ABSTRACT
In this study, we pioneerly developed a portfolio of GO-Col 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.

Keywords: biomechanics, graphene, collagen, scaffolds, biomimetic, bioreactor.

INTRODUCTION
Nowadays, the in vitro engineering of complex tissues and organs remains one of the major goals to be achieved in regenerative medicine. Graphene and its derivatives have been extraordinarily explored in several biomedical applications with very promising results [1]. The atomic flat structure based on an aromatic carbon macromolecular structure with different degree of oxygen functional groups make graphene-based materials particularly interesting as a template to house stem cells. Graphene-based materials are able to successfully mimic complex cellular microenvironments in 2D or 3D forms, including films, electrospun fibers, hydrogels and 3D layer by-layer assembly structures. Indeed, recently it was shown that the mechanical stability and physiochemical properties of graphene substrates can be beneficial for stem cells, with the capability to influence their basic life activities such as cell attachment, proliferation and differentiation [2].

RESULTS AND CONCLUSIONS
The structural integrity of the GO-Col scaffolds is intimately related with the network of repulsion and bonding forces among the two materials, 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 (Table 1). 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 potentiathate a suitable cellular microenvironment and therefore enhance the cell-material interactions.

The GO-Col scaffold was also exposed to several dynamic compression-recovery cycles assays performed inside a bioreactor apparatus [3] (Fig. 1) 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, and revealing compression-recovery features compatible with dynamic cell culture protocols [4].

Table 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.

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

Fig. 1 - (a) detail of the in vitro cell culture bioareactor apparatus where the scaffold is dynamically stimulated; (b) schematic representation of the scaffold/cells interactions inside the bioreactor.

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