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Research Article

# Low-Intensity Electrical Stimulation Improved the Mineralization of Adipose-Derived Mesenchymal Stem Cells

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## Abstract

Multipotent mesenchymal stromal cells (MSCs) derived from adipose tissue have been increasingly studied due their less-invasive extraction surgery, self-renew and differentiation potential. The literature has not been reported the comparison of different sources of MSCs derived from distinct adipose tissues and the use of electrical stimulation (ES) for tooth and bone tissue regeneration. The present study evaluated *in vitro* cell viability by MTT, cell proliferation by CCK-8 and the osteogenic differentiation by alizarin red-S staining of MSCs from Wistar rats adipose tissue, abdominal (ABD) and inguinal (ING), submitted to ES therapy. ES was applied during 60s, 150s and 300s at 10μA-intensity 3x/week. The ES therapy was not cytotoxic at any time of the experimentation. MSCs from ING presented higher percentage of cell viability at 60s of ES than MSCs from ABD stimulated for 150s and 300s. Moreover, MSCs from ING presented higher proliferation rate than ABD at all three ES time-application. The use of ES therapy during the osteogenic differentiation contributed positively to improve the mineralization process. MSCs from ING presented 30% greater mineralization at 150s, and 50% at 300s, while ABD showed 50% greater at 60s of ES. In conclusion, MSCs from ING and ABD are able to increase cell proliferation and improve osteogenic mineralization, but with different responses depending on the ES stimuli at the same experimental condition, which reinforce the needing for further investigation on the use of MSCs derived from fat tissue and ES to optimize their use for cell proliferation and mineralization in regenerative medicine.

## Introduction

Tissue senescence is one of the main factors that can compromise the bone repair process, increasing the incidence of morbidity and calcification, as a consequence of population aging [1]. Orthopedic traumas spend healthcare cost and time, in addition to depend on approaches, such as transplants and long-hospitality, which share numerous disadvantages: high costs, secondary surgeries, greater patient discomfort, risk of infections and rejections [2]. Clinical cases without the capacity for spontaneous regeneration continue to be a challenge for the medical community. The understanding of new approaches that can contribute to tissue repair and replacement of injured tissues is necessary, mainly for the elderly population. Efforts have been done to explore alternative treatments for bone regeneration and bone hemostasis. Electrical stimulation and regenerative medicine are therapies that have been explored to improve fracture healing and [2-4]. Tissue engineering and regenerative medicine are based on the use of living cells to replace and regenerate damaged tissues and organs. Multipotent mesenchymal stromal cells (MSCs), also known as mesenchymal stem cells, are the most widely used cells in tissue engineering due to their capacity for self-renewal, release of paracrine factors, and multipotent differentiation, which ensures the replacement of cells of specific lineages [5,6]. MSCs derived from adipose tissue (AD-MSCs - adipose-derived mesenchymal stem cells) have been explored cause their less invasive extraction procedure. In addition, as demonstrated by some studies, AD-MSCs have better proliferation capacity compared to bone marrow-derived MSCs [6,7].

In animal models, the most commonly used source of AD-MSCs for regenerative medicine *in vitro* study is the inguinal adipose tissue because of its better capacity for cell differentiation. Nevertheless, the supply of adipose tissue from the abdominal region is higher, easier and faster tissue collection, as well as a better comparison with AD-MSCs from human adipose tissue already employed in clinical trials and/or therapies [6,7]. The use of *in vitro* MSCs for osteogenic differentiation is also followed by potential mineralization optimizers: three-dimensional supports, growth factors and non-invasive therapies such as electrical stimulation (ES) [3,8,9]. The use of ES in MSCs cultures has presented promising outcomes, once the therapy may accelerate the osteogenic differentiation, cell proliferation, cytokines and growth factors synthesis in attempt to create reproducible and improved strategies craniofacial reconstruction and odontology [9-11]. The use of adipose-derived stem cells is reported as an alternative approach for viable source of MSCs for osteogenic differentiation combined with and ES application. Previously, we already demonstrated the ES use in osteoblast. We reported the use of low-intensity electrical stimulation did not present any cytotoxic effect, and enhanced the mineralization [9]. The present study aimed to evaluate the viability, proliferation, and differentiation of multipotent mesenchymal stromal cells derived from two regions of adipose tissue (abdominal and inguinal) of Wistar rats submitted to different time-application of electrical stimulation at the lowest intensity (10 μA).

