Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 9s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

VEGF-A and TGF-β1 Release in Bovine Bone Scaffolds via Secretome Application

Annete Nabila[1], Coen Pramono Danudiningrat[*2]

[1]Email ID: annetenabila@gmail.com

[*2]**Corresponding Author:**

Email ID: coen-p-d@fkg.unair.ac.id

Cite this paper as: Annete Nabila, Coen Pramono Danudiningrat, (2025) VEGF-A and TGF-β1 Release in Bovine Bone Scaffolds via Secretome Application. *Journal of Neonatal Surgery*, 14 (9s), 379-384.

ABSTRACT

Freeze-Dried Bovine Bone (FDBB) and *Decellularized- Freeze Dried Bovine Bone* (dc-FDBB) have level low *growth* factor. Culture of *Mesenchymal Stem Cells* (MSCs) can used as *cell free therapy* in form *secretome* Then combined in biomaterial *scaffold* for support development engineering network. VEGF-A and TGF-β1 are *growth factors* that are important in the angiogenesis process in regeneration bones. Compare TGF-β1 and VEGF-A *release* levels in DBBM, DBBM *secretome,* FDBB *secretome* and dc-FDBB *secretome*. VEGF-A and TGF-β1 *release* done quantification with ELISA on DBBM, DBBM *secretome,* FDBB *secretome* and dc-FDBB *secretome.* Statistical test Data normality tests (Shapiro-Wilk), homogeneity (Levene), then comparison tests were carried out with *one-way* Anova and post hoc/ tukey HSD, anova *same subject* and *paired t test.* The highest levels of VEGF-A and TGF-β1 *release* found in FDBB *secretome* compared to with DBBM, DBBM *secretome,* and dc-FDBB *secretome.* There is no difference VEGF-A and TGF-β1 *release* levels in a way significant between FDBB *secretome* with dc-FDBB *secretome.* There is difference VEGF-A and TGF-β1 *release* levels in a way significant between FDBB *secretome* with DBBM and DBBM *secretome.*

Keywords: *secretome, VEGF-A, TGF-β1*

1. INTRODUCTION

Large bone defects can be caused by aging, traffic accidents, fractures, bone tumor resection and can be a serious problem affecting health and quality of life.[1] The types of bone graft materials used for bone defect reconstruction are autograft, allograft, xenograft and alloplastic. The most remarkable advantage of xenografts is that there is a possibility that unlimited amounts of material can be obtained from bovine bone. The structure and morphology of bovine cancellous bone are similar to human cancellous bone, and it also has osteoconductivity.[2]

Tissue engineering aims to replace or regenerate human tissues or organs to restore or establish normal function.[3] Current bone tissue engineering approaches are based on scaffolds that release growth factors required for bone regeneration. Bone scaffolds are 3D matrices that allow and stimulate the attachment and proliferation of osteoinductive cells on their surfaces.[4] Bovine bone scaffold is a biomaterial derived from cow bones that is widely developed because of its abundant availability and has good osteoconductive and osteoinductive components that can overcome several weaknesses of autografts.[5]

Some type of bovine-derived scaffolds are Deproteinized Bovine Bone Mineral (DBBM), Freeze-Dried Bovine Bone (FDBB) and Decellularized Freeze-Dried Bovine Bone (dc-FDBB) scaffolds. DBBM has been used for a long time but is not osteoinductive. FDBB is an alternative in tissue engineering for mandibular defect reconstruction and still maintains organic components and inorganic materials.[6] Scaffolds are also widely developed by combining with Mesenchymal Stem Cells (MSCs) to increase signalling factors in bone substitute materials to make them more similar to autografts.[7]

MSCs culture can be used in the form of a secretome or stem cell conditioned medium (CM), then combined in a biomaterial scaffold to support the development of tissue engineering.[7] Secretome contains many growth factors, such as Insulin-Like Growth Factor (IGF)-1, Transforming Growth Factor (TGF)-β1, and Vascular Endothelial Growth Factor (VEGF), which can affect cellular characteristics and bone cell regeneration behaviour.[8] Osteoinductive growth factors such as TGFs-ß and VEGFs have shown great potential applications in bone healing and osteogenesis to regulate cell behaviour, including recruitment, migration, adhesion, proliferation, and differentiation.[9]

