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Wollastonite-Poly(Ethylmethacrylate-Co-Vinylpyrrolydone) Nanostructured Materials: Mechanical Properties and Biocompatibility

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Abstract.
Synthetic pseudowollastonite (psW) and a nanostructured copolymer made of a biostable component, Poly(ethylmethacrylate) (PEMA) and a bioresorbable component, vinylpyrrolidone (VP) are used in this work for the preparation of a new family of bone substitutes that allow osseointegration and mechanical stability. Composites are prepared by bulk polymerization of the desired composition in 15 mm diameter cylindrical plastic moulds. Polymerization was induced thermally at 50°C using 1wt% azobis(isobutyronitrile) (AIBN) as free-radical initiator. The moulds were filled to a height of 100 mm and 1 mm height discs were cut with a diamond saw. Specimens with a ceramic/polymer ratio 58/42, 33/67,17/83 and 0/100 were obtained. Compression stress in the range 39-59 MPa and elastic modulus between 2.64 and 4.14 GPa are obtained where the greater values correspond to the specimens prepared with a 60% ceramic load. Degradation in SBF produces a porous nanostructure in the polymeric component indicating microdomains of different solubility and the formation of an apatite-like layer on the surface of the wollastonite component. All the compositions assayed present a biocompatibility at least of the level or even superior than the Thermanox® control used.

Introduction

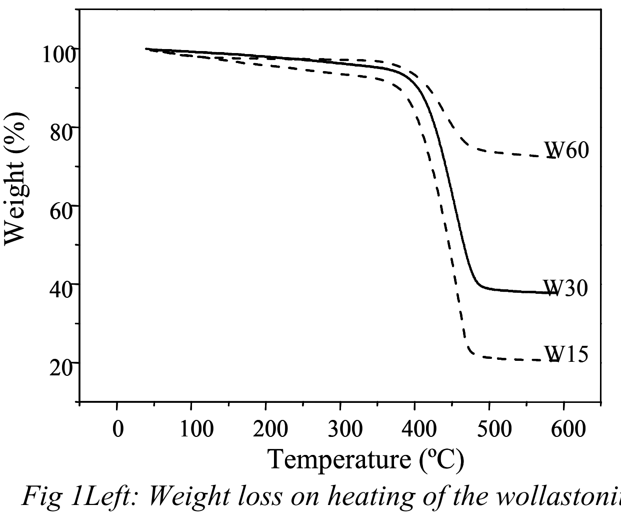
Ability for osseointegration and mechanical stability of a device are the key features for the success of a bone substitute. Osseointegration can be achieved with an interconnected pore system with pore diameters over 100 microns [1]. Nevertheless, such a pore network provides artificial bone substitutes with a very low mechanical strength that restricts the viability of these devices.
Several approaches have been taken to overcome that shortcoming. One of the most promising methods proposed involves the use of a several phases composite where one of the phases is a resorbable polymer [2] The approach assayed in this work involves the manufacturing of a composite made of a bioactive ceramic, synthetic pseudowollastonite (psW) and a nanostructured copolymer made of a biostable component, Poly(ethylmethacrylate) (PEMA) and a bioresorbable component, vinylpyrrolidone (VP). Wollastonite containing materials have shown the ability to create porosity in situ when they are in contact with physiological fluids[3] not producing significant cytotoxic effect but a quick osteoblastic cells adhesion in vitro and integration with bone tissue in vivo[4]. PEMA was selected based on its high ductility, low toxicity and low exotherm [5] while VP may act as a biodegradable support that help on creating porosity while can also be used for the delivering of antibiotics or growth factors where the release rate can be controlled by the resorption profile of the copolymer composition.
The purpose of this paper is to determine the amount of ceramic that may be load into a VP/PEMA 60/40 wt % copolymer. Compression strength, elasticity modulus, degradation behaviour, bioactivity and biocompatibility in vitro are assessed.

Materials and Methods

A natural Wollastonite (W-2M) (Vansil® W40, Vanderbilt, Co., Inc.), with an average particle size of 13.2 µm and 0.99 SiO_2/CaO molar ratio, was selected as the ceramic component and 1-Vinyl-2-pyrrolidone (VP) (Aldrich) and Ethylmethacrylate (EM) (Acros) were chosen as the organic components for the preparation of the copolymer system. Prior polymerization, (VP) monomer was vacuum distilled. The monomers were mixed in a 60/40 weight ratio and the ceramic component was added in four different ceramic/polymer weight ratio: 0/100, 15/85, 30/70 and 60/40. The resulting specimens will be called *W0*, *W15, W30* and *W60* hereafter. A bulk polymerization was induced thermally at 50°C using 1wt% azobis(isobutyronitrile) (AIBN) as free-radical initiator by introducing the desired composition into a cylindrical plastic mould with a diameter of 15 mm. The moulds were filled to a height of 100 mm. Discs of 1 mm height were cut with a diamond saw using alcohol as lubricant.
The nanocomposite specimens were characterized by thermogravimetric analysis in a Perkin Elmer TGA 7, differential scanning calorimetry in a Perkin Elmer DSC7, ^{1}H NMR spectroscopy were performed in a Bruker Advance-300 working at 300 MHz and ATR-FTIR spectroscopy with a Perkin Elmer Spectrum one spectrophotometer. X-ray diffraction patterns were recorded in a glazing angle mode in a Siemens D5000 diffractometer, Scanning micrographs were taken with an environmental microscope Phillips ESEM XL30 and a field emission microscope Hitachi S-4700. Mechanical properties were measured on 12x6 mm cylinders in compression mode with an Instrom universal testing machine. Bioresorption and bioactivity were tested by immersion of 10 mm x 1mm disks into a Kokubo simulated body solution (SBF). Cytotoxicity was tested with a primary culture of human fibroblasts. The cells were seeded in a 96 well-tissue culture plate with a cell density of $11x10^4$ cell/mL. The cytotoxicity was determined using the MTT assay.

