

Comparative Analysis of the Osteoinductive Potential of Commercially Available Allografts: An In-vitro Study

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Background:

- Allografts are commonly used as one of the many types of bone substitute materials for reconstruction of alveolar bone and socket preservation following tooth extraction [1].
- Various types of allografts are commercially available: mineralized, demineralized or combination thereof. While mineralized allografts are only osteoconductive, the demineralized bone matrix (DBM) is claimed to be both osteoconductive and osteoinductive. However, the degree of osteoinductivity of DBM products may differ based on their manufacturing process [2,3].

Objective:

- The aim of this study is to investigate the osteoinductive potential of various mineralized/demineralized allografts obtained with different manufacturing processes using Alkaline Phosphatase (ALP) activity assay.

[1] Ten Heggeler, J.M.; Slot, D.E.; van der Weijden, G.A. Effect of socket preservation therapies following tooth extraction in non-molar regions in humans: A systematic review. Clin. Oral Implants Res. 2011, 22, 779–788.

[2] AlGhamdi, A.S.; Shibly, O.; Ciancio, S.G. Osseous grafting part I: Autografts and allografts for periodontal regeneration—A literature review. J. Int. Acad. Periodontol. 2010, 12, 34–38.C.

[3] A. Jamjoom; Robert E. Cohen. Grafts for Ridge Preservation, J. Funct. Biomater. 2015, 6, 833-848.

Materials & Methods

- Allografts:**

Three commercially available mineralized/demineralized allografts with different manufacturing processes were included.[†]

Allograft	Manufacturer	Particle Size	Demineralized/Mineralized	Preservation Method	Abbreviation
Puros [®] Ci	RTI Surgical Inc. (Marketed by Zimmer Biomet)	250-1000 µm	55% / 45%	Tutoplast [®] + Cancellle SP [®]	TC
Creos [®]	Nobel Biocare	250-1000 µm	30% / 70%	Freeze-drying	FD1
OraGRAFT [®]	LifeNet Health	250-1000 µm	30% / 70%	Freeze-drying	FD2

[†] Third party trademarks and product names are the property of their respective owners.

- Characterization of the DBM:**

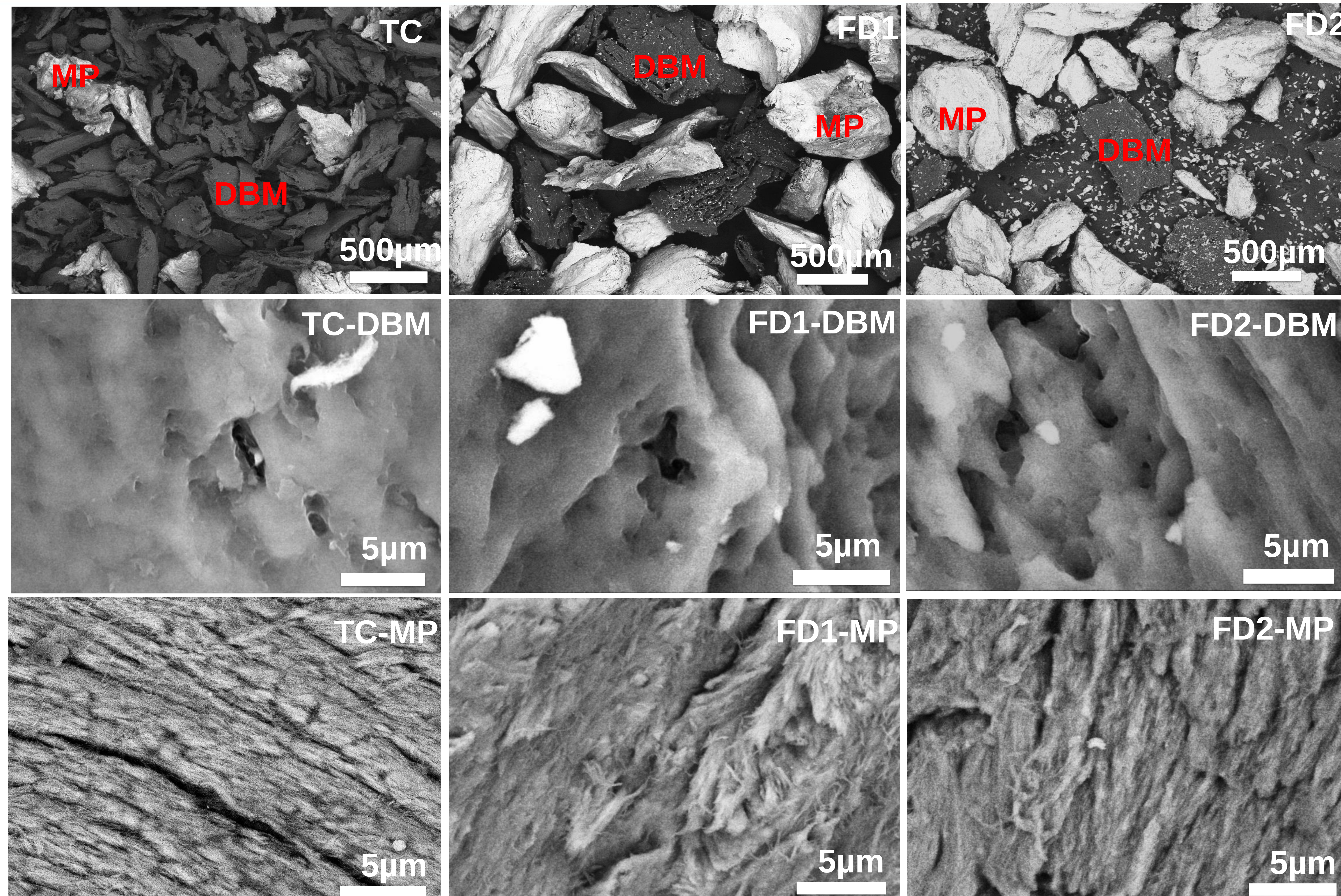
The surface morphology of the DBM component of each allograft was imaged using a scanning electron microscope (SEM; JEOL Model JSM 6460LV).

