Influence of flakes-like nanofillers on the microstructure, mechanical, thermal and biological properties of biopolymer based nanocomposite; an application in tissue engineering

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Abstract

Various nanofillers belonging to the group of ceramics and carbons have been used successfully in the modification of polymer matrices and have been applied in the medical field. New nanocomposite materials are characterised by superior properties compared to those made of pure polymer or conventional composite materials. Nanofillers should have high specific surface areas with exposed chemically active groups capable of interaction with polymer chains. The forms and chemical structures of silicate and carbonous nanofillers are similar. The flake-shaped form of silicate corresponds structurally with that of graphite oxide: both are characterised by the presence of various oxygen groups located at the edges of flakes (for silicate; Si-O-Si, Si-OH; for carbons: C=O, C-OH, COOH).

In the present study, both types of flake-shaped forms, i.e. silicate (MMT, or montmorillonite) and carbon (GO, or graphite oxide), were used for the modification of biopolymer matrices based on chitosan (CS) and sodium alginate (NA). Nanocomposite scaffolds were obtained via the freeze-drying method. Microscopic observations showed that both types of nanofiller affected the microstructure of the scaffolds. The presence of MMT in both biopolymer matrices guaranteed pores of a similar shape and size, while GO created larger and much more disordered pores in the scaffolds. These effects of the nanofillers were confirmed during a compression test. Chitosan-based scaffolds with GO were weaker than scaffolds with MMT nanofillers, but the CS/GO nanocomposite was more rigid and survived the test. FT-Raman spectra showed that MMT interacted with the polymer chain more strongly than GO. This effect was also visible in the biological properties of the nanocomposites: only CS/MMT showed bioactivity and water uptake.

Keywords: flake-shaped nanofillers, montmorillonite (MMT), graphite oxide (GO), chitosan (CS), sodium alginate (NA), tissue engineering

1. INTRODUCTION

Recently, nanocomposite materials based on polymers have been used successfully in various technical and medical fields. Nanoparticles have been used extensively in polymer matrix composites as reinforcements in view of several properties deemed favourable in comparison to corresponding microfillers [1–3]. In biomedical applications, nanofillers not only guarantee superior mechanical properties but also promise new features, such as bioactivity in *in vitro* and *in vivo* conditions (nano-HAp, nano-SiO_2) and electric (MWCNT, GO), magnetic (nano-Fe_3O_4), or bacteriostatic properties (nano-Ag) in final products [4–6]. These effects are possible because of the specific structure and morphology of nanofillers. It is well known that the most promising nanofillers have high specific surface areas with exposed chemically active groups capable of interaction with polymer chains and thus of creating new electrostatic and chemical bonding, e.g. van der

Waals or hydrogen bonds [4–5]. Several authors have reported the preparation and characterisation of biopolymer nanocomposites with modified nanofillers prepared by means of *in situ* polymerisation or the solvent-casting method, achieving remarkable biological and physicochemical improvements in polymer properties with nanofiller contents as low as 3–5 wt% [6–8].

Another important but difficult aspect of nanocomposite preparation is the repeatability of these materials. The process of dispersion of nanofillers into a polymer matrix does not guarantee the homogenous distribution of nanoadditives. The same features responsible for new chemical bonding and electrostatic interaction cause repeated agglomeration and inducted some artefacts into the material [9-10]. For this reason, nanofillers of similar shapes, geometries and sizes, but with different chemical compositions and structures which create different properties, will be used in the current experiment to compare their influence on a biopolymer matrix. The forms and morphology of silicate and carbonous nanofillers are similar. Both groups may take the form of flakes (silicates: montmorillonite, kaolinite, halloysite, laponite; carbons: graphite, graphene) or tubes (silicates: kaolinite nanotubes, or KNTs; carbons: single-wall or multi-wall carbon nanotubes). The silicate flake-shaped form structurally corresponds with the graphite or graphene oxide form: both are characterised by rich oxygen groups located at the flakes' edges (for silicates the groups are Si-O-Si and Si-OH; for carbons, C=O, C-OH and COOH). Like silicate nanofillers, graphite nanofillers can be dispersed in different forms in the polymer matrix: they can be flocculated, intercalated or exfoliated [11].