Results and Discussion

Thermal analysis of the wollastonite containing specimens has shown a weight loss between 316 and 480ºC that corresponds to the thermal decomposition and volatilization of the polymeric component as displayed in figure 1 left. The ceramic/polymer weight ratio can be extracted from that weight loss. The analysis has yielded 58/42, 33/67 and 17/83 for the specimens *W60, W30* and *W15*. Scanning micrographs and element mapping enable to appreciate that the composites are made of discrete acicular particles of W-2M distributed over a homogeneous polymeric matrix. A *W15* specimen micrograph is displayed in figure 1 right as an example.



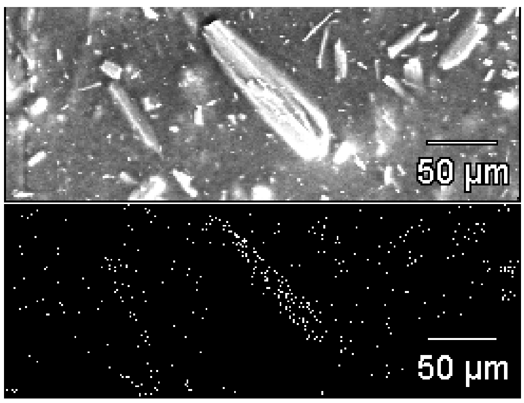


Fig 1Left: Weight loss on heating of the wollastonite containing specimens. Righ: SEM picture and SI mapping of a W15 specimen

The analysis of the compressive strength of the composite specimens has yielded a value of 42 $\pm$ 8 MPA (where $\pm$ 8 represents the standard deviation) for the non reinforced specimens and values of 39 $\pm$ 14 , 40 $\pm$15 and 59 $\pm$ 9 for the reinforced specimens *W15*, *W30* an *W60*. Therefore a greater resistance under compression is obtained only when the ceramic load gets up to 60 % of the total weight. Some studies in particulate filled resins have shown that a low degree of adhesion between the particles and the matrix produces a decrease in the yield stress of a specimen [6]. Other studies in particulate filled PMMA cements have shown that the poor adhesion between the filler and the matrix can be compensated by larger amounts of filler particles resulting in constant values of the yield stress [7]. The studied compositions seem to reproduce this compensation mechanism where only the effects of the greater amount of ceramic load totally overcome the effect of the lack of adhesion resulting in a greater compression resistance. The elasticity modulus range from 2.83 $\pm$ 0.2 GPa for the non reinforced specimens to 2.64 $\pm$ 0.5, 3.39 $\pm$ 1 and 4.15 $\pm$ 1 for the *W15, W30* and *W60* specimens. This is a 47% and 20 % increase for the 58/42 and 33/67 specimens in comparison to the copolymer specimens while the *W15* composition does not show a significant increase in elastic modulus compared with the non reinforced specimen. These values are all greater than the elastic modulus of PMMA cements determined to be 1.6 GPa[8] and closer to the compressive modulus of compact bone for femur reported to be up to 17 GPa [9] and they are greater than the values obtained for several calcium phosphate cements, reported to range between 0 and 51 MPa[10]. As a reference, the compressive strength of femur compact bone varies around 200 MPa where cancellous bone is much weaker having a compressive strength of 1-12 MPa.[9].

After soaking the specimens in SBF for a week, two different phenomena were observed ***(a)*** in the polymeric matrix, a porous nanostructure is formed indicating microdomains of different solubility. Those zones that appeared to be dissolved must be related to the more resorbable VP rich microdomains, whereas those zones that seems to be unaffected by the immersion should correspond to the more stable EM rich domains. This microdomain segregation can be related with a dramatic difference in the reactivity of both monomers in free radical polymerization. ***(b)*** On the W-2M surfaces, the formation of a ceramic layer was observed by SEM. The thickness of this layer is greater for greater percentages of the wollastonite component. ATR-FTIR spectra after the soaking display bands at 1019, 871 and 1417-1506 cm^{-1} that hide the bands of the polymeric blend and wollastonite component appreciated before the soaking. These bands must be attributed to the formation of a carbohydroxyapatite layer on the surface and this interpretation is confirmed by glazing angle XRD patterns where peaks compatible with the hydroxyapatite pattern hide the wollastonite peaks. The soaking for three weeks results in the thickening of the apatite layer