- Cell Culture and Differentiation:**

- Human Mesenchymal stem cells (hMSCs) were seeded in the tissue culture wells containing equal amounts of allografts, and cultured in non-osteogenic medium. The ALP activity of total cells in each well (both on the grafts and inside the well) was measured by Alkaline Phosphatase Assay Kit (Abcam) at 7 and 14 days. Total protein was detected by Pierce[™] Rapid Gold BCA Protein Assay Kit (Thermo Scientific) and was used to normalize the corresponding ALP activity. The hMSCs morphology on DBM was examined by scanning electron microscopy (JEOL JSM 6460LV) after 7 and 14 days cell culture.
- One way ANOVA with Dunnett’s multiple comparison tests were used to determine statistical differences between the groups at 7 and 14 days (TC as control). The p<0.05 were considered significant.

Results

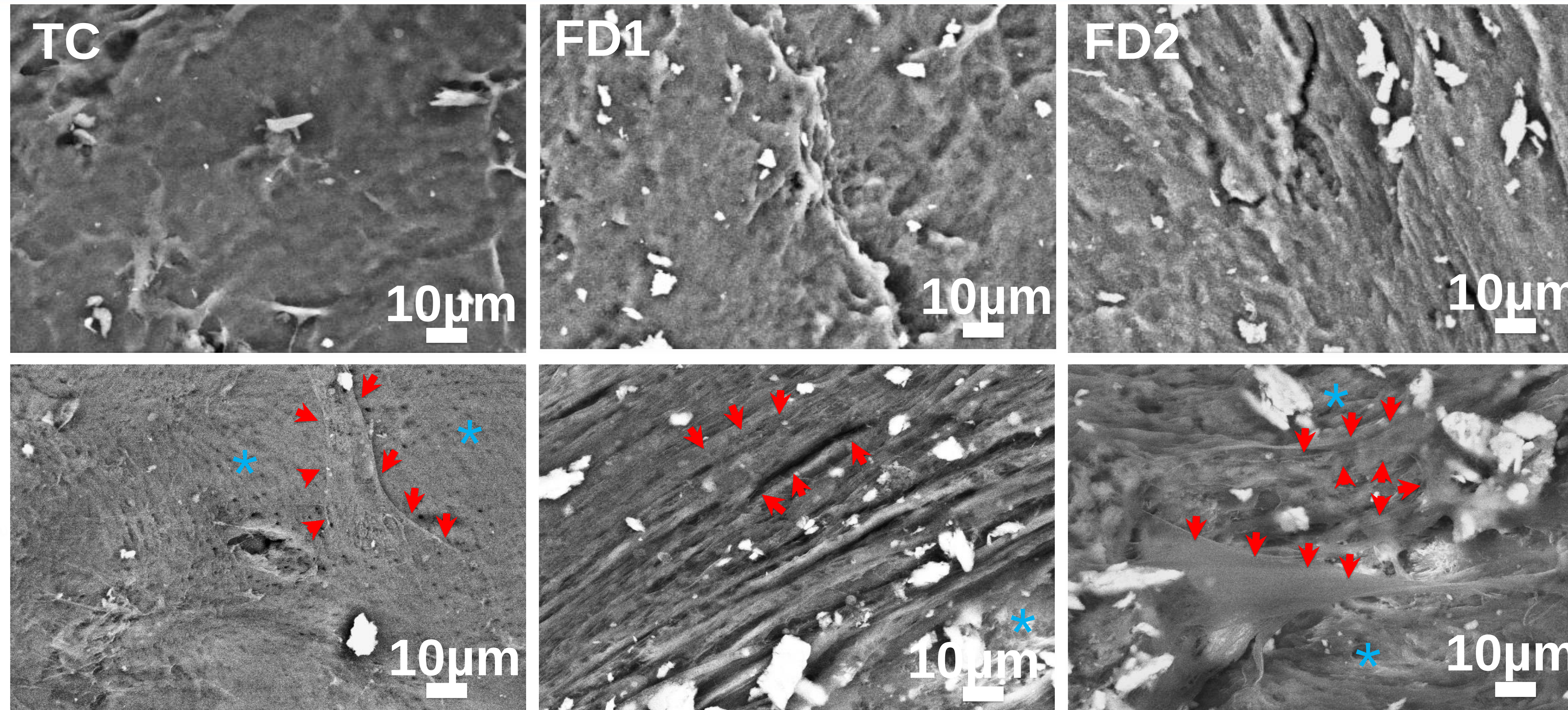
Surface Morphology



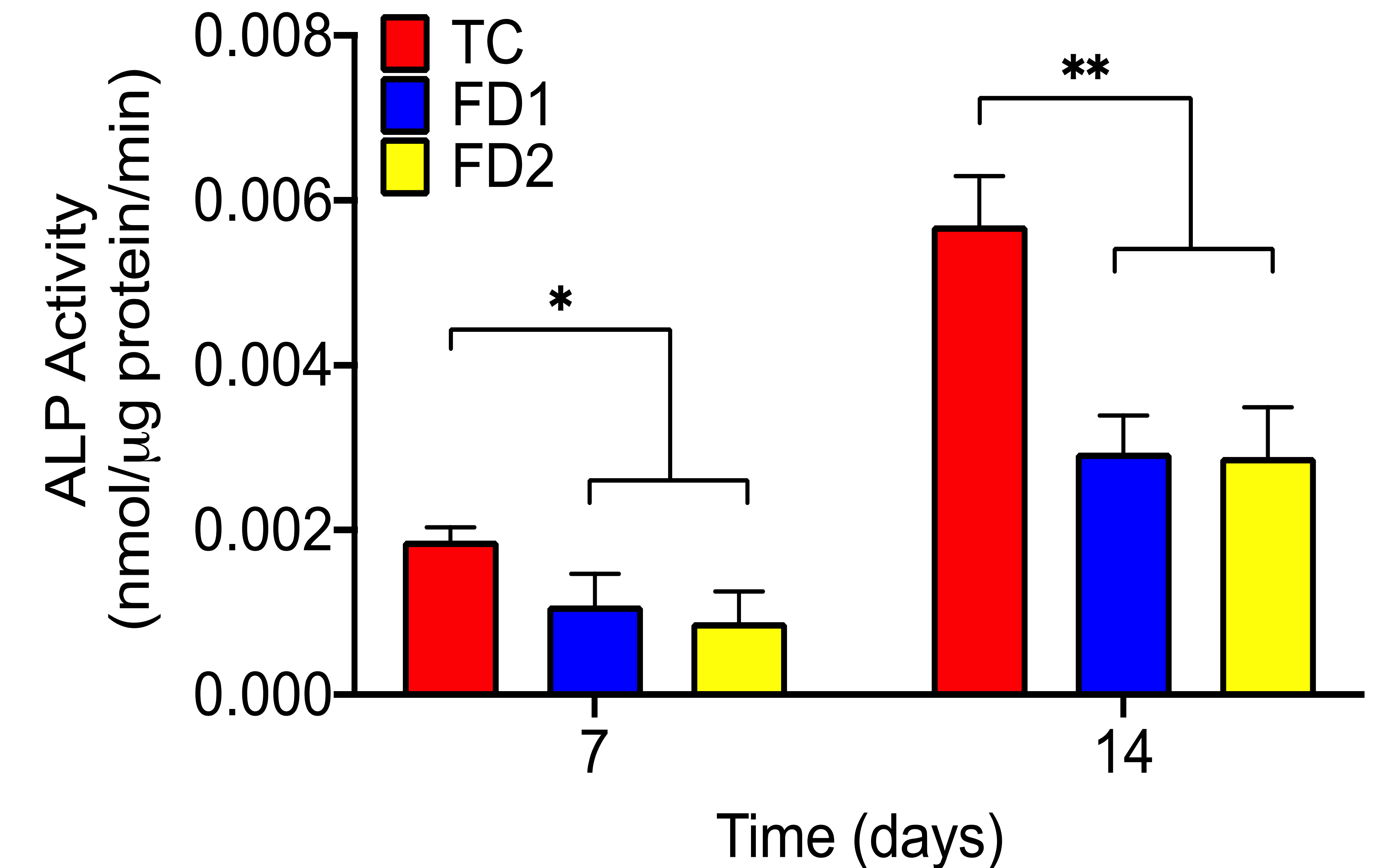
- All the allografts exhibited both demineralized bone matrix (DBM, shown as dark grey color) and mineralized particulates (MP, shown as white color).
- Higher amount of DBM than MP content was observed in TC allograft as compared to FD1 and FD2.
- Higher magnification SEM images exhibited similar surface morphology of the DBM component between all the allografts.
