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Intracutaneous irritation assessment of a zirconia barrier material (Zirbone) for bone augmentation: rabbit study according to ISO 10993-10:2010 and ISO 10993-12:2021

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Abstract

Zirconia barriers have been proposed as an alternative to titanium meshes for guided bone regeneration (GBR) and bone augmentation; however, comprehensive biocompatibility testing is essential before their clinical application. This study assessed the intracutaneous irritation potential of a zirconia barrier device (Zirbone) in accordance with ISO 10993 10. Polar (physiological saline) and non-polar (cottonseed oil) extracts of Zirbone were prepared following ISO 10993 10 and ISO 10993 12 at an extraction ratio of 3 cm²/mL for 72 ± 2 hours at 37 ± 1 °C with gentle agitation. Three young, healthy New Zealand albino rabbits (of the same sex, weighing ≥ 2 kg) were used. For each animal, 0.2 mL of polar and non-polar extracts were injected intracutaneously at five sites on one flank, and 0.2 mL of the corresponding extraction media alone were injected at five sites on the contralateral flank. Erythema and edema were evaluated at 24, 48, and 72 hours using standardized scales, and the primary irritation index (PII) was calculated for each extract by comparison with its control solvent. No erythema or edema was observed at any sites injected with the polar solvent or polar Zirbone extract during the 72-hour observation period. Conversely, very slight to well-defined erythema without edema was observed at sites injected with the non-polar solvent and non-polar Zirbone extract at all time points across all animals. The PII was 0.00 for the polar extract and 0.07 for the non-polar extract, both significantly below the ISO 10993 10 acceptance threshold of 1.0 for non-irritant materials. These results indicate that the polar and non-polar extracts of the zirconia barrier device Zirbone are non-irritant in accordance with ISO 10993 10, thereby supporting its local biocompatibility and potential safe application as a barrier material in GBR and bone augmentation procedures.

Keywords: Zirconia barrier; Intracutaneous irritation; Guided bone regeneration; ISO 10993-10.

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Background

Guided bone regeneration (GBR) and bone augmentation procedures typically employ barrier membranes or shields to stabilize grafts, safeguard regeneration sites, and facilitate selective cell repopulation[1,2]. These barriers are indispensable for achieving adequate bone volume and contour to support implant placement and ensure long-term functionality[3]. Conventional GBR membranes include resorbable collagen and non-resorbable, titanium-reinforced materials; however, each category presents limitations such as handling characteristics, risk of exposure, or the necessity for removal[4,5]. Titanium meshes and titanium-reinforced membranes have been extensively utilized for horizontal and vertical ridge augmentation and are capable of attaining substantial bone gain. Nonetheless, mesh exposure remains a prevalent complication, with reported rates varying from 5% to 60%, depending on the defect type, flap management, and mesh design[5–9]. Early or extensive exposures may result in bacterial contamination, graft resorption, and the premature removal of the mesh, thereby potentially compromising regenerative outcomes[5–8].

Customized zirconia barriers have been introduced as a potential alternative, offering high mechanical strength, dimensional stability, lower biofilm adhesion, and favorable soft-tissue integration, with promising preclinical and early clinical results in horizontal ridge augmentation and maxillary defects [10–13]. Recent experimental studies have shown that customized zirconia barriers can achieve comparable or greater new bone formation than titanium meshes and d-PTFE membranes, without evidence of foreign-body reactions [10,11,14].

Prior to clinical application, these devices are required to undergo comprehensive biological evaluation in accordance with the ISO 10993 series, which includes irritation and sensitization assessments (ISO 10993-10) as well as standardized extraction procedures (ISO 10993-12) [15,16]. Intracutaneous irritation tests conducted in rabbits evaluate the potential of leachables from medical devices to provoke local erythema and edema subsequent to the injection of polar and non-polar extracts [15].

Zirbone is a zirconia-based medical device designed as a barrier for bone augmentation and GBR. As part of its preclinical safety evaluation, we conducted an intracutaneous irritation test in rabbits using polar and non-polar extracts of Zirbone prepared according to ISO 10993-10 and ISO 10993-12.

The aim of this study was to determine the intracutaneous irritation potential of polar and non-polar Zirbone extracts in rabbits, and to assess whether the device meets the ISO 10993-10 acceptance criteria for non-irritant materials.

Methods

Test material

The test item consisted of a zirconia-based barrier device (Zirbone) designed for use in bone augmentation and Guided Bone Regeneration (GBR) procedures. Test extracts were obtained from samples with a total surface area of 4.7 cm², accurately representing the clinically pertinent configuration of the device.

