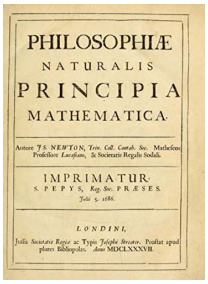
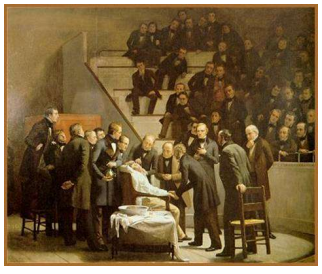
Primum non nocere

Primum non nocere

- Věda vychází ze **systematického** studia založeného na **pozorování**, tvorbě **teorií** a jejich **ověřování experimentem**.

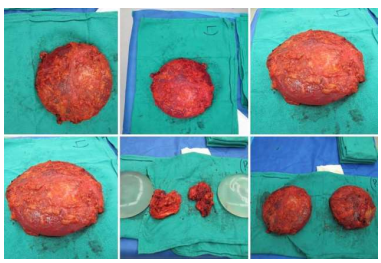


Ignác Filip Semmelweis: Horečka omladnic. 1847 zavedl na oddělení povinné mytí rukou chlorovým vápnem



William Morton: První operace v celkové narkoze - 1846

PIP scandal



Murphy's Law

- "Může-li se něco pokazit, pokazí se to."
- "Jestli někdo může udělat něco špatně, udělá to tak".
  - Nekompatibilita konektorů vedla k smrti dítěte – dodán špatný kabel.

Wieringa. AVOIDING PITFALLS IN THE ROAD FROM IDEA TO CERTIFIED PRODUCT (AND THE HARSH CLINICAL ENVIRONMENT THEREAFTER) WHEN INNOVATING MEDICAL DEVICES. Belgian Day on Biomedical Engineering

PIP (Poly Implant Prothèse) scandal

- What is a breast implant?
  - A medical prosthesis used to augment, reconstruct, or create the physical form of breasts
  - Modern (so-called fifth generation) breast implants usually contain either saline or a viscous silicone gel
  - The outer casing, or shell, is composed of durable elastic silicone manufactured through a chemical process called vulcanization in which sulphur is added to the silicone to increase durability
    - Due to the production method, commercial silicone products will contain variable concentrations of molecular weights and sizes including a subgroup of small-sized molecules referred to as D4, D5, D6. In addition, the normal manufacturing process may result in traces of platinum, used as an essential catalyst.
- <u>The PIP implants were found to contain a higher proportion of small-sized molecules D4, D5, D6 than the norm.</u>

https://doi.org/10.1177/0141076813480994

PIP (Company Poly Implant Prosthese) scandal

- **Timeline:**
- 1991: PIP launched by Jean-Claude Mas
- 1997: PIP authorized to produce **medical-grade silicone** implants
- 2000: FDA warns about deviations from “good manufacturing practices” found at the PIP plant. Additionally, the company withdraws its hydrogel implants from the market when it cannot show they are safe.
- 2001: PIP starts using **unapproved**, (“in-house” formula) **industrial-grade silicone** in their implants
- 2009: Concerns surfaced in France when surgeons started reporting abnormally high rupture rates
- 2010: PIP was placed into liquidation after the French medical safety agency recalled its implants.
- 2011: The French government recommended that 30,000 women with PIP implants seek removal of the implants as a precaution
- 2013: Mas sent to prison for four years, fined 75,000 euros, and banned for life from working in medical services or running a company.


Ruptured PiP Implant

FDA - 2016 Medical Device Recalls

Greatbatch Medical Recalls Standard Offset Cup Impactor Used for Hip Joint Replacement due to Inadequate Sterilization
Centurion Recalls Multi-Med Single Lumen Catheters due to Excess Material that May Split or Separate
Medtronic Recalls Neurovascular Products due to Potential Separation and Detachment of Polytetrafluoroethylene (PTFE) Coating
SentreHeart Recalls FindrWIRZ Guidewire System due to Coating Separation
HeartWare Recalls Ventricular Assist Device Controllers Due to Loose Connector Ports
HeartWare Recalls Ventricular Assist Device Pumps Due to Contamination Causing Electrical Issues
Customer Letter for the Class II Teleflex LMA Mucosal Atomization Devices. (PDF - 76KB)
St. Jude Medical Recalls Implantable Cardioverter Defibrillators (ICD) and Cardiac Resynchronization Therapy Defibrillators (CRT-D) Due to Premature Battery Depletion
TeleFlex Medical Recalls Tracheostomy Tube Set Due to Possible Disconnection During Patient Use
Leonhard Lang Multi-function Defibrillation Electrodes DF29N Will Not Work with Welch Allyn Automatic External Defibrillator model AED 10
Baxter Corporation Recalls 50 mm 0.2 Micron Filter Due to the Potential for a Missing Filter Membrane and Possible Particulate Matter Contamination
DePuy Synthes Recalls Power Tool System Battery Adaptors Due to Possible Explosion Risk
Cook Medical Recalls Roadrunner® UniGlide® Hydrophilic Wire Guide Because of Potential Coating Contamination
Alere Recalls INRatio® and INRatio2® PT/INR Monitoring System Due to Incorrect Test Results
BioMerieux SA Alerts Customers about Potential Inaccurate Test Results When using NucliSENS® easyMAG® Magnetic Silica for Nucleic Acid Extraction
Dräger Recalls VentStar Oxylog 3000 Pediatric Patient Breathing Circuit Due to Potential Valve Leakage
CareFusion Recalls AVEA Ventilator Due to an Electrical Issue Which May Cause an Unexpected Shutdown
Stryker Sustainability Solutions (formerly Ascent Healthcare Solutions) Recalls Flush Angiographic Catheter Due to Tip Separation
HeartWare Inc. Extends Recall to Include Batteries Used in the Ventricular Assist Device Due to Premature Power Depletion

Medtronic Respiratory and Monitoring Solutions Recalls Battery Pack Used on Patient Monitors Due to Potential Fire Risk
Hummingbird Med Devices Inc. Recalls Hummi Micro-Draw Blood Transfer Device Due to Potential for Parts to Disconnect
B. Braun Medical Inc. Recalls Dialog+ Hemodialysis Systems Due Defective Conductivity Sensors
Boston Scientific Corporation Recalls Fetch 2 Aspiration Catheter Due to Shaft Breakage
Vascular Solutions Recalls Guardian II Hemostasis Valve Due to Low Pressure Seal Defect
Focus Diagnostics Recalls Laboratory Examination Kits Due to Inaccurate Test Results
Dexcom Inc. Recalls G4 Platinum and G5 Mobile Continuous Glucose Monitoring System Receivers Due to Audible Alarm Failure
Cook Medical Recalls Central Venous Catheter and Pressure Monitoring Sets and Trays due to Tips that May Split or Separate
Verathon Inc. Recalls GlideScope Titanium Single-Use Video Laryngoscope Due to Potential Video Feed Disruption
Arrow International Inc. Recalls Intra-Aortic Balloon Catheter Kits and Percutaneous Insertion Kits Due to Sheath Separation Issue
Abbott Vascular Recalls MitraClip Clip Delivery System Due to Issue with Delivery System Deployment Process
Dräger Evita V500 and Babylog VN500 Ventilators - Recall Expanded to Include Optional PS500 Batteries with New Power Supply Firmware
Cook Medical Expands Recall for Beacon Tip Angiographic Catheters to Include Additional Product Lots
Dräger Medical Inc. Recalls Emergency Transport Ventilators Due to a System Error that may lead to a Halt in Ventilation Therapy
Arkray Recalls SPOTCHEM II Test Strips Due to Inaccurate Blood Sugar Readings
Thornhill Research Inc. Recalls MOVES Ventilator System Due to Battery Problem
St. Jude Medical Recalls Optisure Dual Coil Defibrillation Leads Due to Damage that May Prevent Patient Therapy
Brainlab Cranial Image-Guided Surgery (IGS) System - Navigation Inaccuracy
Stryker Fuhrman Pleural and Pneumopericardial Drainage Sets - Catheter May Break During Insertion
Dräger Evita V500 and Babylog VN500 Ventilators - Issue with Optional PS500 Battery Power Supply May Cause Ventilators to Shut Down Unexpectedly

Medical devices – avoiding of pitfalls

- **R&D costs and time to market**
  - Large R&D costs and long time to market are very common for even relatively simple medical devices. This is partly due to numerous strict regulations
  - 1960:
    - Within six weeks Earl Bakken modified a metronome circuit from Popular Electronics into a wearable battery powered pacemaker.
    - 3 years later Bakken's company, Medtronic, was selling implantable pacemakers for $375 each.
      - Today, silver-dollar sized pacemakers sell for about $8,000.
  - Today it takes 10 to 15 years and millions of dollars from the "*gleam in the inventor's eye*" for a product to reach the marketplace.

Wieringa. AVOIDING PITFALLS IN THE ROAD FROM IDEA TO CERTIFIED PRODUCT (AND THE HARSH CLINICAL ENVIRONMENT THEREAFTER) WHEN INNOVATING MEDICAL DEVICES. Belgian Day on Biomedical Engineering

Regenerative medicine

- Encyclopedia Britanica:

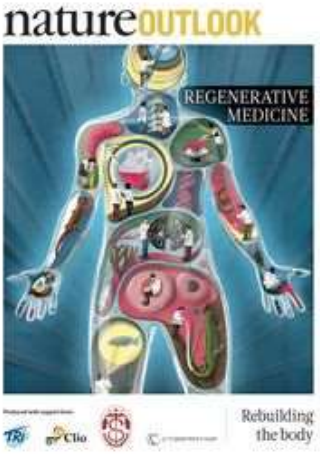
„application of treatments developed to replace tissues damaged by injury or disease.

These treatments may involve the use of:

- *biochemical techniques to induce tissue regeneration directly at the site of damage*
- *or the use of transplantation techniques employing differentiated cells or stem cells, either alone or as part of a bioartificial tissue.“*

- Nature Journal:

„collection of techniques and technologies that aim to restore our physiology to something that resembles its original condition.“



Biomedical engineering

- Nature Journal

„branch of engineering that applies principles and design concepts of engineering to healthcare.

Biomedical engineers deal with:

- *medical devices such as imaging equipment,*
- *biocompatible materials such as prostheses or therapeutic biologicals, or*
- *processes such as regenerative tissue growth.“*

Tissue Engineering

- Nature Journal:

„set of methods that can replace or repair damaged or diseased tissues with natural, synthetic, or semisynthetic tissue mimics.

These mimics can either be fully functional or will grow into the required functionality.“

„Tissue engineering is a branch of regenerative medicine, itself a branch of biomedical engineering.

Tissue engineering and regenerative medicine are concerned with the replacement or regeneration of cells, tissues (the focus of tissue engineers) or organs to restore normal biological function.“

- Encyclopedia Britanica:

„Tissue engineering, scientific field concerned with the development of biological substitutes capable of replacing diseased or damaged tissue in humans.“

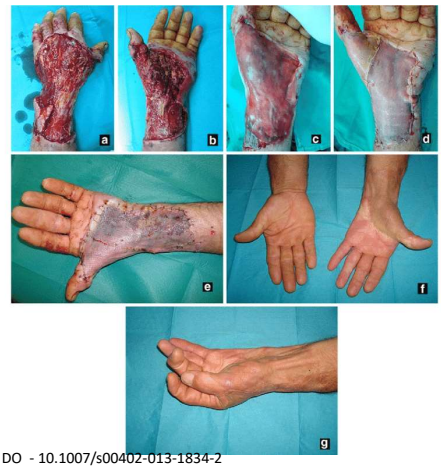
Tissue Engineering

- The idea of tissue engineering emerged in 1988.
- Decade later when the infamous "Vacanti mouse"
  - The ear was actually a mesh of biodegradable plastic that was moulded into the desired shape, sprinkled with cartilage cells collected from a cow, and implanted under the skin of the mouse. The researchers removed the 'ears' after 12 weeks, and found that some new cartilage had been generated within the structure.
    - the reaction was one of excitement and wonder. "If we can grow a human ear, why not a kidney, a liver, an eye?"

30 years of tissue engineering, what has been achieved? Daniel Heath, Lecturer, Biomedical Engineering, University of Melbourne

Tissue Engineering

- Most succesfull examples:



DO - 10.1007/s00402-013-1834-2

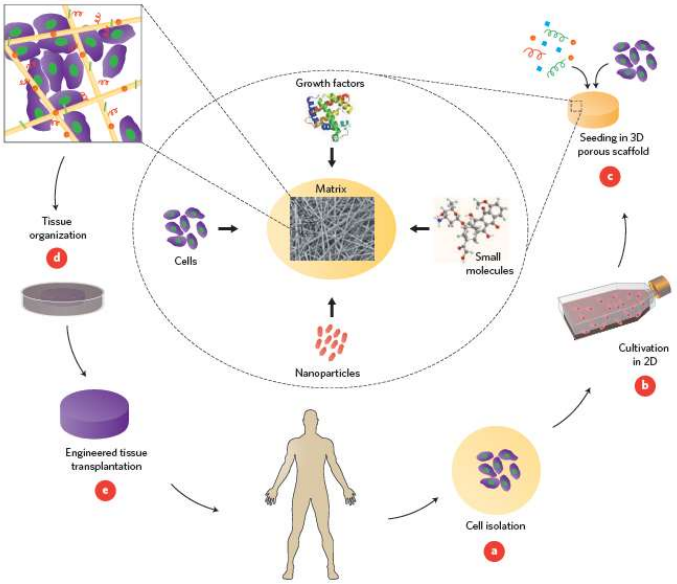
30 years of tissue engineering, what has been achieved? Daniel Heath, Lecturer, Biomedical Engineering, University of Melbourne

Tissue Engineering

- Most succesfull examples:

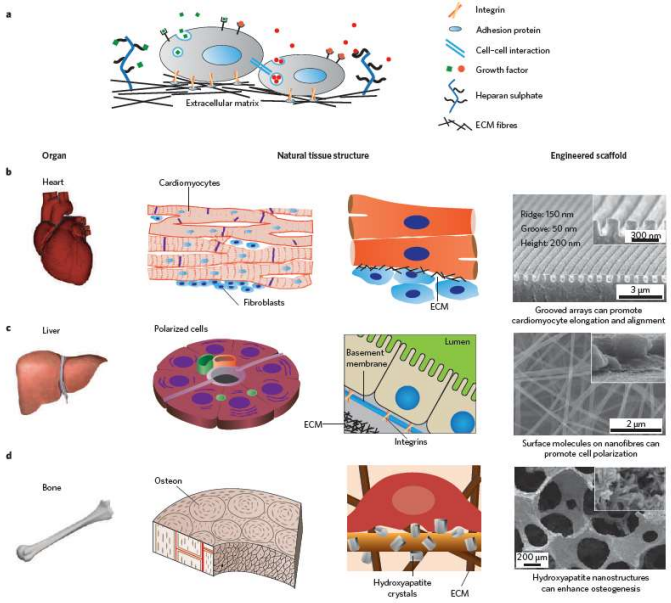
30 years of tissue engineering, what has been achieved? Daniel Heath, Lecturer, Biomedical Engineering, University of Melbourne

Tissue Engineering



Nature nanotechnology | VOL 6 | JANUARY 2011. Doi: 10.1038/nnano.2010.246

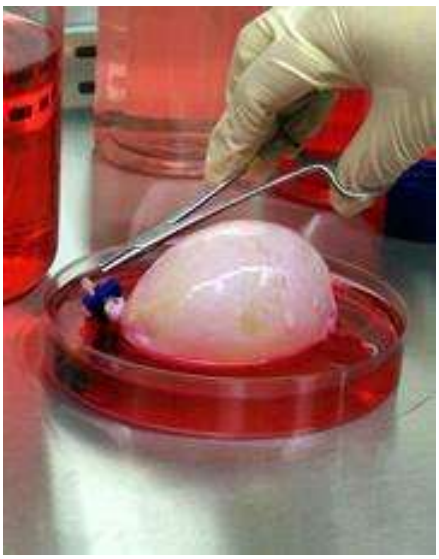
Tissue Engineering



Nature nanotechnology | VOL 6 | JANUARY 2011. Doi: 10.1038/nnano.2010.246

Tissue Engineering

Urinary bladder - 2006

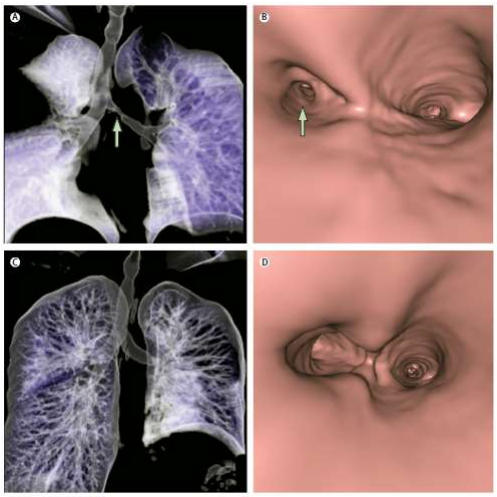


Nature | doi:10.1038/news060403-3

Lancet 2006; 367: 1241–46. DOI:10.1016/S0140-6736(06)68438-9

Tissue Engineering

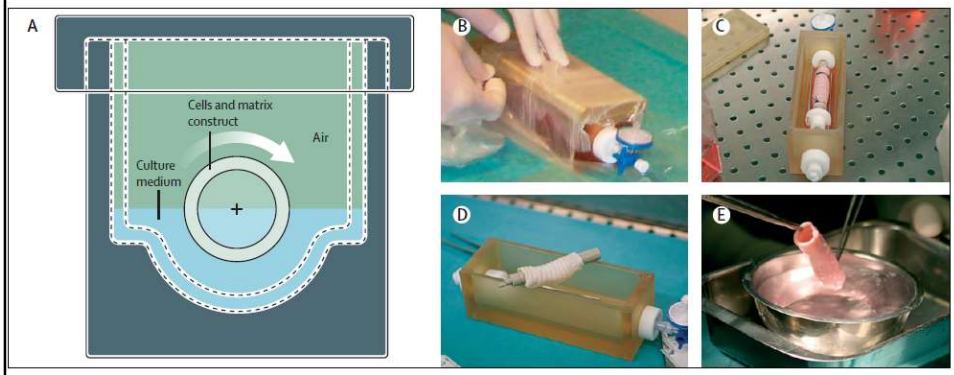
Trachea – decellularised 2008



Lancet 2008; 372: 2023–30. DOI:10.1016/S0140-6736(08)61598-6

Tissue Engineering

Trachea -2008



Lancet 2008; 372: 2023–30. DOI:10.1016/S0140-6736(08)61598-6

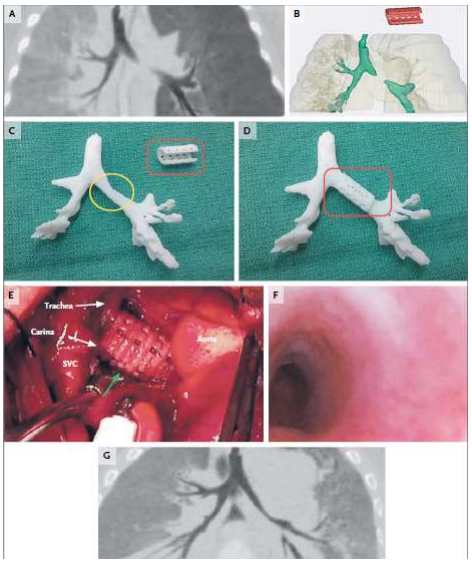
Tissue Engineering

2014 – nasal cartilage

http://dx.doi.org/10.1016/ S0140-6736(14)60544-4

Tissue Engineering

Resorbovatelná dlaha:



N Engl j Med 368;212013; DOI: 10.1056/NEJMc1301237

Biomaterials

- American National Institute of Health:

„Any substance or combination of substances, other than drugs, synthetic or natural in origin, which can be used for any period of time, which augments or replaces partially or totally any tissue, organ or function of the body, in order to maintain or improve the quality of life of the individual.“

History

- 32 000 years ago – first sutures (NATNEWS, 1983, **20**(5): 15–7)
- 1829 – first study of bioreactivity of steels (H.S.Levert)
- 1891 – first Hip and Knee Prostheses, but succesfully developed in period 1968-1972.
- 1912 - anastomose (suture) of blood vessels (Nobel Prize in medicine)
- 1941 – first study of implantation of polymers (cellophane) - wrapping for blood vessels.
- 1959 - first fully implantable pacemaker developed by engineer Wilson Greatbatch and cardiologist W. M. Chardack
- 1960s - first silicone breast implant
- 1969 – first implanted a polyurethane total artificial heart

Figure 4 The *Cairo Toe* was attached to an ancient Egyptian mummy (between 1550 and 700 BC), an elder woman believed to have lost her right big-toe to complications from diabetes was constructed of wood and leather. It was definitely worn by the patient during her lifetime.

Figure 11 The wooden leg of the ar
(1777–1831) preserved in the Polish
HFH.

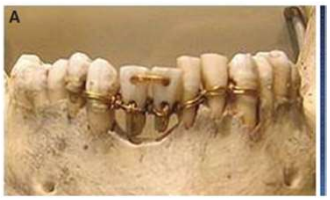
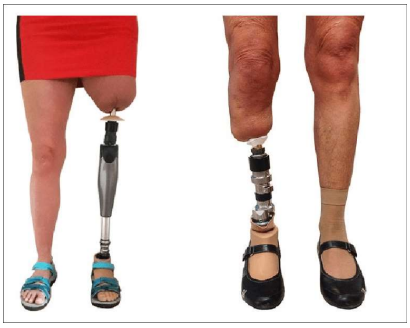
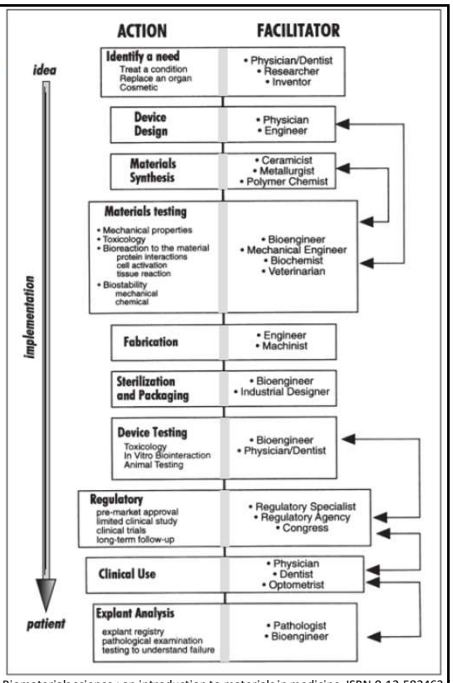


Figure 20 Typical Phoenician dental restoration by anch
work composed of carved dog or calf teeth joined by gold

Biomaterials science - multidisciplinarity



DOI:10.1177/0269215518815215



Biomaterials science : an introduction to materials in medicine. ISBN 0-12-582463-

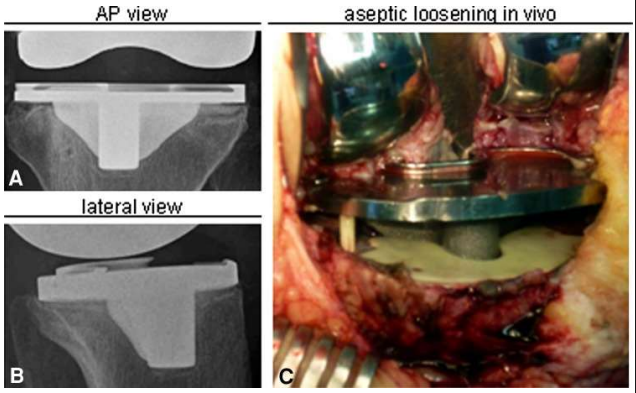
FIG. 6. Disciplines involved in biomaterials science and the path from a need to a manufactured medical device.

Biocompatibility

- Williams Dictionary of Biomaterials (Williams 1999)

„Ability of a material to perform with an appropriate host response in a specific situation."

A cement penetration of 3 to 5 mm is considered optimal for implant fixation. All patients in our study had the tibial surfaces prepared with pulsatile lavage before the tibial baseplate was placed. Pulsatile lavage allows for better cement penetration that increases the tensile and shear strength of the cement-bone interface and therefore the probability of successful implantation. Despite observing these standards, five patients (6.3%) with short-keeled tibial baseplates underwent revision surgery as a result of **aseptic loosening**. The failure in four of the five cases was related to the implant-cement interface. Debonding of the roughened baseplate from the underlying cement mantle causes wear debris between the two surfaces. The produced particulate metal and cement debris lead to rapid osteolysis and early failure.



DOI: 10.1007/s11999-012-2630-y

Personalized medicine

- Nature Journal:

„Personalized medicine is a therapeutic approach involving the use of an individual's genetic and epigenetic information to tailor drug therapy or preventive care."

Biomedicine

- The branch of medicine that deals with the application of the biological sciences, especially biochemistry, molecular biology, and genetics, to the understanding, treatment, and prevention of disease.

