11º CONGRESSO NACIONAL DE BIOMECÂNICA
A. P. G. Castro, C. Pina, P. R. Fernandes, C. Santos, J. Folgado & A. Amaro (Eds)
Sesimbra, Portugal, 21-22 de Fevereiro, 2025

PRELIMINARY EVALUATION OF OSTEOBLASTIC-LIKE CELL RESPONSE TO BONE GRAFT SUBSTITUTES IN THE CONTEXT OF IMPLANT BIOMATERIALS

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KEY WORDS: Biomaterials; bone regeneration; osteoconduction; implant biomaterial

1 INTRODUCTION

The science of implantology is a multi-faceted field that integrates biological, mechanical, morphological, and physico-chemical considerations, all of which are constantly evolving to optimize the treatment protocols. An important biological aspect of implant dentistry is the dependence on sufficient bone volume, as patients indicated for implant therapy often require bone regenerative procedures [1]. As a result, bone substitutes are frequently subjected to research and development to optimize regenerative outcomes. Bone substitutes can be classified broadly based on their origin: autografts, allografts, xenografts, or synthetic grafts; or by their regenerative capacities: osteoconductive, osteoinductive, or osteogenic; serving different purposes based on the desired treatment outcomes [2]. At the same time, advancements in implant biomaterials, such as titanium alloys, hybrid titanium-zirconia, and zirconia, offer a wide range of choices for clinicians [3]. However, the biological response to various combinations of bone graft substitutes and implant biomaterials remains largely unexplored, presenting a critical gap in the literature. In accordance, the present research aims to access the biological response of distinct combinations of bone graft substitutes and implant biomaterials, through an *in vitro* model of human osteoblastic-like cells. In this preliminary assay, different bone grafts were primarily evaluated for their cytocompatibility.

2 MATERIALS AND METHODS

For this study, a xenograft (XENO) (Cerabone™, Botiss, Germany); a synthetic biphasic calcium phosphate (BCP) (Maxresorb™, Botiss, Germany); and a synthetic bioactive glass (BG) (Novabone Dental Putty™, Osteogenics, USA) were used. The materials underwent surface characterization with SEM (scanning electron microscopy) and EDS (energy dispersive spectroscopy) to analyze their morphological and elemental properties, respectively. Following, human osteoblastic-like cells (MG63) cultured in polystyrene tissue culture plates, were indirectly exposed to the graft materials. A control condition, in the absence of graft materials, was established. Subsequently, the cultures were characterized, at various timepoints, as follows:

- Metabolic activity assay (on day 1,4 and 7), to assess the cellular viability by using the AlamarBlue® assay. This assay is based on a reduction reaction carried out by metabolically active cells, providing an insight into the cellular viability and functionality.

- Alkaline phosphatase activity assay (on day 7), to access the cellular differentiation. Alkaline phosphate (ALP) serves as an important biomarker during early-stage osteoblastic differentiation.

The results were analyzed with one-way ANOVA, followed by Tukey test as the post hoc (alpha=0.05).

3 RESULTS

3.1 MATERIAL CHARACTERIZATION

The XENO and BCP materials exhibited a more granular organization with highly irregular surfaces, showcasing varying degrees of microporosity - noticeably higher in the BCP (Figure 1). Elemental analysis of these materials confirmed high levels of calcium and phosphorus. In contrast, BG displayed a more uniform amorphous appearance (Figure 1). Elemental analysis identified not only the calcium and phosphorus peaks, but also significant levels of silicone.

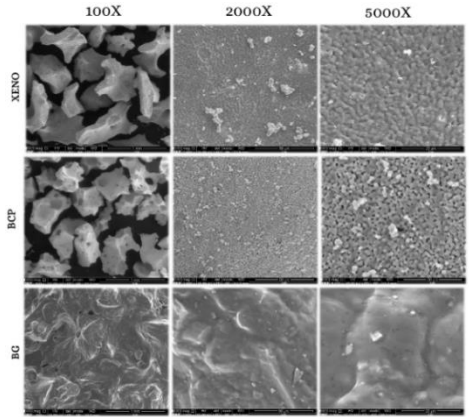

Figure 1: Representative micrographs of bone graft substitutes at various magnifications

3.2 CYTOCOMPATIBILITY ASSAYS

Metabolic activity assay revealed a general upward trend for all groups on day 1, 4 and 7 with significantly lower activity in BG on days 4 and 7, when compared to control. ALP activity assay on day 7 revealed significantly lower activity in BG and XENO, when compared to control.
The results obtained were in agreements with works of Günther [4] and Kubler [5].

4 CONCLUSIONS

This study was conducted to characterize the bone graft materials and analyse their biological response with osteoblastic-like cells, prior to the evaluation of the cell response within the more complex microenvironment of implant biomaterials combined with graft materials. Based on the attained data, it can be inferred that the differences in the origin and physico-chemical properties of the bone graft materials can significantly influence their biological outcomes.

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