FORTH

Foundation for Research & Technology - Hellas

2.5D and 3D printing of biomedical implants

Maria Farsari,
IESL-FORTH
mfarsari@iesl.forth.gr

Outlook

- Principles of Direct Laser Writing
- Materials for Direct Laser Writing
- Applications
    - Microfluidic Medical Implants
    - Scaffolds for Cell Growth and Tissue Engineering
    - Scaffold-less Tissue Engineering

Principles of Direct Laser Writing

Laser: a tool for 3D photopolymer nanostructuring.

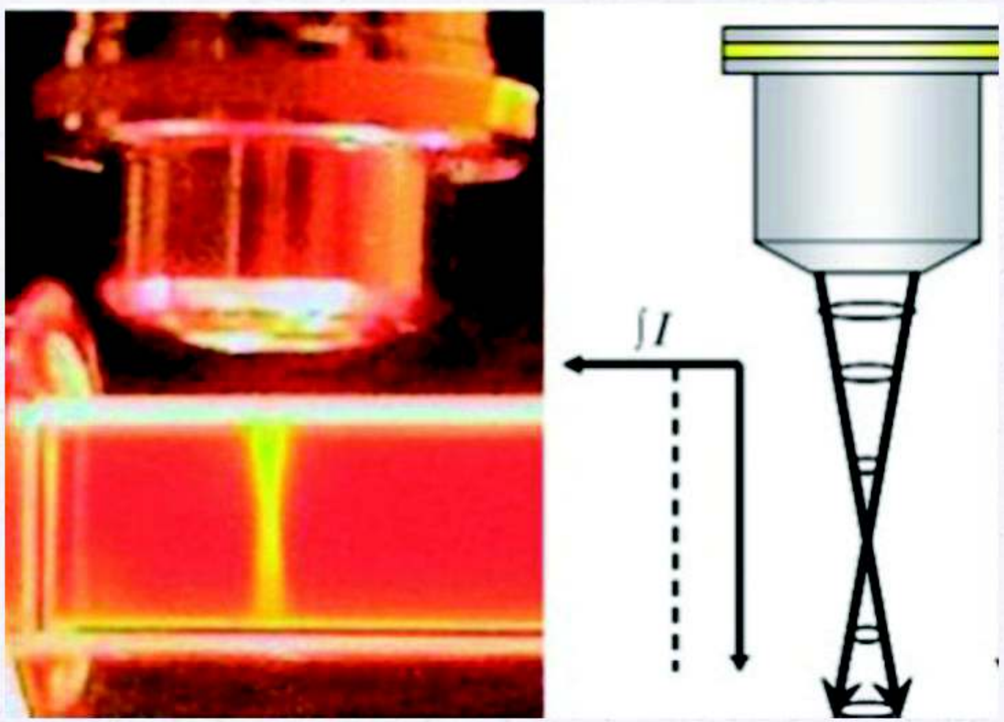
- Photopolymer: a liquid or gel which becomes solid when exposed to appropriate light.

- The use of lasers in 3D structuring is limited by
  - Light absorption at the surface of the material
    - Beer's law: $I(x)=I_0e^{-acx}$
    - can be overcome by nonlinear multiphoton absorption

  - The diffraction limit
    - $d=a*\lambda/(N.A.)$
    - can be overcome by employing materials with well-defined photo-polymerization threshold

Multi-photon polymerization

One-photon polymerization

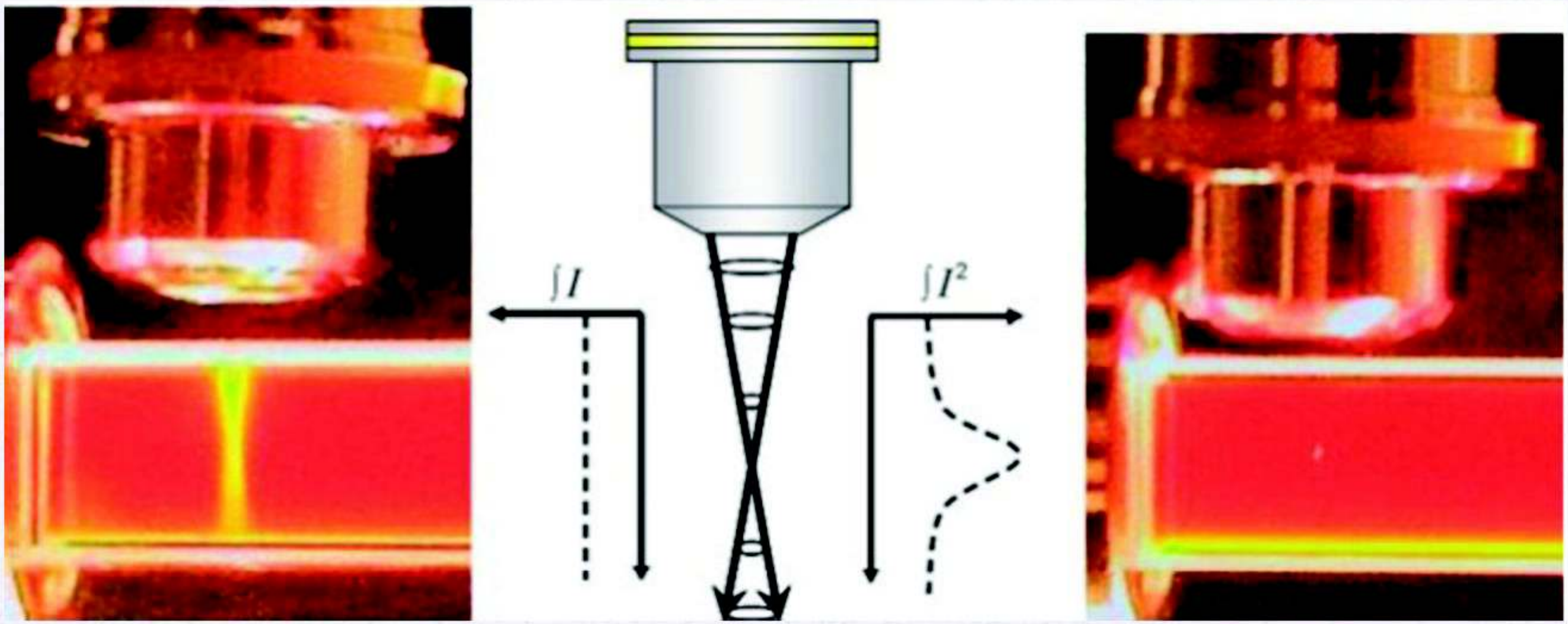


Picture: C.N. LaFatta et al., Angew. Chem. Int. Ed. 2007, 46, 6238 – 6258.

Multi-photon polymerization

One-photon polymerization

Multi-photon polymerization

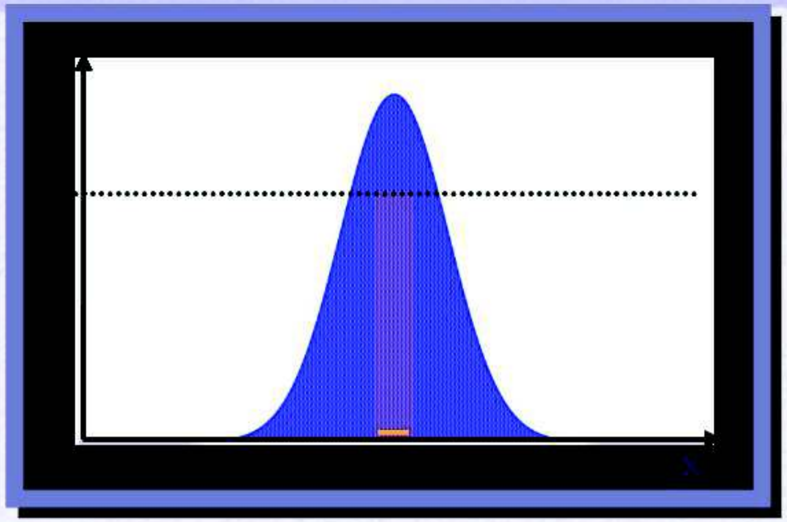


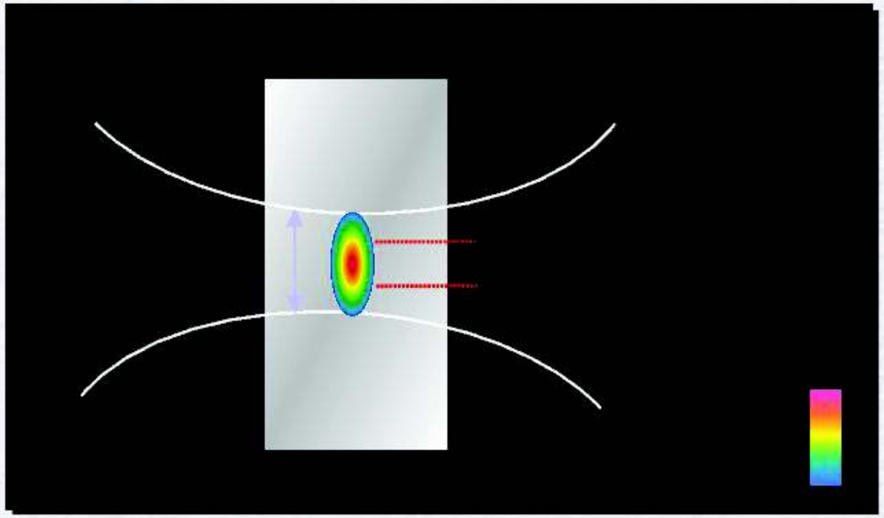
Picture: C.N. LaFatta et al., Angew. Chem. Int. Ed. 2007, 46, 6238 – 6258.

3D structuring

"Ballerina"
by Mary Manoussidaki,
IESL-FORTH, 2011.

Sub-diffraction limit structuring





$$W_{(2)} \sim \sigma_{(2)} I^2$$

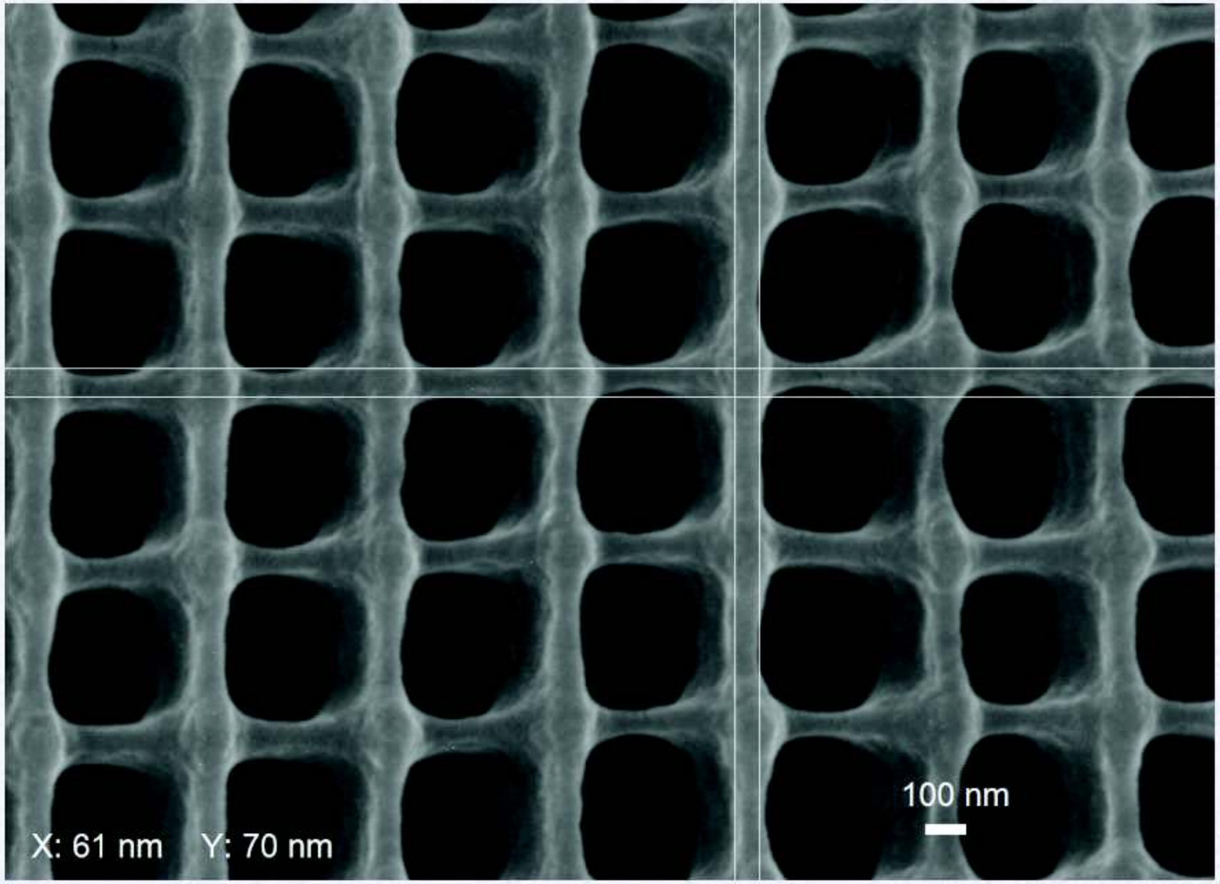
$W_{(2)}$: 2-photon absorption probability
$\sigma_{(2)}$: 2-photon absorption cross-section
I: Intensity of the Gaussian pulse

