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Synthesis and Characterization of a Polymeric Material Blended to Bone Forming Elements

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Abstract

A synthetizing material blended with two distinct proteins (collagen and casein) and mineral mixture, was developed in order to evaluate their properties suitable for possible applications in the biomedical such as inducing the regeneration of damaged bone, either due to an accident or illness. Samples were evaluated by 1) Mechanical properties tests under the bending, 2) Scanning electronic microscopy and 3) Infrared spectroscopy were carried out. The results showed that the developed material has breaking strength and structure characteristics associated with the protein used in their composition. This fact suggests that the used protein determines the resistance of the material, in such a way according to the required use, being able to choose appropriate strength and duration either short or long time. The material composition for specific use, in order to find the most suitable mixture for bone replacement, or induce bone recovery, according to the required properties similar to those of damaged living tissue.

Keywords

Minerals, Bone, Polymethylmethacrylate, Casein, Collagen, Mechanical Properties, Infrared, Scanning Electronic Microscopy

1. Introduction

In order to find the most suitable material for tissue replacement, being two types, soft tissue, such as the skin, tendons, ligaments and some internal organs,

and hard tissue as bone sand teeth. The bone tissue is affected by illnesses or accidents, and even wars have caused many people to lose limbs or suffer bone injuries. The bone tissue provides support to the body and protection of the internal organs, the extremities are of great importance to lead a better quality life and independence. To solve certain bone damage, it is of great interest to analyze the material properties for the bone replacement, according to their mechanical requirements, in order to choose the more adequate material [1] The materials used as a replacement for bone tissue must have a balance between flexibility and resistance, they must not suffer plastic deformation and they must not have flexural memory, because the bone tissue functions for supporting the loads. Their density is of great importance, recommended a material of similar density to that of the tissue to be replaced, in order to prevent decubitus injuries, related to pressure on the soft tissues [2] [3] [4]. Proteins are polymeric molecules present in living organisms, performing functions of transport, structure, catalysis, regulators of biochemical processes and in the immune system. Casein has a great application in the production for the food industry [5]. Biomaterials of natural origin bring different benefits, such as zero toxicity, the possibility of having a beneficial function [5] [6]. The variety of casein types vary in amino acid composition; their structure depends on the physic-chemical environment [7] [8] [9] [10].

Although there are several collagen varieties, type I collagen is one of the most abundant, can form intertwined chains that generate compact solids, form gels of interest in the cosmetics industry. Collagen is obtained from skin, cartilage, tendons and animal bones [11] [12] [13] [14]. The mostly applied area for protein-based materials is targeted as drug delivery, which offers a local potential, increased dose of the drug while lowering systemic and non-specific drug administration [15]. Taking as reference the bone composition, up about 65% minerals and 35% organic matrix, being the 90% collagen and others proteins, cells, water and blood vessels [16].

In order to find the most suitable material for medical replacement, the type of tissue that needs to be repaired must be considered, such as soft tissue such as skin, tendons, ligaments and some internal organs and hard tissue such as bones and teeth. The experimental study is based on the organic and inorganic composition of bone tissue (**Figure 1**).

The goal of the current study was characterizing a synthetized material that resembles the bone composition by mechanical and structural properties for medical application or museum pieces' restoration, such as art objects.

2. Material and Methods

2.1. Samples

Sol-gel synthesis was used to prepare the samples with a rectangular form (measures), following the processes (see **Figure 2**), mixed the precursors and monomer of PMMA methyl methacrylate, in the described steps such as hydrolysis, condensation formation de la solution (sol), and solid formation (gel-liquid),

