ANALISIS OF CHITOSAN/ HYDROXYAPATITE SPIN-COATED FS MICROSTRUCTURED POLY LACTIC ACID (PLA) TEMPORARY CELL SCAFFOLDS

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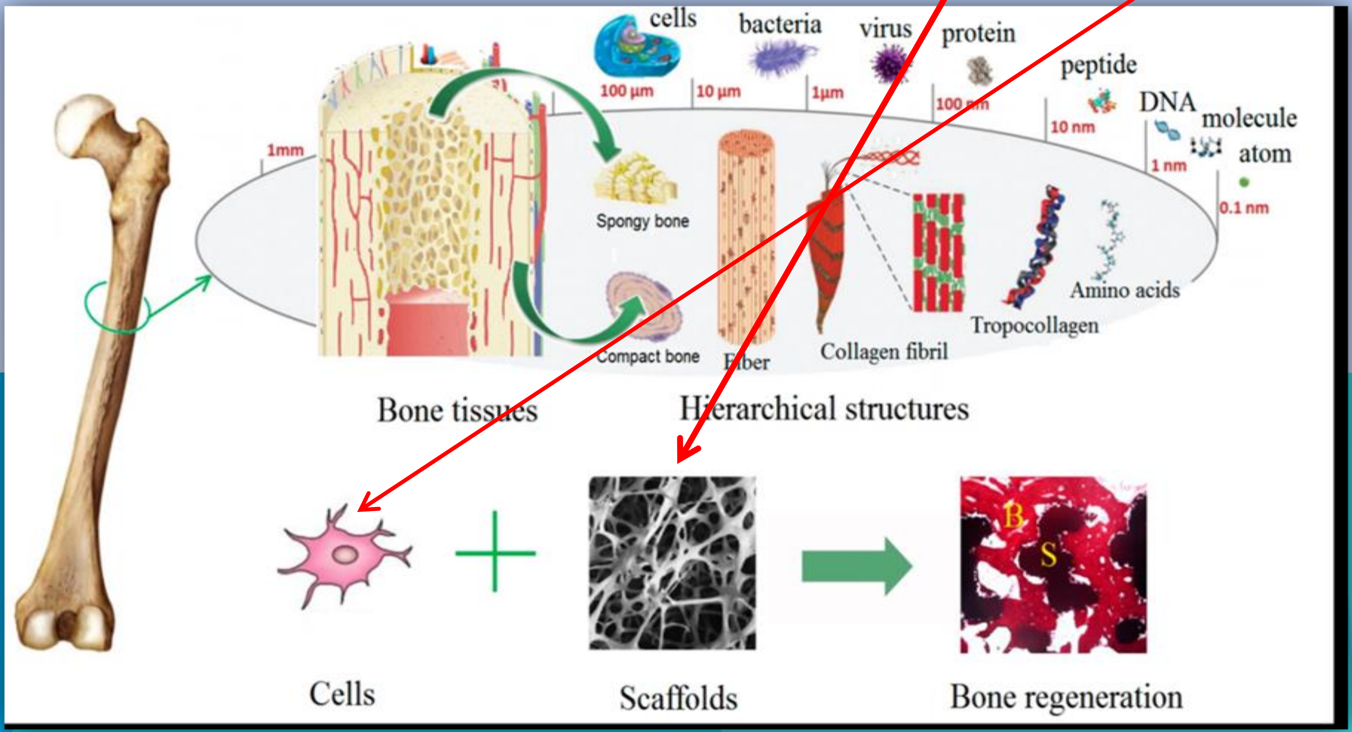
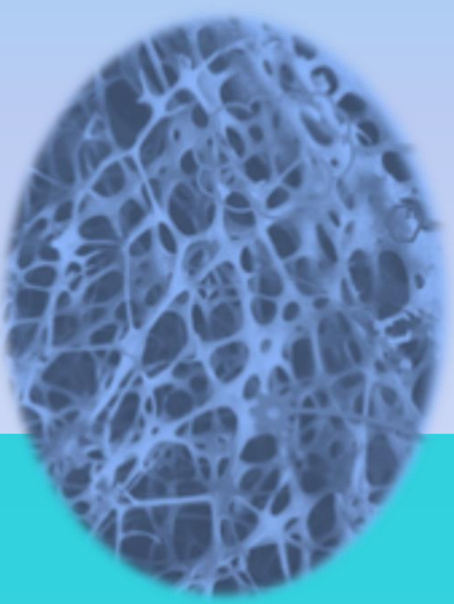
BONE REGENERATION IN TISSUE ENGINEERING – TEMPORARY CELL SCAFFOLDS

In the field of bone tissue engineering, **temporary cell scaffolds**, based on biocompatible and degradable biopolymers, are emerging as one of the most powerful tools for guided self-regeneration of injured, diseased or malfunctioning tissues. These cell environmental structures serve as mechanically stable supporting platforms for **patient's own cells** attachment and proliferation and are gradually displaced by the newly formed bone tissue in an absolutely natural way.





