanium
	Magnesium Alloy
	Zinc (doping)
Bioglass	Silicon
	Calcium-silicate (CS)
Thermoresponsive	N-isopropylacrylamide (NIPAA)
	poly(polyethylene glycol citrate-co-N-isopropylacrylamide) (PPCN)

Table 2: Biomaterials for tissue engineering

- **Osteoconduction** = capability to provide template for bone growth
- **Osteoinduction** = recruitment of mesenchymal stell cells and differentiation to osteoblasts
- **Biocompatibility**: no inflammatory response!
- **Biodegradability**: scaffold = temporary framework, not prosthesis! Full resorption necessary!
- **Vascularization!**

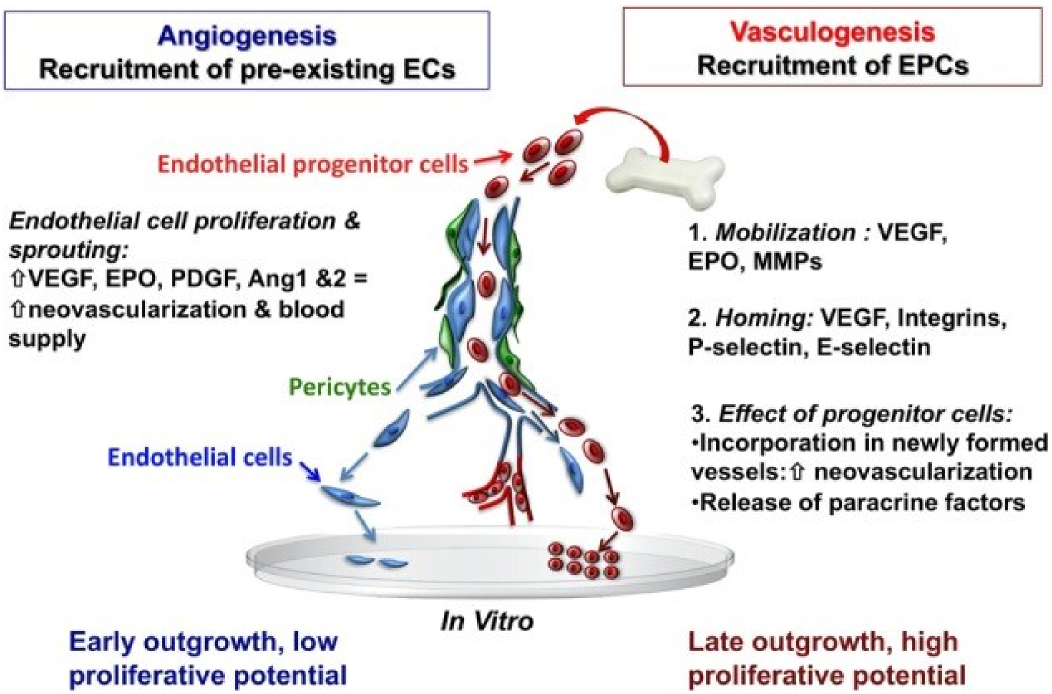
Structure and composition of natural bone



Bone Res. 2017 Dec 21;5:17059. doi: 10.1038/boneres.2017.59. eCollection 2017.
Bone biomaterials and interactions with stem cells. Gao C1, Peng S2,3, Feng P1, Shuai C1,4,5.

Neovascularization

- Incorporation of endothelial progenitor cells (EPCs)
- Neovascularization after response to ischemia
- Mediated by pro-angiogenic factor VEGF (vascular endothelial growth factor
- Porous scaffol structure (150-500ym) necessary ←→ material strength!
- Problem: radiotherapy in head –neck cancer



Front Neurosci. 2013 Oct 24;7:194. doi: 10.3389/fnins.2013.00194.
Stem cell therapy to protect and repair the developing brain: a review of mechanisms of action of cord blood and amnion epithelial derived cells.
Castillo-Melendez M1, Yawno T, Jenkin G, Miller SL.

