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Instituto de Investigación Sanitaria Gregorio Marañon

Hospital General Universitario Gregorio Marañón

uc3m Universidad Carlos III de Madrid

Study of the biocompatibility of biodegradable metals in vivo using CT and flow cytometry

Cussó L[1, 2], **Millán-Casas M**[1], de Francisco A[1, 2], Limones B[3], Castro A[3], Sevostianova V[3], Tang S[3], Bell O[3], Corchado C[3], Li M[3], Pöstges S[4], Kopp A[4], Molina J[3], Patterson J[3], Desco M[1, 2, 5]

[1] Unidad de Medicina y Cirugía Experimental. Instituto de Investigación Sanitaria Gregorio Marañón, Madrid, Spain

[2]CIBER de Salud Mental, Instituto de Salud Carlos III, Madrid, Spain

[3]Fundación IMDEA Materiales (IMDEA) , Madrid, Spain

[4]MEOTEC GMBH (MEO), Aachen, Germany

[5]Departamento de Bioingeniería. Universidad Carlos III de Madrid, Madrid, Spain

Email: lcusso@hggm.es

Web: https://image.hggm.es

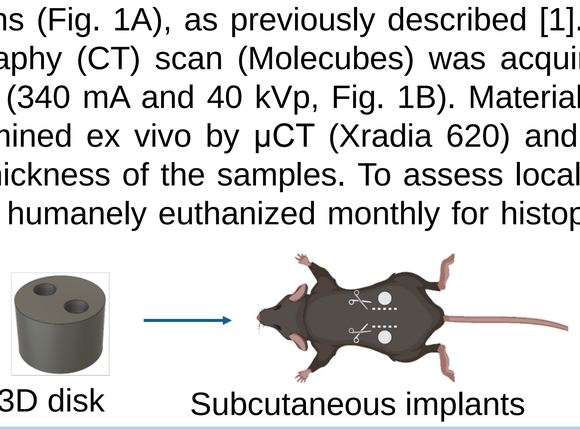
INTRODUCTION

Craniosynostosis is characterized by the premature fusion of the calvarial sutures, which can lead to increased intracranial pressure, permanent brain injury, mid-facial hypoplasia, and orbital deformation. New devices are needed to minimize risks associated with large open surgeries and also to reduce the number of surgeries performed on patients. Therefore, the need to search for new materials that are capable of in vivo degradation to develop 4D smart metallic actuators for expansion therapy is critical. Such an advance would represent a technological improvement, enhancing patient quality of life and reducing healthcare costs. **The main objective of this study was to assess the in vivo biocompatibility of biodegradable metals for shape-morphing devices for the treatment of craniosynostosis using CT and flow cytometry.**

METHODS

Experimental design. A total of 72 male C57BL/6 mice were subcutaneously implanted with 3 mm disks bilaterally on their back flanks. Implants included two types: single-material disks fabricated using either conventional manufacturing (extrusion and turning) or additive manufacturing (AM). The multi-material disks (Groups A: Conventional WE43 PEO + AM Zn1Mg PEO; B: AM nitinol + AM WE43 PEO; C: AM nitinol + Conventional WE43 PEO) were created by suturing separately fabricated disks of each material together using braided 6/0 non-absorbable silk (Lorca Marin, S.A.).

The systemic immune response was evaluated via flow cytometry (FC) on peripheral blood samples collected at baseline (Day 0), 15 days, and monthly thereafter up to 3 months (Fig. 1A), as previously described [1]. An in vivo whole-body computed tomography (CT) scan (Molecubes) was acquired monthly for all groups up to 3 months, (340 mA and 40 kVp, Fig. 1B). Material degradation rates were additionally determined ex vivo by µCT (Xradia 620) and by measuring the change in weight and thickness of the samples. To assess local toxicity, two to six animals per group were humanely euthanized monthly for histopathology and ICP-OES analyses (Fig. 1C).



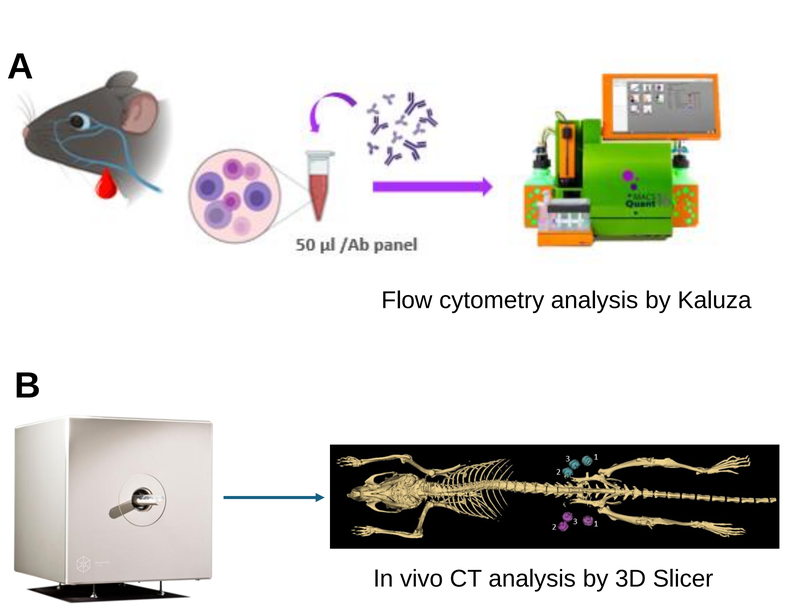


Figure 1. Experimental design. (A) FC was analyzed before implantation (day 0), 15, 30 60- and 90-days post-implantation. (B) CT was acquired at 30, 60 and 90 days. (C) Histology and inductively coupled plasma optical emission spectroscopy (IPC-OES)

RESULTS

The animals showed no clinical indications of discomfort following implantation or throughout the 90-day follow-up period. Animal weight increased significantly over the study duration, with no differences observed between groups. In vivo CT (Fig 2) showed no differences in disk volume or radiological density over time. Ex vivo high-resolution µCT of the explanted disks, however, revealed some degradation of the WE43 samples.

