

Biological Characterization of Three Commercially Available Bovine Derived Xenografts: An *In vitro* study

Xuesong Wang, Ph.D., Olga Sanchez, Elnaz Ajami, Ph.D., Kaushal Kumar, Hai Bo Wen, Ph.D.
Zimmer Biomet Dental, Palm Beach Gardens, FL, USA

Background

- Xenograft bone substitutes are used as alternatives to allografts for alveolar bone defect reconstruction due to the low cost and abundant resources, and their compositional and microarchitectural similarities to human bone.¹
- Manufacturers apply different processing methodologies without or with heat treatment at different temperatures, which may result in differences in the physicochemical properties of the final product and alter the subsequent biological responses, therefore leading to different clinical outcomes.²
- We hypothesized that the differences in the processing of the tissue differentially affect the physicochemical characteristics of the final graft product, and thus potentially impact their angiogenic and osteogenic potential.

Objective

- We aim to evaluate the response of both human umbilical vein endothelial cells (HUVECs) and human bone marrow derived mesenchymal stem cells (hBMSCs) cultured with three differently processed bovine-derived xenografts in vitro.

1. Stavropoulos, A., Deproteinized Bovine Bone Xenograft, in Musculoskeletal Tissue Regeneration: Biological Materials and Methods, W.S. Pietrzak, Editor. 2008, Humana Press: Totowa, NJ. p. 119-151.

2. Perić Kačarević Z, Kavehei F, Houshmand A, Franke J, Smeets R, Rimashevskiy D, Wenisch S, Schnettler R, Jung O, Barbeck M. Purification processes of xenogeneic bone substitutes and their impact on tissue reactions and regeneration. Int J Artif Organs. 2018 Nov;41(11):789-800.

Materials & Methods

Xenografts

Three commercially available bovine-derived xenografts processed under different temperatures were included.[†]

Xenograft	Manufacturer	Particle Size (µm)	Origin	Heat Treatment (°C)	Abbreviation
CopiOs®	Tutogen Medical GmbH (Marketed by Zimmer Biomet)	1000 - 2000	Bovine	None	BDX1
Endobon®	Biomet France Sarl (Marketed by Zimmer Biomet)	1000 - 2000	Bovine	1200	BDX2
Bio-Oss®	Geistlich Pharma AG	1000 - 2000	Bovine	350 - 500	BDX3

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

Biological Characterizations

- The HUVECs and hBMSCs were cultured on the xenografts, and their growth were quantified with a cell counting kit (Dojindo Molecular Technologies) at 1, 3, and 7 days.
- Adherent cells on the xenografts were stained with LIVE/DEAD™ Viability/Cytotoxicity Kit (Invitrogen), and imaged with a fluorescent microscope (Olympus BX53) at 1, 3, and 7 days. Adherent cells at 7 days were further imaged with a SEM (JEOL Model JSM-IT500 HR) to examine their morphology.
- The differentiation of hBMSCs in response to different xenografts were measured through Alkaline Phosphatase (ALP) activity. The hBMSCs were cultured with xenografts in a non-osteogenic medium. The ALP activity of total adherent cells on the xenografts was measured by ALP Assay Kit (Abcam) at 14 days. Total protein was detected with Pierce™ Rapid Gold BCA Protein Assay Kit (Thermo Scientific) to normalize the corresponding ALP activity.

Statistical analysis

- One way ANOVA with Tukey’s multiple comparison tests were used to determine statistical differences between the groups at each time point. The p<0.05 were considered significant.

Results

BDX1 and BDX2 promoted HUVECs and hBMSCs growth *in vitro*.