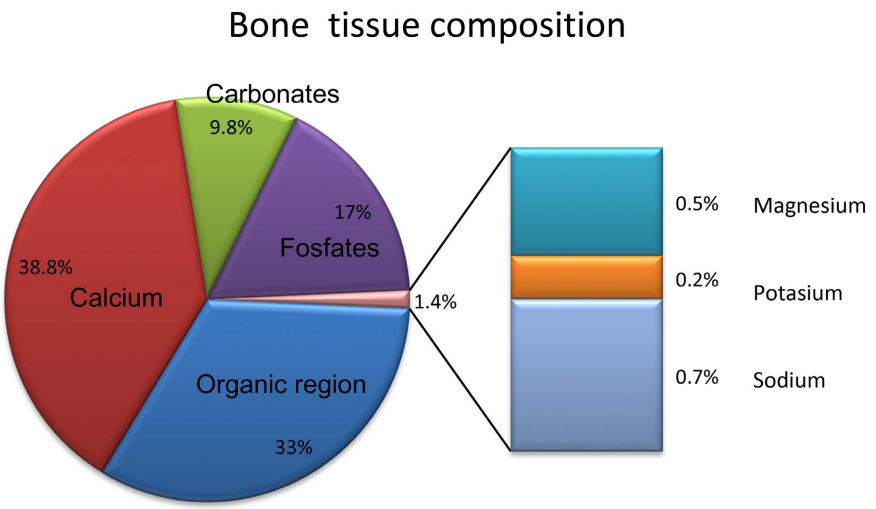


Figure 1. Bone composition, inorganic region includes mineral composition, organic regions correspond to protein [17].

as the viscosity rapidly increase, the solvent is bordered inside the gel. The structure may change considerably along time, the gel is aging, wet gel, dried by solvent evaporation (at environmental temperature), then the capillarity forces will produce in contraction, the gel network will collapse and the xerogel (solid and gas) is formed at environmental conditions. A sol consists of a liquid with colloidal particles which are not dissolved, but do not agglomerate or sediment. Agglomeration of small particles is due to van der Waals forces and tendency to decrease the total surface energy. Van der Waals forces are weak, and cover only for a few nanometers. In order to counter van der Waals interactions, repulsive forces must be established, the liquid phase even contains sol particles and agglomerates, which will continue to react and will condense as the gel dries. The gel is originally flexible and then groups on around branches will condense, making the gel even more viscous. This will enfold out the liquid from the interior of the gel, end shrinkage occurs, this process will continue as long as there is flexibility in the gel [18].

Using the sol-gel method, three wo different samples of materials were obtained each sample contents a thirth portion of mineral mix, casein or collagen and PMMA (organic region), 1) Mixture of collagen, a mixture of minerals (Col-Mx) and polymethylmethacrylate (PMMA) (as reference), 2) Mixture of casein (Cas-Mx), and 3) PMMA samples, which were used as reference points. Dried samples, were stored in closed plastic bags in a controlled environment, **Table 1**.

2.2. Density

In order to carry out the calculation of the different materials density (n = 5), we obtained the average mass of samples prepared in the containers corresponding to the bending test, this procedure was to obtain uniform specimens, which measurements are 79 mm long, 10 mm wide and 5 mm thick, and from which we obtained a 3.95 cm^3 volume.

