Wollastonite/TCP composites for bone regeneration: systematic review and meta-analysis

(Compósitos de wollastonita/TCP para regeneração óssea: revisão sistemática e metanálise)

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Abstract

Composite biomaterials have gained notoriety in recent decades due to the ability to combine desirable properties of each material. Thus, associating bioactivity of wollastonite (W) with biodegradability of tricalcium phosphate (TCP) becomes promising for bone repair. Therefore, this study investigated, through systematic review and meta-analysis, *in vivo* studies that evaluated histomorphometrically the bone repair after implantation of W/TCP composites. The searches were performed in the PubMed/MEDLINE, LILACS/BIREME/Virtual Health Library (VHL), and Scientific Electronic Library Online (SciELO) databases. A total of 312 studies were identified in the databases, of which 6 were included. In data comparison, it was considered the percentage of neoformed bone (NB). Composites with a higher percentage of W and/or in the scaffold format presented higher NB. These results suggested that the association of these two materials, as well as the porous scaffold format, was determinant on NB, which makes these new composites potential for clinical use.

Keywords: biomaterials, calcium phosphates, bone regeneration, systematic review, calcium silicates.

Resumo

Biomateriais compósitos têm ganhado notoriedade nas últimas décadas, devido à capacidade de combinar propriedades desejáveis de cada material. Assim, associar a bioatividade da wollastonita (W) com a biodegradabilidade do fosfato tricálcico (TCP) torna-se promissor para o reparo ósseo. Deste modo, este trabalho investigou, por meio de revisão sistemática e metanálise, estudos in vivo que avaliaram histomorfometricamente o reparo ósseo após implantação de compósitos de W/TCP. As buscas foram realizadas nas bases de dados PubMed/MEDLINE, LILACS/BIREME/Biblioteca Virtual em Saúde (BVS) e Scientific Electronic Library Online (SciELO). Dos 312 estudos encontrados, 6 foram incluídos. Na comparação dos dados considerou-se o percentual de neoformação óssea (NB). Compósitos com maior percentual de W e/ou no formato de arcabouços apresentaram maior NB. Estes resultados sugerem que a associação destes dois materiais bem como o formato de arcabouço poroso foram determinantes na NB, o que tornam estes novos compósitos profícuos para o uso clínico.

***Palavras-chave**: biomateriais, fosfatos de cálcio, regeneração óssea, revisão sistemática, silicatos de cálcio.*

INTRODUCTION

Traumas, surgical resections, infections, neoplasms, congenital diseases, and other disorders of bone tissue, often result in extensive tissue loss [1]. In these conditions, the tissue repair is not consolidated by regeneration, due to the lack of adequate blood supply and a three-dimensional (3D) scaffold that enable cellular events [2-4], resulting in fibrosis. Therefore, it becomes essential to use biomaterials or regenerative techniques to restore, in the shortest possible time, the lost structure and functions, considering that these situations, in addition to causing morbidity, generate high costs for the public health sector, since they require multiple long-term surgical repair procedures [5,6] and, consequently, burden the public health and social security system. In these conditions, given the excellent osteogenic potential of the autogenous graft, this would be the first choice. However, in some cases, its use is restricted, particularly in the absence and/or limited extension of the donor area, risk of cross-infection, dimensions, and morphology of bone loss. Thus, in recent decades, researchers of bone tissue bioengineering have developed new techniques and biomaterials for different applications in bone tissue. This has demanded extensive experimental studies, *in vitro* and *in vivo*, and preclinical and clinical trials, to highlight the biocompatibility of biomaterials through their interactions with tissue fluids, cells and tissues, their mechanical stability, osteogenic

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potential, safety, and efficacy. In this context, composite biomaterials have been extensively studied, considering that they combine the desirable physicochemical properties of each material individually.