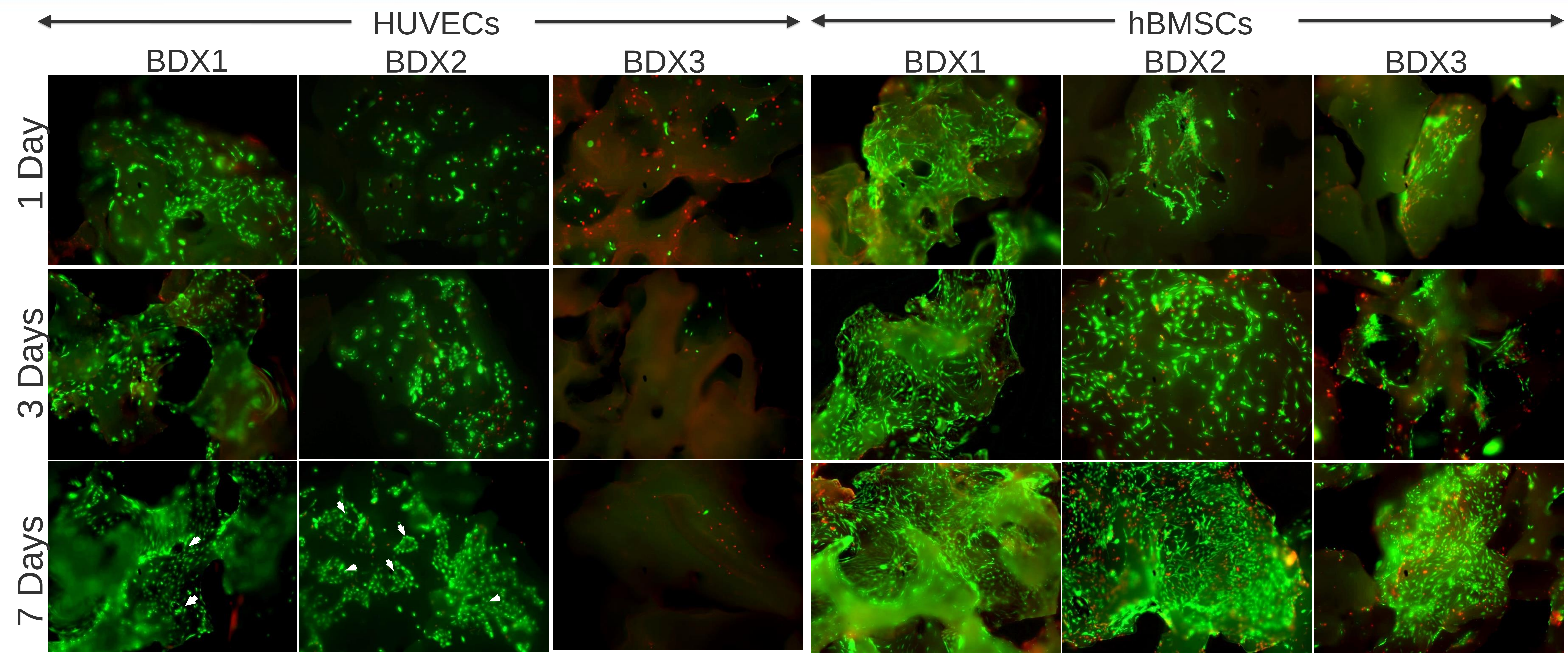


Figure. 1: Fluorescence LIVE (green) / DEAD (red) images of HUVECs and hBMSCs growth on different xenografts at 1,3 and 7 days. White arrows show possible tube-like structures formed by HUVECs at 7 days.

- Considerably more HUVECs were observed on both BDX1 and BDX2 compared to BDX3 at all time points. BDX1 and BDX2 promoted HUVECs to form tube-like structures at 7 days. Hardly any live HUVECs were detected at 7 days on BDX3 (Fig.1).
- Markedly less hBMSCs were visualized on BDX3 than on BDX1 and BDX2 at 1 and 3 days. The hBMSCs growth was exponential only on BDX1 and BDX2, and fully covered the particulates. More dead cells were observed on BDX3 at all time points (Fig.1).
- Quantitative results demonstrated a significantly higher number of HUVECs (Fig. 2A) and hBMSCs (Fig. 2B) on BDX1 and BDX2 compared with BDX3 at all time points. Both BDX1 and BDX2 supported the adhesion and exponential growth of both cell types, but BDX3 was only favorable for the hBMSCs.