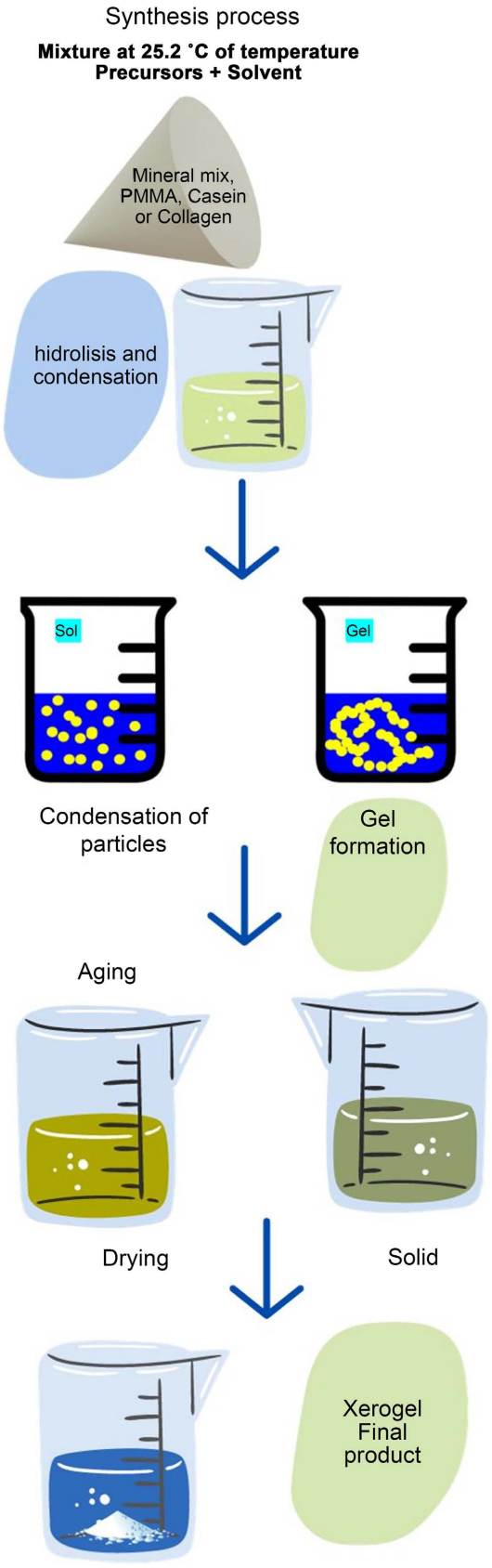


Figure 2. Synthesis process of material samples by sol-gel method [19].

Table 1. Composition of the material used for samples elaboration. composition.

Samples	Components	Acronym
1	Polymethylmethacrylate	PMMA
2	PMMA + Mineral mix + Collagen	Col-Mx
3	PMMA + Mineral mix + Casein	Cas-Mx

2.3. Mechanical Test

Flexibility is the property of a material to undergo large deformations in its elastic zone, it is directly related to Young's modulus, if this is small the material will be flexible, however, if Young's modulus has a high value, the material will be rigid, that is, it will need a great effort to be deformed.

The samples (n = 10) were placed horizontally, on two horizontal supports spaced; the center load point of contact with the rear surface of the midpoint of the material, following the rules of ASTM 2007 and ISO 5833 [20] [21]. The accessory to carry out the bending test moves downwards at a constant speed of 1 mm/min until it breaks in accordance with the official ISO 178:1993 standard. The specimens were made with measurements of 4 mm thick, 10 mm wide and 79 mm long, based on 3-point bending tests [22].

Bending evaluation was carried out by three-point bending test, the samples were placed on two supports and a force was applied in the center. The tests were carried out at a 25.2˚C temperature and a 46% humidity using a Zwick/Roell model Z005 universal testing machine. The test samples were placed with their front face downwards on two horizontal supports spaced 22 mm apart; the central load point of contact with the rear surface of the midpoint of the material. The accessory to carry out the bending test moves downwards at a constant speed of 1mm/min until it breaks in accordance with the official ISO 178:2003, standard American National Standards Institute ANSI/ASAE S459 (1988) [23] [24] [25]. The tests were carried out under the established standards, using samples 4 mm thick, 10 mm wide and 79 mm long, based on the 3-point bending tests [25].

2.4. Infrared Spectroscopy

Infrared spectroscopy test, was used to determine the composition, as well as the purity level. The different samples were evaluated under IR test, in a Bruker Tensor 37 equipment, in transmittance mode, 32 scans were made with a resolution of 1 cm^{-1} [26].

2.5. Scanning Electron Microscopy

Scanning electron microscopy (SEM) was implemented to observe the internal structure of material at the nanometric scale, with a resolution close to 0.5 nm.

The tests were carried out in a Carl Zeiss Scanning Electron Microscope, model EVO-50, which works at high and low vacuum, and has SE1, BSD and EDX (X-ray) detectors. The equipment has critical point dryers (EM CPD300,

Leica; SAMDRI-PTV-3D, Tousimis) and gold, palladium and carbon coaters (DESK II, Denton vacuum; EM ACE200, Leica; SC7620, Quorum).

