University Politehnica of Bucharest – Romania
Reykjavik University - Iceland

Faculty of Medical Engineering

Regenerative medicine biomaterials and biomolecules

MATERIALS AND BIO(REACTIV) MOLECULES

Supporting materials and molecules are needed to create the proper microenvironment for cell proliferation and differentiation into functional tissue.

Suitable scaffold - mimic native tissue, providing cues for cell attachment, migration, growth, and differentiation, and allows cells for reorganization into a functional 3D network.

Biomolecules - cell proliferation and differentiation into functional tissue.

SCAFFOLDS / MATRICES

Materials

Extracellular matrix (ECM): scaffold for native tissue, providing structural integrity, functionality, and ideal conditions for cell growth.

Hydrogels –suitable biochemically and mechanically,
suitable cell deposition,
suitable cell growth.

Organic hydrogels
Synthetic hydrogels

Organic polymers - isolated from animal or human tissue, inherent bioactivity and similarity to native ECM, most cell friendly, mechanical properties are weak.

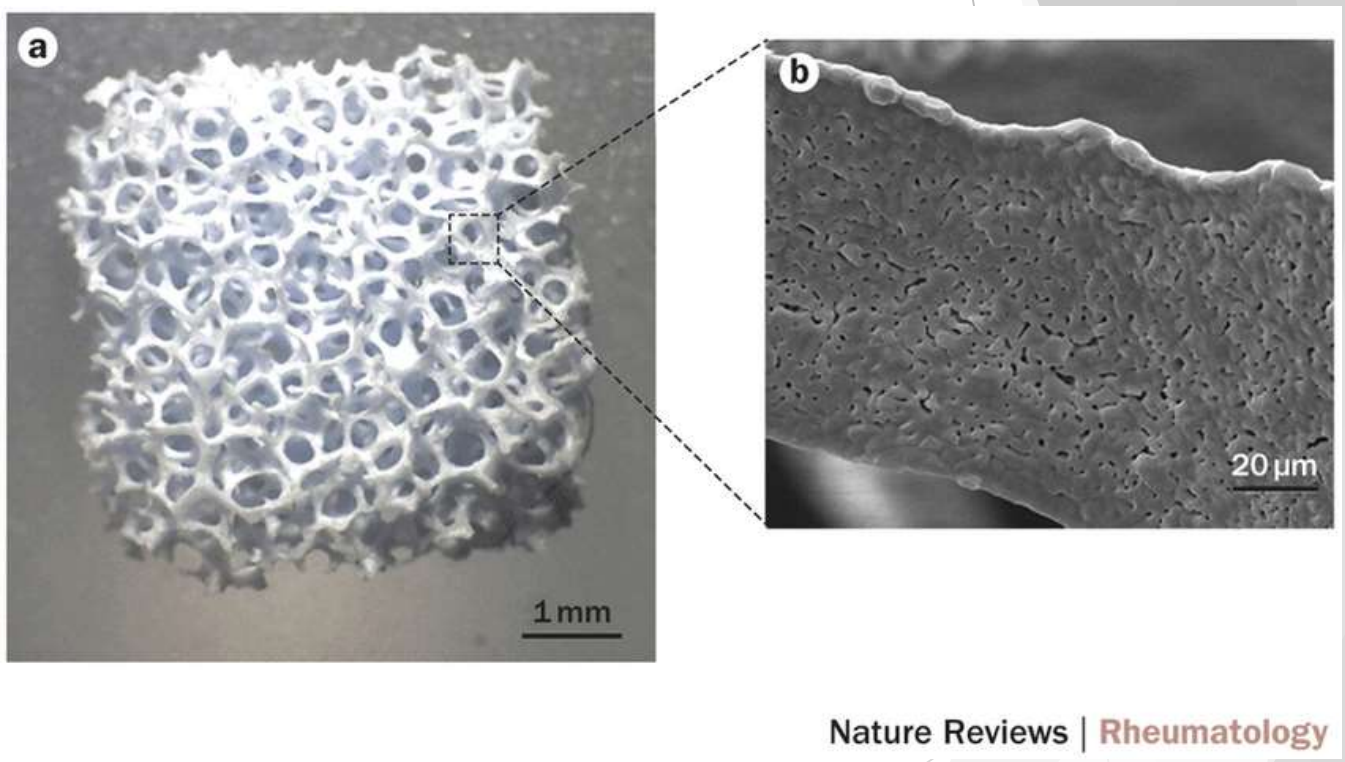
Synthetic hydrogels -stronger mechanical and structural properties (ability to be tailored by chemical modification), poorer biocompatibility, with increased chances of toxic.

Each has benefits and disadvantages, depending on the specific tissue being constructed, e.g. degradation and loss of mechanical properties during tissue degradation in vivo.

SCAFFOLDS / MATRICES

Variety of materials (biologically or synthetic based)

1. POLYMER SCAFFOLDS
2. CERAMIC SCAFFOLDS
3. COMPOSITE SCAFFOLDS
4. *IN VITRO* OBTAINED SCAFFOLDS
5. ECM SCAFFOLDS
6. DECELLULARIZED ORGANS SCAFFOLDS



IDEAL SCAFFOLDS / MATRICES

- biocompatibility,
- non-toxic,
- biodegradability,
- appropriate degradation rate
- mechanical properties,
- mechanically stable,
- allow manufacturing technology,
- architecture,
- support for cell attachment,
- proliferation and differentiation,
- forming an extracellular matrix (ECM),
- facilitates complex cell-to-cell and cell-ECM interactions,

