

New hybrid agarose/Cu-Bioglass[®] biomaterials for antibacterial coatings

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(Received 12 December 2016 • accepted 14 May 2017)

Abstract—Agarose hydrogels, combined with 45S5 Bioglass[®], were elaborated to serve as copper delivery systems. Our aim was to study the antibacterial properties of these hydrogels. The results show that the amount of glass does not influence the stiffness properties, but it improves the hydrophilicity and the swelling profile of agarose hydrogel. Two bacterial strains, *Bacillus* sp. 4J6 and *Pseudomonas aeruginosa* sp. PAO1, were chosen. Their retention on the substrates was analyzed by confocal laser scanning microscopy. The mechanical characteristics and the release of copper have an effect on the bacterial adhesion and the biofilm formation. All the obtained results indicate that these hydrogels could be adapted to a potential application to the antibacterial coatings.

Keywords: Hydrogels, Agarose, Bioglass, Copper Delivery, Anti-adhesion Activity, Biofilm

INTRODUCTION

With an increase in the average age of the population, the problem of diseased tissues or biological malfunctions is more and more frequent. Synthetic or natural biomaterials are used to ensure the care of patients. Indeed, researcher work on the development of bio-materials for the health field and alternative approaches has already been proposed [1]. Numerous biomaterials are synthesized with different biodegradable polymers--alginate [2], collagen, chitosan [3]--and other synthetic polymers such as polyvinyl alcohol [4]. They are used not only as implants in particular to repair livers [5], nerves [6] bones [7], but also to elaborate medical supplies such as catheter, syringes or endotracheal tubes.

These materials, in contact with biological substances and matters, can bring viruses or bacterial infections. The predominant form of life for the majority of microorganisms in any hydrated biologic system is a cooperative community termed "biofilm" [8]. In the medical field, the use of different materials in contact with patients can favor the formation of these biofilms. Their mode of life conveys a survival advantage to the microorganisms associated with it and, thus, biofilm on materials (urinary catheters for example) results in persistent infections that are resistant to antimicrobial therapy [8].

Hydrogels are highly-hydrated materials obtained from natural or synthetic polymers. Their networks can be held by physical and chemical crosslinks. Hydrogels can be biodegradable and can react to different stimuli such as temperature or pH [9]. There is also a surge of interest in elaborating hydrogel systems based on natural biopolymers [10,11]. Indeed, they are already used as cells or drug delivery systems for their biocompatible and biodegradable prop-

erties [12].

This paper presents the study of agarose/45S5 Bioglass[®] hydrogels doped with copper element. Antimicrobial polymer composites based on copper element have been much less studied than those based on silver, for example. Agarose is a polysaccharide extracted from marine red algae. It reacts to the temperature and forms a thermoreversible gel upon cooling agarose aqueous solution. The biocompatibility of agarose and the conditions of its gelation make agarose suitable for applications in tissue engineering [13,14]. Bioactive glasses are particularly reactive when they are immersed in a simulated body fluid (SBF). These glasses are interesting materials because they can degrade and release different chemical elements: silicon, calcium, phosphorus, silver, copper, zinc [15]. These elements can present interesting biological properties [15] and an antibacterial character. Moreover, according to the chemical composition of the glasses, the kinetic of release of these chemical elements can be adapted to a clinical situation. In this work, we chose copper because it presents antibacterial properties and participates in the formation of new biological tissues [16-19].

Our aim was the development of different hydrogels for applications in copper delivery systems to fight against bacterial adhesion. The paper gives a particular attention to (i) the preparation and the mechanical studies of the hydrogels, (ii) the controlled release of copper, and (iii) the effect of copper on the anti-adhesion character.

MATERIALS AND METHODS

1. Materials

1-1. Agarose and Bioactive Glasses

The agarose powder used to prepare the hydrogels was provided by Sigma-Aldrich. It is an agarose of type I-A. This powder presents a melting point of 88 °C (±1.5 °C), a gel point of 36 °C (±1.5 °C), a moisture inferior to 7% and anion traces (SO₄²⁻) inferior to 0.2%.

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Bioactive glasses, containing different amounts of copper, were elaborated by a melting process from silicon oxide (SiO₂, Aldrich, 99.8%), sodium metasilicate pentahydrate (Na₂SiO₃·5H₂O, Aldrich, ≥95%) calcium metasilicate (CaSiO₃, Aldrich, 99%), sodium metaphosphate (Na₃P₃O₉, Aldrich, ≥95%) and copper oxide (CuO, Aldrich, 99.9%). These powders were weighed and mixed using a planetary mixer. These premixed mixtures were melted in platinum crucibles that were placed in an electric furnace at 1,350 °C for 3 hours. The samples were cast in preheated brass cylindrical molds and annealed at 565 °C for four hours near the glass transition temperature of each glass. Obtained glasses were crushed using a mechanical crusher (Retsch Fisher Block Scientific RM100). The obtained powder was sieved using Retsch Fisher Block Scientific AS200 sieve in order to have a powder presenting a granulometry inferior to 40 μm. Finally, three bioactive glasses, named xCu-45S5 (where x=0, 0.1 and 1 wt%), were prepared.