2.6. Statistical Analysis

For the statistical analysis, Statistical Package for the Social Science (SPSS) version 15 was used and the variance test was performed for the comparison between the variables studied in the different samples. All data were analyzed by means of the using ANOVA and Pos Hoc "Tukey" test. A statistically difference was established at "p" values less than 5% ($p \leq 0.05$).

Experimental data were compared, to examine the score differences of composition among samples the following separate statistical analyses were used: (a) density were compared with a ANOVA with repeated measures, 3 (samples composition) × 4 (parameters evaluated. Additionally, a comparison between samples with a one-way ANOVA was obtained analysis of variance ANOVA with repeated measures. Additionally, a post- hoc Tukey test was conducted when pertinent, to detect significant differences between samples throughout the experiment.

3. Results and Discussion

3.1. Results of Density

As it can be seen in Table 2 PMMA (a material for commercial uses) is taken as a reference, however, there are notable differences between the two, the Col-Mx mixture has a significant difference with respect to both, which shows us that it has less rigidity and greater elasticity, while the Cas-Mx mixture has a performance similar to that of PMMA. Due to the structure of the Cas-Mx sample, as for their density, there is no significant variation. Additionally, in the bending test, the specimens, only presented a partial rupture, similar to the case of the white PMMA specimens. This indicates that this material has higher strength and lower weight, consequently makes it ideal for applications where a strong and lightweight material is required [27].

3.2. Results of Mechanical Test

The mechanical test demonstrates as shown in Figures 3(A)-(D), Wistar rat bone and PMMA were taken as a reference, the Col-Mx mixture has a significant difference with respect to both, presenting us that it has less rigidity and greater

Table 2. Mean density ± error values.

Number	Samples	Density (g/cm^3)	Comparisons	P values
1	PMMA	1.1712 ± 0.0103	1 vs 2	0.0022*
2	Col-Mx	1.4817 ± 0.0340	1 vs 3	0.0022*
3	Cas-Mx	1.3831 ± 0.0202	2 vs 3	0.7605 NS

ANOVA gl (1, 14) F, 5.182. Significant differences were at $p \leq 0.05$ level, non-significant differences (NS).

elasticity, while the Cas-Mx mixture has a performance similar to that of PMMA (Table 3 and Table 4).

Flexibility as a property of a material to present large deformations in elastic zone, is directly related to the Young's modulus, a small value the material will be flexible, however, if the Young's modulus has a high value, the material will be rigid, that is, it will require a great effort to be deformed [20]. When exist the necessity of recover a structure or function of bone tissue, requires a material with similar properties, in order to prevent abrasions in the tissue to be repaired or in the surrounding tissues. For each particular situation, the selection of the material must be according to the type of tissue and the use, for short or long term. In the present study, a material obtained showed similar hardness rat bone. Therefore, to replace harder bone, it is necessary to modify its mechanical properties, which can be more or less hard depending on the specific case.