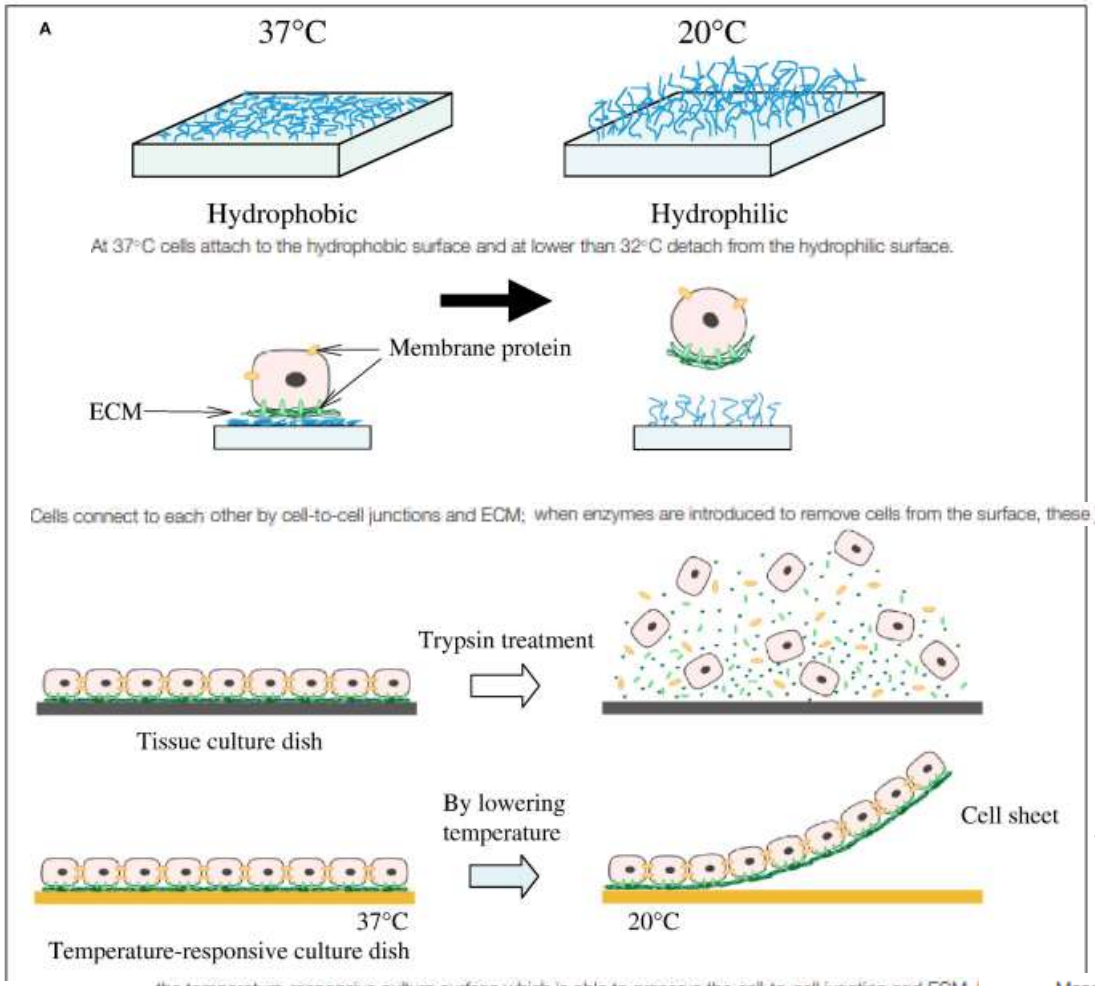
IDEAL SCAFFOLDS / MATRICES

Architecture / Porosity

- cell penetration and ingrowth
- in 3D conditions aggregate,
- diffusion gradient occurs (oxygen delivery and metabolite removal both in vivo and in vitro),
- impact vascularization,
- impact the overall stiffness (dense scaffold versus more porous) which impact stem cell differentiation potential.

Extension of 3D cultured scaffolds towards cell-sheet technologies

Principals of the method: multiple layers of cells aregrown, transferred en bloc, and combined with different cells to develop thicker and more complex tissue grafts (without scaffold use).



Moschouris et al.

doi: 10.3389/fsurg.2021.731031

CELLS GROWTH AND GROWTH FACTORS

Growth factors are biomolecules that provide signals for maturation and differentiation of cells.

Over 300 ECM proteins, ECM-modifying enzymes and ECM binding growth factors have been identified in mammalian cells as pivotal to growth, proliferation and regeneration processes.

e.g. collagens, proteoglycans and glycoproteins serve to provide strength, bind important growth factors, regulate protein complexes within tissues, promote cell adhesion, and participate in cellular signaling.

- Autocrine
- Paracrine

- Intrinsic (Matrigel)
- Extrinsic (various cytokines, miRNAs, loaded to affect cell growth)

GROWTH FACTORS

Fibroblast growth factors (FGF),
Epidermal growth factors (EGF),
Vascular endothelial growth factors (VEGF),
Transforming growth factor beta (TGF-beta),
Platelet-derived growth factors (PDGF).

MATERIAL DESIGN FOR BONE REGENERATION

“*No ideal material for bone repair*”

“*Primum non nocere*” **“First, do no harm”**

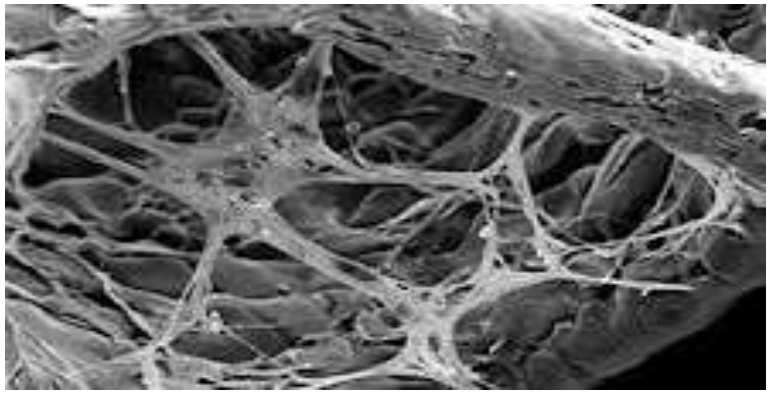
“*A more active life style*”

Aspects to be considered

- ✓ Similar mechanical features with the tissue
- ✓ Permissive
- ✓ Biocompatibility
- ✓ Antimicrobial activity
- ✓ Degradability

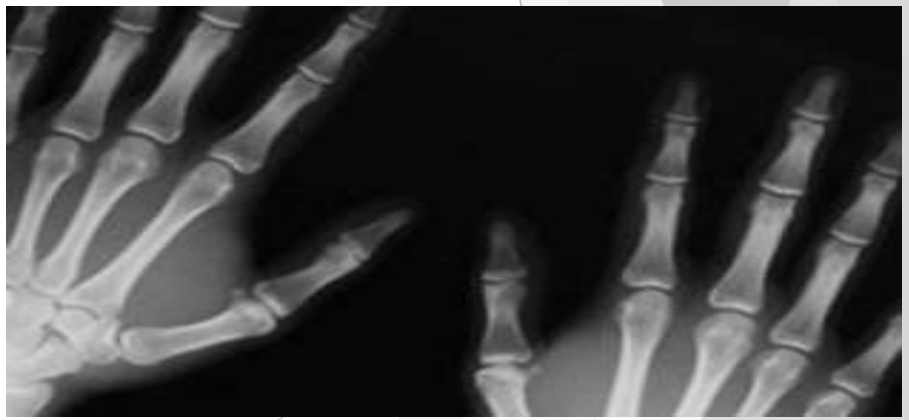
Materials Used for Bone Regeneration

- ✓ Bioceramics
- ✓ Biological / Synthetic Polymers
- ✓ Composite materials



RESEARCH GOAL

Achieving a breakthrough solution for bone repair!



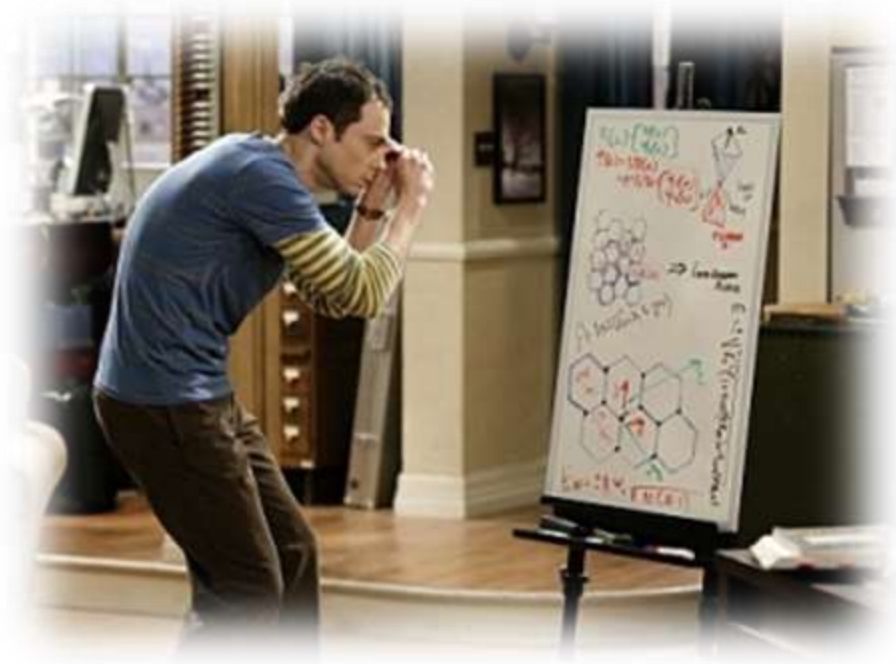
MATERIALS

CHITOSAN

SODIUM ALGINATE

GELATIN

POLY(VINYL ALCOHOL)



