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A partially demineralized allogeneic bone graft: in vitro osteogenic potential and preclinical evaluation in two different intramembranous bone healing models

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In skeletal surgical procedures, bone regeneration in irregular and hard-to-reach areas may present clinical challenges. In order to overcome the limitations of traditional autologous bone grafts and bone substitutes, an extrudable and easy-to-handle innovative partially demineralized allogenic bone graft in the form of a paste has been developed. In this study, the regenerative potential of this paste was assessed and compared to its clinically used precursor form allogenic bone particles. Compared to the particular bone graft, the bone paste allowed better attachment of human mesenchymal stromal cells and their commitment towards the osteoblastic lineage, and it induced a pro-regenerative phenotype of human monocytes/macrophages. The bone paste also supported bone healing in vivo in a guide bone regeneration model and, more interestingly, exhibited a substantial bone-forming ability when implanted in a critical-size defect model in rat calvaria. Thus, these findings indicate that this novel partially demineralized allogeneic bone paste that combines substantial bone healing properties and rapid and ease-of-use may be a promising alternative to allogeneic bone grafts for bone regeneration in several clinical contexts of oral and maxillofacial bone grafting.

In oral and maxillofacial bone grafting procedures, autologous bone grafting is still considered to be the reference technique. This technique has a high success rate as a result of the combination of osteoconductivity (the ability to provide a structural support for bone growth), osteogenicity (the promotion of osteoblastic differentiation of progenitor cells), and osteoinductivity (the induction of bone growth in large bone defects or heterotopic sites, mainly due to growth factors in the bone and marrow environment)[1–4]. Despite these advantages, autologous bone grafts (ABGs) suffer from numerous serious peri- and post-operative drawbacks. Among these, the potential donor site morbidities (e.g., pain, hematoma, blood loss, nerve injury, the risk of bone fracture), the additional operative time, the limited available bone (e.g., iliac crest, calvarial bone), and the occasional need for a second surgical team are the main limitations of this procedure and consequently hindering the clinical benefits. To

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overcome these limitations, several alternatives such as alloplastic and xenogeneic bone substitutes or allogeneic bone grafts are used in oral and maxillofacial surgeries[5–7].

Allogeneic bone grafts are reliable and have been used extensively for decades as an alternative to ABGs[8,9]. The amount of allogeneic bone is virtually unlimited, and it can be obtained in various sizes and shapes (e.g., blocks, blades, paste, putty, powder, chips, injectable forms). The duration of the surgery is shortened compared to ABG-based reconstructions because there is no need for a bone harvesting procedure. Moreover, allogeneic bone grafts exhibit the same bone healing capacities as ABGs in multiple indications such as bone augmentation before dental implant placement (e.g., sinus floor elevation, ridge augmentation)[10,11].

Although allogeneic bone grafts are mainly used in powder or block forms, they exhibit a lack of versatility and/or handling, especially in specific indications related to pre-implant surgery, such as sinus floor elevation, guided bone regeneration in horizontal/vertical augmentation, and alveolar socket preservation. To address these unmet clinical needs, a novel ready-to-use, easy-to-handle, extrudable, and moldable human bone graft for bone regenerative medicine has been developed[12]. This allogeneic bone paste is made of partially demineralized bone particles, consisting of a mineralized core (Sup. Fig. 1, upper panel, white arrows) surrounded by a demineralized bone matrix (Sup. Fig. 1, upper panel, black arrows). This bone paste does not require any mixing, rehydration, or reconstitution prior to its use, which is a key feature for clinical use. Such a bone paste could replace the bone substitutes that are in powder form, particularly for the irregular or hard-to-reach areas frequently encountered in oral and maxillofacial bone surgery. However, aside from its handling and physical characteristics, proof of concept of its bone regenerative capacity should be established prior to its clinical transfer. In this context, the present work aimed to provide a thorough assessment of the bone healing capacity of this allogeneic bone paste compared with an allogeneic particular bone graft before its partial demineralization.

