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Synthesis and Antibacterial Study of New Microporous Zinc Phosphate Bioceramic

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Abstract

In a ternary system composed of aluminum phosphate, zinc oxide, and orthophosphoric acid, we have prepared microporous ceramics for orthopedic and dental uses. Firstly, we have prepared the ceramics with uncontrollable porosity by sintering blocs of the zinc phosphate cement drawn from the ternary system. Then, based on a binary system consisted of aluminum phosphate, zinc oxide, and some porogenic additives, we have prepared ceramics with good and homogeneous porosity structure. In the second issue, the preparation is made by a simple mixture of powders. Thus, after homogenization, compaction and sintering at high temperatures, the resulted ceramics developed high mechanical resistances. Besides, antibacterial tests on the ceramics have been conducted and shown good biological performances. The mechanical behavior as well as the biological properties candidate the ceramics to be a good alternative to the hard tissue. Eventually, all preparations are controlled and investigated using several technics such as FTIR, XRD, SEM, TAG-TAD, mechanical test, and an antibacterial activity test..

Keywords: *Ceramic; zinc phosphate cement; orthopedic material; dental material; sintering; porosity, antibacterial test*

1.Introduction

Ceramics have been used since the dawn of human civilization. They were related to the use of the fire. Then, they have progressed in uses due to the development of needs and art. For instance, pottery and rudimentary tools have been used for thousands of years. An up to date, the ceramics constitute a major field of scientific research and technological innovation and will continue to do so throughout the present century. They are now finding their use in several fields: Environment, energy, medicine, civil engineering, space technology… etc. Two categories of ceramics are involved such as ceramics obtained by the sintering process at high temperature, and alternatively, others obtained by chemical bonding, mostly at ordinary conditions [1]. The first issue is more demanding in terms of energy while the second is carried out at room temperature. Furthermore, both processes provide differences in structure and properties [2]. The sintering process has superior mechanical properties and is far more stable in an acidic environment and at high-temperature than the second one. However, both materials fail to fulfill some needs of recent technological fields. Recently, chemically bonded ceramics are a new generation of materials that fill the gap between both categories [1]. On the other hand, they satisfy modern technological needs. For instance, they have been introduced for the stabilization of hazardous and radioactive wastes, architectural products, and for some complex medical practices. Eventually, they take a growing place in the market, beside other products. Among chemically bonded ceramics, the phosphate-based ceramics are widely used [3], particularly for orthopedic and dental uses. The main use of them is to restore the defect of hard tissues, to full cavities of teeth and bones and to prepare orthopedic and orthodontic parts. They are formed by acid-base reactions between an acid phosphate and a metal oxide (such as that of magnesium, calcium, or zinc). Our work concerns the ternary system $AlPO_4$, nH_2O-ZnO-H_3PO_4, to prepare zinc phosphate cement and ceramics [4,5]. Then, we create porosity inside these products in order to enhance their uses. For instance to prepare spongy bones as an alternative to the defected mineral tissue, and produce filters and membranes for other applications [6]. Historically, zinc phosphate cement was developed as dental cement and was in use during the first half of the twentieth century. It is obtained by sintering powder of zinc oxide at high temperature and then mixed as a powder at room temperature with orthophosphoric acid [7, 8]. After a mixture of both products, the paste sets and hardens rapidly. It is mostly used as a luting cement to fill the dental cavity as liners and put as cement under metal or ceramic [9]. In a previous work [3], we have prepared bioceramics with high biological performances such as biocompatibility and bioactivity [10, 11]. Basing on the fact that the mineral tissue of the body consists of the calcium and the phosphate, we have produced a ceramic based on a mixture of β tricalcium phosphate $Ca_3(PO_4)_2$ and hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$. This ceramic developed a good mechanical profile by adding some fillers of silica and zirconia. In the present study, we have adopted a chemical route to form diverse shapes from the paste of zinc phosphate cement drawn from the ternary system. Then, after hardening, we sinter the consolidated material in a furnace to produce zinc phosphate ceramics. Other ceramics are obtained from the binary system $AlPO_4$-ZnO, in which the porosity is created by adding some porogenic additives such as fluoride compounds [12] and glycerin. Our purpose is to prepare ceramics that have a good chemical similitude with zinc phosphate cement, in order to have common use in orthopedic and dentistry.

