

OBȚINEREA, CARACTERIZAREA STRUCTURALĂ ȘI EVALUAREA BIOACTIVITĂȚII /BIOCOMPATIBILITĂȚII CIMENTURILOR ORTOPEDICE PMMA/Mg₃Al₂(SiO₄)₃ PMMA/Mg₃Al₂(SiO₄)₃ BONE CEMENTS: PRODUCTION, STRUCTURAL CHARACTERIZATION, BIOACTIVITY AND BIOCOMPATIBILITY EVALUATION

SIMONA CAVALU, CRISTIAN RAȚIU *

University of Oradea, Faculty of Medicine and Pharmacy, P-ta 1 Decembrie 10, 410068 Oradea, Romania.

PMMA (poly methyl methacrylate)- based biomaterials still have many shortcomings. Over the last two decades, additives have been developed to address these shortcomings. In our approach, acrylic bone cements were prepared by reinforcing commercial PMMA-based bone cements with 5% Mg₃Al₂(SiO₄)₃. Structural investigation performed by ATR FTIR (Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy) spectroscopy and XRD (X-Rays Diffraction) demonstrated the inclusion of the crystalline phase in polymeric matrix. The SEM (Scanning Electron Microscopy) images (surface and fracture) revealed that the addition of 5% Mg₃Al₂(SiO₄)₃ create a porous structure in PMMA matrix, and facilitate the hydroxyapatite formation in vitro. The biocompatibility tests using human fibroblasts and SEM micrographs revealed the cells attachment and spreading on the surface of both specimens (with and without magnesium aluminosilicate). The fibroblasts showed a wide variety of shapes after 24 hours incubation: spread multipolar or round, as well as spindle shaped or elongated cells. No significant cytotoxic effect was noticed by MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium reduction); moreover, the proliferation rate seems to be favored by the presence of 5% Mg₃Al₂(SiO₄)₃.

Biomaterialele pe bază de PMMA (poly methyl methacrylate) prezintă la ora actuală anumite dezavantaje care, în ultimele două decenii, au fost minimalizate prin folosirea unor aditivi anorganici. În cadrul acestui studiu au fost preparate cimente acrilice ortopedice pe bază de PMMA cu un conținut de 5% Mg₃Al₂(SiO₄)₃ adăugat, care au fost analizate din punct de vedere structural prin spectroscopie ATR- FTIR (Atenuated Total Reflectance Fourier Transform Infrared Spectroscopy) și XRD (X-Rays Diffraction), demonstrându-se incluziunea fazei cristaline în matricea polimerică. Prin microscopie SEM s-a demonstrat faptul că adaosul de 5% Mg₃Al₂(SiO₄)₃ crează o structură poroasă în cimentul acrilic polimerizat și favorizează formarea de hidroxiapatită in vitro. Biocompatibilitatea noului ciment acrilic PMMA/ Mg₃Al₂(SiO₄)₃ a fost investigată in vitro utilizând culturi de fibroblaste. După 24 de ore, imaginile SEM au demonstrat o bună aderență pe suprafața speciemenelor, având o diversitate de forme. Conform analizei MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium), nu s-au observat efecte citotoxice, iar proliferarea celulelor a fost favorizată de prezența Mg₃Al₂(SiO₄)₃ în matricea polimerică.

Keywords: PMMA bone cement, magnesium aluminosilicate, FTIR, XRD, SEM, biocompatibility

1. Introduction

Acrylic bone cements (ABC) are self-polymerizing methyl methacrylate (MMA)-based materials still used in the fixation of orthopedic implants, after a long period of success and popularity, since 1960, serving as a mechanical interlock between the metallic prosthesis and the bone, and a load distributor between the artificial implant and the bone [1]. Commercial bone cements consist of a powder and a liquid component. Typically, the powder constituent is composed of pre-polymerized beads of poly methyl methacrylate (PMMA) and/or MMA-based copolymers, benzoyl peroxide (an initiator for the polymerization), and barium sulfate or zirconium dioxide (a radiopacifier). The liquid constituent is composed of MMA monomer, N, N dimethyl - p - toluidine (an

activator), and hydroquinone (a stabilizer). Although commercial bone cements consist of similar formulations, they differ in composition and in the working and setting properties [2]. The expanding number of potential applications has led to the development of new cements with a wide variety of physical and biochemical properties. Several factors such as their chemical composition, viscosity, porosity, radiopacifiers and antibiotic additives, mixing methods, sterilization, temperature during handling, mechanical properties, and biocompatibility, affect the clinical performance of bone cements. This material still had many shortcomings. Over the last two decades, additives have been developed to address these shortcomings [3]. The focus of bone cement research is to obtain a better mechanical quality