  *The American Heritage® Medical Dictionary Copyright © 2007, 2004 by Houghton Mifflin Company. Published by **Houghton Mifflin Company**.*

- **A highly nonspecific term** for a broad field of study which borrows elements from the history of human and veterinary medicine, anatomy, physiology, genetics, pathology, zoology, botanical sciences, chemistry, biochemistry, biology and microbiology. While traditional medicine is concerned with the direct practical application of medical knowledge,biomedicine looks at its history and involves itself in new research to push the limits of what medicine is able to accomplish.Biomedicine may also refer to a specific type of treatment, generally seen as more 'natural' than others, and often availablein a less regulated context.

  Segen's Medical Dictionary. © 2012 Farlex, Inc.

Nanomaterials

- Defining what we mean by a nanomaterial is never straightforward:
  - for some, the size of the material should be a few nanometres,
  - for others it should be smaller than a few tens of nanometres,
  - for still others anything less than a micrometre will do,
  - for some, one dimension at the nanoscale is enough; for others it should be at least two or even all three.
- For different compounds, the properties that distinguish a nanoscale specimen from its bulk correspondent occur at different sizes.
  - The transition is rarely abrupt, and the properties evolve from bulk to nanoscale in a **continuous** way, so that establishing a threshold size is arbitrary.
- The physical and chemical properties of nanomaterials, as well as environmental and health toxicity, depend on the precise shape and composition as well as size.

Nature Nanotechnology **volume 14**, page193 (2019)

Nanomaterials

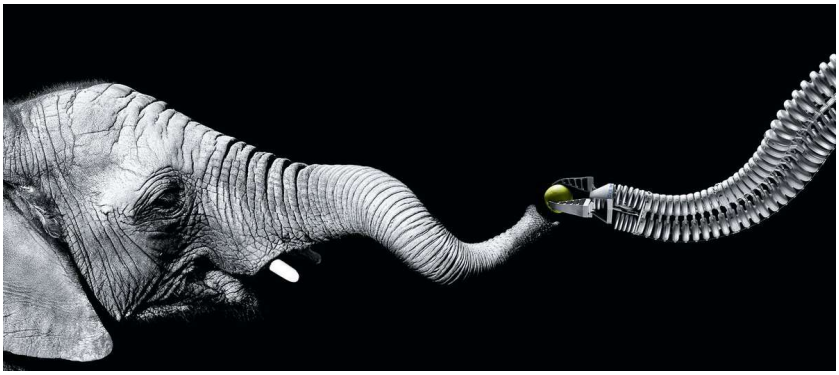
- REGULATION (EU) 2017/745 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL
  - nanomaterial' means a natural, incidental or manufactured material containing particles in an unbound state or as an aggregate or as an agglomerate and where, for 50 % or more of the particles in the number size distribution, one or more external dimensions is in the size range 1-100 nm;

Bioinspired

- Nature Journal:

„Bioinspired materials are synthetic materials whose structure, properties or function mimic those of natural materials or living matter. „

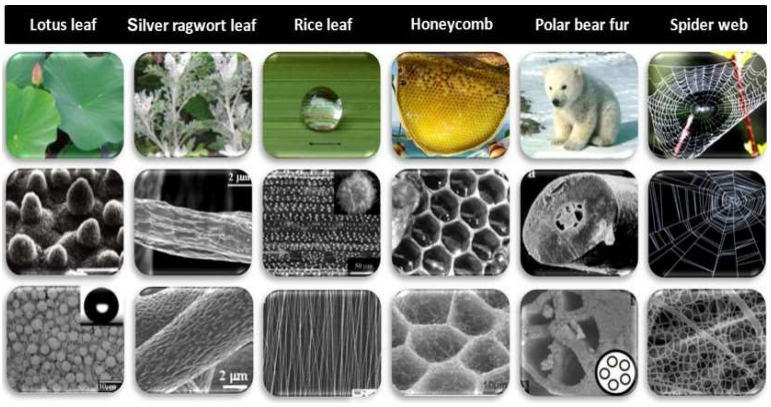
Examples of bioinspired materials are light-harvesting photonic materials that mimic photosynthesis, structural composites that imitate the structure of nacre, and metal actuators inspired by the movements of jellyfish.



Biomimetic

- Nature Journal:

„Biomimetics is an interdisciplinary field in which principles from engineering, chemistry and biology are applied to the synthesis of materials, synthetic systems or machines that have functions that mimic biological processes.„



DOI: 10.1016/j.mattod.2013.06.005

Biofabrication

„Biofabrication refers to the combination of cells, biomaterials, and bioactive factors with advanced fabrication techniques to generate functional tissue constructs, with a level of complexity exceeding simple 2D or 3D cultures.“

Biomaterials 198 (2019) 78–94

Medical device

- REGULATION (EU) 2017/745 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL

„Any instrument, apparatus, appliance, software, implant, reagent, material or other article intended by the manufacturer to be used, alone or in combination, for human beings for one or more of the following specific medical purposes:

- *diagnosis, prevention, monitoring, prediction, prognosis, treatment or alleviation of disease,*
- *diagnosis, monitoring, treatment, alleviation of, or compensation for, an injury or disability,*
- *investigation, replacement or modification of the anatomy or of a physiological or pathological process or state,*
- *providing information by means of in vitro examination of specimens derived from the human body, including organ, blood and tissue donations,*

*and which **does not** achieve its principal intended action by pharmacological, immunological or metabolic means, in or on the human body, but which may be assisted in its function by such means.“*

Medical device – custom made

- REGULATION (EU) 2017/745 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL

*„Any device **specifically made in accordance with a written prescription** of any person authorised by national law by virtue of that person's professional qualifications which gives, **under that person's responsibility**, specific design characteristics, and is intended for the sole use of a particular patient exclusively to meet their **individual conditions and needs**."*

However, mass-produced devices which need to be adapted to meet the specific requirements of any professional user and devices which are mass-produced by means of industrial manufacturing processes in accordance with the written prescriptions of any authorised person shall not be considered to be custom-made devices;

LaserImplants™

LaserImplants are metal 3D printed craniomaxillofacial (CMF) patient specific implants (PSIs) and surgical guides which can be provided with optional polycarbonate anatomical models.

Medical device

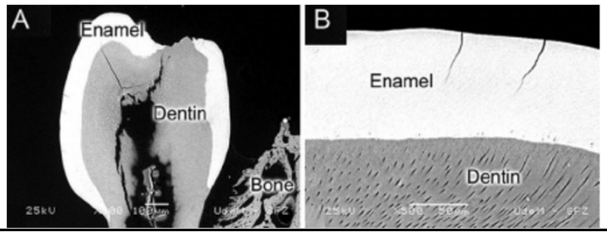
- REGULATION (EU) 2017/745 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL
  - *„clinical evaluation means a systematic and planned process to continuously generate, collect, analyse and assess the clinical data pertaining to a device in order to verify the safety and performance, including clinical benefits, of the device when used as intended by the manufacturer"*
  - *„clinical investigation means any systematic investigation involving one or more human subjects, undertake n to assess the safety or performance of a device"*
  - *„clinical evidence means clinical data and clinical evaluation results pertaining to a device of a sufficient amount and quality to allow a qualified assessment of whether the device is safe and achieves the intended clinical benefit(s), when used as intended by the manufacturer"*
  - *„clinical performance means the ability of a device, resulting from any direct or indirect medical effects which stem from its technical or functional characteristics, including diagnostic characteristics, to achieve its intended purpose as claimed by the manufacturer, thereby leading to a clinical benefit for patients, when used as intended by the manufacturer"*
  - *„clinical benefit means the positive impact of a device on the health of an individual, expressed in terms of a meaningful, measurable, patient-relevant clinical outcome(s), including outcome(s) related to diagnosis, or a positive impact on patient management or public health"*

Cosmetics

- REGULATION (EC) No 1223/2009 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL
  - *„cosmetic product means any substance or mixture intended to be placed in contact with the external parts of the human body (epidermis, hair system, nails, lips and external genital organs) or with the teeth and the mucous membranes of the oral cavity with a view exclusively or mainly to cleaning them, perfuming them, changing their appearance, protecting them, keeping them in good condition or correcting body odours“*
  - *„substance means a chemical element and its compounds in the natural state or obtained by any manufacturing process, including any additive necessary to preserve its stability and any impurity deriving from the process used but excluding any solvent which may be separated without affecting the stability of the substance or changing its composition“*
  - *„mixture means a mixture or solution composed of two or more substances“*

Bioinspired structural materials

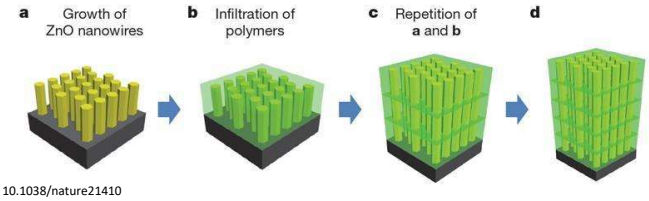
- Materials-design problem - the two key structural properties (strength and toughness) tend to be mutually exclusive:
  - strong materials are invariably brittle,
  - tough materials are frequently weak.
- Bioinspiration
  - Highly mineralized, mostly ceramic, natural structures, such as tooth minimize wear and provide protection.
  - A unique aspect of these materials is that they utilize different structures or structural orientations:
    - Tooth
      - hard surface layers so as to resist wear and/or penetration,
      - tough subsurface to accommodate the increased deformation.



DOI: 10.1038/NMAT4089

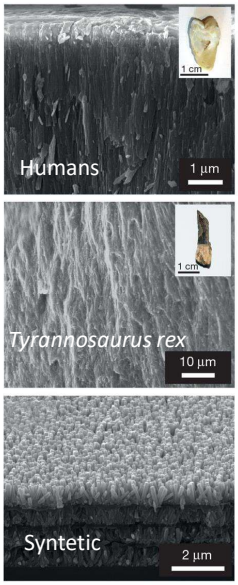
Bioinspired structural materials

- Tooth enamel is remarkably well conserved throughout the natural world.
  - Vertical columns of stiff, inorganic material stand embedded in a polymer matrix.
  - The energy from the vibrations the tooth is subjected to during chewing is dissipated thanks to friction, as the flexible polymer pillars of the tooth oscillate more than the stiffer ones.
- **Natural materials often combine stiff and soft components in hierarchical structures.**



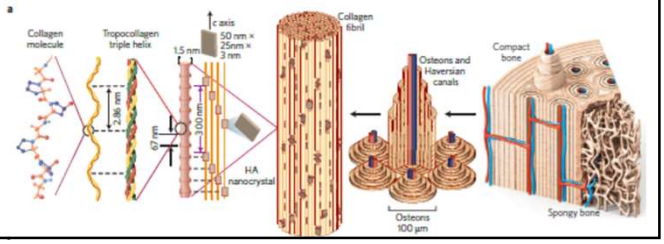
DOI: 10.1038/nature21410

The structure of the enamel



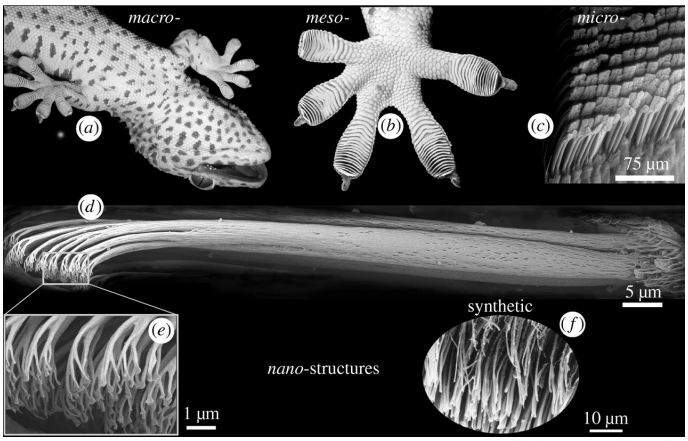
Bioinspired structural materials

- Natural structural materials are built at ambient temperature from a fairly limited selection of components.
  - Limited number of components that have relatively poor intrinsic properties.
  - Superior traits stem from naturally complex architectures that encompass multiple length scales.
- They usually comprise hard and soft phases arranged in complex hierarchical architectures, with characteristic dimensions spanning from the nanoscale to the macroscale.
  - The resulting materials are lightweight and often display unique combinations of strength and toughness, but have proven difficult to mimic synthetically.
  - Any rational strategy must incorporate nano-, micro- and macroscale features, and thus involve the so-called **mesoscale** approach.



DOI: 10.1038/NMAT4089

Structural hierarchy of the gecko adhesive system



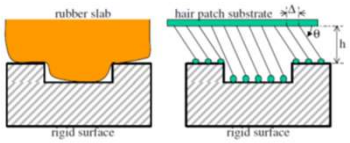


Fig. 1. Partial contact in the case of a solid elastic materials (left) and a hair-like one (right).

Kellar Autumn, and Nick Gravish Phil. Trans. R. Soc. A 2008;366:1575-1590

Bioinspired structural materials

- In the composites, the interface are crucial:
  - For instance, the enamel/dentin interface combines compositional gradients with scalloped interfaces, which ensures stability.

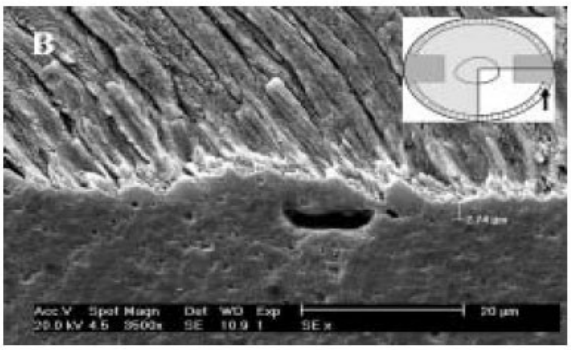


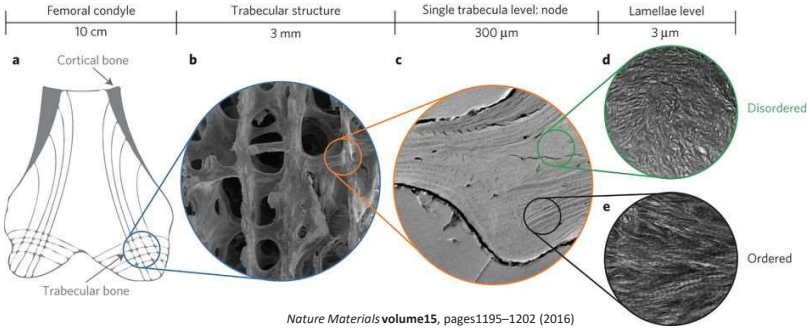
Figure 6b. Adaptation at enamel/resin interface. Clearfil SE Bond (3500X). The arrow indicates the enamel area that was observed on the section.

doi.org/10.1590/S0103-64402006000300001

DOI: 10.1038/NMAT4089

Bioinspired structural materials

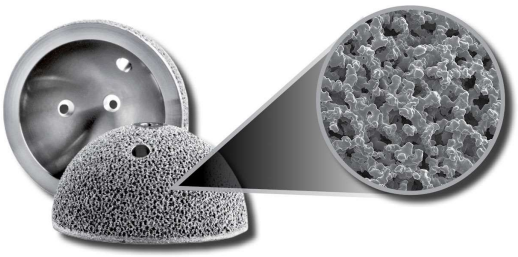
- At the microscopic level, natural composites are usually complex and **anisotropic**.
  - They can have layered, columnar or fibrous motifs.
  - Quite often, the same structure can exhibit distinct layers with different motifs.
  - These motifs are usually orchestrated in sophisticated patterns, such as columns of circular layers in bone.



Nature Materials **volume15**, pages1195–1202 (2016)

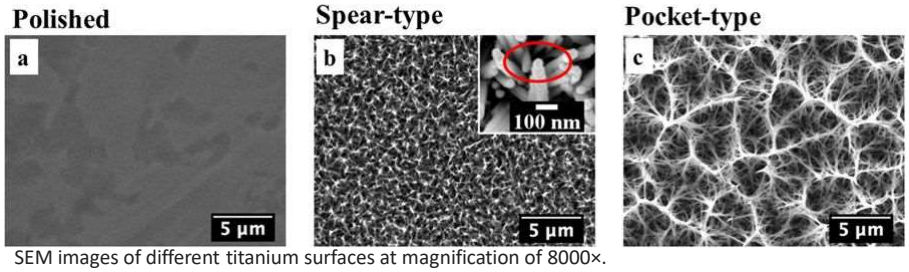
DOI: 10.1038/NMAT4089

Bulk vs Surface



Metal on Polyethylene | Metal on Metal | Ceramic on Ceramic

Bulk vs Surface – titanium surfaces – hip replacement

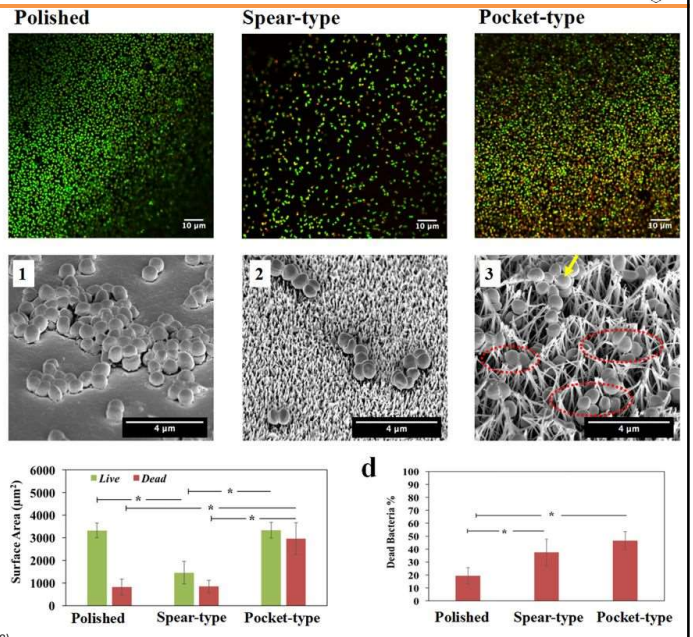


SEM images of different titanium surfaces at magnification of 8000×.
(a) Polished titanium surface.
(b) Spear-type surface showing a dense nanostructure comprising short and thin spears. The inset shows a SEM image of a nano-spear with a cone-shaped cap taken at 100,000×magnification.
(c) Pocket-type surface formed by intertwining of the longer and wider nano-spears.

Scientific Reports **8**, Article number: 1071 (2018)

Bulk vs Surface – titanium surfaces – hip replacement

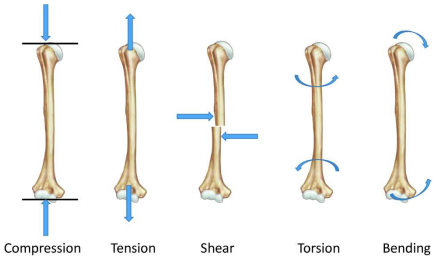
Adherence of *S. epidermidis* on different titanium surfaces after 2 hours' incubation. (**a**) Representative CLSM images with LIVE/DEAD staining of *S. epidermidis* on polished, spear-type and pocket-type titanium surfaces (left to right). (**b**) Different contact mechanisms of *S. epidermidis* on different titanium surfaces as visualized by SEM (Images were taken at 40° tilt with magnification of 25000×). (b1) *S. epidermidis* cells rested on the polished titanium surfaces; (b2) for spear-type surfaces, *S. epidermidis* cells settled on the top of spears resulting in point contacts; (b3) for pocket-type surfaces, *S. epidermidis*settled inside the pocket-like nanostructures (outlines of selected pockets are marked by dashed red lines). The longer, intertwined nano-spears were also observed to provide colonization sites for bacteria (arrow). (**c**) Surface area covered by both live and dead bacteria in the field of view for each titanium surface. (**d**) Bactericidal efficiency of the different titanium surfaces, as indicated by the proportions of dead cells among the adherent bacteria. *Statistically significant difference ($p < 0.05$). Three independent experiments were performed for each substrate type.



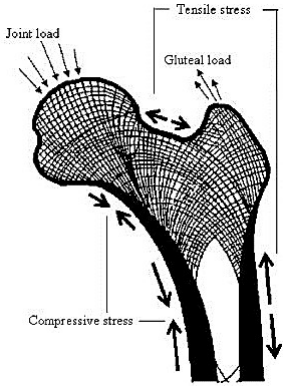
Scientific Reports **8**, Article number: 1071 (2018)

Bulk: Stres (napětí) and strain (deformace)

- **Stress** (**napětí**) is defined as the force experienced by the object which causes a change in the object while a **strain** (**deformace**, přetvoření, veličina – *nezaměňovat s „deformation" – jakožto jevem, procesem*) is defined as the change in the shape of an object when **stress** is applied. **Stress** is measurable and has a unit (n/m^2) while a **strain** is a dimensionless quantity and has no unit.
  - Compression (tlak) stress
  - Tensile (tah) stress
  - Shear (smyk) stress
  - Torsion (kroucení) stress



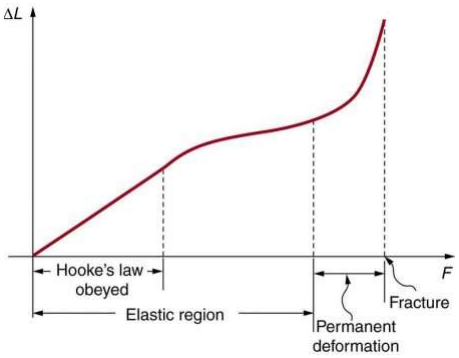
doi.org/10.1007/978-981-32-9971-9_28



DO - 10.1088/1742-6596/908/1/012019

Bulk: Stress / strain curve

- **HOOKE'S LAW** $F = k\Delta L$, where ΔL is the amount of deformation (the change in length, for example) produced by the force F, and k is a proportionality constant that depends on the shape and composition of the object and the direction of the force $\Delta L = \frac{F}{k}$



A graph of deformation ΔL versus applied force F.
The straight segment is the linear region where Hooke's law is obeyed.
The slope of the straight region is 1/k.
For larger forces, the graph is curved but the deformation is still elastic—ΔL will return to zero if the force is removed.
Still greater forces permanently deform the object until it finally fractures.

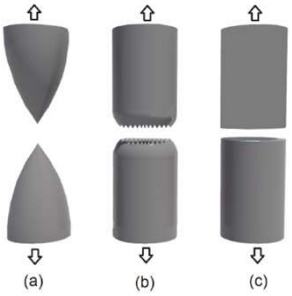
https://courses.lumenlearning.com/physics/chapter/5-3-elasticity-stress-and-strain/

Bulk: Toughness (houževnatost)

- Measures the energy required to crack a material.
- **Materials can be:**
  - **Ductile (tažný) — deforms before it breaks**
  - **Brittle (křehký) — breaks before it deforms**
    - Brittle materials break often without warning. They have little elasticity and do not stretch easily.

Brittle

Brittle vs. Ductile fracture (**a**) Very ductile, soft metals (e.g. Pb, Au) at room temperature, other metals, polymers, glasses at high temperature. (**b**) Moderately ductile fracture, typical metals (**c**) Brittle fracture, cold metals and ceramics.



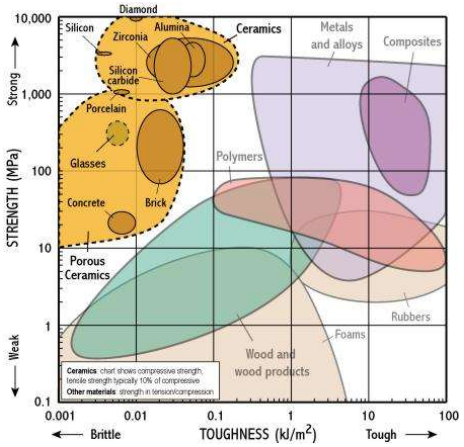
DOI - 10.5772/18127

Bulk: Strength (pevnost)

- **Strength** of a **material** is its ability to withstand an applied stress without failure or plastic deformation
  - Strong
  - Weak

Bulk: Strength (pevnost) vsToughness (houževnatost)

- **Strength** measures the resistance of a material to failure, given by the applied stress (or load per unit area)
- **Toughness** measures the energy required to crack a material; it is important for things which suffer impact
- **Increasing strength usually leads to decreased toughness**



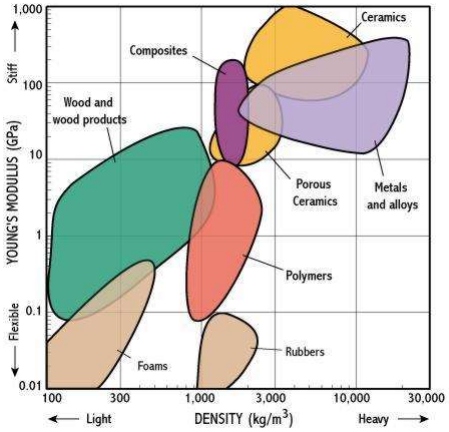
http://www-materials.eng.cam.ac.uk/

Bulk: Stiffness (tuhost) vs flexibility (pružnost)

- **Stiffness** is the extent to which an object resists deformation in response to an applied force.
- The complementary concept is **flexibility**: the more **flexible** an object is, the less **stiff** it is.