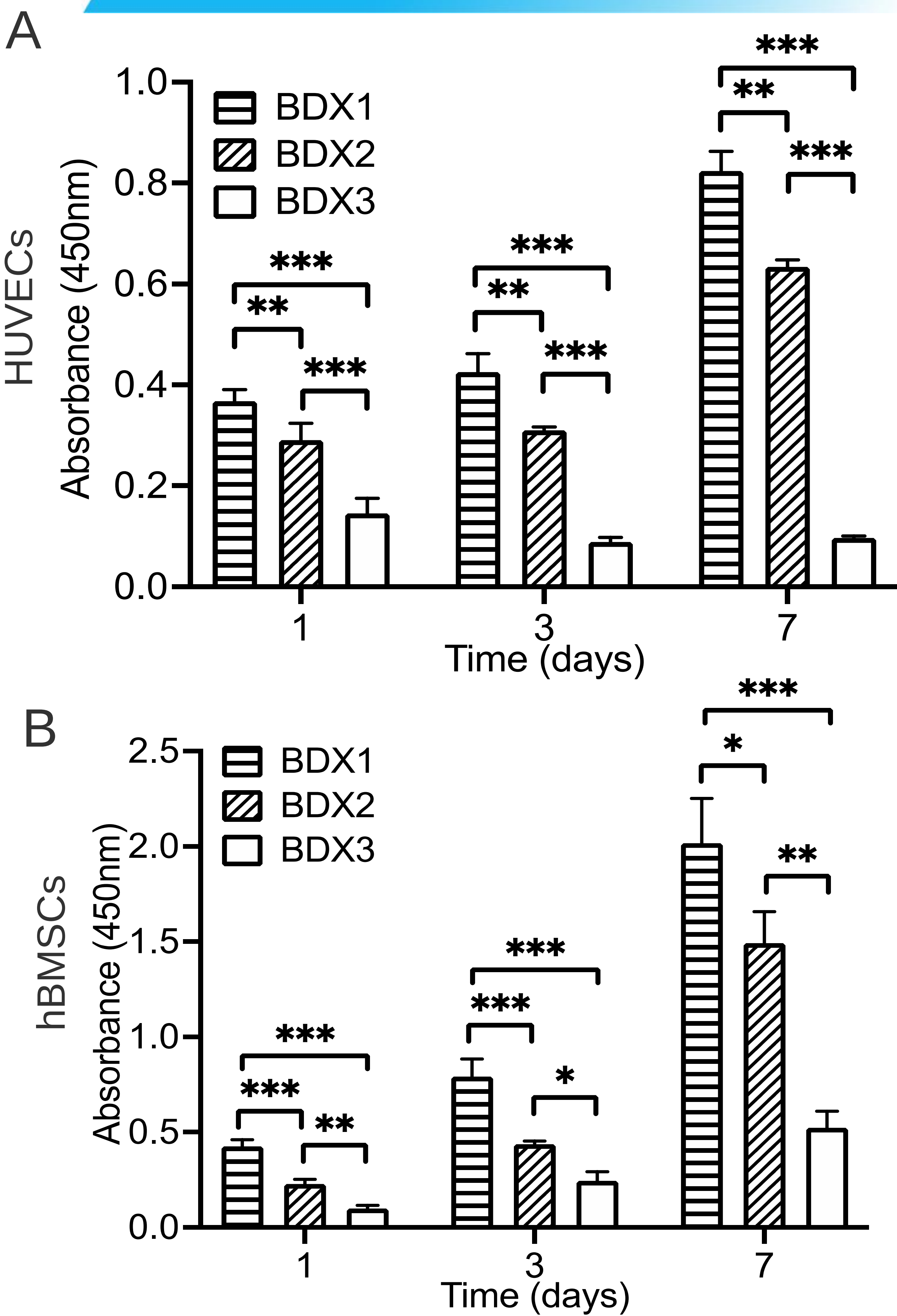


Figure 2: Quantitative analysis of (A) HUVECs and (B) hBMSCs growth at 1, 3 and 7 days.

Results

BDX1 and BDX2 stimulated hBMSCs differentiation *in vitro*.