* Autor corespondent/Corresponding author,
E-mail: ratiu_cristian@yahoo.com

quality, curing time and biocompatibility. One of the problems related to the conventional types of bone cement is their low mechanical and exothermic reaction properties. The general problems regarding PMMA cement include the biological response, leakage of the MMA monomer and a high curing temperature, which can damage cell activity. In an ideal case, a bone cement must functionally match the mechanical behavior of the tissue to be replaced, it must be able to form a stable interface with the surrounding natural tissue and be effective in guided tissue regenerative procedures, it should be easy to handle, biologically compatible, non-supporting of microbial growth, and non-allergenic. In order to improve the mechanical properties of different ABC, reinforcement with selected particles was applied in many research studies, including nano-hydroxyapatite (nHA) particles and micro-zirconia (ZrO₂) particles added with different volume fractions (1%, 2% and 3%) to poly methyl methacrylate (PMMA) as a matrix [4], multiwalled carbon nanotubes [5], or nano-sized titania fibers [6]. On the other hand, bone cements can function as a matrix for the local application of antibiotics such as gentamicin, vancomycin, tobramycin and others [7]. As an alternative to antibiotics, the antimicrobial properties of silver has been demonstrated in several studies [8,9]. It is well known that PMMA cements cannot adhere to existing bone, as it cannot induce new bone formation [10]. Recent studies demonstrated that some new formulations show promise not only in terms of bone growth but also in terms of improved physical and mechanical properties. PMMA reinforced with silanized aluminum borate whiskers [11] improved the flexural strength, surface hardness, and thermal stability of PMMA, while hydroxyapatite-reinforced polymer composites has the ability to reach a bone-mimetic reinforcement level of 40–50 vol.%, improving not only the mechanical properties but also biological behavior (bioactivity) [12]. Hence, in this context, it is essential to examine the role of various compatibilizers on the properties of PMMA composites. The goal of our study is to investigate the structural properties of acrylic bone cements reinforced with Mg₃Al₂(SiO₄)₃, and to evaluate the bioactivity and biocompatibility from *in vitro* studies. By this approach, we aim to improve the mechanical characteristics and the bioactivity of existing commercial acrylic bone cements, by incorporating magnesium aluminosilicate, which is commonly used in the pharmaceutical industry, providing effective drug delivery systems, or as coating, in combination with different polymers [13].

2. Experimental

2.1. Preparation of specimens and structural characterization

Commercial acrylic bone cement produced by

Biomechanica (Spain) has the following composition: 1) Liquide total 20 mL: methylmethacrylate (monomer) 98.2%, N,N- Dimethyl -p-Toluidine 0.816 %, ethyl alcool 0.945 % , acid ascorbic 0.022% and hydroquinone 0.002%. 2) Powder total 40 g: polymethyl-methacrylate 87.5%, barium sulfate 10% and peroxide of benzoil 2.5%. Mg₃Al₂(SiO₄)₃ was supplied by Fluka and mixed with the powder PMMA in a concentration of 5% w/w, then allowed to polymerize for 5 min until the mixture became a paste with high viscosity. The curing parameters were registered according to the ASTM Standard [14]. Time and temperature were measured from the onset of the mixing powder with the liquid. For comparison, specimens without magnesium aluminosilicate were prepared. The structural characterization of the samples with and without magnesium aluminosilicate was performed by ATR-FTIR (Attenuated Total Reflectance Fourier Transform Infrared spectroscopy, Perkin Elmer Spectrum BXII equipped with MIRacle devices) in the range 400-4000 cm⁻¹ and XRD (X-Rays Diffraction) pattern were recorded with D8 Advance, equipped with a Ge (111) monochromator in the incident beam in order to obtain only Cu Kα1 radiation with λ=1.5405 Å). The microstructure was evidenced by Scanning Electron Microscopy (SEM) using a Jeol JSM 5510 LV microscope, equipped with energy dispersive X ray spectroscopy (Oxford Instruments). The samples were coated with a thin layer of gold, using an agar automatic sputter coater, in argon atmosphere. The mechanical behavior of the samples was evaluated in a standard three point bending test (EZ20, Lloyd Instruments). For this purpose, specimens were prepared in triplicate, as bar shaped using a template 50x10x3 mm and a loading rate of 5 mm/min was applied. The flexural strength (S) and flexural modulus (E) were calculated using the following formulas:

(1) $S [MPa] = \frac{3FL}{2bd^2}$, where F is the load at break, L=50 mm is the span of specimen between supports, b=3 mm is the width, d=3 mm is the thickness.

(2) $E [GPa] = \frac{FL^3}{4Db^3}$, where F is the force at deflection, L=50 mm is the span of specimen between supports, b=3 mm is the width, d=3 mm is the thickness, D is the deflection at linear region of load deflection curve.

2.2. Evaluation of bioactivity

The specimens of acrylic cements with and without magnesium aluminosilicate were incubated for 34 days in simulated body fluids (SBF) following the procedure describe by Kokubo *et al* [15]. The mineralization process upon incubation was confirmed by the surface morphology analyses (SEM) combined with XRD pattern.

2.3. In vitro evaluation of biocompatibility

The biocompatibility tests of acrylic bone specimens with and without magnesium aluminosilicate was evaluated with respect to human fibroblasts adhesion and proliferation. Human fibroblasts HFL-1 cell line were maintained in a mixture of Dulbecco's Modified Eagle Medium (DMEM) containing 4.5 g/L glucose and Ham's F12 nutrient medium supplemented with 10% fetal bovine serum, 2 mM glutamine, 1% penicillin/streptomycin, 1% nonessential aminoacids at 37°C, 5% CO₂, 95% relative humidity. The upper surfaces of the sterile specimens (discs) were coated with Poly-L-Lysine (Sigma, P4707) for 2h, and then washed with phosphate buffered saline (PBS). A drop (50 μL) containing 5·10⁴ HFL-1 cells in culture medium was added on the coated surface of the material for 3h in order to promote cell adhesion. The fixation procedure of the cells attached onto the tested material was done after 3, 7 and 24h using paraformaldehyde (4%) for 15 min at room temperature. Three steps of washing using sterile PBS were done prior to the microscopy analysis (SEM). The morphology of fibroblasts cells after 3,7 and 24h incubation time was investigated by SEM. Cytotoxicity test was performed by using MTT assay (3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide) according to Mosmann method [16].

3. Results and discussions

3.1. Structural characterization

Figure 1 present the ATR FTIR spectra recorded in the region 550-2000 cm⁻¹ for the powder PMMA component of acrylic cement, the polymerized acrylic cement specimen, Mg₃Al₂(SiO₄)₃ and polymerized acrylic cement containing 5% Mg₃Al₂(SiO₄)₃.