VEGF is one of the most important growth factors for the regulation of blood vessel development and angiogenesis. VEGF contributes to the coupling of osteogenesis with angiogenesis and directly controls the differentiation and function of

osteoblasts and osteoclasts.[10] TGF-β regulates angiogenesis through proliferation and maturation of blood vessels by signalling two cascades alternately with opposing effects, namely Activin Receptor-like Kinase 1 (ALK1) and Activin Receptor-like Kinase 5 (ALK5). The expression level of TGF-β at low doses can promote the angiogenesis process.[11]

Biomechanical stability and biological activity, which create the ideal conditions for new bone formation, are crucial for the success of growth factor therapy in bone tissue engineering. Therefore, this study aims to assess the osteogenic and angiogenic potential by analyzing VEGF-A and TGF-β1 release ability of scaffold that have undergone freeze-drying and decellularization processes after secretome application

2. METHODS

Research Design, Research Samples, and Experimental Groups

This in vitro laboratory experimental study used a posttest-only control group design to analyze TGF-β1 release ability of DBBM, DBBM secretome, FDBB secretome, and dc-FDBB secretome using ELISA. The research sample comprised of three scaffolds per group, calculated using the Federer formula. Four treatment groups were established: DBBM, DBBM secretome, FDBB secretome, and dc-FDBB secretome, each observed at three-time points: day 1, day 3, and day 7.

The production of DBBM, FDBB and dc-FDBB *scaffolds* were conducted at BRIN. Applications *secretome* to DBBM, FDBB and dc-FDBB *scaffolds*, and measurements level *growth factors release* were conducted at the Research and Development Center *Stem Cell* ITD Airlangga University.

Preparation of Deproteinized Bovine Bone Mineral (DBBM) Scaffold

The DBBM scaffold is made from cancellous bovine bone formed into 5x5x5 mm blocks. The bone is chemically cleaned with 3% hydrogen peroxide to remove blood, fat, and bone marrow, then rinsed with sterile water. It is then burned at 1000°C to remove inorganic mineral components, rewashed with sterile water, and dried in an oven until the water content is below 10%. Furthermore, the scaffold is packed and sterilized with gamma-ray radiation.

Preparation of Freeze Dried Bovine Bone (FDBB) Scaffold

The FDBB scaffold is a bone substitute material made from bovine femur bones cut into 5x5x5 mm blocks. The bones are washed with 3% hydrogen peroxide and rinsed with sterile water. They then underwent a freeze-drying process at -80°C and are dried with a lyophilizer until the water content is below 10%. Furthermore, the scaffold is packed and sterilized with gamma-ray radiation.

Preparation of Decellularized Freeze Dried Bovine Bone (dc-FDBB) Scaffold

The dc-FDBB scaffold, measuring 5x5x5 mm, undergoes a process beginning with washing using 3% hydrogen peroxide followed by rinsing with sterile distilled water. Subsequently, the bone is freeze-dried at -80°C until the water content is less than 10%. The scaffold is then washed with sodium lauryl ether sulfate (SLES) and sterilized using gamma-ray radiation.

Preparation of hUC-MSCs secretome

Validated hUC-MSCs are cultured in a growth medium until reaching 80% confluency. The medium is then replaced with fresh serum-free α-MEM and incubated for 48 hours. After incubation, 8 mL of conditioned medium is collected and centrifuged at 407g for 5 minutes to remove debris, yielding 7.5 mL of supernatant. A second centrifugation at 1630g for 3 minutes further clarifies the supernatant to 7 mL, which is stored at -20°C in sterile Eppendorf tubes for future use.

Preparation of hUC-MSCs secretome application to scaffold

The secretome produced from the secretome preparation process is applied to the scaffold by immersing the scaffold in 400 µl of the secretome. The scaffold with the applied secretome is then incubated for 24 hours at 37°C, with 98% humidity and 5% CO_2.