In the present study, both types of flake-shaped forms typical for silicate (montmorillonite, or MMT) and carbon (graphite oxide, or GO) were used for the modification of polysaccharide matrices: chitosan (CS) and sodium alginate (NA). The first step of the experiment consisted of the optimisation of the nanofiller concentration and dispersion method. In the second step, nanocomposite scaffolds were obtained using the freeze-drying method. Both types of nanofiller (GO and MMT) affected the microstructure of the scaffolds (SEM analysis): the presence of MMT in both biopolymer matrices guaranteed pores of a similar shape and size, while GO created larger and much more disordered pores in the scaffolds. A stiffer porous microstrucuture characterised the nanocomposite with GO and a chitosan matrix, while the materials modified with MMT showed bioactivity and water uptake, which are important properties in tissue engineering. To understand this behaviour, spectral analysis (FT-Raman) was performed. The result of this investigation showed that MMT interacted with the polymer chain more strongly than GO. The carbonyl and carboxyl group present at the edge of a graphite sheet can play an important role in electrostatic interaction, as observed during the compression test - the distance between the polymer chain and graphene was reduced and the chemical group was able to interact. Both flake-shaped nanofillers have the potential for use as fulfilling material with a porous microstructure to support cells in the proliferation and regeneration process.

2. MATERIALS

In the experiment, two kinds of biopolymer matrices were used: chitosan (Sigma-Aldrich) and sodium alginate (FMC BioPolymer). The chitosan (CS) was characterised by medium molecular weight, a degree of deacetylation under 75–85%, and a viscosity of 200–800 cP (1 wt% in 1% CH_3COOH solution, 25°C). The sodium alginate (NA) was characterised by medium molecular weight and a viscosity greater than 2,000 cP (2 wt% in water at 25°C). As nanoadditives, commercial flake-shaped fillers were used. Characteristics of nanofillers used in the study (manufacturer data) are presented below (Table 1).

Table 1 Characteristics of nanofillers used in the experiment: MMT and GO

Name of particles	Particle size [nm]	Specific surface area [m^2/g]
MMT K-10 (Sigma-Aldrich)	flake thickness 8 nm, flake size 50-300 nm	220-270
AO-4 (Graphene Supermarket)	flake thickness 60 nm, flake size 3-7 μm	<15

The shapes and sizes of flake-shaped particles were confirmed by means of TEM observation (Nikon Epiphot 300) and DLS technique (Mastersizer 2000). The morphology of flake-shaped nanoparticles is similar (Fig. 1),

but graphite oxide was characterised by larger flakes, with most in the range 2–15 μm, whereas MMT was characterised by flakes about 0.5–1 μm (Fig. 2). Both nanofillers showed a tendency to agglomerate in a water solution. The homogenisation effect improves when the viscosity of the solution is increased (1 wt% biopolymers dissolve in water for NA or 3% acetic acid for CS). The distribution of nanofillers is most effective when homogenisation is done in two steps: stirring with a magnetic stirrer for 24 h at room temperature and then again for 5 min with an ultrasonic stirrer at a temperature under 10°C (to protect the biopolymer from degradation processes).

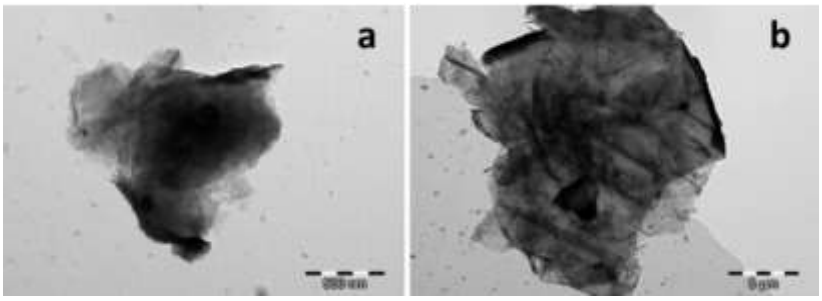


Fig. 1 Morphology of flake-shaped nanofillers: MMT (a) and GO (b)