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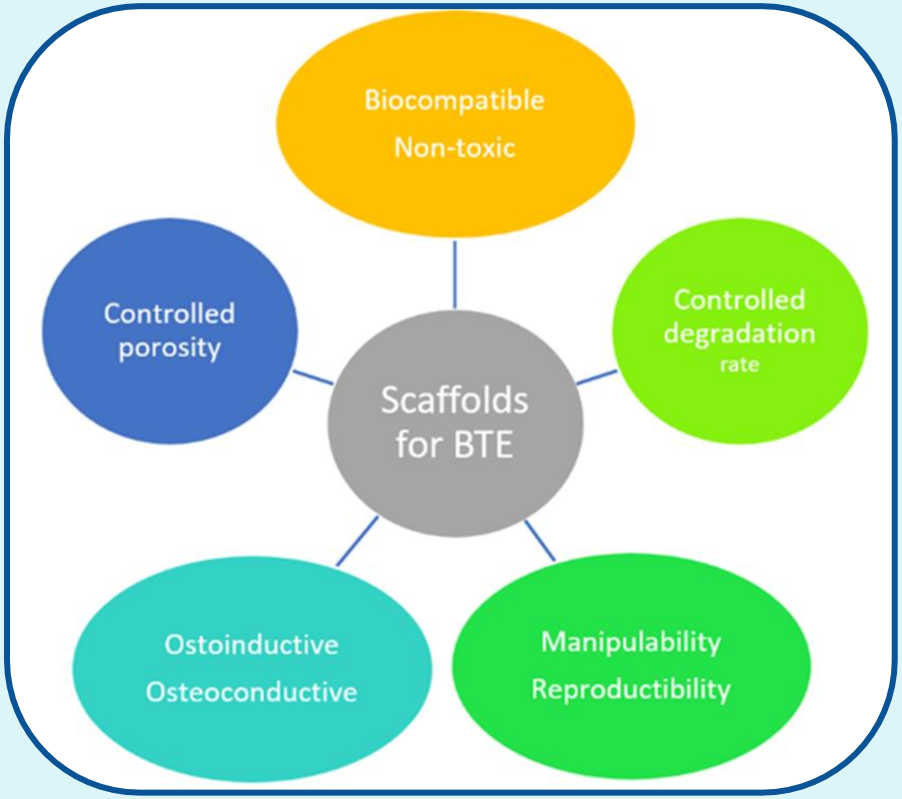
THE "IDEAL CELLULAR MATRIX" IN BONE TISSUE ENGINEERING ...

In recent years scientists are in constant search and optimization of the best materials for **restoring, maintaining and improving** cell scaffold function - their biocompatibility and “extracellular matrix qualities”, such as surface roughness, wettability, hierarchical interconnected porosity, anti-inflammatory properties, and at the same time, avoiding cell cytotoxicity changes in their chemical composition.



multipotent mesenchymal stem cells (MSCs) → **mature osteoblasts**

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MATERIALS AND METHODS
PLA – POLY LACTIC ACID

Tissue	Produced by	Previous alternative	PLA scaffolds advantages
cartilage	chondrocytes	collagen scaffolds	better mechanical properties,
bone	osteocytes, osteoblasts	titan screws, bone grafting	better biocompatibility, no need for scaffold removal, do not contain magnetic metals (e.g. patient can still be scanned via MRI)
peripheral nervous system	Schwann cells in neurons	autografting	no need for many procedures and traumatizing donor tissue
cardiovascular	blood cells: erythrocytes, leukocytes	bypass grafts	vessels less than 5 mm in diameter can be cured

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- biocompatibility and biodegradability.
- ideal contact surface material
- good interaction with the host bone cells at implantation
- nontoxic and mechanically stable, bio absorbable polyester

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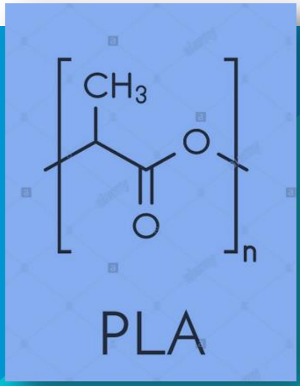
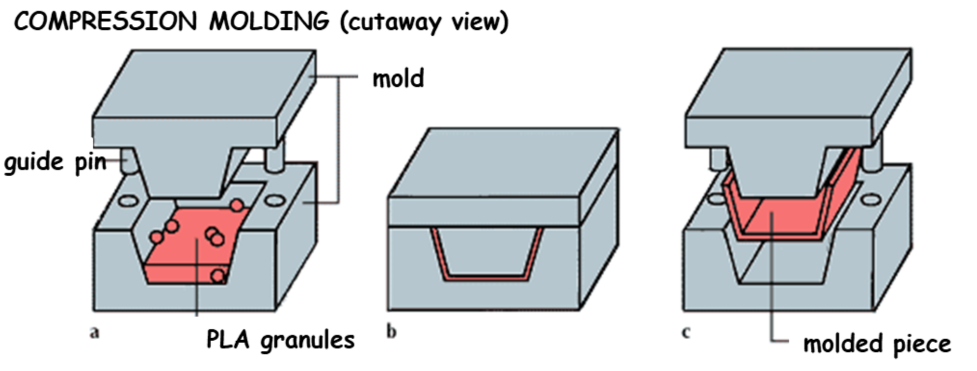
- additional structuring
- hydrophobic nature
- poor cell adhesion





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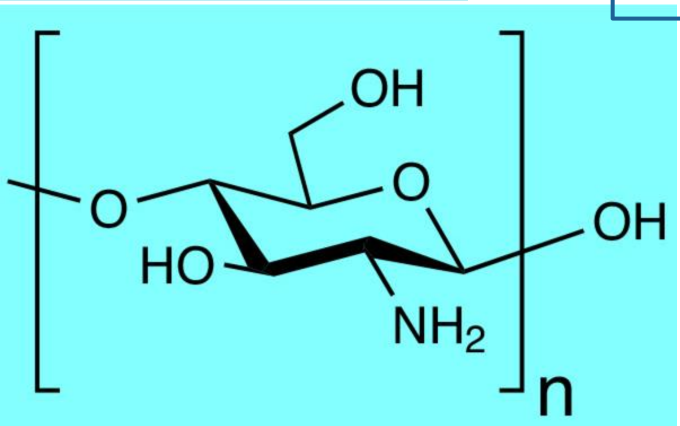
MATERIALS AND METHODS

CHITOSAN

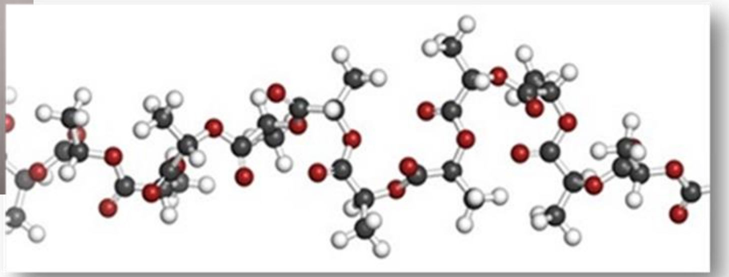
Chitosan is a natural biopolymer with high biocompatibility, antimicrobial activity and superior affinity to proteins.