## Materials and Methods

Every experimental procedure involving cell culture, electrical stimulation, animal care, surgical protocols and adipose tissue extraction were approved by the Institution's Ethics Committee on Animal Use (087/2018 and 064/2019) of the University Center of Hermínio Ometto Foundation - FHO/Araras. The experimental design is shown in Figure 1.

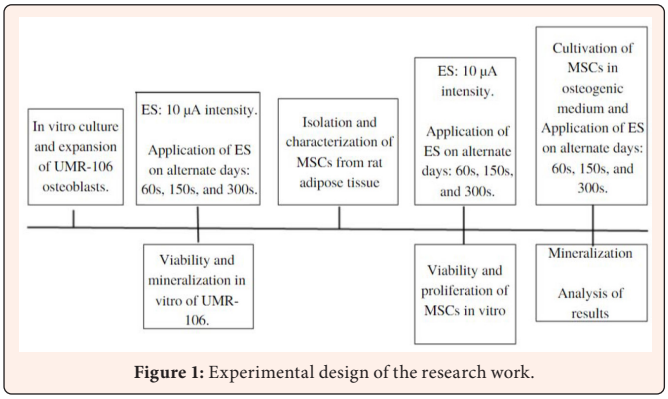


**Figure 1:** Experimental design of the research work.

### Isolation and culture of MSCs derived from inguinal (ING) and abdominal (ABD) tissues

MSCs were obtained from the inguinal (ING) and abdominal (ABD) adipose tissue of 4 Wistar rats aged approximately 90 days. The animals were anesthetized with 10% Ketamine hydrochloride (30 mg/kg body weight) and 2% Xylazine hydrochloride (10 mg/kg body weight) until deep anesthesia. The extracted tissue was kept separately in PBS (phosphate buffered saline) solution supplemented with 2% penicillin/streptomycin. After tissue extraction, the animals received another shot of anesthetic solution and submitted to cervical dislocation. Cells were obtained by enzymatic digestion of abdominal and inguinal tissues using a buffer solution (PBS) containing 0.03% collagenase enzyme type 1 (Sigma-Aldrich) at 37 °C for 30 minutes under stirring. Enzyme activity was subsequently neutralized with culture medium (α-MEM low glucose, 1% glutamine, 1% penicillin/streptomycin) supplemented with 10% SBF (Gibco-Invitrogen). The solution was centrifuged and the sediment resuspended in culture medium (the same as mentioned above) for cell growth in 75 $cm^2$ culture flasks under 5% $CO_2$ at 37 °C. The culture medium was changed twice a week and passages were performed when the culture reached 80% confluence. MSCs on the $4^{th}$ and $5^{th}$ passages were cultivated for experimentation [8].

### Immunophenotypic characterization of MSCs derived from inguinal and abdominal tissue

MSCs were immunophenotypically characterized by flux cytometry to evaluate the expression of surface markers using monoclonal antibodies: anti-CD90, anti-CD45, anti-CD44 and anti-CD34 (Santa Cruz Biotechnology, Santa Cruz, CA, USA) [8]. The assays were performed with 30 minutes exposure at room temperature, protected from light, repeated for 5 times at different passages. Flow cytometry was performed in the FACSCantoTM System (BD Biosciences) by the responsible technical professional.