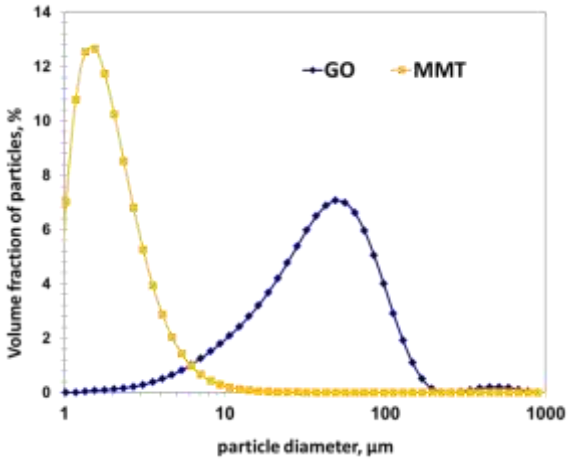


Fig. 2 Size of nanofillers used in the experiment: MMT and GO (in H_2O) estimated using the DLS technique

3. METHODS

Nanocomposite porous scaffolds were obtained using the freeze-drying method. First, the biopolymer solutions were prepared (1% NA in H_2O and 1% CS in 3% CH_3COOH), then 2 wt% of flake-shaped nanofillers (MMT or GO) was homogenised into the biopolymer and frozen at –80°C for 24 h. The freeze-drying process was carried out using a Labconco 5.0 lyophiliser for 24 h at 0.03 torr/–80°C. The final samples took the shape of a cylinder, 10 × 20 mm. The influence of flake-shaped nanofillers on the microstructure of porous samples was observed under an FEI Nova NanoSEM scanning electron microscope. Mechanical properties were measured by means of a compression test (elongation of samples was observed under a tensile strength of 3N) using a Zwick 1435 universal testing machine. The influence on structure composition was investigated using Raman spectroscopy (FT-Raman, Renishaw inVia). The bioactivity test used an artificial SBF mixture [10]. After 7 days of incubation in an *in vitro* condition (37°C/SBF/5% CO_2), the microstructure of samples CS/GO and CS/MMT was observed using SEM/EDS microscopic analysis (Nova NanoSEM, FEI). Water uptake was measured after 24 h of incubation in an *in vitro* condition (H_2O/37°C/24 h) as in a previous study [11].

4. RESULTS AND DISCUSSION

Microscopic observation showed that both types of nanofiller influence the microstructure of scaffolds (Fig. 3). Pure porous scaffolds based on CS or NA are characterised by different pore diameters and shapes: in CS scaffolds, pores are characterised by regular architecture, whereas in NA scaffolds they are larger and less regular. The addition of smaller flake-shaped nanoadditives such as MMT into both biopolymer matrices guaranteed pores of a similar shape and size irrespective of the biopolymer matrix. In chitosan, the size of pores increased compared to pure CS; in an alginate matrix, MMT nanofillers reduced the size of pores compared to pure NA (Fig. 3). Larger flake-shaped fillers such as GO created larger and much more disordered pores in scaffolds. The stronger effect of GO modification is observed in the chitosan matrix: the porosity of this material increased by about 40% over pure chitosan scaffolds.

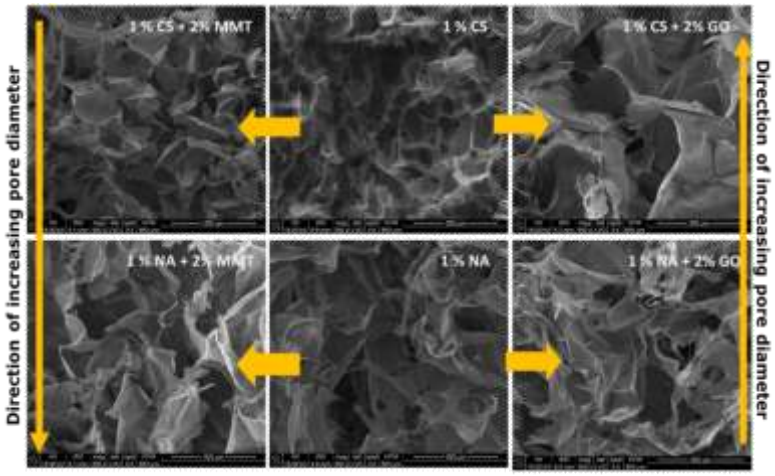


Fig. 3. Influence of nanofillers GO and MMT on the microstrucuture of scaffold samples: NA/MMT, NA/GO and CS/MMT, CS/GO

