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ORIGINAL RESEARCH ARTICLE

Demineralized dentin and enamel matrices as suitable substrates for bone regeneration

Nina Bono[1], Paolo Tarsini[2], Gabriele Candiani[1,2]

[1] Polytechnic of Milan Research Unit, National Interuniversity Consortium of Materials Science and Technology (INSTM), Milan - Italy
[2] Department of Chemistry, Materials and Chemical Engineering G. Natta, Polytechnic of Milan, Milan - Italy

ABSTRACT

Background: In recent decades, tooth derivatives such as dentin (D) and enamel (E) have been considered as potential graft biomaterials to treat bone defects. This study aimed to investigate the effects of demineralization on the physical-chemical and biological behavior of D and E.

Methods: Human D and E were minced into particles (Ø<1 mm), demineralized and sterilized. Thorough physical-chemical and biochemical characterizations of native and demineralized materials were performed by SEM and EDS analysis and ELISA kits to determine mineral, collagen type I and BMP-2 contents. In addition, MG63 and SAOS-2 cells were seeded on tooth-derived materials and Bio-Oss®, and a comparison of cell responses in terms of adhesion and proliferation was carried out.

Results: The sterilization process, as a combination of chemical and thermal treatments, was found to be effective for all materials. On the other hand, D demineralization allowed preserving the collagen content, while increasing BMP-2 bioavailability. D and demineralized D (dD) displayed excellent biocompatibility, even greater than Bio-Oss®. Conversely, the high mineral content displayed by E, as confirmed by EDS analysis, inhibited cell proliferation. Of note, even though the demineralization process was somehow less effective in E than in D, demineralized E (dE) displayed increased BMP-2 bioavailability and improved performance in vitro compared with native E.

Conclusions: Our results substantiate the idea that the demineralization process lead to an increase of BMP-2 bioavailability, thus paving the way toward development of more effective, osteoinductive tooth-derived materials for bone regeneration and replacement.

Keywords: BMP-2, Demineralization, Dentin, Enamel, Osteoblast cell lines

Introduction

The need for off-the-shelf, readily available materials suitable for bone regeneration has inspired much research in the field of regenerative medicine. Nowadays, the commercial anorganic deproteinized bovine substitute Bio-Oss® represents the gold standard for regenerative dentistry worldwide (1, 2), because of its osteoconductive properties and biofunctionality leading to effective and reliable bone regeneration. Despite the widespread use of such a gold standard graft material in alveolar bone augmentations, great interest has recently been focused on autologous bone-like materials as suitable substrates for bone regeneration of alveolar defects. However, although autogenous bone grafts are currently used in dentistry, the major drawbacks of bone-harvesting procedures, such as the limited availability of bone tissue and the need for second surgery, have hindered their efficacy in replacement therapy (3). These issues, together with the shortcomings ensuing from allogeneic transplantation, such as the transmission of possible infections and the lack of osteointegration with host tissues, have spurred on researchers and clinicians to look for novel graft solutions by means of challenging biomaterial techniques. More specifically, the use of tooth-derived materials has recently attracted much interest due to the inherent wide availability of teeth that are extracted every day and discarded as waste.

Dentin (D), the bulk material of the tooth which closely resembles the chemical composition of bone (≈70% w/w of mineral phase, ≈20% of organic matrix and ≈10% of water) (4-7), is considered as a viable bone substitute. In fact, D, in the form of native D (8, 9), and D derivatives, such as demineralized (10-13) and deproteinized D (14), have been used as graft materials in bone repair processes. Some experimental

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Corresponding author:
Prof. Dr. Gabriele Candiani
Department of Chemistry,
Materials and Chemical Engineering "Giulio Natta"
Politecnico di Milano
Via Mancinelli 7
20131 Milan, Italy
gabriele.candiani@polimi.it

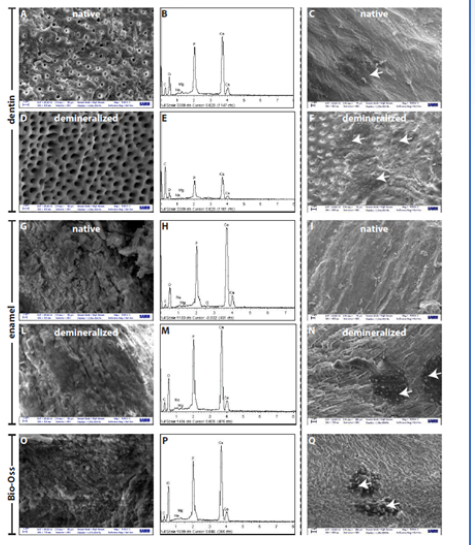

Fig. 2. SEM images (magnification: 5,000×) and the corresponding EDS spectra of: D particles (A-B); dD particles (D-E); E particles (G-H); dE particles (L-M); Bio-Oss®(O-P). SEM micrographs (magnification: 5,000×) after 7 days of SAOS-2 cell culture onto: D particles (C); dD particles (F); E particles (I); dE particles (N); Bio-Oss®(Q).