The Big Bang Theory, Jan. 2010

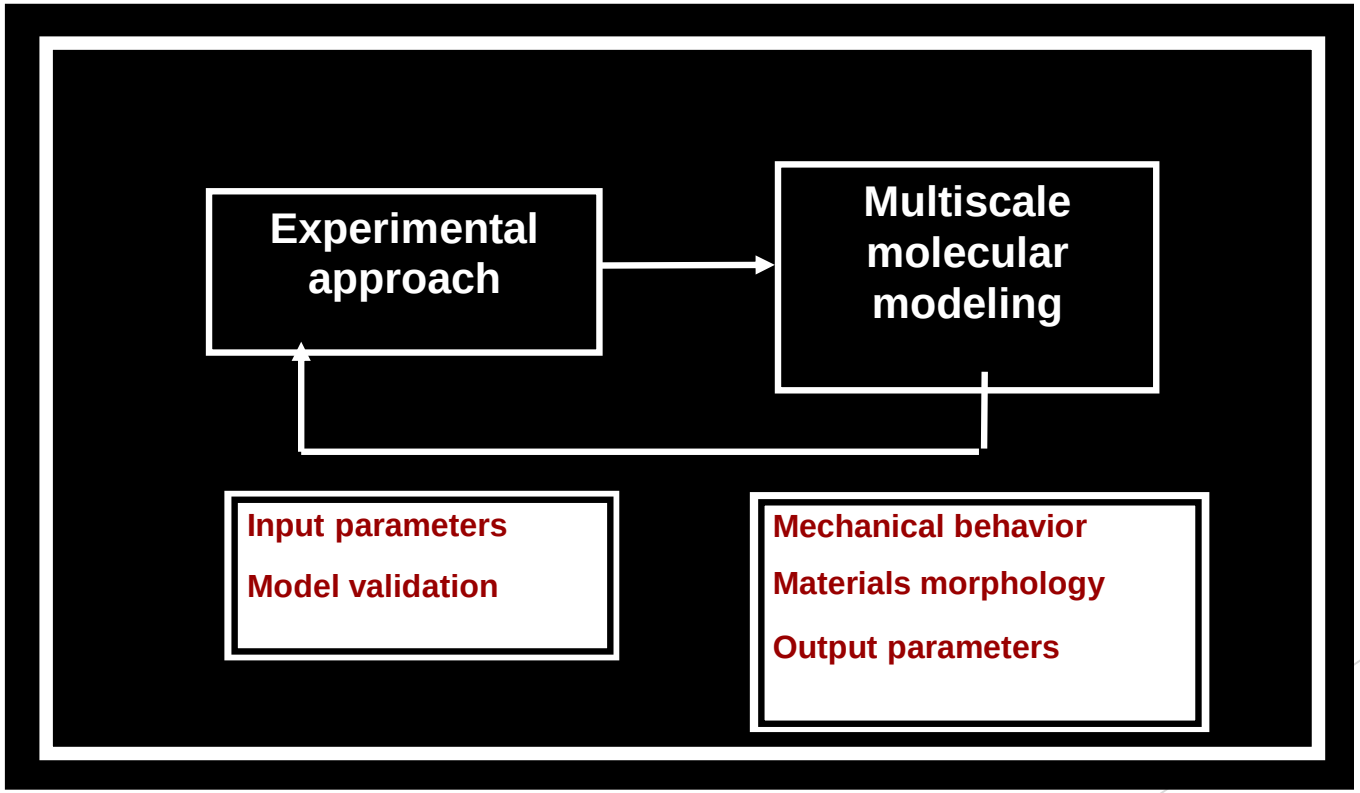
GRAPHENE

- ✓ Thinnest;
- ✓ Most impermeable;
- ✓ Strongest;
  (E = 1 TPa, σ = 130 GPa);
- ✓ Low density, high surface area;
- ✓ Hydrophobic;
- ✓ Antimicrobial.

Novoselov, Geim *et al.*, Science 2004
Novoselov *et al.*, Nature 2012

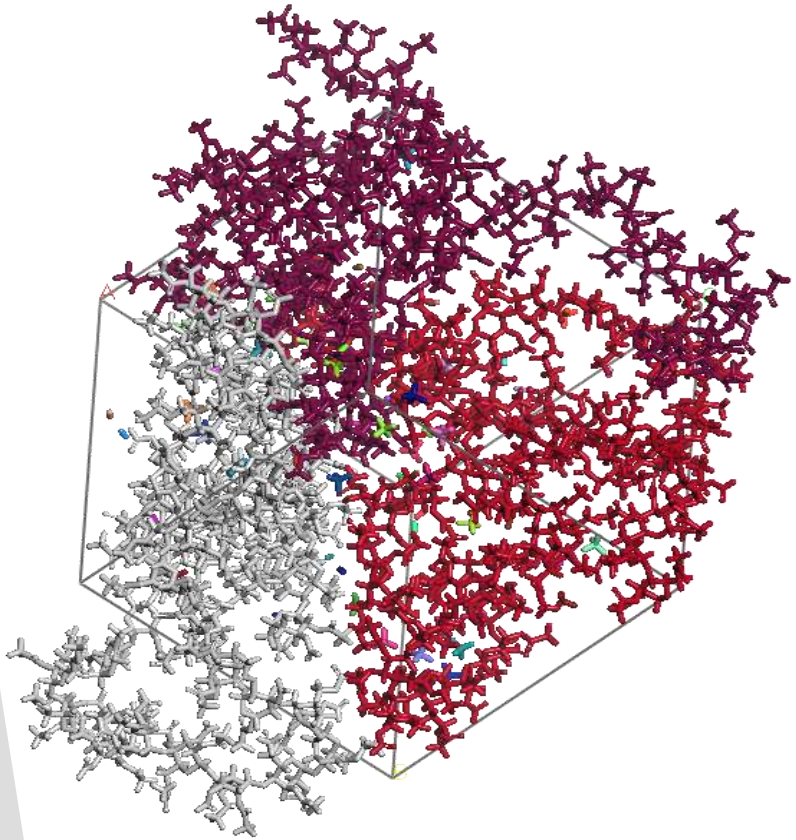
APPROACH

Knowlaged based design of new improved materials biopolymer / graphene for bone regeneration