Bulk: Young's Modulus - Density

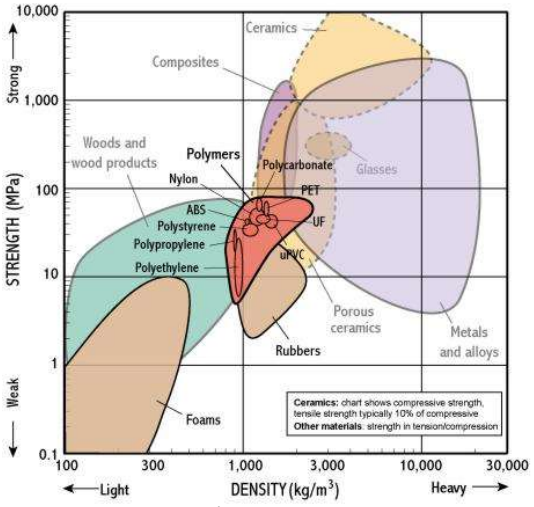
- 'Stiffness' measures how much something stretches when a load is applied. Young's modulus measures stiffness and is a material constant, i.e. it is the same whatever the size of the test-piece.
- Many applications require **stiff** materials, e.g. roof beams, bicycle frames - these materials lie at the **top** of the chart
- Many applications require **low density** materials, e.g. packaging foams - these materials lie to the **left** of the chart.
- **Stiff lightweight materials are hard to find** - composites appear to offer a good compromise, but they are usually quite expensive.



http://www-materials.eng.cam.ac.uk/

Bulk: Strength - Density

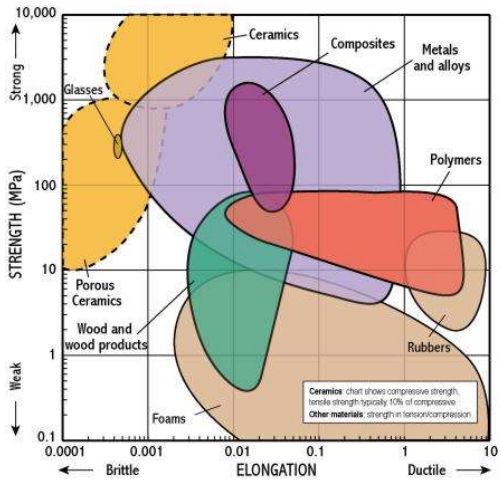
- Strength measures the resistance of a material to failure, given by the applied stress (or load per unit area)



http://www-materials.eng.cam.ac.uk/

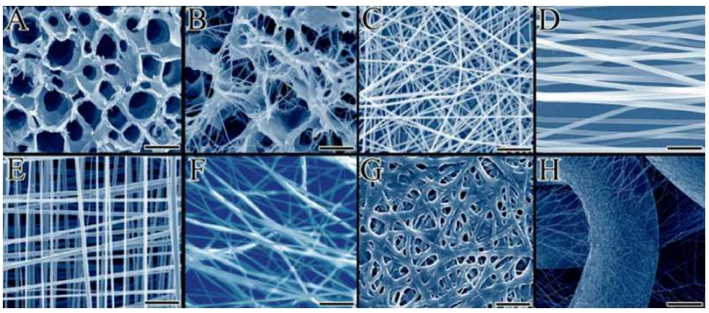
Bulk: Strength - Elongation

- Strength measures the resistance of a material to failure, given by the applied stress (or load per unit area)
- Elongation measures the percentage change in length before fracture
- Elongation to failure is a measure of **ductility**



http://www-materials.eng.cam.ac.uk/

Bulk: Architecture



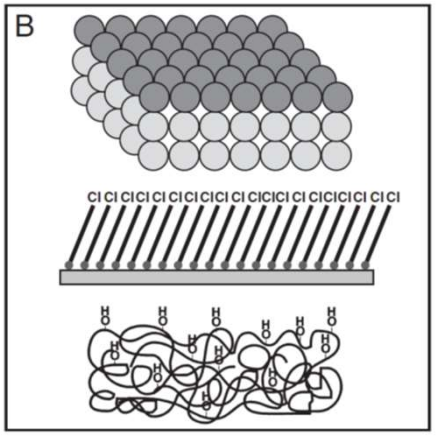
https://www.omicsonline.org/articles-images/2161-0673-3-126-g002.html

Figure 2: Ultramicrographs by SEM of different tissue engineered based scaffolds. (A and B) are porous scaffolds. These types of scaffolds are porous in nature and their porosity is high. The pore size is usually between 10 to 100 µm but it may be larger. No fiber structure is visible in these scaffolds and the molecules are not arranged as fibers. The density of the pores is more than 50% in these types of scaffolds.
(C to F) are fibrous or fiber based scaffolds. (C) is an amorphous scaffold which has some porosity between its fibers while (D) is a highly aligned scaffold which has lower porosity than (C).
(E) is a unique highly aligned scaffold but the fibers have been polymerized and aligned in two different directions so that some of the fibers are perpendicular to others. This unique configuration of the fibers has produced large pores between the fibers.
(F) is a moderately aligned scaffold in which the collagen fibers are mostly aligned but an acceptable porosity exists between the fibers.
(G) is a fibrous scaffold which have been cross-linked so that the fibers have been fused together but some porosity is still preserved in the scaffold. (H) is a hybrid scaffold in which both the micro and nano structured fibers are present in the scaffold. Scale bar for A: 1 µm, B: 500 nm, C to F: 1 µm, G: 2 µm, H: 2.4 µm

Surfaces: Chemistry

Surfaces can be composed of different chemistries:

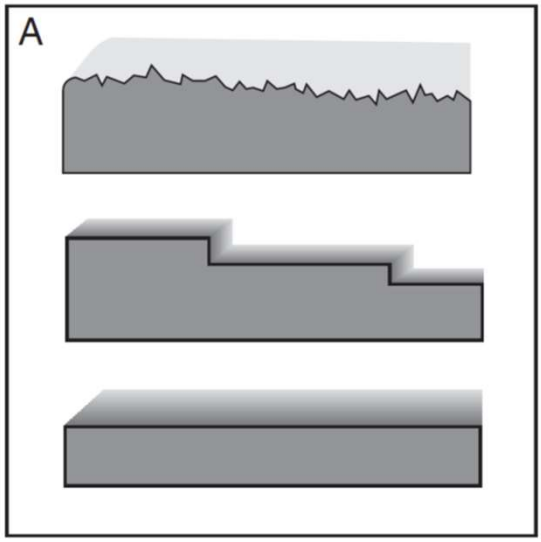
- Atomic.
- Supramolecular (entities of greater complexity than individual molecules — assemblies of molecules that bond and organize through intermolecular interactions).
- Macromolecular (very large molecule, as a colloidal particle, protein, or especially a polymer, composed of hundreds or thousands of atoms).



Biomaterials science : an introduction to materials in medicine. ISBN 0-12-582463-7

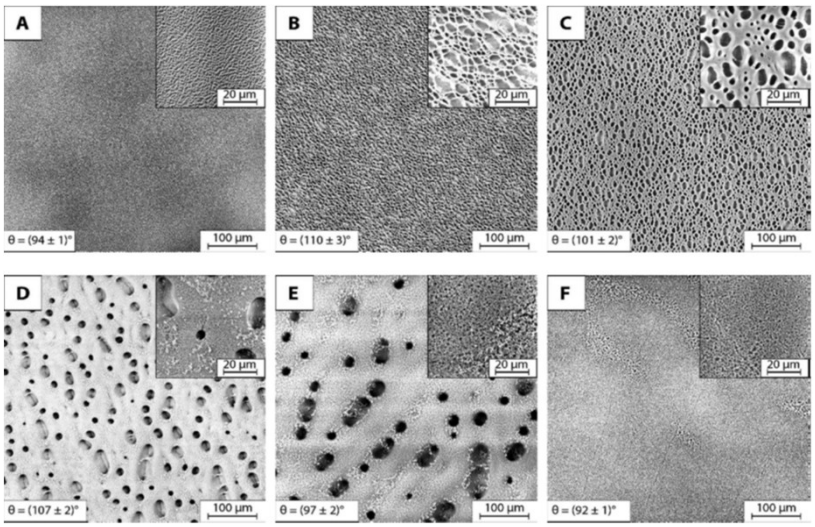
Surfaces: Topography

- Rough.
- Stepped.
- Smooth.



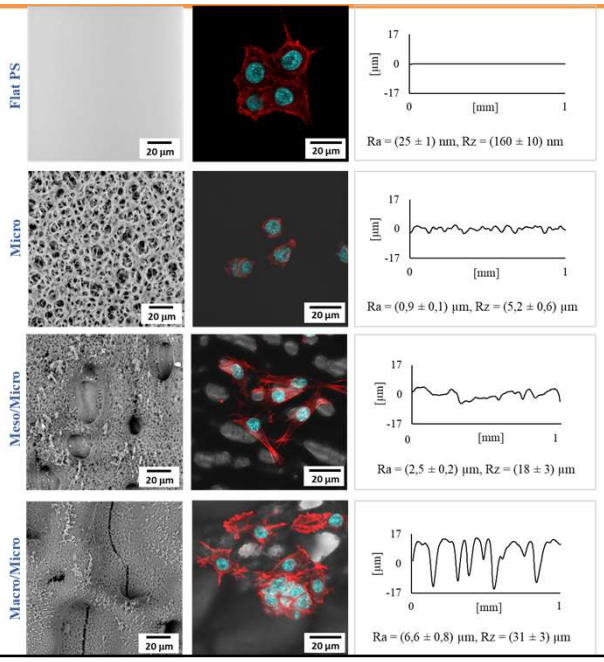
Biomaterials science : an introduction to materials in medicine. ISBN 0-12-582463-7

Surfaces: Topography



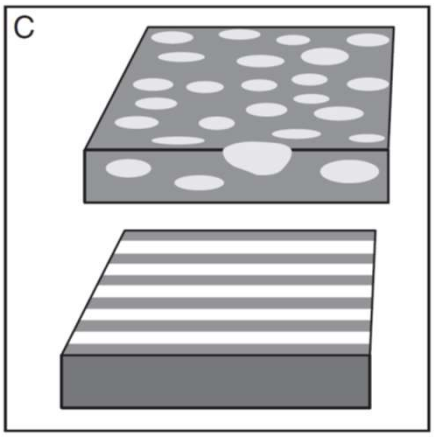
Wrzecionko et al. 2017. DOI: 10.1021/acsami.6b15774

Surfaces: Topography



Surfaces: Homogeneity

- Structurally or compositionally inhomogeneous in the plane of the surface such as phase-separated domains or microcontact printed lanes.



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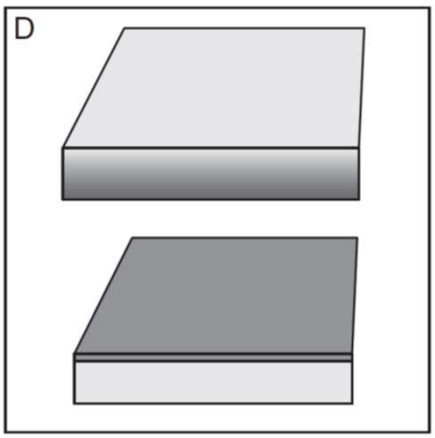
Surfaces: Homogeneity

- Inhomogeneous with depth into the specimen or simply overlayered with a thin film.



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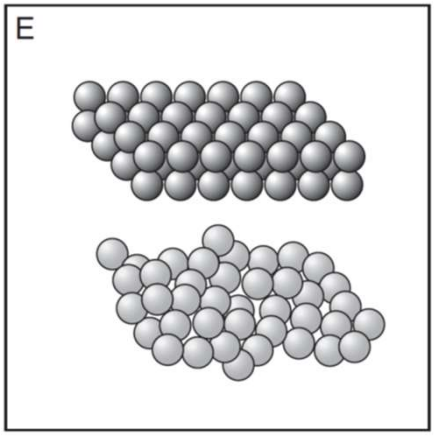
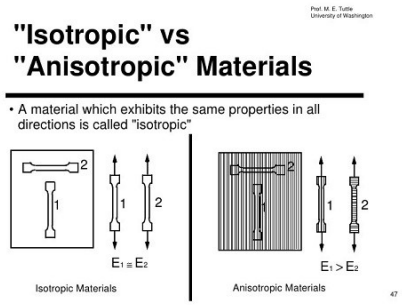
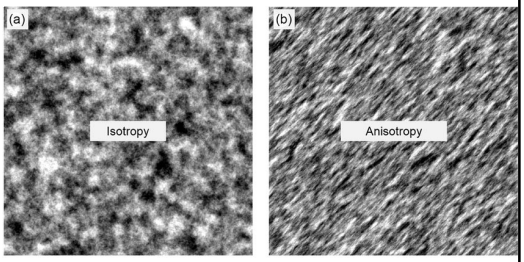
- Highly crystalline or disordered



Biomaterials science : an introduction to materials in medicine. ISBN 0-12-582463-7

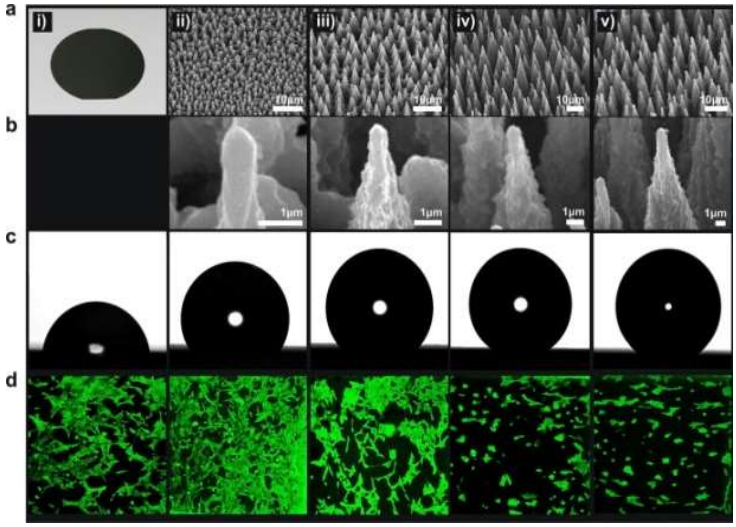
Surfaces: Isotropy





DOI: 10.5194/esurfd-3-1399-2015

Hydrofilicity

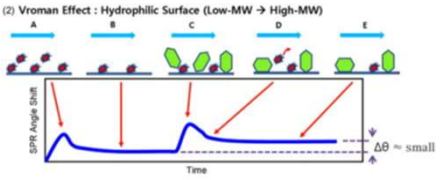


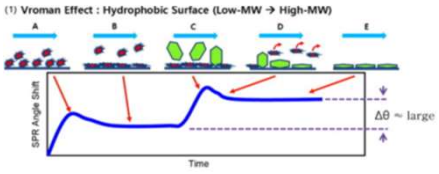
Acta Biomaterialia (2010) doi:10.1016/j.actbio.2010.01.016

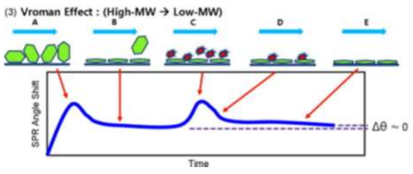
Protein–surface interactions

- Cellular responses to materials in a biological medium reflect greatly the adsorbed biomolecular layer.

Diffusion and binding kinetics underlie the ''**Vroman effect**", which describes the exchange of proteins adsorbed on surfaces. Earlier adsorbed proteins (mostly low MW) by other proteins with stronger binding affinities







doi.org/10.1016/j.bios.2008.07.036

Protein–surface interactions

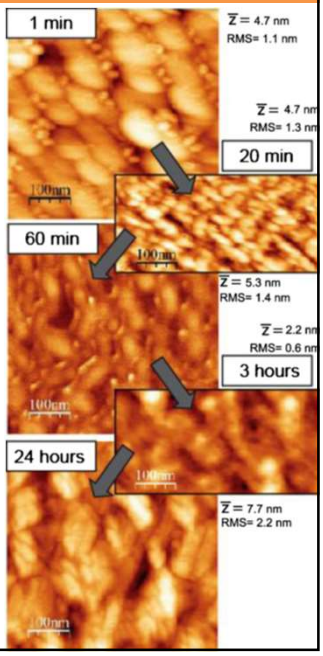
Topographic atomic force microscopy images that illustrate the structural evolution of an adsorbed **cellulase layer on polystyrene** as a function of incubation time (shown in the figure labels).

Large aggregates and unaggregated proteins are observed at 1 min (top-left).

The aggregates reduce in size at 20 min (top-right) and, at 60 min (middle), they form a taller and less dense network structure.

At 3 h (bottom-right), protein aggregates are discernible in a denser layer of reduced height.

At 24 h (bottom-left), the adsorbed proteins have formed taller rod-like structures. The average height and root mean square (RMS) roughness are given for each image.

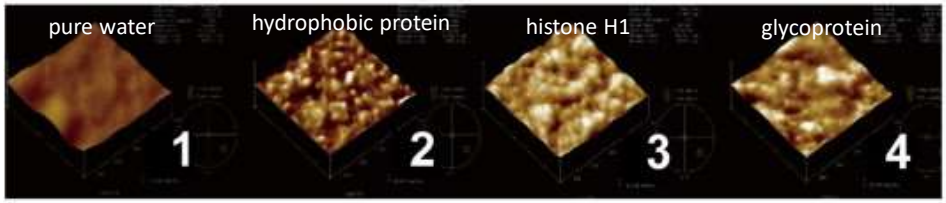


http://dx.doi.org/10.1016/j.colsurfb.2012.10.039

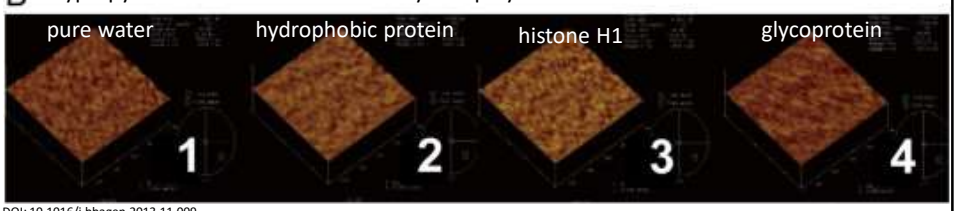
Protein–surface interactions

- AFM views of non-specific protein adsorption to a base plastic material (naked polypropylene slides) (A) and the surface after coating with PC-methacrylic copolymer (B).
- Silds were exposed to pure water (1; negative control), or 10nM aqueous solutions of TGF-β (2; a hydrophobic protein), histone H1 (3; a basic protein), and erythropoietin (4; a glycoprotein hormone) as typical model proteins. After incubating with the solution for 1h at 37°C, the slides were washed three times with 0.05% (w/v) Tween 20 in deionized water and then air-dried.

A Polypropylene - hydrophobic



B Polypropylene coated with PC-methacrylic copolymer



DOI: 10.1016/j.bbagen.2013.11.009

Protein–surface interactions

- Protein–surface interactions depend on the properties of both the protein and the surface.
- **Protein properties:**
  - Size, net charge, stability and unfolding rate regulate the affinity of proteins to surfaces.
    - Smaller proteins adsorb faster to surfaces because of faster diffusion.
    - Larger proteins present a higher surface for interactions with material surfaces, but diffuse slower and take a longer time for surface adsorption.
      - Consider in context of mixtures of proteins, such as serum .
    - Proteins with stronger and more stable interactions replace proteins with low surface affinity.
  - The overall combination of protein properties is what dictates the stability of the protein's adhesion to and stability of interaction with the surface

doi.org/10.1016/j.ymeth.2015.08.005

Protein–surface interactions

- **Surface properties**
  - Topography, chemical composition, hydrophobicity, heterogeneity and surface potential affect protein adsorption and adhesion.
    - Proteins expose hydrophilic amino acids, which are readily available to interact with material surfaces because physiological conditions involve aqueous environments.
    - However, the interactions of hydrophobic surfaces with the hydrophobic domains of proteins lead to the strongest protein adsorption states.
    - Protein affinity to charged surfaces is stronger when the pH of the environment is near the protein's isoelectric point due to decreased protein–protein interactions in solution, as proteins assume a neutral net charge.
    - The distribution of charged residues on proteins and protein–protein interactions in solution therefore affect these adsorption events and their stability easily varies with the pH and ionic state of the culture media.
    - The rate of protein unfolding accelerates protein–material interactions.
    - Yet unfolding can also lead to protein denaturing and loss of protein stability.

doi.org/10.1016/j.ymeth.2015.08.005

Protein–surface interactions

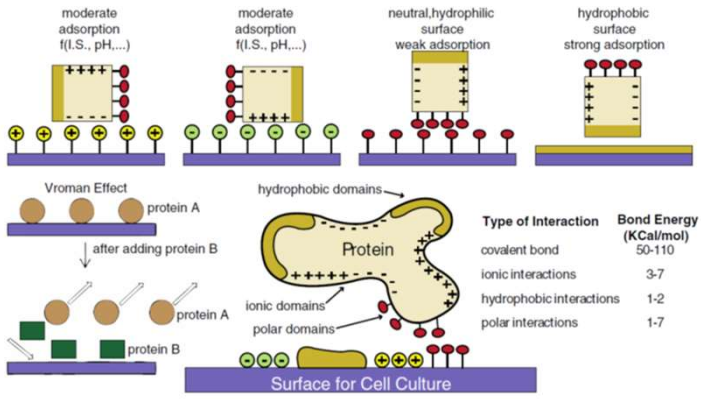
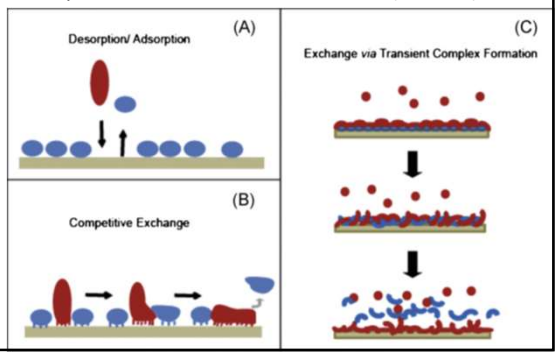


Fig. 5. General mechanisms that govern protein–surface interactions based on surface chemistry. Proteins interact with material surfaces and these interactions are regulated by both protein and surface properties. Interactions between ionic domains result in moderate protein adsorption, which is unstable because it relies on the ionic state (I.S) and the pH of the culture media. Interactions between hydrophilic polar domains are weak and also unstable. Interactions between hydrophobic domains lead to strong adsorption. Based on the mechanisms that regulate protein interaction (the Vroman effect), a protein B with higher affinity to a surface can replace a protein A of lower surface affinity previously adsorbed to the surface. Ideally, to maximize surface–protein interactions, surfaces should have different engineered regions with different chemical properties to match the properties of the protein of interest. Covalent interactions are the strongest among all types of interactions between proteins and surfaces. Figure adapted with permission from [86,87].

doi.org/10.1016/j.ymeth.2015.08.005 & Philos. Trans. A: Math. Phys. Eng. Sci. 370 (2012) 2321–2347 & J. Mater. Chem. 17 (2007) 943–951.