### Morphological characterization of MSCs derived from inguinal and abdominal tissue

To evaluate the multipotency of MSCs from inguinal and abdominal tissue, *in vitro* differentiation into adipocytes and osteoblasts was induced. MSCs from both regions of adipose tissue were distributed in 24-wells culture plates at a concentration of 1 x $10^4$ cells/well, separately. Adipogenesis-inducing medium (α-MEM medium supplemented with 10% SFB, 1 µM dexamethasone, 10 µg/mL insulin, 100 µM indomethacin, 1% glutamine, and 1% penicillin/streptomycin) was added to the wells destined for differentiation into adipocytes, while for osteoblasts differentiation, osteogenesis-inducing medium was applied (α-MEM medium supplemented with 7.5% SBF, 0.1 µM dexamethasone, 200 µM ascorbic acid, 10 mM β-glycerolphosphate, 1% glutamine, and 1% penicillin/streptomycin)[8]. Some wells were kept with growing medium for control (α-MEM medium supplemented with 7.5% SBF, 1% glutamine, 1% penicillin/streptomycin). The culture medium for both differentiations was changed every 3 days. After 14 days, cells were washed with PBS and fixed in 4% paraformaldehyde. The adipogenic differentiation was stained with 2% Sudan IV and the osteogenic differentiation 0.2% Alizarin Red (ARS).

### Electrical stimulation application

Direct and continuous ES was applied using a transcutaneous low intensity equipment (Physiotonus Microcurrent, BIOSET®, Indústria de Tecnologia Eletrônica Ltda., Rio Claro, SP, Brazil). Two metal electrodes attached to the equipment were previously immersed in 70% alcohol for 10 minutes, dried with sterile gauze and submerged carefully in the culture medium. Approximately 1 x $10^4$ MSCs (ABD and ING) suspended each one in 1 mL of supplemented α-MEM medium (7.5% SBF, 1% L-glutamine, and 1% antibiotic-antifungal) were seeded into 24-well culture plates. After 24 h cells were treated with different ES application time (60 s, 150 s and 300 s), three times a week, at 10 µA-intensity, based on our group data using parameters employed *in vivo* [9,12,13]. Cells without ES application was considered for comparative effect.

### Cell viability and proliferation evaluation

The mitochondrial activity of osteoblasts and MSCs was determined using the colorimetric MTT assay (Sigma-Aldrich). Metabolically active cells are able to reduce the chemical compound 3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium into purple formazan crystals, soluble in dimethyl sulfoxide (DMSO). The number of viable cells is proportional to the absorbance value [14]. After 120 min incubation with MTT, the optical density was obtained using ELISA reader (Biotek) at wavelength of 540 nm. The results were obtained considering cells without ES as 100% viability. Cell proliferation was performed using the Cell Counting Kit 8 (CCK8, Sigma-Aldrich). The method uses tetrazolium salt soluble in water to evaluate metabolically viable cells through its bioreduction by electron transporters, resulting in orange formazan compound [15]. After 30 min of incubation, the optical density was obtained at a wavelength of 450 nm. The results were obtained considering cells without ES as 100% proliferation.

### Differentiation of MSCs under ES-application therapy

ABD and ING MSCs were cultivated in 24-well culture plates at the concentration of 1 x $10^4$ cells/well, separately, in 1 mL of osteogenesis-inducing medium, as described before. After 24 h, ES application was done as reported before. MSCs without ES application was considered for comparative effect (control). Evaluation of osteogenic differentiation occurred on day $14^{th}$ staining with Alizarin Red (ARS) 0.2%. The intensity of the dye was evaluated at 450nm to assess the mineralization potential [8].

### Statistical Analysis

The cell viability, proliferation and differentiation assays were carried out in triplicate, repeated three times. One-way ANOVA with Tukey post-test was used to determine the differences between the experimental groups. The differences were considered statistically significant when the p value was less than 5% ($p<0.05$). The GraphpadPrism 8.0 software (USA) was used for the statistical tests and graph construction.

## Results

### Characterization of MSCs derived from abdominal and inguinal tissue

**Immunolabeling:** The characterization of MSCs derived from abdominal and inguinal adipose tissue was first performed by immunolabeling by flow cytometry. As expected, for both cell cultures, anti-CD90 showed high expression (positive marker), while the other markers showed low expression (negative markers). The results strengthen the correct isolation and cultivation of MSCs from inguinal and abdominal adipose tissue.

**Morphological characterization:** The *in vitro* differentiation capacity of MSCs derived from adipose tissue (inguinal an and abdominal) is demonstrated in Figure 2. MSCs derived from inguinal and abdominal sources when subjected to osteogenic and adipogenic inducing medium for 14 days were able to differentiate into osteoblasts and adipocytes, respectively. Cells groups of inguinal and abdominal MSCs were cultivated in basal medium for control. Both characterization results (morphological and immunolabeling) strengthen the isolation and correct employment of these cells.

