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Preparation of a Mixture of Bioglass/ Sodium Alginate Doped with Silver Incorporated with Graphene Oxide.

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OUR RESEARCH'S significance is to synthesize bioactive materials Bioglass/ Sodium alginate (BG/SA) and mechanically improved them with Graphene oxide. In addition to the improvement of anti-microbial by different ratios of silver nanoparticles (Ag NPs).Using the sol-gel method, a glass of chemical composition (٦٠SiO٣٥-٢CaO٥-P٢O٥) was prepared, and sodium alginate polymer was added at a ratio of ١:١ to all samples named as (BG, BG/SA, Control, ٪٣ ,٪٢ ,٪١, and ٪٤). The proportion of graphene oxide was also fixed at ٪٠,٥ of the bio-glass. The samples were examined by UV spectroscopy.The bioactivity of all samples was determined after the investigation in thesimulated body fluid (SBF). Both the pH and the concentration ofsome ions such as calcium and phosphorus were measured.Theresults showed a layer of hydroxyapatite (HA) was deposited onthe surface of all samples.

Keywords: Silver nanoparticles, Graphene oxide, Sodium alginate, SBF, Bioactivity

Introduction

The essential features of biomaterial implants are their biodegradability corresponding to the stage of reconstruction. Moreover, appropriate mechanical properties similar to the bone properties in the area of reconstruction, as well as easy accessibility. In addition to the well-known need for biomaterials to improve the course of therapeutic procedures. However, it is still difficult to create a biomaterial that exhibits biological activity. When a biomaterial facilitates the therapy and regeneration of injured tissue in addition to serving as a matrix for cells and a filler for a defective bone[1]. Natural chemical compounds, such as polymers, have important biological properties, such as gradients in biodegradable and transformation into a series of hydrocarbons that the body can digest and benefit from. Therefore, polymers such as sodium alginate are widely used in various biomedical applications[2].The sodium alginates can be combined with bioactiveglass, ceramic, and carbon-based materials like graphene oxide (GO) and some types of polymers to improve or enhancethe mechanical properties [3].The bioactive glass (BG) and sodium alginate (SA) have good degradability and biocompatibility[4]. Some organisms have been a major bacterial pathogen causative agent of infection-induced osteomyelitis[5], [6].Silver nanoparticles (NPs) revealed wide-spectrum antibacterial properties contra both Gram-positive and Gram-negative bacteria[7]. In this study,we will try to synthesize a multi-functional (biodegradable, bioactivity, good mechanically and antimicrobial) material. So,composites of BG/SA/GO (1:1:0.5, wt.) with Ag- substituted were prepared bythe sol-gel method. Where a chemical composition of glass is (60SiO2-35CaO-5P2O5) doped with 1%, 2%, 3%, and 4% of (Ag) asantimicrobial agent. The addition of GO is to improve the mechanical properties of the prepared composites. The obtained composites were characterized before and after the in-vitro test by the UV-vis technique. The bioactivity of all samples was studied by the in-vitro test (SBF) for 33 days.

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Materials and methods

Preparation of (BG/Ag) composite.

A solution of bioactive glass was prepared with the chemical composition of 60%SiO2, 5% P2O5, and 35% CaO; were purchased for which tetraethyl orthosilicate (TEOS; Si (OC2H5)4, were purchasedfrom Sigma-Aldrich andtriethyl phosphate (TEP; C6H15O4P, were purchasedfrom Alfa-Aesar, and distilled water in ethanol (Merck), The source of the (Ca(NO3)2.4H2O) that was bought from (Panreac PRS), were till the hydrolysis process was refined and polycondensation, adhering to the sol-gel method's suggested procedure as illustrated in Figure 1[8], all samples were calcined at 600 c to eliminate the nitrate.The chemical composition of silver nitrate (AgNO3),was purchased from Acros Organic Ltd.Sodium alginate (SA):C6H9NaO7, polymerizationdegree is: 476, was bought from SDFCL.

All samples named areillustrated in a Table1the flowchart for BG/GO/SA/Ag composites preparation stepsisshown in Figure 2.

.xedni seman selpmas .١ ELBAT

selpmaS	(GB) ssalgoiB	(AS) etaniglA muidoS	(OG) edixOenehparG	(gA) revliS
GB	٪٠٠١	-	-	-
AS/GB	٪٠٥	٪٠٥	-	-
lortnoC	٪٥,٩٤	٪٠٥	٪٥,٠	-
٪١	٪٥,٨٤	٪٠٥	٪٥,٠	٪١
٪٢	٪٥,٧٤	٪٠٥	٪٥,٠	٪٢
٪٣	٪٥,٦٤	٪٠٥	٪٥,٠	٪٣

