

RESEARCH

[Translated article] Design and comparison of bone substitutes. Study of in vivo behaviour in a rabbit model



L. García-Lamas^{a,*}, S. Sánchez-Salcedo^{b,c}, V. Jiménez-Díaz^a, B. Bravo-Giménez^a, M.V. Cabañas^b, J. Peña^b, J. Román^b, J. Jiménez-Holguín^b, M. Abella^d, M. Desco^d, D. Lozano^{b,c}, D. Cecilia-López^a, A.J. Salinas^{b,c}

^a Servicio de Cirugía Ortopédica y Traumatología, Hospital Universitario 12 de Octubre, Instituto de Investigación Hospital 12 de Octubre, imas12, Madrid, Spain

^b Departamento de Química en Ciencias Farmacéuticas, Universidad Complutense de Madrid (UCM), Instituto de Investigación Hospital 12 de Octubre, imas12, Madrid, Spain

^c CIBER de Bioingeniería, Biomateriales y Nanomedicina, Instituto de Salud Carlos III, Madrid, Spain

^d Instituto de Investigación Sanitaria Gregorio Marañón, Departamento de Bioingeniería, Universidad Carlos III de Madrid, Madrid, Spain

Received 28 November 2022; accepted 3 December 2022

Available online 20 March 2023

KEYWORDS

Biomaterials;
Bone regeneration;
Bioactive mesoporous
glass;
Hydroxyapatite;
Rabbit

Abstract

Aim: To compare the in vivo bone formation capacity of of biomaterials designed as bone substitutes with respect to iliac crest autograft, one based on carbonate hydroxyapatite and the other one on bioactive mesoporous glass.

Materials and methods: Experimental study consisting on 14 adult female New Zeland rabbits where a critical defect was made in the rabbit radius bone. The sample was divided into four groups: defect without material, with iliac crest autograft, with carbonatehydroxyapatite scaffold, and with bioactive mesoporous glass scaffold. Serial X-ray studies were carried out at 2, 4, 6 and 12 weeks and a microCT study at euthanasia at 6 and 12 weeks.

Results: In the X-ray study, autograft group showed the highest bone formation scores. Both groups of biomaterials presented bone formation similar and greater than the defect without material, but always less than in the autograft group. The results of the microCT study showed the largest bone volume in the study area in the autograft group. The groups with bone substitutes presented greater bone volume than the group without material but always less than the autograft group.

DOI of original article: <https://doi.org/10.1016/j.recot.2022.12.002>

* Corresponding author.

E-mail address: lgarcialamastr@gmail.com (L. García-Lamas).

<https://doi.org/10.1016/j.recot.2023.03.011>

1888-4415/© 2023 SECOT. Published by Elsevier España, S.L.U. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Conclusion: Both scaffolds seem to promote bone formation but are not capable of reproducing the characteristics of autograft. Due to their different macroscopic characteristics, each one could be suitable for a different type of defect.

© 2023 SECOT. Published by Elsevier España, S.L.U. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

PALABRAS CLAVE

Biomateriales;
Regeneración ósea;
Vidrio mesoporoso
bioactivo;
Carbonato-
hidroxiapatita;
Conejo

Diseño y comparativa de biomateriales para el tratamiento de defectos óseos. Estudio de su comportamiento *in vivo* en modelo animal de conejo

Resumen

Objetivo: Comparar *in vivo* la capacidad de formación ósea de 2 tipos de biomateriales diseñados como sustitutivos óseos respecto a autoinjerto de cresta ilíaca, uno basado en carbonato-hidroxiapatita y otro en vidrio mesoporoso bioactivo.

Material y método: Estudio experimental compuesto por 14 conejos hembras adultas de Nueva Zelanda donde se realizó un defecto crítico en hueso radio. La muestra fue dividida en 4 grupos: defecto sin material, con autoinjerto de cresta ilíaca, con soporte de carbonato-hidroxiapatita y con soporte de vidrio mesoporoso bioactivo. Se realizaron estudios seriados de radiología simple a las 2, 4, 6 y 12 semanas y estudio de micro-TC a eutanasia a las 6 y 12 semanas.

Resultados: En el estudio de radiología simple, el grupo de autoinjerto mostró las mayores puntuaciones de formación ósea (7,5 puntos). Ambos grupos de biomateriales presentaron formación ósea similar (5,3 y 6 puntos, respectivamente) y mayor al defecto sin material (4 puntos), pero siempre menor que el grupo de autoinjerto. Los resultados del estudio de micro-TC mostraron el mayor volumen de hueso en el área de estudio en el grupo de autoinjerto. Los grupos con sustitutivos óseos presentaron mayor volumen de hueso que el grupo sin material, pero siempre menor que en el grupo de autoinjerto.

Conclusiones: Ambos soportes parecen favorecer la formación ósea, pero no son capaces de reproducir las características del autoinjerto. Por sus diferentes características macroscópicas cada uno podría ser adecuado para un tipo diferente de defecto.

© 2023 SECOT. Publicado por Elsevier España, S.L.U. Este es un artículo Open Access bajo la licencia CC BY-NC-ND (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Introduction

The treatment of bone defects is one of the most demanding challenges in the field of orthopaedic surgery. In this context, bone autograft is the best solution to treat bone defects and stimulate new bone formation and is therefore considered the gold standard,¹ given its osteoconductive, osteoinductive and osteogenic capacity. However, its use is limited by factors such as increased intraoperative time, morbidity in the donor site and the limited amount of autograft that can be obtained. Within this framework, the need arises within the field of tissue engineering to create constructs capable of carrying out the function of the autograft without the limitations derived from its use.