Protein–surface interactions

- Processes for the change in composition of a layer adsorbed from a mixture solution by exchange of earlier adsorbed proteins with other proteins.
- (A) Initially adsorbed protein 1 (blue) desorbs, leaving a vacancy for protein 2 (red) to adsorb.
- (B) Initially adsorbed protein 1 is displaced by protein 2 which has a stronger binding affinity to the surface.
- (C) Protein 2 embeds itself in previously adsorbed protein 1 to form a transient complex (top); the complex then turns, exposing protein 1 to solution (middle); protein 1 desorbs into the solution and protein 2 remains on the surface (bottom).



http://dx.doi.org/10.1016/j.colsurfb.2012.10.039

Protein–surface interactions

- For example:
  - Hydrophobic surfaces (e.g., Teflon) with low surface energy denature proteins.
  - Very hydrophilic surfaces (e.g. polyacrylamide hydrogels) sterically hinders protein adsorption.
  - Hydrophobic surfaces with low surface energy (e.g. PDMS - Polydimethylsiloxane) promotes weak interactions with proteins.
- Protocols to attach proteins to these materials first modify the undesirable material surface properties and then functionalize the material surface to promote stable and strong protein attachment using polar interactions, hydrophobic interactions, ionic bonds, and/or covalent bonds
  - Of these, covalent bonds are the strongest and least dependent on the electrochemistry of the extracellular environment

doi.org/10.1016/j.ymeth.2015.08.005

Proteins

- The protein interface is a mediator of cell/material interactions.
- ECM proteins, adsorbed onto the material surfaces after implantation *in vivo* and from the culture media *in vitro*, are recognised by cells through integrins.
- Initial interaction leads to:
  - integrin clustering,
  - internal recruitment of cytoplasm proteins,
  - forming focal adhesions and mediating cell adhesion and contractility.
- Physicochemical properties (chemistry, topography and mechanics) play an important role on the adsorption of proteins onto the material surface and influence:
  - protein surface density,
  - Protein conformation,
  - Protein distribution.

doi.org/10.1016/j.actbio.2018.07.016

Proteins

- Above mentioned directing cell response:
  - early events of cell attachment and spreading,
  - controlling later events such as proliferation, matrix reorganization and differentiation.
- The mechanical organization and reorganization of ECM proteins is important physiological event that happens after the initial cell/protein interaction.
  - In vivo cells secrete and reorganize proteins into fibrils to form their own ECM, which provides them with mechanical support and local growth factors delivery.

doi.org/10.1016/j.actbio.2018.07.016

ECM

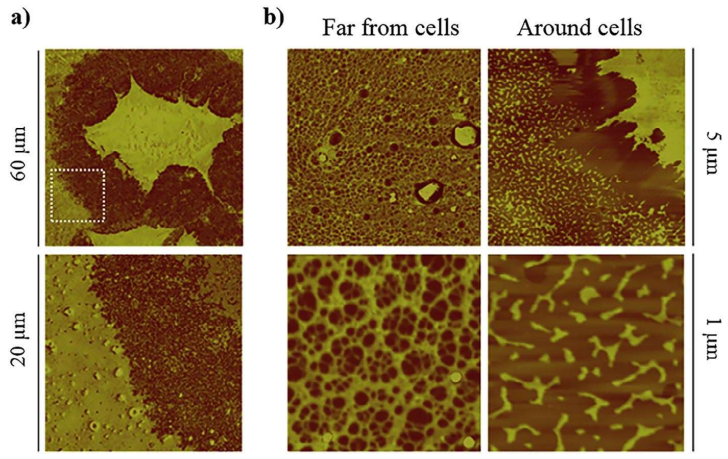
- Cell interactions with the ECM are highly dynamic *in vivo*:
  - Cells receive information from specific cues in the ECM,
  - Respond to these inputs by **remodeling** the surrounding matrix and/or secreting new components.
- Proteolytic degradation for the removal of excess ECM:
  - Mostly active during development, **wound healing and regeneration of tissues**.
  - When misregulated, can contribute to diseases such as fibrosis, arthritis and cancer invasion.

Fibronectin

- One of ECM protein is fibronectin (FN) that plays a key role in cell adhesion and proliferation, controls the availability of growth factors, and so contributes to cell differentiation.
  - FN is synthesized by various anchorage-dependent cells, which then assemble it into a fibrillar network through an integrin dependent mechanism.
  - FN assembly is the initial step which orchestrates the assembly of further ECM proteins such as collagen.

doi.org/10.1016/j.actbio.2018.07.016

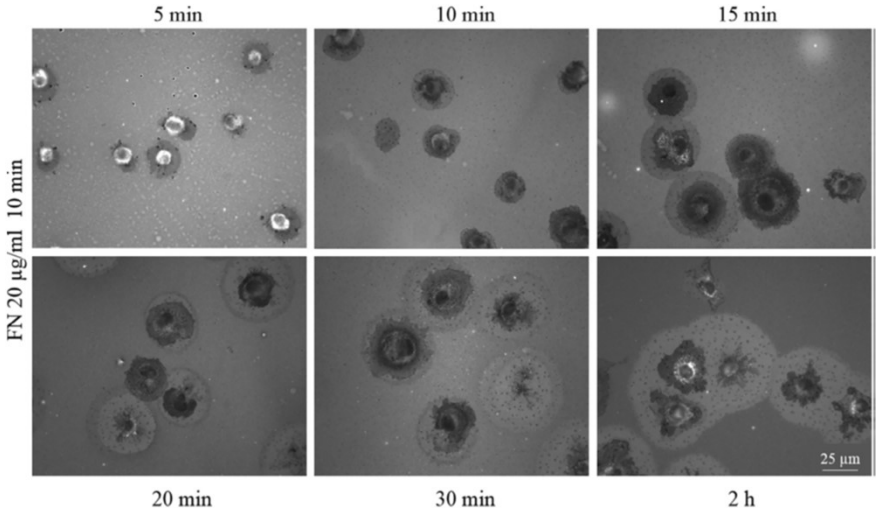
Fibronectin – degradation



Cell-mediated FN matrix degradation on PEA surfaces coated with FN at 20 mg/ml for 1 h. a) Cell-mediated FN degradation at the nanoscale. AFM phase images show different protein patterns in dependence of the proximity (far vs around) to cells. FN distributions in areas around cells and far from cells are showed at higher magnification in b).

doi.org/10.1016/j.actbio.2018.07.016

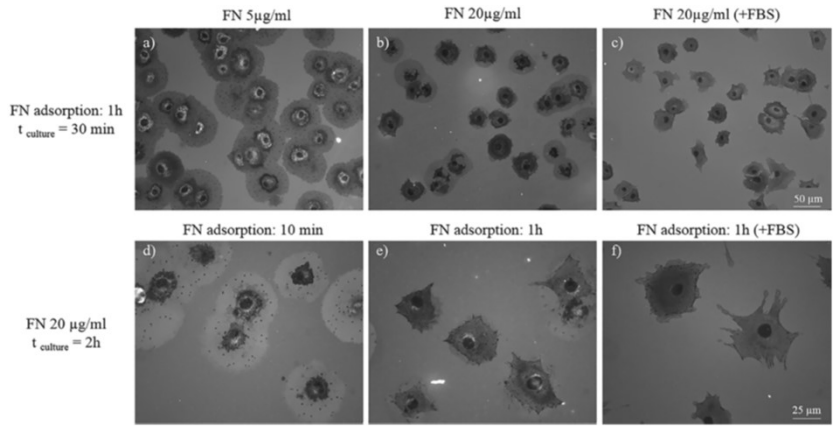
Fibronectin – degradation



Protein matrix degradation during increasing culture time on FN-coated PEA substrates. Cells start degrading the FN layer from short culture times. Scale bar: 25 mm.

doi.org/10.1016/j.actbio.2018.07.016

Fibronectin – degradation

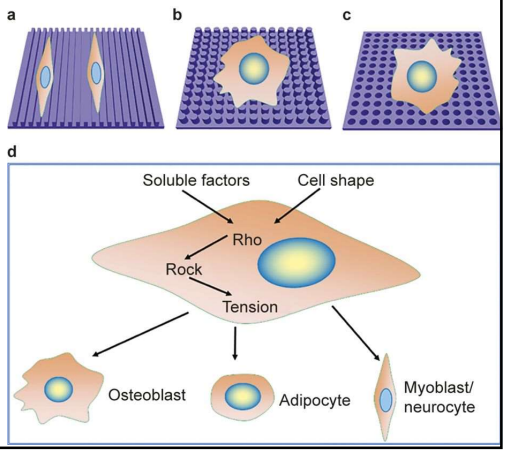


Cell-mediated FN reorganization on FN-coated PEA after 30 min and 2 h incubation. FN degradation is observed regardless of the culture time (a-b, d-e), FN concentration (a-b) and adsorption time (d-e). However, less evidences of degradation are observed with higher FN concentration, which results in better spread cells. In the presence of serum (c,f) no signs of FN degradation are observed and cells present a spread morphology. Top scale bar: 50 mm, bottom scale bar: 25 mm.

doi.org/10.1016/j.actbio.2018.07.016

Differentiation and nanotopography geometries

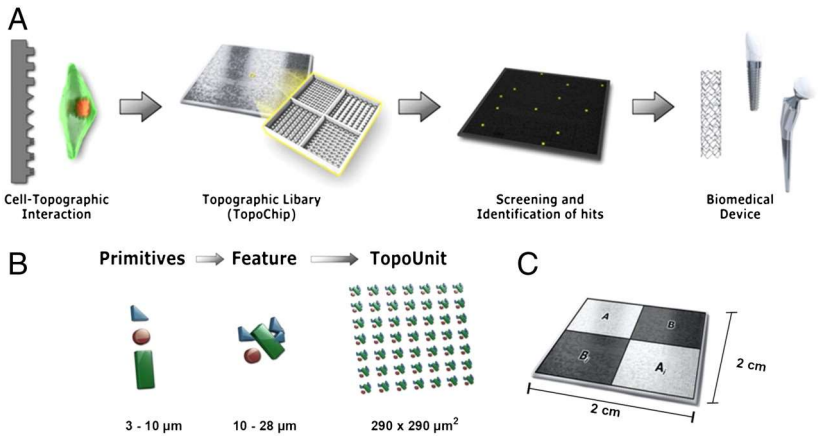
- Three basic nanotopography geometries include nanogrooves (**a**), nanopost array (**b**), and nanopit array (**c**).
- The speculative pathways (**d**) for cell-shape-directed osteogenic and adipogenic differentiations of MSCs were examined in growth medium. RhoA, Ras homolog gene family member A; ROCK, Rho-associated protein kinase.



https://doi.org/10.1038/boneres.2015.29

TopoChip design.

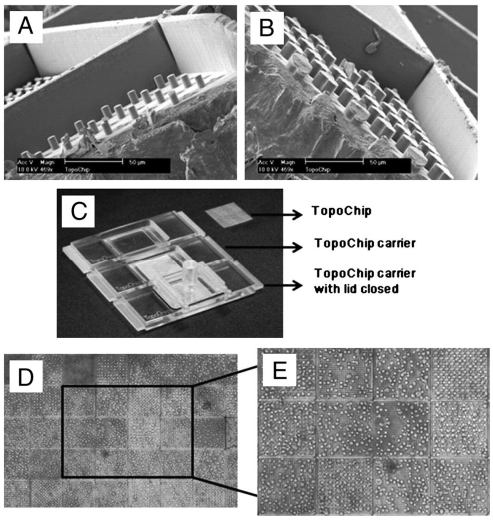
Aplikace High-throughput technologie pro „screening“ biologických vlastností biomateriálů.



Hemant V. Unadkat et al. PNAS 2011;108:16565-16570

PNAS

TopoChip fabrication and characterization.



Hemant V. Unadkat et al. PNAS 2011;108:16565-16570

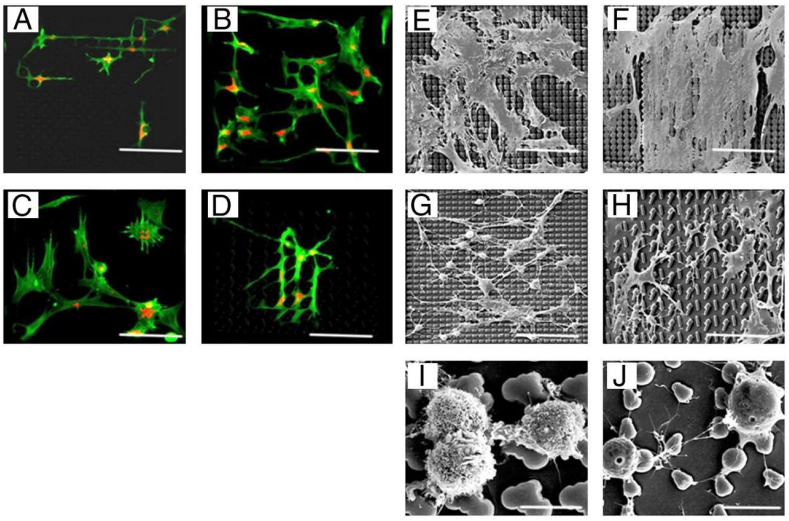
PNAS

Topografie - vliv na morfologii

- TopoChip tvořený jedním materiálem poly(DL-lactic acid).
- 2 176 různých povrchových topografií.
- Primary human mesenchymal stromal cells (hMSCs) byly vysazeny na povrchy.
- Po 8 hodinách fixace a barvení aktinu.

doi/10.1073/pnas.1109861108

Morphology of hMSCs on different TopoUnits.



Hemant V. Unadkat et al. PNAS 2011;108:16565-16570

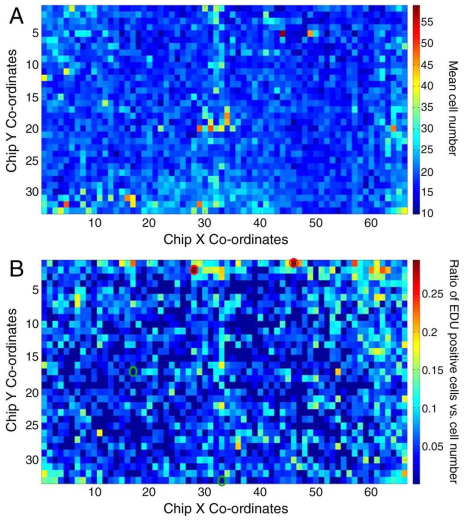
PNAS

Topografie – vliv na proliferaci

- Synchronizace buněčného cyklu – deprivace séra.
- Přidání séra + analogu nukleotidu (EdU).
- 8 hodin kultivace.
- Barvení DNA.

doi/10.1073/pnas.1109861108

Cell proliferation assay.

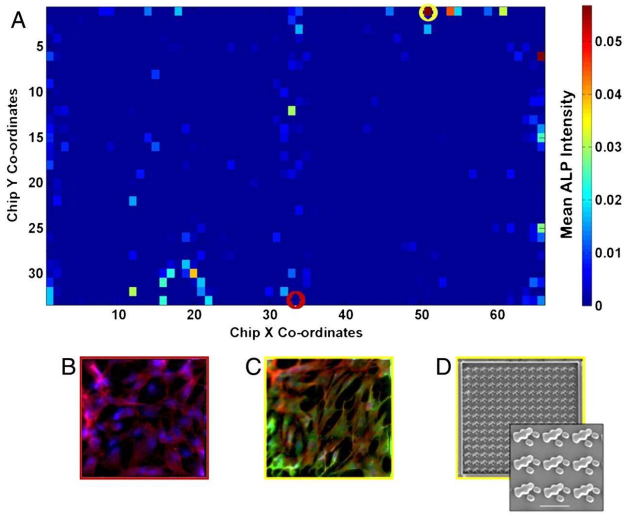


Hemant V. Unadkat et al. PNAS 2011;108:16565-16570

PNAS

Topografie - diferenciace

- Kultivace bez přítomnosti růstových faktorů.
- Alkaline phosphatase – marker rané osteogenní diferenciace.



doi/10.1073/pnas.1109861108

TopoChipy s dalšími materiály

Fig. S2. Conventional PLA TopoChip (left) as well as TopoChips coated with calcium phosphate (middle) and titanium (right).

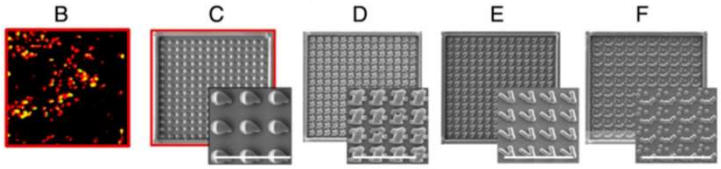
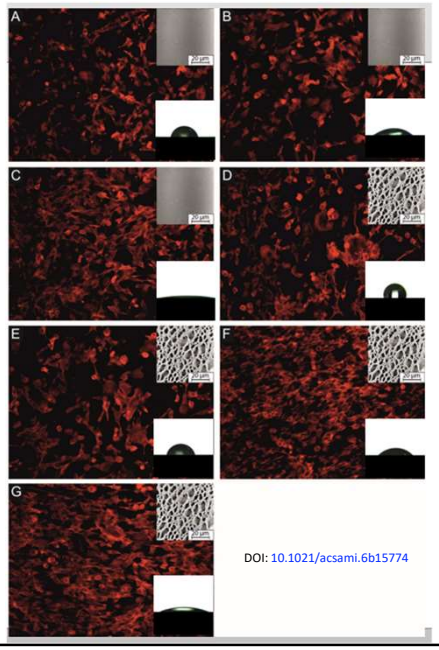


Fig. S4. Cell proliferation assay. (*A*) Heat map of mean proliferating cell count of cells grown on different topographies. The TopoUnit showing the maximum number of proliferating cells is marked with a red circle. (*B*) Fluorescently stained pseudocolored merged image of hMSCs on the highest scoring TopoUnit. In this image the Alexa Fluor 488 EdU (representing proliferating cells) is pseudocolored yellow whereas TOTO-3 (nucleic acid stain representing all the nuclei) is pseudocolored red. (*C–F*) SEM images of the TopoUnits showing the highest score for proliferating cells. The insets show higher magnification views of features. (Scale bar: 50 μm.)

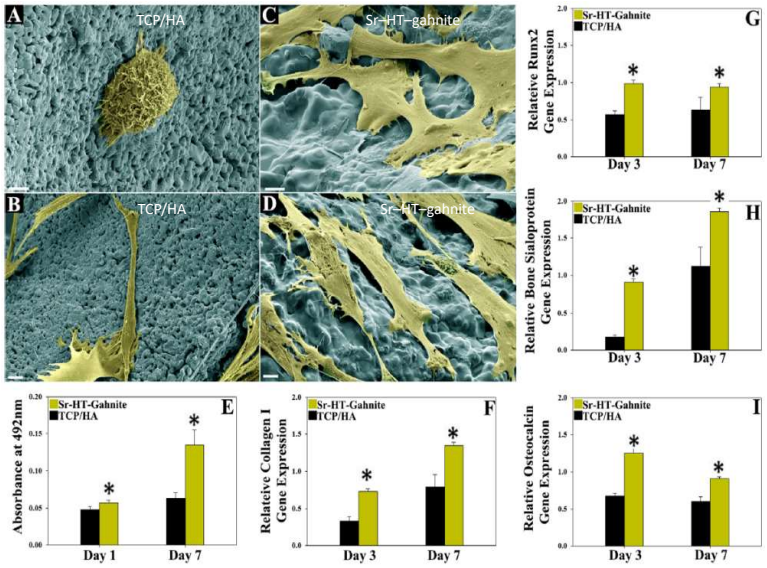
doi/10.1073/pnas.1109861108

Topography & hydrophilicty



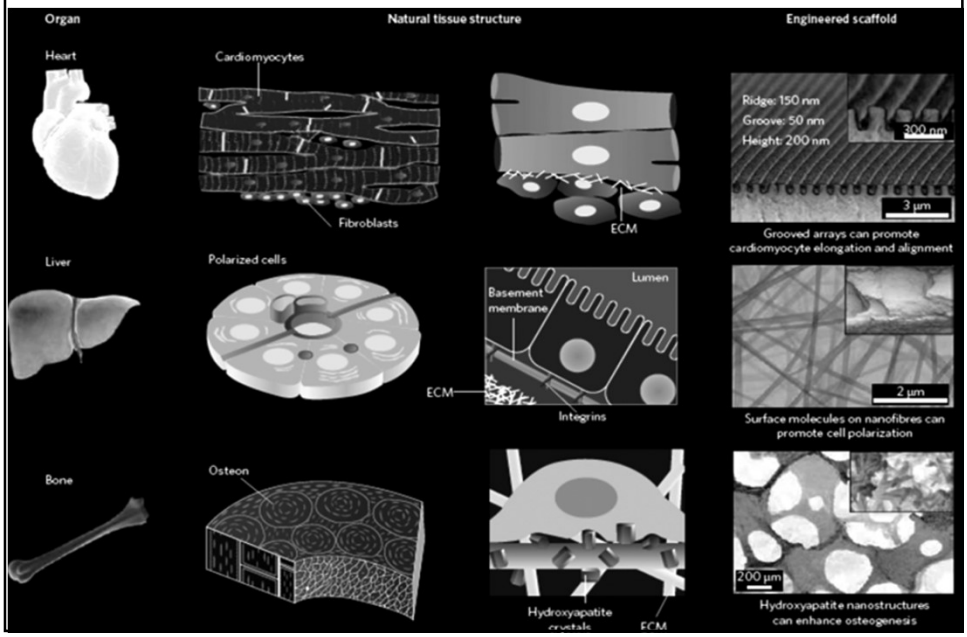
DOI: 10.1021/acsami.6b15774

Biointerfaces – TOPOGRAPHY + COMPOSITION

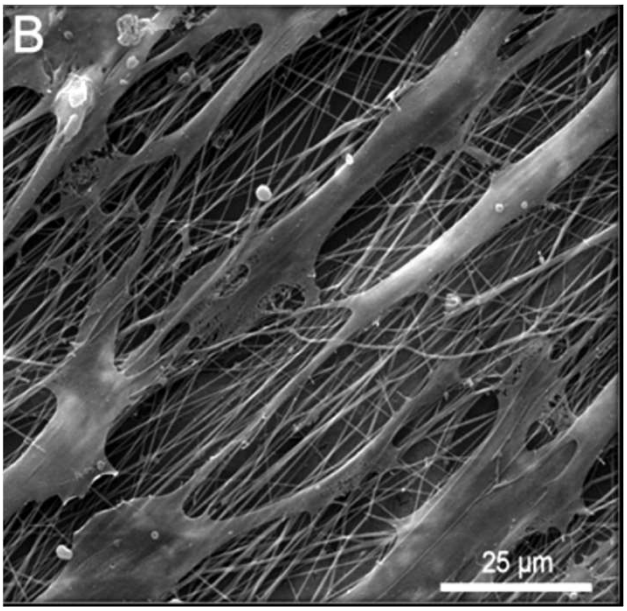


Acta Biomaterialia 9 (2013) 7014–7024. doi:10.1016/j.actbio.2013.02.039

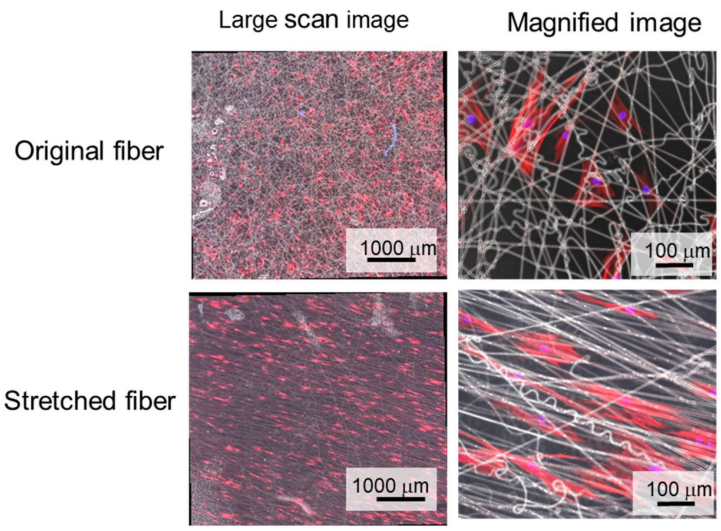
Isotropy - biomimetic



Isotropy – fiber alignment



Isotropy – fiber alignment



Adhesion morphology and alignment of human mesenchymal stem cells (hMSCs) on original (left) and stretched (right) PCL-9.2 nanofibers. hMSCs on each fiber type were stained with f-actin (red) and nucleus (blue). hMSCs were cultured at 37 °C for 24 h. Large scan (left) and magnified (right) images show global and local alignment of hMSCs.
https://doi.org/10.3390/fib7030020

Isotropy

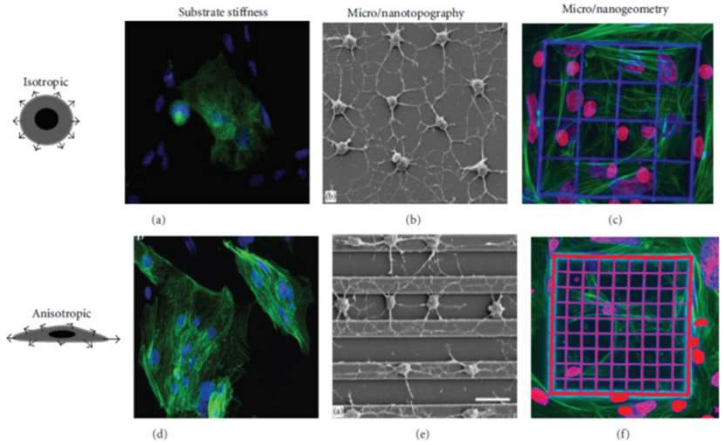


FIGURE 3: Examples of engineering strategies used to study the effects of anisotropic versus isotropic cytoskeletal tension on cultured stem cells. Substrates that mimic (a) soft versus (d) stiff ECM induce differential fates in human embryonic stem cell-derived cardiomyocytes, due to differential patterns of focal adhesions and actin-myosin stress fibres (in green, nuclei in blue). Reprinted and adapted from [8] with permission from John Wiley and Sons. In rat neural progenitor stem cells, (b) nonpatterned substrates induce formation of cell protrusions oriented randomly, while (e) micropatterned surfaces that mimic the native presentation and orientation of ECM proteins to cells induce alignment of elaborated processes in the direction of the grooves. Reprinted and adapted from [9] with permission from Elsevier. In rat mesenchymal stem cells, ultraprecise microscaffolds (c) with pore dimension of 20 microns allow for cell migration and isotropic attachment to the internal 3D lattice, whereas (f) for pores below 10 microns in size, cell migration into the scaffold pores is limited, inducing cell spreading on the top surface of the micro-scaffold (dividing nuclei in pink, cytoskeletal actin in green). Reprinted and adapted from [10] [illegible] Elsevier.

doi:10.1155/2012/797410

Isotropy

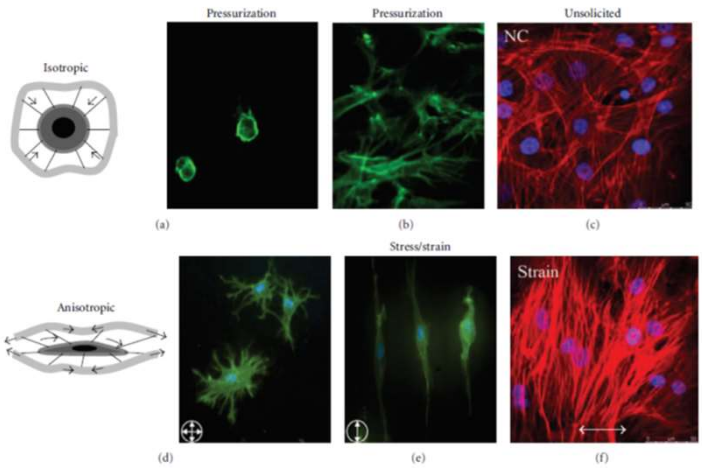
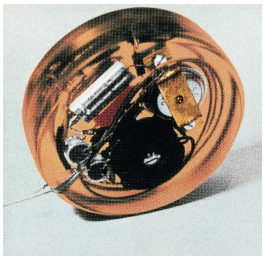


FIGURE 4: Examples of engineering strategies used to study the effects of anisotropic versus isotropic extracellular forces on cultured stem cells. Pig bone marrow-derived stem cells, subjected to cyclic hydrostatic pressure, (a) retain a spherical morphology when encapsulated in softer agarose, whereas (b) spread when encapsulated in stiffer fibrin hydrogels. Reprinted and adapted from [11] with permission from Elsevier. Rat bone marrow-derived stem cells (c) appear randomly distributed in unsolicited culture conditions, whereas (f) anisotropic strain induces cell elongation and upregulation of cardiomyocyte-related markers. Adapted with permission from [12]. In human bone marrow-derived mesenchymal stem cells and meniscal fibrochondrocytes cultured on a randomly organized nanofibrous scaffold, (d) biaxial strain induces a roundish shape of the cell nucleus, with small protrusion in all directions, while (e) uniaxial strain determines an elongated [illegible] nucleus. Reprinted and adapted from [13] with permission from Elsevier.