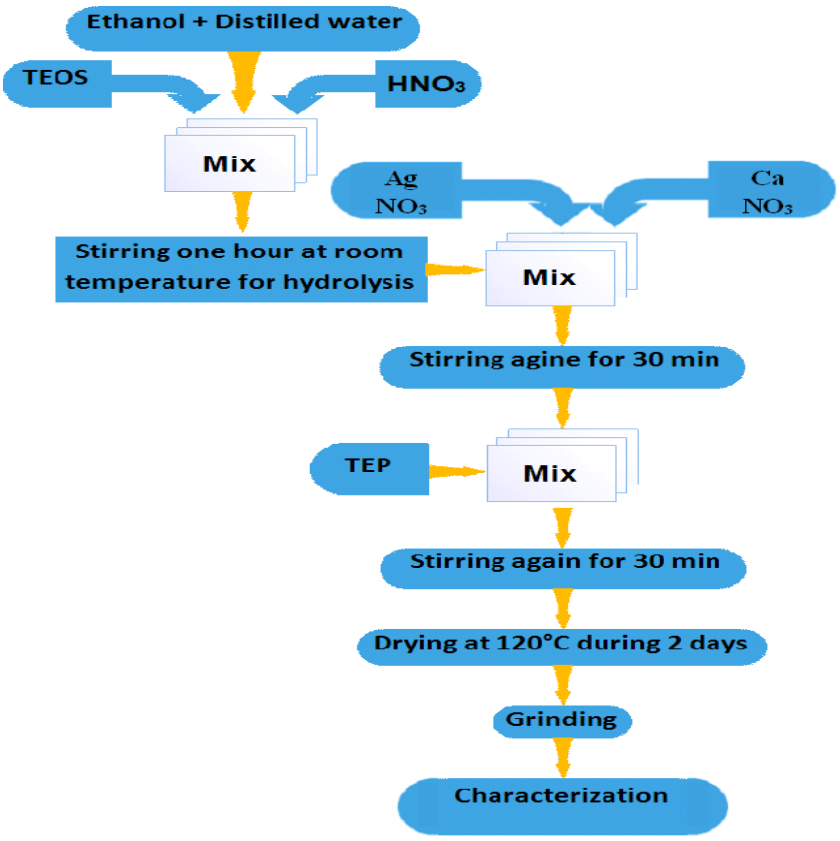


Fig.1. Flowchart for Bioglass/silver composite preparation steps.

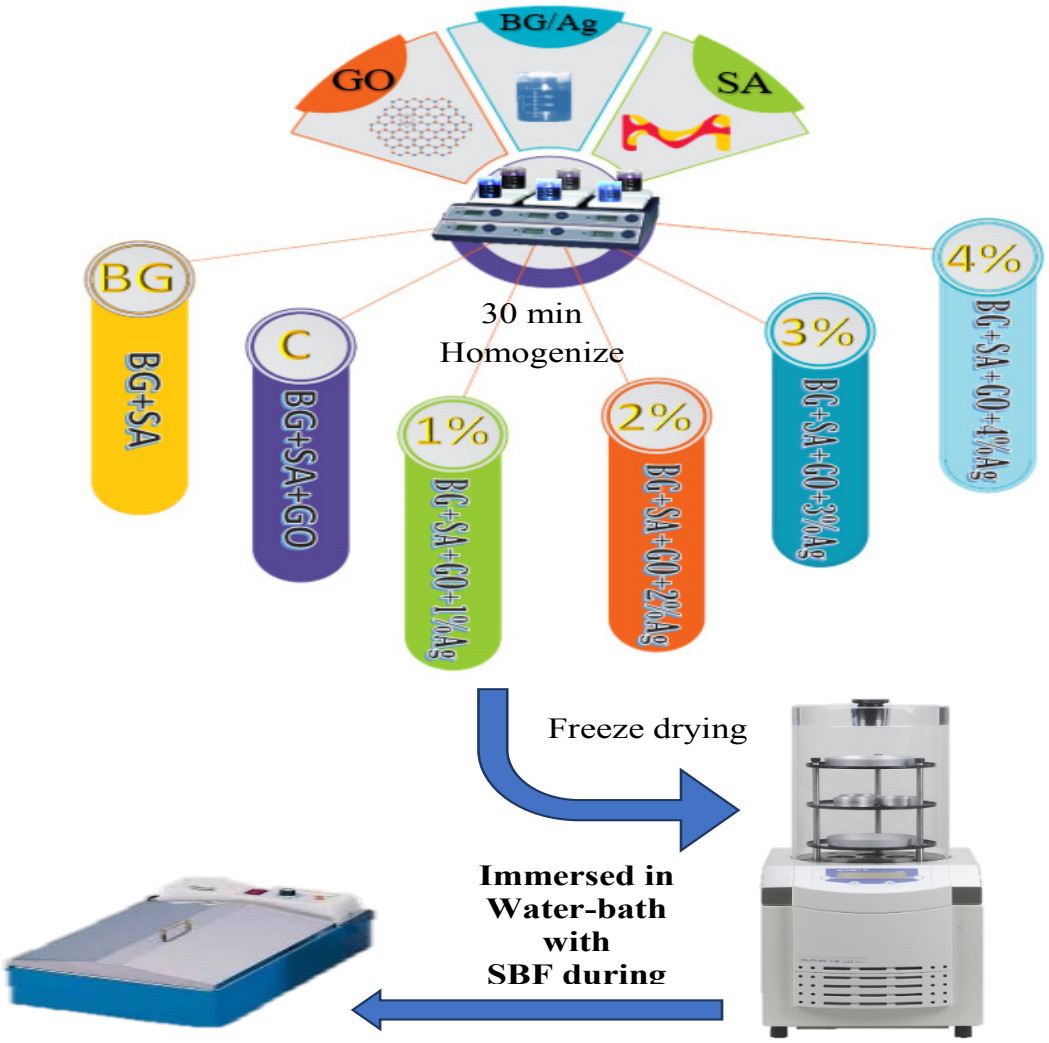


Fig. 2. Flowchart for BG/GO/SA/Ag composites preparation steps.

٪٤	٪٥,٥٤	٪٠٥	٪٥,٠	٪٤

.selpmas gA/ GB fo noitisopmoc lacimehC .٢ ELBAT

	AS/GB	lortnoC	gA٪١	gA٪٢	gA٪٣	gA٪٤
GB	٪٠٠١	٪٠٠١	٪٩٩	٪٨٩	٪٧٩	٪٦٩

Graphene oxide mixing with BG and Sodium alginate samples.

The chemical composition of the bioactive glasses along with a different percentage of silver with the constant ratio of graphene oxide (0.5%) and sodium alginate (1:1) wt.% is presented in Table 2.