1-2. Preparation of Agarose/Bioactive Glass Hydrogels

Each hydrogel was elaborated in the ratio 1/1 to obtain final gel concentration of 3 w/v% of agarose and 3 w/v% of xCu-45S5. The ratio was chosen to observe only the effect of the amount of copper introduced in bioactive glasses. The fabrication consisted of a mix of 3 g of agarose powder in 100 mL of deionized water (3 w/v%) and 3 g of glass powder (3 w/v%) in a Pyrex[®] bottle. This bottle was autoclaved during 20 min at 120 °C. The obtained solution was cast in a Petri dish, previously frozen in order to block immediately the glass grains in the agarose matrix, in sterile conditions. Pellets of hydrogels, Fig. 1, were studied directly after their preparation.

2. Methods

2-1. Rheological Methods

2-1-1. Stiffness Measurements

To measure the stiffness of hydrogels, dynamic oscillatory assays were undertaken using a rheometer (Gemini, Bohlin) at room temperature. Compression tests were also performed. Pellets of hydrogels, having 3 mm of thickness and with parallel faces, were analyzed. Strain sweeps for hydrogels were performed at a constant frequency of 1 Hz from 0.01% to 100% to identify the linear viscoelastic region [20]. For each chemical composition, assays were realized three times on three different pellets. In this study, only the influence of bioactive glass amount (and not the copper amount) was observed.

2-1-2. Contact Angle Measurements

To obtain the homogeneity of the surface at the microscopic scale, the technique consists of using a drop with a small volume. The

measurements were undertaken on a Krüss DSA100M. Droplets of 300 pL of ultrapure water were deposited on the surface of the material. A camera equipped with a microscope objective automatically realized obtainments of data. Ten drops of 2 μL of water were deposited on the surface with a microsyringe by automatic moving. The drop image was processed by an image analysis system (Windrop) that calculated the left and the right contact angles from the shape of the drop. Manipulations were performed three times on three different pellets.

2-1-3. Swelling Profile

The swelling ratio of the freeze-dried hydrogels was measured with gravimetric method in PBS (pH=7.4) at 37 °C for 24 hours. PBS was chosen to avoid the bioactivity reaction of the glass grains in the agarose matrix. The swollen hydrogels was removed and immediately weighed with a microbalance after the surface excess of liquid was absorbed with a filter paper. The equilibrium swelling ratio (ESR) of hydrogel was calculated as follows:

$$ESR = \frac{W_s - W_d}{W_d}$$

where W_s is the weight of the swollen hydrogel at 37 °C and W_d is the weight of the freeze-dried hydrogel.

2-2. Copper Concentration Released

The measurements of the concentration of copper were realized in two solutions, SBF and PBS solution. This last solution was chosen to avoid the bioactivity phenomenon which is well observed in the SBF solution. Moreover, like human urine, PBS solution is mostly composed of water and several minerals. Indeed, sodium, chloride and potassium elements are mainly present [3]. Each pellet was immersed in 8 mL of PBS (or SBF) during short and long periods in triplicate for each chemical composition. The samples were maintained under controlled stirring at 60 rpm at body temperature. At the end of soaking time, the pellets were removed and rinsed with the PBS (or SBF) solution. The solutions were conserved in the refrigerator. Inductively coupled plasma emission optical spectroscopy (ICP-OES, Spectro Ciros Vision Ametek) was used to measure the copper concentration of these solutions. Before analyses, each solution was mixed with a nitric acid solution at 1% to eliminate the eventual precipitation phenomenon and impurities. A calibration line was plotted with pure copper solutions at different concentrations.

2-3. Bacterial Strain and Growth Conditions

The terrestrial bacterium *Pseudomonas aeruginosa* PAO1, with a negative Gram, is an ubiquitous environmental bacterium. Its resis-

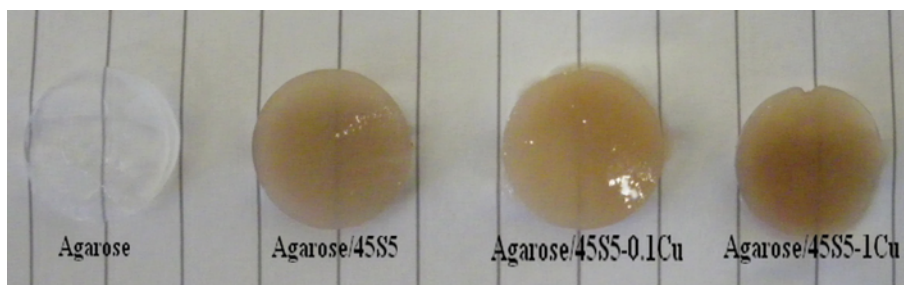


Fig. 1. Photography of studied hydrogels.