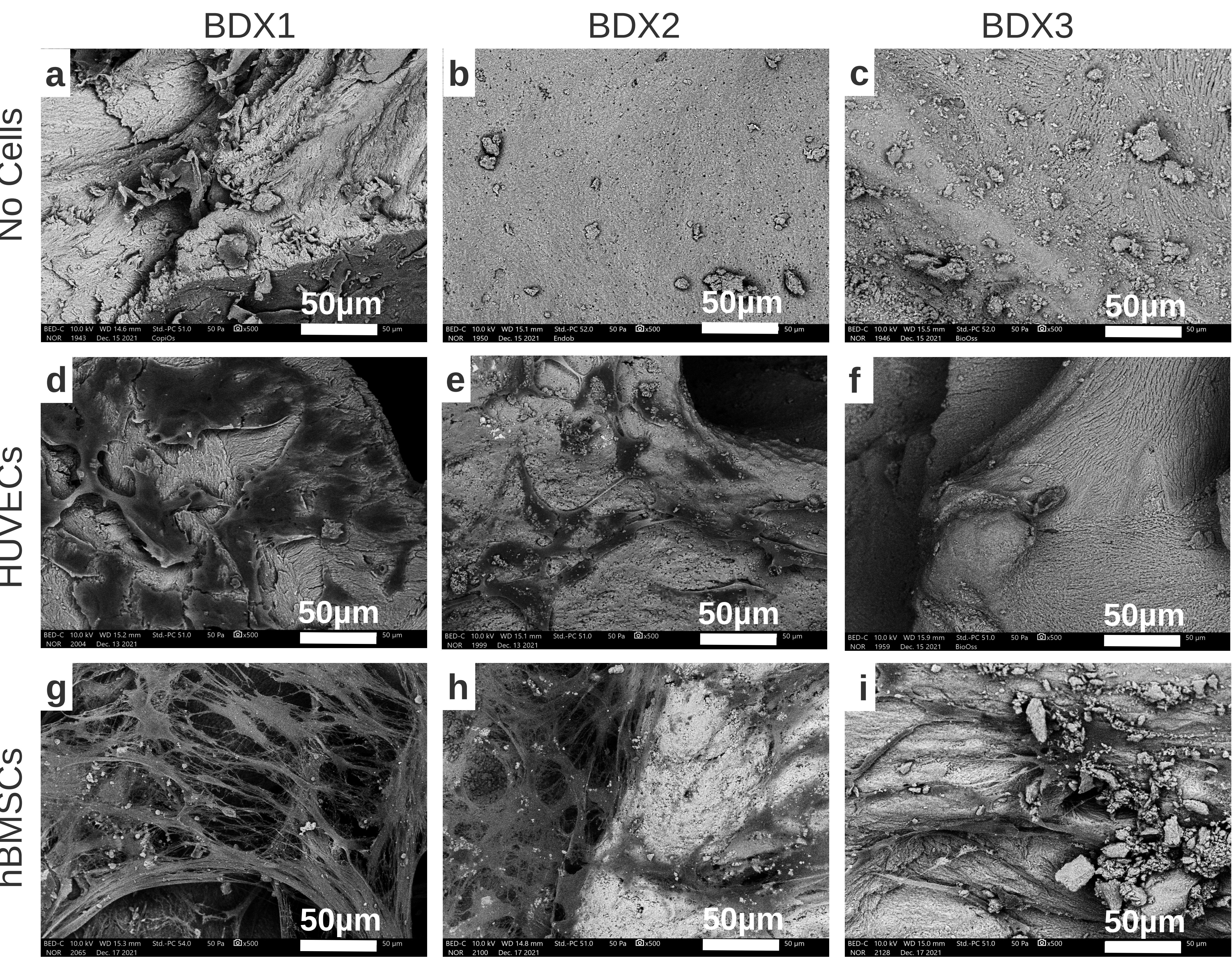


Figure 3: SEM micrographs showing (a-c) surface morphology of xenografts before seeding the cells, (d-f) morphology of adherent HUVECs and (g-i) hBMSCs on different xenografts at 7 days.