Characterization techniques:

Following the addition of a 2 ml sample, each tube was evaluated in SBF at 1, 2, 4, 8, 16, 21, and 33 days from the start of the immersion. And stored frozen until analyzed for determining the concentration of Ca and Pby using UV-Visible Spectrophotometer (Jenway 6305).

The pH value of all samples in the SBF solution was measured by using HM PH-200pH meter.

In-vitro bioactive analysis

Standard Operating Procedure for (SBF) preparation.

Kokubo's[9], [10]the compensation is provided by a metastable solution of calcium and phosphate ions, which are already supersaturated with regard to apatite. Simulated body fluid (SBF). As a result, (SBF) is ready as follows:

√ The pH meter was a calibrated using buffer solution having pH 4 and 7.

√ The temperature is maintained at 37.4˚C throughout the process.

√ The correct amounts of the aforementioned compounds were dissolved in 950 ml of distilled water in the correct sequence (as given in Table 3) to create the SBF solution.

√ Ion concentrations of SBF compared to those of human blood plasma in Figure23after adding each of the chemicals in (Table 3) one at a time until order number (8), after each reagent had completely dissolved.

Table 3 Reagents for preparation of simulated body fluid (SBF).

√ Addition of reagent (9) should be little by little with less than 1gm, to avoid a local increase in pH of the solution.

√ After additions of order number (9), the temperature of the solution is checked, and pHis measured while the temperature is at 37.4˚C. At this point, the pH of the solution should be approximately equal (7.5). HCl solution with a pipette was titrated to adjust the pH at 7.4.

gA	-	-	٪١	٪٢	٪٣	٪٤

.(FBS) diufl ydob detalumis fo noitaraperp rof stnegaeR ٣ ELBAT

redrO	tnegaeR	(L/g) tnuomA	thgiew alumroF
١	sirT	٧٥٠,٦	٦٥٣١,١٢١
٢	lCH	(lm) ٩٣	---
٣	lCaN	٦٠٠,٨	٧٧٢٤٤,٨٥
٤	$_{٣}$OCHaN	٢٥٣,٠	٧٨٦٠٠,٤٨
٥	lCK	٣٢٢,٠	١٥٥,٤٧
٦	$O_{٧}H^{٣},_{٤}OPH_{٧}K$	٨٢٢,٠	٢٢٢,٨٢٢
٧	$_{٢}$lCaC	٧٧٢,٠	٦٨٩,٠١١

√ After adjustment of pH, 50 ml of distilled water was added to the solution, adjusting the total volume of the solution to 1000 ml.

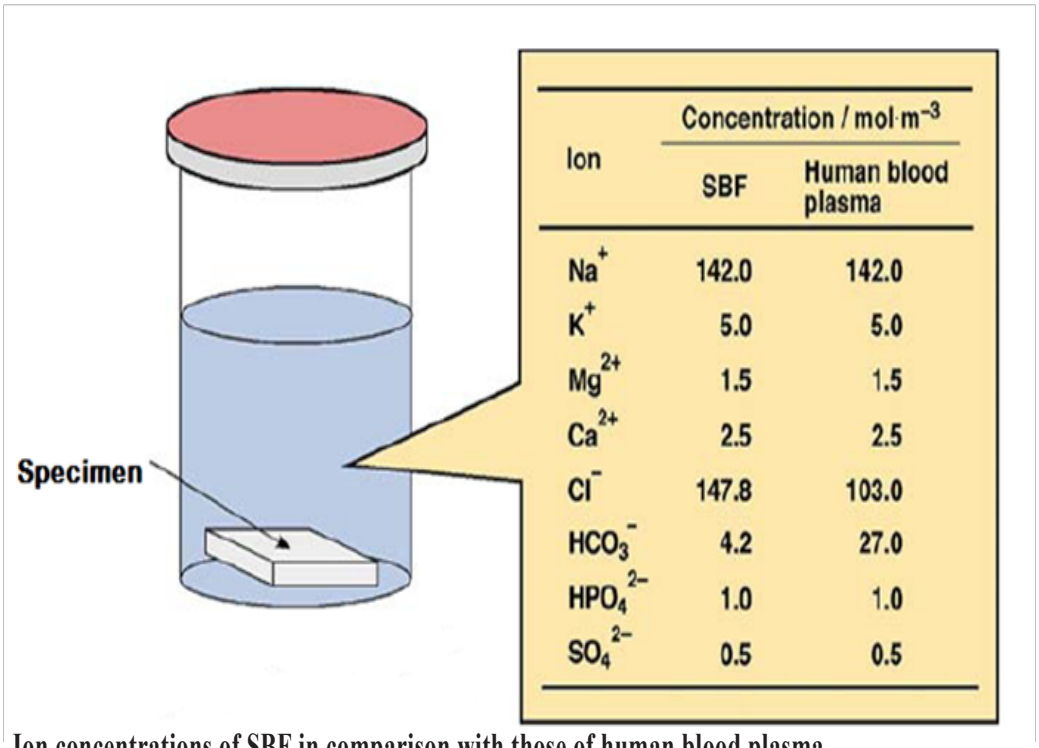
√ Rinse a polyethylene (or polystyrene) bottle of 1000 ml with a bit of the prepared solution (SBF), at least three times. Transfer the solution from the flask to the polyethylene bottle.

√ The bottle was stored in a refrigerator at 5-10 ˚C.

interaction between Bioglass and Graphene Oxide and sodium alginate.

interaction between Bioglass and Graphene Oxide.