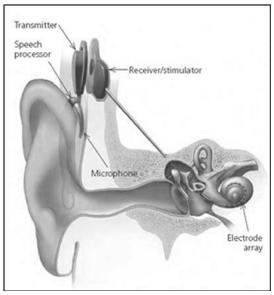
doi:10.1155/2012/797410

Elektricky vodivé polymery – motivace ke studiu

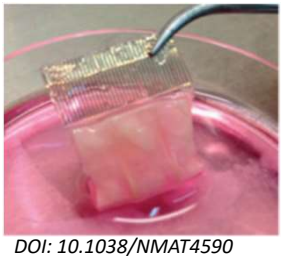
- Rune Elmqvist 1957 – první plně implantovatelný kardiostimulátor
- Cochleární implantát – nejčastěji užívané biologické rozhraní s elektrogenní tkání
- 2016 – „cardiac patch“ umožňující online stimulaci a snímání odezvy při hojení infarktu



www.medmuseum.siemens-healthineers.com/



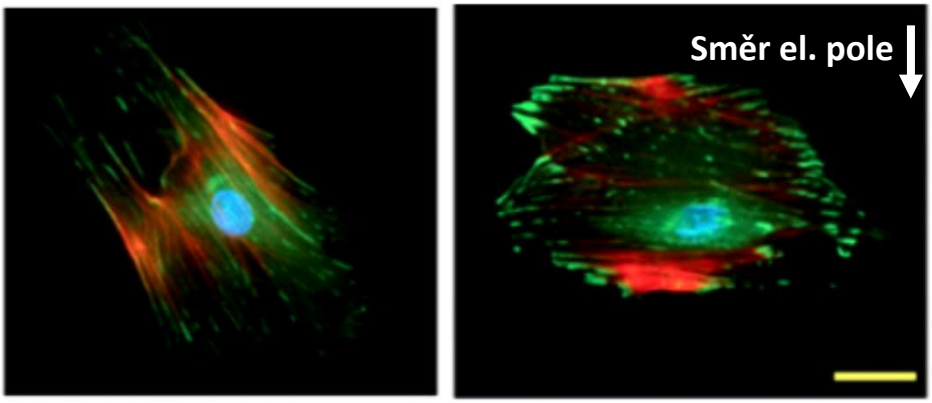
DOI: 10.1186/1471-2482-13-S2-S1



DOI: 10.1038/NMAT4590

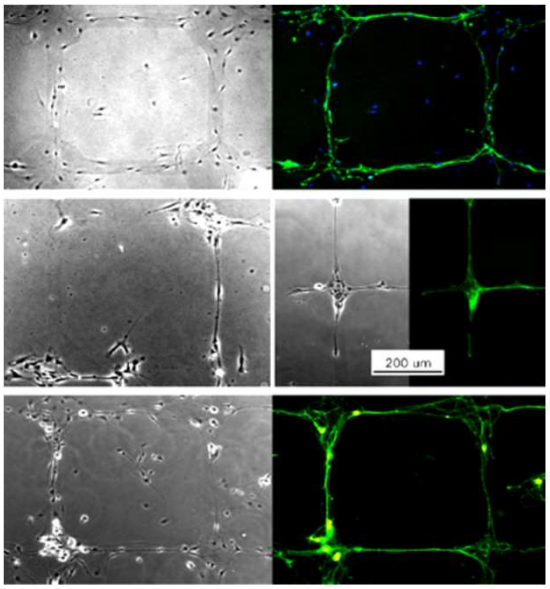
Elektricky vodivé polymery – motivace ke studiu

5h, 0 V/cm **5h, 5 V/cm**



Vliv elektrického pole na eukaryotické buňky – přestavba cytoskeletu DOI: 10.1016/j.proghi.2008.07.001

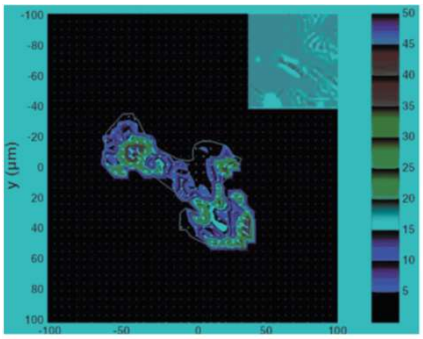
Elektricky vodivé polymery



Biomaterials (2006) 27(3):473-84.

Traction forces

- Cells generally apply traction forces to the networks or the surfaces to which they are bound
  - Cell traction forces is defined as a tangential tension exerted by cells to the ECM or the underlaying layer. It is generated by actomyosin interaction and actin polymerization and regulated by intracellular proteins.

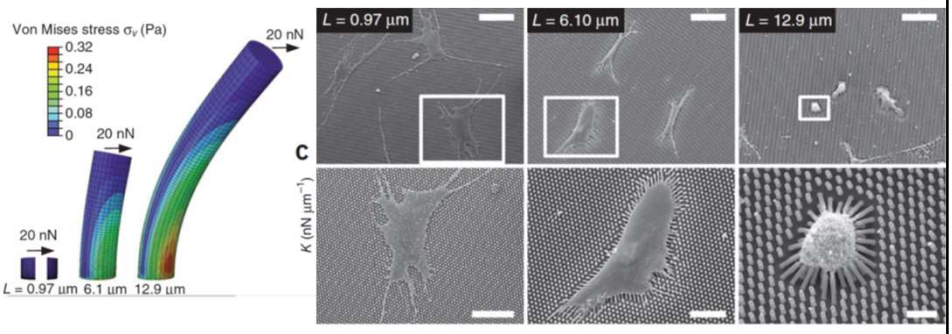


Traction microscopy image: Traction stress field exerted by a rat pulmonary microvascular endothelial cell upon its substrate. Inset: Phase contrast image at reduced magnification. Scale: Shear stress in Pascals.

doi:10.1007/s10439-009-9661-x
Am J Physiol Cell Physiol 2005;289(3):C521–C530.

Traction forces - substrate „nano-rigidity“

- Micropost rigidity impacts cell morphology, focal adhesions, cytoskeletal contractility and stem cell differentiation. Furthermore, early changes in cytoskeletal contractility predicted later stem cell fate decisions in single cells.



doi:10.1038/nmeth.1487

Funkční podjednotky tkání

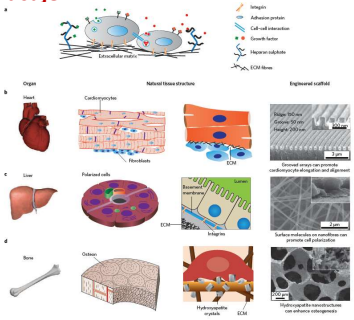
Jedná se o nejmenší jednotky, které zajišťují funkci tkáně či orgánu.

- Funkční jednotky jsou v řádech **~100 µm**.
- Každý orgán je tvořen 10-100 x 10^6 funkčními jednotkami.
- Každá funkční jednotka je tvořena rozdílnými typy buněk a extrucelulární matrix.
- **Rozdělení funkční jednotky na menší části (buňky a ECM) dochází ke ztrátě funkce tkáně či orgánu.**
- Obě části, tedy buňky i ECM jsou nenahraditelné.

Funční jednotka je tedy minimální hladina na které TE pracuje.

Mikro-environment tkáně je dán:

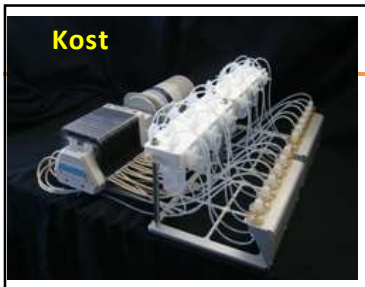
- Hustota buněčného osazení (Cellularity)
- Buněčná komunikace (Cellular Communications)
- Přítomnost živin (Local Chemical Environment)
- Geometrie tkáně (Local Geometry)

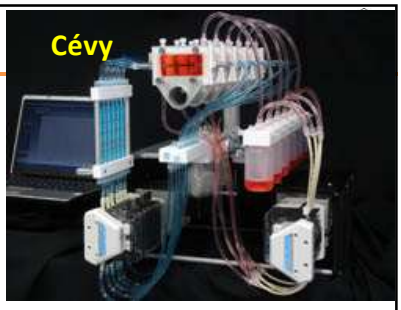


Nature nanotechnology | VOL 6 | JANUARY 2011. Doi: 10.1038/nnano.2010.246

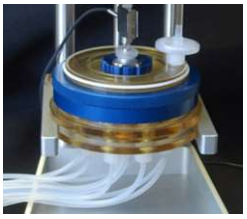
Tkáňové inženýrství (Tissue Engineering)

- Tkáňové inženýrství kombinuje principy a metody inženýrských a biologických věd k vývoji biologických náhrad určených k obnově, udržení či zlepšení funkce tkání (či orgánů).
- Nejběžnější způsob je založen na imobilizaci vhodných buněk v porézních, biodegradibilních a biokompatibilních scaffoldech poskytujících templát k vývoji tkáně a následné kultivaci.
- Klíčovými body jsou:
  - Selekce vhodných buněčných linií
  - Příprava vhodného scaffoldu vytvářejícímu prostor pro buněčné procesy.
  - **Vytvoření vhodných podmínek pro kultivaci buněk v 3D struktuře vedoucí k proliferaci a diferenciaci buněk v cílovou tkáň - bioreaktory.**
    - Jsou dobře zvládnuté techniky pro kultivaci 2D struktur:
      - Kožní náhrady po vředech či popáleninách.
    - Kultivace v 3D struktuře je však velmi odlišná od 2D např.:
      - Nutnost zapojení více buněčných typů.
      - Diferenciace.
      - Dynamičnost procesu – neustále se měnící biologické, fyzikální či mechanické prostředí v *in vivo* podmínkách.
      - Navíc se vše mění v čase – jedná se tedy spíše o **4D**.





BIOREAKTORY.



Chrupavka

Bioreaktory - Úvod

- Lidské buňky jsou velmi náchylné ke změně podmínek prostředí např. kumulace toxických produktů metabolismu, jež jsou v tradičních, **statických**, kultivačních technikách běžné.
- ***In vivo*** je **dynamický** systém vytvářející komplexní síť vzájemných interakcí buňka-buňka, buňka-prostředí.
- Bioreaktory slouží k co nejvěrnější simulaci *in vivo* podmínek.
  - Výběr parametrů jež jsou sledovány je dán cílem kultivace.
    - Množení buněk pro transplantaci – hematopoetické buňky, kmenové buňky, krevní buňky.
    - 3D struktury pro implantace.
    - Orgány.
- Bioreaktory zajišťují také možnost studia vlivu různých mechanických, biochemických a dalších stimulů v 3D struktuře tkáně za *in vitro* podmínek.

Bioreaktory - fermentory

- Bioreaktor je obecný pojem používaný pro uzavřené systémy kultivace umožňující kontrolu jednoho či více faktorů ovlivňujících biologické pochody.
- V **průmyslovém biotechnologickém** využití se jedná především o tzv. **fermentory**.
  - Umožňují růst eukaryotických či prokaryotických buněk ve vysoké hustotě zajišťující vysokou produkci metabolických produktů, enzymů či overexpresi rekombinantních genových produktů.
  - Patří sem:
    - Stirred tank reactors.
    - Packed beds.
    - Membrane bioreactors.
  - Kultivují se monokultury

Bioreaktory pro TE

- V **tkáňovém inženýrství** jsou **bioreaktory** používány k zajištění kontrolované a reprodukovatelné buněčné proliferace.
- Kontrolují se:
  - Teplota
  - pH
  - Koncentrace plynů
  - Míra toku médií
  - Tlak
  - Hydrodynamické a mechanické síly
- Rozdíl oproti jiným kultivačním technikám je v nutnosti zajistit aby buňky proliferovali a diferencovali stejně jako v podmínkách *in vivo*.
- Oproti fermentorům je navíc často požadavek na kultivaci více linií buněk.
- Náročnost se zvyšuje také nutností inkorporace vhodného scaffoldu.
- Cílem tedy není produkce určitého objemu buněk či produktu metabolismu, ale **růst mnoha buněčných linií v organizované 3D struktuře**.

Obecné požadavky na design bioreaktorů pro TE

- Základní podmínky jež by měl splňovat:
  - Vytváření vhodných podmínek srovnatelných s podmínkami *in vivo* (zajištění proliferace a diferenciace).
  - Uniformní rozložení buněk v 3D scaffoldu.
  - Udržování optimální koncentrace živin v celém prostoru.
  - Vytváření vhodných fyzikálních stimulů.
  - Zajištění sterility prostředí (zajištění sterilizace pomůcek, médií, kultivačních technik a s ohledem na délku inkubace také možnost sterilní výměny médií a odběru vzorků).
  - Možnost změny podmínek v čase.
  - Splnění Good Manufacturing Practice a Quality Asurance.

„Osazení“ bioreaktoru

- Pro úspěšnou kultivaci je nezbytná maximální homogenita osazení scaffoldu buňkami.
  - Čím je vyšší hustota buněčného osazení na začátku tím je lepší a uniformní tvorba tkáně.
    - To může být problematické i u malých scaffoldů a s jejich velikostí se tento problém zvyšuje.
- Existují dvě možnosti:
  - Statický systém - přichycení buněk na scaffold a následné přenesení do bioreaktoru.
  - Dynamický systém - okolo statického scaffoldu omýváno médium obsahující buňky.
    - Efektivní pro dosažení vysoké hustoty a zároveň uniformity.
    - Vede však k horšímu osazení uvnitř scaffoldu.
  - Perfusní dynamický systém kdy je opakovaně protlačováno médium skrz scaffold.
    - Nejúčinnější systém.

Transport živin

- *In vivo* jsou tkáně obvykle vzdáleny do **100µm** od kapiláry dodávající živiny.
  - Z toho by se mělo vycházet při přípravě struktur zajišťujících mikroenvironment v rámci jednotlivých podjednotek tkáně.
- Subjednotka by tak měla představovat kostku o straně 100µm což představuje cca 500 – 1000 buněk (chrupavka má jen 1 buňku na 100 μm^3).
- Pokud je cílem tkáň o větších rozměrech pak jsou buňky vzdálené více než 100µm vystaveny nedostatečnému zásobování živinami a odvodem odpadních látek. Důsledkem je vznik hypoxických a nekrotických center.
  - *In vivo* je tento problém řešen pomocí vlásečnic.
  - *In vitro* musí být tento problém řešen konstrukcí scaffoldu (porositou) a bioreaktoru (tokem média).

Vnitřní struktura scaffoldu

- Poréznost ovlivňuje dostupnost živin, odvod odpadních látek a regulačních molekul, mezibuněčnou komunikaci, prostorový růst buněk, fyzikální stres.
  - Vzdálenost - schopnost difuze látek je odvislá od vzdálenosti - buňky bez kultivace v bioreaktorech dokáží růst jen cca do 400 µm od povrchu (v závislosti na porositě).
  - Koncentrace - látky musí být k dispozici ve fyziologické koncentraci (přívod a odvod musí odpovídat jejich spotřebě či produkci) aby efekt nebyl **inhibiční až toxický**.
  - Homogenita - koncentrace látek by měla být stejná v různých místech – tok tekutiny by tedy měl být všude **vyrovnaný**.
  - Koncentrační gradient – u některých látek je třeba vytvářet koncentrační gradient s ohledem na jejich funkci (může být řešeno vazbou v scaffoldu).

Transport živin

- Živiny a plyny jsou transportovány pomocí:
  - Toku živného média.
  - Difuzí.
- Rychlost toku média: *in vivo* je absorpce kyslíku 25 – 250 µmol 0^2/cm^3/h a průtok tekutiny (pefusion rate) 0,07 ml/cm^3/min což je založeno na průměrné koncentraci buněk 500 milionů buněk na cm^3.

Kyslík
- Fyziologická koncentrace ~ 0.2 mM
- Spotřeba 0.05 – 1 µmol/10^6 buněk/hodinu

Glukoza a další primární metabolity
- Spotřeba 0.05 – 1 µmol/10^6 buněk/hodinu

Amino kyseliny, růstové faktory
- Fyziologická koncentrace v řádech nM – µM
- Spotřeba 0.01 – 1.0 nmol/10^6 buněk/hodinu

Odpadní metabolity – kysleina mléčná, amoniak
- Odvod 0.01 – 0.2 µmol/10^6 buněk/hodinu

Průtok by tedy měl být zajištěn zhruba 50 – 400 µL/min/10^6 buněk.

Transport živin

- Příklad kultivace bovinních chondrocytů na scaffoldu z poly(glycolic)acid. Chondrocyty produkují glycosaminoglycany jež jsou barveny červeně.

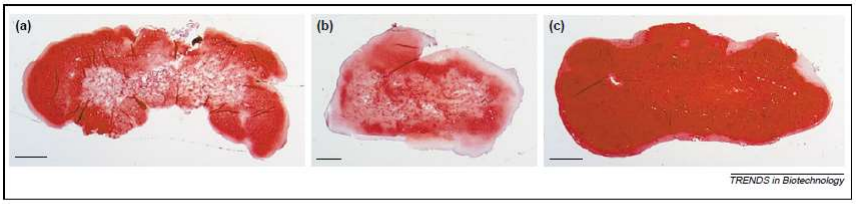
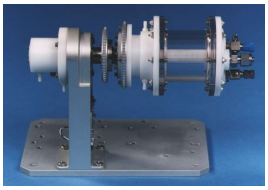
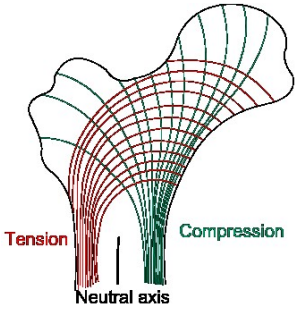


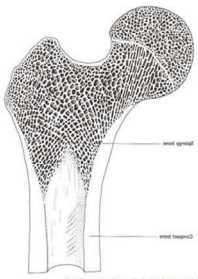
Figure 2. Safranin-O-stained cross-sections of engineered cartilage tissues after six weeks of culture under different hydrodynamic conditions [glycosaminoglycan (GAG) is stained red]. **(a)** Statically cultured constructs contain central regions with negligible GAG deposition. **(b)** Spinner-flask cultured constructs stain more intensely for GAG in their central regions but are encapsulated by fibrous tissue at their periphery. **(c)** Rotating-wall vessel (RWV) cultured constructs stain intensely and homogeneously for GAG throughout their cross-sectional area. The improved structural features following RWV culture (observed histologically and confirmed biochemically) were shown to be related to increased equilibrium modulus, dynamic stiffness and reduced tissue permeability [27,30]. Adapted with permission from [65]. Scale bars, 1 mm.



Biochemechanické stimuly

- Tkáně mají v *in vivo* systémech také mechanické funkce (kosti, chrupavky, vazivo, cévy).
- Působením vhodných mechanických vlivů při *in vitro* kultivaci dochází k tvorbě struktur podobných *in vivo*.
  - Dochází tedy k ovlivnění jak diferenciace tak proliferace a morfologie buněk a ECM.
- Stres daný dynamickým tlakem toku tekutiny (Fluid dynamic stress, or ‘‘shear stress’’) je považován za klíčový parametr vedoucí k aktivaci signálních drah podstatných pro mechanické vlastnosti tkáně.





Biomechanické stimuly

- Každá tkáň vyžaduje odlišné další mechanické stimuly:
  - Pulsující radiální stres tubulárního scaffoldu osazeného **hladkosvalovými buňkami** zlepšuje strukturní organizaci umělých cév. Dále zvyšuje produkci elastinu čímž zlepšuje mechanické vlastnosti graftu.
  - Dynamická deformace stimuluje syntézu glycosaminoglykanů **chondrocyty** a zlepšuje tak mechanické vlastnosti chrupavky.
  - Rotační tlaky **mesenchymálních progenitorových** buněk v kolagenové matrici vedou k spojování buněk a formaci orientovaných kolagenových vláken.
  - Mechanické stlačování a cyklický hydrostatický tlak ovlivňuje významně genovou expresi a produkci extra celulární matrix.
- Vliv na **chrupavku** *in vivo* i *in vitro* v kultuře chondrocytů je ovlivňována:
  - Komprese mění buněčnou proliferaci, metabolismus pojivové tkáně a obsah pojiva.
  - Statický tlak způsobuje trvalou deformaci tkáně zároveň inhibuje syntézu pojiva.
  - Dynamický tlak o určité amplitudě a frekvenci způsobující tok tekutiny v tkáni a její deformaci stimuluje syntézu pojiva.

Typy bioreaktorů

- Existuje celá řada různých typů. V následující části budou uvedeny jen vybrané příklady.
- Uvedené výhody a nevýhody jsou obecné a vždy závisí na konkrétní tkáni jež má být generována a scaffoldu.

In vivo bioreaktory

- Využívají *in vivo* tkáně jako zdroj živin a růstových faktorů.
- Scaffold je umístěn do tkáně a následně jej osadí buňky vlastního těla (především krevní elementy a SC).
- Problém je s nalezením vhodných scaffoldů a jejich povrchových úprav tak aby stimulovali buňky k proliferaci a diferenciaci správným směrem.

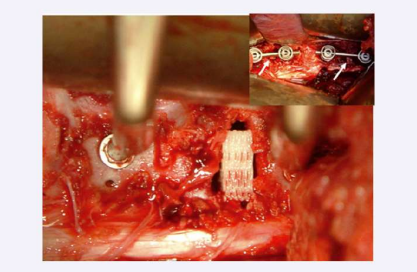


Fig. 8B Implantation of a MSC loaded mPCL-CaP/ scaffold (14x12x5 mm3) into a spinal fusion model (inset, disks were removed on level L1/L2 and L4/L5 and tissue engineered constructs were combined with internal fixation devices) in a 6 month old domestic pig.