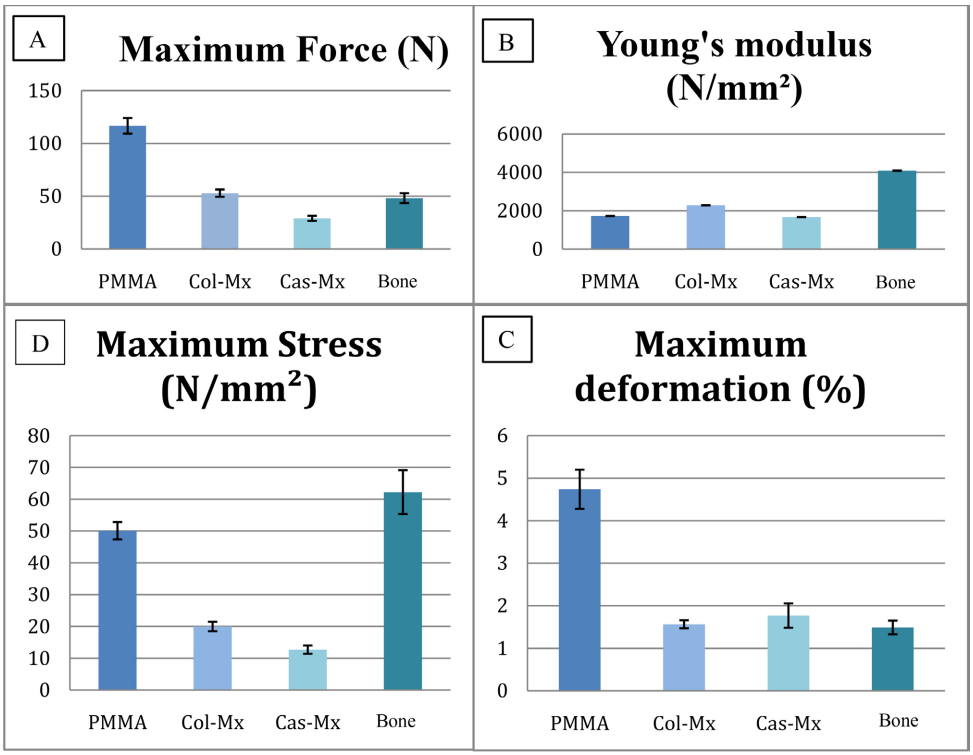


Figure 3. (A-D) Mechanical test comparison between the samples of different composition.

Table 3. Mean of different parameters evaluated (±EE) of mechanical test analyzed by two-way ANOVA, comparisons between different samples composition.

		Samples composition			
Number	Parameter	Col-Mx	PMMA	Cas-Mx	(df) F values
1	Maximum force (N)	52.9129 ± 4.92	116.5367 ± 14.85	29.032 ± 9.967	(1, 29) 45.110
2	Young's modulus (N/mm^2)	2288.0086 ± 141.92	1725.53 ± 142.66	1670.166 ± 142.65	(1, 29) 5.205
3	Maximum stress (N/mm^2)	19.9771 ± 2.51	50.0857 ± 4.58	12.688 ± 1.91	(1, 29) 43.17
4	Maximum deformation (%)	1.5643 ± 0.16	4.7383 ± 0.91	1.768 ± 0.62	(1, 29) 9.764

Table 4. Comparison between different samples of different parameters evaluated of mechanical test, Post hoc analyzed by one-way analysis of variance.

		P values		
Number	Parameter	Col-Mx vs PMMA	Col-Mx vs Casein	PMMA vs Cas-Mx
1	Maximum force (N)	0.0273*	0.0054*	0.0016*
2	Young's modulus (N/mm^2)	0.03623*	0.01049*	0.5332 NS
3	Maximum stress (N/mm^2)	0.0001*	0.0021*	0.0033*
4	Maximum deformation (%)	0.0001*	0.0013*	0.0654 NS

*Significant differences were at $p \leq 0.05$ level, non-significant differences (NS).

3.3. Results of Infrared Spectroscopy

Through IR spectroscopy, it revealed that the used materials have similar patterns as those reported, showed for PMMA a small peak in the region of 2900 cm^{-1} that represents the C-H bonds as well as the one found at 1440 - 1450 cm^{-1}, also at 1730 - 1740 cm^{-1}, we find a band corresponding to the double bonds between C=O, and between 1200 and 1300 cm^{-1}, we observe two small characteristic peaks of the C-O of the ester group as shown in, **Figure 4**, marked with arrows and so on for collagen and casein [28]. In the IR spectroscopy it is observed that the PMMA, collagen and casein that were used in the work have patterns similar to those reported in the literature, specifically, the points of functional groups coincide, which validates the use of the inputs used in this project (**Table 5**).

3.4. Results of Scanning Electronic Microscopy

Images from SEM microscopy, the Cas-Mx mixture generates arrangements of the PMMA spheres, forming bridge-shaped joints between them, deposited in the mixture of protein and minerals. The structure presented some porosity, which could be related to a lower density of the material compared to the Col-Mx mixture. While the PMMA spheres in the collagen and mineral mixture do not have a structured pattern as shown in **Figure 5** and **Figure 6**.