tance to antibiotic and disinfectant is a major factor for the prominence of this strain. This bacterium is one of the top three causes of opportunistic human infections. Indeed, this bacterium is involved in cystic fibrosis [21]. Their cultures were established in Luria Bertani LB (NaCl 10 g/L, Tryptone 10 g/L, Yeast extract 5 g/L). The culture was incubated at 37 °C during 24 hours under agitation. The marine bacteria *Bacillus* sp. 4J6 was chosen because it presents a positive Gram. They were isolated from seawater. For the tests, bacteria were cultured twice for 24 hours and grown in Vääänen nine-salt solution. The cultures were incubated 16 hours at 20 °C under agitation.

2-4. Anti-adhesion Activity

To evaluate anti-adhesion property of hydrogels, experiments were realized in Petri dishes. Agarose, agarose/45S5, agarose/45S5-0.1Cu and agarose/45S5-1Cu were tested. The first experiment concerned the *Pseudomonas aeruginosa* PAO1. Pellets of the hydrogels were dropped in the Petri dish with a minimal medium; the simulated body fluid and bacteria *Pseudomonas aeruginosa* PAO1 were inoculated at 10⁶ CFU/mL. Petri dishes were incubated during 3 hours in static condition at 37 °C to allow the bacterial adhesion. After the incubation, hydrogels were rinsed with SBF. A second experiment concerned the marine bacteria 4J6. Previously, the hydrogel pellets were rinsed with the saline medium ASW. 450 µL of bacteria were inoculated. Petri dishes were incubated during 1.5 hours under continuous agitation at 18 °C. Then, hydrogels were rinsed with ASW solution.

Bacteria were stained with syto9[®] green; it is a nucleic acid stain used at 5 µM ($\lambda_{excitation}=488$ nm, $\lambda_{emission}=498$ nm) to be observed by confocal laser scanning microscopy (LSM, Zeiss 710) by using a ×40 oil immersion objective. The overlap percentage is determined with a JAVA program.

2-5. Biofilm Formation Analysis

The biofilm formation experiments were undertaken with *Bacillus* sp. 4J6. Pellets of the hydrogels were immersed in 10 mL of a nutrient medium Zobell[®]. A volume of 600 µL of 4J6 was inoculated. Petri dishes were incubated during 48 hours in static condition at 18 °C. Experiments were realized three times on each hydrogel. Bacteria were visualized by staining with syto9[®] green. Comstat program was used to analyze the biofilm stacks recorded by confocal laser scanning microscopy (LSM, Zeiss 710). Biomass concentrations were also extracted.

RESULTS AND DISCUSSION

1. Rheological Results

1-1. Stiffness Results

It was proved that the stiffness of a material influences the bacterial adhesion. Indeed, bacterial adhesion tends to increase with the enhancement of stiffness of agarose hydrogels [22]. The aim of this analysis was to study the effect of the amount of bioactive glass introduced in the agarose matrix on the stiffness. With a constant

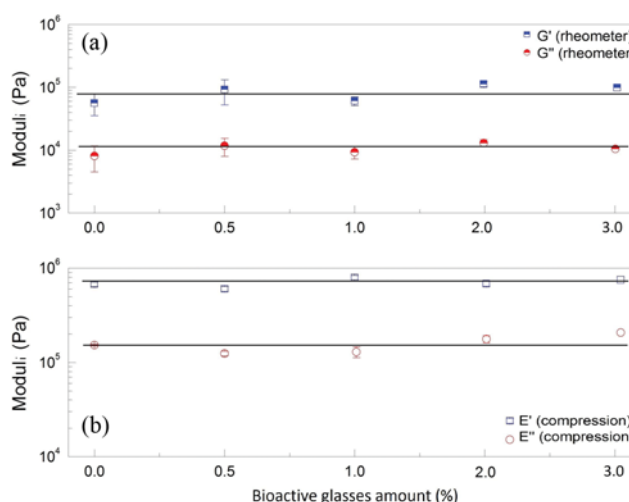


Fig. 2. Storage and loss moduli obtained by rheometry and compression analyses.

concentration of agarose at 3%, different quantities of one single glass, 0, 0.5, 1, 2 and 3%, was added. The storage moduli E' or G', characterizing the elastic behavior of the material; and the loss moduli E'' or G'', characterizing the viscous behavior of the material, were determined and presented in Fig. 2(a) and 2(b).