Biomaterials for scaffold construction

- Gold standard: autologous; donor site morbidity, limited supply, etc.
- Demineralized bone matrix
- Ceramics
- Polymers
- Metals
- Bioglass
- Injectable biomaterials

Demineralized bone matrix (DBM)

- Acid extraction of allogenic bone
- Removes inorganic material, Collagen I framework exposed

  → Osteoinductive factors exposed = good osteoinductivity

  → Poor mechanical strength, porosity
- Poly (lactic acid) PLA/DBM composite scaffolds

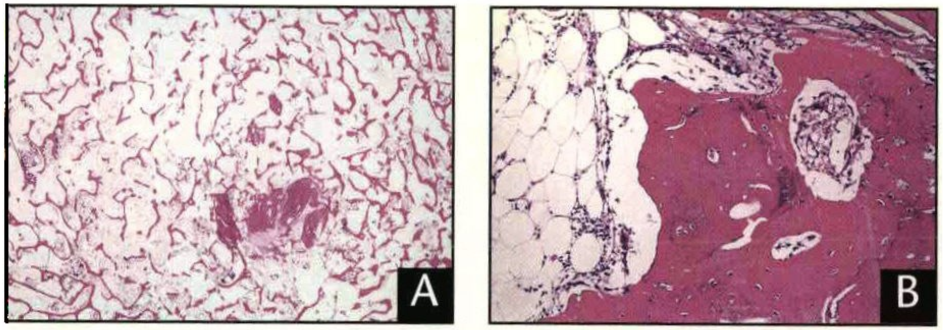
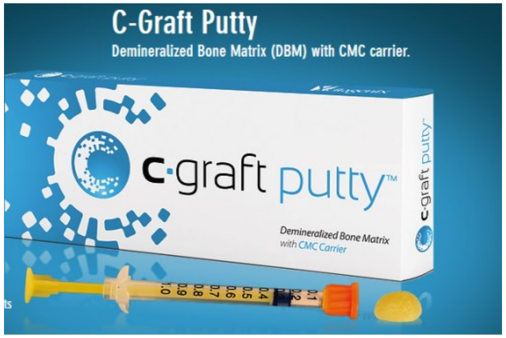


Figure 8: Histological section of putty-treated defect at 26 weeks. Bone formation throughout the defect is complete and the periphery of the defect is difficult to determine. A few DBM particles remain in the center (A). Demineralized bone matrix particles are completely surrounded by woven and lamellar bone (B).

[1] Turner TM, Urban RM, Hall DJ, Cheema N, Lim TH. Restoration of large bone defects using a hard-setting, injectable putty containing demineralized bone particles compared to cancellous autograft bone. Orthopedics. 2003;26:s561-5.



stryker.com

Ceramics

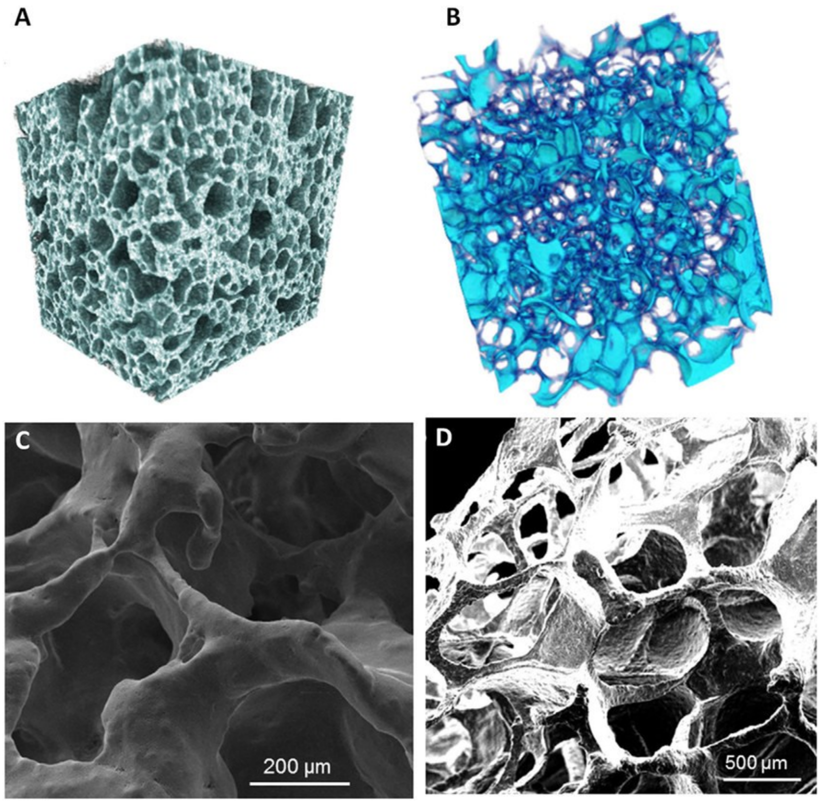
- Hydroxyapatite (calcium phsophate) (HA)