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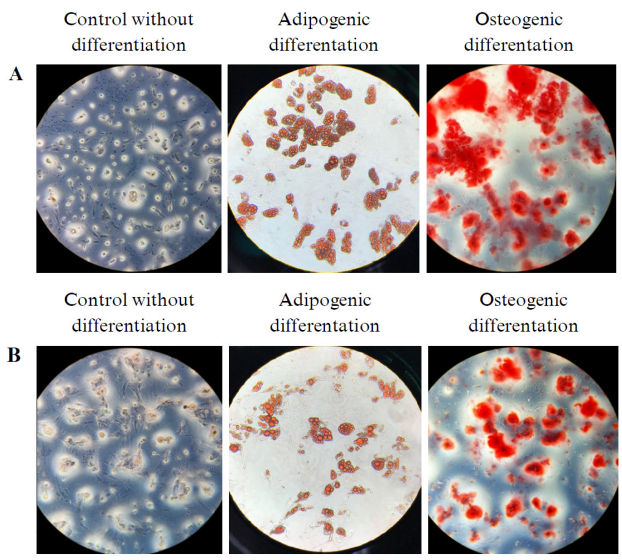
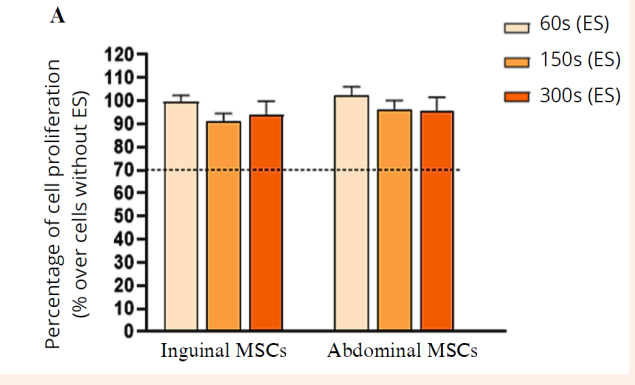



**Figure 2:** *In vitro* differentiation of MSCs derived from adipose tissue. (A) inguinal source of MSCs and (B) abdominal source of MSCs after adipogenic and osteogenic differentiation.

## Evaluation of different MSC profiles

### Viability and proliferation of MSCs from inguinal and abdominal adipose tissue

Initially, the viability and cell proliferation of MSCs from inguinal and abdominal adipose tissue submitted to ES at 10 µA on alternate days, for 60 s, 150 s and 300 s for 4 days was evaluated by MTT and CCK8 protocols, respectively. After 4 days of treating cell of ES (2 ES applications), cell viability and proliferation of both MSCs sources were higher than 70%, compared to the non-stimulated group (control group, considered as 100% viability/proliferation), both for MTT (Figure 3A) and CCK8 assays (Figure 3B). All groups showed results greater than 70% cell viability (implying non-toxicity). After 7 days of treating cell of ES, cell viability and proliferation of both MSCs sources were also higher than 70%, compared to the non-stimulated group (control group, considered as 100% viability/proliferation), both for MTT (Figure 3C) and CCK8 test (Figure 3D). The inguinal adipose tissue-derived MSCs group under ES for 60 s showed 23% higher cell viability than the abdominal adipose tissue-derived MSCs group under ES for 150 s, 18% higher compared to the same cells with ES for 300 s.