In the field of bone substitutes research there are two main families of biomaterials, those formed by apatites and those formed by mesoporous bioactive glasses (MBG). Both types of materials have been shown to be biocompatible, bioactive and resorbable to varying degrees,^{2,3} and they have shown to integrate with the recipient tissue without fibrous tissue interposition in *in vivo* studies.^{4,5}

The purpose of this study was to compare the behaviour of nano-carbonate hydroxyapatite (nCHA) ceramic-based constructs with those based on MBG under the same *in vivo*

conditions and to analyse their bone-forming capacity compared with iliac crest autograft and graftless defect.

Material and method

Preparation of materials

Carbonate hydroxyapatite (CHA) scaffolds were made by the GELPOR3D method^{6,7} by preparing an aqueous solution of agarose (3.5% w/v) which was heated to 80–90 °C, Subsequently, lowering the temperature to 40 °C, at which temperature the gelatine and the corresponding CHA were added (agarose/gelatine/CHA ratio: 45/10/45). Once a homogeneous suspension was obtained, the mixture was poured into a previously designed three-dimensional mould, consisting of rigid stainless-steel filaments of the desired diameter (300–900 µm), which determines the size of the pores in the three dimensions of the space. Once complete gelation was obtained, the mould was removed to obtain the piece, which was subsequently dried by freeze-drying for sterilisation using UV light (Fig. 1A and B).

MBG scaffolds were made following the procedure previously described by Jiménez-Holguín et al.⁸ To fabricate the scaffolds, a 15% PVA suspension (Sigma Aldrich, St. Louis,

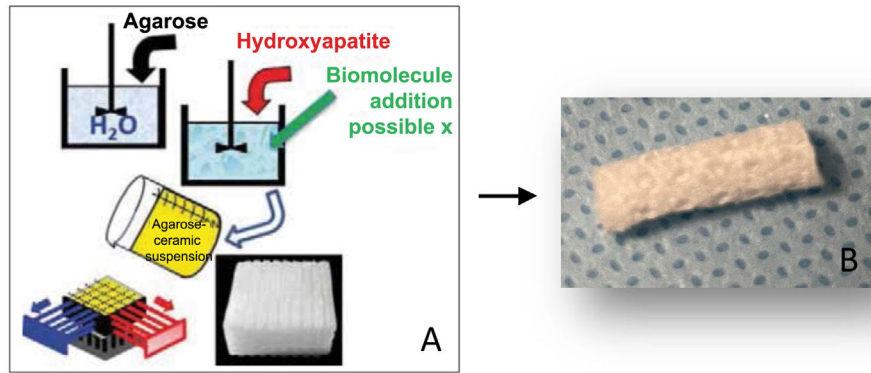


Figure 1 (A) Graph showing the different steps for obtaining CHA-based substrates. (B) Final CHA scaffold prior to implantation with a size of 15 mm × 5 mm.

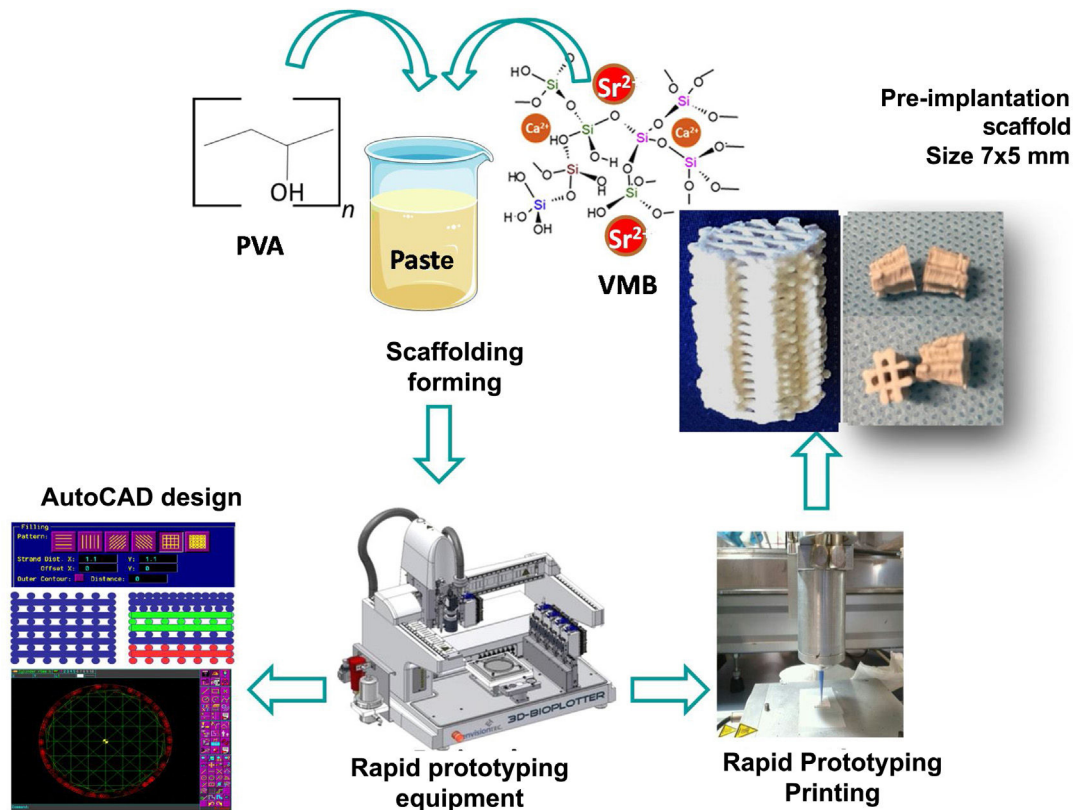


Figure 2 Graph of the synthesis of MBG-based scaffolds. Scaffolds are designed using AutoCAD (BiploterTM 3D printer rapid prototyping equipment [EnvisionTEC, Gladbeck, Germany]) for subsequent printing by rapid prototyping.