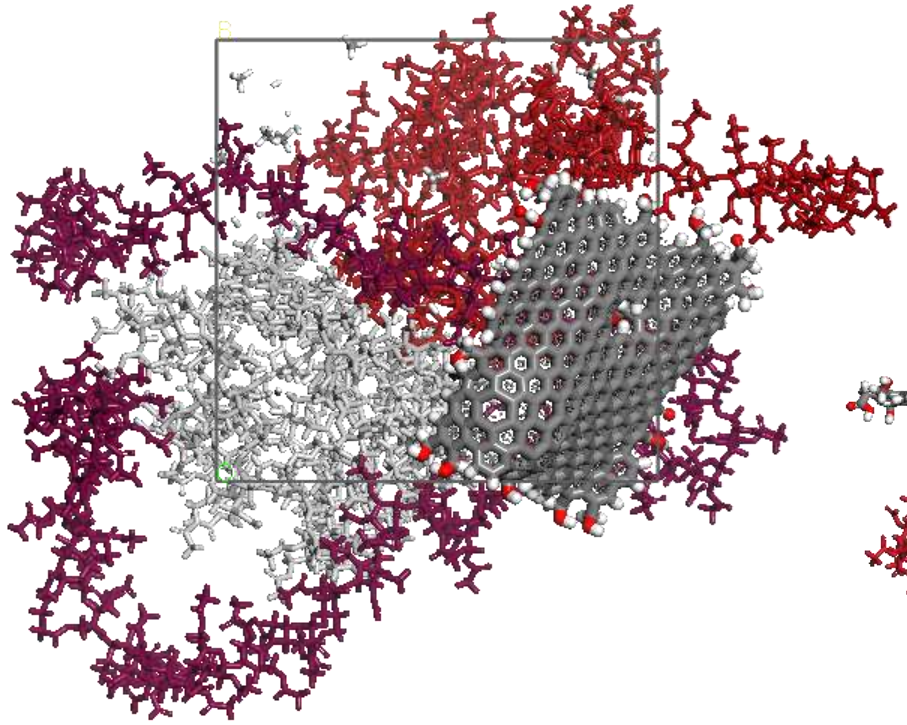


Atomistic Models Investigation

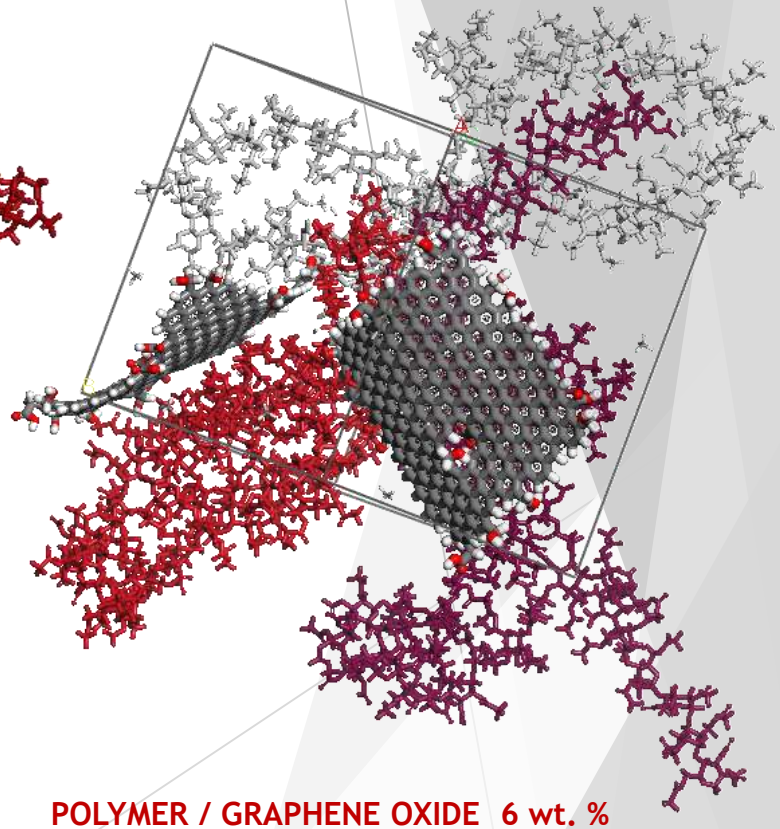
No. of atoms within the model- 6000-7000
Density of bulk model [g/cm^{-3}] -1-1,4
Dimensions of the model [Å] – 38-41



POLYMER

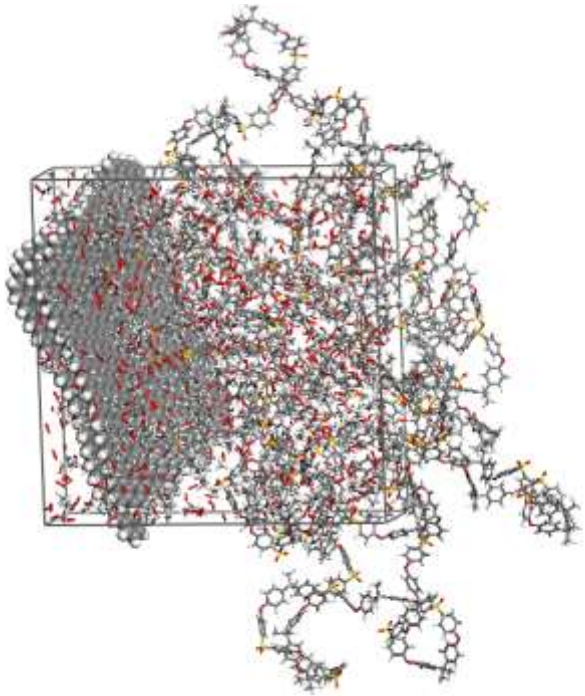


POLYMER / GRAPHENE OXIDE 3 wt. %

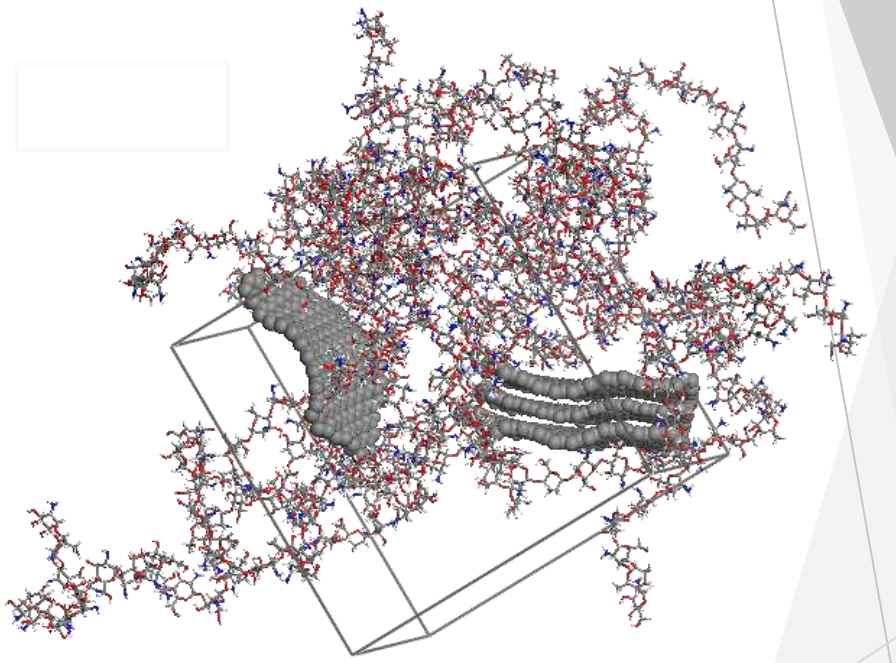


POLYMER / GRAPHENE OXIDE 6 wt. %

Atomistic Models Investigation



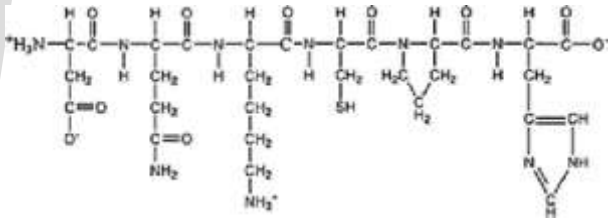
POLYMER / GRAPHENE 4 wt. %



POLYMER / GRAPHENE 8 wt. %

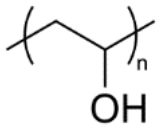
I. MATERIALS

Gelatin (Gel)



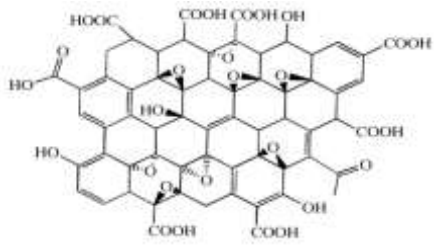
- ✓ Natural polymer
- ✓ Lower antigenicity than collagen

Poly(vinyl alcohol) PVA



- ✓ Synthetic polymer
- ✓ Biocompatible
- ✓ Numerous clinical applications

Graphene oxide (GO)