The increased surface negativity of the Bioglass (BG) due to the amount of hydroxyl group enhanced the reactivity of the BG



Ion	Concentration / mol·m^{-3}	
	SBF	Human blood plasma
Na^+	142.0	142.0
K^+	5.0	5.0
Mg^{2+}	1.5	1.5
Ca^{2+}	2.5	2.5
Cl^-	147.8	103.0
HCO_3^-	4.2	27.0
HPO_4^{2-}	1.0	1.0
SO_4^{2-}	0.5	0.5

Fig. 3. Ion concentrations of SBF in comparison with those of human blood plasma.

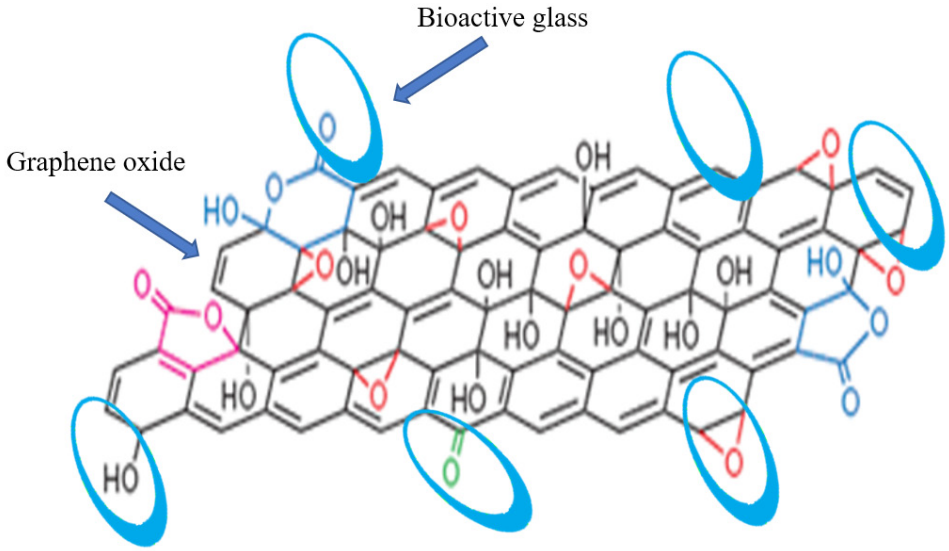


Fig. 4. interaction between Bioglass and Graphene Oxide[11].

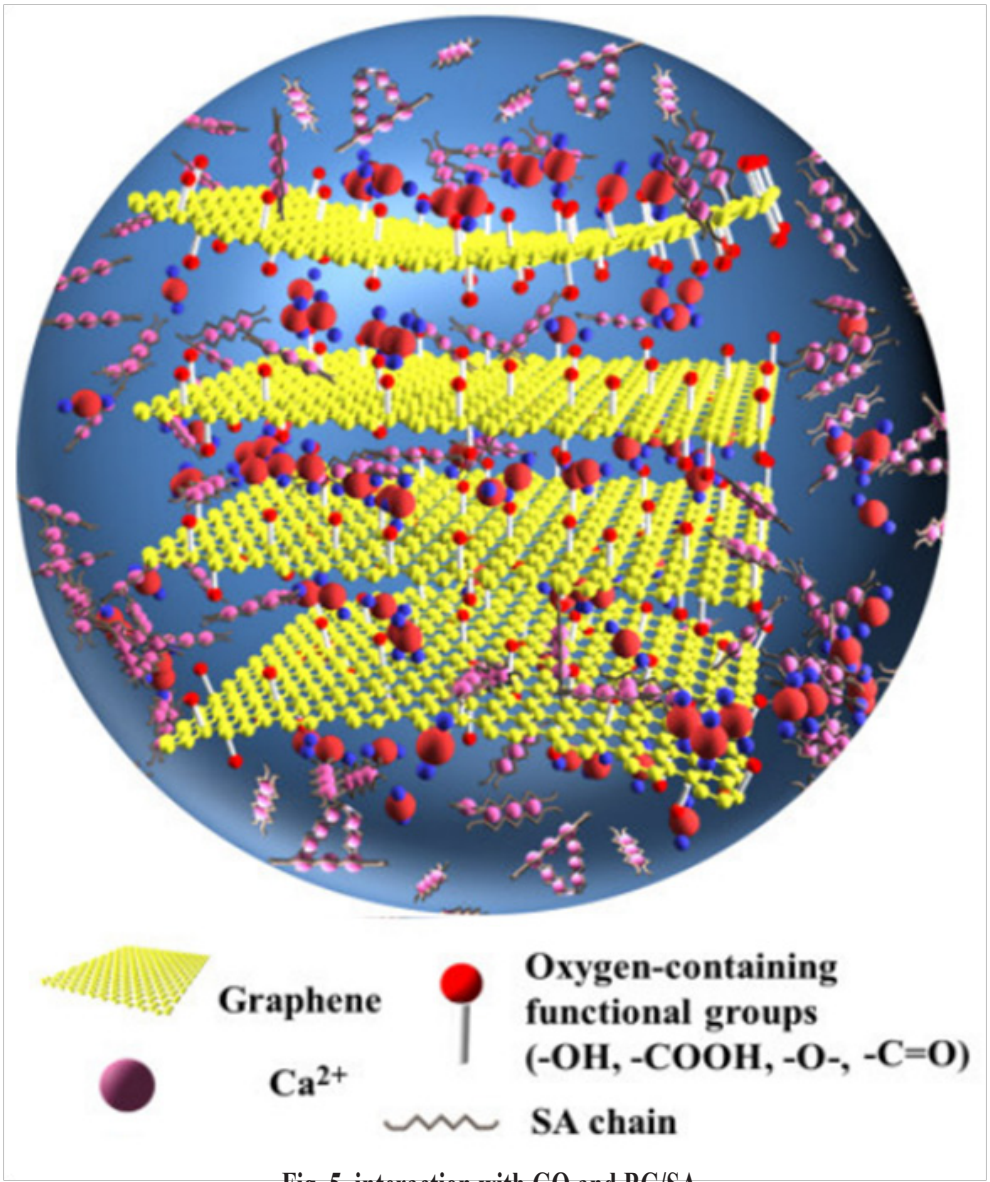


Fig. 5. interaction with GO and BG/SA.