MO, USA) was prepared by mixing 1.25 g of PVA in 10 mL of milliQ H₂O at 90 °C under magnetic stirring and mixed with the MBG generating a PVA/VM paste for subsequent extrusion in the rapid prototyping equipment of the 3D printer (Regemat 3D S.L., Spain). The dimensions of the cylindrical scaffolds obtained were 6 mm in diameter and 7.5 mm in length. The scaffolds were dried at 100 °C for 2 h and sterilised with ultraviolet light for 2 h (Fig. 2).

Animal model

Fourteen adult female New Zealand rabbits weighing $4 \pm .5$ kg were used. In compliance with RD 1090/2015, the study was evaluated and approved by the Ethics Committee

for Research with Experimental Animals of the I+12 Research Institute, the Ethics Committee/Animal Experimentation Subcommittee of the Autonomous University of Madrid and by the Directorate General for Agriculture, Livestock and Food of the Community of Madrid. This experimental animal study adheres to the ARRIVE guidelines and has been conducted in accordance with the UK Animals (Scientific Procedures) Act 1986 and the related recommendations of the EU Directive 2010/63/EU for animal experiments.

Surgical intervention

Pre-anaesthetic sedation was performed with ketamine (40 mg/kg, IM) and xylazine (5 mg/kg, IM). Once the animal

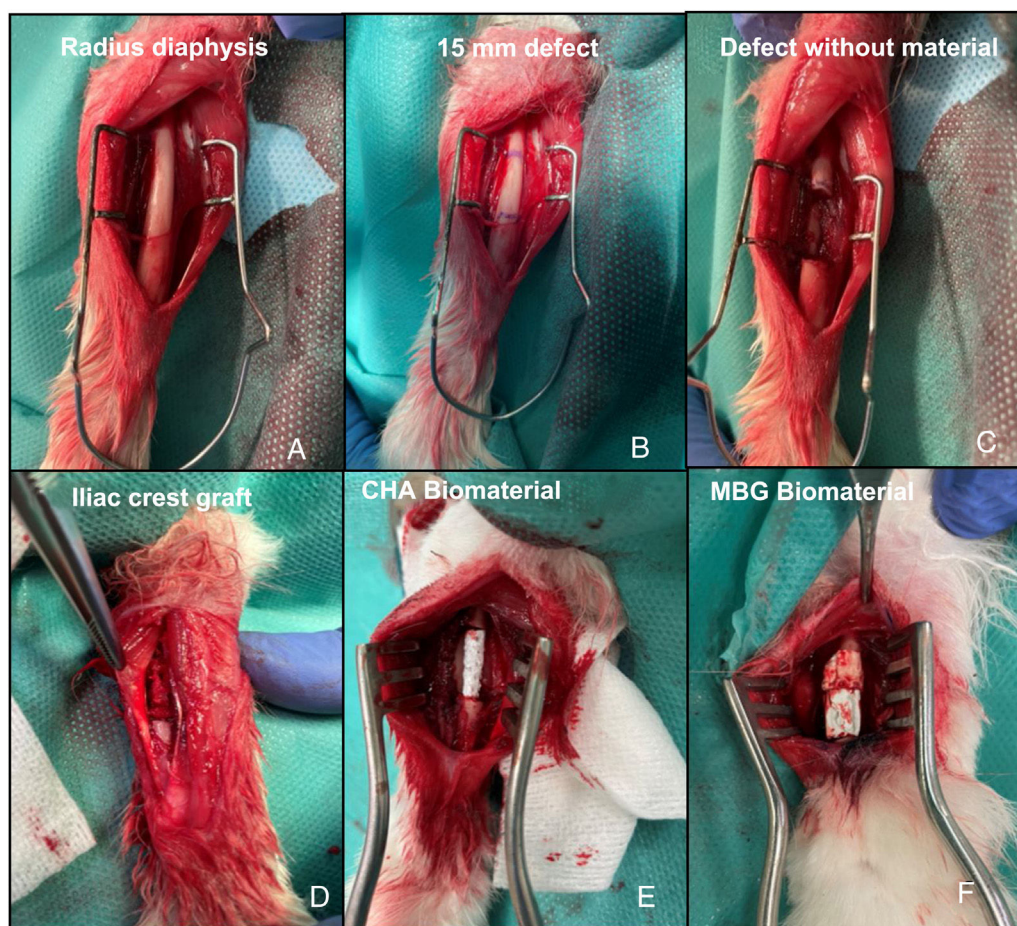


Figure 3 Intraoperative images of the rabbit radius procedure. (A) Exposure of the radial bone diaphysis. (B) Planning of the 15 mm defect to be created. (C) Exposure of the defect without material (G1). (D) Filled defect with autograft of the animal's own iliac crest (G2). (E) Filled defect with CHA scaffold (G3). (F) Filled defect with MBG scaffold (G4).

was sedated, a venous catheter was placed in the marginal vein of the ear. Antibiotic prophylaxis was then administered with cefazolin (50 mg/kg IV) and the forearm and iliac crest were shaved according to the study group, and the skin was aseptically prepared with polyvidone iodine. Anaesthesia was maintained by inhalation with 4% sevoflurane, with continuous monitoring of heart rate and oxygen saturation. A longitudinal incision was made over the dorsal diaphysis of the radius, which was exposed after careful dissection of the adjacent musculature. Subsequently, a 15 mm bone segment with surrounding periosteum was removed to achieve a critical,⁹ with no potential for self-repair, in this bone. The 14 rabbits were divided into four study groups. In the first group (G1) of three rabbits, the defect was not filled to study the behaviour of the defect; in the second group (G2) of three rabbits, the defect was filled with the animal's own iliac crest autograft; in the third group (G3) of four rabbits, the CHA scaffold was implanted; and in the fourth group (G4) of four rabbits, the MBG scaffold was implanted (Fig. 3). Subsequent plane closure was performed by suturing the forearm fascia with 4/0 braided resorbable thread and the skin with 5/0 non-resorbable monofilament. Immediate postoperative loading of the operated limb was allowed. In each group, one rabbit was sacrificed at six weeks and the rest at 12 weeks. Euthanasia was performed

using the previously described sedation protocol followed by intravenous administration of 0.5 g sodium thiopental.