The marker bands of powder PMMA are a sharp and intense peak at 1721 cm⁻¹ due to the presence of ester carbonyl group ν(C=O) stretching vibration, a broad band at 1436 cm⁻¹ due to δ(CH₃) vibration mode and 1237 cm⁻¹ band assigned to torsion of the methylene group (CH₂). The intense peaks at 1189, 1139 and 1073 cm⁻¹ band corresponds to vibration of the O-C-O, C-CH₃ and C-COO stretching vibrations, while C-C stretching bands are located at 981 and 837 cm⁻¹ [9,17]. In the low frequency region, the 748 and 609 cm⁻¹ peak correspond to out of plane bending vibration of sulfate group from barium sulfate. The features of this spectrum are very well preserved after polymerization, as well as the relative intensity of these marker bands, only a few modifications occurs in the range 1073-745 cm⁻¹, as a result of BaSO₄ particles presence in the commercial cement matrix. The vibrational features of Mg₃Al₂(SiO₄)₃ are: Si-O stretching at 1038 cm⁻¹ and 999 cm⁻¹, OH deformation linked to 2Al³⁺ at 912cm⁻¹, and

Si-O-Si bending at 790, 751 and 684 cm⁻¹ due to inter - tetrahedral bridging bonds [18]. The fingerprints of both PMMA and Mg₃Al₂(SiO₄)₃ are evidenced in the final composite (Fig. 1b), with different relative intensities and slowly shifted to higher wavelength, as a result of inorganic particles inclusion in polymeric matrix.

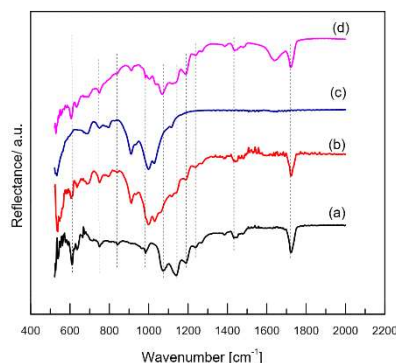


Fig.1 - ATR-FTIR spectra of (a) commercial PMMA powder as received from the supplier; (b) PMMA/Mg₃Al₂(SiO₄)₃ polymerized; (c) powder Mg₃Al₂(SiO₄)₃; (d) polymerized PMMA (commercial) / Spectrele ATR-FTIR corespunzătoare probelor (a) ciment PMMA pulbere comercială, neprelucrată; (b) ciment PMMA/Mg₃Al₂(SiO₄)₃ polimerizat; (c) pulbere Mg₃Al₂(SiO₄)₃; (d) ciment PMMA comercial, polimerizat.

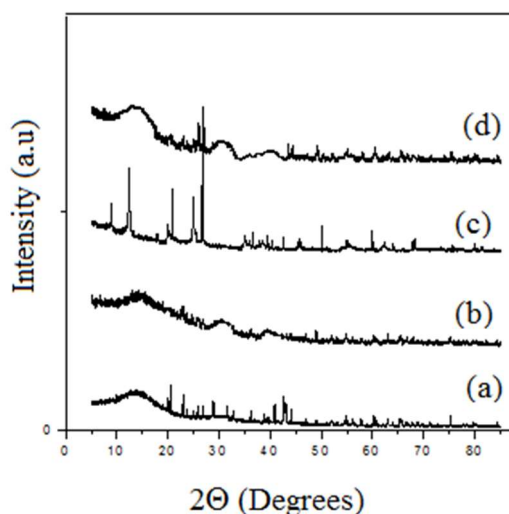


Fig. 2 - XRD pattern of (a) commercial PMMA powder as received from the supplier; (b) polymerized commercial PMMA; (c) powder Mg₃Al₂(SiO₄)₃; (d) polymerized PMMA/Mg₃Al₂(SiO₄)₃ / Spectrele de difracție X ale probelor (a) ciment comercial pulbere PMMA; (b) ciment comercial PMMA polimerizat; (c) pulbere Mg₃Al₂(SiO₄)₃; (d) ciment polimerizat PMMA/Mg₃Al₂(SiO₄)₃

Figure 2 emphasize the XRD pattern of powder and polymerized PMMA with and without addition of 5% Mg₃Al₂(SiO₄)₃, along with the pattern of powder Mg₃Al₂(SiO₄)₃. PMMA is an amorphous polymer and the powder form (as received from