The purpose of this study was hence to assess the effects of the bone paste in vitro (1) on the attachment and osteoblastic commitment of primary human mesenchymal stromal cells from bone marrow (hBM-MSCs), (2) on the polarization of primary human monocytes isolated from circulating blood, and (3) to test and validate the in vivo capacity of this innovative bone paste to support and promote bone healing in two preclinical models: a guided bone regeneration (GBR) and a critical size defect (CSD) model in rat calvaria to mimic intramembranous bone healing of oral and maxillofacial defects.

Results

Attachment and osteoblastic commitment of hBM-MSCs. The attachment of hBM-MSCs was first investigated after short periods (1, 3, and 6 h) of contact with the particular bone graft or the bone paste. After 1 h of contact, the cells on the surface of the particular bone graft exhibited a round shape, while they had an elongated and spread-out shape on the surface of bone paste. Interestingly, at 3 h and 6 h, all of the cells exhibited an elongated and spread-out shape (Fig. 1a) irrespective of the graft. These data strongly suggest that the bone paste allows faster cell adhesion compared to the particular bone graft. Quantification of the DNA content of the adherent cells revealed no difference after 1 h of contact. However, after 3 and 6 h of contact, the DNA content was significantly higher (60% and 70%, respectively) for the cells cultured in contact with the bone paste compared to the cells in contact with the particular bone graft (Fig. 1b).

To determine whether the particular bone graft or the bone paste may affect the osteoblastic differentiation of hBM-MSCs, the cells were cultured in contact with the bone grafts in culture medium with or without osteogenic factors for 14 and 21 days. As seen by the SEM analyses, the osteogenic factors did not influence the colonization of the particular bone graft or the bone paste by the hBM-MSCs. Under all of the culture conditions, the cells had elongated cellular bodies and cytoplasmic expansions (Fig. 1c, white arrows). Of interest, the cohesion between the bone paste allowed the cells to create a network from particle to particle, with or without osteogenic factors, at all of the time points (Fig. 1c). It is also worth noting that this phenomenon was not observed with the cells cultured in contact with the particular bone graft. Finally, to evaluate the osteogenic commitment of the hBM-MSCs cultured in contact with the particular bone graft or the bone paste, the ALP activity was measured in cell lysates, and it was found to be significantly higher in the cells cultured in contact with the bone paste compared to the cells in contact with the particular bone graft: 3.3 and 2.7 times higher, respectively, in the absence of osteogenic factors and 7.3 and 4.6 times higher, respectively, in the presence of osteogenic factors after 14 and 21 days of culture (Fig. 1d).

Intramembranous bone healing in a GBR model. To mimic clinical indications of guided bone regeneration (e.g., alveolar socket preservation), the ability of the bone paste to support bone healing was first assessed in a GBR model in rat calvaria. The µ-CT analyses of the control defects at 7 weeks post-surgery revealed a significant degree of centripetal bone ingrowth from the edges of the defects (Fig. 2a), as expected in this GBR model. In the grafted defects, homogenous bone ingrowth (Fig. 2a, yellow arrows) was observed, but the particular bone graft and the bone paste were still visible in the defects (Fig. 2a, white arrows). The three-dimensional quantification of the MV/TV, increasing over time, indicated that new bone formation occurred in the control and in the grafted defects (Fig. 2b). However, the increase in the MV/TV between 0 and 7 weeks was greater in the group grafted with the bone paste than in the group grafted with the particular bone graft (2.2 vs. 1.6-fold, respectively).

In all of the defects, the histological observations revealed the collagen-rich and layer-organized nature of the newly formed bone (yellow matrix), with numerous osteocytes, that was identical to the native bone outside the defects (Fig. 3). These observations confirmed the µ-CT analyses, with significant bone formation after 7 weeks in the control defects, yet without continuous newly formed bone tissue (i.e., full closure). In the defects implanted with the particular bone graft and the bone paste, the grafts were perfectly osteointegrated and still visible (Fig. 3, *gr*) and recognizable due to their shape and the absence of osteocytes inside the grafts.