Bone regeneration is one of the most important and challenging tissue engineering approaches considered to be an ideal strategy for treating diseases, injuries, in regenerative medicine. The experimental material is an excellent alternative for bone regeneration, because the lost cost in front of traditional used material such is hydroxyapatite. In complement with bases of tissue engineering the developed material can be applied in several cases for induces bone regeneration, because the susceptibility of natural materials to enzymatic degradation or potential allergenicity it can be applied as scaffold while the bone reaches the functional structure. In this case, susceptibility can be a beneficial property, in contrast to replace bone indefinitely, it is necessary to reinforce the material with metallic nanoparticles, carbon nanofibers. For this reason, it is a good option to replace existing materials, due to its low cost. Added antimicrobial substances, are necessary it can be competitive.

Table 5. Main peaks of infrared tests, correspond to the samples components functional groups. The peaks (arrows) indicated in **Figures 4(A)-(C)**. corresponding to the functional groups observed of mixtures components [29] [30].

PMMA		Collagen		Casein	
Band	Bond	Band	Bond	Band	Bond
2900 cm^{-1}	C-H	1657 cm^{-1}	Amina I (N-H)	1750 cm^{-1}	C=O (double)
1440 - 1450 cm^{-1}	C-H	1552 cm^{-1}	Amina II	1650 cm^{-1}	Amida I (N-H)
1370 - 1740 cm^{-1}	C=O (double)	1241 cm^{-1}	Amina III	1450 - 1550 cm^{-1}	Amida II N=H and C=N
1200 - 1300	C-O-C (ester)				