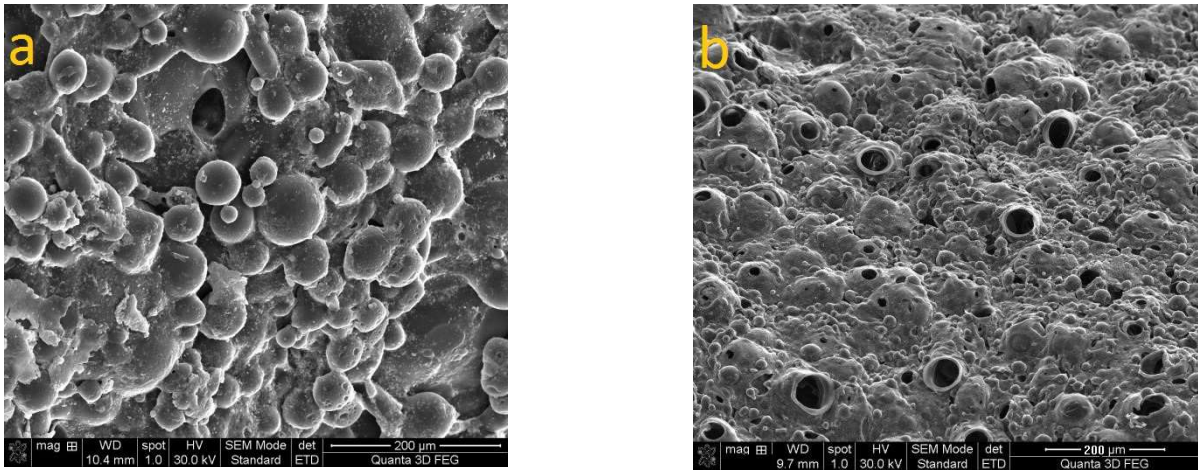


Fig. 3 - SEM micrographs recorded on the surfaces of commercial PMMA (a) and new composite PMMA/Mg₃Al₂(SiO₄)₃(b). / Imagini de microscopie electronică a suprafețelor: (a) ciment comercial; (b) compozit PMMA/Mg₃Al₂(SiO₄)₃

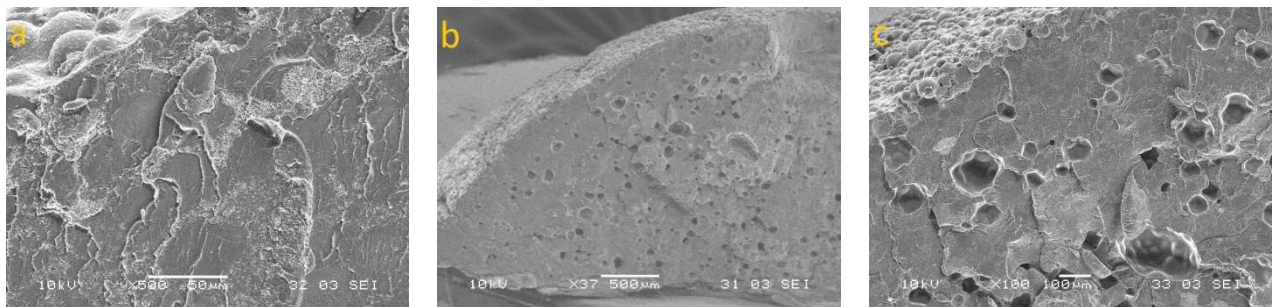


Fig. 4 - SEM images recorded on the fractured surface of the specimens: (a) commercial PMMA bone cement; (b,c) PMMA/Mg₃Al₂(SiO₄)₃ composite, with different details and magnifications. / Imagini de microscopie electronică a fracturilor: (a) ciment PMMA comercial; (b,c) compozit PMMA/Mg₃Al₂(SiO₄)₃, cu diferite detalii.

the supplier) shows a broad band at $2\theta = 13^\circ$, and some low intensity, sharp peaks corresponding to BaSO₄ crystalline phases at $2\theta = 20^\circ; 24.5^\circ; 26^\circ; 29^\circ; 33^\circ; 44^\circ$ (Fig.2a). After polymerization, the fingerprints of PMMA are revealed as broad bands at $2\theta = 13^\circ, 31^\circ$ and 40° (Fig.2b). According to literature, the shape of the first most intense peak reflects the ordered packing of polymer chains while the second peak denotes the ordering inside the main chains [17, 19]. The pattern of Mg₃Al₂(SiO₄)₃ indicates a crystalline structure (Fig. 2c) with the main peaks at $2\theta = 11.5^\circ, 20.5^\circ, 25^\circ$ and 27° , the major phase being SiO₂ according to reference card No [79-1910]. The pattern of PMMA with addition of 5% Mg₃Al₂(SiO₄)₃ indicates the same broad bands characteristic to PMMA, along with the main features of magnesium aluminosilicate, demonstrating the inclusion of crystalline phase in polymeric matrix (Fig. 2d).

In order to get information about the ultrastructure of the composites, SEM images were recorded on the surface of the specimens with and without Mg₃Al₂(SiO₄)₃ but also on the fracture area. The images are presented comparatively in Fig. 3. The PMMA beads retain their shape and are surrounded by the polymerized MMA monomer acting as binding agent. After magnesium aluminosilicate addition, the surface shows some modifications, presenting a crater-like structure. Comparing the fracture images (Fig.4), it can be

notice that addition of 5% Mg₃Al₂(SiO₄)₃ create a porous structure. The development of porosity might be related to polymerization shrinkage of the bone cement, based on density changes, in converting monomer to polymer. Therefore, further investigations are required in order to follow the kinetics of parameters such as polymerization stress, degree of conversion and reaction exotherm, as well as understanding and control of the curing process. According to literature, similar results were obtained by adding β -tricalciumphosphate (β -TCP) to PMMA matrix [20] and the new composite was demonstrated to improve the osteoconductivity, due to its open macroporous structure, allowing the bone cells and the blood vessels to colonize the bone.

Mechanical tests results are presented in Table 1 as mean values of flexural strength (S) and flexural modulus (E), calculated according to the formulas (1) and (2), for both the commercial PMMA and reinforced specimens. The introduction of 5% Mg₃Al₂(SiO₄)₃ in PMMA matrix slightly increased the value of these parameters. Even if the improvement is not spectacular, we need to take into account that, for this type of applications, biomaterials need to have stiffness similar to bone; they do not require high strength, since they will not be responsible for carrying load. In the same time, increasing the elastic modulus can be a favorable attribute in producing materials that combine

bioactive behavior with elastic modulus comparable to natural bone [6,11]

Table 1

Mechanical properties of PMMA and PMMA/Mg₃Al₂(SiO₄)₃ determined by tree point bending test. / *Proprietățile mecanice ale cimentului PMMA comercial și ale compozitului PMMA/Mg₃Al₂(SiO₄)₃, conform testului de rezistență în trei puncte.*