Quantification of Growth Factor Release using ELISA

Growth factor release was quantified using an ELISA kit (Bioassay Technology Laboratory) following the manufacturer's instructions. Briefly, 50 µl of standard reagent was added to the standard well, and 40 µl of sample and 10 µl of anti-growth factor-A antibody were added to the sample well. Then, 50 µl of streptavidin-HRP was mixed thoroughly in both wells. The plate was sealed, shaken gently, and incubated for 60 minutes at 37°C, then washed five times with wash buffer. Next, 50 µl of substrate solutions A and B were added to each well and incubated for 10 minutes at room temperature in the dark. Finally, 50 µl of stop solution was added, and optical density (OD) was measured at 450 nm using a microplate reader within 10 minutes.

Statistical Analysis

The analysis begins with the Shapiro-Wilk test for normality, followed by Levene's test for homogeneity of variance. A between-subjects ANOVA is used for comparison. A significance value (sig. 2-tailed) > 0.05 indicates no significant

difference, while < 0.05 indicates a significant difference. If differences are found, a Tukey HSD test is conducted. A within-subjects ANOVA is used to compare TGF-β1 and VEGF-A levels on days 1, 3, and 7, followed by a paired t-test if differences are present.

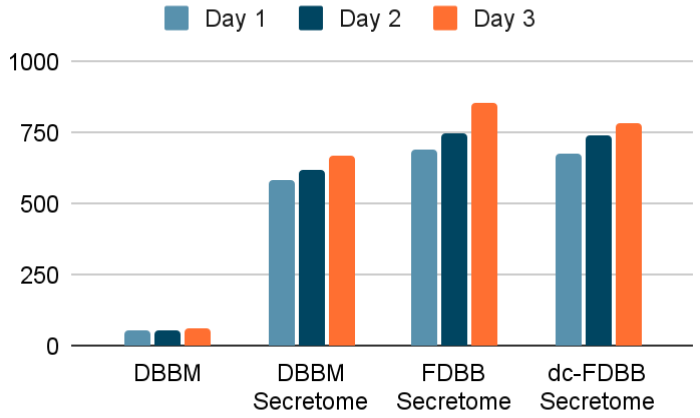
3. RESULTS

The levels of VEGF-A and TGF-β1 release analyzed in this study were based on four groups: DBBM, DBBM secretome, FDBB secretome, and dc-FDBB secretome groups. Each group was divided into three observation times: day 1, day 3, and day 7. Each group used three scaffolds.

ELISA results of VEGF-A Release levels

Our study utilized a sandwich ELISA at 450 nm to quantify VEGF-A release, directly correlating with absorbance. The results shown with distinct patterns across experimental groups. The DBBM group showed a stable VEGF-A levels initially, while the DBBM Secretome group exhibited significantly higher levels from day 1, peaking by day 7. The FDBB Secretome and dc-FDBB Secretome groups displayed increasing VEGF-A release over time, with the highest levels observed by day 7. These findings highlighted differential VEGF-A release profiles among treatments throughout the study. A Shapiro-Wilk normality test was conducted to determine the distribution of VEGF-A release data, confirming normal distribution across all groups. The homogeneity of variances was validated using Levene's test, with results indicating homogeneous variances. One-way ANOVA revealed significant differences between groups on days 1, 3, and 7.

Table 1. VEGF-A release data between observation times on days 1, 3, 7



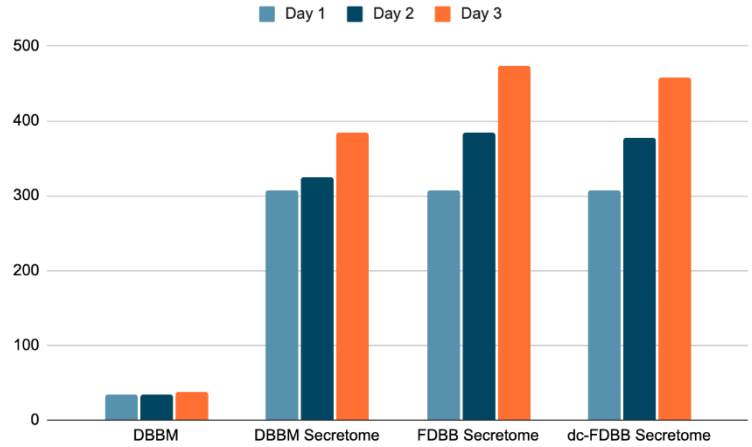
Post hoc Tukey HSD tests further identified that on day 1, significant differences were observed between the DBBM group and all secretome groups. However, no significant differences were found among the secretome groups. On day 3, there were significant differences between DBBM and all secretome groups, DBBM secretome and FDBB secretome and dc-FDBB secretome, but not between FDBB secretome and dc-FDBB secretome. By day 7, significant differences persisted between DBBM and all secretome groups and between DBBM secretome, FDBB secretome, and dc-FDBB secretome. In contrast, FDBB secretome and dc-FDBB secretome remained statistically similar.