2. Material and Methods

2.1. Microporous Ceramics from zinc phosphate cement

The first method used for the preparation of microporous ceramics is based on the firing of hardened blocs of zinc phosphate cement. From the ternary system ($AlPO_4$, nH_2O- ZnO- H_3PO_4) Figure 1, we have defined a zone in which the zinc phosphate cement develops good mechanical properties. The composition rate chosen in weight is: ($AlPO_4$, nH_2O) = 12%, ZnO = 37% and H_3PO_4 (d = 1, 71; 85%) = 51%. The liquid phase of cement is a mixture of H_3PO_4 and

water, and the solid phase is a mixture of $AlPO_4$, nH_2O and ZnO fired at high temperatures. The liquid/solid ratio of the final mixture is 0,3 cm^3/g. cement paste is placed then on molds to prepare some complexional shapes. After setting and hardening, the blocs of cement are sintered at high temperature following a process based on the thermogravimetric analysis TGA and differential thermal analysis DTA curves figure 2.The sintering process of the microporous ceramics is started in the oven (marc Memmert 600 D 06062) by drying the different shapes of hardened cement at 100°C for one hour. At this temperature, the molecules of free water leave the cement matrix. Then, blocs are introduced in a brand (marc Nabertherm N11/H, N° 166434) programmed at 500°C for one hour. After that, the temperature is increased with a range of 50°C/min until reaching the paler of temperature at 900°C which is considered the temperature of sintering. Finally, the mass of cement is fired for one hour at this temperature.

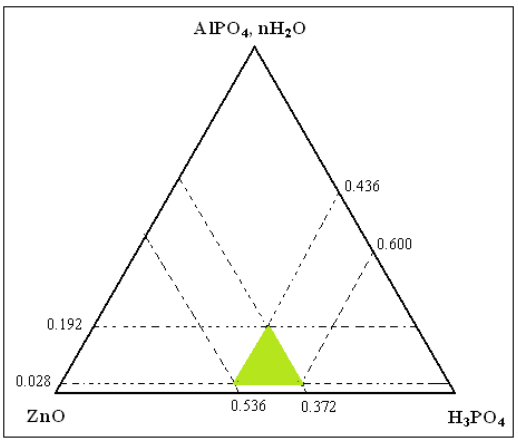

Figure 1: Local region of cement in the tertiary system (AlPO4, nH2O –ZnO–H3PO4)

2.2. Microporous Ceramics from the binary system $AlPO_4$, nH_2O-ZnO

For the second method, we have adopted the sintering process in order to make a porous ceramics. Any chosen composition from the binary system of aluminum phosphate and zinc oxide contributes to a new ceramic. Thus, both compounds are intensively mixed in an Ika labortechnic arm mixer. Then, they underwent a previous calcination process at temperature inferior to its sintering condition. Thus, the obtained aggregates are ground adding glycerine in a Pfaff steel ring mill. Furthermore, in order to improve mechanical and biological properties and create porosity inside the ceramic, some products are added to the powder. Especially, filers of silica SiO_2 and zirconia ZrO_2 for the mechanical aspect, and the calcium phosphate compounds such as the β tricalcium phosphate and the hydroxyapatite for improving biocompatibility and bioactivity. Finally, the porogenic additive such as SnF2 is added to create microporosity. In order to remedy the shrinkage problem of ceramics, we have deflocculated the powder at high temperatures. Again, the aggregates are cooled and ground then compacted by uniaxial pressing to form a cylindrical form. Eventually, the sample is conducted to sintering process at a temperature slightly superior to 850°C. The X-ray diffraction patterns of synthesis sintered ceramics were performed on a Shimadzu XRD-6000, Shimadzu, Japan using the CuKα radiation. The patterns were recorded in a 2θ range of 0°-70° with a step size of 0.02°. Infrared spectra (FTIR) were measured using a SHIMADZU spectrometer. The spectra were obtained in the range of 4000 to 400 cm^{-1}, using the KBr pellet method, with a resolution of 2 cm^{-1}. The pore size and morphology of the sintered ceramics were characterized using a scanning electron microscope (SEM). Energy dispersive spectroscopy (EDS) was also used to investigate the elemental composition of ceramics. For mechanical properties, we have measured the Vickers' hardness Vk on some ceramics. The measure is made with an automatic durometer SHORE brand, which can give the four types of surface hardness (Brinell, Rockwell, Vickers, and Knoop) by a simple shifting inside its program. The

samples have a cylindrical form with 15 mm in diameter and height. These samples are placed to receive the shock of the indentation ball. The measurement of the hardness takes into account the jet force and the penetration of the ball.