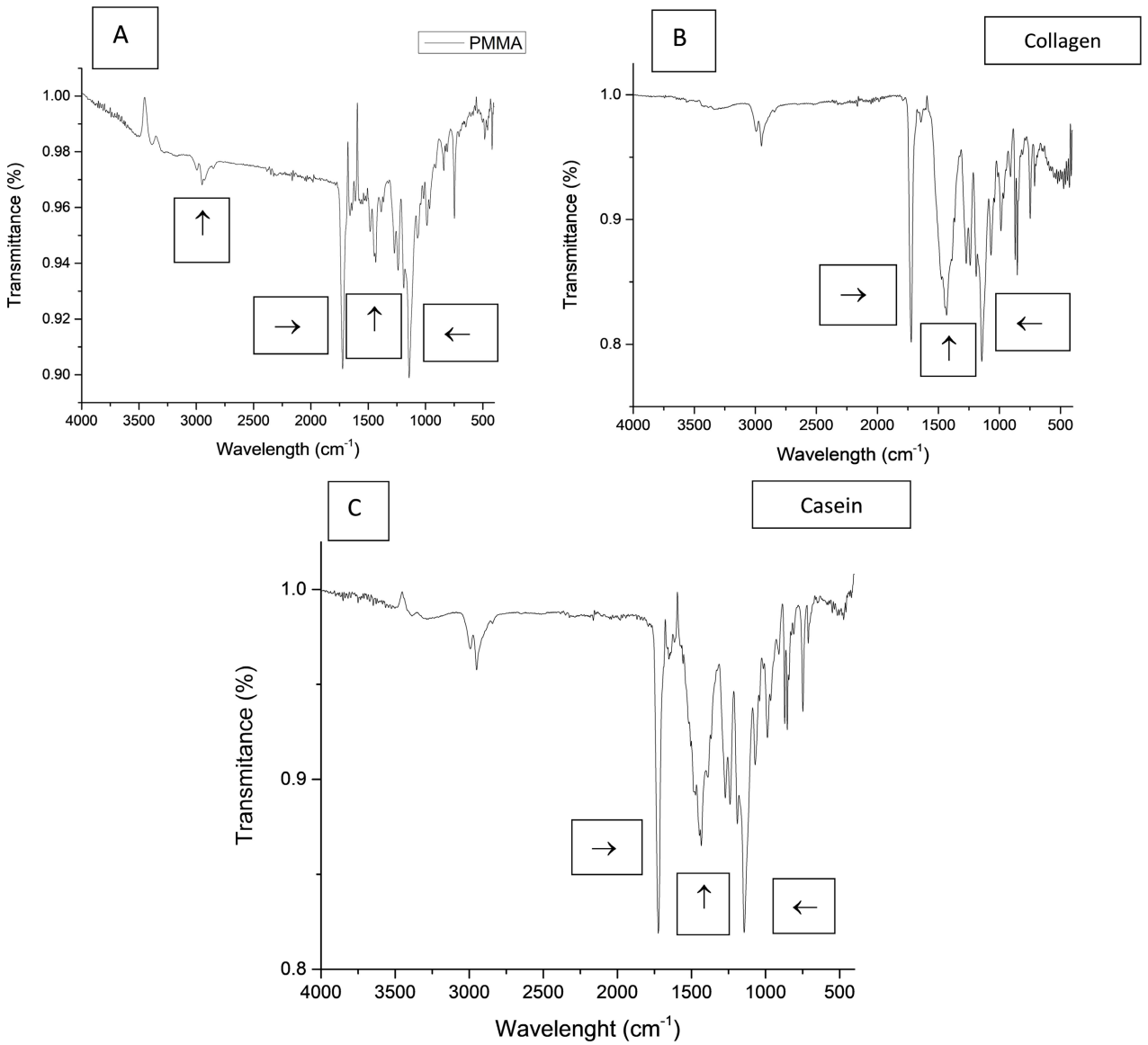
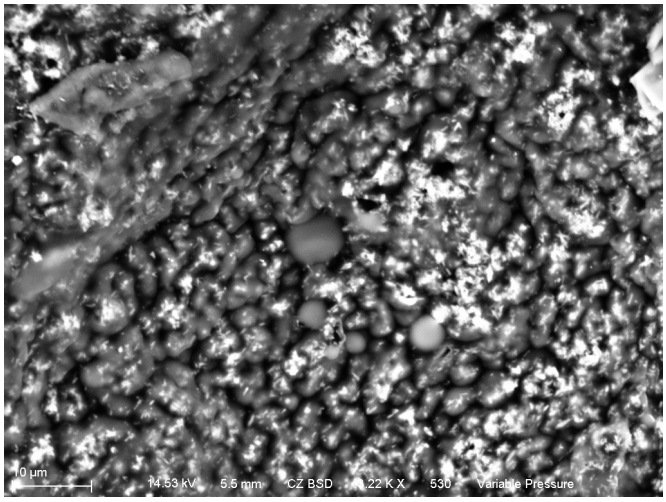
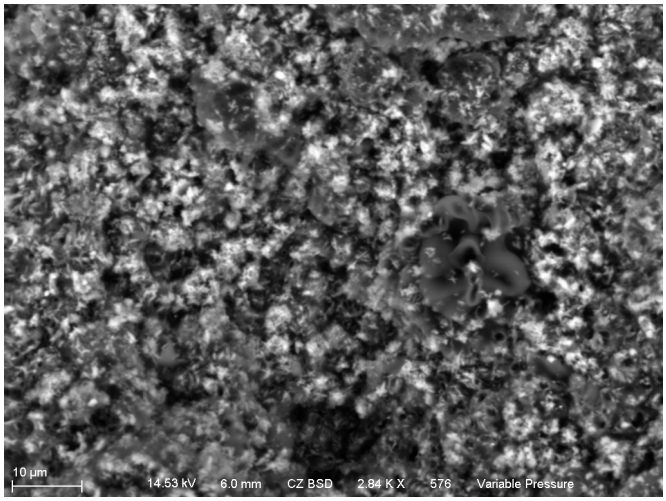


Figure 4. IR spectrum of samples components, PMMA (A), collagen (B) and casein (C). The peaks (arrows) corresponding to the functional groups of mixtures components observed, see **Table 5**.