ANOVA within-subjects analysis revealed significant differences in VEGF-A release across days 1, 3, and 7 for all groups ($p < 0.05$). Paired t-tests demonstrated significant differences between days 1 and 7, and days 3 and 7 for the DBBM group. Significant differences between days 1 and 7 were observed in the DBBM secretome group. The FDBB secretome group showed significant differences between days 1 and 7. For the dc-FDBB secretome group, significant differences were found between all compared days.

ELISA results of TGF-β1 Release

We utilized a sandwich ELISA method at 450 nm to quantify the TGF-β1 release, directly correlating with absorbance. The results revealed distinct patterns across experimental groups. The DBBM group exhibited stable TGF-β1 release levels throughout the study period, whereas the DBBM Secretome group started with significantly higher levels on day 1 and peaked by day 7. Similarly, the FDBB Secretome and dc-FDBB Secretome groups showed increasing trends in TGF-β1 release over time. These findings highlight diverse TGF-β1 release profiles among treatments. The Shapiro-Wilk test confirmed that TGF-β1 release data were normally distributed across all groups. Following this, Levene's Test indicated homogeneity of variances. A One-Way ANOVA revealed significant differences in TGF-β1 release among the groups on days 1, 3, and 7 ($p < 0.05$).

Table 2. TGF-β1 release data between observation times on days 1, 3, 7



Post hoc analysis using Tukey HSD revealed significant differences in TGF-β1 release between DBBM and all secretome groups (DBBM secretome, FDBB secretome, and dc-FDBB secretome) on days 1, 3, and 7 ($p < 0.05$). No significant differences were found between the secretome groups on day 1. Still, on days 3 and 7, significant differences emerged between the DBBM secretome, FDBB secretome, and dc-FDBB secretome.

ANOVA within-subjects analysis indicated significant differences in TGF-β1 release across days 1, 3, and 7 for all groups ($p < 0.05$). Paired t-tests showed significant differences between days 1 and 7, and days 3 and 7 for the DBBM group. For the DBBM secretome group, significant differences were observed between days 1 and 7. The FDBB secretome group showed significant differences between days 1 and 3 and days 1 and 7. The dc-FDBB secretome group exhibited significant differences across all compared days.

4. DISCUSSION

This study compares the levels of VEGF-A and TGF-β1 released from DBBM, DBBM secretome, FDBB secretome, and dc-FDBB secretome. Results showed the highest average VEGF-A release is in the FDBB secretome group, followed by dc-FDBB secretome, DBBM secretome, and the lowest in the DBBM group. The highest average TGF-β1 release on day 1 was in the dc-FDBB secretome group, while on days 3 and 7, it was in the FDBB secretome group. Growth factor release was analyzed over 7 days, as previous studies indicated that growth factor release tends to decline by day 7.[12,13]

Increases in VEGF-A and TGF-β1 release in the DBBM secretome, FDBB secretome, and dc-FDBB secretome groups were similar, showing a rise from days 1 to 7. This suggests an ongoing angiogenesis during this period. VEGF-A is crucial for angiogenesis during bone formation, peaking shortly after bone defect occurrence. It stimulates endothelial cell proliferation and migration, forming tubular blood vessels and promoting the recruitment and survival of bone-forming cells. TGF-β1 promotes the expression of key regulators of vasculogenesis and angiogenesis, including VEGF. It regulates angiogenesis through different mechanisms, involving the proliferation and maturation of blood vessels via alternating signaling cascades with opposing effects (ALK1 and ALK5).[14]