3. Results and discussions

3.1. Microporous Ceramics from zinc phosphate cement

The TGA and DTA analyses Figure 2 show that free water is volatilized between 94 °C and 117 °C with a loss of weight equal to 1,43%. The important loss of weight (7,3%) is done between 166 °C and 185°C and it was due to the dehydration of rehydrated aluminum phosphate $AlPO_4$, nH_2O and then of two molecules of hopeïte $Zn_3(PO_4)_2,4H_2O$. At 315°C, the cement lost 3% of its weight due to the loss of two remaining molecules of hopeïte and from different condensation (dehydroxylation) which are made inside the structure under the heating process. At this temperature, the rate of weight loss is proportional to the quantity of hopeïte produced in hardened cement. At the range of temperature 900-910°C, the DTA curve shows an endothermic peak which may be relied on the hopeïte melting point (900°C). At this temperature, the ceramic develops a very good resistance and keeps partially its porosity and goes on until 1000°C. Then over 1000°C it will be an inflation of the structure by loss of its porosity. Despite that, the ceramic becomes more resistance due to the consolidation of matrix particles by melting particles of aluminum phosphate. In this light, we have concluded that to preserve ceramic with good resistance and porosity, the temperature of sintering by the present way should be inferior or equal to 900°C.

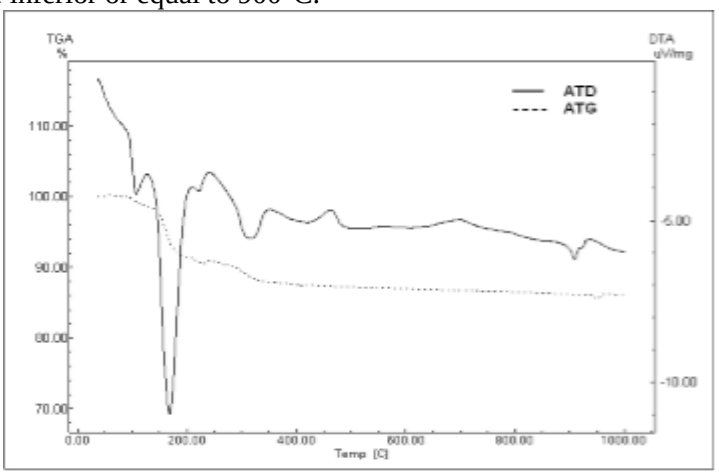

Figure 2: TGA–TDA curves of hardened cement

Inside the zinc phosphate cement domain explored in the ternary system, any composition is possible and involves cement that could be sintered. The difference is in physical properties and mechanical profile such as setting time and strength. Only one formulation is chosen which has good resistance. After setting and hardening, the analysis of cement by XRD and FTIR [4] shows the mixture of zinc phosphate salts in which the hopeïte is the main compound and is eventually, responsible for the consolidation and bonding between molecules. In addition, the matrix of hardened cement consists of a small quantity of non-reacted zinc oxide, aluminum phosphate, the trace of water, and phosphate acid that not have participated in the reaction of setting and hardening. The analysis by scanning electron microscopy Figure 3-a and -b of the cement after hardening and sintering at 900° shows the cement which firstly has a consolidated structure with well-connected and twisted crystals figure 3-a, becomes an open and porous structure with interconnected granules. However, some crystals increase in size and become clear and flattened.