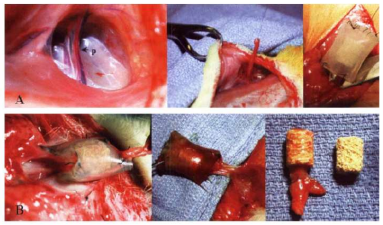


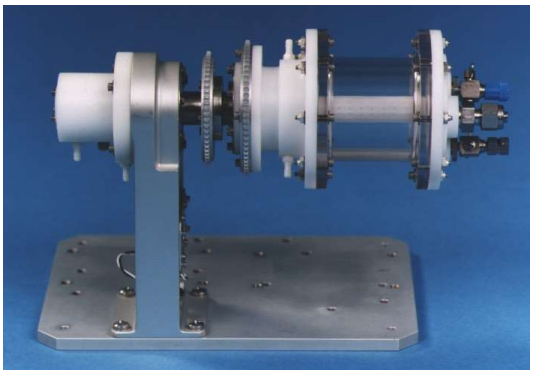
Fig. 1. Surgical technique for implantation and retrieval of in vivo bioreactor. (A) Prior to implantation, a midline ventral incision is made in the rat. Superficial dissection of the skin flap exposes the superficial inferior epigastric artery and vein. The artery and vein are ligated 2 cm distal to their takeoff from the femoral vessels. The pedicle is then threaded through the bioreactor (coral cylinder ± soak loaded with BMP and encased within silicone tubing). The ligation suture is secured to the silicone end suture. The silicone is then secured to the subcutaneous tissues to prevent torsion (*p*) = pedicle. (B) After 6 weeks, the bioreactor is harvested. The pedicle is dissected cleanly, and then transected to ensure pulsatile bleeding. The silicone is cut, and the coral cylinder is retrieved. Scaffold with pedicle (*l*) demonstrates tissue ingrowth, while avascular scaffold (*r*) has no ingrowth.

Spinner flasks

- Buňky jsou transportovány na povrch i do scaffoldu pomocí konvekce.
- Míchání média zvyšuje povrchový transfer média a podporuje růst buněk nicméně generuje turbulence které mohou mít negativní dopad na tvorbu tkáně.

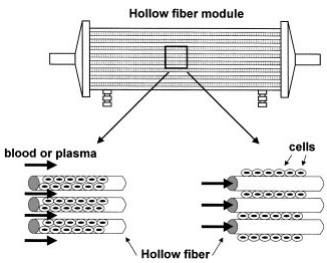
Rotating-wall vessels

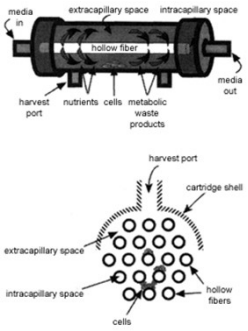
- Poskytuje dynamické prostředí při vysoké dostupnosti živin a odvodu odpadních látek.
- Vytváří nízký smykový stres.
- Jsou velmi vhodné pro tvorbu chrupavky protože indukují chondrogenezi.



Hollow-fiber bioreactors

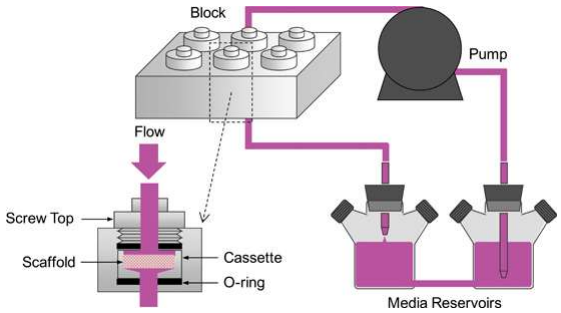
- Využívány k vytvoření dostatečné proliferace u buněk s intenzivním metabolismem (hepatocyty).
- Jsou dvě možnosti uspořádání:
  - Buňky jsou umístěny do gelu uvnitř dutých vláken (o průměru cca 200µm) a médium je protlačováno skrze stěny vláken.
  - Buňky jsou v prostoru mezi vlákny a médium je tlačeno skrze vlákna.



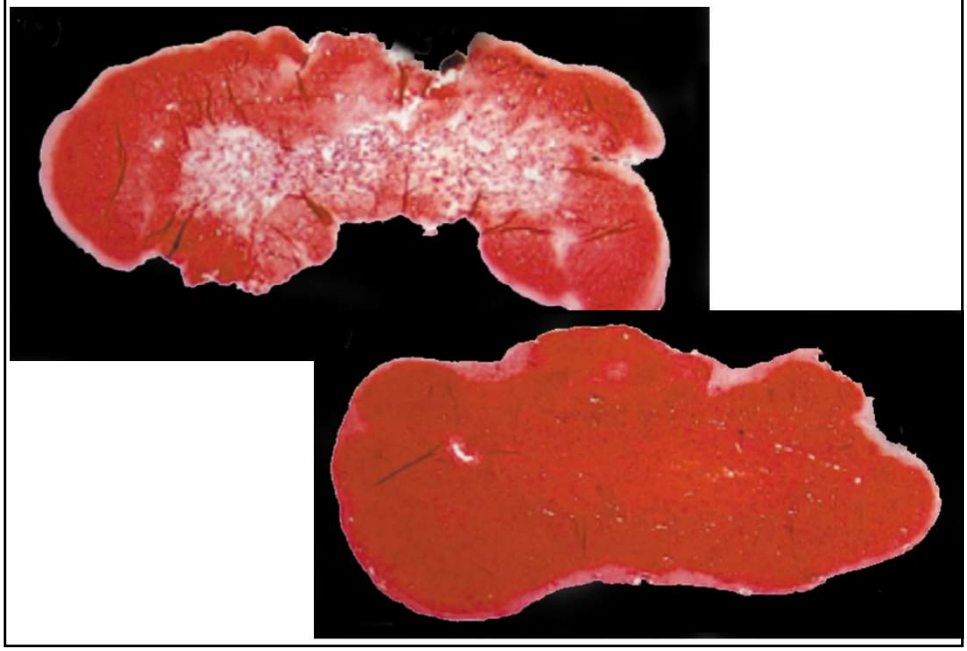


Direct perfusion bioreactors

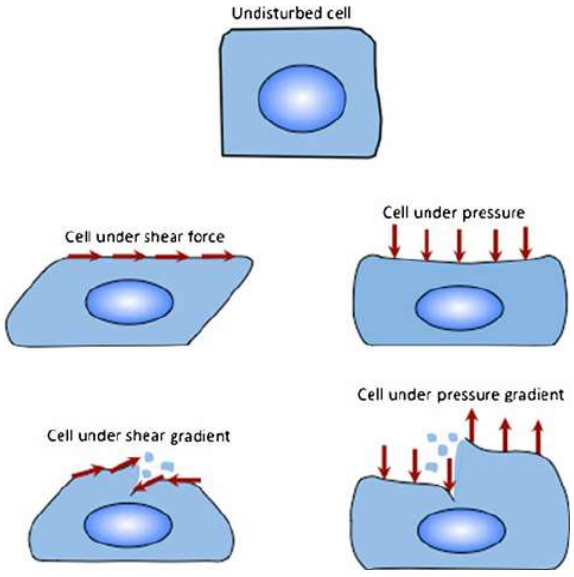
- Médium je tlačeno skrz póry scaffoldu.
- Využíváno pro osazení i následnou kultivaci.
- Při osazování jsou buňky transportovány skrze póry přímo do scaffoldu což zajišťuje vysokou uniformitu.
- V průběhu kultivace tok zajišťuje vysokou tvorbu buněčné hmoty.



CELLS IN 2D OR 3D WORLD – CULTIVATION TECHNIQUES



Schematics of an undisturbed cell, cells under shear stress, pressure, and their gradients



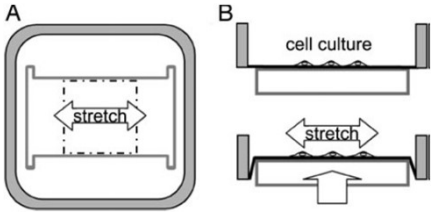
DOI: 10.1007/s10544-011-9543-5

Cyclic uniaxial stretch and stress fibers

- The tension generated by contraction of adherent cells against their underlying surface results in an internal stress field that depends on the organization of the cytoskeleton and the associated adhesive contacts.
- Intracellular forces have an important role in cellular functions such as migration, proliferation, apoptosis, differentiation, and gene expression.
- Actin **stress fibers**, which are formed in response to cell contraction, consist of bundles of actin microfilaments cross-linked by -actinin, myosin, myosin light-chain, tropomyosin, and other proteins arranged in a manner similar to that in muscle sarcomeres.
  - Stress fibers represent the main contractile apparatus in non-muscle cells and are the primary structures associated with intracellular tension.
  - Stress fibers terminate at focal adhesions, which attach the cell to the extracellular matrix.
- Isometric contraction of a cell would result in tension development in the stress fibers, which are anchored at their ends.
- **Cyclic uniaxial stretch induces** the orientation of stress fibers in endothelial cells (ECs) **perpendicular to the principal direction of stretch.**

doi10.1073pnas.0506041102

Stress fibers and Rho pathway

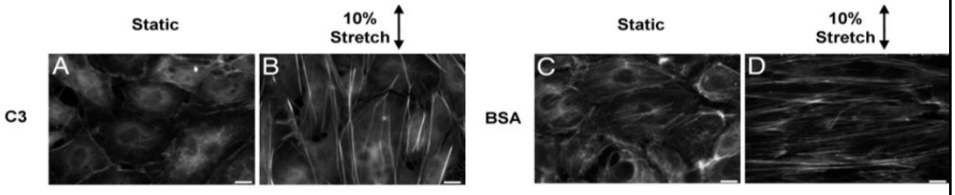


- Top (*A*) and side (*B*) views of a stretch chamber and indenter to illustrate the principle of cell stretching.
- An I-shaped teflon indenter pushed up against a silicone rubber membrane secured to a square frame results in a principal stretch oriented along the long axis of the indenter.
- The small tension generated in the orthogonal direction is opposed by the tendency for the membrane to compress orthogonal to the principal stretch direction. The extensions at the corners of the indenter increase the uniformity of the strain field over the indenter, resulting in a virtually uniaxial stretch. Cells were seeded in the central 4 4-cm region of the membrane where strain was uniform.

doi10.1073pnas.0506041102

Stress fibers and Rho pathway

- The GTPase Rho regulates the formation of actin stress fibers in adherent cells through activation of its effector proteins Rho kinase.
- Study on **aortic endothelial cells.**
- Inhibitions of Rho, Rho-kinase.
  - **Rho activity inhibited with C3 exoenzyme - C3 or by Y27632.**
    - stress fiber formation was almost completely absent under unstretched condition (Fig. *A*);
    - 10% stretch at 1 Hz of these cells caused the formation of linear actin fiber bundles, which were **oriented parallel to the direction of stretch** (Fig. *B*).
  - **Control cells transfected with BSA (do not inhibit Rho activity).**
    - Unstretched contained actin fibers not oriented in any particular direction (Fig. *C*).
    - Stretching resulted in the orientation of stress fibers perpendicular to the direction of stretch (Fig. *D*).



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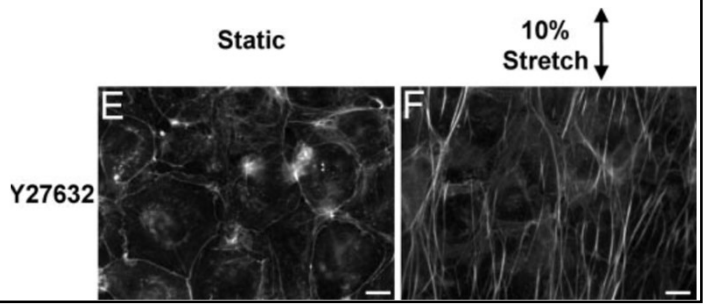
Stress fibers and Rho pathway

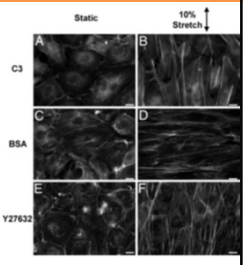
- Rho-kinase activity inhibited by Y27632 - **Y27632.**
  - Effect similar to inhibition with C3 exoenzyme

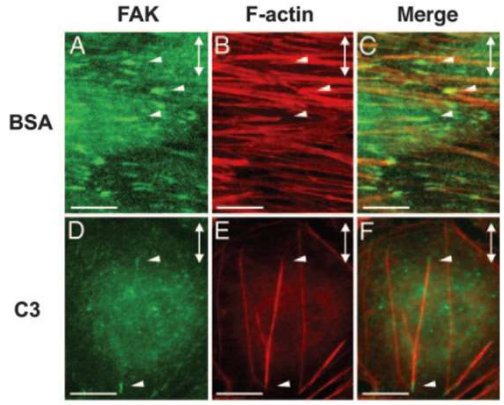


doi10.1073pnas.0506041102

Stress fibers and Rho pathway

- Immunostaining with an antibody specific for focal adhesion kinase (FAK) revealed the association of these fibers with focal adhesion-like structures in a manner similar to that of stress fibers both in the control cells transfected with BSA (Fig. 3*C*) and in cells transfected with C3 (Fig. 3*F*).

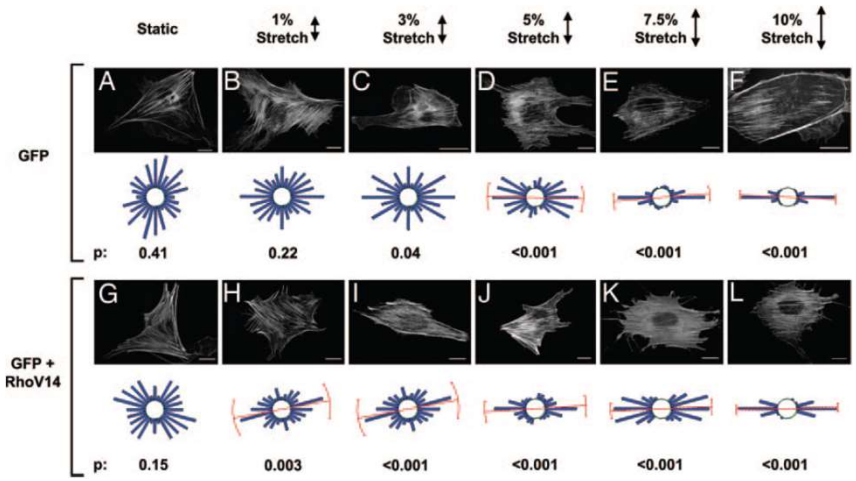




doi10.1073pnas.0506041102

Stress fibers and Rho pathway

- Normally expressed Rho (GFP) vs overexpressed Rho (GFP+RhoV14)
  - Overexpression leads to orientation in lower stretch.



doi10.1073pnas.0506041102

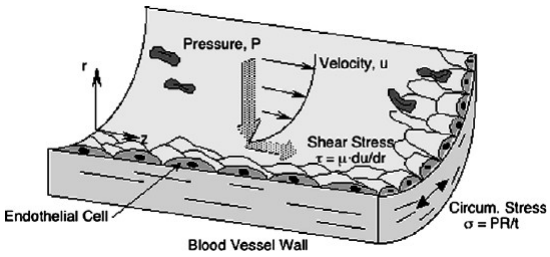
Stress fibers and Rho pathway

- Inhibitions of Rho, Rho-kinase, and mDia:
  - suppressed stress fiber formation, but
  - fibers appeared after 10% cyclic uniaxial stretch (1-Hz frequency).
- In normal cells:
  - predominately perpendicular alignment of stress fibers to the stretch direction
  - the extent of perpendicular orientation of stress fibers depended on the magnitude of stretch
- In cells with Rho pathway inhibition:
  - stress fibers became oriented parallel to the stretch direction.
- The activity of the Rho pathway plays a critical role in determining both the direction and extent of stretch-induced stress fiber orientation in bovine aortic endothelial cells.
- The stretch-induced stress fiber orientation is a function of the interplay between Rho pathway activity and the magnitude of stretching.

doi10.1073pnas.0506041102

Shear stress and hydrostatic pressure – endothelial cells

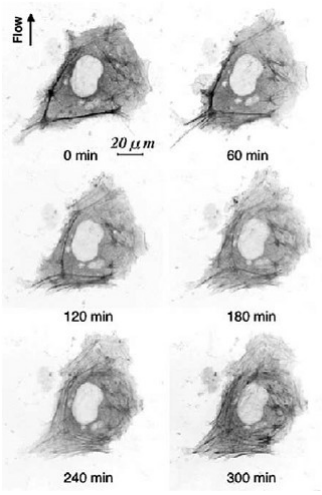
- Three different mechanical forces, shear stress, hydrostatic pressure and cyclic stretch, acting on endothelial cells.



Biorheology, vol. 42, no. 6, pp. 421-441, 2005

Shear stress and hydrostatic pressure – endothelial cells

- Dynamic change in actin filament structure of a single endothelial cells exposed to fluid shear stress up to 300 min. (From Ohashi et al. [50] with permission.)



Biorheology, vol. 42, no. 6, pp. 421-441, 2005

Shear stress and hydrostatic pressure – endothelial cells

Typical fluorescent images of actin filaments in bovine aortic endothelial cells before and after exposure to shear stress of 2 Pa.

(a) Control,

(b) after 20 min,

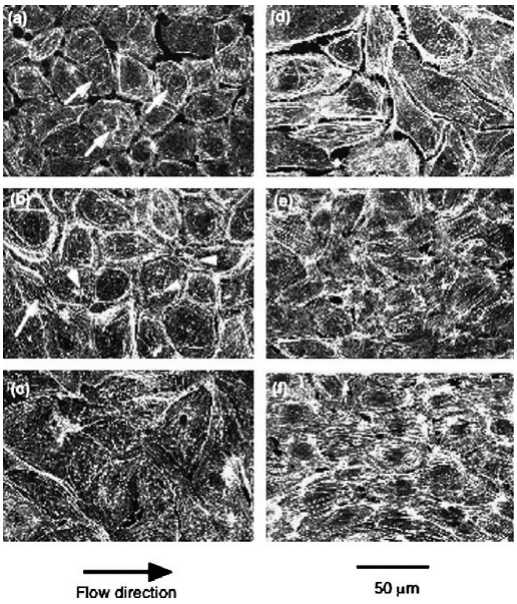
(c) 40 min,

(d) 1 h,

(e) 3 h,

(f) 6 h.

(From Kataoka and Sato [29] with permission.)



Biorheology, vol. 42, no. 6, pp. 421-441, 2005

Shear stress and hydrostatic pressure – endothelial cells

Fluorescence images of actin filaments of EC

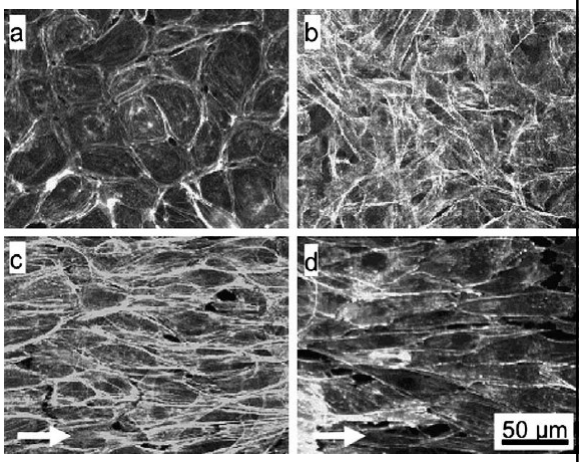
(a) control,

(b) hydrostatic pressure of 100 mmHg,

(c) shear stress of 2 Pa,

(d) hydrostatic pressure of 100 mmHg and shear stress of 3 Pa.

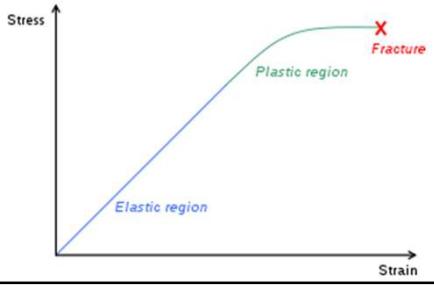
The direction of flow was from left to right.



Biorheology, vol. 42, no. 6, pp. 421-441, 2005

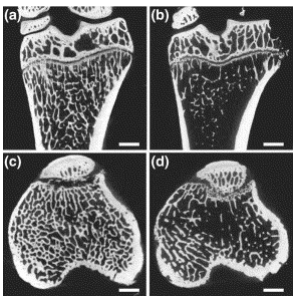
Bones

- The secondary bones are discussed here:
- Critical characteristics of the bone:
  - **Mechanical properties:**
    - **Toughness - HOUŽEVNATOST** (resistance to fracture)
      - Stiffness - TUHOST (resistance to elastic deformation - the material returns to its original shape when the force is removed).
      - Strength - PEVNOST (resistance to plastic deformation - the material does not return to its original shape when the force is removed).
  - **Structural adaptation** to changing external conditions.
  - **Capacity of self-repair.**



Bone – Capacity of self repair

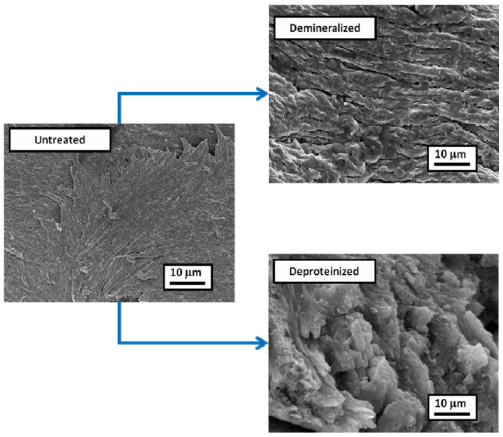
- **Capacity of self-repair:**
  - the combination of growth and remodeling (resorption and replacement of old material),
    - Osteoclasts are permanently removing material.
    - Osteoblasts are depositing new tissue.
  - Continuous remodeling allows for structural adaptation to changing external conditions, as well as the removal and replacement of damaged material.



Trends in Biotechnology Vol. 20 No. 8 (Suppl.), 2002

DOI: 10.1146/annurev-matsci-070909-104427

- Minerals provide hardness and fragility.
- Proteins provide plasticity.



Soft and flexible/ductile.

Hard and fragile.

DOI: 10.1557/opl.2011.94

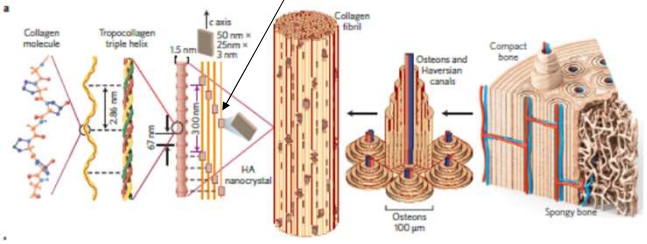
Bone

- Aging-related changes such as excessive remodeling.
  - Consequent fractures can lead to significant mortality.
  - A primary factor is bone quality - characteristics of the bone matrix nano- and microstructure that can influence mechanical properties.
    - Traditional thinking focused on bone quantity described by the bone-mass or bone mineral density (BMD), defined as the amount of bone mineral per unit cross-sectional area.
      - For example, the elevation in bone-remodeling activity, concurrent with menopause in aging women, can lead to osteoporosis, a condition of low bone mass associated with an increased risk of fracture.
    - Reality – more complicated proces involving not only BMD but other factors such as hierarchy.

DOI: 10.1146/annurev-matsci-070909-104427

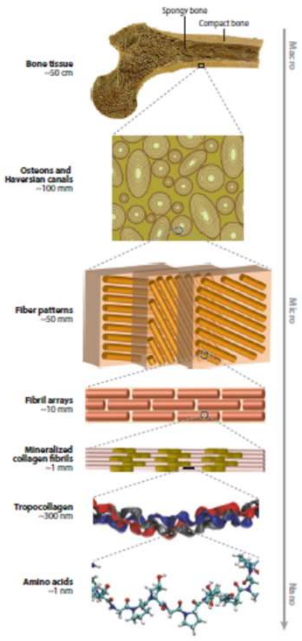
Bioinspired structural materials - Bone

- Bone is composed of cells embedded in an extracellular matrix, which is an ordered network assembled from two major nanophases (95% of the dry weight of bone):
  - Collagen fibrils made from type-I collagen molecules (~300 nm long, ~1.5 nm in diameter).
  - Calcium fosfate based hydroxyapatite (Ca10(PO4)6(OH)2)) nanocrystals (plate-shaped, 50 nm × 25 nm in size, 1.5–4 nm thick) distributed along the collagen fibrils.
    - The hydroxyapatite nanocrystals are preferentially oriented with their *c* axis parallel to the collagen fibrils, and arranged in a periodic, staggered array along the fibrils.



Hierarchical structure of bone.

- The macroscale arrangements of bones are either:
  - Compact/cortical (dense material found at the surface of all bones).
  - Spongy/cancellous (foam-like material whose struts are some 100 µm thick).
- Compact bone is composed of osteons that surround and protect blood vessels. Osteons have a lamellar structure.
- Each individual lamella is composed of fibers arranged in geometrical patterns. These fibers are the result of several collagen fibrils, each linked by an organic phase to form fibril arrays.
- Each array makes up a single collagen fiber.
- The mineralized collagen fibrils are the basic building blocks of bone.
  - They are composed of collagen protein molecules (tropocollagen) formed from three chains of amino acids.