FDBB is derived from freeze-dried bovine femoral cancellous bone, while dc-FDBB undergoes additional decellularization. Decellularization can reduce growth factor levels. Methods include chemical, biological, and physical exposures, such as 1% Triton X-100, *Sodium Dodecyl Sulfate* (SDS), and *Sodium Lauryl Ether Sulfat* (SLES). SDS lyses cells, denatures proteins, and damages collagen and glycosaminoglycans (GAG) in the ECM.[15] GAGs play roles in cell adhesion, growth regulation, and proliferation. SDS requires extensive washing due to its ionic nature. Triton X-100 increases cell permeability and removes cell nuclei from tissues. Das et al. (2021) found TGF-β1 levels decreased by 47% after Triton X-100 treatment.[16]

In this study, dc-FDBB scaffolds were decellularized using 3% H_2O_2 with Sodium Laureth Sulfate (SLES), which supports tissue architecture and retains ECM properties better than SDS. SLES-treated groups showed regular collagen fiber orientation and significantly lower DNA content. Balancing cell removal and ECM retention is essential for effective protocols.[17,18,19] Statistical results showed no significant difference in VEGF-A and TGF-β1 release between FDBB secretome and dc-FDBB secretome groups, indicating SLES effectively retains growth factor levels in scaffolds.

Significant differences in VEGF-A and TGF-β1 release between DBBM secretome, FDBB secretome, and dc-FDBB secretome groups compared to the DBBM group support the secretome's ability to increase growth factor levels in scaffolds. TGF-β1 levels supporting bone formation range between 100-500 ng, while excessively high levels (2 µg or 2000 ng) can disrupt bone regeneration.[14]

Human umbilical cord-derived MSCs can differentiate into osteoblasts, adipocytes, and chondroblasts. MSCs secrete various growth factors and cytokines, known as the secretome, which recruit and proliferate endogenous MSCs.[20] The secretome offers many advantages over MSCs, including better immune compatibility, lower risks of tumorigenesis, embolism formation, and infection transmission. Moreover, the secretome is easier and cheaper to produce, store, and apply.[21]

Mass production with characteristics tailored to needs is a significant advantage of the secretome over MSCs.[22] In animal studies and human applications, MSC implantation enhances fracture healing by recruiting endogenous MSCs to the fracture site through paracrine effects via the secretome, containing various growth factors, pro-inflammatory cytokines, and anti-inflammatory agents like TGF-β, IGF-1, VEGF, and HGF.[23,24,25]

Growth factor release begins within the first 24 hours after wound formation. Thus, VEGF and TGF-β1 release measurements in this study started on day 1.[26] The highest VEGF and TGF-β1 release levels were observed on day 7, consistent with Zwittnig *et al.* (2022), which showed peak release on day 7, declining by day 10. Bagdadi *et al.* (2017) found similar results with peak release on day 7. TGF-β1 release levels in FDBB secretome increased significantly between days 1 and 3 but not between days 3 and 7. In contrast, TGF-β1 release in dc-FDBB secretome increased significantly from day 1 to 3 and from day 3 to 7, indicating dc-FDBB secretome's gradual TGF-β1 release capability until day 7 compared to FDBB secretome's early phase release.

Higher TGF-β expression levels in tumors with higher Gleason scores suggest a role in tumor development and prognosis. Excessive TGF-β levels may be associated with tumor progression, as higher TGF-β levels are found in more aggressive tumors, possibly due to mutations conferring resistance to TGF-β receptors.[27]

5. CONCLUSION

Based on this study findings, there were significant differences in VEGF-A and TGF-β1 release between DBBM secretome, FDBB secretome, and dc-FDBB secretome groups. There was no significant difference in VEGF-A and TGF-β1 release between FDBB secretome and dc-FDBB secretome groups. The highest levels of VEGF-A and TGF-β1 release were found in FDBB secretome compared to DBBM, DBBM secretome, and dc-FDBB secretome on days 1, 3 and 7. dc-FDBB secretome has the ability to release TGF-β1 more gradually until day 7 compared to FDBB secretome. The highest levels of VEGF-A and TGF-β1 release in DBBM secretome, FDBB secretome, dc-FDBB secretome were on day 7. In further research, it is suggested that angiogenic potential can be observed in vivo. Further in vitro research of secretome is needed as a control group.