+

Threshold Material

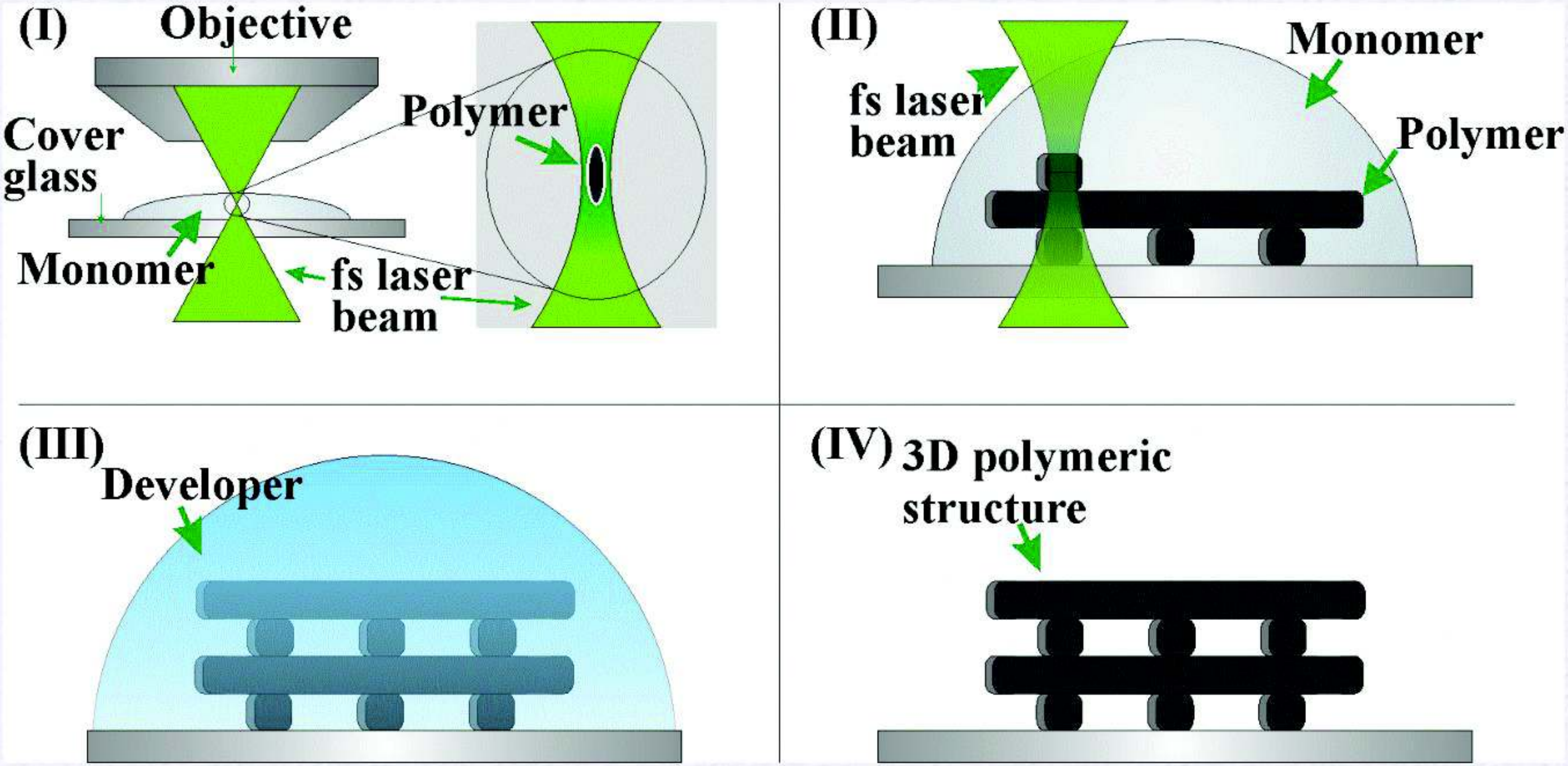
→ High resolution, in-volume structuring

Sub-diffraction limit structuring



Sakellari, I. *et al.* Diffusion-Assisted High-Resolution Direct Femtosecond Laser Writing. *ACS Nano* **6**, 2302-2311, (2012).

NonLinear Lithography

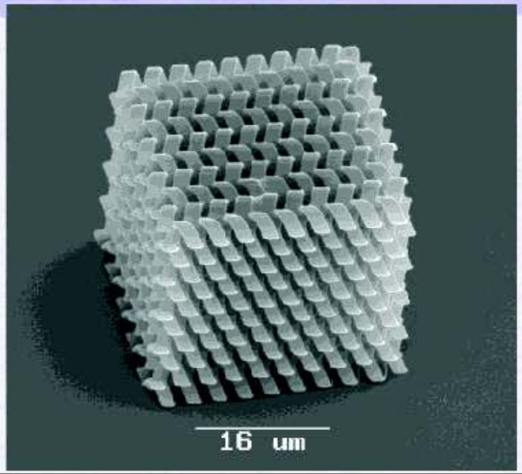


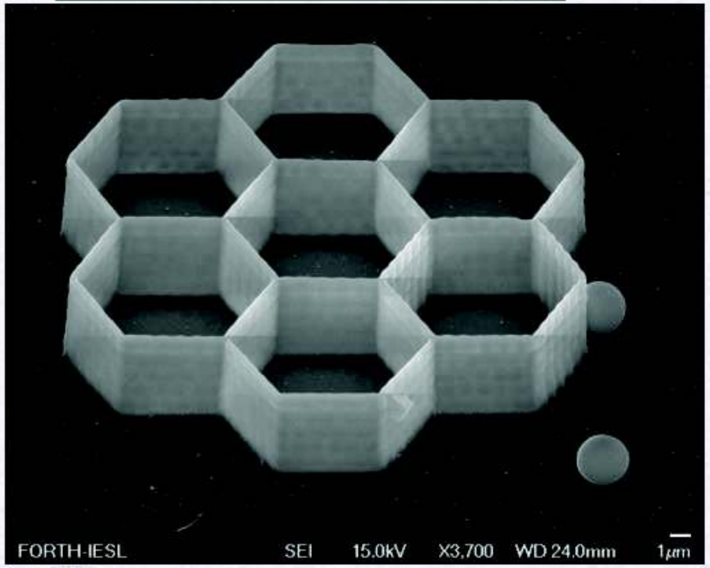
Materials

Hybrid Photosensitive Materials

- Majority of applications involve commercially-available negative photo-resists such as SU8.
- Good structural results but limited flexibility when it comes to bulk or surface functionalization.
- <u>Sol-gel Chemistry:</u> a chemical process for the incorporation of inorganic compounds into organic molecules.
- Photosensitive hybrids undergo through two polymerizations, inorganic and organic.
- Easily functionalized, surface and bulk.

Zirconium, Titanium, Germanium, Ionogel & Graphene, Vanadium Silicates

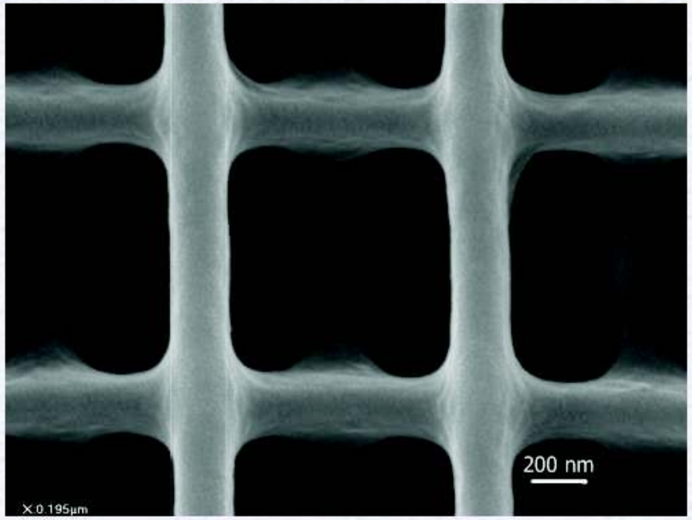


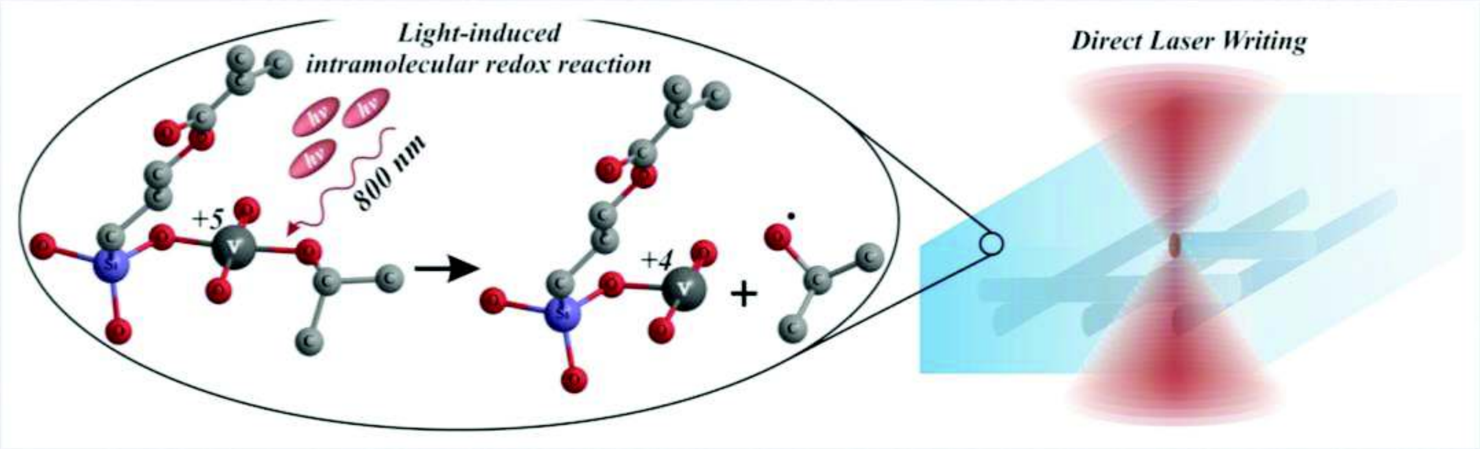


- Ovsianikov, A., et al. (2008). ACS Nano **2**(11): 2257
- Sakellari, I., et al. (2010). Appl.Phys.A **100**: 359
- Terzaki, K., et al. (2011). Opt. Mater. Expr. **1**(4): 586
- Vasilantonakis, N., et al. (2012). Adv.Mater. **24**(8): 1101
- Sakellari, I., et al. (2012). ACS Nano **6**(3): 2302
- Malinauskas, M., et al. (2012). Opt. Lasers Eng. **50**(12): 1785
- Oubaha, M., et al. (2012). J. Mater. Chem.**22**(21): 10552.
- Kabouraki, E., et al. (2013). Nano Letters **13**(8): 3831.