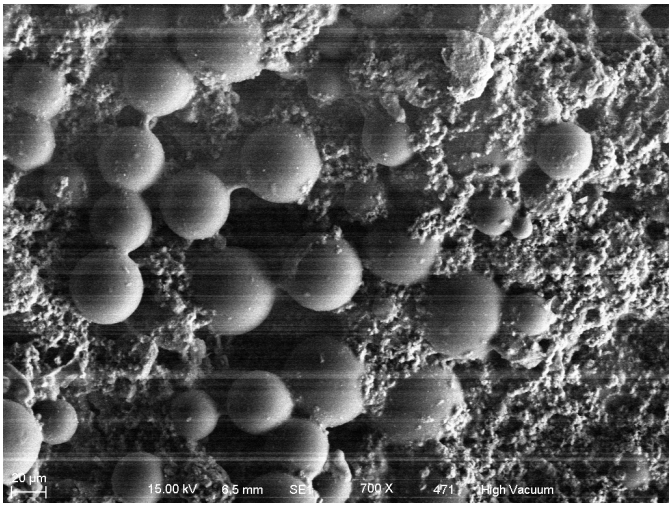


(A)

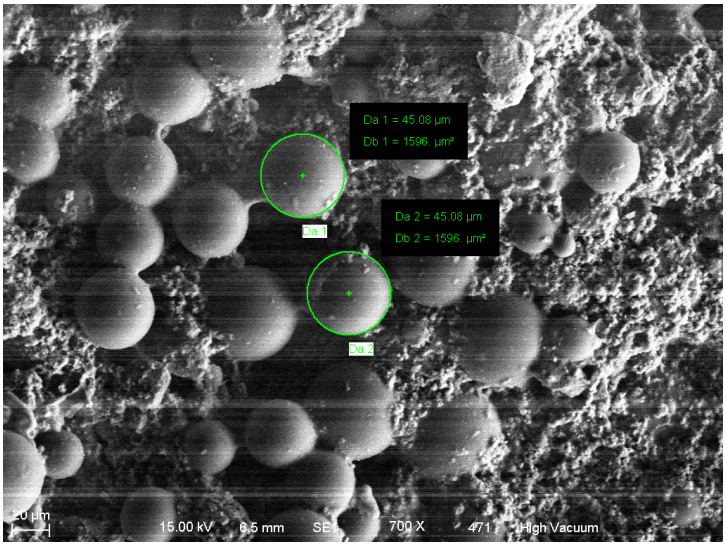


(B)

Figure 5. Images of collagen mineral mixture an polymetylmetacrilate at different magnifications, obtained by SEM. At different magnification (A) and (B).



(A)



(B)

Figure 6. Images of the casein, mineral mixture and polymethylmethacrylate, obtained by SEM, at magnifications (A) 700× and (B) 700×.

4. Conclusions

The results showed that the developed material possess breaking strength and structure characteristics associated with the protein used within their composition. Such factual result suggests that the used protein determines the resistance of the material in such a manner according to the required medical application; thus, being able to choose the appropriate strength and duration for the replacement of living damaged tissue or controlled release of pharmaceutic for a short or long term requirement. Also, it could be used for artistic objects restoration such as bone fossil remains and ceramic restoration.

We concluded that the mixtures obtained are likely to be used for biomedical applications, such as replacement or implants. However, it is suggested that more characterization tests may be carried out to determine the most adequate mixture that presents the necessary mechanical properties for each type of application. The material mixtures have a similar structure pattern, to that generated by hydroxyapatite, which is a material applied for its use for bone implants. We suggest the tested mixtures for their use instead of hydroxyapatite, in order to reduce the cost. The foregoing perspective, is aimed to replace the polymer used in this material with one that is of 100% natural origin, preferably one that can be obtained easily and at low cost. In the hybrid materials (organic-inorganic), the sol-gel method favors the creation of charges, in the presence of a polymer with functional groups, these charges are used to join the polymer and the inorganic phase. With these characteristics, the material could achieve greater biocompatibility. The mixtures used preserved the integrity of the proteins, collagen and casein, since the polymerization was carried out at room temperature, which prevents their denaturalization. Because there are a variety of possibilities for inducing bone regeneration scaffolds, studies are required, in

order to stablish which, type of cells and growth factors are more appropriate to specific cell culture. In order to apply the synthetized material as scaffolds, due use it in agreement to the bone morphology and region of the bone structure damaged.

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Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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