Image analysis

Plain radiology

Radiological controls of the defect were performed using a Xiscan 4400 fluoroscope in the immediate postoperative period and at two, four, six and 12 weeks after surgery depending on the study group to assess bone formation, union and remodelling of the defect. The results were scored using the modified Lane and Sandhu¹⁰ scale as shown in Table 1.

Computerised microtomography study (Micro-TC)

Image analysis and three-dimensional micro-CT reconstruction was performed at the end of the study where the following variables of newly formed bone were determined: BV/TV (bone volume/total volume, %), Tt.Ar (total cancellous bone, mm²), Ct.Ar (total cortical bone, mm²), and Ct.Th (cortical thickness, mm) in the defect area (Fig. 4).

Table 1 Modified Lane and Sandhu radiological scoring scale.

Bone formation	
No evidence of bone formation	0
Bone formation occupying 25% of defect	1
Bone formation occupying 50% of defect	2
Bone formation occupying 75% of defect	3
Bone formation occupying 100% of the defect	4
Union (proximal and distal evaluated separately)	
No union	0
Union possible	1
Radiographic union	2
Total points possible by category	
Bone formation	4
Proximal union	2
Distal union	2
Remodelling	2
Maximum score	10

Statistical analysis

Plain radiology and micro-CT results were compared by mean difference using the non-parametric Kruskal–Wallis test, and when p -values were <0.05 , pairwise comparisons were performed using the Mann–Whitney U -test. A p value <0.05 was considered statistically significant.

The statistical package SPSS Statistics for Windows, version 24.0, was used. Armonk, NY: IBM corp.

Results

Clinical follow-up

No deaths occurred during surgery. An early euthanasia was performed seven weeks post-operatively on a rabbit that suffered a spinal cord injury during a change of location for cleaning of the housing enclosure, belonging to the iliac crest autograft group (G2). Early euthanasia of this animal was decided for humane reasons. There was also a suture dehiscence with no signs of infection in the G2 group which was resolved by serial dressings and an infection in the G1 group which was washed, debrided and treated with antibiotics and resolved after two weeks (Fig. 5A and B).

Results of plain radiology

Bone formation according to the modified Sandhu and Lane scale increased over time in all groups (Fig. 6). At all follow-up times the iliac crest autograft group (G2) had the highest scores, this difference being statistically significant at four ($p=0.03$) and six weeks ($p=0.04$) follow-up. Both the mesoporous glass group and the NCHA group showed higher bone formation after four weeks than the no-material control group (G1), although always lower than in the G2 group. The radiological results are shown in Table 2.

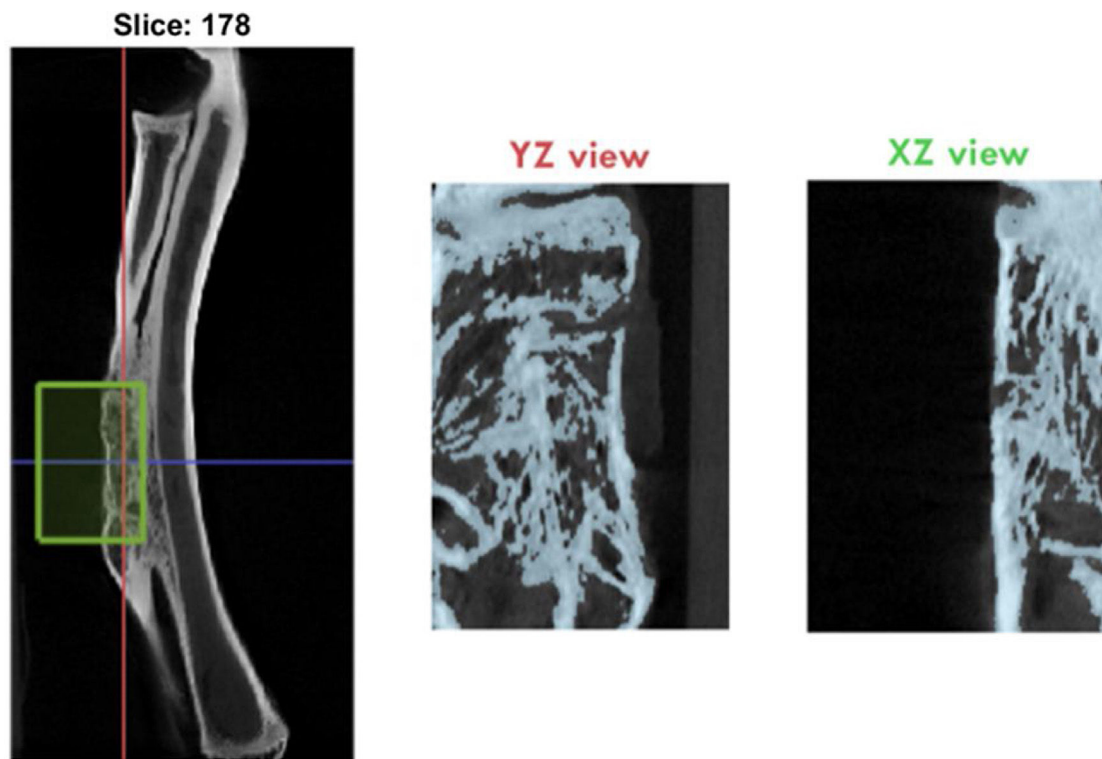


Figure 4 Image of the selection of the defect to be studied in the micro-CT study and visualisation of the same in the different planes of space to adjust the work area.