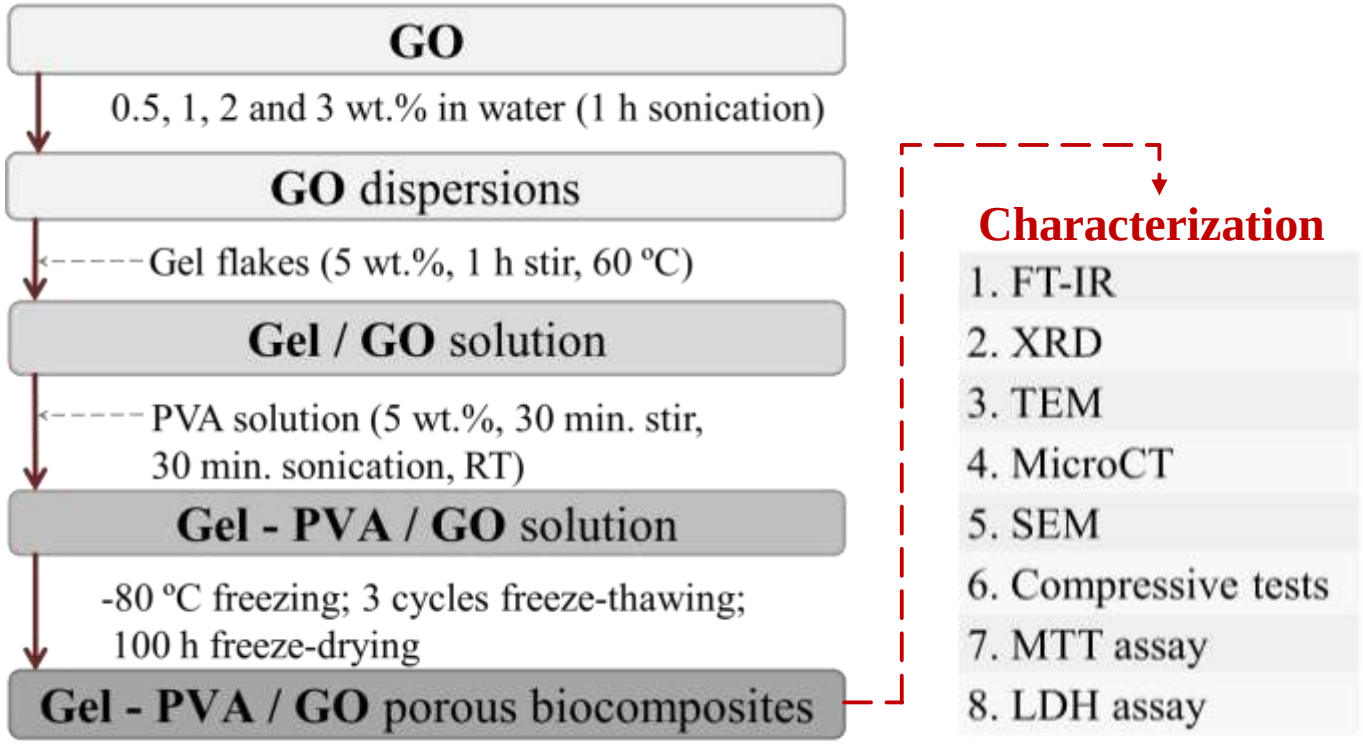


- ✓ Less hydrophobic / dispersable
- ✓ Biodegradable.

Chen *et al.*, Nanoscale 2014
Fiorillo *et al.*, Oncotarget 2015
R. Kurpati, *et al.*, Small 2015
D. Jasim, *et al.*, Applied Materials Today, 2016

II. METHODS

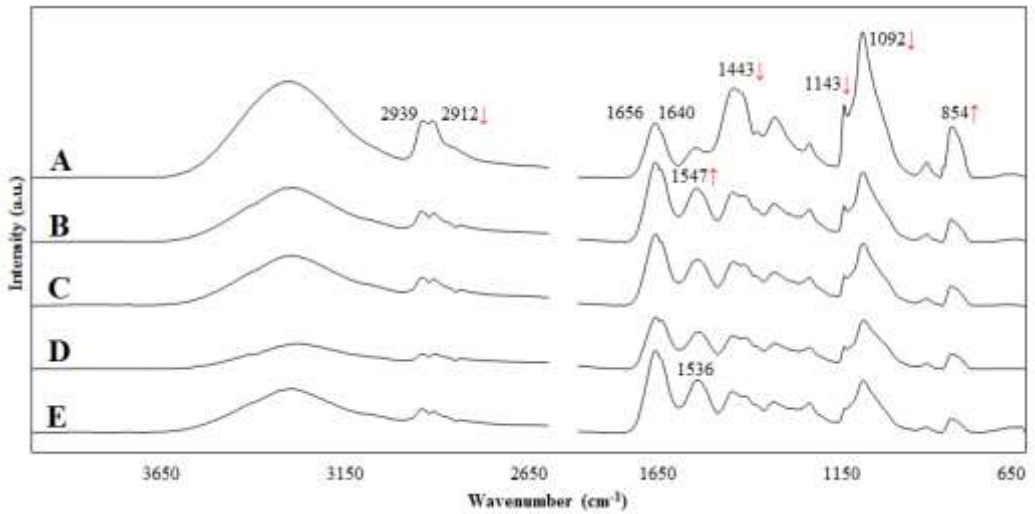
Synthesis flux



Characterization

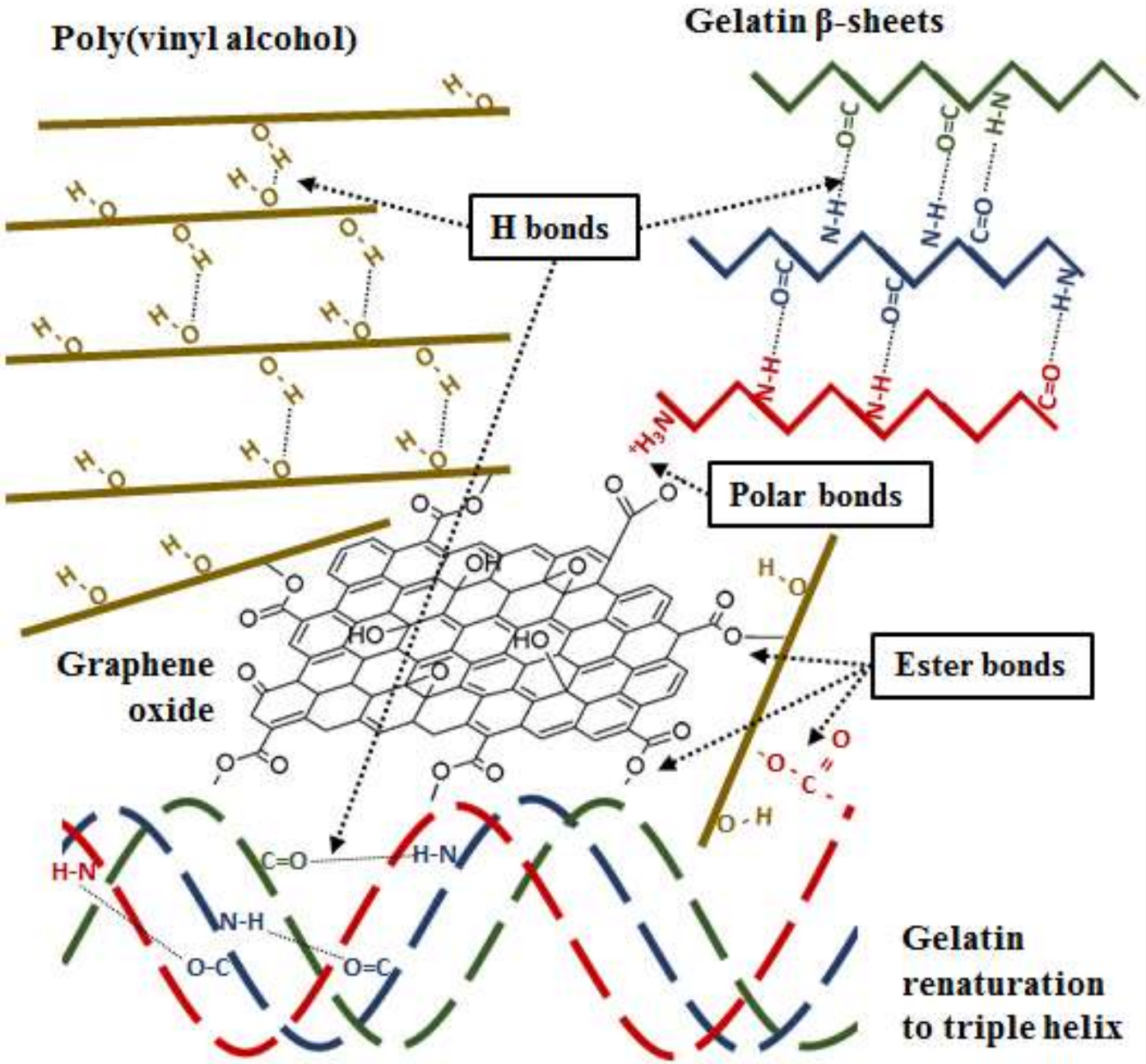
1. FT-IR
2. XRD
3. TEM
4. MicroCT
5. SEM
6. Compressive tests
7. MTT assay
8. LDH assay