Highlights

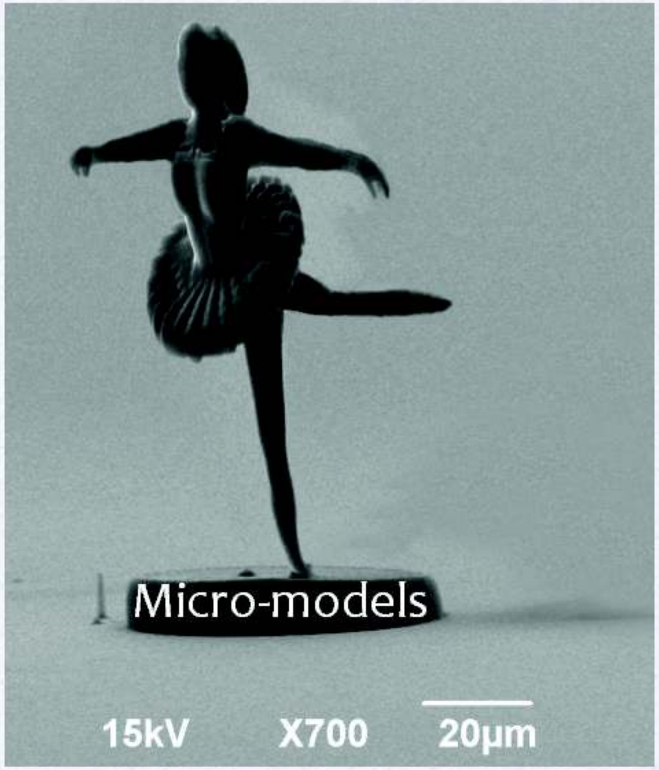
- First Demonstration of Redox Multiphoton Polymerization
- Initiator-free photopolymer
- A third-order material
- Saturation-free structuring

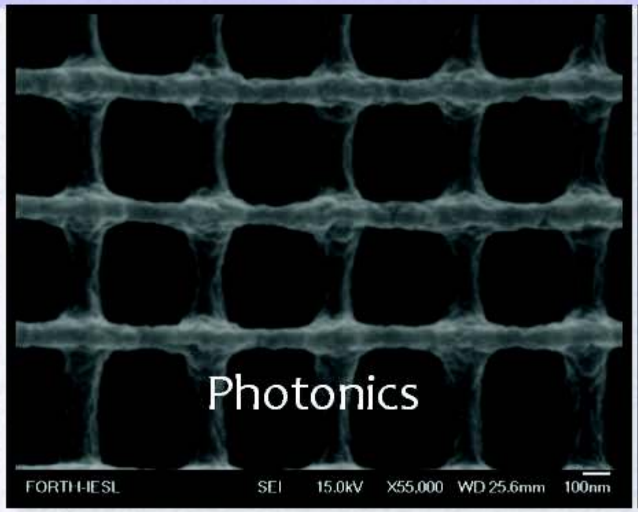


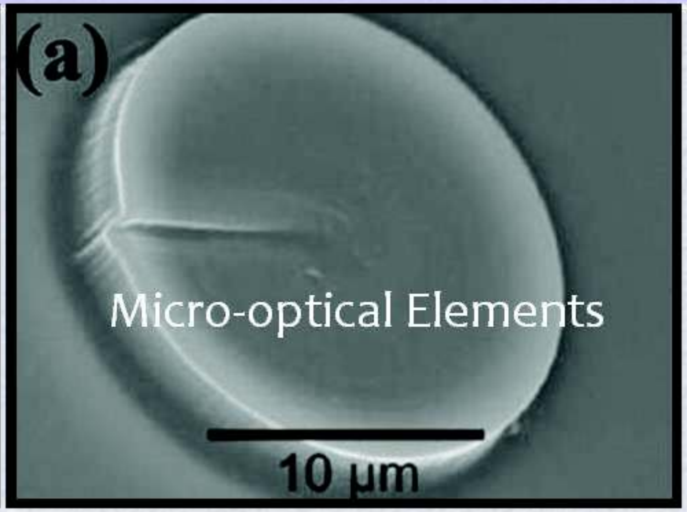


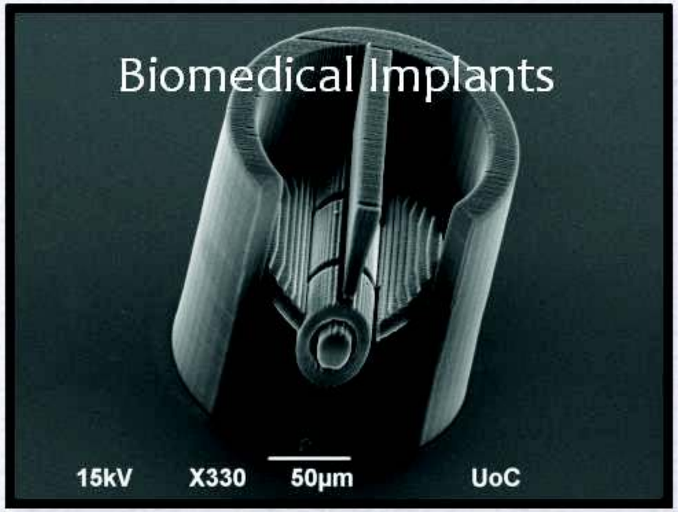
Kabouraki E et al. (2013) Redox Multiphoton Polymerization for 3D Nanofabrication. Nano Letters 13:3831-3835.

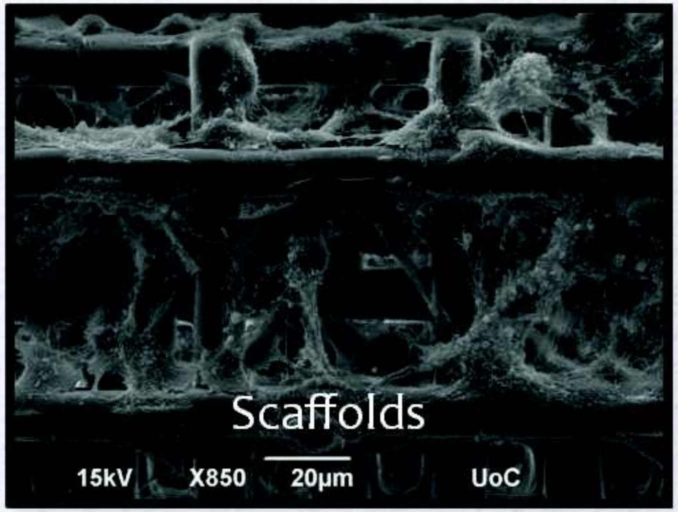
Applications











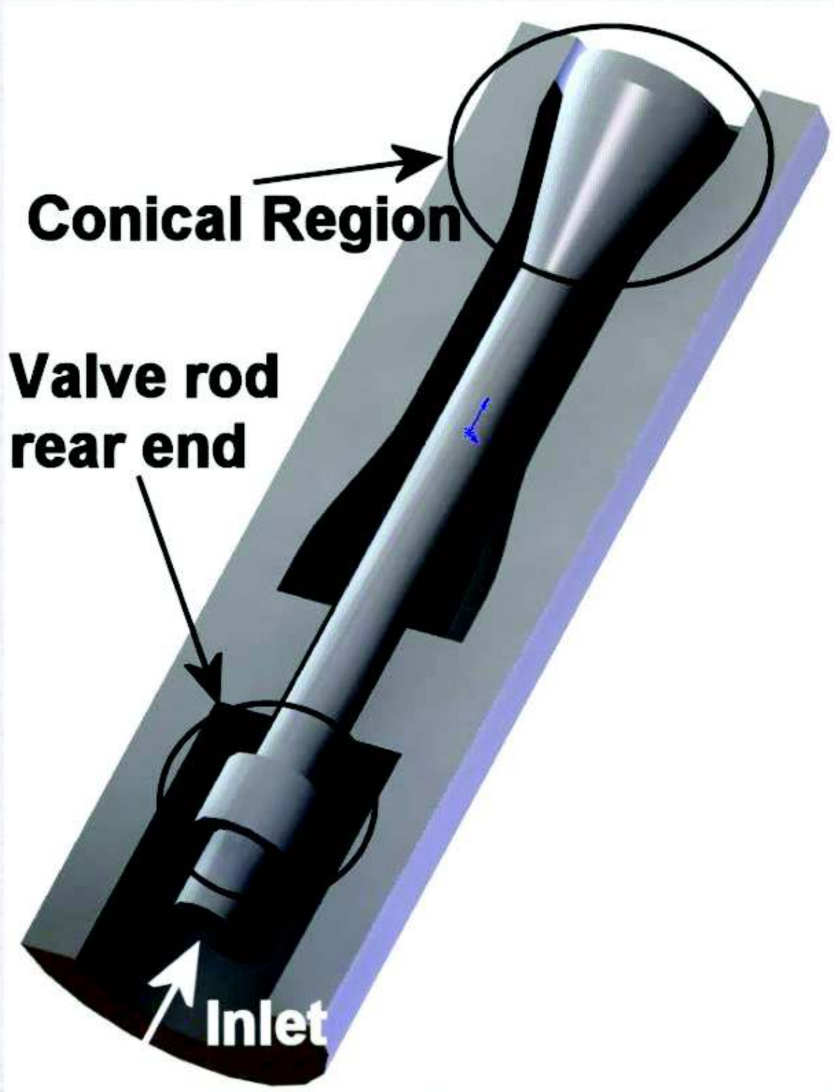
FORTH
Foundation for Research & Technology - Hellas