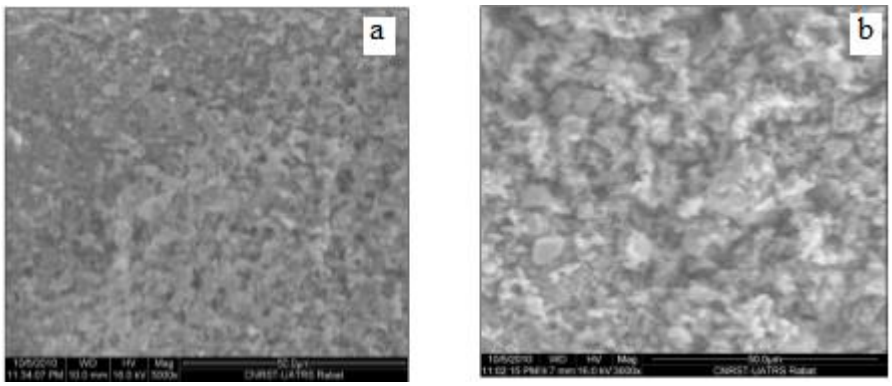

Figure 3: EMS of hardened cement (a) and sintered cement (b), (magnification x3000)

3.2. Microporous Ceramics from the binary system $AlPO_4$, nH2O-ZnO

The X-ray diffraction of the ceramic-based $AlPO_4$,nH_2O - ZnO Figure 4 shows characteristic peaks of zinc oxide and aluminum phosphate AlPO4. However, no phase is involved at the sintering temperature. Only the transformation of the amorphous aluminum phosphate to the mixture of its different crystalline phases such as Berlinite, phosphocristobalite, and phosphotridymite by losing water molecules. The consolidated product at high temperature is due to the entire coverage of zinc oxide particles by the aluminum phosphate. The FTIR analysis Figure 5 shows the characteristic peaks of PO_4 grouping and very weak bands contributed to the hydroxide O-H, which is due to the humidity.

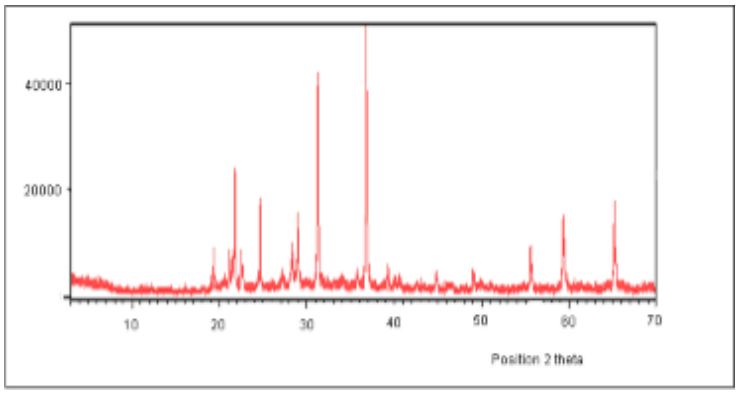

Figure 4: X-ray diffraction diagram of the ceramic-based AlPO4,nH2O – ZnO

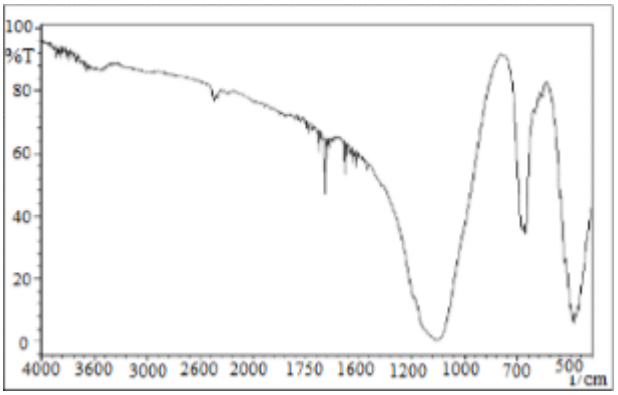

Figure 5: FTIR analysis of the ceramic-based AlPO4,nH2O – ZnO

Figure 6 presents the TGA curves of the binary system ceramic-based $AlPO_4$-ZnO with calcium phosphate additives and SnF_2. The curve (a) is the TGA of the initial powder without deflocculating process at high temperature, and (b)

the powder obtained after deflocculating. In the case of the powder (b) obtained by the previous calcination, there is no loss of the weight. This process facilitates the formation of ceramics and avoids the shrinkage phenomenon during the sintering process. Besides, the previous deflocculating and grinding operations provide homogeneous and perfect powder, which promotes easily the formation of liquid and decreases the temperature of sintering. Furthermore, the temperature of sintering can be decreased with a vid gas furnace.