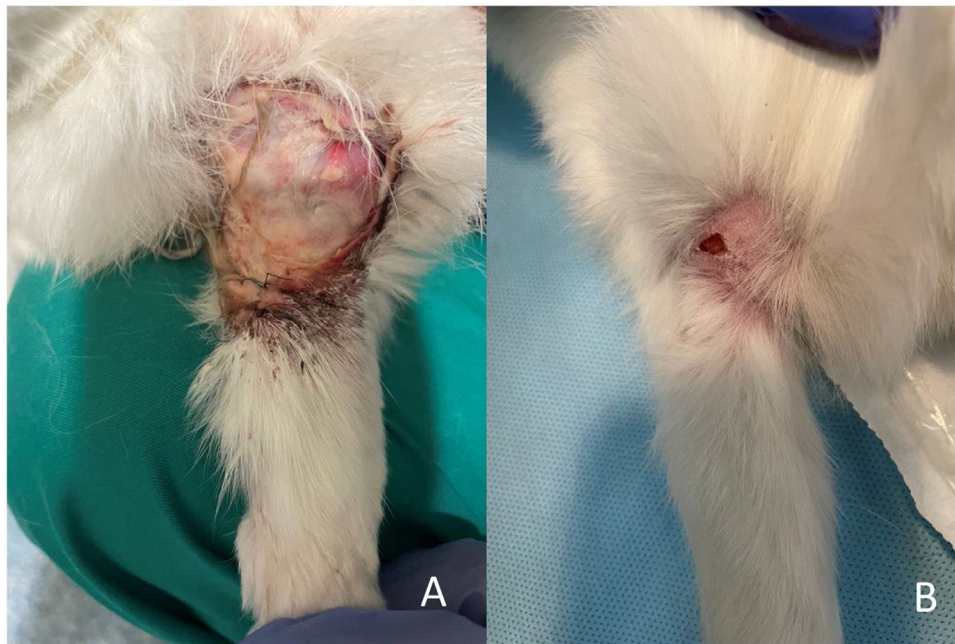


Figure 5 Images corresponding to a postoperative infection in the operated limb. (A) Wound appearance 10 days after surgery. (B) Wound appearance 24 days after surgery after lavage, surgical debridement and antibiotic therapy.

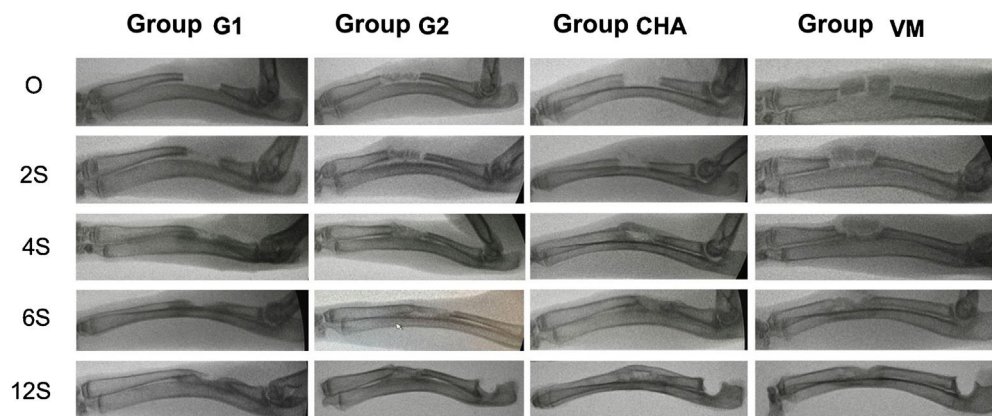


Figure 6 Serial radiological controls of the different groups at 0, 2, 4, 6, and 12 weeks postoperatively. Note the pattern of bone formation in the different study groups, highlighting the lack of bone formation in the G1 group, where a large central area without bone filler can be seen.

Table 2 Results of plain radiology.

Weeks after the operation	Mean (min–max)				p^a
	Control	Gold standard	CHA	VB	
2 weeks	2 (1–3)	3 (2–4)	0 (0–0) ^b	.3 (0–1) ^c	.0005
4 weeks	3 (2–5)	6.3 (6–7) ^d	4.3 (3–6)	4.3 (3–5)	.05
6 weeks	3.3 (2–6)	7.3 (7–8) ^e	4.8 (3–8)	3.7 (1–5)	.13
12 weeks	4 (2–6)	7.5 (7–8)	5.3 (3–8)	6 (5–7)	.49

^a Non parametric Kruskal–Wallis.

^b $p = .09$ (compared to control by Mann–Whitney U -test).

^c $p = .02$ (compared to control by Mann–Whitney U -test).

^d $p = .03$ (compared to control by Mann–Whitney U -test).

^e $p = .04$ (compared to control by Mann–Whitney U -test).

Table 3 Bone volume measurements within the defect by groups.

	BV/TV ROI	SD	Error	T-test vs. G1	T-test vs. G2
G1	.26	.0729	.0421		.0108
G2	.55	.0830	.0479	.0108	
CHA	.38	.1551	.0776	.3127	.1641
MBG	.37	.0314	.0181	.0896	.0226

BV/TV ROI: bone volume in the study area; CHA: carbonate hydroxyapatite group; G1: no material control group; G2: iliac crest autograft control group; MBG: mesoporous bioactive glass group; SD: standard deviation.

Table 4 Trabecular measurements from micro-CT study.