Among the bioceramic substrates most used for the synthesis of composites, wollastonite (W) has recently stood out due to its biocompatibility, osteoconductivity, biodegradability, solubility, and, especially, bioactivity [6-8]. This calcium metasilicate is acicular, non-metallic, and can be of natural or synthetic origin. When it occurs in the natural form, it arises from metamorphic and magmatic mechanisms that involve intrusive carbonate and magmatic rocks, resulting from the variation of heat and pressure of limestone and silica [9]. In nature, it has a theoretical composition of 48% calcium oxide (CaO) and 52% silicon dioxide (SiO_2), sometimes associated with other minerals, containing elements such as aluminum (Al), iron (Fe), magnesium (Mg), titanium (Ti), manganese (Mn), and potassium (K) [9]. When compared to these minerals, W is the only natural one able to organize itself in an acicular form (needle shape) [10-12]. The synthetic W, pyroxene type, is constituted by 3 tetrahedrons, presents higher chemical purity, and has a structure with higher crystallinity in relation to the natural one, main aspects that differentiate the two forms of W [11]. When obtained synthetically, it presents stable physical-chemical characteristics, which allows 3 types found in nature: triclinic form, most common and predominant one; monoclinic parawollastonite (β-$CaSiO_3$), obtained at low temperatures; and triclinic pseudowollastonite (p-W), acquired at high temperatures above 1200 ºC [11, 13, 14], although p-W is a polymorphic silicate stable at temperatures higher than 1030 ºC [15]. For biomedical purposes, wollastonite (W) has been synthesized, processed and used in different formats and geometric shapes, such as metal alloy coatings, microspheres, granules, and sintered or non-sintered porous 3D scaffolds [6, 16] due to its bioactivity, resulting from the substantial release of calcium (Ca^{2+}) and silicate (SiO_3^{2-}) during osteogenesis [6, 17, 18].

In addition to W, bioceramics based on calcium phosphate (CaP), especially hydroxyapatite (HA) and tricalcium phosphate (TCP), have been widely used for decades for the synthesis and processing of bone substitute biomaterials [19-21]. This occurs mainly because of its biocompatibility, the similarity with the inorganic portion of the bone matrix, osteoconductivity, and absence of immune-mediated rejection [18, 19, 21, 22]. The TCP is chemically stable, has 3 polymorphs (low-temperature β-TCP and two high-temperature forms α and α'-TCP) and enables ion substitutions without promoting significant modifications in its spatial arrangement in the β-TCP allotropic form [23-25]. Both forms synthesized at high temperatures have a lower practical interest since they only occur above ~1465±5 °C. On the other hand, the β-TCP is stable at room temperature and is reconstructively transformed at ~1115±10 °C into α-TCP, which can be retained during the cooling to the room temperature [15, 20, 26, 27]. Other highlights of this bioceramic include its biodegradability, even though the fast biodegradation occurs asynchronously with the bone neoformation mechanism and the material is resorbed even before the consolidation of the bone regeneration mechanism, even though it still promotes tissue integration by HA precipitation, followed by bone growth in the newly formed crystal [2, 17, 18, 20, 22, 28-31]. Consequently, this bioceramic is rarely used in clinical applications individually [2, 21, 28, 29], mainly, in extensive tissue losses. Moreover, as well as the HA, TCP is a biomaterial with low mechanical resistance and must be strengthened before *in vivo* implantation [2, 18, 28].

Given the above, the researchers of bone tissue bioengineering have sought to unite different substrates to obtain composite biomaterials, which present the main physical and chemical properties of each raw material [19, 20, 32]. Thus, the synthesis of W/TCP composites has become promising, given that the composites produced are bioactive, osteoconductive, biodegradable, and suitable for bone regeneration. Furthermore, this association allows not only the biodegradation control but also promotes adjustments in the interactions between biomaterials and bone tissue [5, 32-34]. Therefore, the present study carried out a systematic review and meta-analysis of the results obtained by experimental studies published in the last 10 years, which analyzed histomorphometrically the bone repair after *in vivo* implantation of a composite containing W/TCP.

METHODOLOGY

Search strategy: the search was performed on the PubMed/MEDLINE, LILACS/BIREME/Virtual Health Library (VHL), and Scientific Electronic Library Online (SciELO) databases. The search terms used, according to the MeSH (Medical Subject Headings) and DeCS (Health Sciences Descriptors) were: 'wollastonite AND beta-tricalcium phosphate', 'wollastonite AND beta-TCP', 'wollastonite AND tricalcium phosphate', 'wollastonite AND TCP', 'calcium silicate AND beta-tricalcium phosphate', 'calcium silicate AND beta-TCP', 'calcium silicate AND tricalcium phosphate', and 'calcium silicate AND TCP'. The choice of these terms, abbreviated or not, with or without letter β, was based on the lack of standardization of this information observed in the titles of the articles that refer to the same biomaterial.