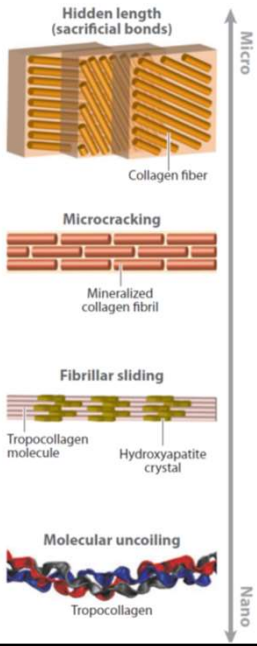
DOI: 10.1146/annurev-matsci-070909-104427

Bones – toughness and hierarchy

- **Submicrometer** deformation mechanisms contribute intrinsically to the fracture toughness of bone by **forming plastic zones** around crack-like defects, thereby protecting the integrity of the entire structure by allowing for localized failure through energy dissipation.
- **Micro- to macroscale dimensions**, the toughness of cortical bone is associated with a **crack-tip shielding**.
  - Toughening that arises during crack growth rather than during crack initiation.
    - Microstructure, especially the interfaces of the osteons, provide microstructurally weak or preferred paths for cracking.
    - As these features have a specific alignment in bone, the osteons provide the basis for the marked anisotropy of the fracture properties of bone (bone is easier to split and to break) and for the fact that the toughness is actually lower in shear than in tension.

Bones – Toughness and collagen

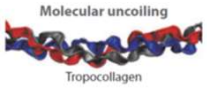
- In collagen fibrils the following mechanism competes:
  - Molecular uncoiling - breaking of weak and strong bonds between tropocollagen molecules,
  - Molecular stretching,
  - Intermolecular sliding,
- Collagen is able to stretch up to 50% tensile strain before breaking while reaching force levels of more than 10 nN(permolecule) or 10–20 Gpa stress.

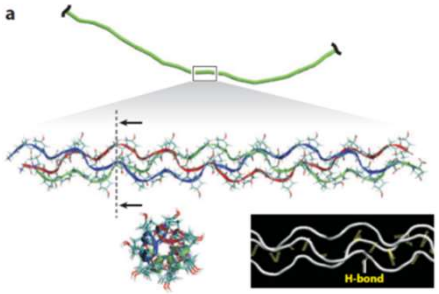


DOI: 10.1146/annurev-matsci-070909-104427

Bones - Toughness and collagen - Molecular Uncoiling

- H-bond breakage at 10% to 20% strain provides one of the major mechanisms that mediate the deformation of collagen fibrils:
  - It is a reversible process and may thus provide a means to dissipate energy through large-deformation behavior of the soft-collagen bone matrix.
  - Aged collagen tends to show a high cross-link density, whereas young collagen features few crosslinks.
  - The larger the cross-link density, the lower is the material's ability to dissipate energy without failure. At large cross-link densities, collagen fibrils tend to involve molecular fracture and breaking of cross-links, leading to increasingly brittle material behavior.

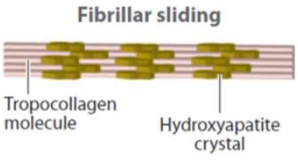


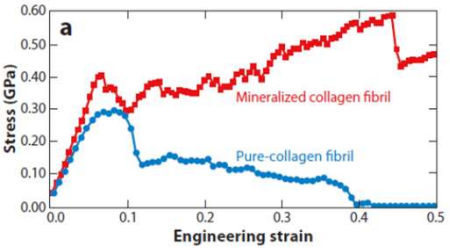


DOI: 10.1146/annurev-matsci-070909-104427

Bones - toughness and collagen – Fibrillar sliding of mineralized collagen fibrils

- Importance of a mineralization of collagen:
  - Figure shows a tensile test of a tropocollagen molecule for deformation up to 40% strain.
  - Mineral phase has an elastic modulus that is more than an order of magnitude higher than that of collagen, the presence of the hydroxyapatite phase is critical to the stiffness of bone.





- Slip at the hydroxyapatite/tropocollagen interface initiate the glide between tropocollagen molecules and between hydroxyapatite particles and tropocollagen molecules.
  - This glide enables a large regime of dissipative deformation once yielding begins, thus effectively increasing the resistance to fracture.

DOI: 10.1146/annurev-matsci-070909-104427

Bones - toughness and collagen – Fibrillar sliding of mineralized collagen fibrils

- Young's modulus, tensile yield strain, and fracture strength for mineralized collagen fibrils are 6.2 GPa, 6.7%, and 0.6 GPa, respectively, as compared with the corresponding values of 4.6 GPa, 5%, and 0.3 GPa, respectively, for pure-collagen fibrils.

DOI: 10.1146/annurev-matsci-070909-104427

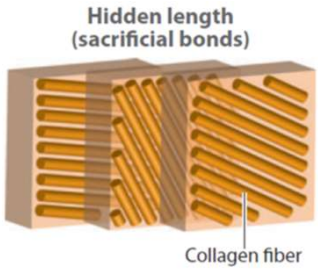
Bones - toughness and collagen – Fibrillar sliding of collagen fiber arrays

- The long (>5–10-µm) and thin (~100-nm) mineralized collagen fibrils are twisted into collagen fibers, which are "glued" together by a thin layer (1–2 nm thick) of extrafibrillar matrix.
  - When the tissue is externally loaded in tension, the load is resolved into tensile deformation of the mineralized fibrils and shearing deformation in the extrafibrillar matrix.

DOI: 10.1146/annurev-matsci-070909-104427

Bones - toughness and collagen – Fibrillar sliding of collagen fiber arrays

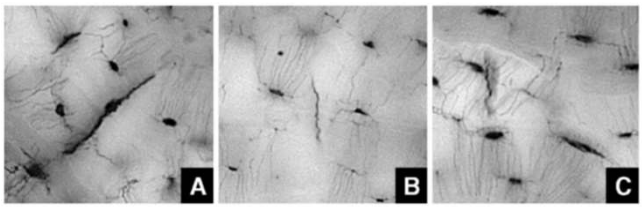
- Extrafibrillar matrix:
  - Composed of noncollagenous proteins, such as osteopontin, and proteoglycans, such as decorin.
  - Properties similar to those of a glue layer between the fibrils—it is relatively weak but ductile and deforms by the successive breaking of a series of sacrificial bonds.
  - Specifically, the separation of individual fibrils and the larger fibers during deformation and fracture is resisted by this macromolecular glue via sacrificial bonds that break at a fraction (~0.1–0.5) of the force required to break the backbone of the macromolecules.
  - The matrix may also be partially calcified, which would increase its shear stiffness and reduce its deformability.



DOI: 10.1146/annurev-matsci-070909-104427

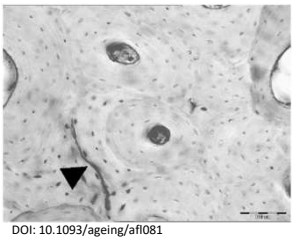
Bones - Toughness – Microcracking

- At several length scales from the submicrometer scale to a scale of tens of micrometers, the proces of microcracking in bone provides the prevalent mechanism of microscale deformation.
- Microcracking effects:
  - Microcracking is a process of plastic deformation.
  - Essential phenomenon for the development of the most potent extrinsic toughening mechanisms, notably crack bridging and crack deflection, that predominate at larger length scales.
  - Crucial role in signaling the remodeling of the bone, which occurs in so-called basic multicellular units (BMUs), i.e., combination of cells that are able to remove (osteoclasts) and form (osteoblasts) bone tissue.



collagen fibril

doi:10.1016/j.bone.2005.01.023

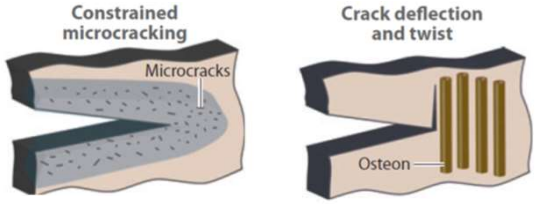


DOI: 10.1093/ageing/afl081

DOI: 10.1146/annurev-matsci-070909-104427

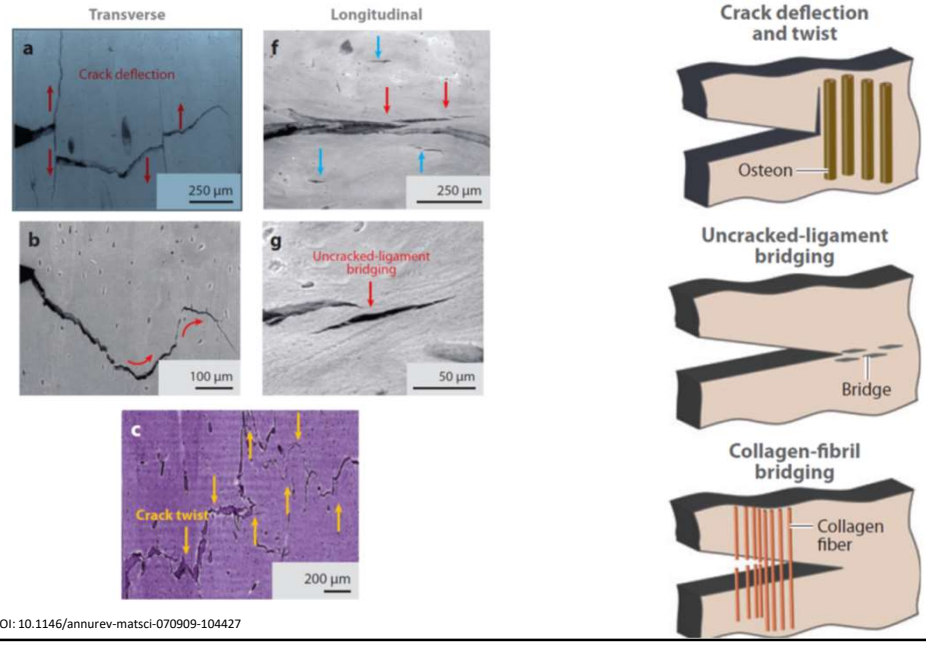
Bones - Toughness – Constrained Microcracking

- In cortical bone, the path of least microstructural resistance is invariably along the cement lines which are the hypermineralized interfaces between the bone matrix and secondary osteon structures.
  - These regions are therefore preferential sites for major microcracks to form, particularly as bone ages and the osteon density increases with remodeling.
  - These microcracks thus have a typical spacing in the tens to hundreds of micrometers and are aligned primarily **along the long axis** of the bone, an orientation that directly results in the strong anisotropy of toughness in bone.
    - Fracture of hydroxyapatite crystals surrounding collagen fibers or delamination at the crystal/fiber interfaces has been suggested as the cause of such microcracking damage.
  - Importance of microcracking extrinsically is that it results in both crack bridging and crack **deflection**, which are the most potent toughening mechanisms in bone.



DOI: 10.1146/annurev-matsci-070909-104427

Bones - Toughness – Crack deflection, twist and bridging



DOI: 10.1146/annurev-matsci-070909-104427

Bone tissue engineering – Critical bone defects

- Critical bone defects need intervention therapy to achieve recovery.
- Critical bone defects is regarded as one that would not heal spontaneously despite surgical stabilization and requires further surgical intervention, such as autologous bone grafting.
  - General guidelines that have been suggested in the literature include defect length greater than 1–2 cm and greater than 50% loss of the circumference of the bone .
    - However, this is impacted upon by the anatomic location of the defect and the state of the soft tissues surrounding it.
      - Segmental defects of the femur often have a good soft-tissue environment and spontaneous healing of segmental defects 6–15-cm long has been reported.
      - By contrast, poor outcomes with the lack of spontaneous healing have been reported with much smaller defects in the tibia, when the defect size is greater than 1–2 cm and greater than 50% of the cortical circumference.

doi.org/10.1016/j.actbio.2018.08.026 & doi: 10.1097/BOT.0000000000000978

Bone tissue engineering – Critical bone defects

- Interventions:
  - ‘‘gold standard” for intervention - graft materials - limited by supply.
  - Fabrication methods with limited control of the pore shape, architecture, porosity, or interconnectivity.
    - Extrusion-based processes - fabrication accuracy on tens and hundreds of micrometers
    - Freeze drying
    - Fiber bonding
    - Particulate/salt leaching
    - Emulsification
    - Phase separation/inversion

doi.org/10.1016/j.actbio.2018.08.026 & doi: 10.1097/BOT.0000000000000978

Materials for bone substitutes

For metal bone substitutes:

- selective laser melting (SLM), selective laser sintering (SLS),
- sintering,
- perforating titanium sheet,
- capsule-free hot isostatic pressing (CF-HIP).

Polymer and ceramic

- Porogen leaching,
- freeze drying,
- 3D printing of successive fibre/strut layers,
- electrospinning,
- gas foaming.

Table 1 Biomaterial abbreviations and material group

Biomaterial abbreviation	Full form of biomaterial	Biomaterial group
CaP	Calcium phosphate	Ceramic
HA	Hydroxyapatite	Ceramic
MBG	Mesoporous bioactive glass	Ceramic
β-TCP	β-Tricalcium phosphate	Ceramic
Ti6Al4V	Titanium	Metal
TiNi	Titanium nickel	Metal
TT	Trabecular titanium	Metal
CSNF	Chitosan network fibres	Polymer
Col	Collagen	Polymer
CG	Collagen–glycosaminoglycan	Polymer
DEF	Diethyl fumarate	Polymer
HFIP	Hexafluoroisopropanol	Polymer
PA	Polyacrylamide	Polymer
PDMS	Poly(dimethylsiloxane)	Polymer
PLGA	Poly(lactide-*co*-glycolide)	Polymer
PPC	Poly(propylene carbonate)	Polymer
PPF	Poly(propylene fumarate)	Polymer
PCL	Poly(ε-caprolactone)	Polymer
SF	Silk fibroin	Polymer
SPCL	Starch poly(ε-caprolactone)	Polymer
TG	Thermoplastic gelatin	Polymer
TPU	Thermoplastic polyurethane	Polymer
PDLLA	Poly(D,L-lactic acid)	Polymer

DOI: 10.1039/c7tb00741h

Markers of bone regeneration

- During bone regeneration, cells proliferate and differentiate into osteoblasts which deposit a collagen matrix that becomes mineralized.
  - Proliferation, takes place in the first days after seeding and consists mainly of cell division. Cells are able to migrate.
  - After proliferation, cells start to differentiate into osteoprogenitor cells until the end of the second week, and the release of alkaline phosphatase (ALP) increases.
  - In two weeks after the differentiation stage, osteocalcin (OCN) and osteopontin (OPN) are produced and secreted by the cells, indicating the presence of osteoblasts.
  - When the collagen matrix is synthesized by osteoblasts, biomineralization is initiated and mineral crystals are formed within the collagen matrix.
  - In parallel with the proliferation and differentiation of cells, blood vessels form from existing vessels (angiogenesis). These vessels create a vascular network to provide oxygen and nutrients to the cells and developing tissue within the bone substitute. This network provides stem cells needed for bone regeneration and direct the differentiation of endothelial cells and preosteoblasts.

Table 2 Osteogenic markers

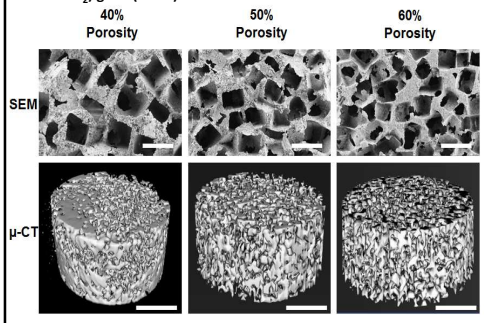
Marker abbreviation	Full form of marker	Expressed by
ALP	Alkaline phosphatase	Osteoprogenitor
RunX-2		Osteoprogenitor, osteoblast
OPN	Osteopontin	Osteoblast
OCN	Osteocalcin	Osteoblast
OPG	Osteoprotegerin	Osteoblast, inhibits bone resorption
Calcium		Osteoblast
Col1	Collagen type 1	Organic matrix of bone, synthesized by osteoblasts
VEGF	Vascular endothelial growth factor	Growth factor blood vessels
BSP	Bone sialoprotein	Mineralized tissue

DOI: 10.1039/c7tb00741h

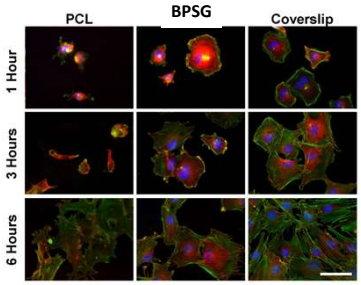
Optimization of scaffold structures

- Porosity
  - Supports cell migration into the scaffold and improves the available surface area for cell–scaffold binding and interaction with the surrounding tissues.
  - Conflicting issues that high porosity (30-50%) cannot simultaneously attain cell migration and growth whilst maintaining optimal mechanical strength.
  - Compromise must be made among strength, bone integration, and porosity.

Polycaprolactone (PCL) and borophosphosilicate (B_2O_3-P_2O_5-SiO_2) glass (BPSG)



https://biomaterials.ca/#!/abstracts/view/114242



Compared to polycaprolactone, cells on the hybrid material displayed enhanced spreading, focal adhesion formation, and cell number, consistent with excellent biocompatibility.

doi.org/10.1016/j.actbio.2018.11.039

Optimization of scaffold structures

- Pore size
  - The minimum pore size for a scaffold is approximately 100 nm because of cell size, transport, and migration requirements.
  - Typically between 100 nm and 300 nm to allow cell penetration, migration, and growth, as well as an optimal tissue vascularization
    - Smaller pores leads to low seeding efficiency and inhomogeneity.
    - Higher pores leads to cells tend to escape from the structure.
  - However, there is not yet a clear consensus on the effect of optimal pore size on optimized mechanical and osteogenic performance.

2008

Q. Zhang et al. / Acta Biomaterialia 10 (2014) 2005–2013

The collagen porous scaffolds prepared with ice particulates in the range of 150–250 lm showed the highest promoting effect on the gene expression and the production of cartilaginous matrix proteins as well as on cartilage regeneration.

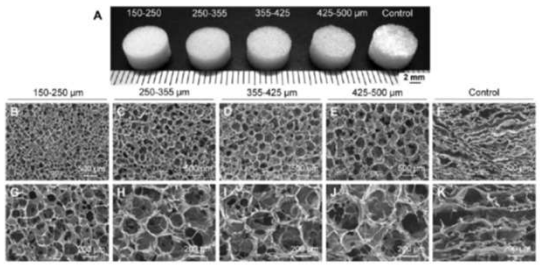


Fig. 1. Gross appearances (A) and SEM photomicrographs (B–K) of the cross-sections of the four types of collagen porous scaffolds prepared with ice particulates having a diameter range of 150–250 (B, G), 250–355 (C, H), 355–425 (D, I) and 425–500 μm (E, J), and a control collagen porous scaffold prepared without ice particulates (F, K) at low (B, C, D, E, F) and high (G, H, I, J, K) magnifications.

doi.org/10.1016/j.actbio.2018.11

https://doi.org/10.1016/j.actbio.2013.12.042

Pore size and Seeding effciency

- The seeding efficiency depends on:
  - The number of attachment sites within a porous biomaterial:
    - Small pore size:
      - Cells aggregate at the seeding surface.
    - With an increased pore size:
      - The surface area within the structure decreases – thus there is less attachment sites for the seeded cells.
      - The permeability of the porous biomaterial increases - this is associated with a higher flow rate, which reduces the time for cell attachment to the surface of the structure during seeding.
  - The available time for cells to attach to the surface.

DOI: 10.1039/c7tb00741h

Pore size and Cell viability, Proliferation and Migration

- Pore size smaller than 100 nm:
  - Cells are more likely to aggregate and block the way for oxygen and nutrients to the centre of the scaffolds.
  - Pores smaller than 100 nm should be avoided to prevent cell death.
- Restricted cell migration was observed in porous biomaterials with small pores, while cells can migrate more easily and distribute homogeneously when a structure contains bigger pores up to 500 um.

DOI: 10.1039/c7tb00741h

Pore size and Cell differentiation, Vascularization and Mineralization

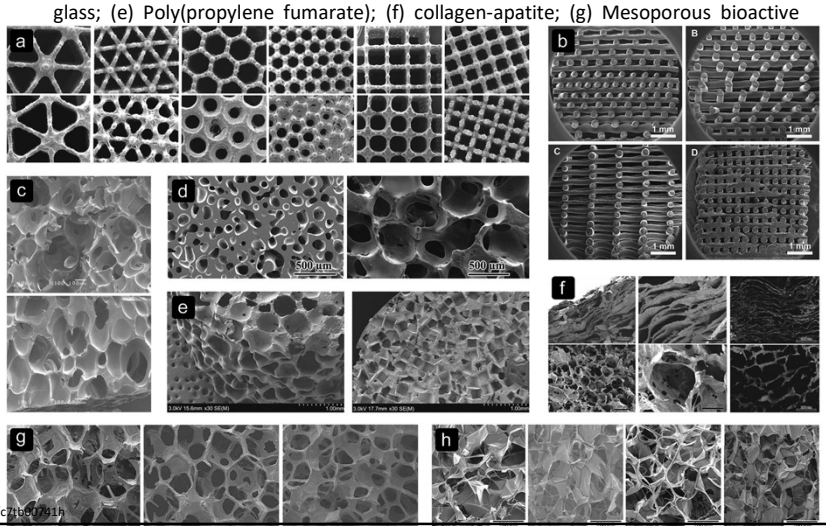
- Osteogenic differentiation occurred more in large pores.
  - The cells tend to be more spread in large pores compared to small pores. This morphology is thought to promote osteogenic differentiation
- Pores larger than 400 µm are preferable for blood vessel formation and consequently for the delivery of oxygen and nutrients to the cells inside the bone substitute.
- Porous biomaterials with larger pores were found to have a better and higher distribution of calcium and mineral deposition parallel to the pore walls in vitro.
  - This could be an effect of the alignment of cells with the pore walls, higher cell viability, distribution, and proliferation rate in structures with large pores.

DOI: 10.1039/c7tb00741h

Pore shape

- The geometry of pores within a bone substitute can be, among others, spherical, rectangular, square, hexagonal or trabecularlike.

(a) Titanium; (b) Starch poly(ε-caprolactone); (c) Poly(lactide-*co*-glycolide); (d) bioactive glass; (e) Poly(propylene fumarate); (f) collagen-apatite; (g) Mesoporous bioactive



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- The pore size and shape affect the mechanical properties of porous biomaterials, as they determine the dimensions and orientation of the struts or fibres and, thus, the stress distribution inside those structural elements.
  - Scaffolds with a ladder-like structure and rectangular pores and scaffolds with large spherical pores collapse more easily than porous biomaterials with smaller uniform round pores.

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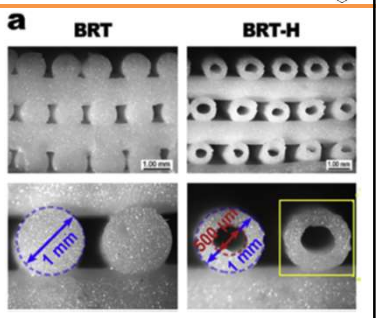
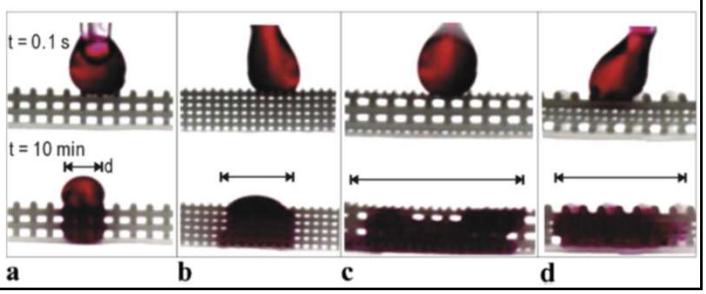
Bone tissue engineering - Macrostructure

- Macrostructure influence :
  - Mechanical property.
  - Degradation – connected to surface area and porosity.
    - e.g. hollow-struts-packed possess a faster degradation rate owing to their large surface area and high porosity.
  - Permeability - water-uptake ability and water movement.





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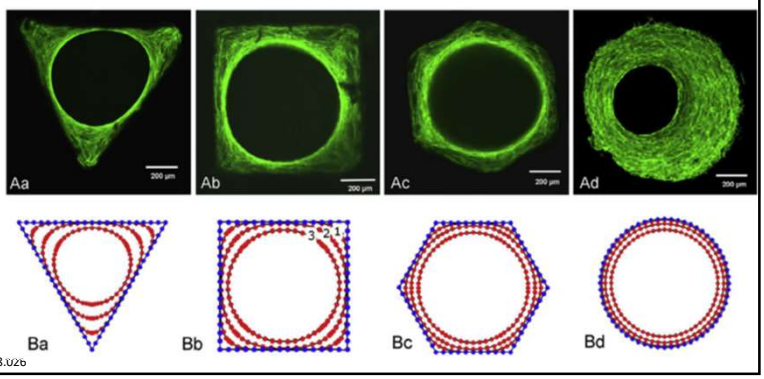
Bone tissue engineering - Macrostructure

- Macropore geometry:
  - Cells prefer a radius of curvature much larger than that of the cells themselves.
    - Murine osteoblast-like cells exhibited curvature driven migration, proliferation, and differentiation, thereby resulting in initial tissue formation occurring at the corners.
    - High curvature leads to mechanical forces in cells, proved by the formation of actin stress fibers at the tissue–fluid interface, which enhance further tissue growth.