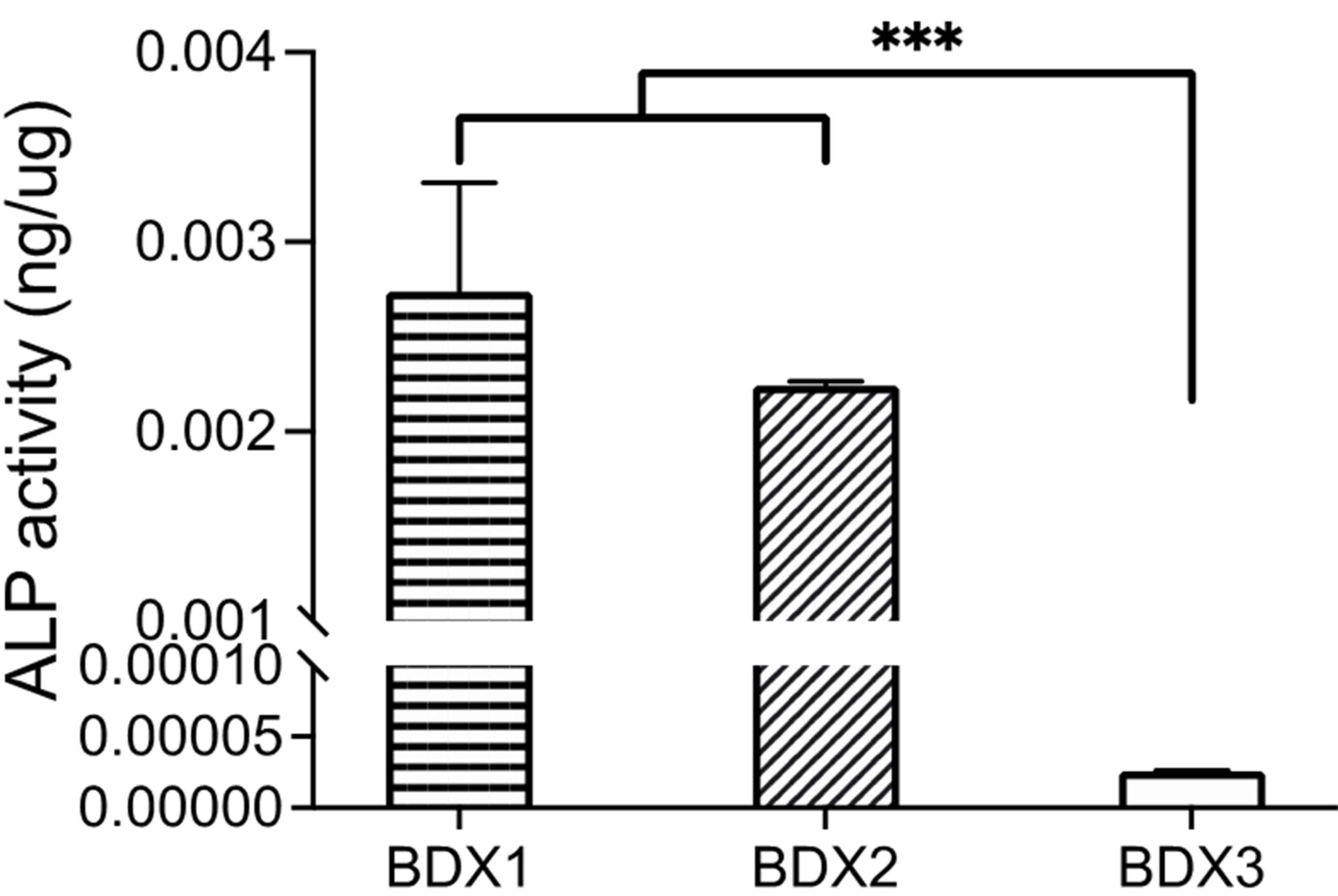


Figure 4: ALP activity of hBMSCs in response to different xenografts at 14 days.

- At 7 days, adherent HUVECs exhibited stretched cell morphology on both BDX1 and BDX2. A higher degree of interconnection between cells on BDX1 than on BDX2 was demonstrated (Fig.3 d,e). Hardly any HUVECs were observed on BDX3 (Fig.3 f).
- The hBMSCs were highly stretched on all xenografts (Fig.3 g,h,i), whereas formed a 3D network structure only on BDX1 and BDX2 at 7 days (Fig. 3 g,h)
- Significantly higher ALP activity of hBMSCs was observed on both BDX1 and BDX2 when compared with BDX3 at 14 days (Fig. 4).

Discussion and Conclusion

Discussion

- Manufacturers conduct different processing methods for the preparation of xenografts. While some apply heat treatment at varying temperatures, others choose processes without heat treatment. This may result in different physicochemical properties of the final product and its subsequent biological responses.
- BDX1 is a partially deproteinized and defatted xenograft without any heat treatment, while BDX2 and BDX 3 are heat treated at considerably different temperatures.
- Both BDX1 and BDX2 promoted HUVECs and hBMSCs growth. In contrast, BDX3 that heat treated at a lower temperature compared to BDX2, exhibited limited potential in favoring HUVECs and hBMSCs growth. The latter may be explained by the negative effects of elutes from BDX3.¹
- Significantly higher ALP activity of hBMSCs on BDX1 and BDX2 indicated their higher osteogenic potential than BDX3.

Conclusions

Different tissue processing methodologies of the xenografts employed by the manufacturers result in variations in their biological performance *in vitro*. Within the limitations of this study, HUVECs and hBMSCs exhibited a different biological response to three types of bovine-derived xenografts. Both BDX1 and BDX2 significantly promoted HUVECs and hBMSCs growth, and hBMSCs differentiation, as compared to BDX3. This may indicate their superior angiogenic and osteogenic potential, thus preferred xenograft candidates for alveolar bone reconstruction.

1. Zimmermann CE, Gierloff M, Hedderich J, Ail Y, Wiltfang J, Terheyden H. Biocompatibility of bone graft substitutes: effects on survival and proliferation of porcine multilineage stem cells in vitro. Folia Morphol (Warsz). 2011 Aug;70(3):154-60.