Inclusion and exclusion criteria: the inclusion criteria used were: i) scope aimed at bone regeneration; ii) *in vivo* studies, after implantation of biomaterials in animal models; and iii) experimental conditions involving the implantation of W/TCP composites. The exclusion criteria were as follows: 1) clinical studies; 2) *in vitro* studies; 3) literature reviews, thesis, and dissertations; 4) experimental conditions involving only the use of TCP or W (wollastonite), in an individualized way; 5) implantation of biomaterials in other tissues; and 6) insufficient data. Duplicate publications, among the search terms, within each database, were excluded, as well as the duplicate papers identified, common among the databases.

RESULTS

The search in the electronic databases found 857 papers. After reading the titles, duplicate studies, available in more than one search term, were identified and excluded within each database, generating a total of 312 studies. Then, the database search results were compared, excluding 81 duplicate studies, common among the databases, selecting 231 studies for analysis. After reading the titles and abstracts, taking into consideration the exclusion criteria previously described, 190 papers were excluded, leaving 41 articles for the complete reading. Finally, after analyzing the experimental conditions of the studies, taking into consideration the inclusion criteria, 35 articles were excluded, and 6 papers were selected to compose the systematic review and meta-analysis, as illustrated in the flowchart of Fig. 1. All 6 studies included (100%) used the rabbit as an experimental animal model and performed a histomorphometric evaluation of bone repair. Of these, in relation to the type of bone defect, 3 studies (50%) used the femur, 2 (33.3%) the tibia, and one (16.7%) the calvaria. Regarding the diameter of the bone defect, 2 studies (33.3%) used the bone defect of 8 mm, 2 (33.3%) used the defect of 6 mm, one (16.7%) used the defect of 5 mm, and one (16.7%) used the defect of 2 mm. In relation to the shape of the biomaterial, 4 articles (66.7%) evaluated scaffolds, and 2 (33.3%) analyzed microspheres (Table I).

For quantitative analyzes, in view of the great variability in the content ratios in W/TCP associated with the chemical compositions of the biomaterials used in the reviewed experiments, a categorical variable called "grouping" was defined to represent 3 experimental conditions and compare the results (percentage of NB - neoformed bone) of the studies: 1) W>TCP: composites with a higher content of W; 2) W<TCP: composites with a higher content of TCP; and 3) W=TCP: composites with the same content of W and TCP. When considering these 3 groups and summarizing all associations of W with TCP, within each study in all biological points adopted, it was observed that: W>TCP was evaluated 4 times (44.4%); W<TCP was evaluated 3 times (33.3%); and W=TCP was evaluated twice (22.2%). One of the 6 studies did not show the percentage of NB, nor did it present sufficient data to obtain this measure. Thus, 5 studies were summarized in the statistical analysis.

Statistical analysis: the data obtained and summarized in Table II did not satisfy the requirements and the assumptions for the application of parametric tests for comparing means, such as the Student's t-test. Therefore, to assess the existence of significant differences ($p<0.05$), a non-parametric equivalent test, Mann-Whitney test, was applied [37, 38]. Taking the biological point of 12 weeks as a reference, the only common one among all studies in the evaluation of the two formats of biomaterials, a higher percentage of bone neoformation was observed after implantation of the scaffolds in relation to the microspheres, 24.6%±9.2% and 20.0%±3.1%, respectively (Table II), although the Mann-Whitney's test result indicated that there was no statistically significant difference ($p=0.286$). For the comparison of the results in the intragroup evaluation (format of the biomaterials), in relation to the 3 previously defined W/TCP