REFERENCES

[1] Que, H., Fu, H., Han, Z., and Sun, Y. Biomaterials for Bone Tissue Engineering Scaffolds: A Review. RSC Advances. 2019;9(45): 26252–26262.

[2] Karalashvili, L., Chichua, N., Menabde, G., Atskvereli, L., and Grdzelidze, T. 2017 Decellularized Bovine Bone Graft for Zygomatic Bone Reconstruction. Medical Case Report, 4(1): 52.

[3] Gilpin, A., and Yang, Y. 2017. Decellularization Strategies for Regenerative Medicine: From Processing Techniques to Applications. Biomed Research International, 9831534.

[4] Oliveira, É. R., Nie, L., Podstawczyk, D., Allahbakhsh, A., Ratnayake, J., Brasil, D. L., and Shavandi, A. 2021. Advances in Growth Factor Delivery for Bone Tissue Engineering. International Journal of Molecular Sciences, 22(2): 903

[5] Sheikh, Z., Hamdan, N., Ikeda, Y., Grynpas, M., Ganss, B., and Glogauer, M. 2017. Natural Graft Tissues and Synthetic Biomaterials for Periodontal and Alveolar Bone Reconstructive Applications: A Review. Biomaterials Research, 21: 9

[6] Kamadjaja, D. B., Satriyo, H., Setyawan, A., Lesmaya, Y. D., Safril, J. W., Sumarta, N. P. M., and Rizqiawan, A. et al. 2022. Analyses of Bone Regeneration Capacity of Freeze-Dried Bovine Bone and Combined Deproteinized-Demineralized Bovine Bone Particles in Mandibular Defects: The Potential Application of Biological Forms of Bovine-Bone Filler. European Journal of Dentistry, 16(2): 403–413

[7] Presen, D. M., Traweger, A., Gimona, M., and Redl, H. 2019. Mesenchymal Stromal Cell-Based Bone Regeneration Therapies: From Cell Transplantation and Tissue Engineering to Therapeutic Secretomes and Extracellular Vesicles. Frontiers in Bioengineering and Biotechnology, 7: 352

[8] Katagiri, W., Kawai, T., Osugi, M., Sugimura-Wakayama, Y., Sakaguchi, K., Kojima, T., and Kobayashi, T. 2017. Angiogenesis in Newly Regenerated Bone by Secretomes of Human Mesenchymal Stem Cells. Maxillofacial Plastic and Reconstructive Surgery, 39(1): 8

[9] Khojasteh, A., Behnia, H., Naghdi, N., Esmaeelinejad, M., Alikhassy, Z., and Stevens, M. 2013. Effects of Different Growth Factors and Carriers on Bone Regeneration: A Systematic Review. Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology, 116(6): e405–e423

[10] Hu, K., and Olsen, B. R. 2017. Vascular Endothelial Growth Factor Control Mechanisms in Skeletal Growth and Repair. Developmental Dynamics: An Official Publication of the American Association of Anatomists, 246(4): 227–234

[11] Guerrero, P. A., and McCarty, J. H. 2017. TGF-β Activation and Signaling in Angiogenesis. InTech.

[12] Zwittnig, K., Kirnbauer, B., Jakse, N., Schlenke, P., Mischak, I., Ghanaati, S., and Al-Maawi, S., et al. 2022. Growth Factor Release within Liquid and Solid PRF. Journal of Clinical Medicine, 11(17): 5070

[13] Al-Maawi S., Herrera-Vizcaino C., Dohle E., Zrnc T.A., Parvini P., Schwarz F., and Sader R. et al. 2018. Homogeneous Pressure Influences the Growth Factor Release Profiles in Solid Platelet-Rich Fibrin Matrices and Enhances Vascular Endothelial Growth Factor Release in the Solid Platelet-Rich Fibrin Plugs. International Journal of Growth Factors and Stem Cells in Dentistry, 1:8-16

[14] Guerrero, P. A., and McCarty, J. H. 2017. TGF-β Activation and Signaling in Angiogenesis. InTech.

