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Original Article

Adverse effect of beta-tricalcium phosphate with zeta potential control in repairing critical defects in rats' calvaria☆

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ABSTRACT

Objective: To evaluate whether a new biphasic cement composed of calcium sulfate and beta tricalcium phosphate with zeta potential control could induce or lead to bone neoformation in critical defects.

Methods: A critical defect of diameter 8 mm was made in the calvaria of forty male Wistar rats. In the Test Group ($n = 20$), the defects were filled with cement. In the Control Group ($n = 20$), the defect was not filled and only coagulum was present. The animals were sacrificed 7, 14, 21 and 42 days after the operation. Calvaria specimens were subjected to microtomography and were then prepared for histological analysis. The analyses included morphological assessment on the histopathology of the repair; comparative morphometric evaluation of the area of formation of bone trabeculae between the groups; and histochemical staining by means of tartrate-resistant phosphatase (TRAP) in order to identify osteoclasts.

Results: Microtomographic images of the defects filled by the cement did not show any decrease in area over the course of postoperative evolution. In the Test Group, the material continued to present a foreign-body response until the last observational periods. Histomorphological analysis showed that there were more significant groupings of giant cells in the Test Group and greater maturity of neoformed bone in the Control Group. Exogenous material was also present. Histomorphometric analysis showed that in the Control Group, the total area of bone neoformation was significantly greater ($p = 0.009$) and grew progressively. The giant cells presented a positive reaction to TRAP but no osteoclasts were observed.

☆ Study carried out at the Department of Surgery, Prosthesis and Oral and Maxillofacial Traumatology, Faculdade de Odontologia, Universidade de São Paulo (USP), São Paulo, SP, Brazil.

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Conclusion: The ceramic cement did not induce or lead to bone neoformation from the microtomographic or histological point of view.

Efeito adverso do beta-fosfato tricálcico com controle de potencial zeta no reparo de defeitos críticos em calvária de ratos

Palavras-chave:
Regeneração óssea
Ratos Wistar
Materiais biocompatíveis
Potencial zeta

RESUMO

Objetivo: Avaliar se um novo cimento bifásico composto por sulfato de cálcio e beta fosfato tricálcico com controle de potencial zeta poderia induzir ou conduzir a neoformação óssea em defeitos críticos.

Métodos: Foi feito um defeito crítico de 8 mm de diâmetro na calvária de 40 ratos Wistar machos. No grupo teste (n = 20) os defeitos foram preenchidos pelo cimento. No grupo controle (n = 20) os defeitos não foram preenchidos, permaneceu apenas o coágulo. Os animais sofreram eutanásia em 7, 14, 21 e 42 dias do pós-operatório. Espécimes da calvária foram microtomografados e posteriormente preparados para análise histológica. As análises incluíram a avaliação morfológica da histopatologia do reparo e a avaliação morfométrica da área de formação das trabéculas ósseas comparativamente entre os grupos e coloração histoquímica por meio da fosfatase tartrato-resistente (TRAP) para identificação de osteoclastos.

Resultados: As imagens microtomográficas dos defeitos preenchidos pelo cimento não apresentaram diminuição da área de acordo com a progressão dos períodos pós-operatórios. No grupo teste houve permanência do material e resposta corpo estranho até os últimos períodos de observação. A histomorfologia mostrou agrupamentos mais expressivos de células gigantes no grupo teste e osso neoformado mais maduro no grupo controle e comprovou a presença de material exógeno. Na histomorfometria, a área total de neoformação óssea foi significativamente maior (p = 0,009) e crescente no grupo controle. As células gigantes apresentaram expressão histoquímica positiva para TRAP e não foram observados osteoclatos.

Conclusão: O cimento cerâmico não induziu ou conduziu a neoformação óssea sob o ponto de vista microtomográfico e histológico.

Introduction

Autogenous graft is still the material of choice for the reconstruction of bone tissue loss in orthopedic and maxillofacial surgery. However, increase in operation time, surgical trauma and possible complications inherent to the donor area approach does not always make it feasible. New biomaterials and substances that can mimic the characteristics of the autogenous bone tissue have been a constant pursuit of bioengineering.

Among the alloplastic materials most often used nowadays are bioceramics, mainly hydroxyapatite and beta-tricalcium phosphate (β-TCP). The latter shows a more rapid biodegradation than hydroxyapatite and in some situations this may be a more advantageous characteristic for a biomaterial, mainly when there is no need for mechanical strength. Moreover, beta-tricalcium phosphate has been widely used as a carrier or scaffold in tissue engineering.

Recently, a biphasic ceramic material consisting of calcium sulfate and beta-tricalcium phosphate with a negative surface charge, called zeta potential control, was launched in the international market with the proposal of making beta-tricalcium phosphate an inductive bone substitute and thus, promote bone regeneration.[1]

According to the manufacturer,[2] this ceramic is fully synthetic and has what they called "intelligent porosity", which facilitates cell growth and nutrient distribution in the extracellular matrix internally in the macroporosities of this compound. Some authors have demonstrated intense bone regeneration capacity in vertebral defects in sheep.[1]

The osteogenic potential of this compound, however, has been questioned. Some authors have shown concern about the biological safety of the product.[3] Other researchers[4] discontinued early clinical trials due to the appearance of unexpected adverse effects, such as aseptic inflammation and delayed repair. The literature is scarce on the analysis of the biological behavior of this new biomaterial in bone repair in *in vivo* studies.

In this context, we propose an *in vivo* study to assess, from the microtomographic and histological point of view, whether this bioceramic compound can induce or lead to bone neoformation in critical defect models in rat calvaria.