indicating that strongly protein adsorption due to the interaction between the amine group NH2 of the bovine serum albumin (BSA) with the OH group of Bioglasssurface and the interaction between Bioglass and Graphene Oxide (GO) formation of BG/GO composites where Ca2+ in BG is interacted to GO through negatively charged COO– groups. The molar percentage of silica decreased after formed the composition.

The negativity of the surface of BG/GO with silver nanoparticles decreased due to the reactivity ofthe carbonyl group of graphene oxide with Bioglass and sodium alginate, which blocked the negative sites on the surface. From the results in Figure 4, it can be concluded that the reactivity of protein with the control sample,1%, 2%, 3%, and 4% decreased gradually due to decreasing negativity of the surface, which decreased by interaction with the carbonyl group of graphene oxide[11], [12].

interaction with GO and BG/SA

But the presence of a percentage of silver nanoparticles gives the mixture more biological properties and gains biological and antimicrobial immunity, so we study different percentages of silver nanoparticles while negating the percentage of graphene oxide.

The interaction between GO and BG/SA with silver nanoparticles illustrate in Figure5.

Results and Discussion

The gauss and Avogadro software can be calculated and draw the shape to illustrate the interaction between Bioglass (without and withsilver) and graphene oxide as shown in Figure 6.

But the presence of a percentage of silver nanoparticles gives the mixture more biological properties and gains biological and antimicrobial immunity, so we study different percentages of silver nanoparticles while negating the percentage of graphene oxide.

Elemental analysis.

Calcium concentration

The change in the concentration of Ca ions can be seen as a reflection of the conflict between the rate of dissolution and precipitation processes. At first, the SBF solution contains around 100 mg/l of calcium. While the Ca concentrations were 100 mg/l, additional Ca was shown to be present, proving that the dissolving rate outpaces the precipitation rate. The Profile Silver percentage (%) as a function of Ca and P concentrationis shown in Figure7.

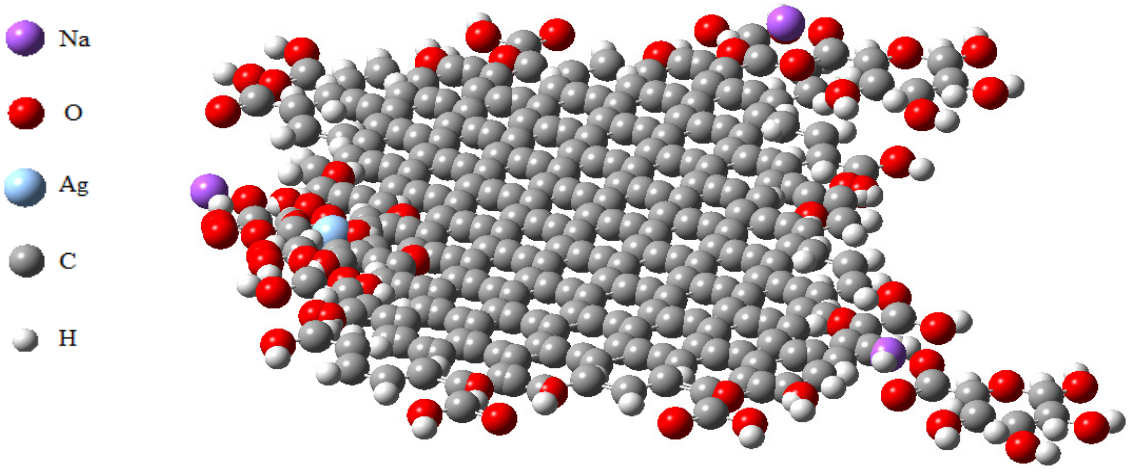


Fig. 6 interaction between Bioglass withsilver and graphene oxide.

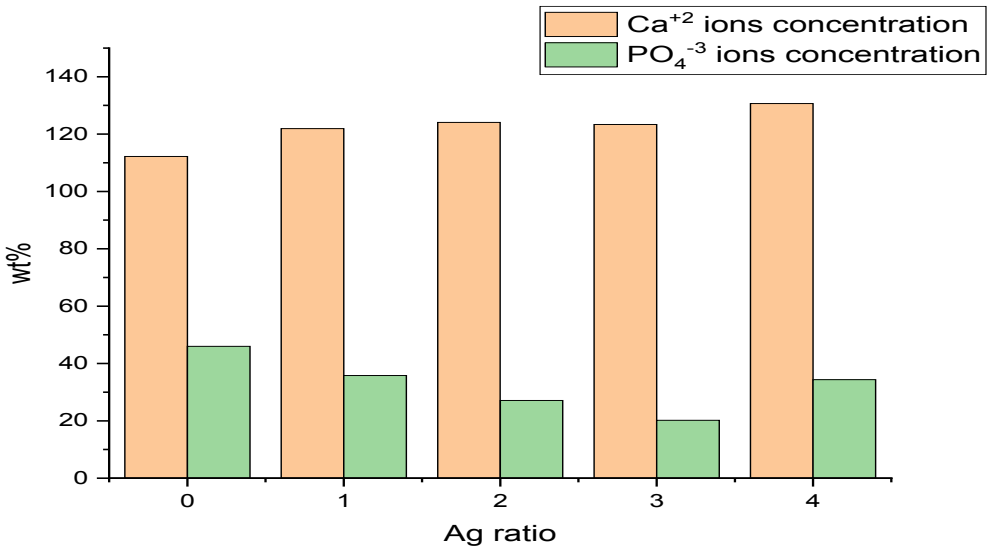


Fig.7. Profile Silver percentage (%) as a function of Ca and P concentration.