Analyses show that agarose hydrogel, without 45S5, presents a storage modulus of 56 kPa (± 22 kPa) and a loss modulus of 8.1 kPa (± 3.6 kPa). The hydrogel, with 3% of glass, presents a storage modulus of 78 kPa (± 33 kPa) and a loss modulus of 9.8 kPa (± 3.1 kPa). These values are very close considering the standard deviation.

Compression analyses gave the same trend. The values of E' modulus approximately ranged between 600 and 800 kPa, and that of E'' modulus between 100 and 200 kPa.

So, the amount of bioactive glass, introduced in the agarose matrix, does not seem to have influences on these characteristics. For the following studies in this paper, the agarose and bioactive glass concentrations in hydrogels were maintained at 3 w/v% to allow a greater contribution of the bioactive glass for the following studies.

1-2. Contact Angle Results

Wettability is an important criterion for biomaterials in a biological system. The static contact angles, using water, were measured at the surface of agarose, agarose/45S5, agarose/45S5-0.1Cu and agarose/45S5-1Cu hydrogels. The aim was to evaluate the hydrophilicity of each material. Table 1 presents the obtained results. Using water, the contact angle is worth 42.9° showing a moderate hydrophilic nature for the agarose hydrogel. The contact angle decreases with the introduction of bioactive glass in the agarose matrix: 38.0 ($\pm 0.5^\circ$) with the introduction of 45S5, 37.2° ($\pm 0.6^\circ$) with 45S5-0.1Cu and 37.9° ($\pm 0.6^\circ$) with 45S5-1Cu. The three bioactive glasses seem to have the same effect. The agarose hydrogels with bioactive glass present lower contact angles compared with

Table 1. Contact angle measurements on pure agarose, agarose/0.1-45S5 and agarose/45S5-1Cu hydrogels

	Pure agarose	Agarose + 45S5	Agarose + 45S5-0.1Cu	Agarose + 45S5-1Cu
Contact angle (°)	42.9±0.6	38.0±0.5	37.2±0.6	37.9±0.6

the pure agarose hydrogel. Also, small amounts of copper, introduced in the 45S5 Bioglass®, do not influence the hydrophilicity. Here, the simple presence of the bioactive glass improves the hydrophilicity of the surface of the pure agarose hydrogel. Indeed, with the presence of a large quantity of silica, bioactive glasses are in general very hydroscopic. This characteristic can explain the improvement of the hydrophilicity.

1-3. Swelling Profile Results

Fig. 3 shows the equilibrium swelling ratios of pure agarose, agarose/45S5, agarose/0.1Cu-45S5 and agarose/1Cu-45S5 hydrogels in

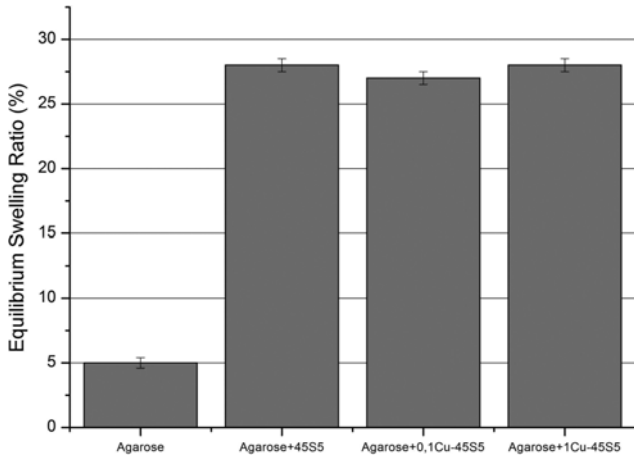


Fig. 3. Equilibrium swelling ratio of hydrogels.

PBS. The swelling ratio increases by a factor 6 with the introduction of a bioactive glass. Indeed, the hygroscopic character of the 45S5 Bioglass® can explain this trend and this gives a high water holding capacity [23-25]. Agarose hydrogel indicates a maximum swelling of 5% and the agarose/bioactive glass hydrogel presents a swelling ratio of about 28% after 24 hours in PBS. The bioactive glass previously studied improves the hydrophilicity of pure agarose hydrogels. It is an important result if the aim is the vectorization of drug or other substances in a liquid medium. Also, the great hydrophilic capacity of agarose/bioactive glass can have a huge influence on the bioactive character, the formation of hydroxyapatite on the glass grain surface and the release of copper element of agarose/xCu-45S5 hydrogels.

2. Copper Delivery

The release of copper was studied in two solutions: SBF and PBS solution. The copper possesses antibacterial properties and its release can provoke an anti-adhesion character.