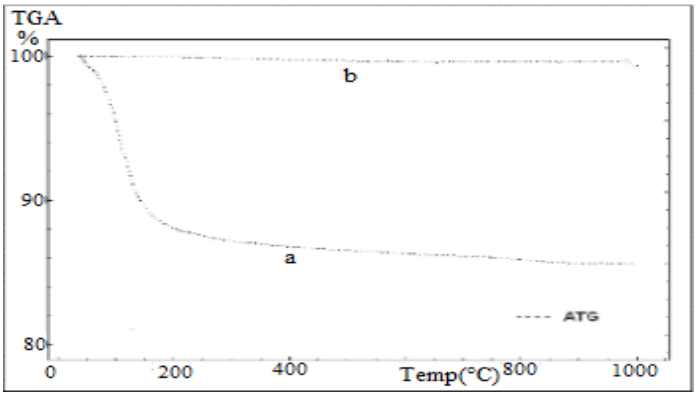

Figure 6: TGA curves of hardened cement

The presence of glycerin traces in the powder during the pressing process favors the good connection of particles and eliminates the intergranular pores. The ultimate contact between grains promotes excellent compaction of the powder. Besides, the sintering of the powders mixed to glycerin allows the creation of pores inside the ceramics Figure 7. This figure shows a bad structure of the created pores which limits the use of this product as a microporous ceramic.

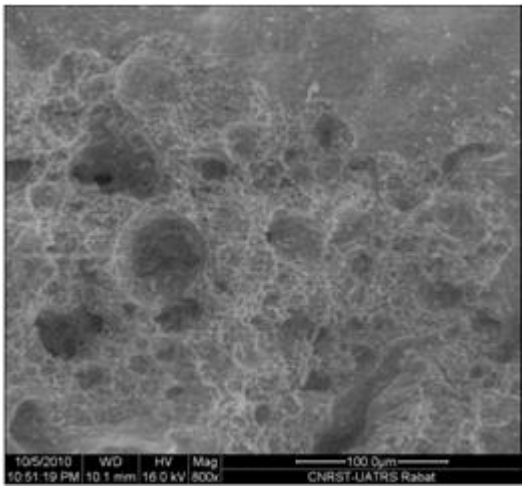

Figure 7: SEM of microporous ceramic shows porosity created by glycerin (x 3000)

To improve the biological properties of the ceramic, we have added a mixture of calcium phosphate compounds such **as** the β tricalcium phosphate and the hydroxyapatite. As a porogenic additive, we have added SnF_2 to create microporosity.

The fact that SnF_2 has a very low melting point around 213° C, its introduction inside powders allows the fluidization of the matrix at this temperature. Besides, the formation of the first liquid drops at this temperature allows the grains of the powder to fill the intergranular spaces. However, this fluoride has a boiling point around 850 ° C which promotes the porous structure of ceramics at temperatures slightly above 850 ° C.

The analysis of SEM images of ceramics Figure 8-a and -b shows a better porous structure which is due to the departure of the fluoride molecules (SnF_2) at temperatures around 850 ° C. the figure shows also that the sintering is carried out, largely, by the cohesion of the particles.

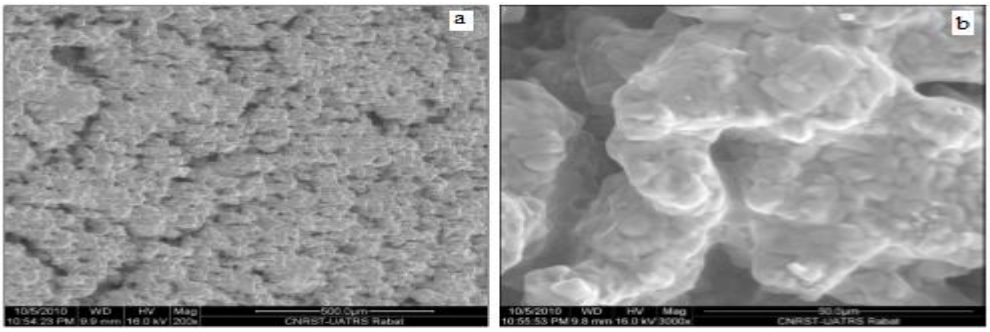

Figure 8: SEM of ceramic shows the homogeneous and connected porosity (a x200, b x3000)

3.3. The measure of the ceramic hardness

Table 1 gathers the Vickers' hardness of some ceramics prepared by the sintering process, on the binary system $AlPO_4$, nH_2O- ZnO at the rate of 55/45 respectively. Then, on ceramics reinforced by adding fillers of silica and zirconia. Furthermore, the porosity is created by adding SnF_2 as an additive. In conclusion, all ceramics have developed good mechanical properties, allows them to be good candidates for the hard tissue replacement and for the preparation of filters and membranes.