HYDROXYAPATITE

Hydroxyapatite (**HAp**) is a mineral naturally found in bones and is used for fabrication of dense and porous bioceramics and composites.

PLA



A combination of the best properties of bioceramics with biodegradable polymers is employed to achieve improved mechanical and biological features of engineered scaffolds.

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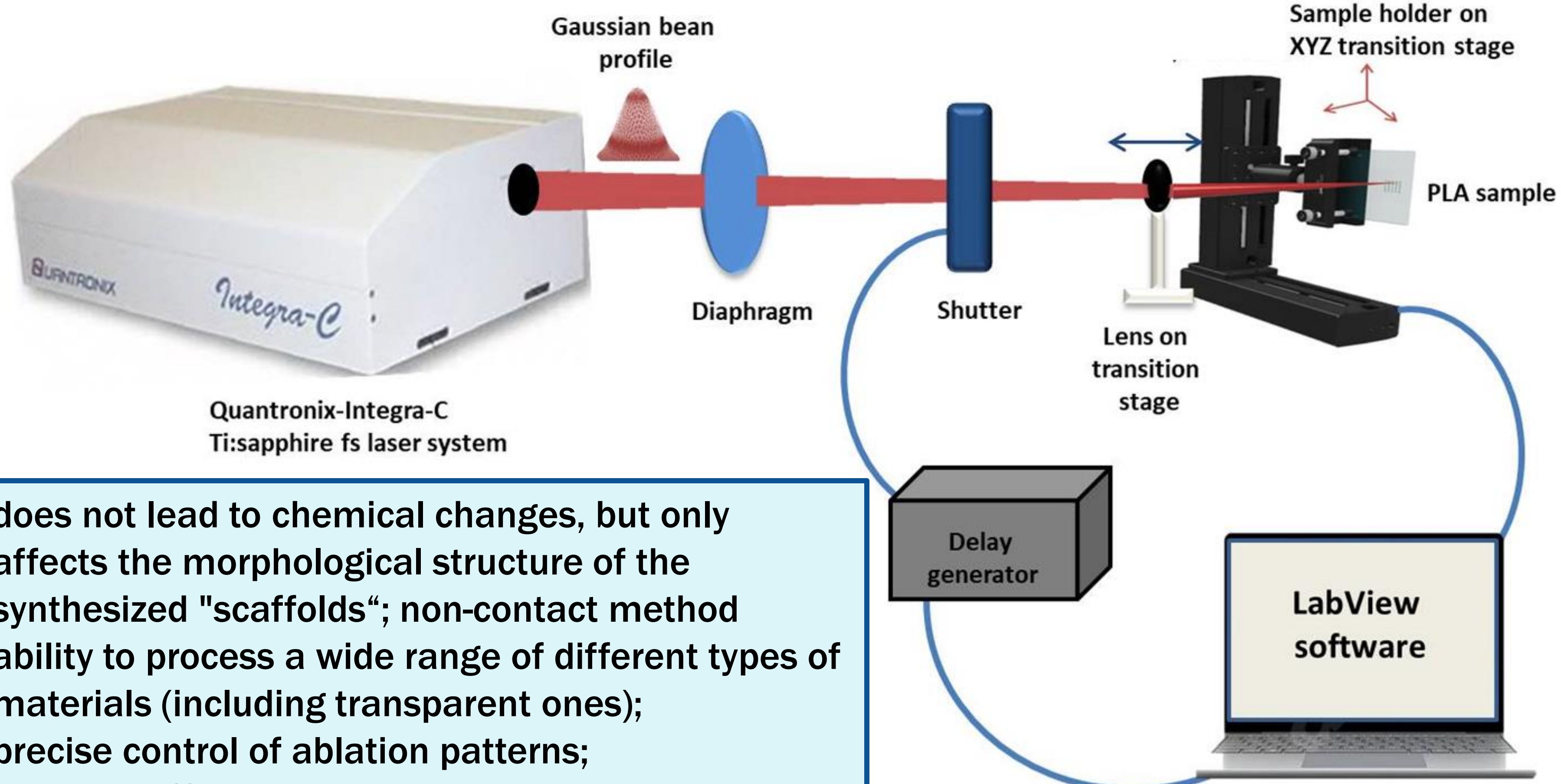
MATERIALS AND METHODS

1. Laser-based functionalization

Ti:sapphire fs laser system (**τ =150fs, λ =800nm, ν=0.5 kHz**) was used for surface modification of PLA samples at fluence **F= 0.8 J/cm²** and scanning velocity **V=3.8mm/s**.



- ✓ does not lead to chemical changes, but only affects the morphological structure of the synthesized "scaffolds“; non-contact method
- ✓ ability to process a wide range of different types of materials (including transparent ones);
- ✓ precise control of ablation patterns;
- ✓ low heat affected zone;
- ✓ absence of molten zones and reduced ablation thresholds as a result, control over the characteristics of biomaterials can be achieved.