The results obtained by ICP-OES are shown in Fig. 4. They present the release of copper during the first hours and after longer delays in the two liquid medium. In the SBF, after 2 hours of immersion, agarose/0.1Cu-45S5 and agarose/1Cu-45S5 released respectively 0.11 ppm and 0.20 ppm. From 4 hours of immersion, a significant difference was observed. Indeed, the concentration of copper delivered by agarose/1Cu-45S5 is 5 times greater than that released by agarose/0.1Cu-45S5, respectively, 0.86 ppm and 0.16 ppm. After 4 hours of immersion, agarose/0.1Cu-45S5 tends to stabilize its copper release with a limit of 0.20 ppm up to 40 days of immersion.

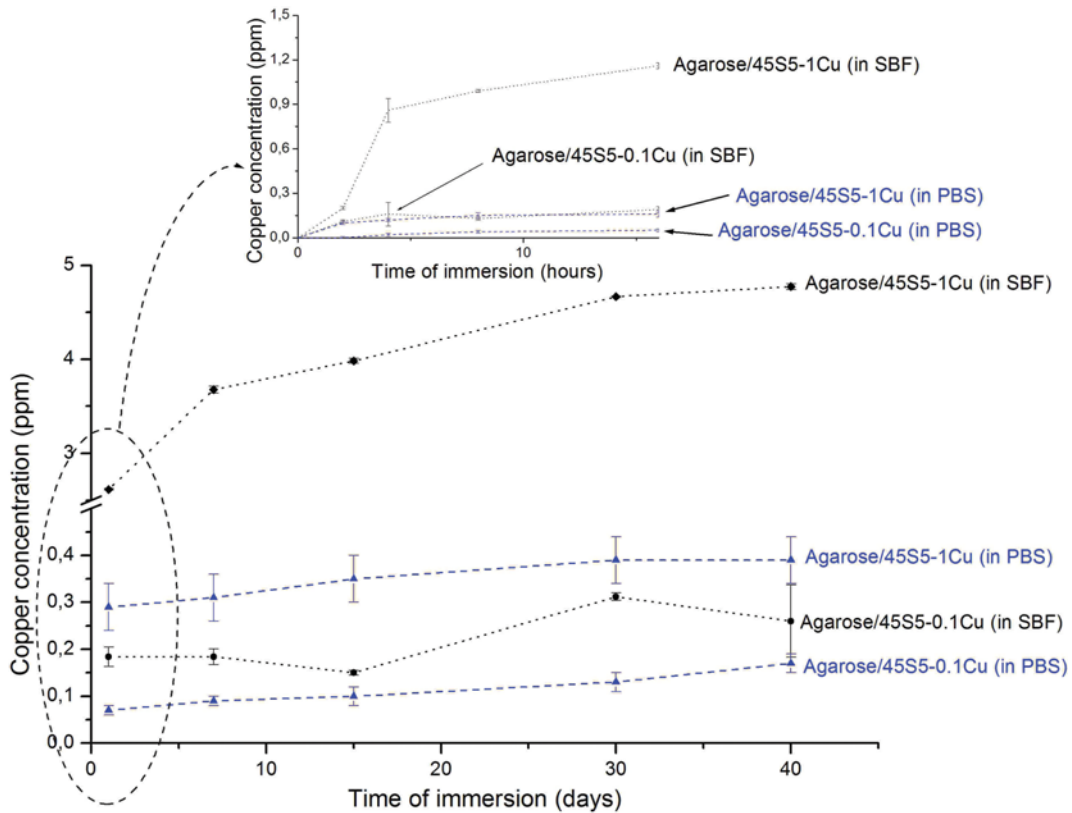


Fig. 4. Copper concentration in SBF after immersion of the hydrogels at different periods.

On the contrary, agarose/1Cu-45S5 continues to releasing up to 40 days immersion to reach a copper concentration of 4.80 ppm.

In the PBS solution, up to 16 hours of immersion, agarose/0.1Cu-45S5 and agarose/1Cu-45S5 release, respectively, 0.05 and 0.16 ppm. After long time, the copper concentration in the liquid medium reaches 0.17 ppm for agarose/0.1Cu-45S5 and 0.39 for agarose/1Cu-45S5 after 40 days of immersion.

These data are important for the anti-adhesion activity study because bacteria were incubated during 1.5 hours for *Bacillus* sp. 4J6 and 3 hours for *Pseudomonas aeruginosa* PAO1 in contact with hydrogels.

Thus, the agarose matrix does not influence the release of copper in the liquid medium. The chemical composition of bioactive glasses and their kinetics of bioactivity are responsible for the copper concentration released. The nature of the liquid medium has an influence on the reactivity of the glasses introduced into the agarose matrix. According to the liquid medium, we can direct the use of these hybrid biomaterials. Also, the obtained copper concentrations in the SBF deserve to be discussed. Indeed, the recommended dietary allowance (RDA) for adult men and women is 900 µg/day [26]. The average intake of copper from food in the United States is approximately 1.0 to 1.6 mg/day for adult men and women. The tolerable upper intake level (UL) for adults is 10,000 µg/day (10 mg/day). In addition, long-term exposure to a copper concentration of 1.3 ppm (e.g. water contamination) is known to cause liver or kidney damage [27]. The use of agarose hydrogels with Cu-Bioglass®, as they are, unusable for introduction into the human body due to the high copper concentration released in the simulated body fluid. However, their use as antibacterial coatings (e.g., catheter) can be interesting and considered.