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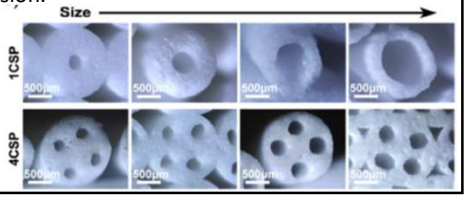
Bone tissue engineering - Macrostructure - Multioriented hollow channel structures

- Multioriented hollow channel structures exhibited improved bone regeneration:
  - Hollow channel structures led to a quick release of ions from the scaffolds.
  - Improved degradation offered more space for the formation of new bone tissue.
  - Hollow tube provided a channel for oxygen and nutrition transport and cell migration, thus benefiting the improvement in bone formation.
  - Bioactive ions released from the hollow-pipe-packed silicate bioceramic scaffolds can also promote angiogenesis by inducing the migration of endothelial cells.
  - Facilitate the infiltration of host blood vessels into the hollow channels and also improve the delivery of stem cells and growth factors, thus contributing to further tissue regeneration.
  - Promote osteoclast/immune cell invasion.



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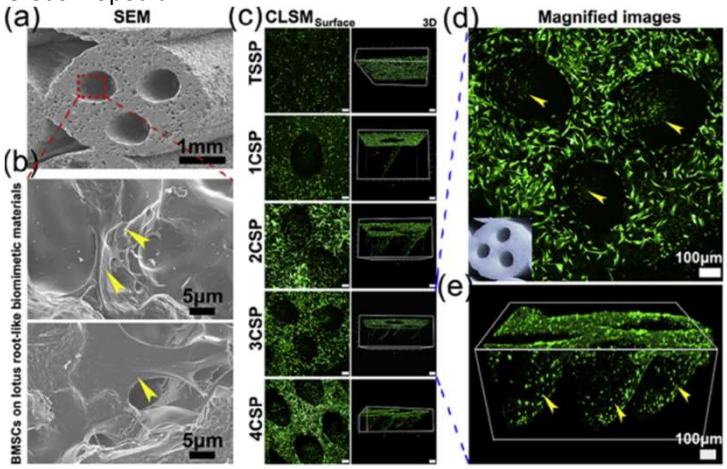
Bone tissue engineering - Macrostructure - Multioriented hollow channel structures

- In vitro and in vivo results of the lotus root-like biomimetic scaffolds. (a, b) SEM images of the attached BMSCs after culturing for 3 days.
- (b) The yellow arrows show that the BMSCs adhered on the scaffolds through numerous filopodia.



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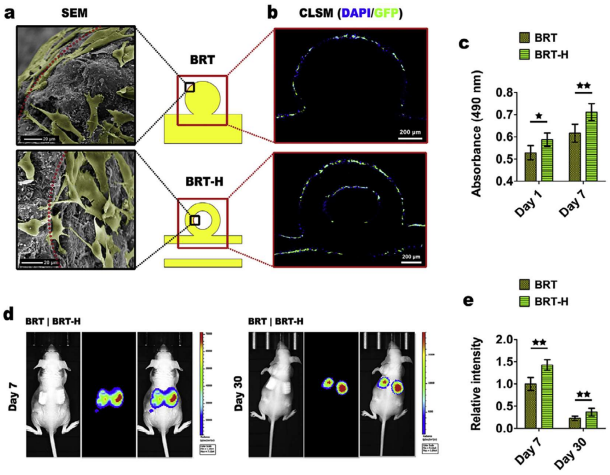
Bone tissue engineering - Macrostructure - Multioriented hollow channel structures

- The effect of hollow channels on stem cell delivery.
- (a) SEM observations of adherent rBMSCs on the scaffold surface. Cells can be observed in the hollow channels of the BRT-H scaffolds.
- (b) The cells in the hollow channels were still alive after 7 days of culture in vitro.
- (c) Cell viability at day 1 and day 7 of rBMSCs seeded in the scaffolds.
- (d) Scaffolds with luciferase-labeled BMSCs were transplanted into mice and detected through in vivo fluorescence imaging.
- (e) Statistical results for the relative fluorescence intensity



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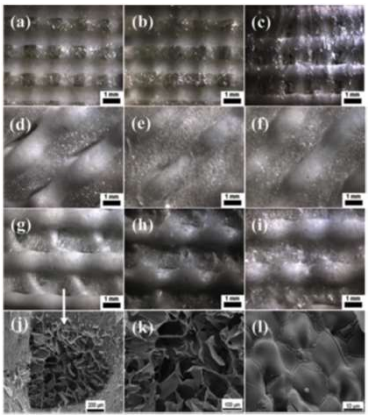
Bone tissue engineering – Microstructure & nanostructure

- 3D printing allow to prepare desirable macrostructure. The micro and nano sized hierarchy must be subsequently be done by some of followed methods:
  - Freeze-drying method
    - Hierarchically composite scaffolds were composed of ordered macropores of the bioceramic scaffolds (first level: 1 mm) and micropores of silk networks (second level: 50–100 μm),



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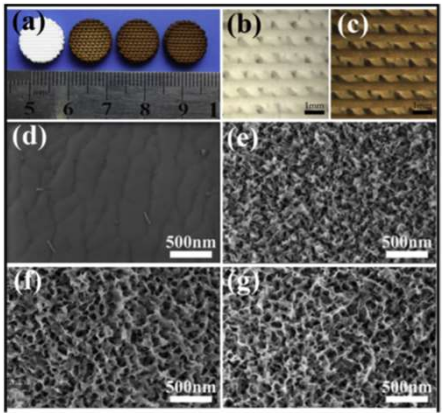
Bone tissue engineering – Microstructure & nanostructure

- **Self-assembly method**
  - (a) Photos of 3D-printed pure bioceramics (BC), DOPA-BC (2 mg/mL), DOPA-BC (4 mg/mL), DOPA-BC (6 mg/mL) scaffolds, respectively; Optical photos of pure BC (b) and 4 mg/mL DOPA-BC (c) scaffolds on the top view; SEM images of pure BC (d) and 2 mg/mL DOPA-BC (e), 4 mg/mL DOPA-BC (f), 6 mg/mL DOPA-BC (g) scaffolds, showing a uniform nanolayer composed of amorphous Ca-P nanoparticles and polydopamine on DOPA-BC surface.



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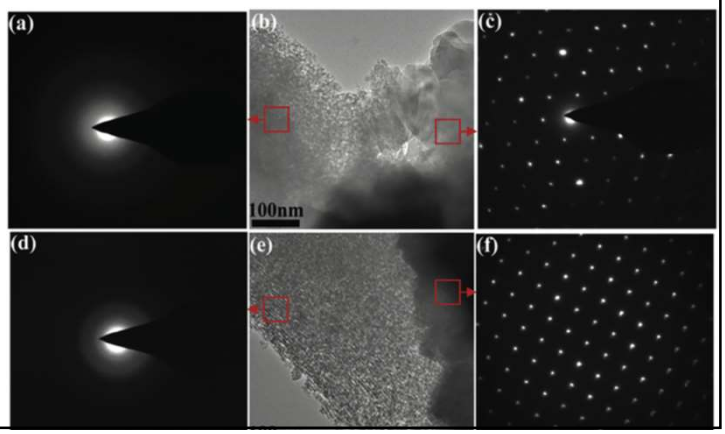
Bone tissue engineering – Microstructure & nanostructure

- Spin coating method
  - TEM images for the coated BG (b) and MBG (e) layers on b-TCP scaffolds. The MBG layer presents a well-ordered mesoporous structure with a mesoporous pore size of 5 nm (e). Electron diffraction patterns reveal the amorphous states of the coated BG (a) and MBG (d) layers as well as the crystalline state of the substrate of b-TCP scaffolds (c, f)



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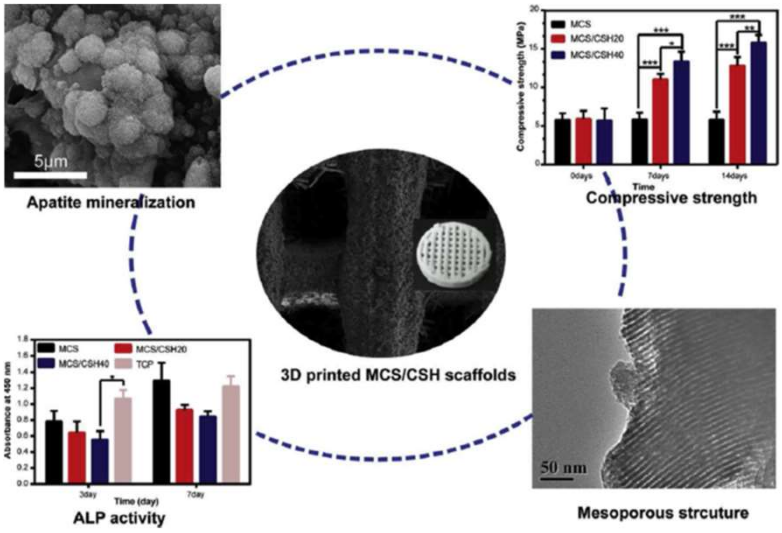
Bone tissue engineering – Microstructure & nanostructure

- Hydration process



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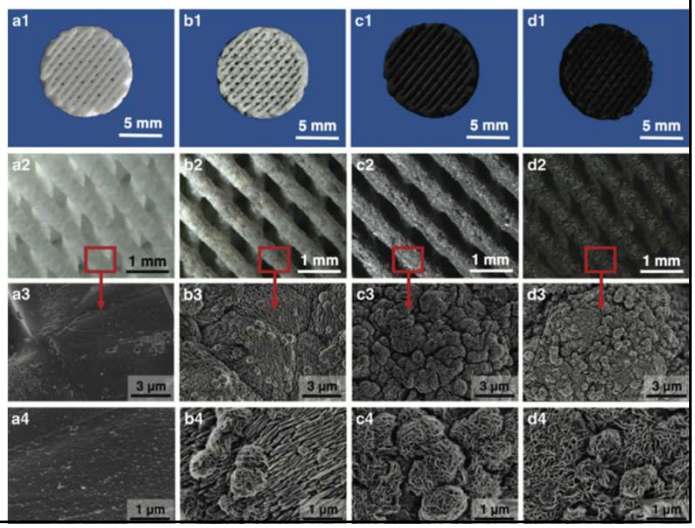
Bone tissue engineering – Microstructure & nanostructure

- Combined a 3DP technique with a hydrothermal method to realize in situ growth of molybdenum dissulfate MoS2 nanosheets on the strut surface of bioceramic.



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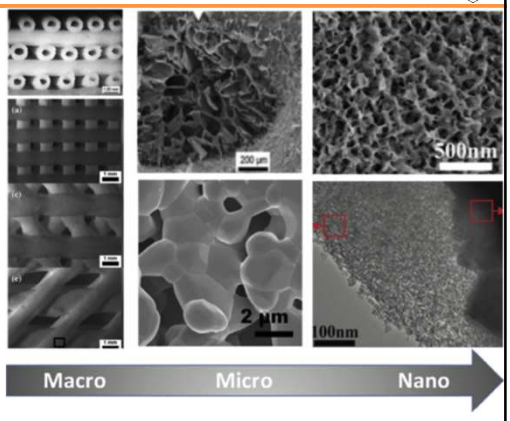
3D printing for bone scaffolds

- Fabrication through 3D printing
  - Stereolithography (SLA)
  - Selective laser sintering (SLS)
  - Fused deposition modeling (FDM)
  - Binder-based 3DP3DP



Characteristics of different 3DP techniques.

Printing techniques	SLA	SLS	FDM	3DP
Forming principle	Photo polymerization	Powder sinter	Melt extrusion	Powder + binder deposition
Material type	Liquid based	Powder based	Solid based	Powder based
Materials	Polymers, ceramics	Metals, ceramics, polymers	Thermoplastic polymer, ceramics	polymers/ceramics
Accuracy	Excellent	Good	Average	Average
Cost	High	High	Low	Low
Post cure	Yes	Yes	No	No
High temperature	No	Yes	Yes	No

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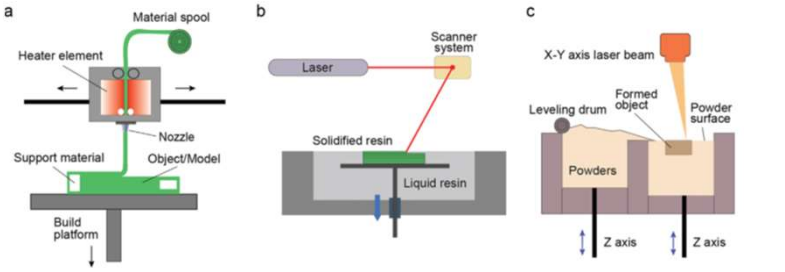
3D printing for bone scaffolds



Fused Deposition Modeling (FDM) | Stereolithography (SLA) | Selective Laser Sintering (SLS)

Comparison of conventional 3D printing technologies of bone engineering.

	Materials for 3D printing	Advantages	Disadvantages
SLA [27,29,30,31,34], [35]	PPF/DEF PPF/DEF-HA PDLLA/HA β-TCP	High accuracy Smooth surface finish Complex internal features can be obtained Growth factors, proteins, and cell patterning is possible	Average build volume Sensitive to long exposure Only applicable for photopolymers
FDM [36,37,38,39,43]	TCP TCP/PP Al_2O_3 PCL TCP/PCL	Fast Low-cost consumer machines and materials No need for platform/support	Low accuracy Low details Limited design compatibility Material restriction due to need for molten phase
SLS [44,45,46,47,50]	PCL Nano HA CaP/PHBV CHAp/PLLA PLLA β-TCP PHBV	Strong functional parts Design freedom No need for support No post processing is needed	Rough surface finish Limited material options Feature resolution depends on laser beam diameter

SLA, Stereolithography; FDM, Fused deposition modeling; SLS, Selective laser sintering; PPF, Poly(propylene fumarate); DEF, Diethyl fumarate; HA, Hydroxyapatite; PDLLA, Poly(D,L-lactic acid); TCP, Tricalcium phosphate; PP, Polypropylene; PCL, Poly-e-caprolactone; PHBV, Poly(hydroxybutyrate–cohydroxyvalerate); CHAp, Carbonated hydroxyapatite; PLLA, Poly(L-lactic acid).

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4D printing for bone scaffolds

- 4D printing is a term referring to printed constructs that are designed to change form and function after 3D printing based on response to an environmental stimuli, thus offering additional capabilities and performance-driven applications.
- Using the same technologies as 3D printing but based on the development of novel stimuli responsive biomaterials which interact with environmental factors (e.g., humidity, temperature, or chemicals), and changes their forms accordingly.

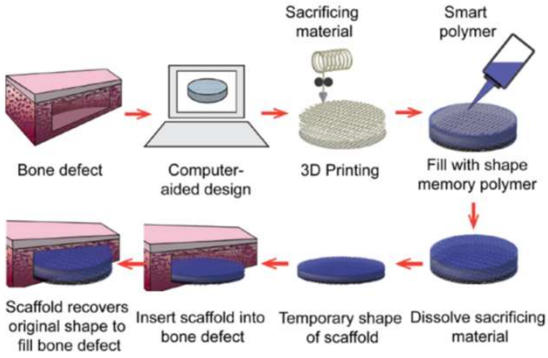

Fig. 2. (Upper row). Workflow of the 4D printing process. (Bottom row). Illustration of using a shape memory material to repair bone defects in which the scaffold size changes after implantation to occupy the void space, commonly referred to as 4D printing.

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Bone tissue engineering – bioceramic scaffolds

- An ideal scaffold for bone tissue regeneration is designed to mimic the structure and biological function of a healthy bone tissue in terms of both chemical compositions and hierarchical structure as well as properties.
- Bioceramic scaffolds (e.g., calcium phosphate ceramics, calcium silicate ceramics, and bioactive glasses)
  - similarity to native bone inorganic components,
  - biocompatibility,
  - hydrophilicity,
  - bioactivity,
  - osteoconductivity, and osteoinductivity.
- Bioceramic scaffolds have been designed with a hierarchical structure consisting of the macro-, micro-, and nanostructures.

Composition of printable inks and printing parameters.

Powder	Binder	Printing parameters
HA + PLGA	1,4-dioxane	Pressure:110 kPa, speed: 18 mm/s
Polycaprolactone (PCL)+HA + CNT	dichloromethane (CH_2Cl_2)	Diameter needle: 0.45 mm
TCP + 1 wt% SrO + 1 wt% MgO	Polyamide	Interconnected pore size: 500, 750, and 1000 μm
HA and β-TCP	Phosphoric acid binder	Powder layer thickness: 0.10 mm, binder spray: 0.30 L/m^2
Tricalcium silicate (Ca_3SiO_5, C_3S)	Hydroxypropylmethylcellulose	Pressure: 200–400 kPa, speed: 10 mm/s.
Apatite–wollastonite glass ceramic + hydroxyapatite	Dextrin	Layer thickness: 0.1 mm
$Sr_5(PO_4)_2SiO_4$	F 127	Pressure: 300–500 kPa, speed: 6 mm/s
Mn-doped TCP + sodium alginate	F 127	Speed: 6 mm/s, pressure: 1.5–2.5 bar.
$Ca_7Si_2P_2O_{16}$ + sodium alginate	F 127	shell/core nozzle: G 16/21, 16/22, 16/23, 18/25, and 20/27.
$Ca_2MgSi_2O_7$ + sodium alginate	F 127	struts diameter: 1.5 mm, channel diameter: 400–600μ m

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Bone tissue engineering – bioceramic scaffolds

- Calcium phosphate bioceramic scaffolds:
  - Certain calcium phosphate ceramics are osteoinductive in the absence of supplements.
  - Unadeqate mechanical property limits the clinical application.
- **Calcium silicate bioceramic scaffolds CS (CaSiO3)**
- **Bioactive glasses**
- **Hydroxyapatite** (HA) (Ca10(PO4)6(OH)2)
  - Chemical composition same as that of the main bone components
    - Leading to positive influences on adhesion and proliferation of osteoblasts.
    - Slow degradation rate and poor mechanical strength as well as fracture toughness of pure HA hinder complete bone formation and possibly increases the risk of infection.
      - combined with different compositions like ZrO_2, carbon fiber, and Al_2O_3 to improve the mechanical characteristics. ZrO_2, carbon fiber, and Al_2O_3 are bioinert materials that reduce the bioactivity of HA significantly.
      - combined with natural polymers – such as polylactide-co-glycolide acid (PLGA) – favorable biodegradability and good biocompatibility.

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Bone tissue engineering – bioceramic scaffolds

- **Tricalcium phosphate** (TCP),
  - First attempt to implant β-TCP as an artificial material to repair surgically created fractures in rabbit bones was made in 1920 by the American surgeon Fred Houdlette Albee.
  - Good biocompatibility and osteoconduction.
  - Ability to form a strong bone–calcium phosphate bond.
  - Better biodegradability than other biomaterial implants including HA.
  - Main mechanism of bioactivity of TCP is the partial dissolution and release of Ca and phosphate ion products thus forming a biological apatite precipitate on the surface of the bioceramic scaffold.
  - The bending strength and fracture toughness of β -TCP bioceramic scaffolds are better than those of HA bioceramic scaffolds but still lower than those of the human cortical bone.
    - Therefore, β-TCP bioceramic scaffolds cannot be used for load-bearing implants.
  - Degradation rate of β-TCP bioceramic scaffolds cannot match the growth rate of the new bone tissue.

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Bone tissue engineering – bioceramic scaffolds

- **Biphasic calcium phosphate** (BCP)
  - Intimate mixture of hydroxyapatite and beta-tricalcium phosphate.
  - Good biocompatibility, bioactivity, and osteoconduction.
  - Combined with HA and β -TCP.
  - Compared with pure HA and pure β-TCP the BCP ceramics exhibit controllable degradation rate, better biocompatibility, and enhanced bone regeneration ability.
  - A large difference between HA and β -TCP scaffolds in sintering temperature leads to poor sintering quality of BCP ceramics.
  - Due to low mechanical properties, BCP ceramics also cannot be used for load-bearing bone tissue regeneration.

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Bone tissue engineering – bioceramic scaffolds

- Doped elements
  - Zinc
    - The incorporation of Zn in HA ceramics improves the dissolution property.
    - Promotes osteoblast differentiation from bone marrow stromal cells in vitro.
    - Ehances new bone formation in vivo by promoting osteoblast mineralization.
    - Suppressing osteoclast differentiation by antagonism toward NF-jB activation driven by TNFa, a suppressor of bone formation in vitro and in vivo.
  - Strontium
    - pure-phase $Sr_5(PO_4)_2SiO_4$ bioactive ceramic scaffold prepared by combining 3DP technique with a solid-state reaction method.
      - Significantly stimulated the proliferation and angiogenesis-related gene expression (KDR, VEGF, eNOS, and HIF1a) of HUVECs.
      - Could activate the Ca-sensing receptor, thus leading to the mitogen-activated protein kinase signaling pathway and the activation of inositol triphosphate production.

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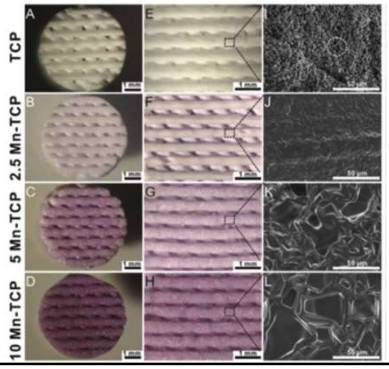
Bone tissue engineering – bioceramic scaffolds

- Manganese
  - Stimulation of cell adhesion and proliferation
  - Enhancement of the bioactivity of cartilage oligomeric matrix protein.
  - Promotion of osteogenic activity in vitro.
  - Especially for the regeneration of both cartilage and subchondral bone tissues owing to the synergetic effect of Mn and Ca ions released from the scaffolds, which can stimulate the maturation of chondrocytes and regeneration of cartilage significantly.



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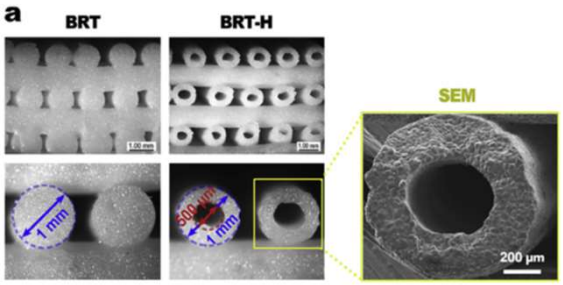
Bone tissue engineering – bioceramic scaffolds

- Magnesium
  - Can affect **vascularization** and bone regeneration.
    - Scaffolds with modified core/shell printing setup by coaxial 3DP for fabricating hollow-pipe-packed silicate bioceramic Ca7MgSi4O16 (BRT-H).
      - The released ions (including Mg, Ca, and Si ions) demonstrated several effects for stimulating angiogenesis and osteogenesis.
      - In addition, the hollow structure of scaffolds showed a synergistic effect with the released ions on promoting vascularization



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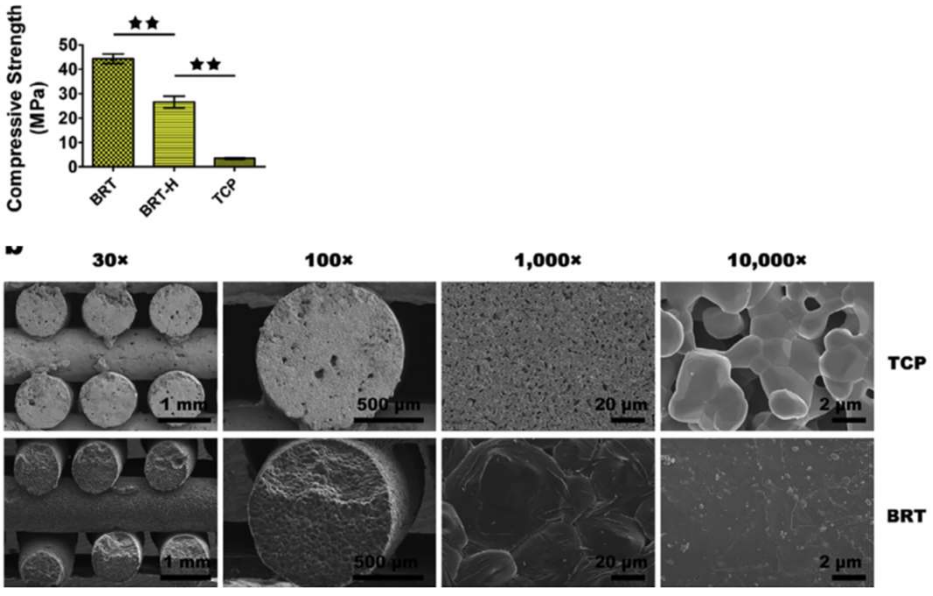
Bone tissue engineering – bioceramic scaffolds



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