Table 1: Vickers's hardness (HV) of some ceramics obtained by the sintering process

Ceramics composition	Vickers's Hardness (HV)
Binary ceramic $AlPO_4$, nH_2O / ZnO at the rate of 55/45 prepared by the sintering process	360
Porous ceramic $AlPO_4$, nH_2O / ZnO at the rate of (55/45) prepared by the sintering process	220
Porous ceramic $AlPO_4$, nH_2O / ZnO at the rate of (55/45) reinforced by adding 10% of SiO_2 and 10% de ZrO_2	280

4. Antimicrobial activity of ceramics

The antimicrobial activity tests are made by screening test on microporous ceramics against selected microorganisms. We have prepared ceramics by both methods such as the chemically bonded ceramic and the sintering process. We have added a mixture of NaF, SnF_2, and CaF_2 as an active ingredient to the row meal of the powder at the rate of 5%. Besides, we have tested eight different microbial strains regrouped into two categories. Tow nutritional lactic bacteria such as *Lactobacillus Plantarum* SG100 and *Lactobacillus pentosus* S41, have been isolated from green olives in natural fermentation. The other six strains with medical interest are isolated from chicken meat such as *E. coli*, *Salmonella sp*, *Klebsiella pneumonia*, *Pseudomonas sp*, *Staphylococcus aureus*, and *Proteus sp*. All tests were performed, in Petri dishes, on sterilized blocs of ceramics under disk forms with a 10 mm diameter (figure 8). The culture medium used for lactic acid bacteria is de Man Rogosa and Sharpe (MRS) while for the other bacteria the Mueller Hinton culture medium is chosen. The agar culture media were inoculated previously by flooding with overnight microbial cultures of target strains, prepared on the same culture medium used previously for the test. Then,

the sterilized ceramic blocs were deposited on the inoculated culture media. The incubation was conducted for 48 hours at 30°C for lactic acid bacteria and 48 hours at 37°C for the others. The results are carried out by measuring the diameter (cm) of the inhibition zone figure 9 developed around ceramic disks (figure 8). Eventually, we have tested a ceramic without adding fluoride compounds.

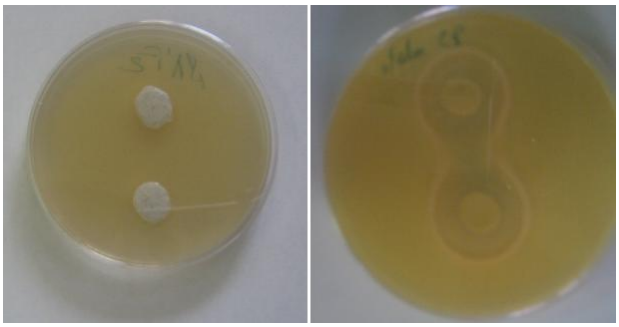

Figure 9: Inhibition zone developed by ceramics

In Figure 9, all microporous ceramics, including ceramic without additives, have developed large inhibition zones, indicating their significant inhibitory effect on all tested bacterial strains. Besides, the activity changes from one strain to another according to bacterial Grams Table 2. In addition, the microporous ceramics have shown great effectiveness against gram-positive bacteria which have a nutritional interest "lactic acid bacteria" in comparison to the gram-negative bacteria that are with hygienic interest. The inhibition zone developed by ceramic without fluoride additives is due to the presence of the Zinc that has a good inhibitory effect against microorganisms. Furthermore, the inhibition zone is increased by adding fluoride compounds. Eventually, the study of the antimicrobial activity of the various ceramics showed an excellent antimicrobial activity on the studied bacteria.

