Bioactive Nanocomposite Spheres with Proved Shape Memory Capability in Bone Defects

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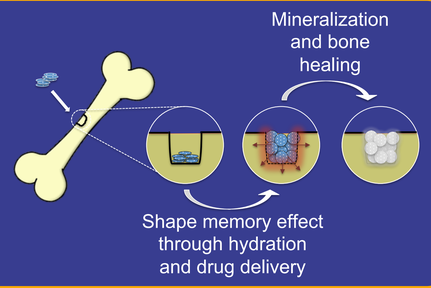
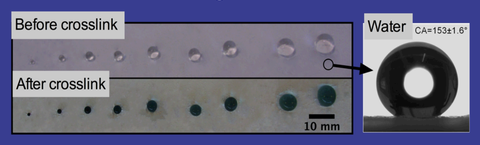
INTRODUCTION

Bioactive glass nanoparticles (BGNPs) promote an apatite surface layer in physiologic conditions that lead to a good interfacial bonding with bone.[1] A strategy to induce bioactivity in non-bioactive polymeric biomaterials is to incorporate BGNPs in the polymer matrix. This combination creates a nanocomposite material with increased osteoconductive properties. Chitosan (CHT) is a polymer obtained by deacetylation of chitin and is biodegradable, non-toxic and biocompatible. The combination of CHT and the BGNPs aims at designing biocompatible spheres promoting the formation of a calcium phosphate layer at the nanocomposite surface, thus enhancing the osteoconductivity behaviour of the biomaterial. Shape memory polymers (SMP) are stimuli-responsive materials that offer mechanical and geometrical action triggered by an external stimulus.[2] They can be deformed and fixed into a temporary shape which remains stable unless exposed to a proper stimulus that triggers recovery of their original shape. This advanced functionality makes such SMPs suitable to be implanted using minimally invasive surgery procedures. Regarding that, the inclusion of therapeutic molecules becomes attractive. We propose the synthesis of shape memory bioactive nanocomposite spheres with drug release capability.[3]



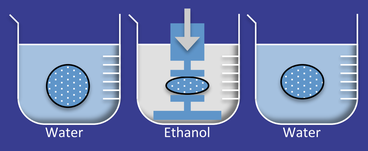
MATERIALS AND METHODS

1% (w/v) CHT was dissolved in an acetic acid solution 2% (v/v). Different contents of GNP (1, 5, 10 and 20% w/w relatively to CHT) and BGNPs (0, 5, 10, 20, 40, 50 and 60% w/w relatively to CHT) were added to the solution. A controlled volume of each formulation was dropped in a superhydrophobic surface. The crosslinking occurred for 24 h.

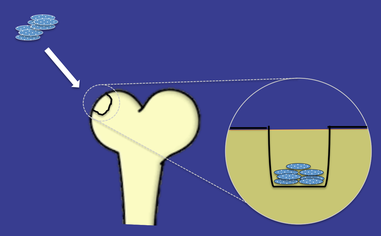


To assess the shape memory capability, hydrated spheres were compressed and the stress was held constant while the sphere was immersed in pure ethanol for 30 min (maximum strain (ε_m) of 75%). The stress was removed, and the sample acquired a temporary shape. R_f characterized the ability of a system to fix the temporary shape and R_r was the recoverability of the permanent shape. They were calculated according to the equations (ε_u was the fixed strain after unloading and ε_p, the permanent strain after induced recovery):

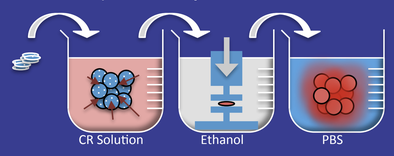
$R_f(\%)=\varepsilon_u/\varepsilon_m \times 100$ $R_r(\%)=1-(\varepsilon_p/\varepsilon_m) \times 100$



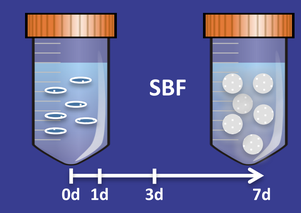
To monitor of the shape memory capability of the spheres; dehydrated BGNPs spheres (10%BGNPs10%GNP) in the temporary shape were placed inside a 4 mm diameter rabbit femur defects.