	TB.N	SD	T-test vs. G1	T-test vs. G2	TB. th	SD	T-test vs. G1	T-test vs. G2	TB.sp	SD	T-test vs. G1	T-test vs. G2
G1	.016	.0027		.1011	1.8	.3578		.2722	1.42	.6108		.9494
G2	.024	.0051	.1011		1.28	.3805	.2772		1.39	.3325	.9494	
CHA	.016	.0079	.4130	.1010	1.77	.5124	.9528	.2557	2.29	1.2299	.3530	.2873
MBG	.020	.0020	.1120	.2396	1.73	.1994	.8468	.1729	1.23	.1853	.6425	.2102

BV/TV ROI: bone volume in the study area; CHA: carbonate hydroxyapatite group; G1: control group without material; G2: iliac crest autograft control group; MBG: mesoporous bioactive glass group; TB.N: number of bone trabeculae; SD: standard deviation.

Table 5 Cortical thickness measurements by micro-CT study.

	Cortical. Th (mm)	SD	Error	T-test vs. G1	T-test vs. G2
G1	.47	.1282	.0740		.8467
G2	.45	.0593	.0342	.8467	
CHA	.45	.0588	.0294	.8442	.5934
MBG	.40	.1853	.1070	.3815	.4090

CHA: carbonate hydroxyapatite group; cortical. Th (mm): cortical thickness in mm; G1: control group without material; G2: iliac crest autograft control group; MBG: mesoporous bioactive glass group; SD: standard deviation.

Results of the micro-CT study

Bone volume in the study area (BV/TV, region of interest [ROI]) was significantly higher ($p = .012$) in the G2 group compared to the rest of the groups. Both the MBG and NCHA groups presented greater bone formation than the G1 group, although this difference was not statistically significant (Table 3).

Regarding the trabecular study, no statistically significant differences were found in any of the parameters studied, which were the number of bone trabeculae (TB.N), thickness (TB.Th mm) and trabecular separation (Tb.sp mm). The results of the statistical study are shown in Table 4.

Regarding the cortical study, no significant differences in cortical thickness were found in any of the study groups. The results are shown in Table 5.

Discussion

To evaluate the bone regeneration potential of the designed biomaterials, some based on MBG and others on CHA, a critical defect was generated on rabbit radial bone. Furthermore, it was compared with the gold standard (iliac crest autograft) and with the biomaterial-free defect.

The New Zealand rabbit was chosen as the study model, as previous studies have shown it to be ideal for bone substitute studies,¹¹ given the similarities between rabbit

and human limbs, its medium size, its relative ease of handling and housing, as well as its affordable cost. It also exhibits an increased rate of bone regeneration, which has led it to be widely used as a model in numerous bone regeneration studies.^{12,13}

A critical defect was defined as “the smallest intraosseous defect in a particular bone and animal species that cannot regenerate itself during the animal’s entire lifetime.”¹⁴ Although the minimum size that a defect must be to be considered critical is not yet well established, it has been defined as a length of 2.5–3 times the diameter of the long bone where the defect is located.¹⁵ The radius bone was chosen as a model for a bone defect in long bone, as its shape is tubular, which allows the creation of a segmental defect. Furthermore, despite the defect, the ulna continues to support the weight of the forearm, allowing immediate postoperative loading and avoiding the need for stabilisation with additional osteosynthesis material. The diameter of the radius in New Zealand rabbits is between 4 and 5 mm, so, according to other authors,^{16,17} a 15 mm radial diaphysis defect was created. Numerous investigators have shown that this size defect is filled with a fibrous scar with no bony connection.^{16,17}

Regarding the composition and design of the biomaterials used in this study, two of the most promising families in the field of biomaterials engineering were chosen. One based on bioglasses and the other based on apatites.¹⁸ The groups developing bioglass-based materials consider these to be the

ideal scaffolds for the development of bone substitutes. The same is promulgated by groups specialising in research in the field of apatite-based substrates. In this work, two types of three-dimensional porous scaffolds developed by the Intelligent Biomaterials Research Group (GIBI) were compared. Tests were performed under the same *in vivo* conditions for both types of biomaterials, as these materials were implanted in the same animal model, same defect, and same surgeon in the same facilities.

Furthermore, Oonishi et al.¹⁹ showed that there are two classes of bioactivity. Materials with class A bioactivity exhibit both osteoconductive and osteoproducer effects, as a result of a rapid surface reaction including the local release of critical concentrations of ions, mainly Si, Ca, P and Na, causing intra- and extracellular reactions with the biological environment. MBGs belong to this class. On the other hand, biomaterials with class B only produce an osteoconductive effect and allow direct bone apposition on the support due to their low rate of interface reactions. CHA belong to this group. Furthermore, the design of the substrates was based in both cases on a three-dimensional architecture of interconnected pores of different sizes in order to simulate the bone structure,²⁰ which has a porosity between 1 and 3500 µm, where different pore sizes imply different functions. Pores larger than 100 µm, (macropores) allow for progenitor cells, tissue growth, nutrition and vascularisation. Pores <2 nm in size (micropores) or those between 2 and 50 nm (mesopores) promote cell adhesion, metabolite uptake and controlled resorption rates of biomaterial necessary for tissue repair.²¹ In addition, this porosity was created in the design phase in an anisotropic manner,²² as it has demonstrated greater bone formation than when designed in an isotropic manner for both materials.

Regarding the behaviour of the MBGs, they show a rapid bioactive response, due to their partial solubility in contact with biological fluids, releasing significant quantities of ions into the surrounding medium, especially Si ions, which enhance angiogenesis, and Ca²⁺ ions which contribute to cell proliferation and also show osteogenic activity.²³ The constitution of the mesoporous glasses used for this work was 78.5% SiO₂–15% CaO–5% P₂O₅–2.5% SrO (mol-%). The MBG mesomacroporous supports were enriched with SrO and showed mesoporosity (6 nm), macroporosity (1–100 µm) and giant channels (1 mm). Under *in vitro* conditions they have shown excellent properties for bone replacement, including ordered mesoporous structure, high textural properties, rapid bioactive response and the ability to release concentrations of strontium ions, which can stimulate the expression of early markers of osteoblastic differentiation.²⁴

For all these reasons, and given the extensive experience of the study group in the design and manufacture of this type of support, they were considered ideal candidates for carrying out this *in vivo* study.