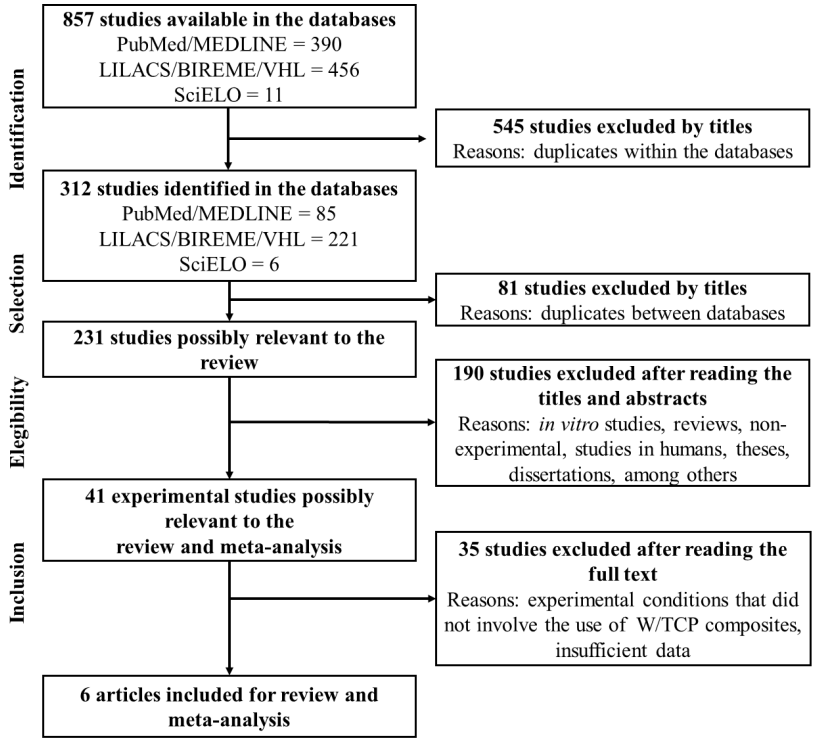

Figure 1: Flowchart of the search and selection of articles in the databases.
[Figura 1: Fluxograma da busca e seleção dos artigos nas bases de dados.]

Table I - *In vivo* studies of W/TCP (wollastonite/tricalcium phosphate) composites.
[Tabela I - Estudos in vivo de compósitos de W/TCP (wollastonita/fosfato tricálcico).]

Study	Animal	Defect type (diameter)	Biomaterial	Preparation method	Composite W/TCP (wt%)	Follow-up (week)	NB (%)
[35]	Rabbit	Tibia (2 mm)	Scaffold	Polymeric mold (impregnation)	W/β-TCP (60/40)	4	$(40\pm14).10^{3}$ #
						8	$(73\pm6).10^{3}$ #
[36]	Rabbit	Tibia (5 mm)	Scaffold	Manufacturing	CSP (45/55)	4	4.79*
						12	9.58*
						20	23.49*
[1]	Rabbit	Calvaria (8 mm)	Microsphere	Dual-shell droplets	CaP/CaSi/CaP (26/73.9)	6	7.50
						12	16.47
						18	23.43
					CaSi/CaP/CaSi (70.1/29.8)	6	~11
						12	19.66
						18	29.65
[5]	Rabbit	Femur (8 mm)	Microsphere	Core-shell droplets	CaP/CaSi (79.7/20.3)	6	~8
						12	20
						18	~27
					CaP/CaSi (24.9/75.1)	6	11
						12	~24
						18	~34
[31]	Rabbit	Femur (6 mm)	Scaffold	Polymeric mold (impregnation)	50% CS (50/50)	4	~20
						12	~23
						26	~28
					80% CS (80/20)	4	~30
						12	~33
						26	~33.5
[22]	Rabbit	Femur (6 mm)	Scaffold	Polymeric mold (impregnation)	β-CS/β-TCP (80/20)	4	15.50
						12	27.62
						26	21.90
					β-CS/β-TCP (50/50)	4	19.54
						12	30.00
						26	23.55

*W, CS, CaSi - calcium silicate or wollastonite; CaP, TCP - calcium phosphate or tricalcium phosphate; CSP - calcium silicate phosphate; NB - neoformed bone; * values calculated based on the percentages of bone neoformation and total bone defect made available by the authors; # values not subject to percentage calculation due to insufficient data made available by the authors; ~ values obtained from published graphs.*

content ratio groupings (W>TCP, W<TCP and W=TCP), it was not possible to use the analysis of variance (ANOVA). Therefore, its non-parametric equivalent test, Kruskal-Wallis test (Table III), was applied [37, 38]. In all the biological points evaluated, it was observed that, regardless of the shape of the biomaterial, composites containing W>TCP content ratio presented a higher percentage of NB in relation to the other defined experimental conditions, although this difference was not statistically significant ($p>0.05$).