Specimen	S [MPa]	E [GPa]
PMMA (commercial)	63.76 ± 2.06	2.44 ± 0.06
PMMA/ Mg ₃ Al ₂ (SiO ₄) ₃	79.73 ± 2.66	3.13 ± 0.1

3.2 Bioactivity evaluation

After 34 days incubation in simulated body fluids (SBF) of both specimens (with and without addition of magnesium aluminosilicate), a mineralization process can be observed on the surface. The SEM images recorded on the surfaces of both specimens are presented comparatively in Fig. 5 (a,b), along with the details with higher magnification in Fig. 5c and the corresponding EDAX spectrum in Fig. 5d. It is obvious that addition of 5% Mg₃Al₂(SiO₄)₃ improve the mineralization process, showing the formation of dense aggregates with different morphology on the surface. The quantitative measurement indicated the ratio Ca/P=1.65, which is an indicative for hydroxyapatite formation.

The mineralization process upon incubation was confirmed also by XRD pattern. In Fig. 6 (a,b) are displayed the XRD patterns of PMMA/Mg₃Al₂(SiO₄)₃ specimens recorded before and after 34 days incubation. The mineralized layer

shows the characteristic fingerprints of hydroxyapatite at 2θ= 23, 26, 27, 32.5 and 34°, according with standard card No [9-432] [21]. This behavior is in accordance with the mechanism of apatite formation described by Hench [22] and other previously reported data in literature, showing that polymer matrices reinforced with bioactive phase can potentially combine the bioactive behavior of composite material with mechanical properties closer to human tissues [23]. Examples of bioactive particles previously used for this purpose are bioactive glasses [24], titania fibers [25], hydroxyapatite particles [12], alumina nanofillers [25,26] and silver nanoparticles [27]. It is generally accepted that the ability to form apatite precipitation layers in SBF or in an animal model can be consider as the evidence of bioactivity for bioceramics and for other types of orthopedic materials. Consequently, the *in vivo* bioactivity of acrylic cements can be predicted from the apatite formation on their surfaces in SBF [28].

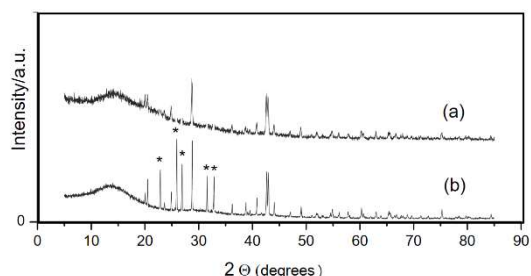


Fig. 6 - XRD pattern of the PMMA/Mg₃Al₂(SiO₄)₃ specimen before (a) and after (b) incubation in SBF for 34 days. Hydroxyapatite phase is marked / *Spectrul de difracție a razelor X înregistrat pe suprafața cimentului PMMA/Mg₃Al₂(SiO₄)₃ înainte (a) și după incubare timp de 34 zile (b). Faza corespunzătoare hidroxiapatitei este marcată cu **.

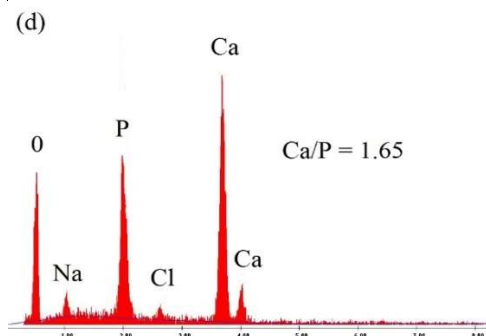
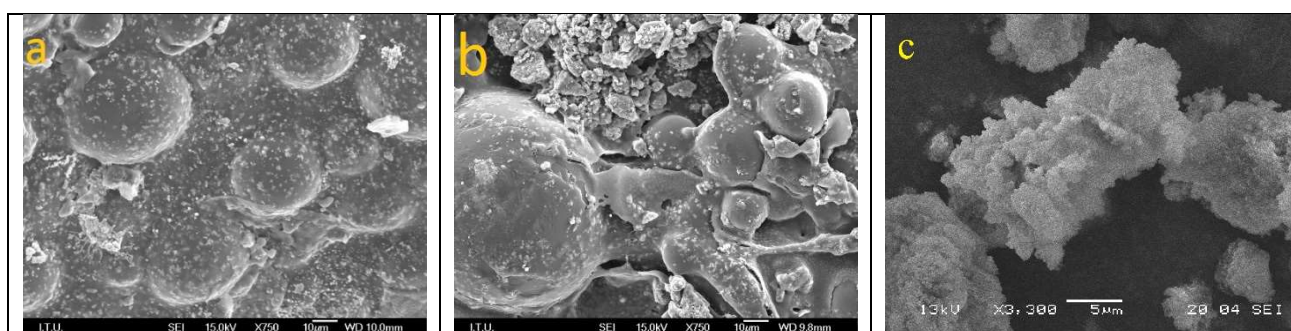


Fig.5 - SEM micrographs recorded on the surface of acrylic bone cements after 34 days incubation in SBF: (a) commercial PMMA cement; (b) PMMA/Mg₃Al₂(SiO₄)₃ cement, with low magnification; (c) high magnification details of mineral phase formed on the surface of PMMA/Mg₃Al₂(SiO₄)₃ cement after 34 days incubation; (d) the corresponding EDAX spectrum of mineral phase. / *Imagini de microscopie electronică înregistrate pe suprafața cimenturilor acrilice după 34 de zile de incubare în SBF: (a) ciment PMMA comercial; (b) compozit PMMA/Mg₃Al₂(SiO₄)₃, imagine de ansamblu; (c) detaliu (magnificație înaltă) al fazei minerale formate pe suprafața compozitului PMMA/Mg₃Al₂(SiO₄)₃, după 34 zile de incubare; (d) spectrul EDAX corespunzător fazei minerale.*