Figure 8 shows the change of concentration of Ca ions after soaking BG/SA with different ratios of silver nanoparticles in SBF solution. The increase in the Ca concentration observed for all specimens from the first day and gradually decreased in the Ca concentration to the eight days in SBF is attributed to the dissolution of the Bioglass phase. The rate of dissolution approximately stopped from day 8 up to 33 days of immersion in SBF.

For all of the BG/SA composites, the initial calcium content appears to rise.The release of calcium ions from the BG/SA composites is what causes this rise in calcium concentration. The consumption of calcium ions through the development of apatite on their surfaces may account for the reduction in calcium content from 2 to 8 days. [13]. The fast development of the apatite nuclei generated on the surface of the BG/ SA composite, which outpaces the release rate of calcium ions to the solution, is responsible for the declines in the amount of Ca2+ ions in SBF.

The pH varied according to Ca2+ concentration variations because Ca2+ ions in the BG/SA composite exchanged with H+ or H3O+ in the SBF[14]. This means that a layer of apatite formed in all samples, but the surface area of it decreased by increasing the ratio of silver nanoparticles concentration.

Phosphorus concentration

Figure 9 shows the concentration of P5+ ions after 33 days of soaking of the composites in SBF solution. Before immersion phosphate ion concentration in SBF is 30 ppm only. After immersion of the samples in SBF for 33days, at 24 h it is noted that phosphate concentration in all BG/SA composites increased up to high values due to the release of phosphate from these samples to the solution, but it was gradually decreased after 24h up to the fourth day to reach low values indicating the consumption of phosphate in the formation the layer of crystalline HCA on the surface of BG/SA composite.

After soaking in SBF phosphorus concentration is approximately a constant from 4 up to 16 days, this saturation in phosphorus concentration maybe due to the consumption of phosphate ions through the formation of apatite on the surfaces of BG/SA composites balanced with the release of the phosphate ions from the samples then it showed slightly increased value from 16 up to 33 days[15].

PH analysis

The variation of pH values relative to soaking time in SBF of BG and SA with different ratios of silver composites are shown in Figure10. The dissolution of Ca2+ ions from the samples would lead to a locally increasingin the pH value of the surrounding fluid. In the start and during the four days, pH changed to a value (sample) of 7.8 Bioglass (BG), 8 (control), 9 (1%), 8.8 (2%), 8.1 (3%), 7.7 (4%).

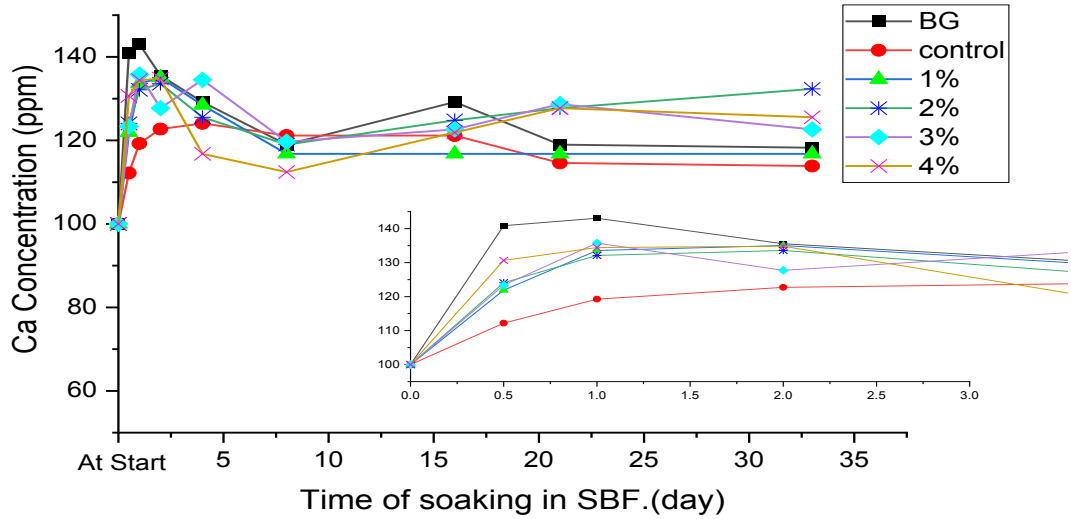


Fig. 8. The concentration of Ca2+ ions after soaking in SBF solution for 33 days.