  → high osteoconductivity, safe, reliable, biocompatible, long shelf life

  → Brittle, slow resorption
- biphasic calcium phosphate
- betaTCP

  → faster resorption
- Calcium carbonate
- → good biodegradation, rapid resorption

  → little research for larger defects
- HA/collagen composite grafts
  → improved stiffness, osteointegration



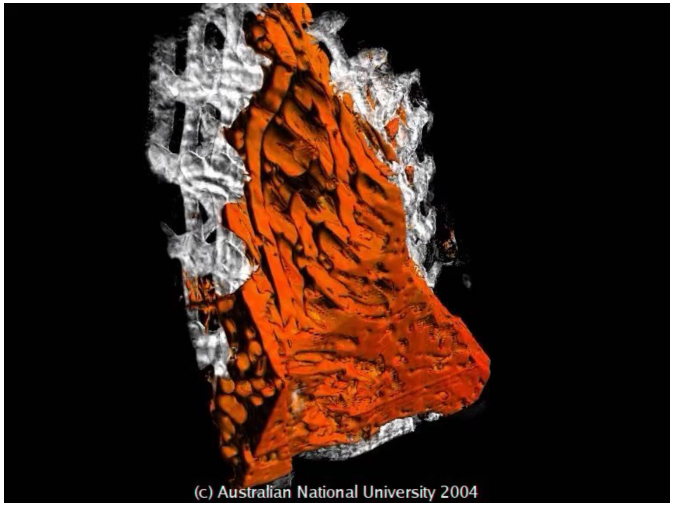
[1] Caddeo S, Boffito M, Sartori S. Tissue Engineering Approaches in the Design of Healthy and Pathological In Vitro Tissue Models2017.

Polymers

Natural (Collagens, Fibrins…):

→ good cell adhesion, functional support properties, biodegradable, biocomptible, porosity

→ Less control over mechanical properties, sometimes immunogenicity; expensive!



https://physics.anu.edu.au/appmaths/capabilities/xct-gallery.php

Synthetic:

PLA (poly lactic acid), PGA (poly glycolic acid)

→ Poor osteoinductivity, PLA/PGA alone not suitable for scaffolds

PPF (poly propylene fumarate), PMMA (polymethyl methacrylate)

Polyamide (PA)

→ Excellent strength, biocompatibility

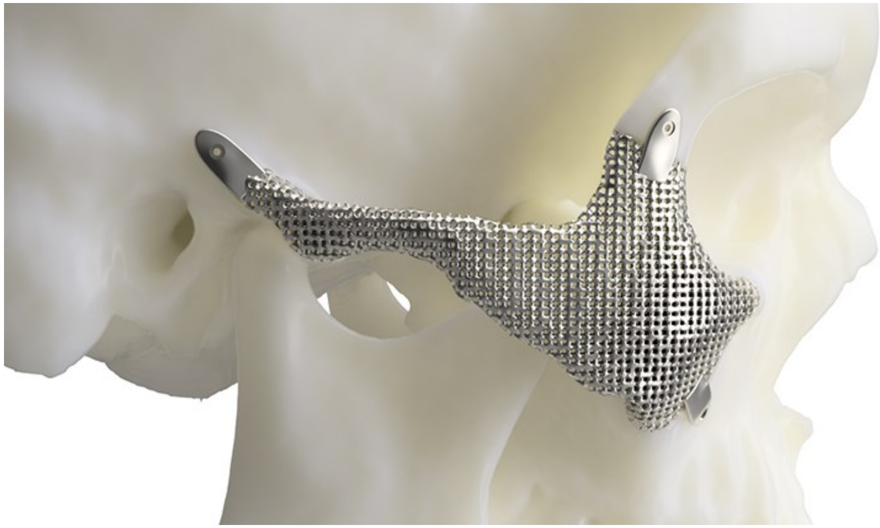
→ **Composite grafts!**

Metals

- Titanium
  → Well established for implants
  → Inert alloplasts, no integration, no stimulationof bone formation
- Magensium alloys
  → Good porosity and mechanical properties, strength, durability, osteoconductivity
- Metal nanoparticles into polymers