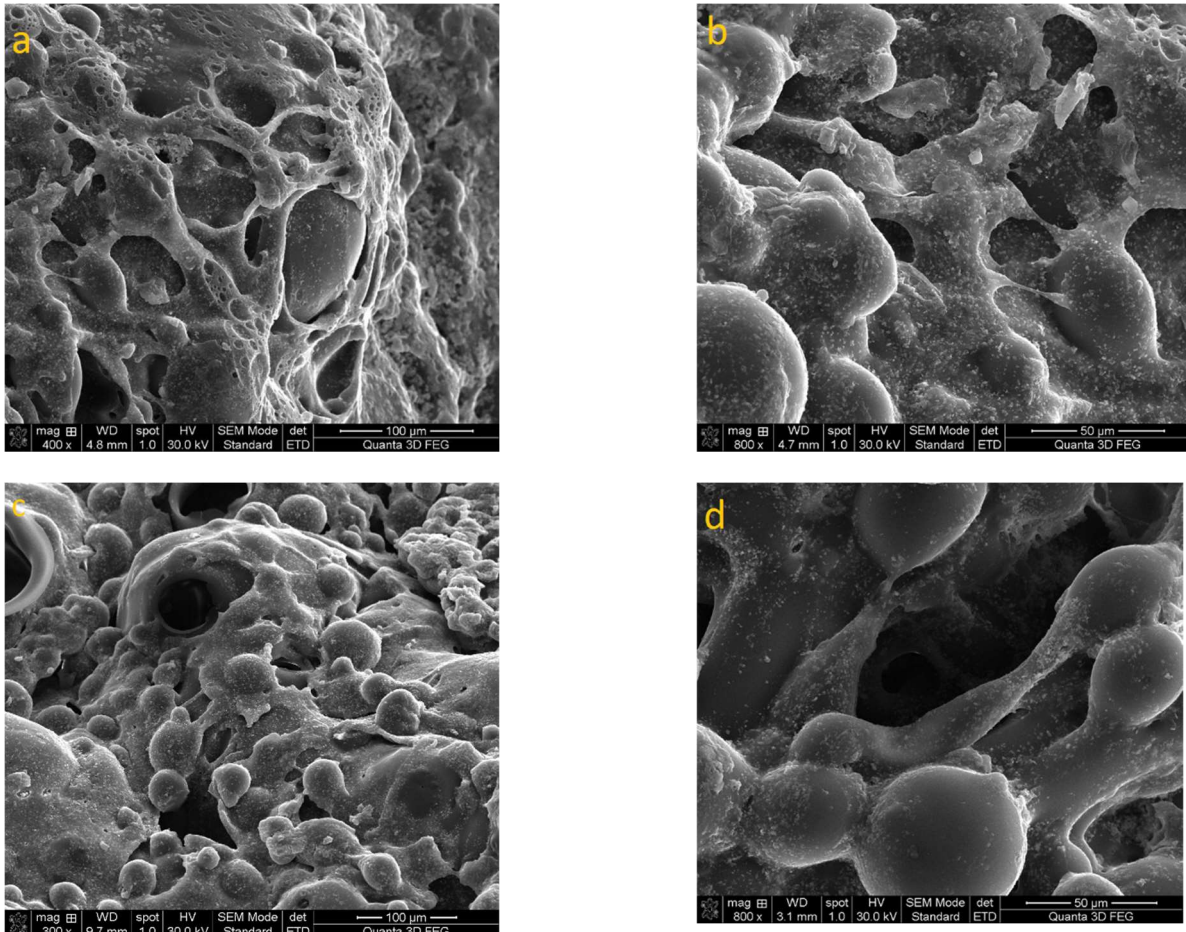


Fig. 7 - SEM micrographs showing the adherence and proliferation of human fibroblasts on the surface of commercial PMMA bone cement (a,b) and PMMA/Mg₃Al₂(SiO₄)₃(c,d) / *Imagini de microscopie electronică demonstrând aderarea și proliferarea fibroblastelor umane pe suprafața cimentului ortopedic comercial (a,b) și pe suprafața compozitului PMMA/Mg₃Al₂(SiO₄)₃ (c,d).*

3.3. Biocompatibility evaluation

The biocompatibility evaluation of the PMMA specimens was investigated *in vitro*, using human fibroblasts (HSFs) in a density of 2×10^4 cells/cm³, seeded upon each specimen substrate and incubated in standard culture conditions for 24 h in order to promote the adhesion and spreading. The SEM micrographs presented in Fig. 7 (a,b,c,d) demonstrates the human fibroblasts attachment and spreading on the surface of both specimens (with and without magnesium aluminosilicate). The fibroblasts show a wide variety of shapes: spread multipolar or round, as well as spindle shaped or elongated cells.

The results are also supported by the MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium reduction), a rapid and sensitive colorimetric method based on the capacity of the mitochondrial dehydrogenase enzymes to convert a yellow-water soluble tetrazolium salt, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) into a purple insoluble formazan product by a reduction reaction. The insoluble crystals are dissolved in DMSO or acidic isopropanol for the measurement of absorbance values in a spectrophotometer. This assay is based on the formation of a visible dark

blue formazan product by the action of cellular mitochondrial dehydrogenases, which acts as a marker for living cells [20,21]. Our result based on MTT assay showed viable fibroblasts cells with respect to PMMA and PMMA/Mg₃Al₂(SiO₄)₃ specimens after 1, 3, 7 and 24 hours of culture. Initial cells attachment was not influenced by the 5% Mg₃Al₂(SiO₄)₃ content in the PMMA, but a progressive increase in optical density was noticed after 3 hours. The results demonstrated no significant cytotoxic effect and showed that both specimens led to cell survival and proliferation, but the proliferation rate seems to be favored by the presence of 5% Mg₃Al₂(SiO₄)₃. Of course, further *in vivo* analyses of this composite interaction with bone tissue leading to bone bonding should be performed to confirm the biocompatibility of this composite.