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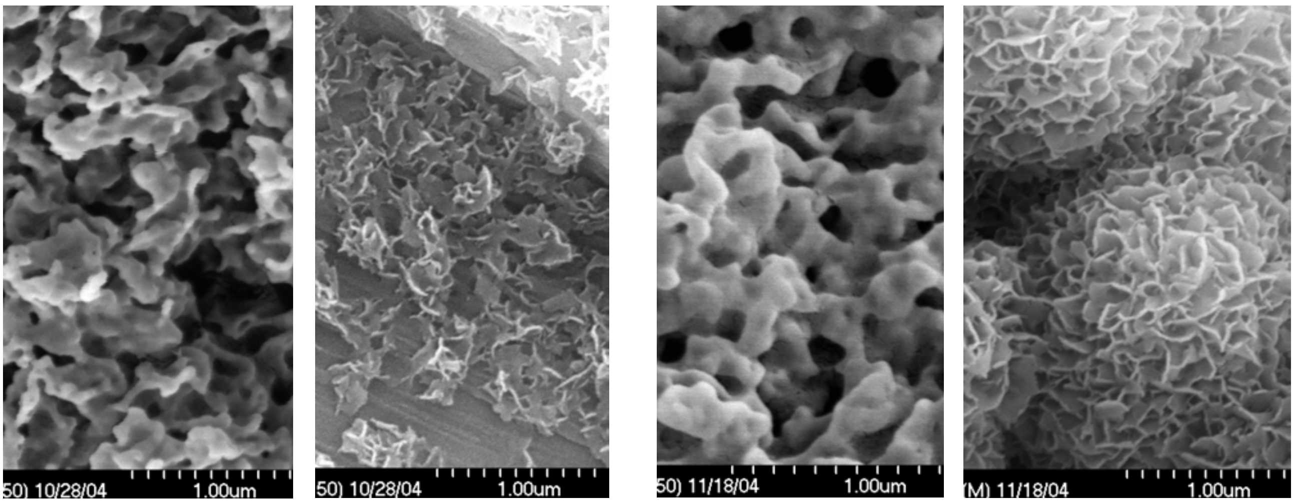

Fig 2: SEM pictures of specimens soaked in sbf . From left to right: W0 soaked for one week, W60 one week, W0 three weeks and W60 three weeks

MTT assay was used to measure cell metabolic function. This test is dependent on the intact activity of a mitochondrial enzyme, succinate dehydrogenase, which may be impaired after exposure of cells to toxic species. MTT results show the absence of mitochondrial damage in any of the experimental formulations. All the compositions present a biocompatibility at least of the level or even superior than the Thermanox® control as can be seen in figure 3

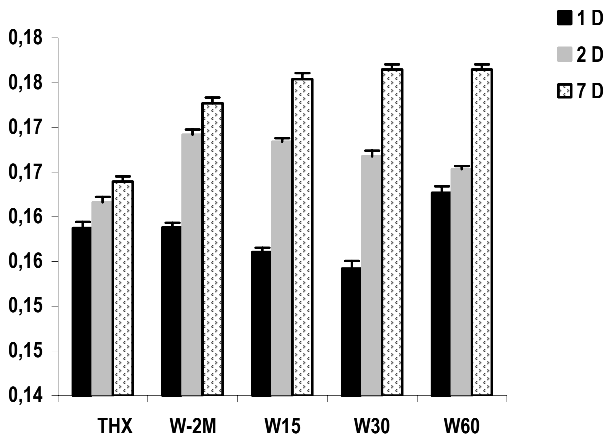

Fig 3: MTT cytotoxicty results for the control THX and the test materials.

Summary

The system W2M-poly (VP-co-EM) provides with a new family of biocompatible materials that are able to develop nanoporous structures and where is possible to control the stiffness and bioactivity of the specimens thus potentially overcoming the problem of poor mechanical properties with enhanced adhesion to bone. A ceramic load of 60 % percent seems to improve both mechanical behaviour and bioactivity of these composites while maintaining the biocompatibility at the level of the control.

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References

[1]W. Pompe, et al., Mater Scie Eng A 362 (2003) p.40
[2]L. Vaz, B. Lopes and M. Almeida, J Mater Sci: Mater Med 10 (1999) p.239
[3]P.N. de Aza, F. Guitian and S. de Aza, Biomaterials 18 (1997) p.1285
[4]P.N. de Aza, et al., J. Microsc. 201 (2001) p.33
[5]E.J. Harper, J.C. Behiri and W. Bonfield, J Mater Sci: Mater Med 6 (1995) p.799
[6]A.C. Moloney, H.H. Kausch and H.R. Stieger, J Mater Sci 19 (1984) p.1125
[7]C.I. Vallo, et al., J Biomed Mater Res (Appl Biomater) 48 (1999) p.150
[8]J. Tamura, et al., J Biomed Mater Res 30 (1996) p.85
[9]J. Vuola, et al., Biomaterials 19 (1998) p.223
[10]E. Fernandez, et al., J Biomed Mater Res 41 (1998) p.560