For drug delivery studies, Congo Red (CR) was loaded into the spheres. The spheres were pre-deformed during 30 min by compression (ε_m=75%). Then, they were immersed in a solution of 10 mg mL^{-1} of CR in PBS for 24 h. After loading, the samples were again compressed (ε_m=75%) and dehydrated for 30 min in ethanol, and dried. After the deformation and fixation of the temporary shape, spheres were immersed in PBS. Aliquots were taken and CR was quantified by absorbance at 498 nm.



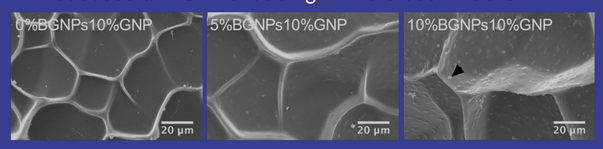
In vitro bioactivity tests were carried out in simulated body fluid (SBF) for 1, 3 and 7 days at 37 ˚C.



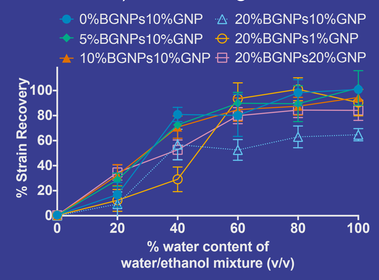
Micro-Computed Tomography (μ-CT) , SEM and EDS were used to assess the composition, morphology and behaviour of the spheres.

RESULTS AND DISCUSSION

The 10 wt % BGNPs content showed a rough surface with evenly distributed BGNPs protuberances. Indicating the successful BGNPs loading in the crosslinked CHT.

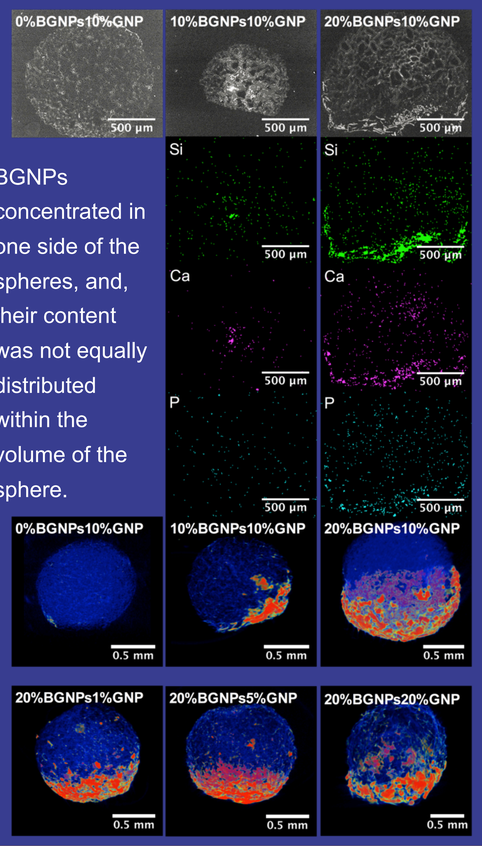


Spheres with no BGNPs content and with lower crosslink (1% GNP content) showed higher strain recoveries.



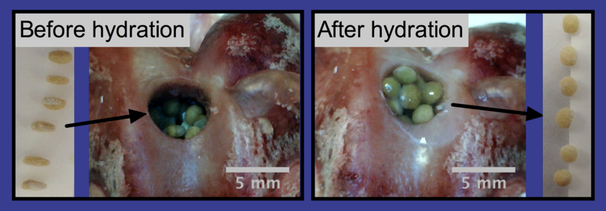
Spheres were capable to maintain the deformation and to recover their permanent shape.