1. FT-IR SPECTROMETRY:

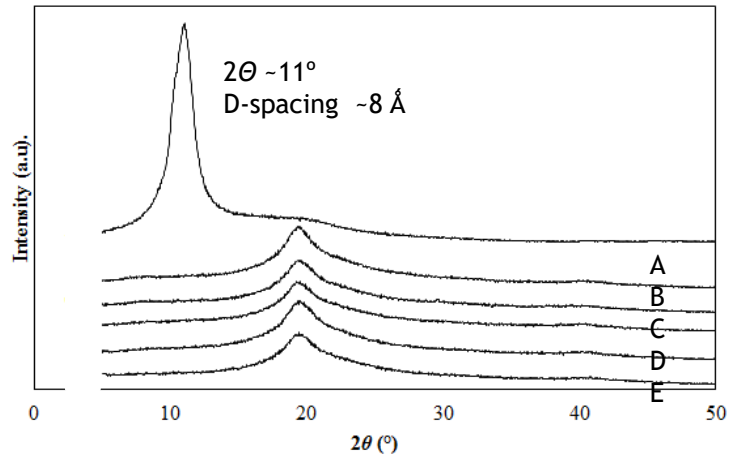


No typical peak around 1680 cm^{-1} COOH of Gelatin

Band	
3000-3600	OH
2939	CH
2912	
3325	Amide A
2925	Amide B
1656-1640	Amide I (alpha vs beta)
1547	Amide II
1238	Amide III
1443	OH in plane wagging
1143	C-O (ordered)
1092	C-O (amorphous)
921	C-C
854	


Poly(vinyl alcohol)
Gelatin β-sheets
H bonds
Polar bonds
Ester bonds
Graphene oxide
Gelatin renaturation to triple helix

2. X RAY DIFFRACTION:



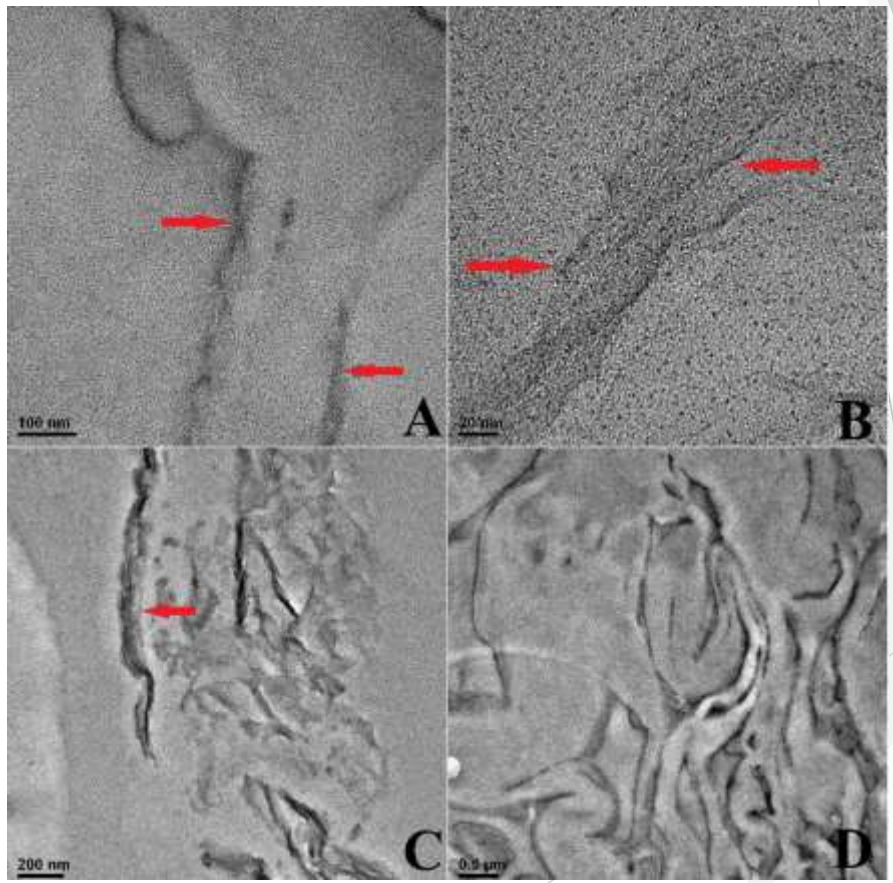
XRD measurements of 2θ and related d-spacing, full width at half maximum (FWHM) and mean size of the ordered domains (τ).

	Sample	2θ (°)	d-spacing (Å)	FWHM (°)	τ (Å)
A	Gel – PVA	19.88	4.46	4.3	13.5
B	Gel - PVA / GO 0.5 wt.%	19.6	4.52	3.19	18.2
C	Gel - PVA / GO 1 wt.%	19.7	4.5	3.8	15.3
D	Gel - PVA / GO 2 wt.%	19.8	4.49	3.43	16.98
E	Gel - PVA / GO 3 wt.%	19.7	4.5	3.51	16.54

IV. RESULTS:

3. TRANSMISSION ELECTRON MICROSCOPY

- Efficiently dispersed GO nanosheets;
- Preferential arrangement;
- GO flexibility;
- Seldom GO agglomerates;
- Microporosity.