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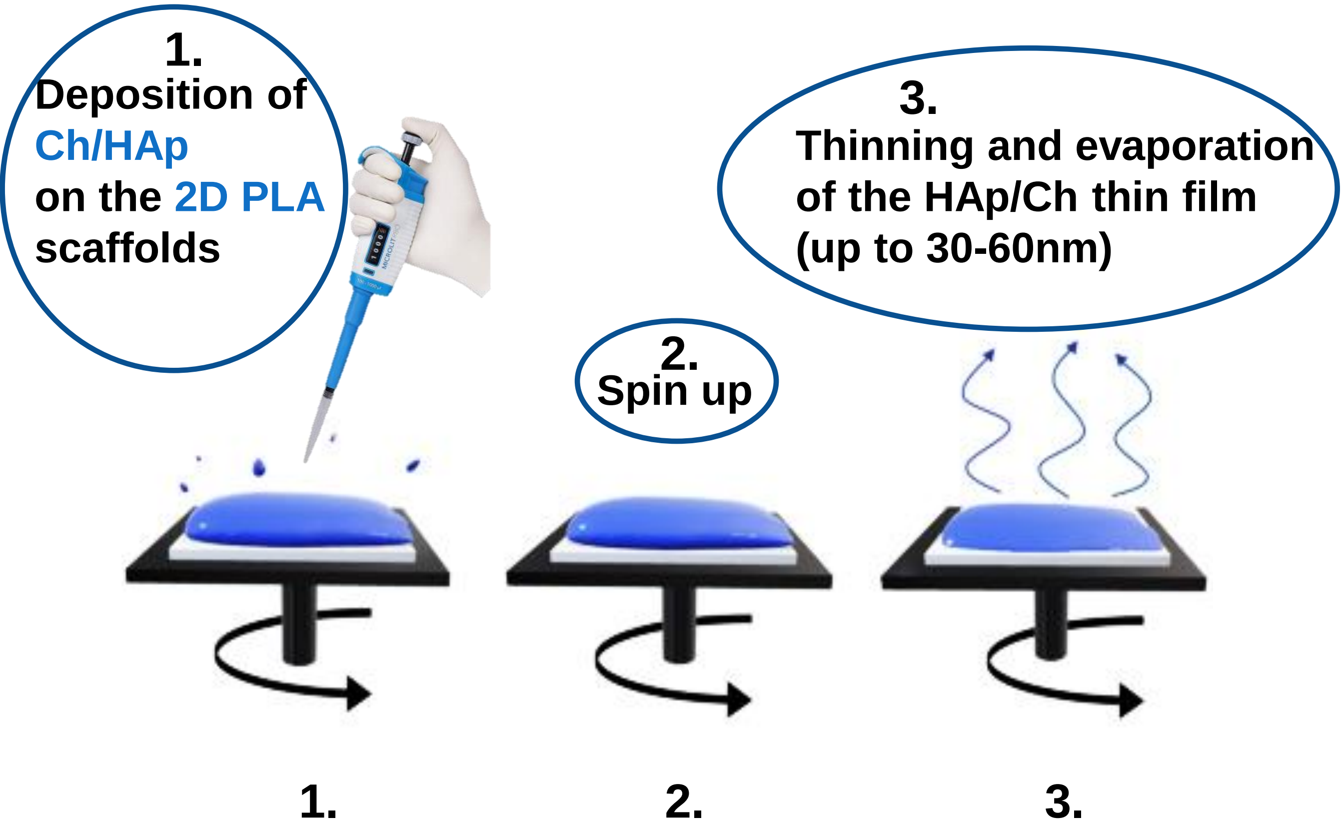
MATERIALS AND METHODS

2. Spin coating of the fs structured/control PLA cell matrices



For each sample two rounds of spin coating were carried out, changing the voltage; the rotation parameters were chosen on the basis of the calibration curve for the used centrifuge movement.

Spin coating	1st round	2nd round
Voltage (V)	8	10
Spinning velocity (rpm)	2500	3000
Time (s)	60	60

In order to monitor their complementary impact on the PLA scaffolds properties, both surface modification methods were applied on the PLA samples, prepared by compression molding. **Each laser processed PLA scaffold was analyzed in respect to control - laser treated and untreated surface, covered with Ch or HAp,** respectively.

