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RESEARCH

Study of the viability and adhesion of osteoblast cells to bone cements mixed with hydroxyapatite at different concentrations to use in vertebral augmentation techniques☆

J. Pino-Mínguez, A. Jorge-Mora*, R. Couceiro-Otero, C. García-Santiago

Servicio de Cirugía Ortopédica y Traumatología, Complejo Hospitalario Universitario de Santiago y Universidad de Santiago de Compostela, Santiago de Compostela, Spain

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KEYWORDS
Polymethylmethacrylate;
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Abstract
Objective: The purpose of this study is to compare the biocompatibility and the effect in osteoblasts of polymethyl methacrylate alone, and mixed with hydroxyapatite in different concentrations of 5, 10, 15 and 20%, without exceeding 20%, as it can alter mechanical properties of the composite.
Materials and methods: Experimental study comparing osteoblast response to polymethyl methacrylate alone and with hydroxyapatite in different concentrations.
Results: Composites at 15 and 20% obtained better osteoblast response, with higher osteoblastic activity markers, and lower apoptosis markers. Electron microscopy images show improved adhesion of osteoblasts.

PALABRAS CLAVE
Polimetilmetacrilato;
Hidroxiapatita;
Osteintegración;
Respuesta celular;
Osteoblasto

Estudio de viabilidad y adhesividad celular de osteoblastos a cementos óseos utilizados en técnicas de refuerzo vertebral, mezclados con hidroxiapatita a distintas concentraciones

Resumen
Objetivo: El objetivo de este estudio es comparar la biocompatibilidad y efecto sobre osteoblastos de polimetilmetacrilato solo y PMMA al que se ha añadido, hidroxiapatita en concentraciones del 5, 10, 15 y 20%, no superando nunca esta cifra del 20%, dado que si se supera esta cifra pueden verse alteradas las propiedades biomecánicas del PMMA.

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* Corresponding author.
E-mail address: aljmora@gmail.com (A. Jorge-Mora).

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Material y métodos: Estudio experimental que consiste en el estudio de la adhesividad, diferenciación y muerte celular sobre discos de PMMA y composite PMMA/HA a diferentes concentraciones.
Resultados: Los composites al 15 y especialmente al 20% presentaron mejor respuesta osteoblástica, mayores marcadores de actividad y menores marcadores de apoptosis. En las imágenes de microcopía electrónica se aprecia una mayor adhesión celular.

Introduction

For over 50 years, polymethylmethacrylate (PMMA) has been used in Traumatology and Orthopedic Surgery to graft implants on the organism,[1] particularly since the 1960s by Charnley and Smoth. A resistant and durable union is achieved between PMMA and the correct implant, but the same is not true between the cement and the bone, as PMMA limits the activity of osteoblasts which are in contact with it. It is common to observe fibroblastic cells in the interface between cement and bone.[2]

In recent years, the use of PMMA has extended to spinal surgery, particularly in reinforcement techniques for the treatment of vertebral fractures. Treatment with these techniques started in 1987, for both traumatic and pathological fractures, and cement also began to be used as a supplement for fixation of pedicular screws in specific cases.

PMMA used in vertebral reinforcement techniques has specific characteristics which differ from those of conventional PMMA, mainly increased viscosity and lower exothermic capacity. Its osseointegration characteristics and the biological response to it are not different from conventional PMMA, but small areas of bone necrosis have been detected in the vertebrae following vertebral reinforcement techniques.[3]

There have been studies of vertebral reinforcement with biocompatible materials, such as hydroxyapatite (HA) and tricalcium phosphate (TCP), but their biomechanical properties in terms of rigidity, strength, resistance and Young modulus are notably inferior to those of PMMA,[4,5] so they are not reliable for the support of loads in the damaged vertebra.

PMMA and HA cements are being investigated using different HA concentrations in order to obtain a composite with similar biomechanical properties to PMMA and biocompatibility with similar osteoconductive properties to HA.[6–13]

The objective of this study is to compare the biocompatibility and effect on osteoblasts of PMMA alone and PMMA with added HA in concentrations of 5%, 10%, and 20%, never exceeding the level of 20% as this could alter the biomechanical properties of PMMA.[14]

Materials and methods

Preparation of materials

Hydroxyapatite was obtained from Keramat (Ames, A Coruña, Spain). The size of HA powder particles ($Ca_{10}[PO_4]_6[OH]_2$, Ca/P = 1.67) was examined with a particle size analyzer (Beckman Coulter, Fullerton, CA, USA), and the microstructure of each powder was examined by scanning electron microscopy (SEM), (SEM, Hitachi S-2400, Japan). Detection mapping and X-ray of the chemical composition of the powders was carried out using an energy dispersive X-ray spectrometer (EDX spectrometer, Kevex MS3 Sigma, USA).

As polymethylmethacrylate cement we used PMMA HV-R Kyphon (Medtronics, Spain), which is commonly used in our Unit for percutaneous vertebral reinforcement techniques.

Powder HA was mixed with the PMMA monomer at different concentrations by weight (5%, 10%, 15% and 20%) in our laboratory under sterile conditions in a vertical laminar flow cabinet. The mix was prepared in a mixing mill (Retsich MM400, Germany) for 10 min with a vibration frequency of 20 s.

The microstructure of the surfaces of the composites created by different HA concentrations was studied using SEM to assess their rugosity (Fig. 1).

The mixture was left to settle for 24 h following the setting times indicated by Medtronics. We then sterilized the discs created using gamma radiation in a dry environment.

A total of 100 discs were used in the project, divided into 5 groups according to the concentration of HA. In addition, a small series of 10 discs was reserved for electron microscopy analysis. The analysis by SEM was conducted at the Microscopy Service of Santiago de Compostela University.

Cellular response

Human osteoblasts of the cellular lineage ATTC Saos-2 were seeded in the specimens in a disc shape (height = 3 mm, diameter = 6 mm) at a density of 6×10^5 cells/ml with Dulbecco's modified Eagle's medium (DMEM-HG, Sigma, St. Louis, MO, USA) with 10% fetal bovine serum (FBS, Gibco BRL, MO, USA) for 72 h (37 °C, 5% CO_2). We then proceeded to study the differences in cellular proliferation on the different samples by means of an MTT assay (Sigma).

The total volume of osteoblasts cultured per sample was 60×10^3 on the discs with different concentrations of PMMA-HA. Prior to the cell culture, the discs were kept in contact with a volume of DMEM for 20 min so as to functionalize the surface of the cements, thus avoiding any possible hydrophobic interactions which could ultimately interfere with cellular adhesion. The discs were cultured in plates with 24 wells (Corning, USA) for 5, 10 and 15 days (Fig. 2). The culture supernatant was methodically kept at −20 °C after the ending times of the culture period. All the