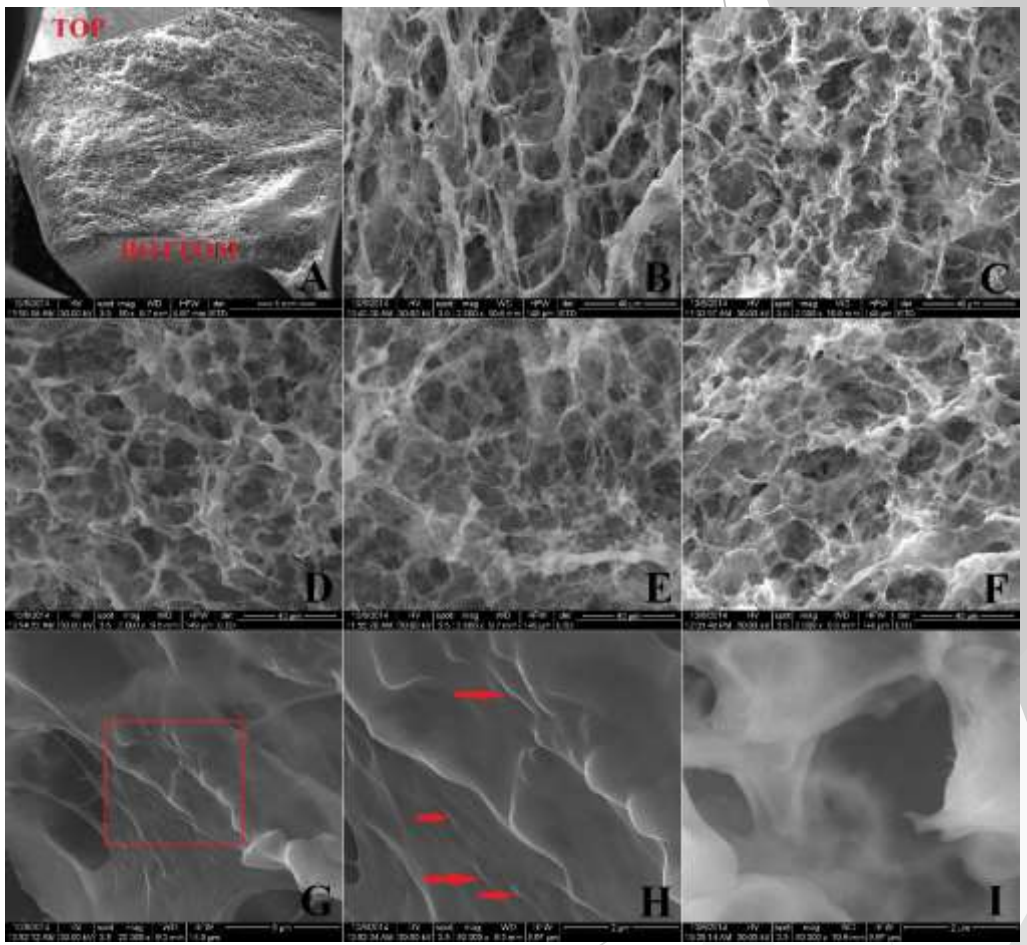


TEM images of 0.5 wt.% containing Gel – PVA / GO composite.

IV. RESULTS:

4. SCANNING ELECTRON MICROSCOPY

- Good compatibility Gel - PVA / GO;
- Macroporosity;
- More homogenous porous architectures;
- Sharp prominences (GO).



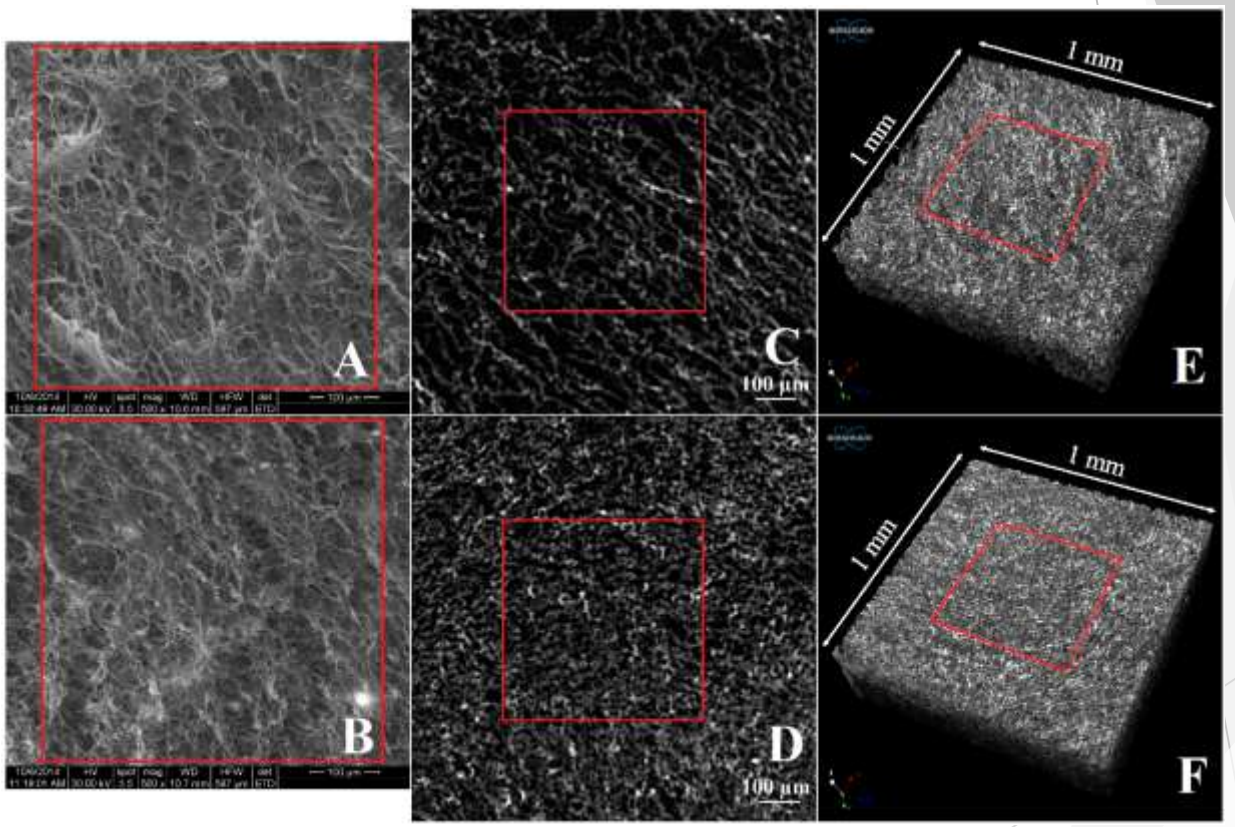
Overall morphology of Gel – PVA (A). Gel – PVA (B) and Gel – PVA / GO composites containing 0.5, 1, 2 and 3 wt.% (C-F). GO prominences onto pore walls of 0.5 wt.% composite (G and H, indicated by arrows) and their absence from Gel – PVA scaffold (I).

IV. RESULTS:

5. X RAY MICROTOMOGRAPHY

Sample	Po(tot) (%)
Gel - PVA	62 ± 1.75[1]
Gel - PVA / GO 0.5 wt.%	51 ± 4.26
Gel – PVA / GO 1 wt.%	64 ± 0.99[1,2]
Gel - PVA / GO 2 wt.%	69 ± 1.96[2]
Gel – PVA / GO 3 wt.%	43 ± 3.75

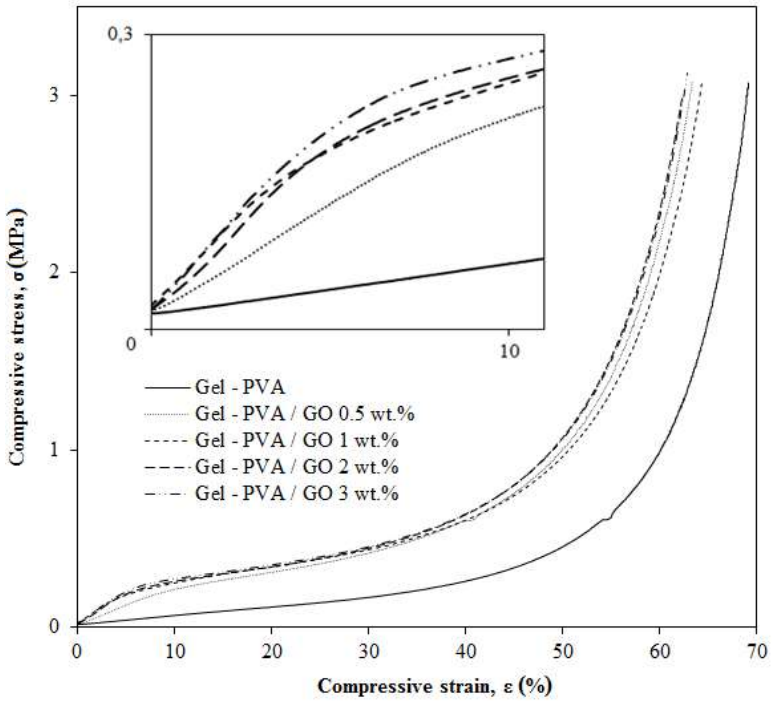
Superscript numbers indicate insignificant differences between the marked values by statistical analysis ($p < 0.05$).