Some studies demonstrated that the increase of contact surface due to the increased porosity of the cement and the formation at the bone cement interface of newly formed trabecular bones with strands of connective tissue going deep into the superficial layer of the cement, may prove a good osteointegration of the cement with the biological structures of the host [20,29].

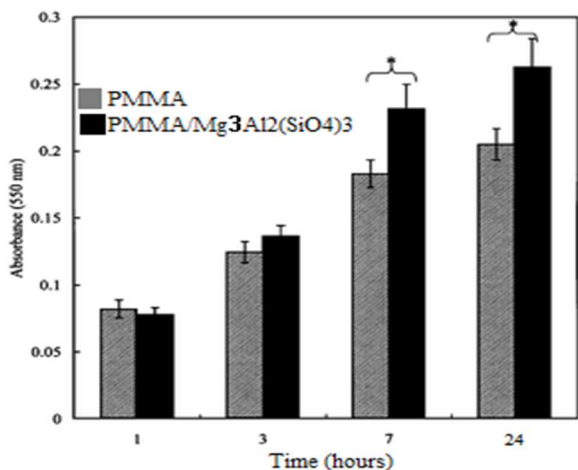


Fig. 8 - MTT assay of viable human fibroblasts with respect to commercial PMMA cement and PMMA/Mg₃Al₂(SiO₄)₃/ Testele de viabilitate celulară MTT a celulelor fibroblaste umane, în urma contactului cu cimentul PMMA comercial, respectiv compozitul PMMA/Mg₃Al₂(SiO₄)₃.

According to literature [6-8], there are many bone cement additives, none of which is perfect as strength, bioactivity and biocompatibility, often being adversely affected with minor additions of an additive. This is why some researches are focusing on the effect of combining various additives. New approaches may yield bone cements that display the beneficial properties of each additive, while still maintaining structural integrity.

4. Conclusions

The goal of our work was to examine the role of Mg₃Al₂(SiO₄)₃ additive with respect to commercial PMMA bone cement from the structural, bioactivity and biocompatibility point of view. The ATR FTIR spectra and XRD pattern demonstrated the inclusion of the crystalline phase in polymeric matrix. The SEM images (surface and fracture) demonstrated that the addition of 5% Mg₃Al₂(SiO₄)₃ create a porous structure in PMMA bone cements and improved bioactivity. The hydroxyapatite formation on the surface of specimens was revealed both by SEM and XRD analysis. The biocompatibility tests using human fibroblasts and SEM micrographs revealed the human fibroblasts attachment and spreading on the surface of both specimens (with and without magnesium aluminosilicate). The fibroblasts showed a wide variety of shapes after 24 hours incubation: spread multipolar or round, as well as spindle shaped or elongated cells. No significant cytotoxic effect was noticed by MTT assay; moreover, the proliferation rate seems to be favored by the presence of 5% Mg₃Al₂(SiO₄)₃.

Acknowledgement:

The authors acknowledge the support by UEFISCDI, PNII ID PCE-2011-3-0411, contract nr. 237/2011.