- Addition of zinc + silicone

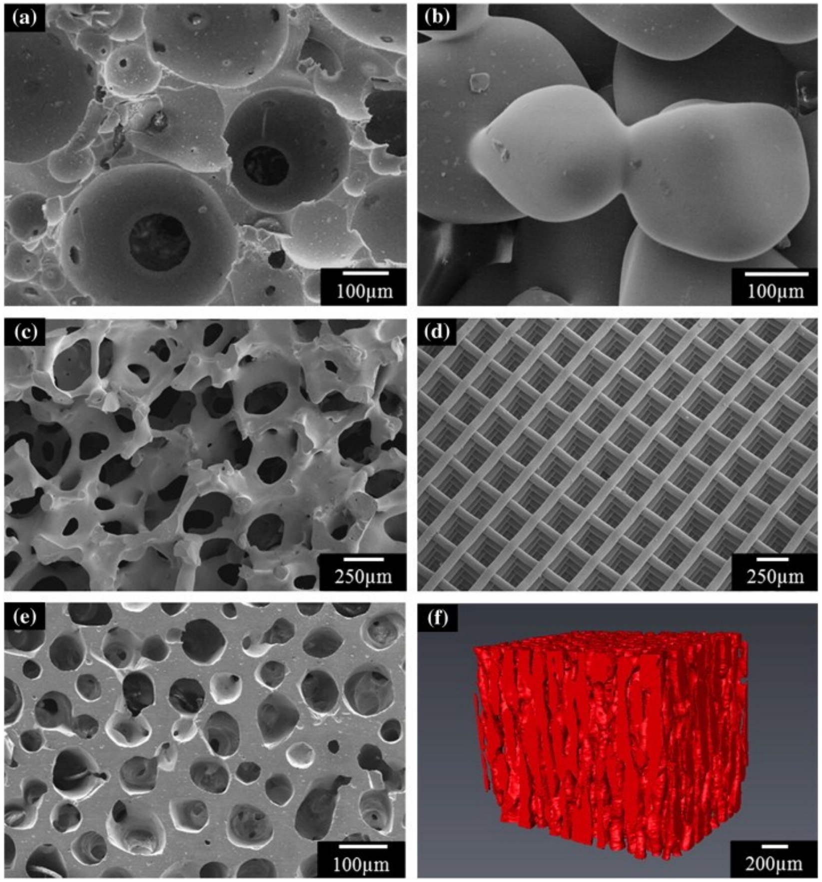
→ Higher Col I-expression, angiogenesis, osteoblast differentiation



https://blog.chirurgia3d.com/en/scaffold-the-future-of-metal-implants/

Bioglass

- Glass-ceramic
- Glass-polymer
- Silicon in glass:
  → Angiogenesis, growth factor production in osteoblasts, stimulation of osteogenesis
  → Scaffold with osteogenesis and angiogenesis without exogenous growth factors!
  → Brittleness, less strength than original bone



[1] Fu Q, Saiz E, Rahaman MN, Tomsia AP. Bioactive glass scaffolds for bone tissue engineering: state of the art and future perspectives. Materials Science and Engineering: C. 2011;31:1245-56.

Injectable biomaterials

→ Can be delivered minimal invasively

→ Mold to shape of complicated defects

→ Less infammation and scarring

- NIPAA
  - → Good thermoresponsive
  - → Toxicity, nondegradability
- PPCN
  - → thermoresponsive, retains viable cells, antioxidant
- Hydrogels

  Water-absorbing matrices of hydrophilic polymers

  - → Well suited to carry growth factors and stem cells
- Hydroxyapatite/calcium sulfate pastes
  - → syneresis, contraction, brittleness
  - → Combination with other materials/composites