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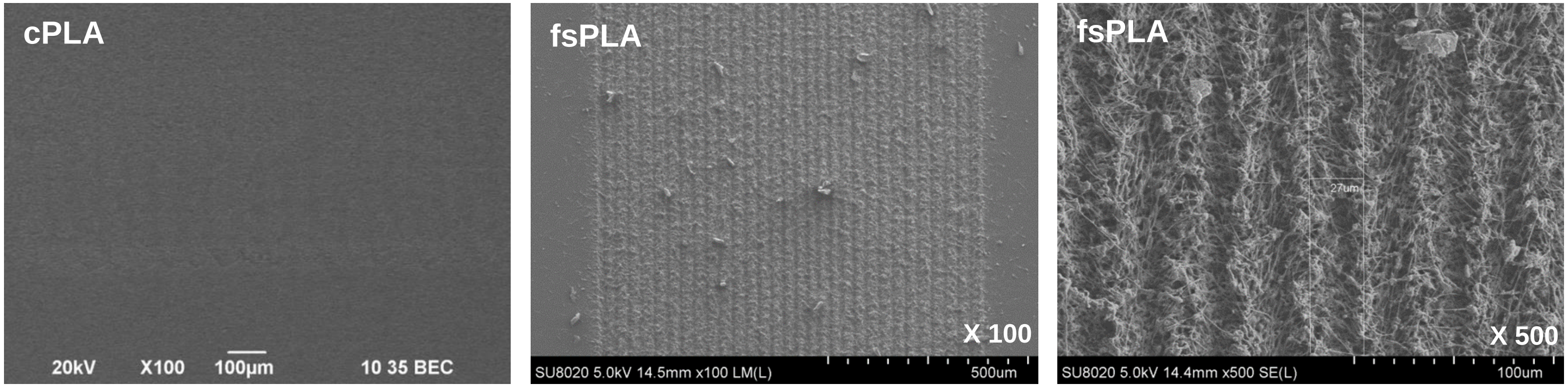
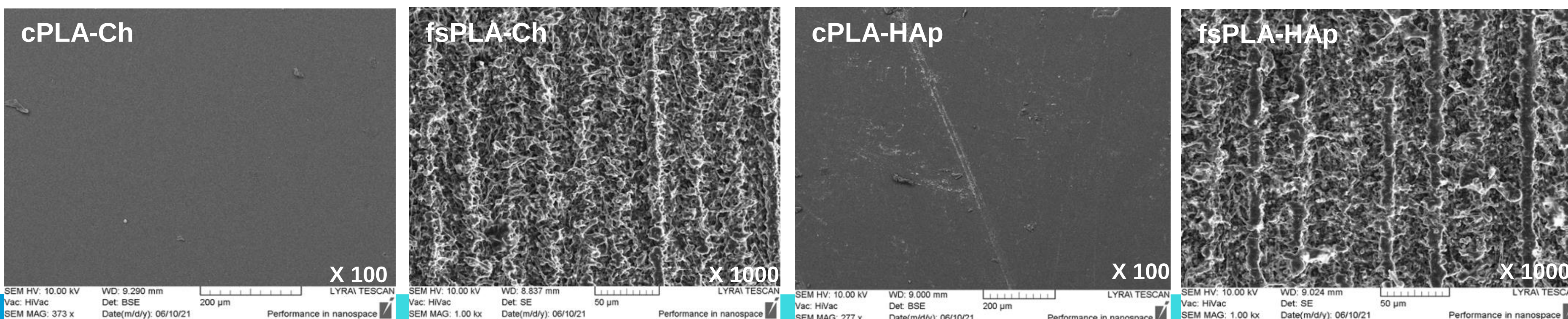
RESULTS AND DISCUSSION - SEM

Control PLA/fsPLA without spin coating



Ra_{cPLA}=0.02µm
Ra_{fsPLA}=1.92µm

Control PLA/fsPLA spin coated with Ch/HAp



- Enhanced surface roughness – fs grooves with precise dimensions → determining the direction of cell growth;
- No change in the morphology and roughness after spin coating, only additional surface functionalization with Ch/HAp;

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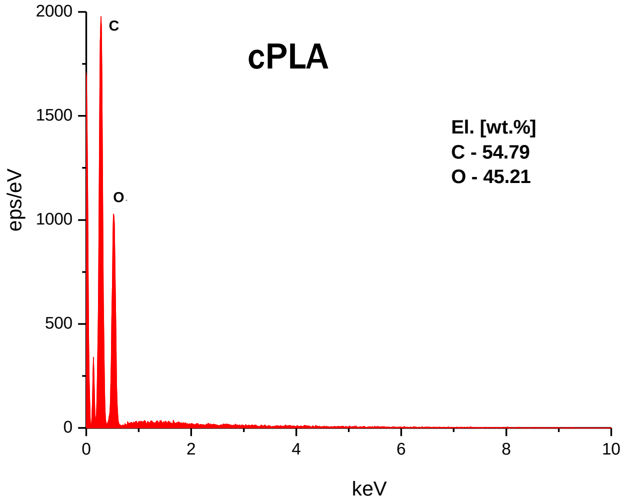
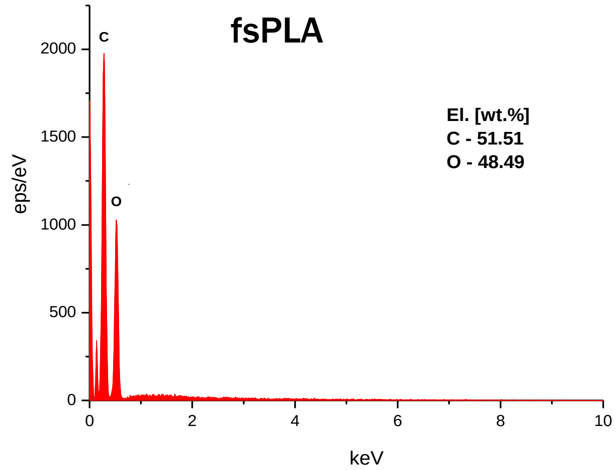
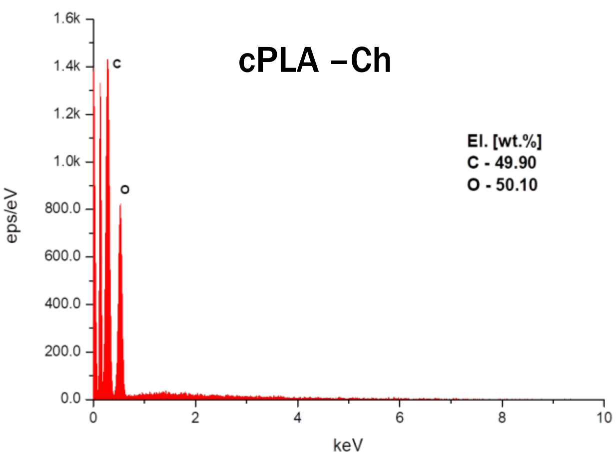
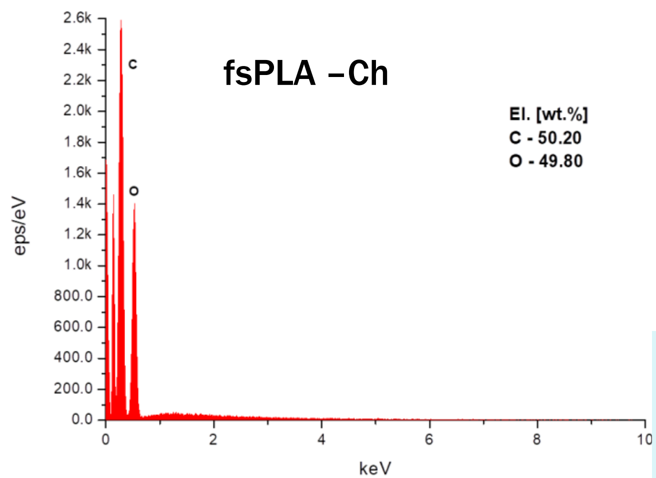
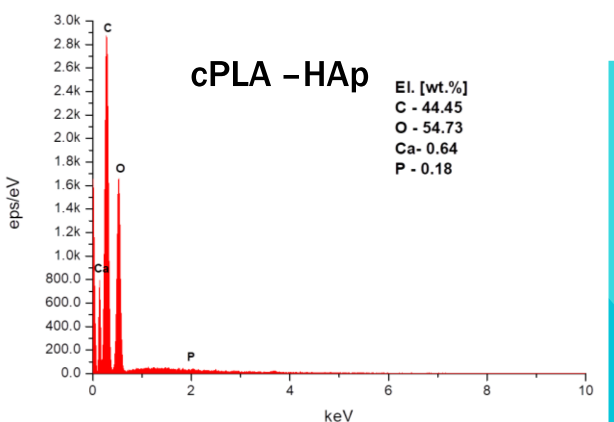
RESULTS AND DISCUSSION - EDX