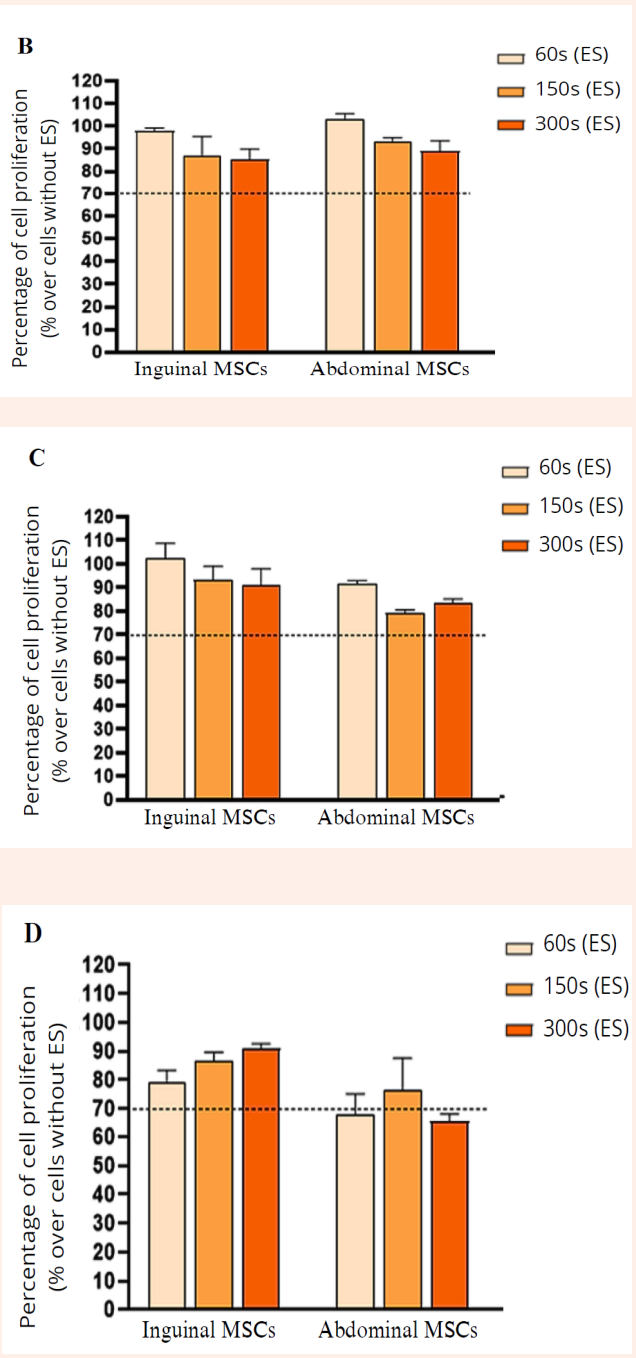




**Figure 3:** Assessment of viability and cell proliferation of MSCs derived from inguinal and abdominal adipose tissue submitted to electrical stimulation. A) Cell viability protocol by MTT after 4 days; B) Cell proliferation protocol by CCK8 after 4 days; C) Cell viability protocol by MTT after 7 days; D) Cell proliferation protocol by CCK8 after 7 days.

## Mineralization assessment

As expected, the osteogenic media contributed positively to the osteogenic differentiation of MSCs from abdominal and inguinal adipose tissue (Figure 4). Regarding the different ES application times on the inguinal cells, the 300 s application presented the greater mineralization than 60 s and 150 s time application groups, in addition to around 50% higher than the cells treated with osteogenic media, but without ES (tressed line as control group). On the other hand, the abdominal cells treated with ES for 60 s showed 43% higher mineralization compared to the control group, meanwhile 300 s and 150 s time application presented opposite results compared to the inguinal ones. These results suggest different responses from the two cellular profiles, even with the same ES application periods.

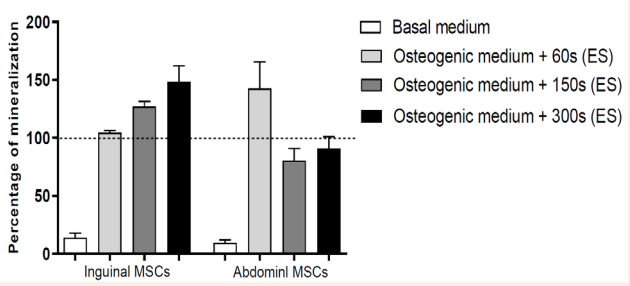



**Figure 4:** Inguinal and abdominal adipose tissue-derived MSCs mineralization assessment after 14 days. Cells were cultivated in osteogenic medium submitted to electrical stimulation application. Data is expressed as percentage of mineralization compared to cells cultivated in osteogenic medium without electrical stimulation.