The freezing conditions proposed in this experiment (–80°C/24h) enabled a slower rate of growth of ice crystals along with a faster rate of nucleation. As a result, the samples were expected to be characterised by small pore size and a high specific surface area [12, 13]. MMT nanofillers present in both biopolymers stabilised this effect: the microstrucuture of CS/MMT and NA/MMT showed homogenous pore distributions. Graphite oxide influenced to the deformation of pores, which was probably the effect of the size of flake-shaped fillers (3–7 μm) and their weaker interaction with a biopolymer matrix (smaller number of chemically active groups in comparison to MMT flakes). In any type of scaffold modified by flake-shaped additives, skin-layer was not observed which is in contrary to the scaffolds based on pure biopolymers (both types: CS and NA) where this layer was clearly identified [14].

Many authors in the field of nanocomposite materials have shown that nanofillers also improve mechanical properties in porous materials [15]. Most biopolymers, such as chitosan and alginate, combine with MMT or CNT or ceramic nanoparticles such as HAp [15–17]. When biopolymers were modified with flake-shaped nanofillers, a dual effect could be observed (Fig. 4). Scaffolds with GO are stiffer than scaffolds with MMT nanofillers, in spite of their larger pores and more irregular porous microstructure. Additionally, CS/GO nanocomposites are not destroyed at the end of the test. It seems to be easier to implant scaffolds modified with GO than those modified by MMT into a human body.

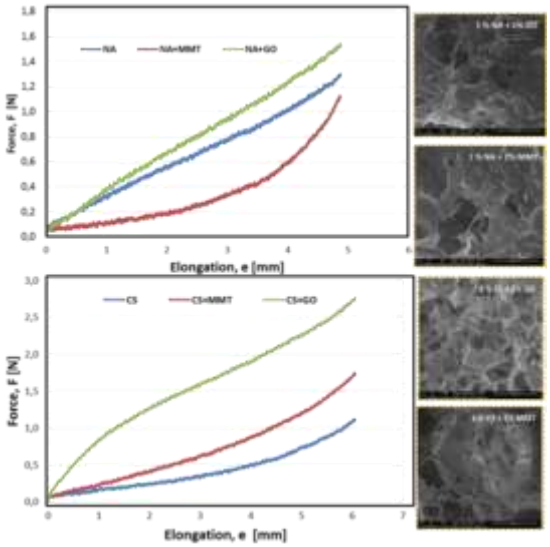
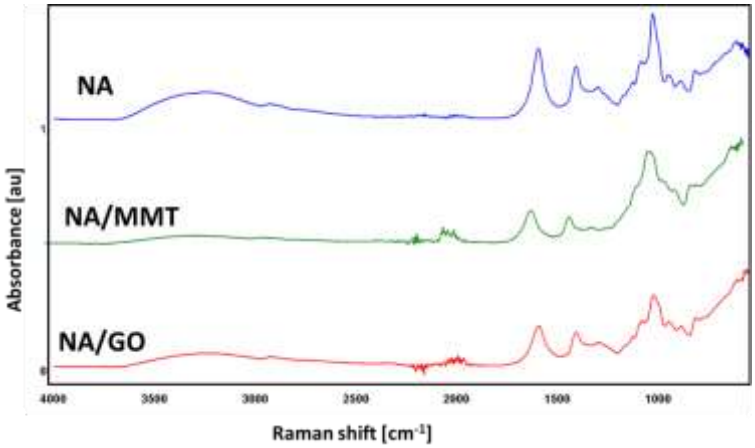


Fig. 4. The mechanical properties of nanocomposite scaffolds based on CS and NA and modified by GO and MMT flake-shaped nanoadditives

The biopolymers selected in the experiment (CS, NA) are characterised by amine, hydroxyl, carboxyl and carbonyl groups easily identified in Raman spectra (Fig. 5a, b). The polysaccharide chain possesses many active sites siutable for modification and/or interaction processes [18]. In FT-Raman spectral analysis, the stronger interaction between MMT and chitosan was observed, comparing to GO and chitosan chain. Chitosan matrices are much more compatible with MMT nanofillers: hydroxyl groups, as well as amine groups, can bond with Si-O-Si groups of the silicone layer of montmorillonite [19-21]. These effects could be stabilised by a CH_3COOH solvent, because a MMT nanofiller in a liquid system of CS/CH_3COOH facilitated the formation of hydrogen bonds [22]. Much more homogenous chemical structure in the sodium alginate chain which is rich only in carbonyl, carboxyl and hydroxyl groups, can result in weaker interaction with both nanofillers (MMT and GO) (Fig. 5a).



