Minocycline-releasing PMMA for craniofacial bone reconstruction - in vitro characterization

Silva T1, Ginho L3, Barros J1, Silva J1, Pinto R3, Colaço B, Fernandes H1, Bettencourt A3, Gomes PS3

1 Laboratory for Bone Metabolism and Regeneration, Faculty of Dental Medicine, U Porto, Porto.
2 INEB-IDIBS - Institute of Investigation and Innovation in Health, Porto.
3 Research Institute for Medicines (Med.U.Lisboa), Faculty of Pharmacy, Lisbon University, Lisbon.
4 Department of Animal Science, University of Trás-os-Montes e Alto Douro, Vila Real.

Introduction/Objective

The repair of complex craniofacial bone defects is challenging and a successful result depends on the defect size, the quality of the soft tissue covering the defect, and the choice of reconstructive method. [1] Defects or deficiencies of the craniofacial skeleton are quite common, and may be due to congenital or acquired causes. Common aetiologies include post-surgical defects following tumour resection or ablative, trauma, and a large number of congenital anomalies. [1, 3] These lesions can be met with the development of infections, loss of local blood supply, and massive tissue necrosis that reduces the tissue viability and difficulty in any kind of successful regeneration. [4-6] Generally, the treatment of these lesions is based on multiple reconstructive stages (staged repair) that despite the difficulties found (e.g., distortion of anatomical references, loss of volume defect and wound contamination) can produce a greater outcome in aesthetic/functional regeneration than the conventional approach of single stage procedures. With an objective to optimize the regenerative approaches and minimise the related complications, a multi-stage approach is envisaged with the addition of a bioceramic that can be used as a space maintainer. [5, 7] The use of space maintainers based in poly(methylmethacrylate) (PMMA) has been applied successfully in craniofacial reconstructions. [8, 9] Successful space maintenance involves the prevention of soft tissue collapse, combined with mechanical support of the defect. [10] Furthermore, ceramics can be combined with bioactive agents, for instance antibacterial agents, in order to further display additional properties – in the case, antibacterial activity. Tetracyclines have been associated with distinct biomaterials for the management or prevention of biomaterial-associated tissue infections and to prospectively enhance the local bone tissue response upon implantation [11, 12].

In the present work, it is aimed the development and biological characterization of a poly methyl methacrylate (PMMA)-based minocycline delivery system, to be used as a space maintainer within craniofacial staged regenerative interventions.

Materials and Methods

Three different bone cement (BC) specimens were obtained: control BC and BC loaded with minocycline at 1% and 2.5%, relative to the weight of BC powder, herein designated minocycline-loaded bone cement (MBC1) and MBC2.5, respectively. BCs were characterized for their mechanical release and assayed in vitro for anti-bacterial and anti-inflammatory activity, and biocompatibility with human bone cells.

Minocycline release: The release study was carried out in a 1:2 HPC system (Shimadzu system LC-6A, Shimadzu Corporation). Chromatographic analysis was performed at a detection wavelength set at 252 nm. The cumulative release (µg/ml) was expressed as the total minocycline released over time.

Antibacterial activity: Reference strains S. epidermidis (ATCC® 29223), slime-producer S. epidermidis R26A (ATCC® 35994) and Escherichia coli (ATCC® 25922) were used and grown in Tryptic Soy Broth, at 37 ºC for 12 h. Bacteria were grown directly over material samples and assayed also on materials effluents, up to 72 hours. Quantitative evaluation were conducted with the renaturation assay using a Leica MPS confocal microscope (n=3).

Cell compatibility assessment: MC3H human osteoblastic cells (ATCC® CRL-1427) were grown in α-MEM (Gibco, Germany) supplemented with foetal bovine serum (10% V/V), 100 IU/mL penicillin and 2.5 μg/ml streptomycin. Cultures were maintained at 37 ºC, in a 95% air and 5% CO2 humidified atmosphere. At adequate confluency, cells were harvested and seeded over the bone cement material samples, at a density of 106 cells/cm², for 7 days.

Results

Attained results converge to support the possible efficacy of the developed MBC systems for the clinical management of complex craniofacial trauma, in which biomaterials with space maintenance properties are necessary for the management of staged reconstructive approaches, thus minimizing the risk of periprosthetic infections and enhancing the local tissue healing. Regarding the release profile of minocycline, both MBC1 and MBC2.5 formulations showed an initial burst release, which is a convenient profile, thus minimizing the risk of infection in the immediate post-operative period. [13] The 72 hour release profile is of interest. Also, increased metabolic activity and high ALP activity were verified for MBC formulations, sustaining an enhanced osteoblastic response. In terms of inflammation-modulatory capacity, MBC were found to significantly reduce the macrophage production of NO and TNF-alpha, following LPS activation. [15] Furthermore, no significant differences were found between the biological activity of MBC1 and MBC2.5 compositions.

Discussion

The developed PMMA systems, with controlled release of minocycline, allowed for an effective antibacterial activity against strains relevant within trauma-related infections. Furthermore, an improved osteoblastic cell response - with enhancement of cell adhesion and cell proliferation - and increased anti-inflammatory activity were verified for MBC, as comparing to control BC.

Conclusions

The developed PMMA systems, with controlled release of minocycline, allowed for an effective antibacterial activity against strains relevant within trauma-related infections. Furthermore, an improved osteoblastic cell response - with enhancement of cell adhesion and cell proliferation - and increased anti-inflammatory activity were verified for MBC, as comparing to control BC.

References

5. Altice Arena, Lisbon
6. Laboratory for Bone Metabolism and Regeneration
7. Minocycline-releasing PMMA for craniofacial bone reconstruction - in vitro characterization
8. Silva T1, Ginho L3, Barros J1, Silva J1, Pinto R3, Colaço B, Fernandes H1, Bettencourt A3, Gomes PS3

Fig. 1 - Cumulative minocycline release over time: A=6 hours; B=2 weeks.

Fig. 2 - Grown bacteria by culturable method (A) and metabolic active bacteria (B) on MBC, at 24 h of culture.

Fig. 3 - C and D - Confluent images of bacteria grown on assayed formulations, following live/dead staining – viable bacteria stained green and nonviable bacteria stained red. Scale bar corresponds to 10 μm.

Fig. 4 - Osteoblastic cytocompatibility assay by CLSM and SEM micrographs - cell area determination.

Fig. 5 - Osteoblastic cytocompatibility by MTT reduction, ALP activity and time course confocal imaging following cytoskeleton (green) and nucleus (red) staining.

Fig. 6 - Determination of nitric oxide - NO (method of Griess - Griess Reagent System, Promega) and TNF-alpha (ELISA Kit, Mouse, Thermo Fisher Scientific) determination, in accordance with the manufacturer's instructions.

Statistical analysis: The non-parametric Kruskal-Wallis test was used to compare the results between samples on the same experimental day, using SPSS software (v 20.0 for Windows; SPSS Inc., Chicago, IL). For all tests, the level of significance was p < 0.05 and represented graphically as * - significant different from BC, p<0.05.