3. Anti-adhesion Activity

Pseudomonas aeruginosa PAO1 and *Bacillus* sp. 4J6 overlap percentages on the different hydrogels are presented Table 2 and Fig. 5. For PAO1, on the agarose hydrogel witness, 57.9% (± 6.5) of bacteria adhere to pure agarose hydrogel. On the agarose/45S5, 13.7%

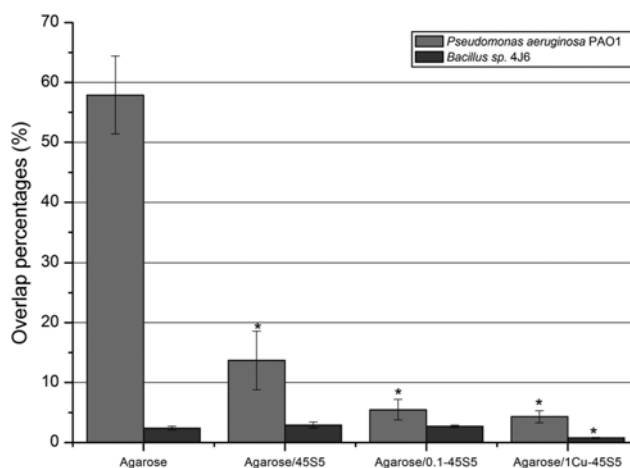


Fig. 5. *Pseudomonas aeruginosa* PAO1 and *Bacillus* sp. 4J6 overlap percentage determined with a JAVA program on four agarose surfaces (ANOVA. * $p < 0.01$).

(± 4.9) of bacteria adhere. This rate decreases at 5.47% (± 1.7) for agarose/0.1Cu-45S5 and at 4.3% (± 1.0) for agarose/45S5-1Cu. These values show that the addition of the glass in the support allows an inhibition of the bacterial adhesion by a factor of 4. The addition of copper was also evaluated; the copper addition induces an inhibition of the bacterial adhesion by a factor of 11 at a copper concentration of 0.1%. For a copper concentration at 1.0%, the bacterial adhesion decreases by a factor of 13.

For 4J6, on the agarose hydrogel witness, 2.4% (± 0.31) of bacteria adhere to pure agarose hydrogel. On the agarose/45S5, 2.90% (± 0.50) of bacteria adhere. This rate decreases at 2.70% (± 0.19) for agarose/0.1Cu-45S5 and at 0.77% (± 0.08) for agarose/45S5-1Cu. In this case, the introduction of 1 wt% of copper in 45S5 decreases by a factor 4 the bacterial adhesion. The addition of 45S5 tends to improve the bacterial proliferation. The presence of 0.1% of copper in 45S5 provokes a decrease of a factor 1.1 of the overlap per-

Table 2. Overlap percentages of PAO1 and 4J6 adhesion on hydrogels surfaces

	Agarose	Agarose/45S5	Agarose/45S5-0.1Cu	Agarose/45S5-1Cu
Overlap percentage PAO1	57.9 \pm 6.5	13.7 \pm 4.9	5.47 \pm 1.70	4.3 \pm 1.0
Overlap percentage 4J6	2.4 \pm 0.3	2.9 \pm 0.5	2.70 \pm 0.19	0.77 \pm 0.08
ANOVA	-	$p < .01$	$p < .01$	$p < .01$