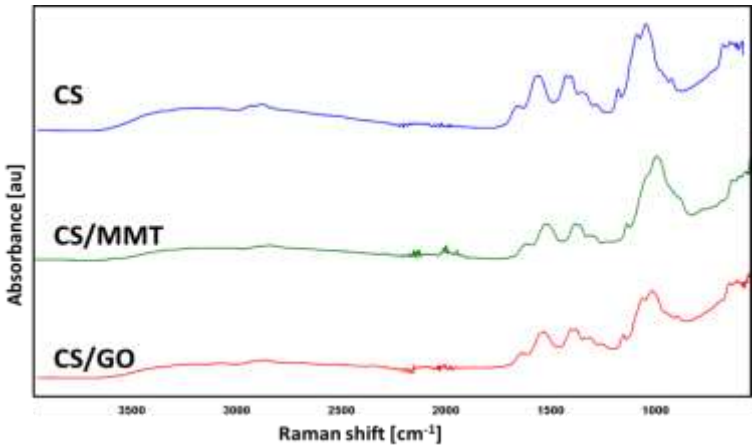


Fig. 5. The FT-Raman spectra of nanocomposite scaffolds based on NA (a) or CS (b) and modified by GO and MMT flake-shaped nanoadditives.

Chitosan belongs to the group of hydrogel polymers characterised by high water uptake. This feature is stronger in porous scaffolds, in which pores are inclined to water accumulation. When hydrogel matrices (CS) were modified by flake-shaped nanofillers, scaffolds showed higher water uptake than a pure CS porous sample. The larger pores present in the CS/GO nanocomposite did not guarantee improved wettability (114°) or consequent higher water uptake (650% for CS and 715% for CS/GO). The smaller and more homogenous pores in CS/MMT scaffolds were characterised by higher wettability (92°) and more significant water uptake than pure chitosan (1050% for CS/MMT). Probably this is a good starting point for the supersaturation process, inducting nucleation and apatite formation in a CS/MMT nanocomposite [23]. These phenomena are observed after 7-day incubation in SBF solution in an *in vitro* condition (Fig. 6). Not even long-term incubation of nanocomposite materials with GO nanofiller (21 days) initiated apatite mineralisation.

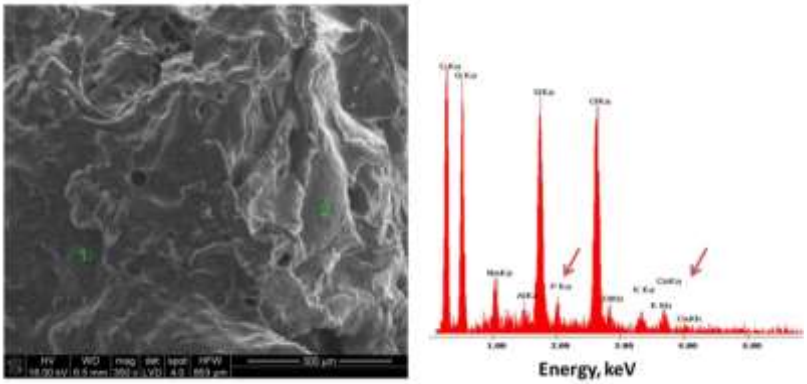


Fig. 6. SEM/EDS analysis of a nanocomposite porous scaffold CS/MMT after a bioactivity test

5. CONCLUSION

The results of the investigation show that a stronger interaction was observed between biopolymers and the MMT flake-shaped nanofiller: a homogenous microstructure and bioactivity which correlated with structural changes. Nanocomposite porous scaffolds with MMT nanofiller are better candidates for bone- tissue engineering (water uptake, faster apatite formation process) but the most practical materials which can be used for scaffold fulfilment seem to be CS/GO nanocomposites. Chitosan modified by GO was characterised by better mechanical properties and guarantees an expanded range of pores in polymer scaffolds. CS/MMT nanocomposite scaffolds seem to constitute a much more compatible system than NA/MMT.

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