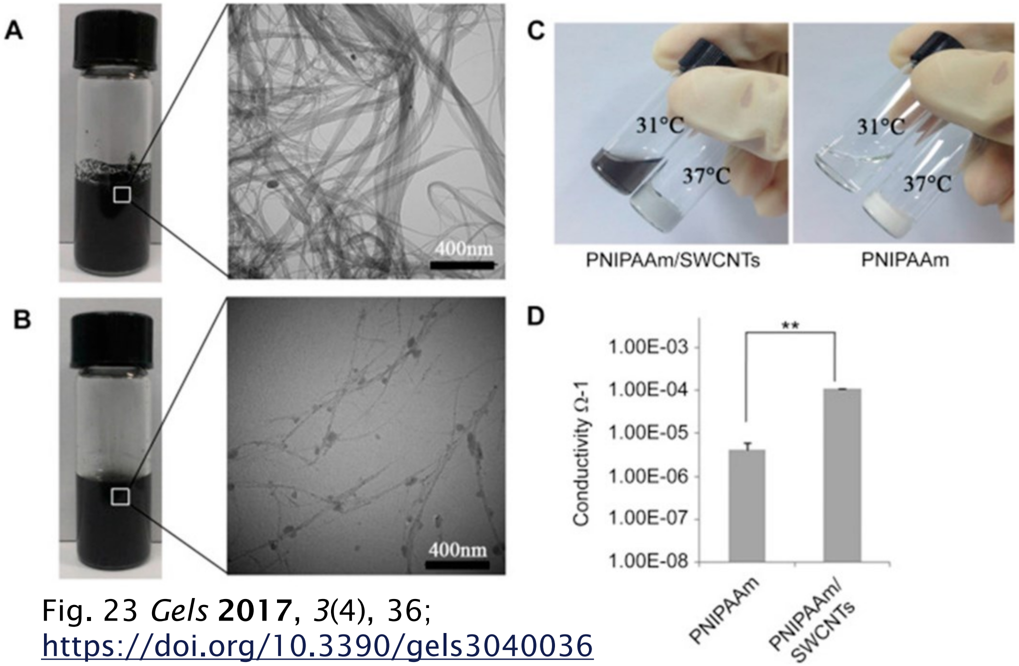


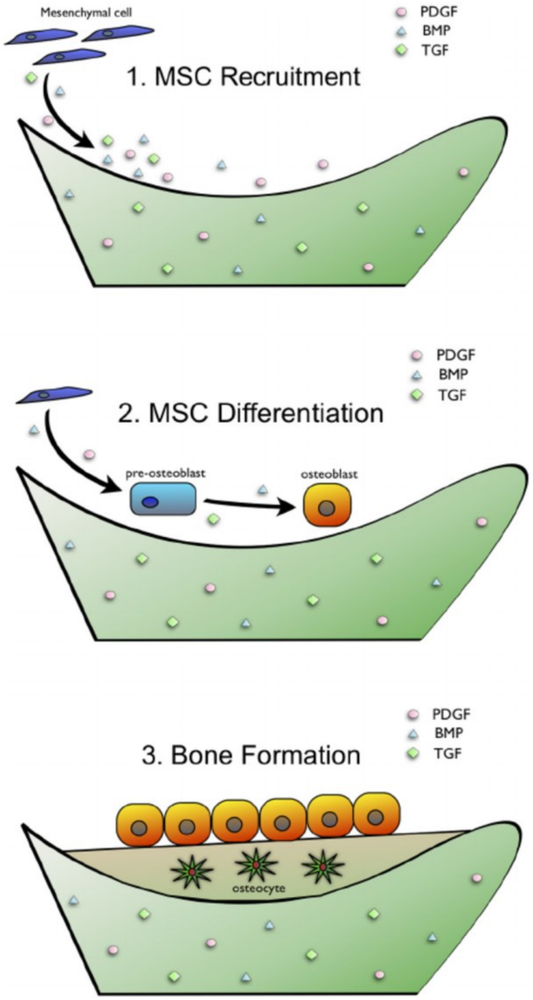
Fig. 23 *Gels* **2017**, *3*(4), 36; https://doi.org/10.3390/gels3040036