Microfluidic Medical Implants*

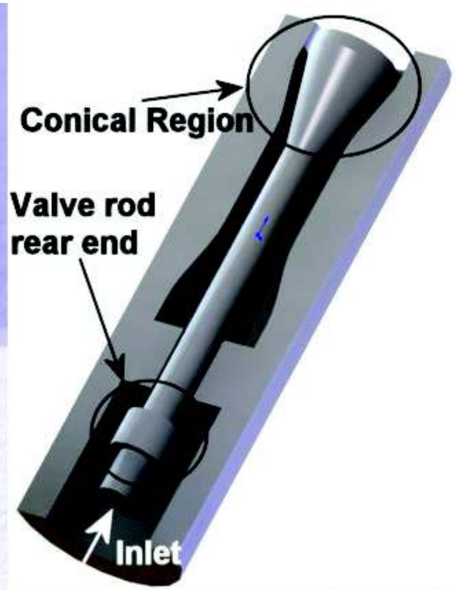
* In collaboration with D. Karalekas

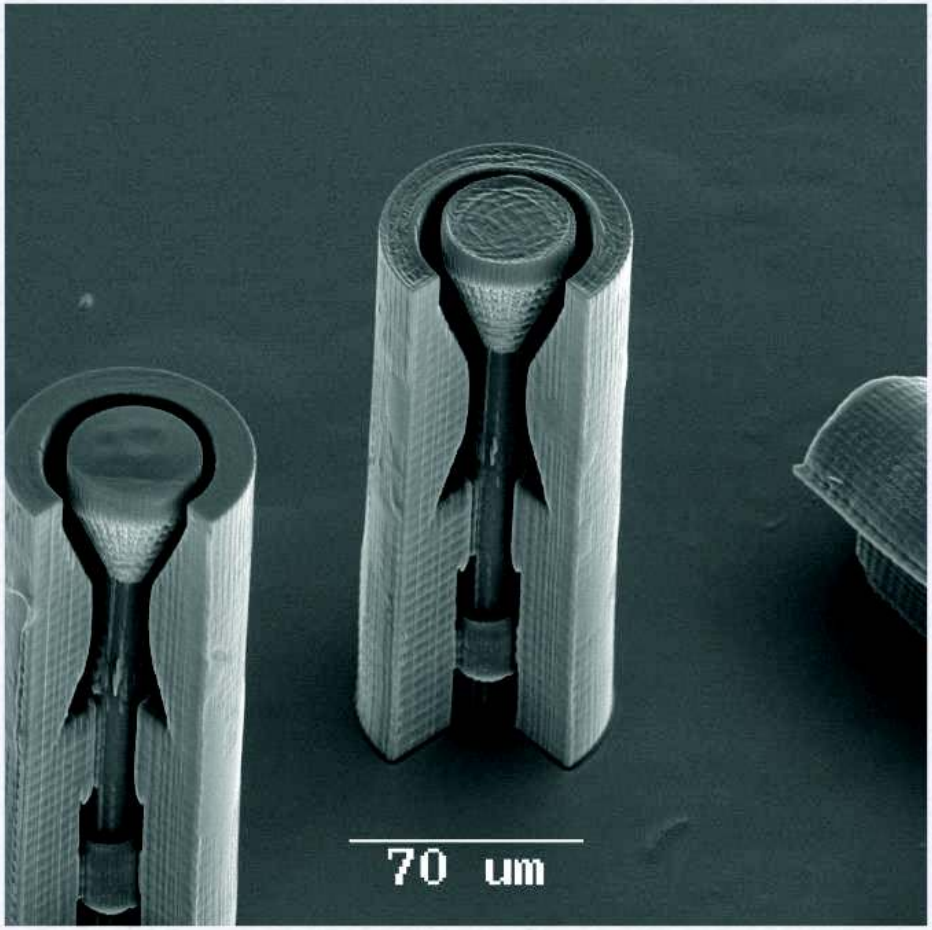
Microfluidics: Blood-flow control

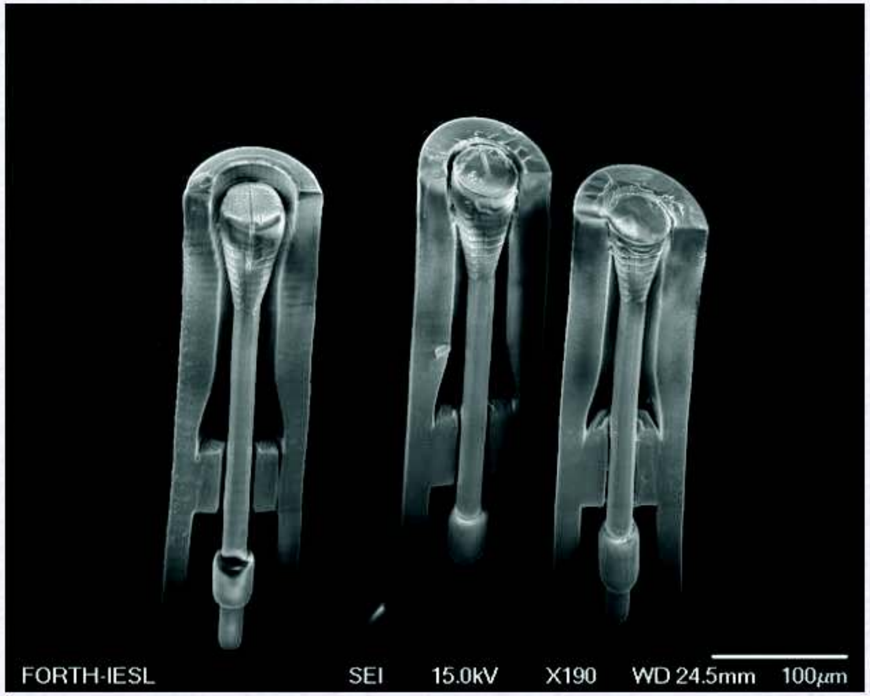
- Containing two non-contact parts:
  - main body
  - moving piston-rod
- Both parts fabricated in a single step
- The valve is designed to open under forward fluid flow and close in backward flow