Animals and ethics

Three young, healthy albino New Zealand rabbits of the same sex, each weighing at least 2 kg at the commencement of the study, were utilized. The animals were sourced from approved breeders (Granja San Bernardo, Tulebras, Navarra, Spain, or CEGAV, France) and were acclimatized for a minimum of five days prior to dosing under standardized housing and feeding conditions.

The study plan received approval from Ethics Committee No. 76 and was conducted in accordance with the European Communities Council Directive 2010/63/EU and French Decree No. 2013-118 concerning the protection of animals used for scientific purposes. Animal husbandry and procedures adhered to institutional standard operating procedures to minimize pain and distress. Upon completion of the observation period, animals were euthanized using sodium pentobarbital (Dolethal).

Preparation of extracts

Extraction preparation adhered to ISO 10993-10 (Appendix A) and ISO 10993-12 [15,16]. Two extraction media were employed:

- Polar solvent: physiological saline solution.
- Non-polar solvent: cottonseed oil.

The extraction ratio was 3 cm² of device surface area per mL of solvent. Samples were incubated at 37 ± 1 °C for 72 ± 2 hours under gentle agitation. In parallel, each extraction medium alone (without device) was processed under identical conditions to provide control solutions.

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Intracutaneous administration

Approximately 24 hours prior to dosing, the dorsal and lateral regions of the trunk of each rabbit were shaved utilizing electric clippers equipped with a fine comb to expose an area of approximately 10 × 20 cm of skin. Animals exhibiting visible skin lesions were omitted from the study and replaced accordingly.

For each rabbit, 0.2 mL of the polar and non-polar Zirbone extracts were administered intracutaneously at five distinct sites on a single flank. Conversely, on the opposite flank, 0.2 mL of the polar solvent and 0.2 mL of the non-polar solvent (serving as controls) were each administered intracutaneously at five sites under identical conditions. Accordingly, each subject functioned as its own control.

Clinical observations and scoring

Animals were observed immediately after dosing and at 24 ± 2, 48 ± 2, and 72 ± 2 hours post-injection. At each time point, a macroscopic examination of the injection sites was conducted under consistent lighting to evaluate erythema, eschar formation, and edema, as well as to document any indications of systemic toxicity, including behavioral changes, neuro-vegetative alterations, and reduced food intake.

Erythema was scored on a 0–4 scale:
- 0: No erythema
- 1: Very slight (barely perceptible) erythema
- 2: Well-defined erythema
- 3: Moderate erythema
- 4: Severe erythema (beet-red) to eschar formation preventing grading

Edema was scored on a 0–4 scale:
- 0: No edema
- 1: Very slight edema
- 2: Slight edema, contour clearly defined by marked swelling
- 3: Moderate edema (thickness approximately 1 mm)
- 4: Severe edema (thickness greater than 1 mm and surface larger than the application zone)

Primary irritation index

For each animal, mean erythema and edema scores were calculated for each set of five test sites (polar extract, non-polar extract) and corresponding control sites (polar solvent, non-polar solvent) at each observation time. The primary irritation index (PII) for each extract was obtained by averaging the differences between test and control scores across animals and time points, in line with ISO 10993-10[15].

Results

General observations

All animals remained in good general condition throughout the study. No behavioral abnormalities, neuro-vegetative alterations, or decreased food intake suggestive of systemic toxicity were observed following intracutaneous injection of test extracts or control solutions.

Cutaneous reactions

No erythema or edema was observed at any sites injected with the polar solvent (physiological saline) at 24, 48, or 72 hours. Similarly, no cutaneous reactions were recorded at any sites injected with the polar Zirbone extract at any observation time. At sites treated with the non-polar solvent (cottonseed oil) and non-polar Zirbone extract, minimal (score 1) to well-defined (score 2) erythema was observed at 24, 48, and 72 hours in all subjects, with no accompanying edema. The degree of erythema did not escalate over time and stayed within the lower ranges of the scoring scale.

Primary irritation index

The PII values derived from erythema and edema scores are summarized in Table 1.

Extract type	PII	Classification (ISO 10993-10)
Polar extract	0.00	Non-irritant
Non-polar extract	0.07	Non-irritant (PII < 1.0)

Table 1. Primary irritation index values and classification according to ISO 10993-23:2021.

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Discussion

This investigation assessed the intracutaneous irritation potential of polar and non-polar extracts derived from a zirconia barrier device (Zirbone) in rabbits, in accordance with ISO 10993-10 and ISO 10993-12[15,16]. The absence of erythema and edema at the injection sites with the polar extract, coupled with a primary irritation index (PII) of 0.00, indicates excellent local tolerance of aqueous leachables from the device. These results are consistent with the favorable soft-tissue compatibility documented for zirconia in oral applications and for customized zirconia barriers utilized in guided bone regeneration (GBR) [10–13].