[15] Xu, K., Kuntz, L. A., Foehr, P., Kuempel, K., Wagner, A., Tuebel, J., and Deimling, C. V. et al. 2017. Efficient Decellularization for Tissue Engineering of the Tendon-Bone Interface with Preservation of Biomechanics. Plos One, 12(2): e0171577

[16] Das, P., Mishra, R., Devi, B., Rajesh, K., Basak, P., Roy, M., and Lahiri, D. et al. 2021. Decellularized Xenogenic Cartilage Extracellular Matrix (ECM) Scaffolds for the Reconstruction of Osteochondral Defects in Rabbits. Journal Of Materials Chemistry, 9(24): 4873-4894

[17] Roosens, A., Somers, P., De Somer, F., Carriel, V., Van Nooten, G., and Cornelissen, R. 2016. Impact of Detergent-Based Decellularization Methods on Porcine Tissues for Heart Valve Engineering. Annals of Biomedical Engineering, 44(9): 2827–2839

[18] Gilpin, A., and Yang, Y. 2017. Decellularization Strategies for Regenerative Medicine: From Processing Techniques to Applications. Biomed Research International, 9831534.

[19] Keshvari, M. A., Afshar, A., Daneshi, S., Khoradmehr, A., Baghban, M., Muhaddesi, M., and Behrouzi, P. et al. 2021. Decellularization of Kidney Tissue: Comparison of Sodium Lauryl Ether Sulfate and Sodium Dodecyl Sulfate for Allotransplantation in Rat. Cell And Tissue Research, 386(2): 365–378

[20] Wang, H. S., Hung, S. C., Peng, S. T., Huang, C. C., Wei, H. M., Guo, Y. J., and Fu, Y. S. et al. 2004. Mesenchymal Stem Cells in The Wharton's Jelly of the Human Umbilical Cord. Stem Cells (Dayton, Ohio), 22(7): 1330–1337

[21] Dilogo, I. H., Fiolin, J., Canintika, A. F., Pawitan, J. A., and Luviah, E. 2022. The Effect of Secretome, Xenogenic Bone Marrow-Derived Mesenchymal Stem Cells, Bone Morphogenetic Protein-2, Hydroxyapatite Granule and Mechanical Fixation in Critical-Size Defects of Rat Models. The Archives of Bone and Joint Surgery, 10(1): 17–22

[22] Vizoso, F. J., Eiro, N., Cid, S., Schneider, J., and Perez-Fernandez, R. 2017. Mesenchymal Stem Cell Secretome: Toward Cell-Free Therapeutic Strategies in Regenerative Medicine. International Journal of Molecular Sciences, 18(9): 1852

[23] Pawitan, J. A. 2009. Prospect of Adipose Tissue Derived Mesenchymal Stem Cells in Regenerative Medicine. Cell and Tissue Transplantation and Therapy, 2:7-9

[24] Kilborn, S. H., Trudel, G., and Uhthoff, H. 2002. Review of Growth Plate Closure Compared with Age at Sexual Maturity and Lifespan in Laboratory Animals. Contemporary Topics in Laboratory Animal Science, 41(5): 21–26

[25] Marcucio, R. S., Nauth, A., Giannoudis, P. V., Bahney, C., Piuzzi, N. S., Muschler, G., and Miclau, T. 2015. Stem Cell Therapies in Orthopaedic Trauma. Journal of Orthopaedic Trauma, 29(12): S24–S27

[26] Sahin, I. O., Gokmenoglu, C., and Kara, C. 2018. Effect of Concentrated Growth Factor on Osteoblast Cell Response. Journal of Stomatology, Oral and Maxillofacial Surgery, 119(6): 477–481

[27] Ries, C., Egea, V., Karow, M., Kolb, H., Jochum, M., and Neth, P. 2007. MMP-2, MT1-MMP, and TIMP-2 are Essential for the Invasive Capacity of Human Mesenchymal Stem Cells: Differential Regulation by Inflammatory Cytokines. Blood, 109(9): 4055–4063