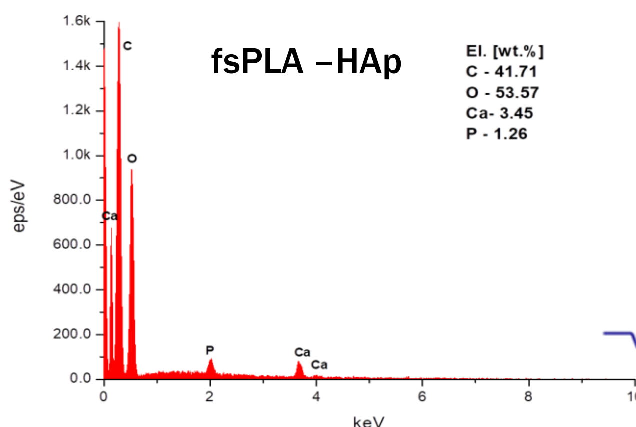












Sample	C [wt.%]	O [wt.%]	Ca [wt.%]	P [wt.%]
cPLA	54.79	45.21	-	-
fsPLA	51.51	48.49	-	-
cPLA-Ch	49.90	50.10	-	-
fsPLA-Ch	50.20	49.80	-	-
cPLA-HA	44.45	54.73	0.64	0.18
fsPLA-HA	41.71	53.57	3.45	1.26

- No chemical alternations, only [wt.%] variations.
- Higher C [wt.%] of fsPLA-Ch compared to cPLA-Ch
- Ca and P [wt.%] of HAp was **5-7 times higher** on fs modified PLA.

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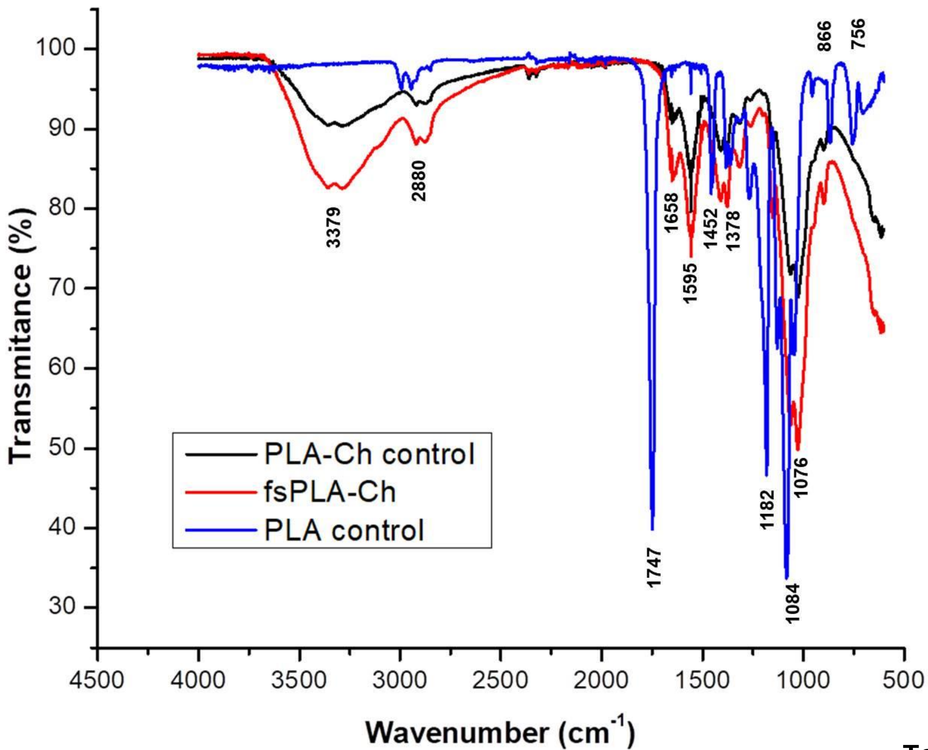
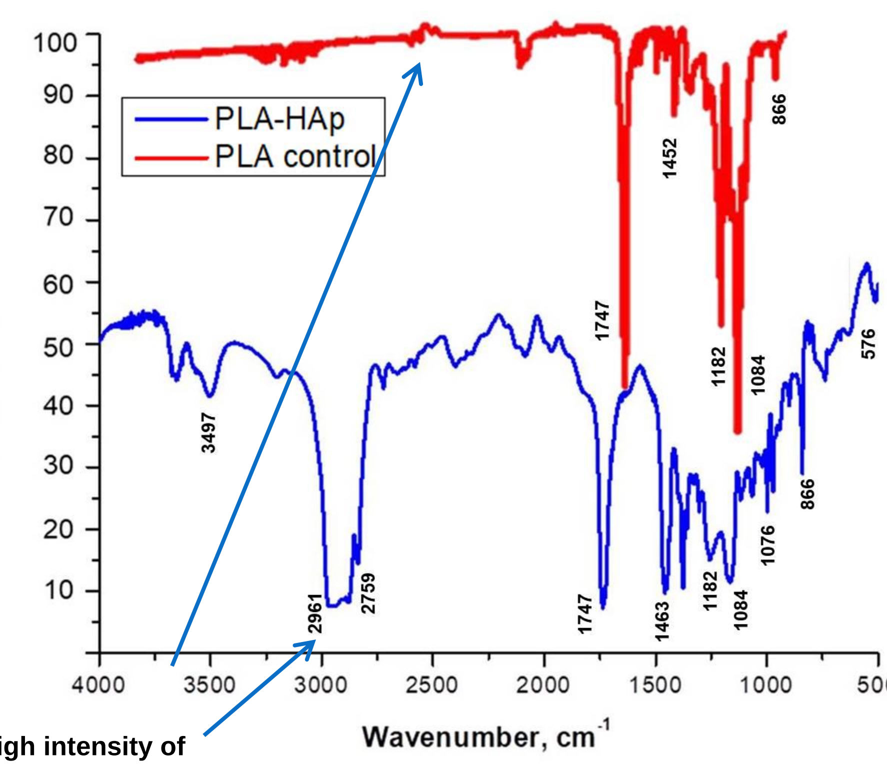
RESULTS AND DISCUSSION - FTIR