REFERENCES

1. J. Charnley, The bonding of prostheses to bone by cement, Journal of Bone and Joint Surgery (British), 1964, **46**, 518.
2. Y. Imai, A. Ohyama, Characterization of powder component of commercial bone cements, Dental Materials Journal, 2001, **20** (4), 345.
3. A.T. Cucuruz, E. Andronescu, C.D. Ghitulica, A. Ilie, Synthesis and characterization of a new composite material based on poly(methyl methacrylate) and silica for dental applications, Romanian Journal of Materials, 2014, **44**(1) 54.
4. S.I. Salih, J.K. Olewi, Q.A. Hamad, Investigation of Fatigue and Compression Strength for the PMMA Reinforced by Different System for Denture Applications, International Journal of Biomedical Materials Research, 2015, **3**(1) 5.
5. M. K. Singh, T. Shokuhfar, J. J. de Almeida Gracio, A. C. Mendes de Sousa, J. M. Da Fonte Ferreira, H. Garmestani, S. Ahzi, Hydroxyapatite Modified with Carbon Nanotube-Reinforced Poly(methyl methacrylate): A Novel Nanocomposite Material for Biomedical Applications, Advanced Functional Materials, 2008, **9999**, 1.
6. S. M. Z. Khaled, P. A. Charpentier, A. S. Rizkalla, Physical and Mechanical Properties of PMMA Bone Cement Reinforced with Nano-sized Titania Fibers, Journal of Biomaterials Applications, 2011, **25**, 515.
7. M. Arora, E. K. S. Chan, S. Gupta, A. D. Diwan, Polymethylmethacrylate bone cements and additives: A review of the literature, World Journal of Orthopedics, 2013, **4**(2) 67.
8. T. Ghaffari, F. Hamedirad, B. Ezzati, In Vitro Comparison of Compressive and Tensile Strengths of Acrylic Resins Reinforced by Silver Nanoparticles at 2% and 0.2% Concentrations, Journal of Dental Research Dental Clinics Dental Prospect, 2014, **8**(4) 204.
9. S. Cavalu, V. Simon, G. Goller, I. Akin, Bioactivity and antimicrobial properties of PMMA/Ag₂O acrylic bone cement collagen coated, Digest Journal of Nanomaterials and Biostructures, 2011, **6**(2) 779.
10. M. J. Provenzano, K. P. J. Murphy, L. H. Riley, Bone cements: review of their physiochemical and biochemical properties in percutaneous vertebroplasty, American Journal of Neuroradiology, 2004, **25**, 1286.
11. X. Zhang, Xiu Zhang, B. Zhu, K. Lin, J. Chang, Mechanical and thermal properties of denture PMMA reinforced with silanized aluminum borate whiskers, Dental Materials Journal, 2012, **31**(6), 903.
12. R. K. Roeder, G. L. Converse, R. J. Kane, W. Yue, Hydroxyapatite-reinforced polymer biocomposites for synthetic bone substitutes, JOM (Overview Biological Materials Science), 2008, **60**(3) 38.
13. M. Jafarbeglou, M. Abdouss, A. M. Shoushtari, M. Jafarbeglou, Clay nanocomposites as engineered drug delivery systems, 2016, RSC Advances, **6**, 50002.
14. *** American Society for Testing and Materials (ASTM), Standard F451-99a, Annual Book of ASTM Standard, 2000, Vol. 13.01, 55.
15. T. Kokubo, S. Ito, Z. T. Huang, T. Hayashi, S. Sakka, T. Kitsugi, T. Yamamuro, Ca,P-rich layer formed on high-strength bioactive glass-ceramic A-W, Journal of Biomedical Materials Research, 1990, **24**, 331.
16. T. Mosmann, Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, Journal of Immunology Methods, 1983, **65**, 55.
17. D. E. Baci, J. Simitzis, D. Giannakopoulos, Synthesis and characterization of acrylic bone cement reinforced with zirconia bioceramic, Digest Journal of Nanomaterials and Biostructures, 2012, **7**(4) 1779.
18. B. J. Saikia, G. Parthasarathy, Fourier Transform Infrared spectroscopic characterization of kaolinite from Assam and Meghalaya, Northeastern India, Journal of Modern Physics, 2010, **1**, 206.
19. S. Ahmad, S. Ahmad, S. A. Agnihotry, Synthesis and characterization of in situ prepared poly (methyl methacrylate) nanocomposites, Bulletin of Materials Science, 2007, **30**(1), 31.

20. C. Dall'Oca, T. Maluta, F. Cavani, G.P. Morbioli, P. Bernardi, A. Sbarbati, D. Degl'Innocenti, B. Magnan, The biocompatibility of porous vs non-porous bone cements: a new methodological approach, *European Journal of Histochemistry*, 2014, **58**, 2255.
21. K. Agrawal, G. Singh, D. Puri, S. Prakash, Synthesis and characterization of hydroxyapatite powder by sol-gel method for biomedical application, *Journal of Minerals & Materials Characterization & Engineering*, 2011, **10**(8), 727.
22. L. L. Hench, *Bioceramics: from concept to clinic*, in *Journal of American Ceramics Society*, 1991, **74**, 1487.
23. M. de Magalhaes Pereira, R. L. Orefice, H. S. Mansur, M. T. P. Lopez, R. M. de Marco, A. C. Vasconcelos, Preparation and biocompatibility of poly(methyl methacrylate) reinforced with bioactive particles, *Materials Research*, 2003, **6**(1), doi: 10.1590/S1516-14392003000300002.
24. L. Floroian, I. Mihailescu, F. Sima, G. Stanciu, B. Savu, Evaluation of biocompatibility and bioactivity for polymethyl methacrylate – bioactive glass nanocomposite films obtained by matrix assisted pulsed laser evaporation, *University Politehnica Bucharest Scientific Bulletin Series A*, 2010, **72**(2) 134.
25. S. M. Z. Khaled, P. A. Charpentier, A. S. Rizkalla, Physical and mechanical properties of PMMA bone cement reinforced with nano-sized titania fibers, *Journal of Biomaterials Applications*, 2011, **25**, 515.
26. S. A. B. Hasan, M. M. Dimitrijević, A. Kojović, D. B. Stojanović, K. Obradović-Duričić, R. M. J. Heinemann, R. Aleksić, The effect of the size and shape of alumina nanofillers on the mechanical behavior of PMMA matrix composites, *Journal of Serbian Chemistry Society*, 2014, **79**(10), 1295.
27. T. Ghaffari, F. Hamedirad, B. Ezzati, In vitro comparison of compressive and tensile strengths of acrylic resins reinforced by silver nanoparticles at 2% and 0.2% concentrations, *Journal of Dental Research, Dental Clinics, Dental Prospects*, 2014; **8**(4), 204.
28. S. Cavalu, V. Simon, Microstructure and bioactivity of acrylic bone cements for prosthetic surgery, *Journal of Optoelectronics and Advanced Materials*, 2006, **8**(4), 1520.
29. J. L. Gilbert, J. M. Hasenwinkel, R. L. Wixson, E.P. Lautenschlager, A theoretical and experimental analysis of polymerization shrinkage of bone cement: A potential major source of porosity, *Journal of Biomedical Materials Research*, 2000 (52), 210.

MANIFESTĂRI ȘTIINȚIFICE / SCIENTIFIC EVENTS

Second Central American Drymix Mortar Meeting, Mexico City

Tuesday, June 27, 2017, 09:00 - 15:00



Event Description

drymix.info is uniting the drymix mortar industry by staging mortar meetings all around the globe. The meeting features competent lectures by the mortar industry, an Industry showcase and provides ample communication time for Delegates from Mexico, Central America, Northern South America and the Caribbean. The Central American Drymix Mortar Meeting is THE Place for the Global Drymix Mortar Community to re- unite and network.

More information

Please send an E- Mail to info@drymix.info in case of questions