DISCUSSION

Aiming to gather scientific data and current evidence on the performance of composite biomaterials containing W (wollastonite) and TCP (tricalcium phosphate), the present study investigated, through systematic review and meta-analysis, *in vivo* studies that evaluated histomorphometrically the bone repair after implantation of these composites. All studies included in the meta-analysis of this paper used rabbits as an experimental animal model. The choice of this

Table II - Statistical analysis according to the shape of the biomaterial.
[Tabela II - Análise estatística em função do formato do biomaterial.]

Follow up (week)	Biomaterial format	Assessment frequency	NB* (%)
4	Scaffold	5	18.0±9.1
	Microsphere	-	-
6	Scaffold	-	-
	Microsphere	4	9.4±1.9
12	Scaffold	5	24.6±9.2
	Microsphere	4	20.0±3.1
18	Scaffold	-	-
	Microsphere	4	28.5±4.4
20	Scaffold	1	23.49± -
	Microsphere	-	-
26	Scaffold	5	26.1±4.7
	Microsphere	-	-

*NB - neoformed bone; * mean ± standard deviation.*

Table III - Statistical analysis according to the content ratio of W/TCP association and the format of the biomaterial.
[Tabela III - Análise estatística em função do teor de associação de W/TCP e do formato do biomaterial.]

Follow up (week)	Content ratio	Assessment frequency	NB* (%)	Kruskal-Wallis
		Scaffold		
4	W>TCP	2	22.7±10.2	p= 0.368
	W<TCP	1	4.79± -	
	W=TCP	2	19.8±0.3	
12	W>TCP	2	30.3±3.8	p= 0.301
	W<TCP	1	9.6± -	
	W=TCP	2	26.5±4.9	
26	W>TCP	2	27.7±8.2	p= 0.741
	W<TCP	1	23.5± -	
	W=TCP	2	25.8±3.1	
		Microsphere		
6	W>TCP	2	11.0± -	p= 0.333
	W<TCP	2	7.7±0.3	
	W=TCP	-	-	
12	W>TCP	2	21.8±3.1	p= 0.667
	W<TCP	2	18.2±2.5	
	W=TCP	-	-	
18	W>TCP	2	31.8±3.1	p= 0.333
	W<TCP	2	25.2±2.5	
	W=TCP	-	-	

** mean ± standard deviation.*

animal has as its main advantage the greater similarity with the human bone anatomy and physiology, mainly regarding the macro and microstructure of the bone tissue of the femur concerning the human maxillary bone [39], in relation to rodents. In spite of presenting some differences with the human bone anatomy and physiology, the rodents have been widely used in experimental research and contributed significantly to the development of new biomaterials and regenerative techniques, especially those aimed at bone regeneration [40, 41]. This has been a model of choice for research that investigate biocompatibility and safety in the use of materials, such as [42, 43]: biodegradation and bioresorption; carrying of drugs and/or active substances; osteoinduction, osteoconduction, and osteostimulation; osteogenic potential; and cell therapies associated with biomaterials. Despite the differences between rabbits and rodents, it is known that the use of small animals has numerous advantages over large ones, such as: better cost-benefit; relatively short time of observation and rehabilitation; easy handling and manipulation of animals; standardization of experimental conditions among genetically similar individuals; easy reproducibility of study methods; and greater control of experimental conditions [40-42].

Regarding the experimental surgical model, the studies included in our meta-analysis used different sites to the confection of the bone defect (femur, calvaria, and tibia) with varying dimensions (8, 6, 5, and 2 mm) [1, 5, 22, 31, 35, 36]. Despite the variety of experimental models available and used to assess bone repair, defects made in calvaria present morphological characteristics that enable the generation of essential data to comprehend the mechanisms involved in the associated tissue response after the implantation of biomaterials, especially the osteogenic potential. In this surgical site, due to the predominant compact and tiny spongy portion, the regenerative capacity of bone tissue is limited, which shows the osteogenic potential of biomaterials or regenerative techniques, especially in critical bone defect [44]. Moreover, the calvaria presents: a) anatomical location that facilitates the surgical access and transoperative manipulation; b) bone structure that enables the establishment of uniform, reproducible and standardized defects, easily assessed by imaging, histomorphologic and morphometric examinations; and c) fixation of the biomaterial by the repositioned flap [40, 45], even though biomaterials in the microsphere format move more easily from the bone defect [46].