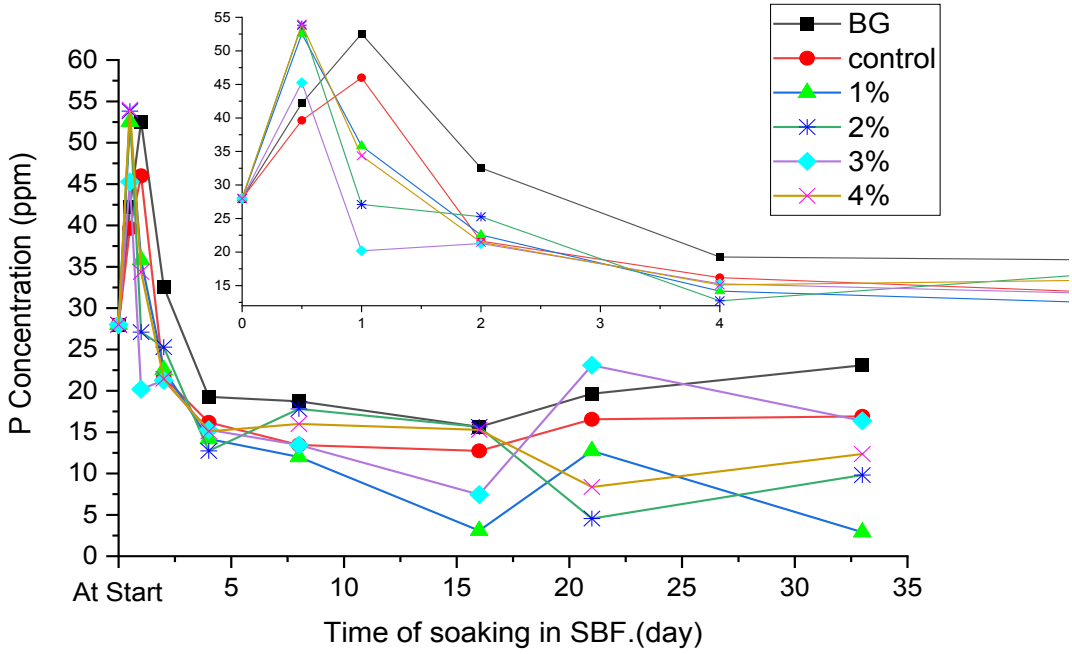


Fig. 9. The concentration of P5+ ions in SBF solution after soaking for 33 days.

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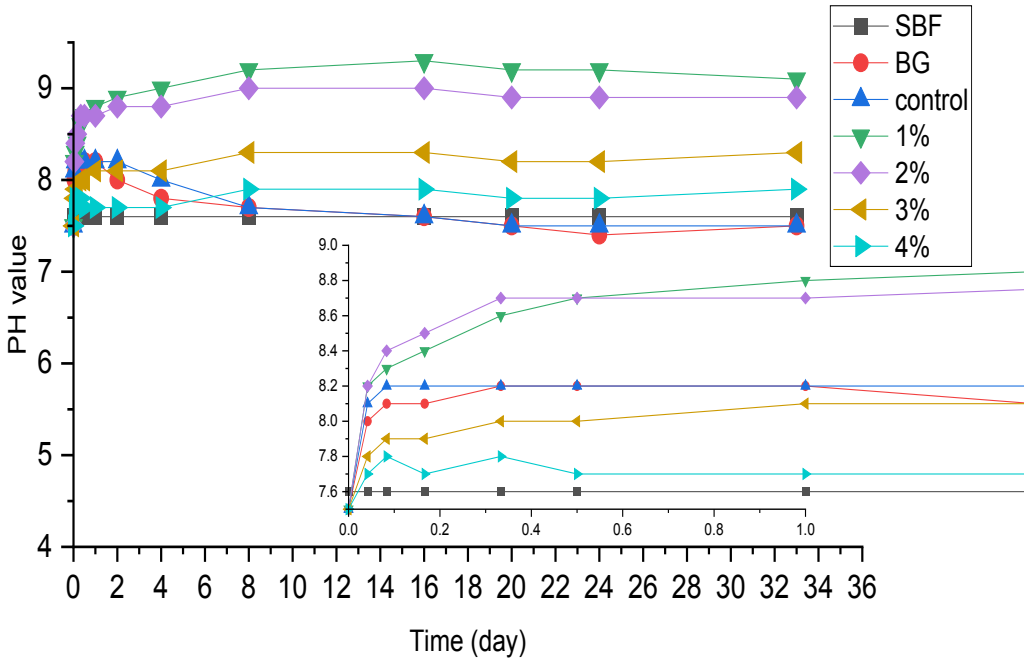

Fig. 10. The change in PH results of all samples during immersion in SBF solution for 33days.

The pH goes up. The partial dissolving process from the result after 4 to 16 days provides evidence of the materials' high reactivity. These results are compatible with the (Ca2+ and H3O+) ion exchange process described in the BG/SA Composite's apatite layer production processes. Due to these interactions, the pH increases, which promotes the growth of apatite nuclei on the silanol groups of the BG/SA Composite surface. The nest extract with AgNPs catalyzed the bio-reduction of silver ions to produce metallic AgNPs with a pH of 8.3 within 1hore[16].

The formation of apatite in SBF is strongly pH dependent; the increase in pH signifies the reduction in the concentration of H+ due to the replacement of metal ions in the BG/SA Composite and subsequent production of OH- groups due to the breaking of siloxane bond. From the 16 days up to 33-day the pH value is approximately constant until reach 7.6 Bioglass (BG), 7.6 (control), 9.3 (1%), 9 (2%), 8.3 (3%), 7.9 (4%).

The fluctuation in pH value of all samples may be explained when considering the result of two opposite processes: the release of Ca2+ from the BG/SA Composite, and the consumption of Ca2+ due to the formation of the apatite layer. Therefore, when the releasing rate of Ca2+ is higher than the consumption rate, the pH will increase, or else, the pH will decrease. It is found from the results that the substitution of Ca ions in the BG/SA composition increase the solubility of the BG/SA Composite, which subsequently increases the interchange of Ca2+ and H3O+ ions from the solution and increases the pH value.

The silver ions liberated from the samples containing different percentages of silver helped to lead the pH in all the samples, and a high increase in the pH was noted, which decreases with the increase in the silver concentration in the samples.