The results obtained in the MBG group, both in the case of the simple radiology study and in the micro-CT study, showed that there was greater bone formation than in the case of the control without material, although not as much as in the bone marrow autograft group. This suggests that these scaffolds help in the formation of new bone, but are not sufficient on their own to replace bone marrow autograft.

Gil-Albarova et al.²⁵ reported in a study on a defect in a rabbit femur that bioactive glasses are capable of generating bone, although given their high bioactivity and porosity, could also be ideal for antibiotic release.

The interest in apatite-based materials for the development of bone substitutes is marked by their high acceptance as a biocompatible material given their similarity to the mineral component of bone,³ their osteoconductive capacity²⁶ and their good integration into the bone defect. The CHA component that makes up this scaffold, integrated within an agarose matrix, can be easily degraded, favouring its progressive replacement by neoformed tissue. The porosity of these supports is between 50 and 800 µm. This type of biomaterial, in turn, has been shown to present a surface suitable for the adhesion of pre-osteoblastic cells that could grow on the support without toxicity from any of the components that make up this material.³ For these reasons, this material was considered ideal for this *in vivo* study.

As for the results obtained from the radiological and micro-CT studies in the CHA group, they were essentially similar to those obtained in the case of the MBG. Again, more bone formation was shown than in the no-material control group, but not as much as in the bone marrow autograft group. These data are in agreement with those published by Oryan et al.²⁷ in which a study with the same model comparing hydroxyapatite-based scaffolds with control and PRP-enriched hydroxyapatite showed that the hydroxyapatite scaffolds showed greater bone formation than the controls without material, although less than in the case of the PRP-enriched hydroxyapatite scaffolds. In another *in vivo* study on rabbit femur using this type of scaffold, it was concluded that they allow osteoconduction with an appropriate range of resorption and with wide intraoperative adaptability, obtaining bone formation in all defects.²⁸

Once again, the scaffolds studied seem to improve bone formation with respect to the control group, but not enough to be considered bone substitutes with characteristics similar to autograft. This response may be due to the lack of osteoinductivity of this type of scaffold, which has been previously reported,²⁹ even with the same experimental model.³⁰

Although in terms of composition and macroscopic characteristics they are very different, no differences have been found in this preliminary study with regard to bone formation between the MBG-based biomaterials and those based on CHA. However, these macroscopic characteristics could make each of them more suitable for a specific type of defect. In the case of MBG-based scaffolds, they have greater initial strength, although on the other hand, they have less intraoperative adaptability to the defect, and the size of the defect must be planned in advance for the scaffold to fit it. For these reasons, this type of scaffold would be more suitable for defects involving cortical bone as well as medullary bone. However more *in vivo* studies are needed in this respect to confirm this assertion. In the case CHA-based scaffolds, they have a coralline consistency, which makes them less resistant initially, although they can be adapted to the defect intraoperatively, with the possibility of cutting the scaffold in the surgical act to adapt it to the defect. These characteristics, *a priori*, would position this type of scaffold as a more suitable option for defects that mainly involve cancellous bone.

In conclusion, we observed that both CHA-based and MBG-based scaffolds favour bone formation, but it is still far from being affirmed that they alone are capable of reproducing the characteristics of bone autograft. Our research group is working on the enrichment of these scaffolds with osteoinductive substances and progenitor cells to optimise the biological behaviour of these biomaterials and get closer to our ultimate goal of being able to use them as a substitute for bone autograft in our routine medical practice.

Level of evidence

Level of evidence I.

Funding

This project was funded by the Foundation of the Spanish Society of Orthopaedic Surgery and Trauma by means of the grant for research initiation projects 2020 and by the Carlos III Health Institute with the FIS grant number PI20/01384, IP co-financed with European Regional Development Fund (ERDF) funds from the European Union.

Conflict of interests

The authors have no conflict of interests to declare.

Ethics committee approval

In compliance with RD 1090/2015, the study was evaluated and approved by the Ethics Committee in research with experimental animals of the I+12 Research Institute, the Ethics Committee/ animal experimentation subcommittee of the Autonomous University of Madrid, registration number: ES 28079-0001164 and by the Directorate General of Agriculture, Livestock and Food of the Community of Madrid, registration number: ES280790001164.