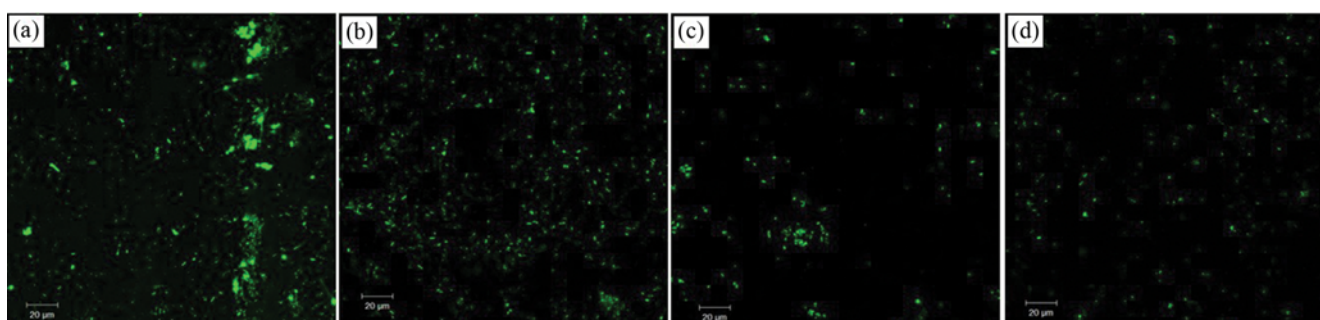


Fig. 6. Confocal laser scanning microscopy of PAO1 adhesion after 3 hours on four surfaces ((a) agarose hydrogel. (b) agarose/45S5. (c) agarose/45S5-0.1Cu and (d) agarose/45S5-1.0Cu).

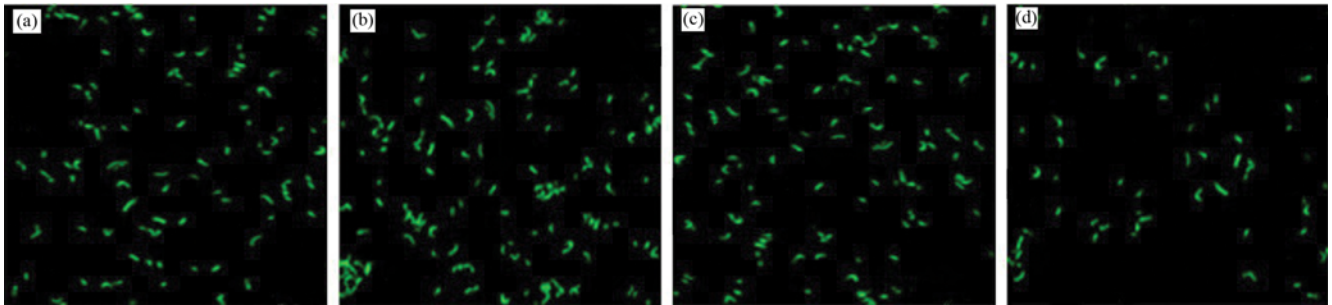


Fig. 7. Confocal laser scanning microscopy of 4J6 adhesion after 1.5 hours on four surfaces ((a) agarose hydrogel. (b) agarose/45S5. (c) agarose/45S5-0.1Cu and (d) agarose/45S5-1.0Cu).

centage. Thus, the increase of copper induces a variation of the surface activity. But the anti-adhesion property is improved with the addition of copper. The addition of glass in the agarose matrix shows a slow decrease of the 4J6 adhesion. But the presence of glass allows a high inhibition of the adhesion of PAO1. These results are in agreement with the obtained results by ICP-OES.

Fig. 6 presents the observations of PAO1 adhesion obtained by confocal laser scanning microscopy. A progressive decrease of the bacterial adhesion is clearly visible for PAO1. It is possible that the change of the hydrophilicity of the surface participates at the inhibition of the bacterial adhesion. Indeed, the hydrophilicity allows a better release of copper. Between 2 and 4 hours of immersion in the SBE, the concentration of copper delivered by agarose/1Cu-45S5 is five-times greater than that released by agarose/0.1Cu-45S5 involving a decrease of the bacterial adhesion.

For 4J6, in Fig. 7, the decrease of the bacterial adhesion is less visible. Except on the image showing the agarose/1Cu-45S5 hydrogel where the bacteria density is clearly smaller. Here, the use of the PBS solution does not allow a total reactivity of the particles of bioactive glass. The release of copper is lesser and its effect on the bacterial adhesion is less visible. So, a study of the biofilm formation was undertaken with 4J6. Thus, the introduction of copper into the glass matrix allows an important anti-adhesion character of agarose hydrogels when the bioactive glass can highly interact with the liquid medium, but the effects are less visible when the bioactive glass cannot totally react with its medium.

4. Biofilm Formation

Bacillus sp. 4J6 biofilm and the biomass concentration are, respectively, presented in Fig. 8 and Table 3. On the agarose hydrogel witness, 4J6 biofilm is well distributed over the entire surface of