Conclusion

A composite prepared via the sol-gel method formed from BG/SA/GO (1:1:0.5, wt.) and Ag- substituted BG/SA/GO. The chemical composition of glass is (60SiO2-35CaO-5P2O5) doped with (1%, 2%, 3%, and 4%)silver. In this research, Sodium alginate polymer was added with graphene oxide to improve the mechanical properties of the samples in fixed proportions in all samples and to enhance the biological activity of the samples by adding different percentages of silver nanoparticles and using them as an antimicrobial (anti-bacterial - anti-fungal) so that the mixture is suitable for biological applications and automatically providing the anti-infective in the organic matter Alternative to reduce the use of drugs after transplants and surgery. All prepared composites were soaking in SBF for 33 days, and we measured after 1, 2, 4, 8, 16, 21 and 33 days from the beginning of the immersion. And stored frozen until analyzed for determining the concentration of Ca and P by using UV-Visible Spectrophotometer. All of the composites' surfaces had a coating of (HA) layer them. It is noted that this layer increased from (BG/SA) sample without silver up to (2%) sample, but decreased gradually in (3% Ag) and (4%) samples. So, we can say the optimum bioactive composite effective concentration of silver Ag was (2%). properties and antibacterial material, which can be used successfully in bone tissue engineering application and bone mineralization biocomposite.

Declarations

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*Availability of Data and Material*Not applicable.

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*Conflict of interest:*The authors affirm that they do not have any competing interests.

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تحضير مزيج من الزجاج الحيوي وبوليمر الجينات الصوديوم المطعم بالفضة واكسيد الجرافين

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[ج] شعبة الاطياف – قسم الفيزياء – المركز القومي للبحوث .

تعد المواد العضوية البديلة من افضل الحلول في الاجزاء التعويضية وهندسة الانسجه الا ان الزجاج الحيوي علي الرغم من تمتعه بالنشاط الحيوي والتوافق النسيجي إلا انه هش اي لا يمتلك اي صلابه مقارنة بالمعادن.

- في هذه الرسالة يتم تدعيم الزجاج الحيوي النشط بيولوجيا بأكسيد الجرافين كأحد الحلول المهمة لزيادة الخواص الميكانيكية للزجاج.
- باســـتخدام طريقـة السـول- جيل تـم تحضير عينة حاكمة من الزجاج الحيوي (Bioglass) في التركيب (٦٠٪ SiO_2, ٣٥٪ CaO, ٥٪ P_2O_5)
- تم التخلص من النيتريت في جميع عينات الزجاج بالمعالجة الحرارية عند ٠٠٦ م° كما تم التخلص من الرطوبة والايثانول الموجود بالعينات التى تم تحضيرها وذلك بالمعالجه الحرارية عند ٠٢١م° لمدة يومين.
- تم تحضير عدد (٤) عينات بالاضافة الي العينة الحاكمة , حيث تتكون كل عينة من الزجاج الحيوي واكسيد الجرافين بنسب (٠,٥٪) التي حققت افضل نتائج ميكانيكية ونسب مختلفة من الفضة النانومترية علي حساب الكالسيوم ٥٣٪ الموجود في التركيب الكيميائي للعينات والنسب هي (١, ٢, ٣, ٤)٪ و تسميتها كالتالي (gA٪١+BG),(Ag٪٢+BG),(gA٪٣+BG)و(gA٪٤+BG)بالاضافة الي العينة الحاكمةوعولجت جميع العينات حراريا عند ٠٠٦ م° ثم دمجت معاكسيد الجرافين بالطريقة الميكانيكية.
- تم تذويب بوليمر الجينات الصوديوم ليتم دمجة الي الخمس عينات المحضرة سابقا والمحتوية كلها علي نفس النسبة (٠,٥) من اكسيد الجرافين ونسب مختلفة من الفضة النانومترية ليكون اسمائها (C),(١٪),(٢٪),(٣٪) و(٤٪)بالاضافة الي عينة اخري لا تحتوي علي اكسيد الجرافين فقط ٠٥٪ زجاج حيوي و ٠٥٪ بوليمر الجينات الصوديوم والتي سميت (BG)
- تم تجفيف العينات بطريقة التجفيف بالتجميد (Freeze draying) عن طريق رفع الضغط وسحب الماء من العينات للحصول علي القوام الصلب الاسفنجي.
- تم دراسة النشاط الحيوى لهذه العينات باختبارها خارج جسم الكائن الحى (In -ortiv tset) حيث تم غمس ٨,٠ جرام من كل عينة لمدة ٣٣ يوم فى ٠٨ ميلي لتر من سائل محضر كيميائيا بتركيزات مشابهة لتركيب الأيونات الموجودة في بلازما دم الإنسان (SBF) داخل حمام مائي عند ٧٣م° .
- تمت دراســة وتوصيــف جميع العينات التي تم تحضيرها ,والنشـــاط الحيوي لها بعدد من الاجهزة مثل (UV ypocsortceps, retem Hp) اثناء الغمر في السائل المحاكي لبلازما الدم (SBF) وعلي فترات مختلفة في اليوم الاول ثم الثاني الي ٣٣ يوم.
- وأخيرا , النتائج الحالية تقترح أن أفضل سطح في النشاط الحيوي هو سطح العينة ٢٪ حيث تحتوي علي كلا من الخليط العضوي والغير عضويا (الزجاج الحيوي,بوليمر الجينات الصوديوم) كما تحتوي علي نسبة (٠,٥) من اكسيد الجرافين والتي حققت افضل القياسات الميكانيكية وأيضا احتوت علي نسبة ٢٪ من جزيئات الفضة النانومترية والتي اكسبت العينة مقاومه بكتيريه وفطرية كما سمحت بتكون نسبة مقبولة من الاباتيت اذ ان الزياده عن هذه النسبه تقلل من تكون الاباتيت كما في العينة ٤٪.