- SEM of mineralized particles revealed collagen compartment of the bone, which seems to be better preserved in TC given less disruption to the microarchitecture of the bundles.

Results

ALP activity assay



Red arrows are showing the edge of a cell layer formed over another fully grown cell layer that is shown by blue asterisks.



- Significantly higher ALP activity of hMSCs was seen with TC as compared to FD1 and FD2 allografts at 7 days ($P < 0.05$), which became even more pronounced at 14 days ($p < 0.01$).
- The ALP activity was increased with culture time for all the allografts.
- No significant differences in the ALP activity were observed between FD1 and FD2 at any time points.
- SEM images showed that the DBM component of all the allografts was fully covered with layers of cells after 7 days, which was proven by the differences seen in the surface morphology of DBM before and after cell attachment.

Summary

- The DBM component of allografts contains native proteins and growth factors. Biological activity of these proteins is highly influenced by the manufacturing processes, which will in turn impact the in vivo performance of the allografts.
- The ALP activity of the total hMSCs in culture in the presence of allografts was measured regardless of their attachment to the graft. Thus, measured ALP activity here is highly related to the amount of released proteins from the allografts into the surrounding environment and its impact on the total cell differentiation in culture.
- A significantly higher ALP activity of hMSCs was measured in the presence of TC as compared to FD1 and FD2 allografts at both 7 days ($P < 0.05$), and 14 days ($p < 0.01$), indicating higher osteoinductive potential of TC.
- The SEM images showed similar morphology for the DBM component of all the allografts as well as comparable cell coverage on all at both time points.

Conclusions:

Within the limitations of this study, significantly higher ALP activity in the presence of TC is indicative of TC's higher osteoinductive potential as compared to FD1 and FD2. Since the morphology of the DBM component as well as the cell coverage was comparable on all the allografts, such distinguished osteoinductive potential of TC could be due to better preservation of native proteins and growth factors during the Tutoplast® and Cancell SP® process, and/or higher amount of DBM mixed with mineralized particulates in TC compared to FD1 and FD2 allografts.