STUDY OF THE MORPHOLOGY AND STRUCTURE OF HYBRID BIODEGRADABLE 3D SCAFFOLDS BASED ON PIEZOELECTRIC POLY(L-LACTIC ACID) AND rGO/GO FOR BONE TISSUE ENGINEERING

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Introduction. Tissue engineering and regenerative medicine (TERM) aim to repair or replace damaged or diseased tissues/organs using artificial materials, including biodegradable ones in intended cases. Also, a bioactive charged surface can provide enhanced cell adhesion/proliferation [1]. Poly(l-lactic acid) (PLLA) polymer is well studied for diverse biomedical applications due to its biocompatibility, biodegradability and simple manufacturing [2]. In addition, PLLA possesses piezoelectric response, which is lower compared to piezoelectric ceramics or non-biodegradable polymers. However, dielectric graphene oxide (GO) and reduced graphene oxide (rGO) nanofillers can improve piezoelectric properties of polymers, as shown for PVDF [3]. Moreover, GO and rGO possess unique physicochemical properties, flexibility and biocompatibility [4]. Thus, the present study aims to investigate the influence of GO/rGO fillers on the morphology and structure of piezoelectric biodegradable PLLA scaffolds.

Materials and methods. The rGO and GO nanoflakes were fabricated by improved Hummers method [4]. To fabricate electrospun hybrid scaffolds (**Table 1**), the different weight concentrations (0.2%, 0.7% and 1.0 wt.%) of rGO and GO were dispersed by ultrasonication (Skymen Cleaning Equipment Shenzhen Co. Ltd, China) at room temperature for 2 hours in chloroform. Afterwards, 10 wt.% of PLLA powder (PL-18, Corbion) was added into rGO/GO solution and subjected under shaking at room temperature for 3 hours for a homogenous mixture. Then, 1 ml of acetone was added into solutions to increase conductivity and viscosity, and mixed for uniform dispersion. Electrospinning was performed using the following parameters: voltage 5-7 kV, tip-collector distance 6-8 cm, room temperature.

The morphology of the prepared scaffolds was examined using scanning electron microscopy (SEM) Quanta 600 (Thermo Fisher, Japan) operated at an accelerating voltage of 10 kV. To study the crystalline structure changes induced by rGO/GO dopants, X-ray diffraction analysis was performed using XRD–6000 diffractometer (Shimadzu Corporation, Japan) operated at 40 kV / 30 mA. XRD patterns were recorded in the automatic mode in the range from 5 to 80°.

Results and discussion. SEM analysis revealed that the addition of GO/rGO led to the formation of defect-free scaffolds (e.g. without beads), thereby resulting in achieved GO/rGO homogenous distribution in fibers. Furthermore, it was found that a high concentration of GO/rGO led to the formation of thinner fibers (**Table 1**). The decrease of the fiber diameter could be attributed to the charge accumulation on the GO/rGO surface in a polymer solution jet while electrospinning, i.e. leading to electrostatic repulsions [5].

Table 1 - The average fiber diameters for GO/rGO-PLLA scaffolds

Scaffold composite	Mean fiber diameter ± standard deviation
PLLA	1.64 ± 0.36 μm
0.2%GO/PLLA	1.35 ± 0.37 μm
0.7%GO/PLLA	1.26 ± 0.37 μm
1.0%GO/PLLA	1.12 ± 0.25 μm
0.2%rGO/PLLA	1.56 ± 0.33 μm
0.7%rGO/PLLA	1.73 ± 0.34 μm
1.0%rGO/PLLA	1.02 ± 0.40 μm

The analysis of XRD patterns of PLLA scaffolds revealed the absence of sharp peaks (**Figure 1a-b**), likely indicating the presence of amorphous or nanocrystalline structure [6]. In

turn, the addition of 0.7 wt.% and 1 wt.% rGO/GO nanofillers in scaffolds led to the appearance of the crystalline reflections of PLLA. The observed reflections at 15.0°, 16.6° and 18.9-19.1º was assigned to (010), (200/110) and (014/203) planes of α-phase, respectively. While reflections located at 16.7-16.8º, 19.2-19.3º and 22.3º can be assigned to (200/110), (201/111) and (210) planes of β-phase, respectively [7]. Thus, the addition of both rGO and GO nanofillers at 0.7 wt.% and 1 wt.% indicate a possible presence of reorganization and formation of new crystal structure in PLLA scaffolds [3]. However, it is worth mentioning that the intensity of XRD peaks was higher in the case of 1 wt.% addition of rGO/GO. This can be explained by the increased number of sites for crystallization or the stretching of fiber upon ES due to enhanced electrostatic repulsion, which resulted in decreased fiber diameter (**Table 1**).

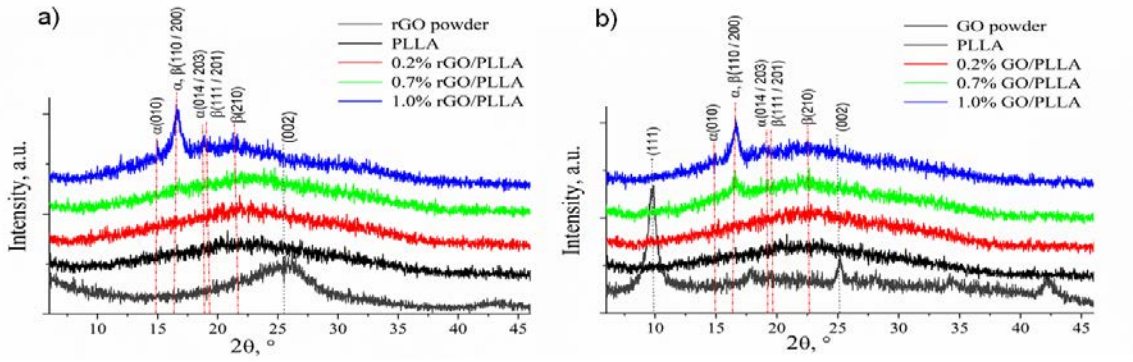

Figure 1 - XRD patterns of (a) rGO/PLLA and (b) GO/PLLA scaffolds

Conclusion. Defect-free PLLA fibers with GO/rGO doping up 1.0 wt.% were sucessfully fabricated using electrospinning. SEM analysis showed that the increase of GO/rGO content resulted in the formation of thinner fibers due to the electrostatic repulsion. In turn, XRD analysis demonstrated that both GO/rGO dopants at 0.7 wt.% and 1 wt.% induce the formation of the α-phase and β-phase in the electrospun PLLA scaffolds.

In the future, the characterization of the piezoelectric properties of fabricated hybrid GO/rGO-PLAA scaffolds via piezoresponse force microscopy will be performed.

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