New bioactive PMMA-Hydroxyapatite based bone cement reinforced with graphene oxide

Gil Gonçalves[a], Sandra M.A. Cruz[a], José Grácio[a], Paula A.A.P Marques[a], Cecilia Ramírez-Santillán[b], María Vallet-Regí[c,d], María-Teresa Portolés[b]

[a]Nanotechnology Research Division, Center for Mechanical Technology & Automation, University of Aveiro, 3810-193 Aveiro, Portugal.
[b]Department of Biochemistry and Molecular Biology I, Faculty of Chemistry, Universidad Complutense, 28040-Madrid, Spain
[c]Department of Inorganic and Bioinorganic Chemistry, Faculty of Pharmacy, Universidad Complutense, 28040-Madrid, Spain
[d]Networking Research Center on Bioengineering, Biomaterials and Nanomedicine, Madrid, Spain

ggoncalves@ua.pt

The technologies associated with production of graphene have evolved greatly in recent times and there is no doubt that graphene has risen as a shining star in the horizon on the path of the scientists searching for new materials for future electronic and composite industry.[1, 2] One of the most interesting applications is the use of graphene as a mechanical reinforcing agent in polymer matrices. The ease chemical manipulation of graphene surface makes it one of the most attractive reinforcements of polymer matrixes,[3] since it is possible to match the interfaces to achieve a seamless integration of all constituents at atomic level.[4]

Polymethylmethacrylate (PMMA) bone cement is widely used for prosthetic fixation in orthopaedic surgery; however, the interface between bone and cement is considered a weak zone. The addition of hydroxyapatite (HA) enhances the connection to the bone since HA is the main inorganic constituent of bone tissue. However, its addition to the PMMA cement formulation lowers the mechanical properties of the composite. One possibility to overcome this problem can be the addition of a second nano-filler that works as a mechanical reinforcement.

In the present study graphene oxide (GO) was added to a composite matrix of PMMA/HA bone cement in the concentration range between 0.01 and 1.0 wt%. The preparation method consisted in the addition of the nano-fillers to the solid fraction of the cement followed by homogenization in aqueous suspension, that was then freeze granulated to maintain the uniform distribution of all the components and dried by liophilization. The nanocomposite cements were then prepared by the traditional technique of mixing solid and liquid phases promoting the PMMA in situ radical polymerization, keeping the solid to liquid concentrations relation of the traditional bone cement unchanged.

The nanocomposite materials were then chemically and structurally characterized showing a very good distribution of the fillers inside the polymeric matrix (Figure 1). Concerning the mechanical testing, the initial results, although positive, were not as high as expected. This has prompted us to realize further studies concerning the determination of the chain size distribution and chemical structure of the polymer extracted from the composite. The data indicate that GO has an active intervention during the PMMA radical polymerization by acting as radical scavenger during the PMMA polymerization reaction due to the delocalized π-bonds. As a consequence, there is inhibition and retardation of the polymerization, which reflects on the final mechanical properties of the nanocomposite.

In order to suppress this drawback we decided to increase the radical concentration added during the PMMA bulk polymerization to overcome the percentage of radicals inactivated by GO. We found that by doubling the initial radicalar agent concentration, the mechanical properties of the final nanocomposites show an enormous increase, being the best results obtained for a GO reinforce of 0.5 wt%.

Considering the final envisaged application of the novel nanocomposite as bone cement, in vitro bioactivity and biocompatibility studies were performed. The ability of this nanocomposite to promote the growth of a calcium phosphate layer at their surface in a simulated body fluid (SBF) was demonstrated. We show the importance of the HA presence in the nanocomposite to promote the bioactive behaviour.

The biocompatibility of this material was evaluated in vitro with mouse L929 fibroblasts and human Saos-2 osteoblasts cultured for 3 days in contact with smooth and rough surfaces of disks prepared with

the novel composite. Both cell types adhere and grow on all these surfaces with high cell viability assessed by propidium iodide exclusion (80-85% in fibroblasts and 90% in osteoblasts) and low apoptosis levels evaluated by flow cytometric analysis of subG1 phase (1% in fibroblasts and 4% in osteoblasts). The morphology of L929 fibroblasts and Saos-2 osteoblasts cultured on disks was analyzed by Scanning Electron Microscopy (SEM) and Confocal Microscopy (CM). Both cell types colonize the disk surfaces exhibiting their characteristic morphology with a distinctive actin network and no apoptotic nuclei. Figure 2 shows the morphology of L929 fibroblasts by SEM (2a) and Saos-2 osteoblasts by CM (2b) after culture on this novel material.

In conclusion, PMMA based bone cements reinforced with GO are potential candidates to be used as bone cements with augmented mechanical properties and appropriated biological behaviour.

References

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Figures:

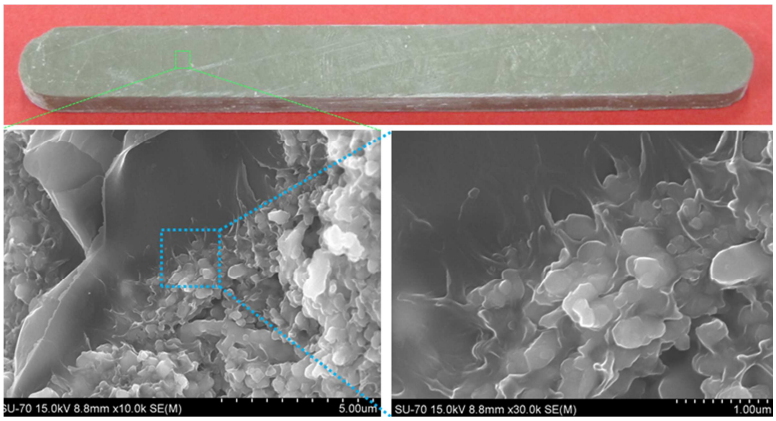

Figure1: Photograph of nanocomposite specimen prepared with 0.5 wt% of GO. SEM images show a good integration of the fillers with the polymer.

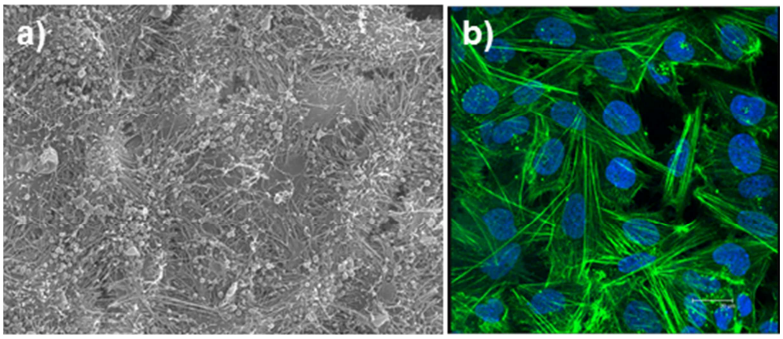

Figure 2: Morphology of L929 fibroblasts (a) and Saos-2 osteoblasts (b) cultured on nanocomposite disks evaluated by Scanning Electron Microscopy and Confocal Microscopy respectively.