Comparative view between unloaded and 0.5 wt.% GO loaded composites through SEM images (A and B), microCT tomograms (C and D) and VOIs 3D rendering (E and F). Scale bars are 100 µm for figures A - D. The red squares frame areas of 500 µm.

IV. RESULTS:

6. COMPRESSIVE TESTS



Compressive tests characteristic curves of Gel-PVA and Gel-PVA/GO 0.5-3 wt%.

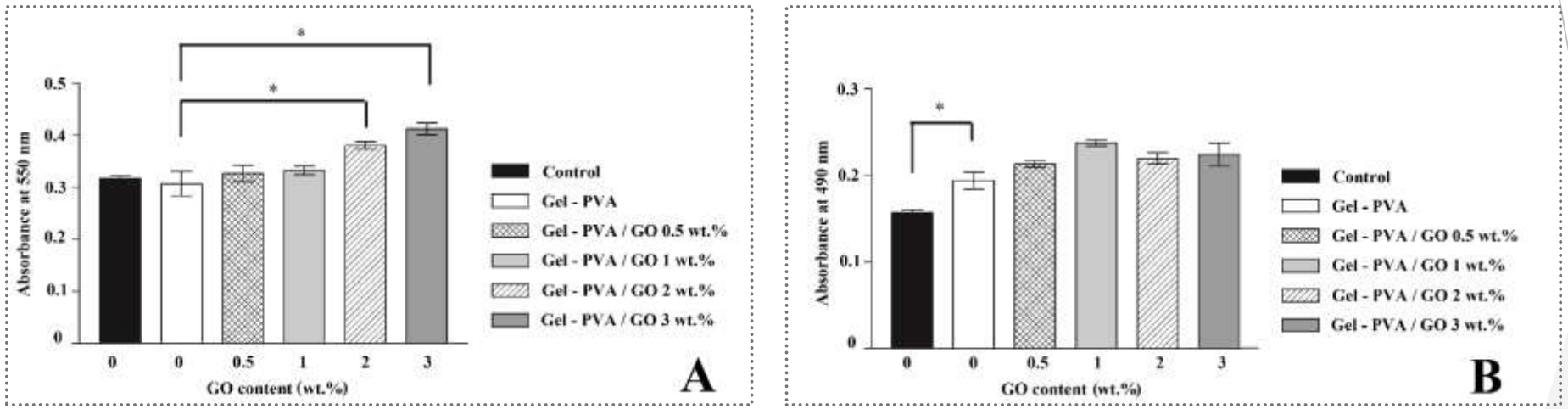
Compressive strength (σ), elastic modulus (E) and their percentage increase.

Sample	σ (MPa)	σ increase* (%)	E (MPa)	E increase* (%)
Gel – PVA	0.111 ± 0.026	-	0.012 ± 0.005[2]	-
Gel - PVA / GO 0.5 wt.%	0.219 ± 0.049[1]	97	0.020 ± 0.008[2,3]	67
Gel - PVA / GO 1 wt.%	0.221 ± 0.021[1]	99	0.025 ± 0.007[3]	108
Gel - PVA / GO 2 wt.%	0.226 ± 0.031[1]	104	0.026 ± 0.007[3]	117
Gel - PVA / GO 3 wt.%	0.233 ± 0.022[1]	110	0.028 ± 0.008[3]	133

*increase related to Gel – PVA (control sample). The superscript numbers indicate insignificant differences between the marked values by statistical analysis ($p < 0.05$).

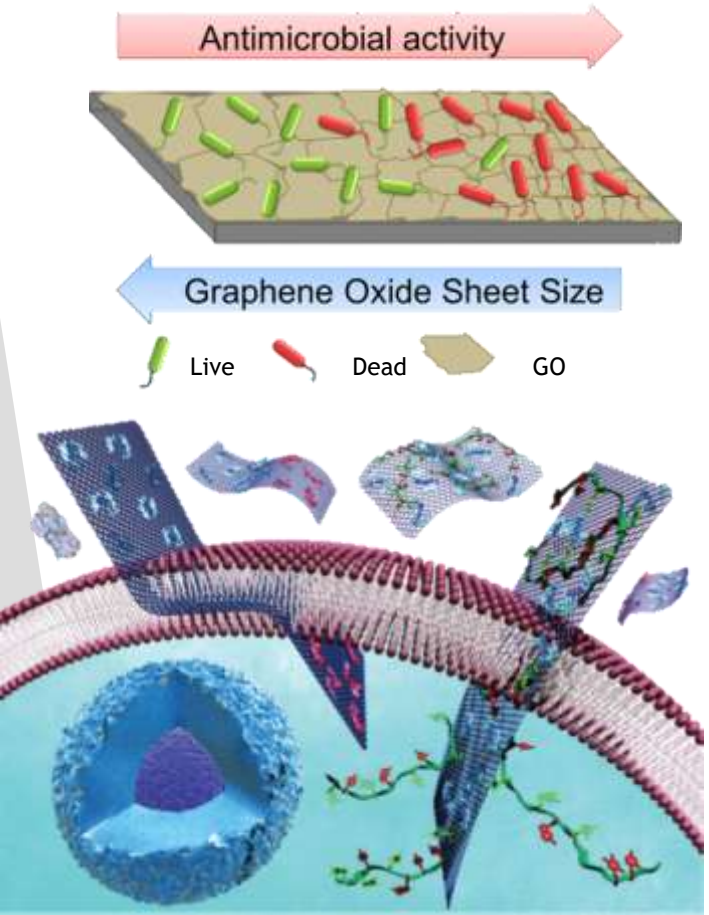
IV. RESULTS:

7. BIOCOMPATIBILITY TESTS



Preosteoblasts viability assessed after 24 h contact with PVA - Gel / GO 0.5 - 3 wt.% composites extracts by MTT assay (A). PVA – Gel / GO 0.5 - 3 wt.% composites cytotoxic potential exerted on preosteoblasts after 24 h of indirect contact, as revealed by LDH assay (B).

ANTIMICROBIAL / ANTIFUNGAL FEATURES



- **RGO *vs. E. coli*** (Qi *et al.*, Scientific Reports 2015)
- **GO *vs. E. coli*** (Perreault *et al.*, ACS Nano, 2015)
- **GO-based hydrogels *vs. E. coli*** (Wang *et al.*, Chemical Engineering Journal, 2015)
- **G@Cu and G@Ge *vs. S. aureus and E. coli*** (Li *et al.*, Scientific Reports 2014)
- **GO *vs. P. syringae, X. campestris pv. Undulosa , F. graminearum* and *F. oxysporum*** (Chen *et al.*, Nanoscale 2014)
- **GO *vs. C. albicans, E. coli, P. aeruginosa, S. faecalis* and *S. aureus*** (Al-Thani *et al.*, Online Journal of Biological Sciences 2014)
- **Gt, GtO, GO, RGO *vs. E. Coli*** (Liu *et al.*, ACS Nano 2011)
- **PVK-GO composite films *vs. E. coli*** (Chemical Communications, 2011)
- **G, GO *vs. E. coli*** (ACS Nano, 2010).

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