Formulations	Shape Fixity ratio (R_f) (%)	Shape Recovery ratio (R_r) (%)
0%BGNPs10%GEN	72.67 ± 5.20	95.85 ± 9.14
5%BGNPs10%GEN	64.43 ± 11.00	99.19 ± 7.78
10%BGNPs10%GEN	71.47 ± 2.80	92.72 ± 4.63
20%BGNPs10%GEN	17.96 ± 4.90	93.17 ± 3.80
20%BGNPs1%GEN	70.55 ± 7.00	94.85 ± 5.44
20%BGNPs20%GEN	81.57 ± 8.70	88.76 ± 6.70

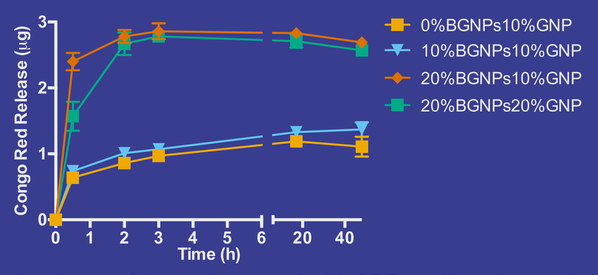


BGNPs concentrated in one side of the spheres, and, their content was not equally distributed within the volume of the sphere.

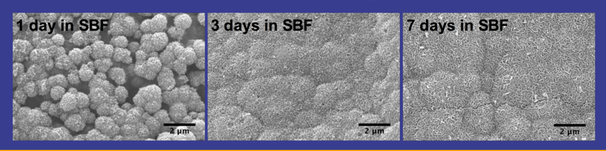
Spheres completely occupied the bone defect, and accommodated trough a press-fitting effect.



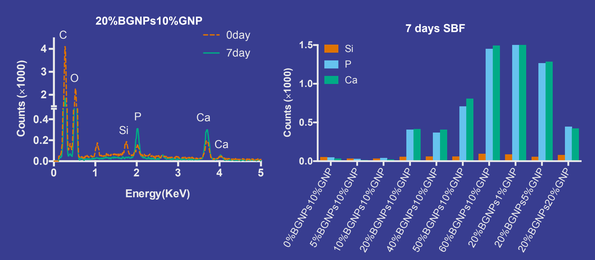
Higher release was achieved for the higher BGNPs content, while higher GNP content slowed the CR release.



Apatite precipitates increased with SBF soaking time and revealed the cauliflower morphology of hydroxyapatite.



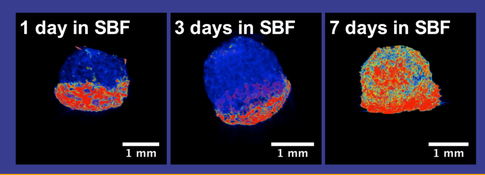
As the amount of BGNPs increased, the relative intensity of the P and Ca peaks also increased, indicating a densification of the apatite layer.



Ca/P ratios were close to the hydroxyapatite stoichiometric theoretical value (1.67).

Formulations	Ca/P
10%BGNPs10%GNP	1.55
20%BGNPs10%GNP	1.54
40%BGNPs10%GNP	1.67
50%BGNPs10%GNP	1.69
60%BGNPs10%GNP	1.59
20%BGNPs1%GNP	1.56
20%BGNPs5%GNP	1.58
20%BGNPs20%GNP	1.62

After 7 days in SBF the apatite depots covered the entire surface of the spheres.



CONCLUSIONS

The composite spheres presented a bioactive behaviour and high values of shape fixity and shape recovery confirmed the shape memory behaviour of the spheres triggered by hydration. The spheres were able to incorporate and release a drug model molecule. These spheres demonstrated to be attractive as bioactive multifunctional biomaterial for bone-related therapies.

References:

1. L. L. Hench, *Am. Ceram. Soc. Bull.*, 1993, **72**, 93-98.
2. A. Lendlein and S. Kelch, *Angew Chem Int Edit*, 2002, **41**, 2034-2057.
3. Á. J. Leite, S. G. Caridade and J. F. Mano, Journal of Non-Crystalline Solids (*in Press*)

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