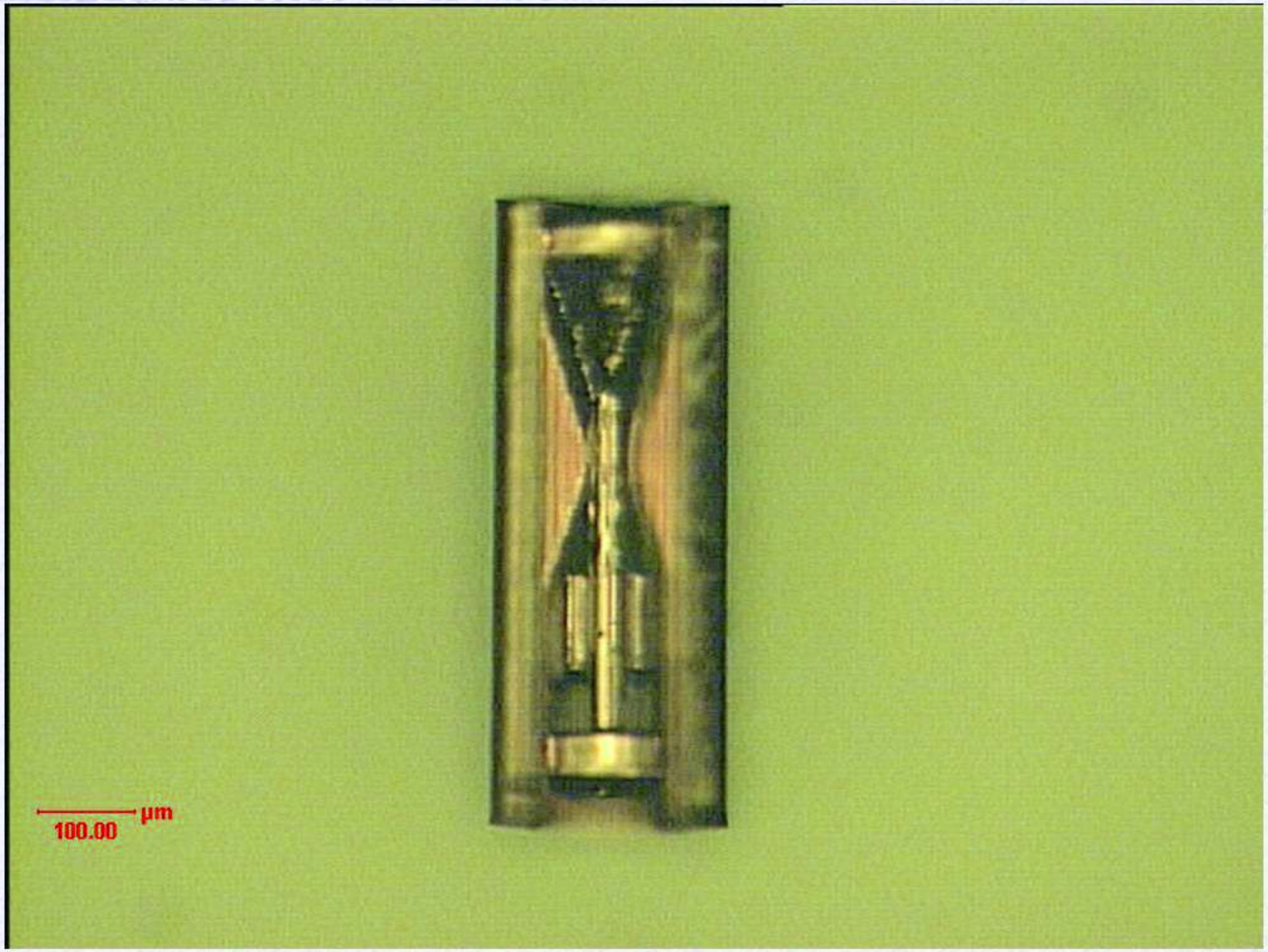
The valve


Conical Region
Valve rod
rear end
Inlet


70 um


FORTH-IESL SEI 15.0kV X190 WD 24.5mm 100μm

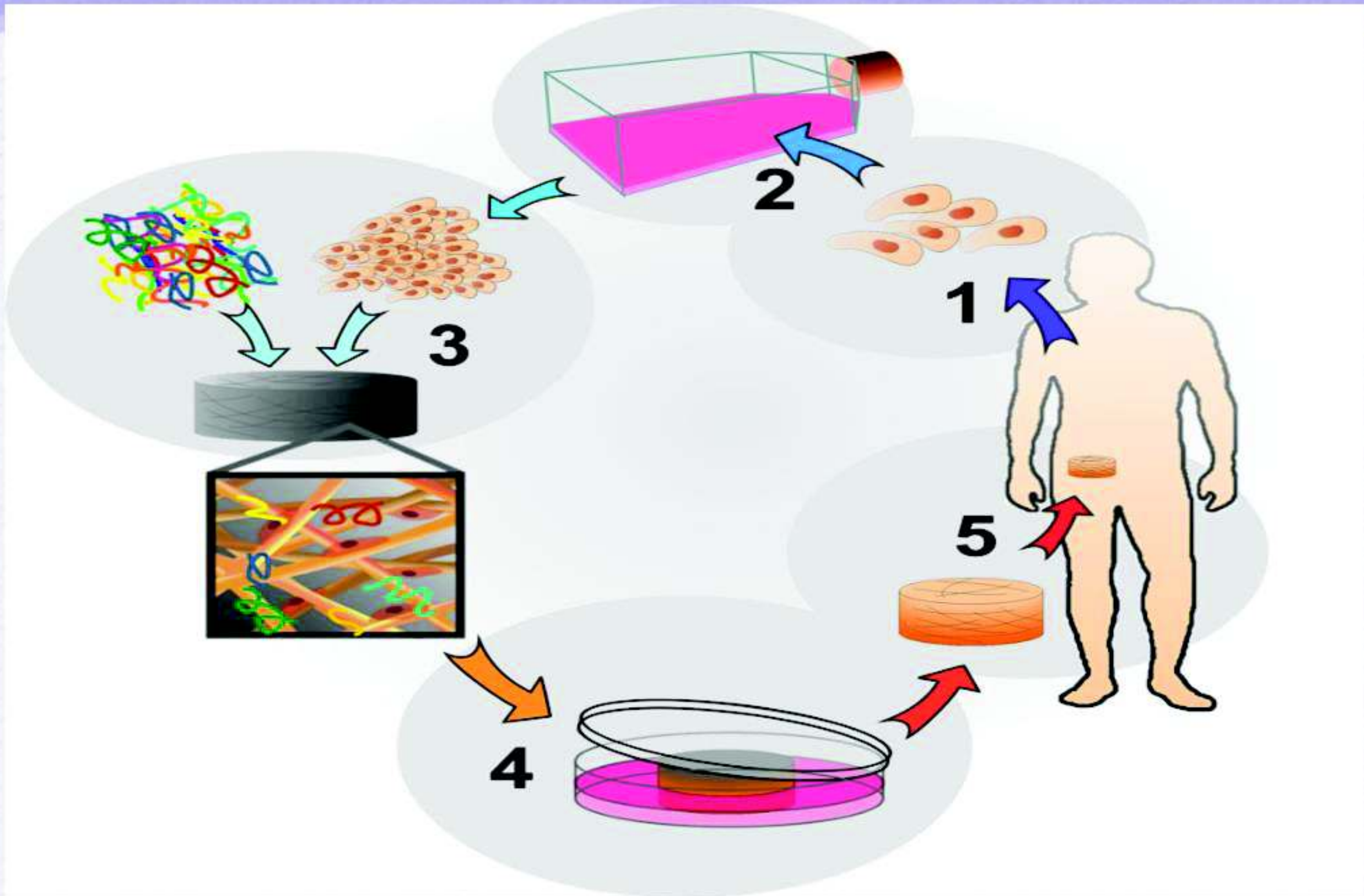
Microvalve: Open



Microvalve: Closed

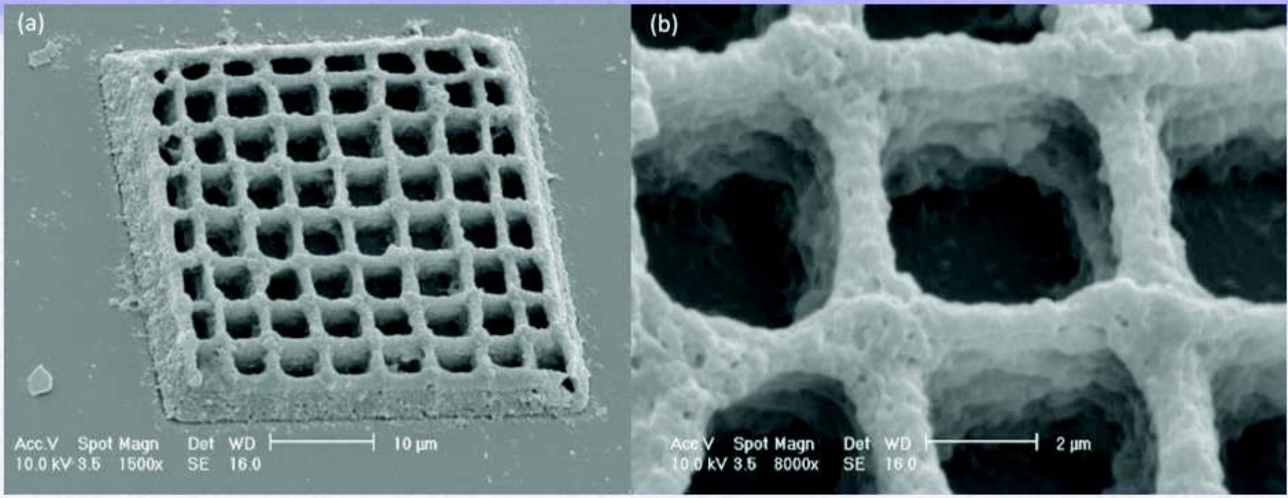
Scaffolds for Cell Growth and Tissue Engineering

Tissue Engineering using scaffolds



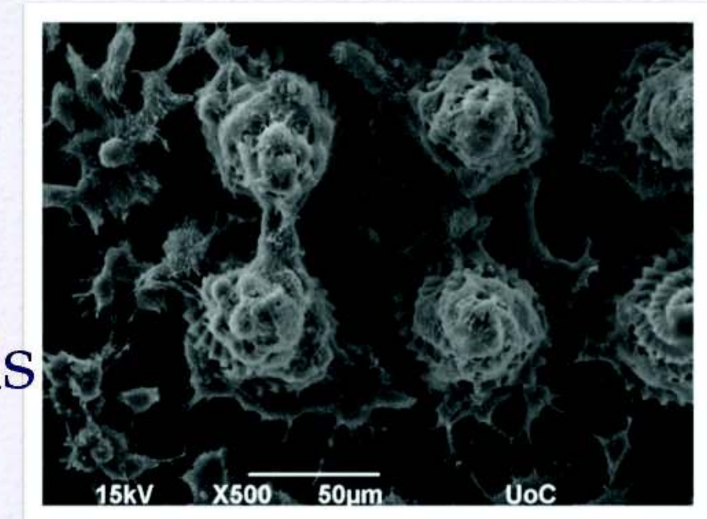
http://biomed.brown.edu/Courses/BI108/BI108_2007_Groups/group12/Homepage.html

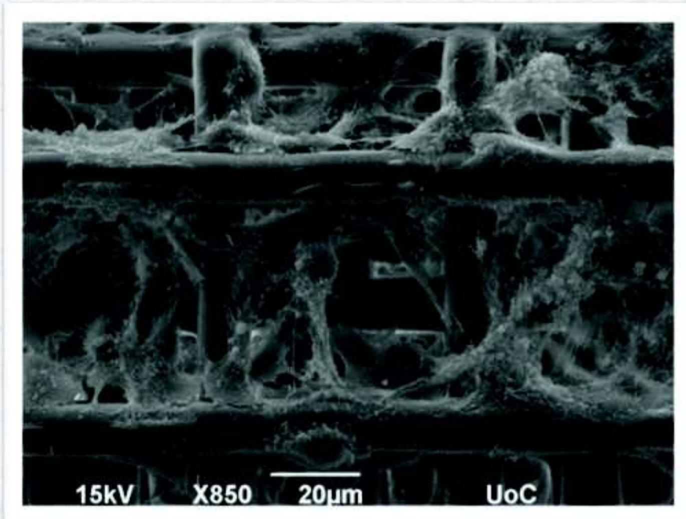
3D Scaffolds



BSA with flavin mononucleotide

Biodegradable PLA with neural cells



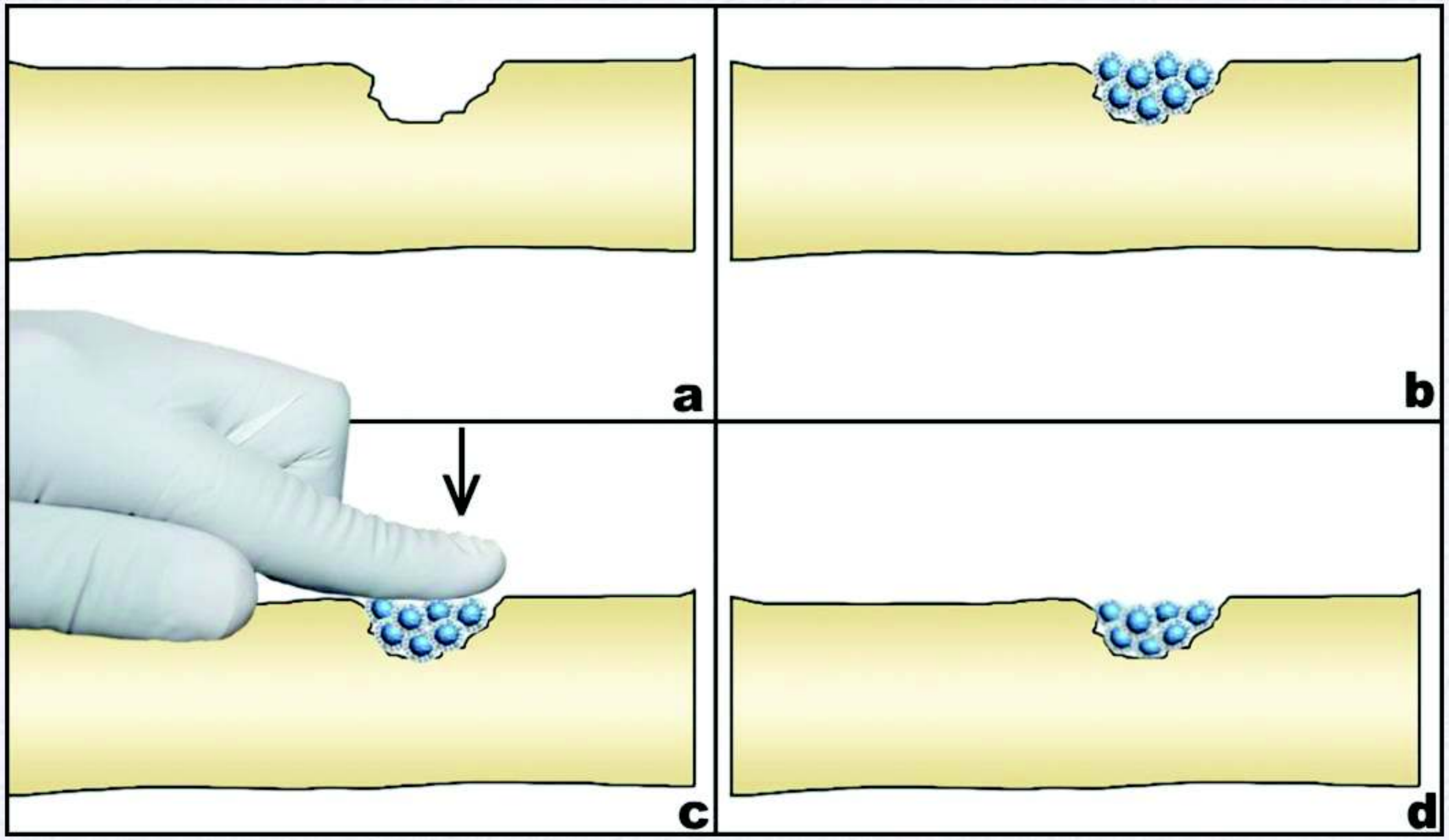


FORTH
Foundation for Research & Technology - Hellas

Lasers for Life, London, 2014.

Maria Farsari

Scaffold-free Tissue Engineering



3D bone tissue biofabrication using tissue spheroids

A third strategy …

"… there is a growing consensus that a third strategy based on the integration of a directed tissue self-assembly approach with a conventional solid scaffold-based approach could be a potential optimal solution …"

Kachouie NN, Du YA, Bae H, Khabiry M, Ahari AF, Zamanian B, Fukuda J, Khademhosseini A (2010) Directed assembly of cell-laden hydrogels for engineering functional tissues. Organogenesis 6:234-244.

Bone Regeneration: Motivation

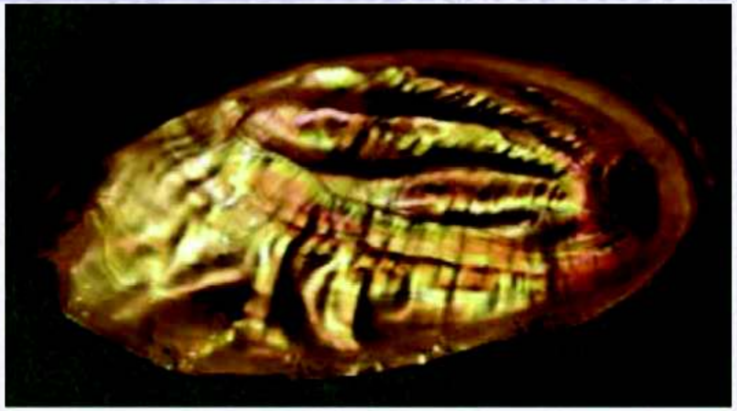
- The most common chronic diseases for elderly people are related to failure of bones and joints.
- Future health care will rely on replacement of ill and injured bone tissues.
- Rapidly developing stem cell research, material science and nanotechnology have already generated products used in medicine.