Too high intensity of CH_2 and CH_3 (out of scale).

	Hydroxyapatite					Chitosan					PLA				
Band (cm-1)	578	1056	1463	2759-2961	3497	1076	1378	1595 and 1658	2880	3379	1084	1182	1747	2925	3750
Group	PO_4 asymmetric stretching	PO_4 n4 symmetric stretching	CO_2 absorption	C-H stretching	O-H stretching	C-O stretching	bridge O stretching	N-H stretching	C-H stretching	O-H stretching	COOH	C–O–C stretching	C=O stretching	CH_3	O-H stretching

- All transmittance bands of PLA, Ch and HAp are clearly observed;
- No appearance of any uncommon functional groups in the laser ablated samples could be detected

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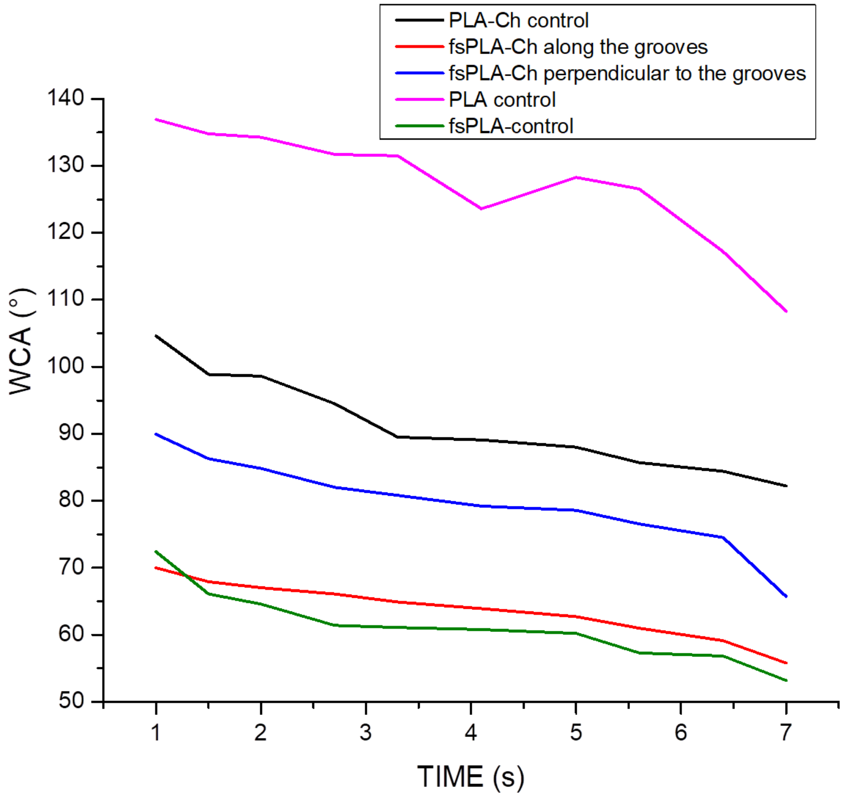
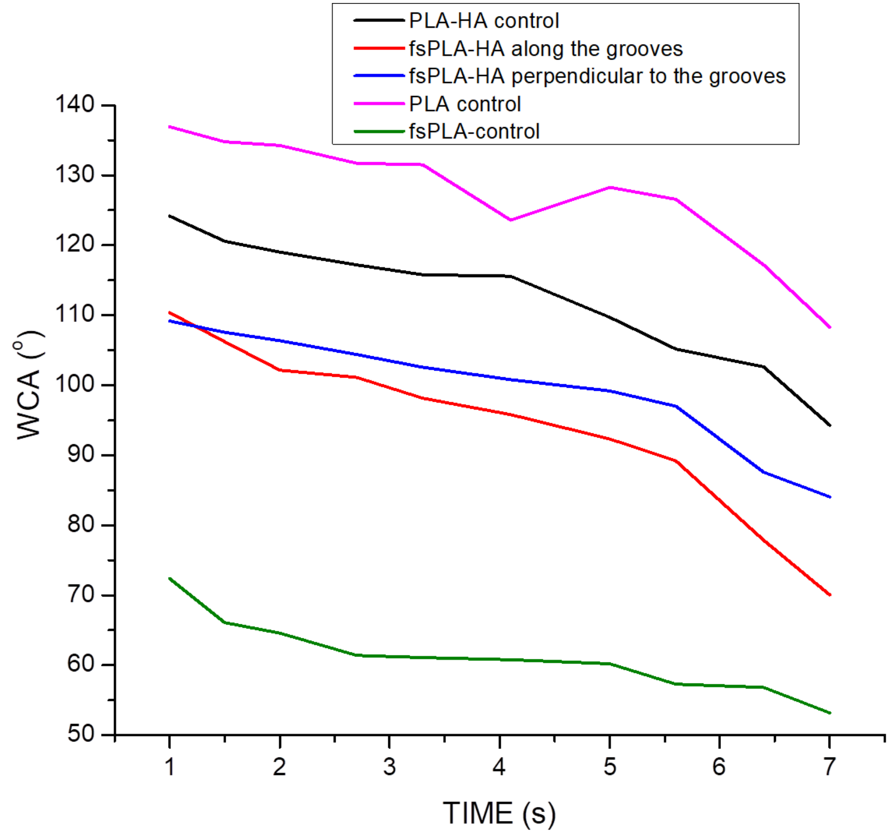
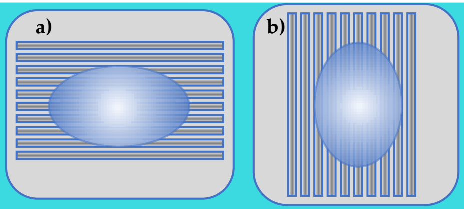
RESULTS AND DISCUSSION – WCA EVALUATION





