7º CONGRESSO NACIONAL DE BIOMECÂNICA
Paulo Flores *et al*. (Eds)
Guimarães, Portugal, 10 e 11 de fevereiro de 2017

GRAPHENE OXIDE-COLLAGEN SCAFFOLD AS A VERSATILE MICROENVIRONMENT FOR MECHANICAL STIMULATION ON TISSUE ENGINEERING

André F. Girão[1], Gil Gonçalves[1], Sofia S. Almeida[1, 2], António Completo[1] and Paula A.A.P. Marques[1]

[1] TEMA, Department of Mechanical Engineering, University of Aveiro, Portugal;

[2] Faculty of Engineering, University of Porto, Portugal.

KEYWORDS: Tissue Engineering, 3D composite scaffolds, Graphene Oxide, Collagen, Bioreactor

SUMMARY: *In this study, we pioneerly develop a portfolio of Graphene Oxide – Collagen (GO-Col) composite scaffolds with distinct mechanical properties in order to select the most suitable candidate for tissue engineering (TE) applications. The further analysis of the biological, chemical and mechanical features of the optimal GO-Col scaffold revealed suitable properties for both static and dynamic cell-material protocols.*

1 INTRUDUCTION

The outstanding biochemical and physical properties of GO has placed this nano-biomaterial as a front runner regarding TE strategies since its capability to be used as mechanical reinforcement agent or to enhance cell response [1]. Additionally, due to its highly functional surface, GO sheets can be easily combined with other biomaterials such as proteins and polymers in order to work as building blocks and consequently generate versatile self-assembled next generation 3D structures [2].

In this regard, we tested the potential of collagen, the principal component of the extracellular matrix, as physical crosslinker for GO sheets with the purpose of developing a 3D cellular microenvironment able to associate suitable levels of biocompatibility with enhanced mechanical properties. Our final goal is to be able to modulate cell-material interactions not only via the biochemical and structural features of the scaffold, but also by applying specific mechanical stimuli via a bioreactor and consequently mimic different cellular niches.

2 METHODOLOGY

The electrostatic interactions between the negatively charged GO nanosheets and the positively charged collagen particles were explored at different pH levels and Col/GO (w/w) ratios in order to fabricate an optimal self-assembled hydrogel like structure able to provide a suitable porous network for cell culture protocols after a lyophilisation process. The GO-Col scaffold was thoroughly studied via chemical analysis (XPS and FTIR) and its structural integrity was also evaluated by SEM analysis, swelling tests and via static and dynamic (via a bioreactor) compressive mechanical tests. To evaluate the biocompatibility of the GO-Col scaffold, a Rat Schwann cell line was used.

3 RESULTS AND DISCUSSION

As we have hypothesized, the structural integrity of the GO-Col scaffold is

intimately related with the network of repulsion and bonding forces among the two materials (Fig. 1), which can be modulated by changing the pH of the synthesis medium and the Col/GO ratio (w/w) used. In fact, the evaluation of its mechanical and swelling properties showed that the optimal GO-Col scaffold was obtained using a pH level of 2 and 24% Col/Go (w/w) ratio (Tab. 1).

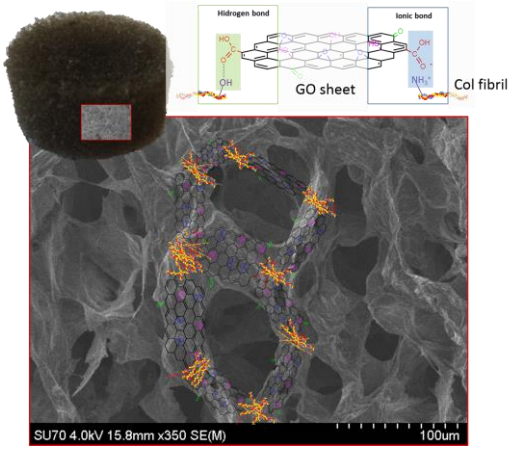

Fig. 1. 3D GO-Col porous network.

Tab. 1. Properties of the GO-Col Scaffold. The scaffolds synthesis parameters are identified as *a.b*, where *a* is the pH of the synthesis medium and *b* is the %Col/GO ratio (%w/w) used.

Scaffold *(a.b)*	Swelling ratio (after 24h)	Compressive modulus at dry state (kPa)	Compressive modulus at wet state (kPa)
2.18	54.52 ± 2.68	12.58 ± 0.55	12.58 ± 0.55
2.24	44.23 ± 4.00	15.75 ± 0.64	15.75 ± 0.64
4.18	63.98 ± 5.18	15.20 ± 1.84	15.20 ± 1.84
4.24	50.13 ± 2.96	17.70 ± 0.64	17.70 ± 0.64
6.12	70.60 ± 10.07	17.52 ± 1.44	17.52 ± 1.44

Complementary, the XPS and FTIR analysis confirmed a successful ionic bonding between GO and collagen, which was a critical factor to assemble a heterogeneous porous network able to potentiate a suitable cellular microenvironment and therefore enhance the cell-material interactions (Fig. 2).

The GO-Col scaffold was also exposed to several dynamic compression-recovery cycles assays performed inside a bioreactor apparatus (Fig. 3) in order to evaluate its potential to integrate TE approaches that include *in vitro* mechanical stimulation. Results showed that independently of the degree of deformation applied (1%, 3% and 7%), the structural integrity of the scaffold was not affected, revealing compression-recovery features compatible with dynamic cell culture protocols [3].

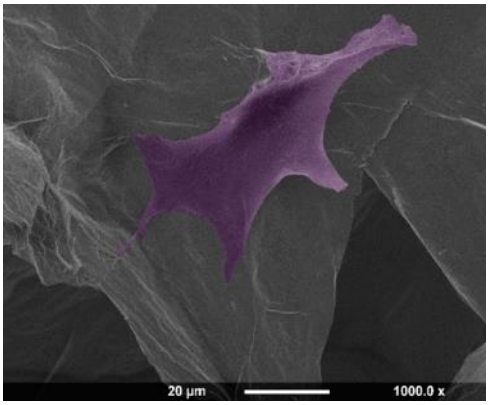

Fig. 2. Cell-material interactions: SEM analysis.

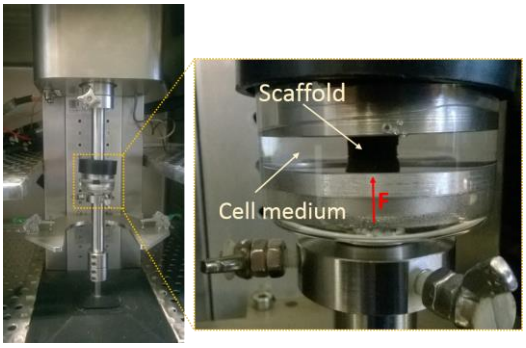

Fig. 3. Mechanical stimulation using the bioreactor technology.

4 REFERENCES

[1] S. Goenka, V. Sant, S. Sant, "Graphene-based nanomaterials for drug delivery and tissue engineering", *J Control Release*, Vol. 10, nº 173, 75-88, 2014

[2] H. Bai, C. Li, X. Wang, G. Shi, "On the gelation of Graphene Oxide", *J. Phys. Chem. C.*, Vol. 115, nº 13, 5545-51, 2011

[3] Y. Jung, S.H. Kim, Y.H. Kim, S.H. Kim, "The effects of dynamic and three-dimensional environments on chondrogenic differentiation of bone marrow stromal cells", Biomed Mater., Vol. 4, nº 5, 1-7, 2009