Table 2: Inhibition zones (cm) of ceramics

Bacteria	**Ceramic without fluor additives (cm)**	**Ceramic with chemically bonded method +fluor (cm)**	**Ceramic by sintering method (cm)+ fluor**
Escherichia coli	1.2	2.1	1.8
Salmonella sp	1.8	2.5	2.6
Klebsiella pneumoniae	1.0	1.9	1.8
Pseudomonas sp	1.2	2.0	1.8
Staphylococcus aureus	2.3	3.4	3.0
Proteus sp	1.0	2.0	1.8
Lactobacillus Plantarum	2.0	2.9	2.4
Lactobacillus pentosus S41	1.6	2.9	2.4

5. Conclusion

In this work, we have prepared microporous ceramics using a chemically bonded ceramic and sintering process. We have obtained ceramics with interconnected pores, which can be good alternatives to spongy bones in case of defection. Besides, all ceramics developed a good mechanical profile especially when they are combined with fillers of silica and zirconia. Finally, we have conducted an antimicrobial test of the various ceramics on several microorganisms. The test showed excellent antimicrobial activity.

References

[1] S. Y. Jeong, A. S. Wagh, Phosphate Ceramics: Cementing the Gap between Ceramics, Cements, and Polymers, (2002), https://www.researchgate.net/publication/228391636.

[2] A. S. Wagh, Chemically bonded phosphate ceramics, twenty-first-century materials with diverse application, *Elsevier* (2004), vii Edition.

[3] M. Jabri, E. Mejdoubi, M. El Gadi, B. Hammouti, Synthesis and optimization of a new calcium phosphate ceramic using a design of experiments, *Res Chem Intermed*, DOI 10.1007/s11164-012-0587-9.

[4] M. Jabri, E. Mejdoubi, M. El Gadi, B. Hammouti, Hydratation mechanism of a zinc phosphate cement and development of its mechanical profile, *Res Chem Intermed*, DOI 10.1007/s11164-012-0824-2.

[5] M. Jabri, E. Mejdoubi, M. El Gadi, B. Hammouti, Optimisation of hardness and setting time of dental zinc phosphate cement using a design of experiments, *Arabian Journal of Chemistry* (2012)5, 347-351.

[6] K. Burugaa, H. Songb , J. Shangc , N. Boland, J. T. Kalathia , K. H. Kimd, A review on functional polymer-clay based nanocomposite membranes for treatment of water, *Journal of Hazardous Materials*, 379 (2019) 120584 7. CPK. Wadhwani, Implant luting cements, *New York, Springer*, (2015) 47-82.

[8] CPK Wadhwani, ER. Schwedhelm, DY. Tarica, KH. Chung, Cementation in Dental Implantology - An Evidence-Based Guide, *New York, Springer*, (2015), 47-82.

[9] D. Flanagan, Zinc phosphate as a definitive cement for implant-supported crowns and fixed dentures, *Clinical, Cosmetic and Investigational Dentistry*, (2017) 9, 93-97.

[10] E. Sahin, Synthesis and characterization of calcium phosphate cement based macroporous scaffolds, A Thesis Submitted to *in Chemical Engineering, PhD Thesis*, July, (2012).

[11] A. J. Ambard, L. Mueninghoff, Calcium phosphate cement: review of mechanical and biological properties, *J. Prosthodont*, 15 (5), 321-8.

[10] Jabri, M., Mejdoubi, E., Elgadi, M., Ghabbour, N., Asehraou, A.,
Hammouti, B., 2010. Fluoride release by zinc phosphate cement:
kinetic and antibacteriological studies. J. Mater. Environ. Sci. 1, 52.

Jabri, M., Mejdoubi, E., Elgadi, M., Ghabbour, N., Asehraou, A.,
Hammouti, B., 2010. Fluoride release by zinc phosphate cement:
kinetic and antibacteriological studies. J. Mater. Environ. Sci. 1, 52.
[10] Jabri, M., Mejdoubi, E., Elgadi, M., Ghabbour, N., Asehraou, A.,
Hammouti, B., 2010. Fluoride release by zinc phosphate cement:
kinetic and antibacteriological studies. J. Mater. Environ. Sci. 1, 52.

[12] M. Jabri, E. Mejdoubi, M. Elgadi, N. Ghabbour, A. Asehraou, B. Hammouti, Fluoride release by zinc phosphate cement: Kinetic and antibacteriological studies. *J. Mater. Environ. Sci. 1 (1) (2010) 52-57.*