- PLA has hydrophobic properties;
- Fs laser treatment enhances the wettability of PLA - WCA drops from 138.6º to 52º;
- Ch and HAp also lead to hydrophilic surface of the 2D scaffolds – WCA drops from 108.5º to 55.3º for Ch spin coated fsPLA and WCA drops from 124º to 72.8º for HAp spin coated fsPLA (measured along the laser created grooves);
- enhancement of cell adhesion is one of the key factors for successful scaffold fabrication in tissue engineering;



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fs microstructuring could improve the biological properties of PLA:

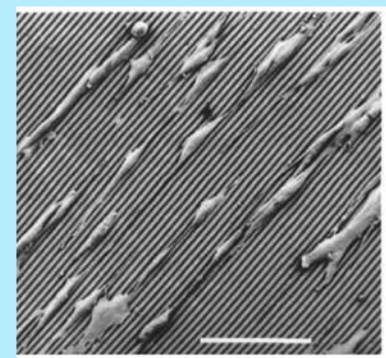
osteoconductivity
osteointegration

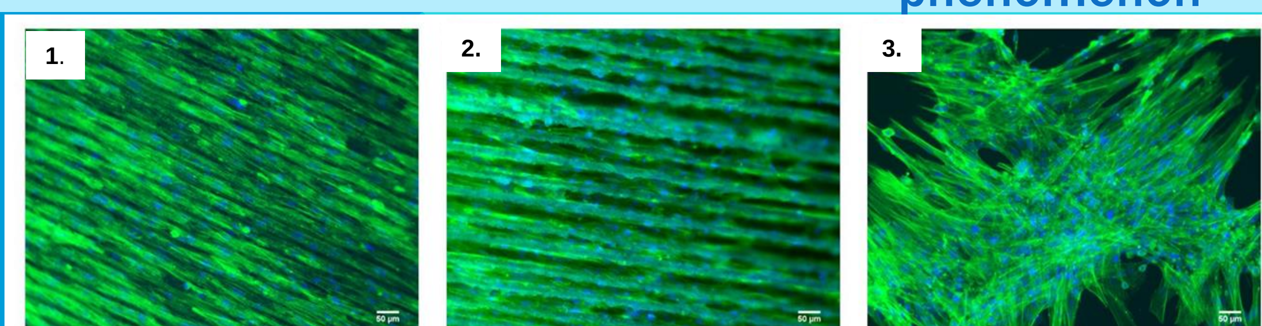
at the same time

Precise control of:

- porosity,
- roughness (Ra, Sa)
- wettability,
- dimensions of the created structures (depth/width of grooves)
- without chemical structure alterations

Tuning the cell seeding conditions by proper optimization of the **fs** laser parameters used; cell orientation and migration phenomenon





Morphology of MSCS at day 14
1 and 2-fsPLA
3-cPLA

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CONCLUSION:

In this study both surface modification methods described above were applied on PLA 2D scaffolds, in order to monitor their complementary impact on the scaffolds properties for optimizing their application in tissue engineering. The microstructured scaffolds were investigated by SEM, EDX, FTIR and WCA analyses. Such combined functionalization of tissue scaffolds can essentially improve bioactivity properties of hybrid 2D matrices.

As a next step, further biological evaluation and cell viability studies will be performed for investigating the influence over cellular dynamics and monitoring a disordered spreading on smooth surfaces to a tendency of orientation along the grooves, in respect to Ch/HAp effect on the cells behavior which could additionally enhance the fs functionalization of the PLA 2D cell matrices studied.

ACKNOWLEDGMENTS:

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