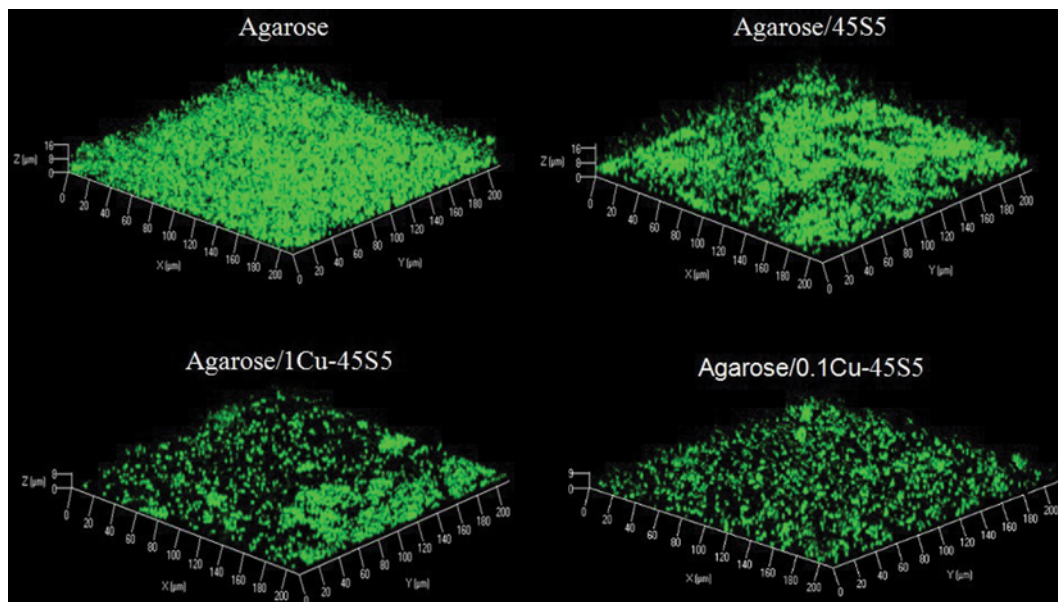


Fig. 8. Confocal laser scanning microscopy of biofilm formation by 4J6 on four hydrogels.

Table 3. Obtained biovolumes on the hydrogels using COMSTAT analyses

Hydrogels	Agarose	Agarose + 45S5	Agarose + 0.1Cu-45S5	Agarose + 1Cu-45S5
Biovolume ($\mu\text{m}^3/\mu\text{m}^2$)	0.73	1.05	0.65	0.32

the pellet. It shows a homogeneous surface with a biovolume of $0.73 \mu\text{m}^3/\mu\text{m}^2$. With the adding of 45S5 into the agarose matrix, as for the anti-adhesion activity, the biovolume increases ($1.05 \mu\text{m}^3/\mu\text{m}^2$), but the biofilm is less homogeneous. By introducing 0.1 wt% of copper in the glass matrix, a decrease of the biovolume is observed ($0.65 \mu\text{m}^3/\mu\text{m}^2$). The hydrogel surface containing 0.1Cu-45S5 shows a non-homogeneous surface with some areas presenting a high concentration of biofilm. The biovolume of agarose/1Cu-45S5 has been halved compared to the agarose/0.1Cu-45S45 hydrogel. Indeed, the agarose/1Cu-45S5 hydrogel presents a biovolume of $0.32 \mu\text{m}^3/\mu\text{m}^2$. Thus, the effect of hydrogels on the formation of biofilm by 4J6 is clearly visible. In a nutrient medium and in contact with 4J6 bacteria after 48 hours, bioactive glasses doped with copper delay the biofilm formation.

CONCLUSION

The purpose of this paper was to form biomaterials able to prevent the bacterial adhesion for an eventual application for antibacterial coatings. The mechanical studies showed that the amount of the same bioactive glass introduced into the agarose matrix does not change the values of the storage and loss moduli, unlike agarose concentration. The adding of bioactive glass powder in the agarose matrix decreases the contact angle, improving the hydrophilicity and the swelling profile.

Hydrogels, immersed in a simulated body fluid, showed a great interaction, increasing the copper concentration in the liquid medium, unlike the immersion in PBS solution. The anti-adhesion character was investigated on *Pseudomonas aeruginosa* PAO1 in SBF and *Bacillus* sp. 4J6 in PBS. The percentage of adhesion decreased up to a factor 13 for PAO1 and up to a factor 3.5 for 4J6 by adding of 1% of copper in the 45S5 bioactive glass. Moreover, the introduction of Cu-bioactive glass in the agarose matrix slowed the formation of biofilm at the surface of the biomaterial. Finally, the results illustrate that the released copper improves the anti-adhesion character and inhibits the formation of biofilm. In SBF a toxic concentration in copper was measured. In PBS, the copper was less released, the anti-adhesion character was less marked and copper concentrations in the liquid medium were acceptable.

All these results are encouraging to improve the use of these hydrogels as implantable biomaterials. However, we consider these hydrogels as a future candidate for antibacterial coatings on medical tools (urinary catheter...).

ACKNOWLEDGEMENTS

The authors acknowledge T. Le Norcy, I. Linossier and K. Réhel for their great support. The authors also thank N. Kerisit for the proof reading of this paper. E.Wers warmly thanks his co-author B. Lefeuvre for his implication in all the experiments and for his precious help. E.Wers would like to assure him of his friendship and sincere respect.

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