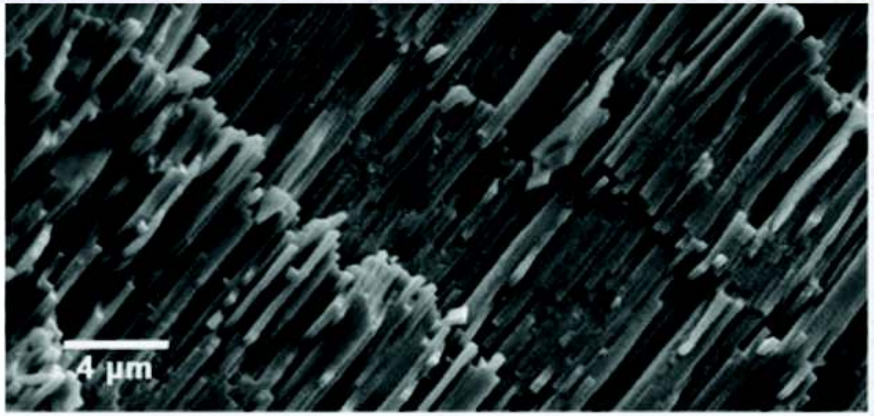
Mineralization of biomimetic 3D scaffolds: a new route to bone tissue engineering

+

A combination of
“Top-down” and **“Bottom-up”** technologies

Mimicking Nature to Create Hard Tissue

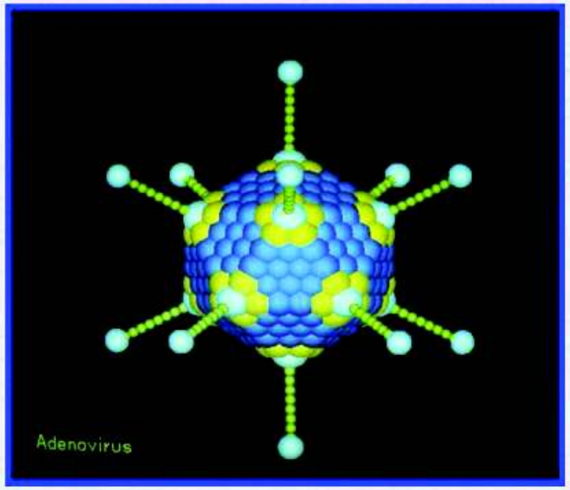




epitaxial growth of aragonite crystals between the organic matrix

- outer layer of calcite crystals
- inner layer (nacre) composed of a 'brick-wall' arrangement of plate–like aragonite crystals
- proteins direct the growth of the inorganic phase
- organic matrix mediates in order to absorb the vibrations

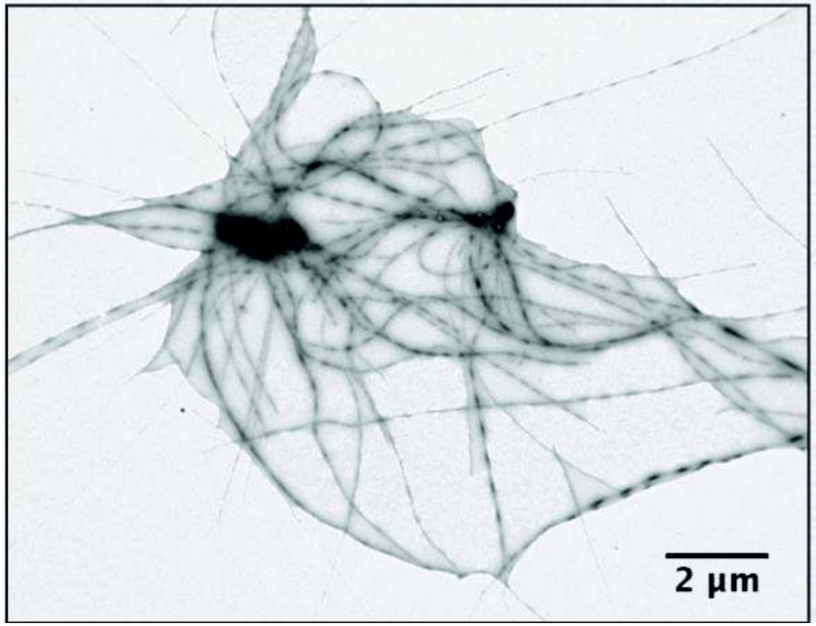
Amyloid-Type Fibrils



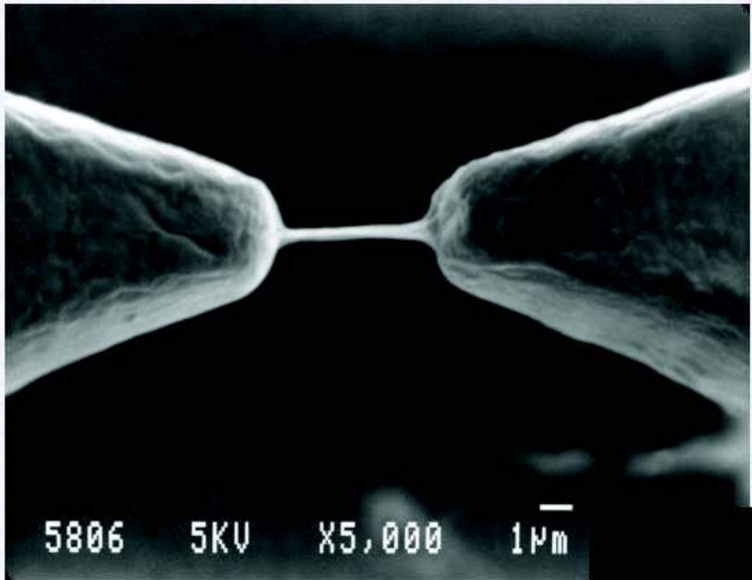
Amyloid fibrils are fibrillar aggregates that have specific morphological and structural properties. They are associated with a large group of neurodegenerative diseases.

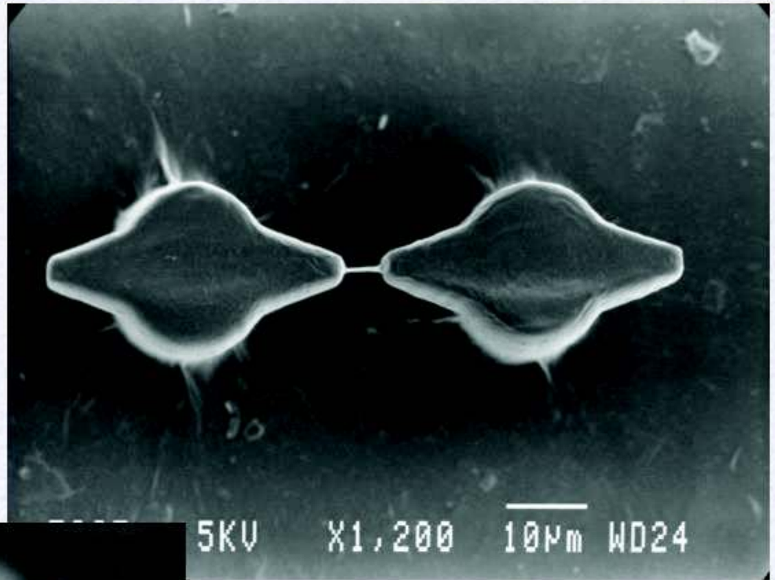
Self assembled nanostructures:

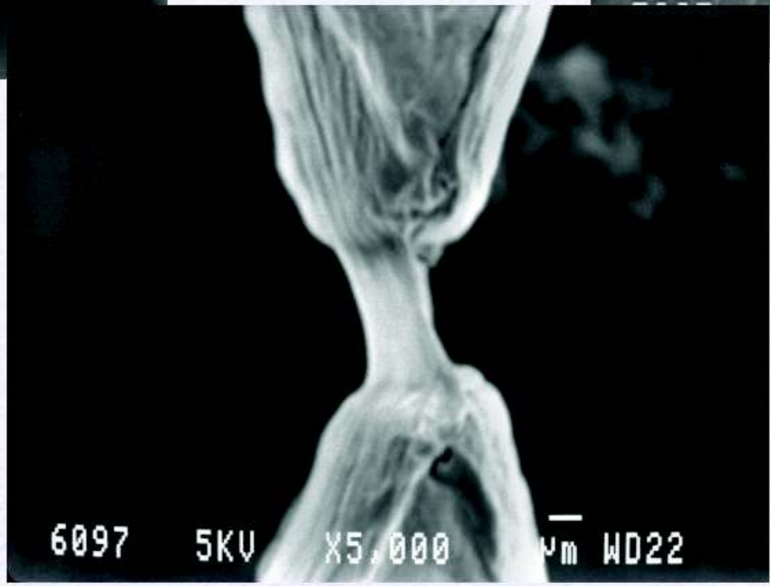
- controllable sequence
- stable
- excellent thermal and mechanical properties
- form following protein misfolding and miss assembly events
- Backbone of nacre, oysters, sponges.



Directed self assembly of peptide fibrils







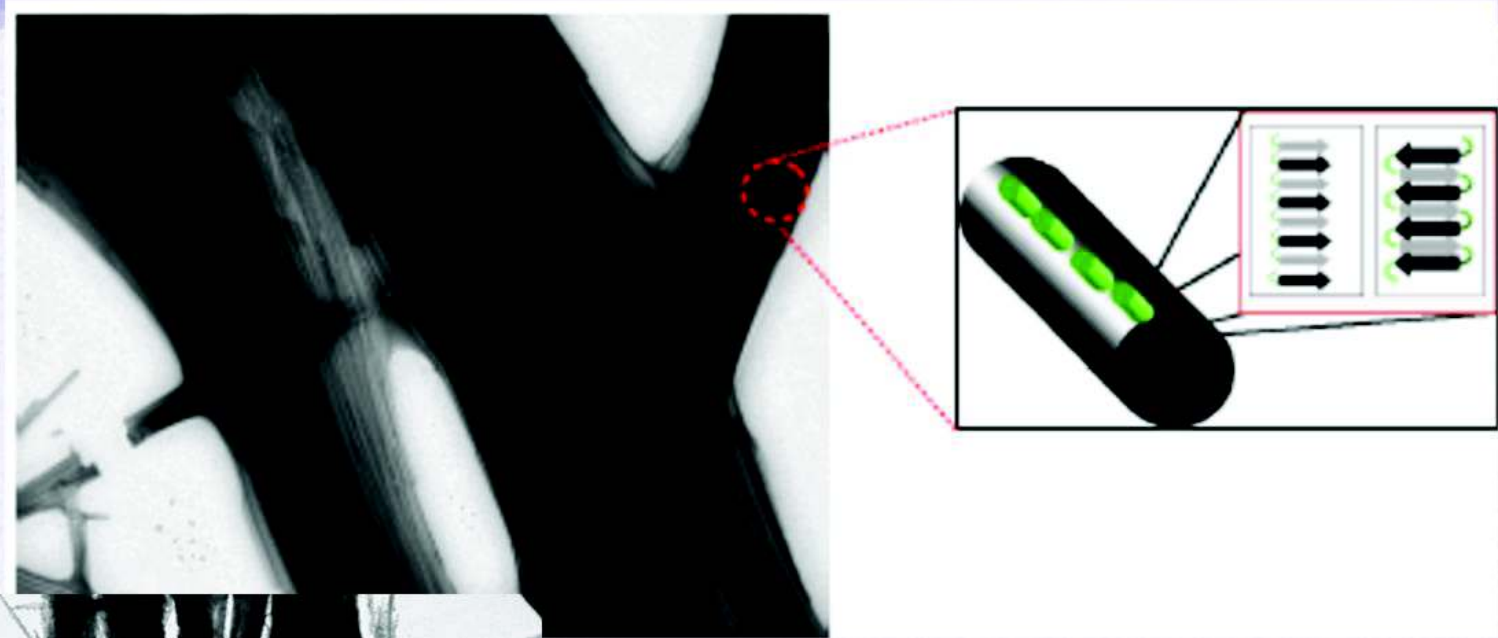
Design of calcium binding peptides for use as scaffolds for hard tissue regeneration

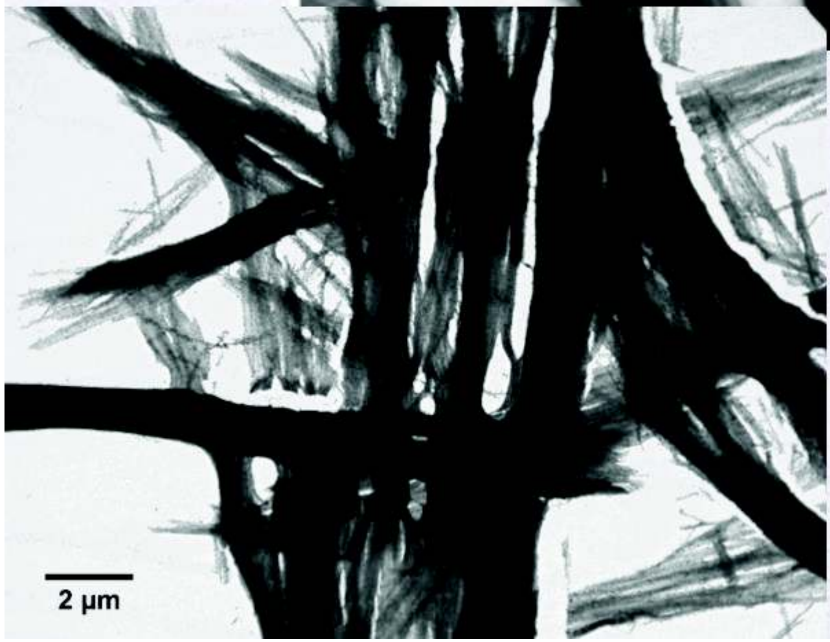
- **aspartic acid (D)** offers a strong ability for calcium binding
- strong Ca^{2+} binding requires at least **2 ligands** carrying negative charges (to have equal but opposite charges)