In contrast, non-polar injections (cottonseed oil and non-polar Zirbone extract) produced only very slight to well-defined erythema without edema, yielding a very low PII of 0.07 for the non-polar extract, well below the ISO 10993-10 acceptance limit of 1.0[15]. Mild erythema associated with oily vehicles is a recognized finding in intracutaneous irritation tests and often reflects the vehicle rather than the test article itself[15]. The similarity between non-polar solvent and non-polar extract reactions supports the conclusion that Zirbone does not add relevant irritation beyond the inherent effect of the cottonseed oil vehicle.

Titanium meshes and titanium-reinforced membranes are extensively employed as non-resorbable barriers for both horizontal and vertical ridge augmentation procedures, and are regarded as effective in facilitating significant bone gain [5–9,17]. However, the exposure of titanium meshes remains a common and clinically significant complication. Reported rates of exposure vary from approximately 5–20% in certain series to 50–60% in others, influenced by factors such as defect type, flap management, and mesh design [5–9]. Early or extensive exposures may result in bacterial contamination, graft resorption, and the premature removal of the mesh, thereby potentially jeopardizing the regenerative outcome [5–8]. Even when adequate bone augmentation is ultimately attained, practitioners often need to manage prolonged wound care and increased patient morbidity associated with mesh exposure and soft-tissue dehiscence [5–9,17].

Recent experimental and clinical investigations suggest that customized zirconia barriers may provide both biological and technical benefits in this setting[10–14]. Zirconia surfaces demonstrate lower biofilm adhesion and elicit a more favorable fibroblast response compared to titanium, which potentially contributes to decreased inflammation and enhanced soft-tissue stability surrounding the barrier[10,14]. In vivo studies comparing customized zirconia barriers with titanium meshes and d-PTFE membranes have reported equal or superior new bone formation in the zirconia groups, without evidence of foreign-body reactions or necrosis[10,11,14]. Furthermore, a randomized clinical trial and case series have indicated that customized zirconia barriers can achieve clinically significant bone augmentation and may serve as a viable alternative to titanium-based devices in selected defects[11–13].

The current irritation data complement these findings by addressing a specific aspect of biological safety: the potential of device extracts to induce local skin irritation under exaggerated exposure conditions. Although the present study does not directly compare Zirbone with titanium meshes in vivo, the non-irritant profile of Zirbone extracts, together with the reported soft-tissue advantages of zirconia barriers, suggests that zirconia may help mitigate some soft-tissue complications associated with titanium meshes, particularly in cases with thin biotypes and elevated exposure risk[5–10,14]. Nevertheless, long-term clinical data and controlled comparative trials are necessary to ascertain whether zirconia barriers can reliably reduce exposure rates and enhance overall GBR predictability in comparison with titanium meshes[5,9,17].

Standardized extraction and irritation testing in accordance with ISO 10993-10 and ISO 10993-12 are essential components of the evaluation process for medical devices, particularly for innovative barrier designs intended for intraoral application [15,16]. Future research should focus on implantation studies and controlled Guided Bone Regeneration (GBR) models that directly compare zirconia barriers with titanium meshes, in order to provide a comprehensive assessment of the associated risks and benefits [5,9,17,18]. Within the constraints of the current study, our results support the local biocompatibility of Zirbone and align with the concept of zirconia serving as a biologically acceptable alternative to titanium-based meshes in bone augmentation procedures [5–14,17].

Conclusions

The polar and non-polar extracts of the zirconia barrier device Zirbone exhibited no edema and only minimal erythema during an intracutaneous irritation test conducted on rabbits. The primary irritation indices recorded were 0.00 for the polar extract and 0.07 for the non-polar extract, both of which are below the ISO 10993-10 threshold of 1.0 and therefore indicate a non-irritant classification. These findings substantiate the local biocompatibility of Zirbone and its potential safety for use as a barrier material in guided bone regeneration and bone augmentation procedures. Additional comparative studies in-

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volving titanium meshes within clinical GBR models are recommended to assess the relative benefits of zirconia barriers in minimizing soft-tissue complications and enhancing regenerative outcomes.

Abbreviations

GBR: Guided bone regeneration; PII: Primary irritation index; d-PTFE: Dense polytetrafluoroethylene

Declarations

Ethics approval and consent to participate

The study received approval from the registered Ethics Committee No. 76 and was conducted in strict accordance with the European Communities Council Directive 2010/63/EU and French Decree No. 2013-118 concerning the protection of animals used for scientific purposes.

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Joseph A. NAMMOUR and Nabih A. NADER: Conceptualization, study design, supervision of animal experiments, data analysis, manuscript drafting and critical revision. Both authors read and approved the final manuscript.

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