Nevertheless, there is no consensus in the literature on the exact diameter of the bone defect to be confectioned, neither in relation to the periods of analysis during the studies. Thus, having as reference the biological point of 12 weeks, the only one common to all studies in the evaluation of the two formats of biomaterials, a higher percentage of NB (neoformed bone) was noticed after implantation of the scaffolds in relation to the microspheres (Table II). In addition, when comparing the NB averages between the

conditions W>TCP and W<TCP content ratios, a greater discrepancy was noticed in the percentages obtained with scaffolds than with microspheres. These differences can be justified by the influence of the 3D structural characteristics of the scaffolds that are decisive in the biological behavior and, consequently, in the regenerative potential of the biomaterials [5, 35, 40, 47, 48]. These characteristics highlight the potentiality of the polymeric mold impregnation method, also known as sponge replica method, considered highly effective and reproducible in the processing of 3D scaffolds, adopted by the studies that presented the highest percentages of NB in this meta-analysis (Table I) [12, 13, 18]. This method permits to obtain 3D scaffolds with a porous structure and a microarchitecture that enable the adhesion, migration, proliferation, and differentiation of distinct cell types, beyond promoting neovascularization at the implantation site, which favors the osteogenesis inside its 3D structure [1, 2, 4, 5, 22, 42, 49]. For this purpose, they need to present pores with at least 100 μm, although 300 μm is the most appropriate size for the aforementioned events, during bone neoformation inside the scaffolds [48]. Beyond these properties, scaffolds can also modulate the inflammatory response at the implantation site [4, 36], which reduces the chances of rejection of the biomaterial by the body.

The contrast noticed in relation to the percentage of NB related to the contents of W and TCP association may be related to the fact that, according to some studies, the presence of W associated with TCP can adapt the chemical and mechanical properties of the biomaterial, which allows consolidating bone neoformation more quickly inside these composites [5,16,18,19,47]. On the other hand, Liu et al. [31] observed a decrease in compressive strength in biomaterials with the highest percentage of W and suggested that smaller amounts of W keep the scaffold with stable density. Thus, in relation to the aforementioned findings, it is emphasized that excessive loads should be avoided in ceramic biomaterials during the initial stages of regeneration [19]. Bioactive biomaterials containing silicon (Si) are capable of inducing the nucleation, precipitation, and formation of a layer of amorphous CaP (apatite) on the surface of these materials and, thus, amplify the mechanism of bone neoformation *in vivo* [1, 17-20, 22, 29]. In addition to this factor, the higher NB percentage of composites containing W>TCP content ratio can be attributed to the fact that, when compared to other bone substitutes, W presents greater bioactivity by the effect of the release of calcium (Ca^{2+}) and silicate (SiO_3^{2-}) ions, substantial during the osteogenesis mechanism [1, 5, 6, 17, 18]. Moreover, the presence of SiO_3^{2-} also induces proliferation, differentiation, and increased activity of osteoblasts and, consequently, promotes biomineralization, elementary events during bone repair [2, 16, 19, 20, 31, 50]. However, high concentrations of Si appear to cause cell death [20, 43]. Thus, the relationship between concentration and response to SiO_3^{2-} is still debated, and many questions remain unanswered so far [33].

The main limitation found in our paper was the fact that none of the included studies had a percentage of NB with mean and standard deviation, which made it impossible to construct the graph known as "forest plot", recommended in the scientific literature during the elaboration of the meta-analysis [51]. One of the studies selected for the review did not compose the statistical analysis since it did not present a percentage of NB. Despite the different methodological approaches observed in the studies analyzed, it was noticed that the W/TCP composites show promising osteogenic potential. Given this, it is evident that further studies need to be carried out in order to elucidate the uncertain points that still exist about the ideal Si content and the best content of association between W and TCP as well as to assess the potential of these biomaterials in extensive bones losses. For this purpose, it is essential that these studies share the same biological points of observation, in order to facilitate the understanding and enable a better comparison of results, especially in relation to the inflammatory response, bone neoformation and the maturation of mineralized tissue associated with these composites.

CONCLUSIONS

The results observed in this review demonstrated that the porous structure of the scaffolds was determinant in the neoformed bone (NB) of the composites containing wollastonite (W) and tricalcium phosphate (TCP), higher than the percentage of NB obtained with the use of the composites in the form of microspheres. The association of these two bioceramics showed promising osteogenic potential for clinical use.

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