References

- Bishop JA, Palanca AA, Bellino MJ, Lowenberg DW. Assessment of compromised fracture healing. *J Am Acad Orthop Surg.* 2012;20:273–82.
- Heras C, Jiménez-Holguín J, Doadrio AL, Vallet-Regí M, Sánchez-Salcedo S, Salinas AJ. Multifunctional antibiotic- and zinc-containing mesoporous bioactive glass scaffolds to fight bone infection. *Acta Biomater.* 2020;114:395–406.
- Paris JL, Lafuente-Gómez N, Cabañas MV, Román J, Peña J, Vallet-Regí M. Fabrication of a nanoparticle-containing 3D porous bone scaffold with proangiogenic and antibacterial properties. *Acta Biomater.* 2019;86:441–9.
- Trejo CG, Lozano D, Manzano M, Doadrio JC, Salinas AJ, Dapía S, et al. The osteoinductive properties of mesoporous silicate coated with osteostatin in a rabbit femur cavity defect model. *Biomaterials.* 2010;31:8564–73.
- Nandi SK, Kundu B, Ghosh SK, De DK, Basu D. Efficacy of nano-hydroxyapatite prepared by an aqueous solution combustion technique in healing bone defects of goat. *J Vet Sci.* 2008;9:183–91.
- Peña J, Román J, Victoria Cabañas M, Vallet-Regí M. An alternative technique to shape scaffolds with hierarchical porosity at physiological temperature. *Acta Biomater.* 2010;6:1288–96.
- Paris JL, Román J, Manzano M, Cabañas MV, Vallet-Regí M. Tuning dual-drug release from composite scaffolds for bone regeneration. *Int J Pharm.* 2015;486:30–7.
- Jiménez-Holguín J, Sánchez-Salcedo S, Vallet-Regí M, Salinas AJ. Development and evaluation of copper-containing mesoporous bioactive glasses for bone defects therapy. *Microporous Mesoporous Mater.* 2020;308:110454.
- Reichert JC, Saifzadeh S, Wullschlegel ME, Epari DR, Schütz MA, Duda GN, et al. The challenge of establishing preclinical models for segmental bone defect research. *Biomaterials.* 2009;30:2149–63.
- Lane JM, Sandhu HS. Current approaches to experimental bone grafting. *Orthop Clin North Am.* 1987;18:213–25.
- Lin JP, Shi ZJ, Shen NJ, Wang J, Li ZM, Xiao J. Serum N-terminal telopeptide of type I collagen as an early marker of fracture nonunion in rabbits. *Exp Ther Med.* 2016;12:3595–601.
- El-Rashidy AA, Roether JA, Harhaus L, Kneser U, Boccaccini AR. Regenerating bone with bioactive glass scaffolds: a review of in vivo studies in bone defect models. *Acta Biomater.* 2017;62:1–28.
- Emilov-Velev K, Clemente-de-Arriba C, Alobera-García MÁ, Moreno-Sansalvador EM, Campo-Loarte J. Regeneración ósea en animales de experimentación, mediante cemento de fosfato cálcico en combinación con factores de crecimiento plaquetarios y hormona de crecimiento humana. *Rev Esp Cir Ortop Traumatol.* 2015;59:200–10.
- Gugala Z, Gogolewski S. Regeneration of segmental diaphyseal defects in sheep tibiae using resorbable polymeric membranes: a preliminary study. *J Orthop Trauma.* 1999;13:187–95.
- Gugala Z, Lindsey R, Gogolewski S. New approaches in the treatment of critical-size segmental defects in long bones. *Macromol Symp.* 2007;253:147–61.
- Kasten P, Vogel J, Geiger F, Niemeyer P, Luginbühl R, Szalay K. The effect of platelet-rich plasma on healing in critical-size long-bone defects. *Biomaterials.* 2008;29:3983–92.
- Niemeyer P, Szalay K, Luginbühl R, Südkamp NP, Kasten P. Transplantation of human mesenchymal stem cells in a non-autogenous setting for bone regeneration in a rabbit critical-size defect model. *Acta Biomater.* 2010;6:900–8.
- García A, Cabañas MV, Peña J, Sánchez-Salcedo S. Design of 3D scaffolds for hard tissue engineering: from apatites to silicon mesoporous materials. *Pharmaceutics.* 2021;13:1981.
- Oonishi H, Hench LL, Wilson J, Sugihara F, Tsuji E, Matsuura M, et al. Quantitative comparison of bone growth behavior in granules of bioglass, A-W glass-ceramic, and hydroxyapatite. *J Biomed Mater Res.* 2000;51:37–46.
- Arcos D, Vallet-Regí M. Sol-gel silica-based biomaterials and bone tissue regeneration. *Acta Biomater.* 2010;6:2874–88.
- Gerhardt LC, Boccaccini AR. Bioactive glass and glass-ceramic scaffolds for bone tissue engineering. *Materials.* 2010;3:3867–910.
- Li J, You F, Li Y, Zuo Y, Li L, Jiang J, et al. Bone regeneration and infiltration of an anisotropic composite scaffold: an experimental study of rabbit cranial defect repair. *J Biomater Sci Polym Ed.* 2016;27:327–38.
- Vallet-Regí M, Salinas AJ. Mesoporous bioactive glasses for regenerative medicine. *Mater Today Bio.* 2021;11:100121.
- Jiménez-Holguín J, López-Hidalgo A, Sánchez-Salcedo S, Peña J, Vallet-Regí M, Salinas AJ. Strontium-modified scaffolds based on mesoporous bioactive glasses/polyvinyl alcohol composites for bone regeneration. *Materials (Basel).* 2020;13:5526.
- Gil-Albarova J, Salinas AJ, Bueno-Lozano AL, Giavaresi G, Fini M, Giardino R, et al. The in vivo behaviour of a sol-gel glass and

- a glass-ceramic during critical diaphyseal bone defects healing. *Biomaterials*. 2005;26:4374–82.
26. Dorozhkin SV. Nanosized and nanocrystalline calcium orthophosphates. *Acta Biomater*. 2010;6:715–34.
27. Oryan A, Meimandi Parizi A, Shafiei-Sarvestani Z, Bigham AS. Effects of combined hydroxyapatite and human platelet rich plasma on bone healing in rabbit model: radiological, macroscopical, histopathological and biomechanical evaluation. *Cell Tissue Bank*. 2012;13:639–51.
28. Gil-Albarova J, Vila M, Badiola-Vargas J, Sánchez-Salcedo S, Herrera A, Vallet-Regí M. In vivo osteointegration of three-dimensional crosslinked gelatin-coated hydroxyapatite foams. *Acta Biomater*. 2012;8:3777–83.
29. den Boer FC, Wippermann BW, Blokhuis TJ, Patka P, Bakker FC, Haarman HJTM. Healing of segmental bone defects with granular porous hydroxyapatite augmented with recombinant human osteogenic protein-1 or autologous bone marrow. *J Orthop Res*. 2003;21:521–8.
30. Yassine KA, Mokhtar B, Houari H, Karim A, Mohamed M. Repair of segmental radial defect with autologous bone marrow aspirate and hydroxyapatite in rabbit radius: a clinical and radiographic evaluation. *Vet World*. 2017;10:752–7.