↓

DDS	D-D-S-G-A-I-T-I-G	H_2N-Asp-Asp-Ser-Gly-Ala-Ile-Thr-Ile-Gly-$CONH_2$
AS	A-S-G-A-I-T-I-G	H_2N-Ala-Ser-Gly-Ala-Ile-Thr-Ile-Gly-$CONH_2$

Bi-functional Peptide Fibril



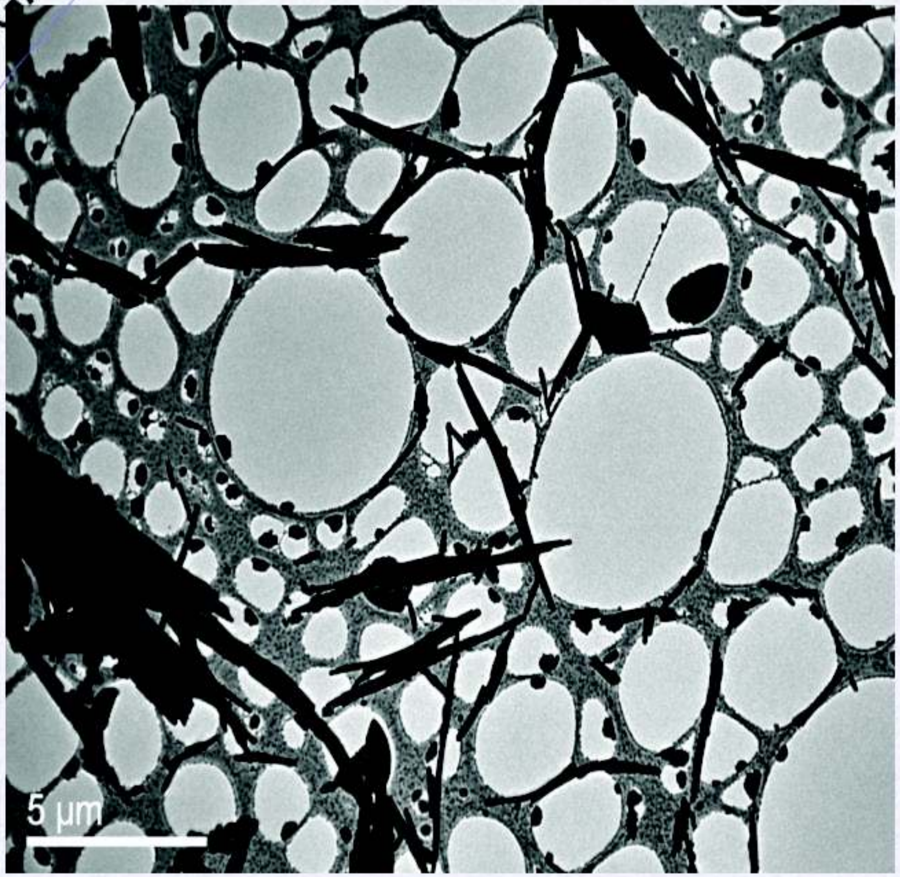


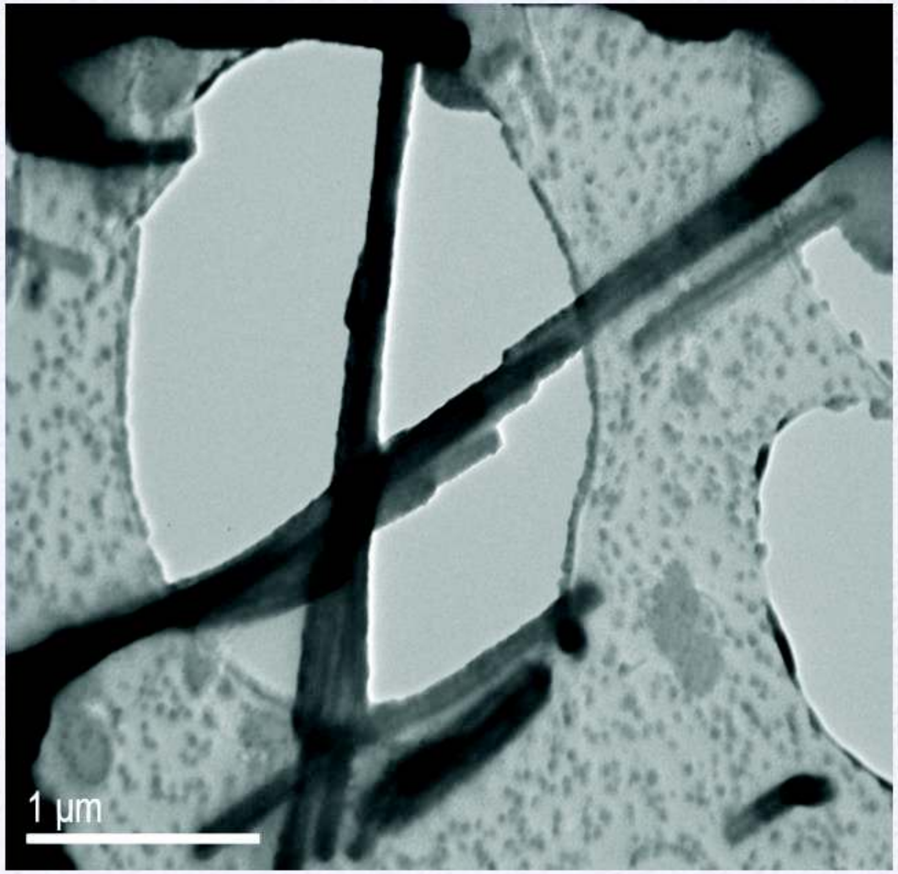
TEM micrograph of peptide fibrils containing acidic amino acids negatively stained

peptide fibrils templated with **gold nanoparticles.**
No negative staining was used