Peripheral blood FC (Fig 3) demonstrated a significant effect of Time on the immunological patterns (both lymphoid and myeloid subsets). However, the interaction between Time and Group (i.e., material type) was nonsignificant for all variables. This indicates that the immunological patterns evolved similarly across all groups, consistent with the normal aging process of the animals and not attributable to the implants.

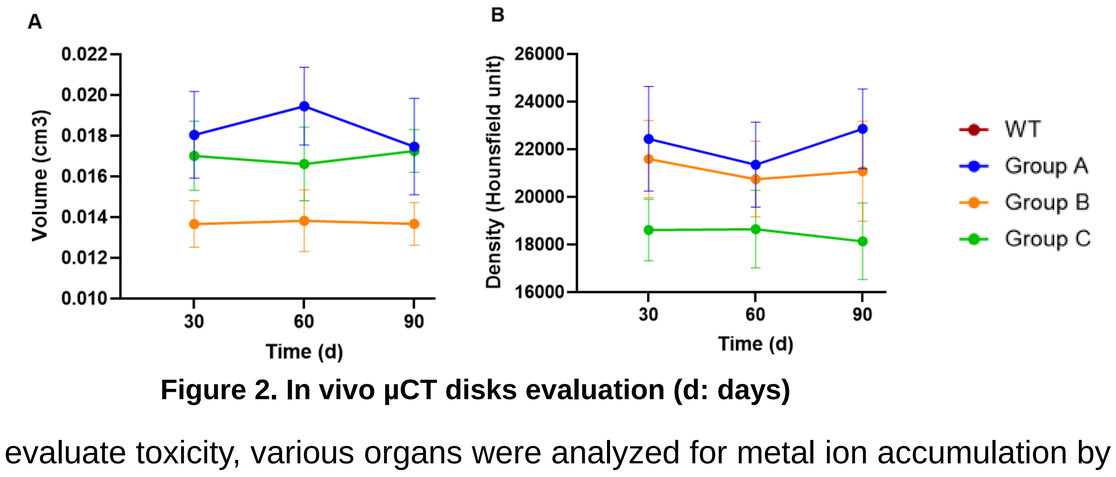


Figure 2. In vivo µCT disks evaluation (d: days)

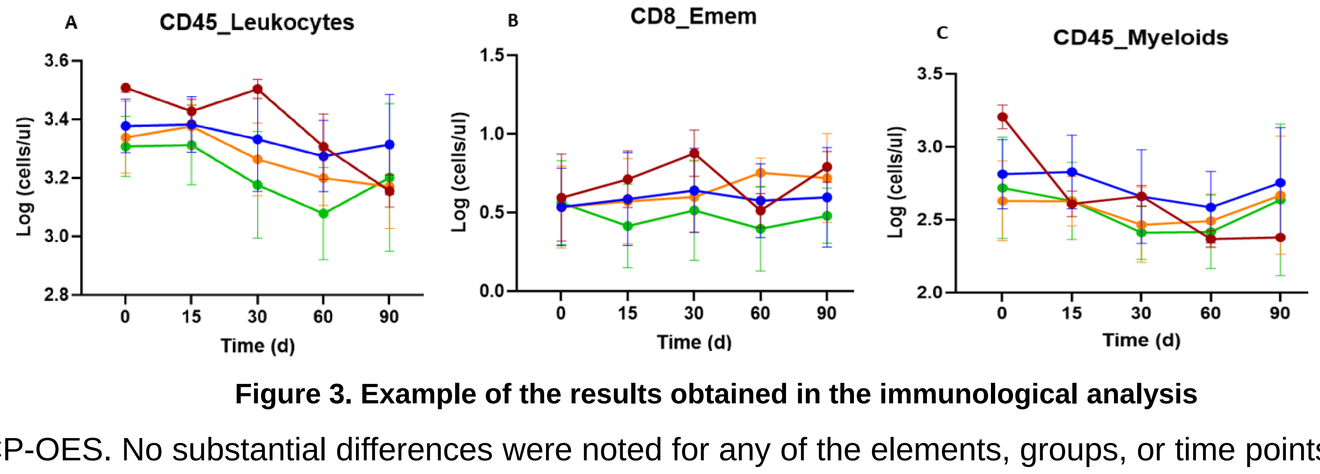


Figure 3. Example of the results obtained in the immunological analysis

To evaluate toxicity, various organs were analyzed for metal ion accumulation by ICP-OES. No substantial differences were noted for any of the elements, groups, or time points compared to healthy animals (WT). Furthermore, H&E tissue staining of the explanted organs showed no evidence of inflammation, necrosis, vascularity alterations, or changes in organ size. Skin from the subcutaneous implantation site exhibited normal tissue morphology and no signs of local inflammation.

[1] Barco A., et al Front. Immunol. 2024

CONCLUSIONS

In consequence**, this study concludes that no evidence of altered biocompatibility, as defined by an immune or local response to the implants, was found for any of the materials tested.**

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