Osteoinductive molecular structure

= design priority to optimize osteoconductive and osteoinductive

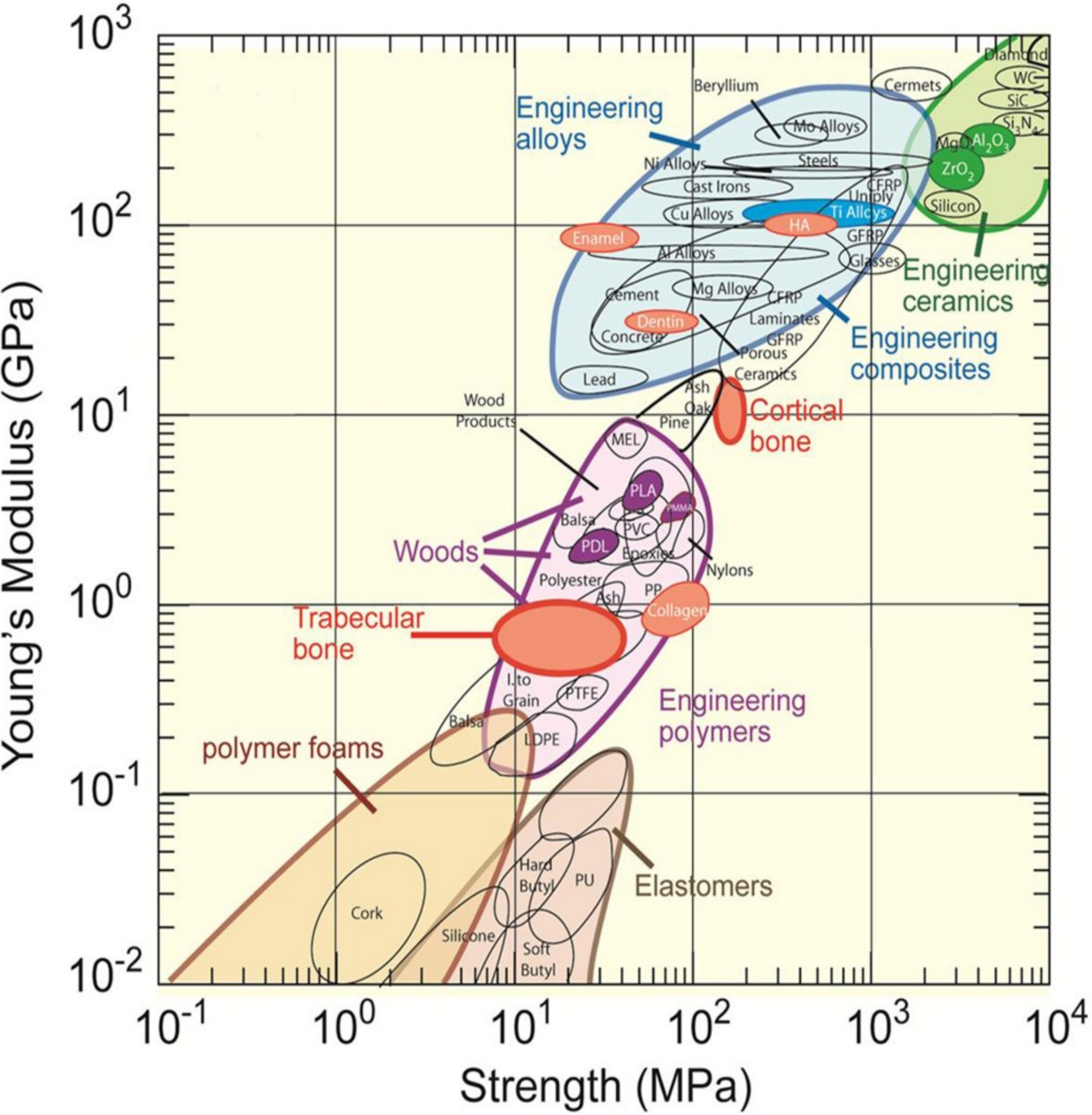
- Optimum: closely mimic natural healing
- Basic structure of scaffold: long cylindrical unit in line with bone´s axis
- Osteoclasts on leading end, osteoblasts at lagging end

→ Initiate bone formation without exogenous molecular signals



[1] Miron R, Zhang Y. Osteoinduction: A Review of Old Concepts with New Standards2012.

Mechanical properties of scaffolds



"Young's modulus, also known as the elastic modulus, is a measure of the stiffness of a solid material. It is a mechanical property of linear elastic solid materials (…)" (Wikipedia)

- The lower Young´s module, the more elastic the material
- Strength: "the strength of material is the amount of force it can withstand and still recover its original shape" (Wikipedia)

[1] Fu Q, Saiz E, Rahaman MN, Tomsia AP. Bioactive glass scaffolds for bone tissue engineering: state of the art and future perspectives. Materials Science and Engineering: C. 2011;31:1245-56.

Conclusion and future directions

- Close collaboration of material science and molecular biology
- Combination of materials for optimal scaffolds
- New stem cell resources (e.g. urine-derived)
- Advances still complicated by drawbacks: scarring, osteomyelitis, osteonecrosis, radiation damage
- Little research addressing tissue engineering with medical comorbidities/comprised wound healing

Discussion

- Incidence of large defects requiring (synthetic) coverage?
- Application of scaffolds in daily clinic?
- Which scaffolds are approved in Austria?
  - HA, DBM?
- Does tissue engineering play a role in clinics yet?
- Customized scaffolds via 3D-printing?

Thank you!

Questions?