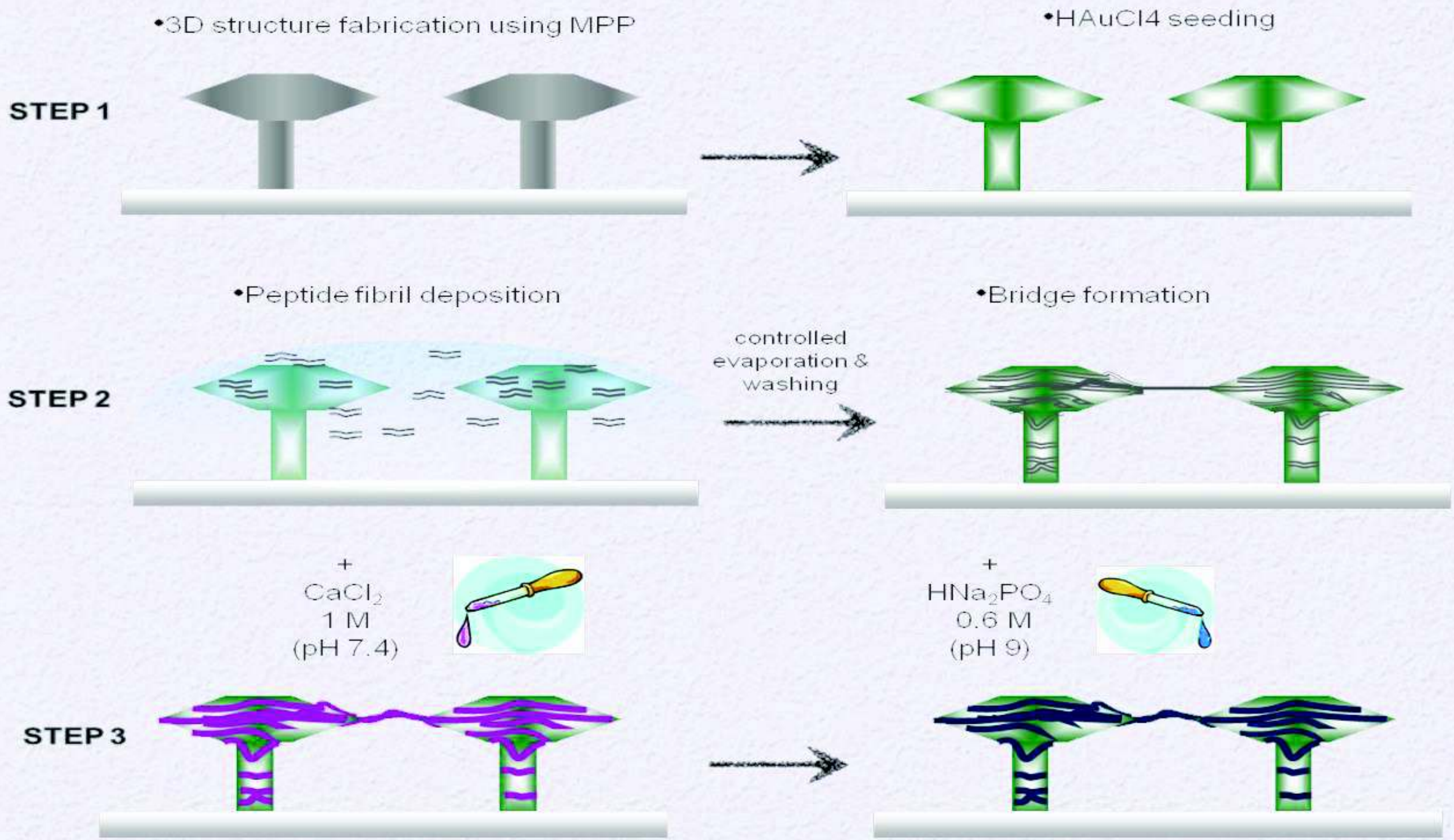
Mineralization of Peptide Fibrils

DDSCAITIG

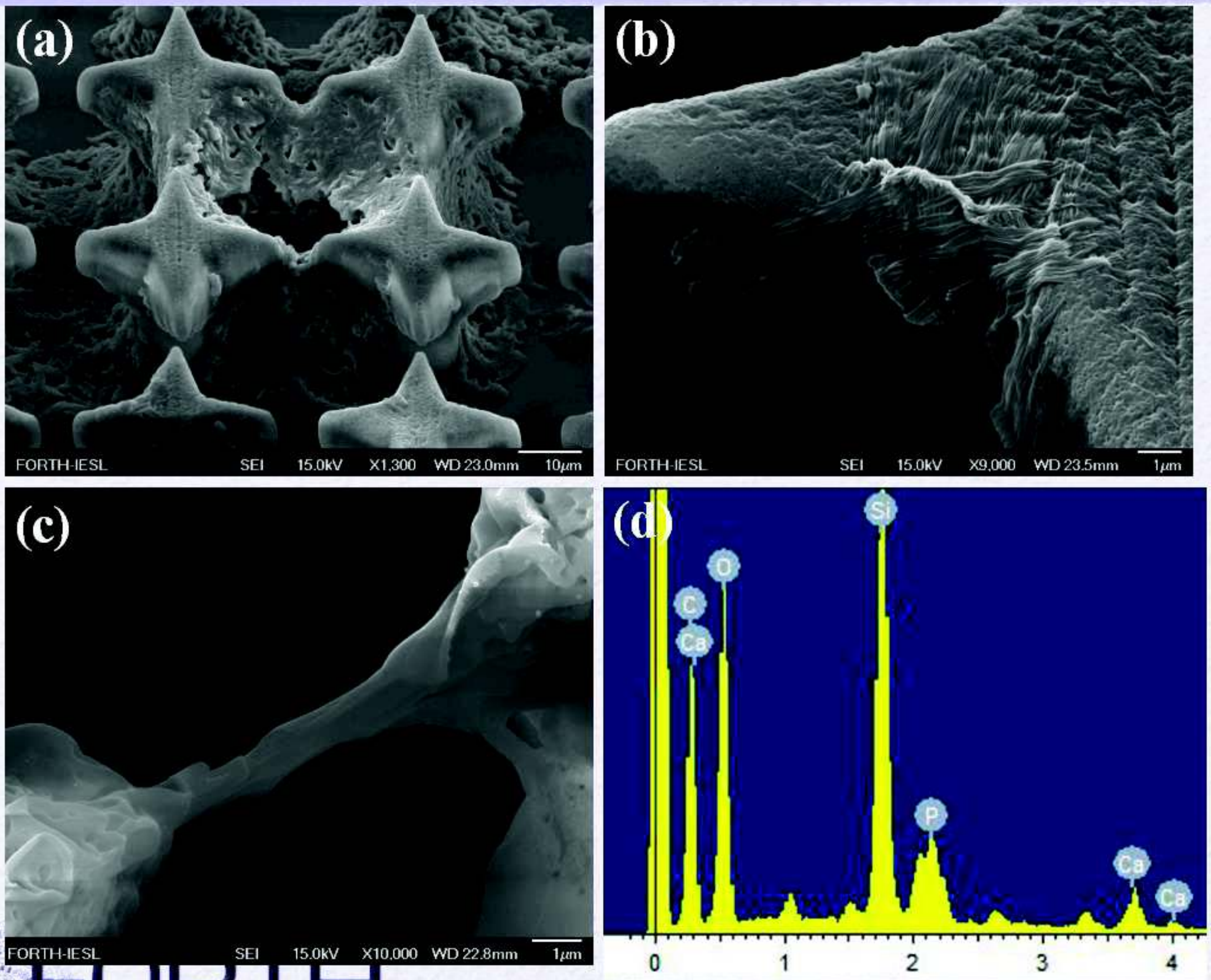




'Scaffold-on-Scaffold' Strategy



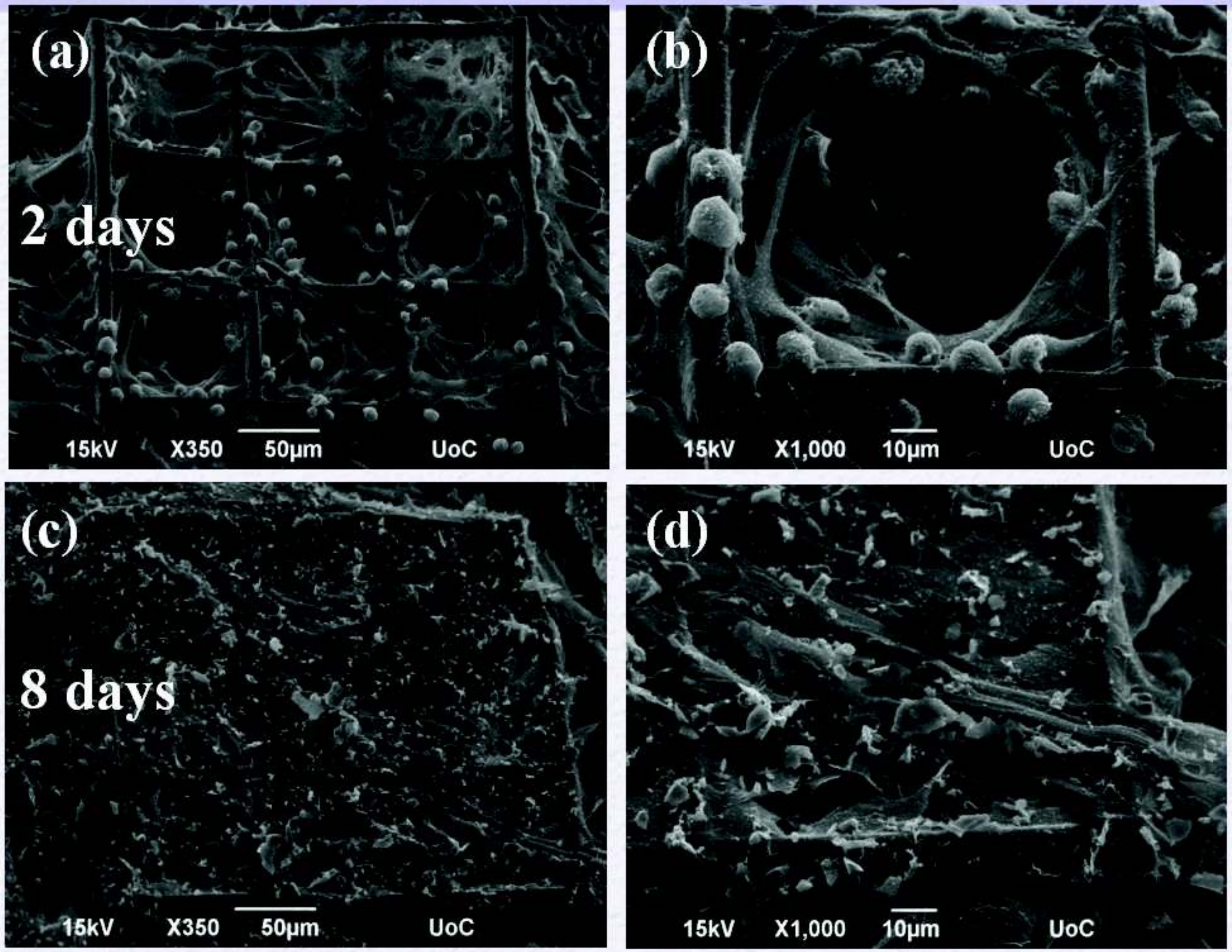
Calcium Phosphate 3D structures



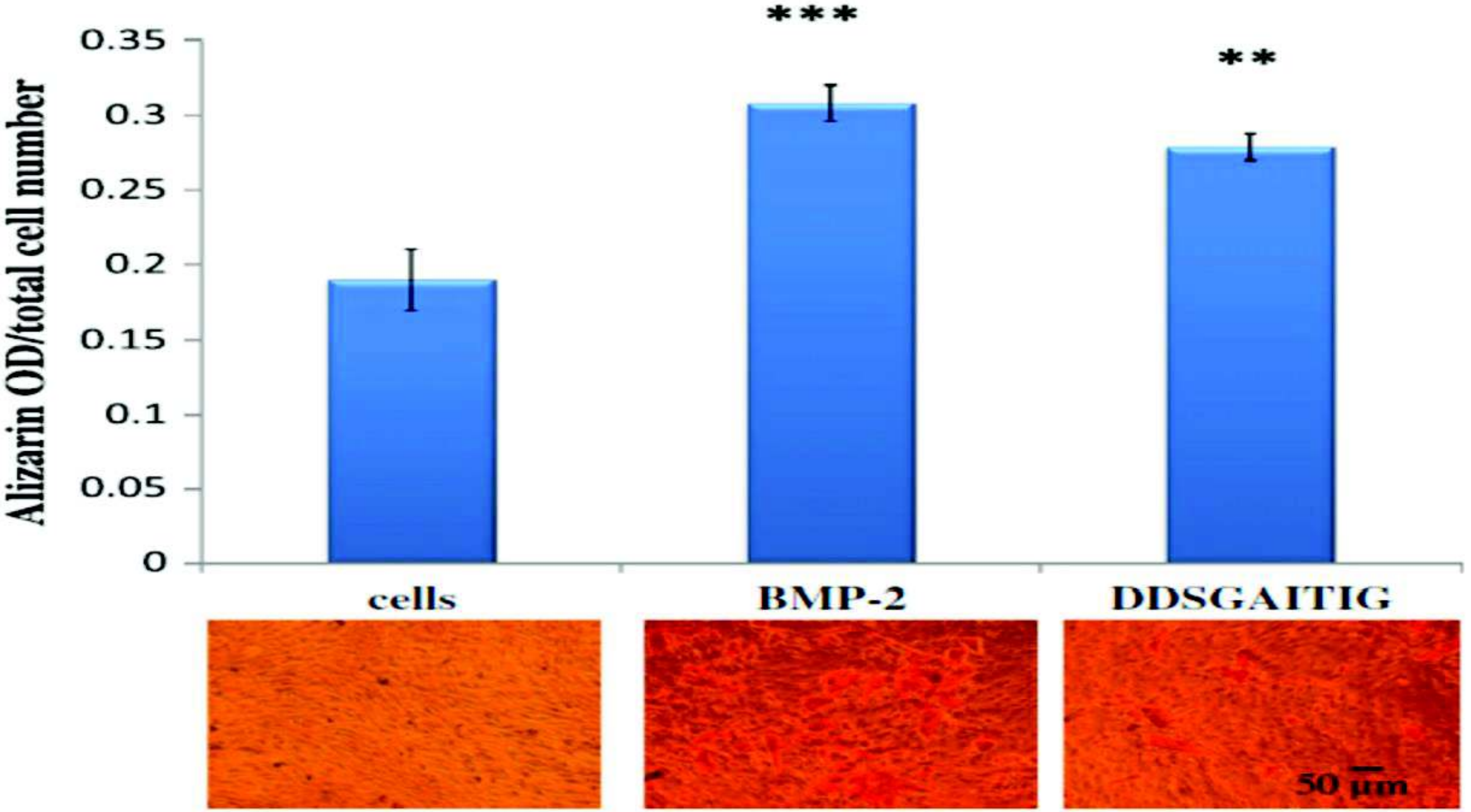
Stoichiometric ratio: Ca/P =1.35

(Ca/P = 1.33 of octacalcium phosphate)

Pre-osteoblastic Cell Adhesion onto Mineralized Scaffolds



Biomineralization increase of pre-osteoblasts cultured for 2 weeks on material surfaces



visualization of stained cells with Alizarin Red S prior extraction to quantification

Conclusions

- NonLinear Lithography is a technology which allows the fabrication of fully 3D structures with sub-100 nm resolution
- Materials engineering can be crucial in achieving functional high-resolution devices
- The combination of 3D structuring with self-assembly can open new avenues in tissue regeneration

Contributors

Students

- Elmina Kabouraki
- Aggelos Xomalis
- Argyro Giakoumaki

Former students

- Ioanna Sakellari
- Nikos Vasilantonakis
- Dina Terzaki
- Paulius Danilevicius †

FORTH & UoC

- Alexandros Selimis
- Maria Vamvakaki
- David Gray
- Anna Mitraki
- Maria Kafesaki
- Costas Soukoulis
- Maria Chatzinikolaidou
- Costas Fotakis

- Vladimir Mironov MD, Campinas, Brazil.

- Funding: ITNs TOPBIO, Thales Program 3DSET, EOARD, LaserLab Europe.

Further Reading

- I. Sakellari *et al*. "Diffusion-Assisted High Resolution Direct Femtosecond Laser Writing". ACS Nano. 2012;6:2302-11.
- N. Vasilantonakis, *et al*. Three-Dimensional Metallic Photonic Crystals with Optical Bandgaps. Advanced Materials. 2012;24:1101-5.
- E. Kabouraki *et al.*, "Redox Multiphoton Polymerization for 3D Nanofabrication" *Nano Letters.* 2013;13:3831-5.
- K. Terzaki *et al.*, "Mineralized self-assembled peptides on 3D laser-made scaffolds: A new route towards 'scaffold on scaffold' hard tissue engineering". *Biofabrication* **5** 2013 045002
- M. Malinauskas *et al.*, "Ultrafast laser nano-structuring of photopolymers: a decade of advances". *Physics Reports* http://dx.doi.org/10.1016/j.physrep.2013.07.005

THANK YOU FOR LISTENING!

FORTH
Foundation for Research & Technology - Hellas