## Discussion

The ES therapy has been used for a long time. However, its therapeutical application still needs further studies [16,17]. The ES promotes bone formation by the activation of different cell signaling, which stimulate the releasing of growth factors and the up-regulation of osteogenic gene expression [18]. Several research works aimed to comprehend the effects of such stimulation on cells in a wide range of experimental protocols, trying to understand the change on cells behavior and the mechanisms induced by ES. A deep understanding of its use on isolated bone cells, and posterior association to scaffolds for local and guided bone repair will definitely be an important clue for its clinically use, however, it is far away from completely comprehension [19]. The use of continuous current at 100 mA demonstrated great results regarding proliferation and elongation of osteoblasts [20]. High intensity ES (>100 V/cm) showed favorable results as well for cell proliferation in a short period of time, yet a higher intensity might induce cell death [21]. It has been also proposed that low ES intensity application (ranging from 10 µA to 50 µA) *in vitro*, as direct current, might stimulate the repair process by cell stimulation [13,16,22]. Observed enhanced proliferation and mineralization for 4 hours on *in vitro* osteoblasts by applying capacitive coupling current [23]. Nevertheless, there are disagreement on this matter, varying from protocols to cells lineages. The stimulating mechanisms are not fully understood. Different effects may be observed depending on the cellular lineage used, tissue or organs from which cells are isolated, cell culture medium, ES-time application, intensity [3].

Many are the aspects presented by the literature regarding the ES, which impairs the process of results comparison and the establishment of an adequate protocol for the clinic [2,3]. It is more than necessary establishing the right parameters for intensity, frequency, and application time, accordingly to each model of study. Initially, this study aimed to evaluate *in vitro* the application periods and frequency (daily or alternate) on osteoblasts, considering data from our research group, which used ES at 10 µA [9,12,13]. Our group reported the use of osteoblasts *in vitro* for ES application. The choice for the UMR-106 osteoblasts (rat osteoblasts) was due its good availability and use in several previous studies which employed the ES into bone repair protocols, indicating no-cytotoxicity over 7 days [9,10,23,24].

MSCs are undifferentiated and unspecialized cells with the ability of generating new cells, and under either physiological or experimental environments, differentiate into other cellular lineages, a very important role for tissue formation and regeneration [5,7]. Many works have compared the results from bone marrow MSCs and adipose tissue MSCs, which could be harvested by human or animal abdominal liposuction, or inguinal tissue from rats. However, few studies analyzed the adipose tissue-derived MSCs cellular profile from different sources [3,5]. MSCs have been characterized, expressing positive markers such CD90, CD105, and CD73, and do not express markers from hematopoietic or endothelial lineages such CD44, CD34, CD45, CD14, or CD11b [25]. MSCs also must have the ability to differentiate into *in vitro* adipocytes, chondrocytes, and osteoblasts [26]. The results are under those criteria and reinforce the fine MSCs isolation and proliferation.

According to some authors, one of the effects of *in vitro* ES therapy on adipose tissue MSCs is its influence on bone cells differentiation and/or function, such as migration, proliferation, differentiation, mineralization, and extracellular matrix production. Different effects of ES are attrubuted to the use of different cell lines, application period, medium condition [3]. It was noticed higher differentiation on the MSCs from ING and ABD under 300s and 60s of ES, respectively. However, less mineralization was observed for 150s and 300s on the ABD MSCs. Data different MSCs responses for the same experimental environment.

There is a great variety of ES intensity and application protocols in the literature, showing mainly about cellular migration and osteogenic differentiation of MSCs derived from adipose tissue, but without comparing the different sources of this tissue [24,27-29]. When used 200 µA of continuum current on Wistar mice for 4h for 21 days, and observed a 100% improvement of calcium deposition when compared to the group without ES [2]. When ES was applied along to voltage dependent calcium blockers, the previous defects were restored. This data shows a need of calcium channels in the process of cellular differentiation and a positive stimulus of ES during that process [2]. The use of ES during the initial processes of osteogenic differentiation of MSCs (until the 7$^{th}$ day) is enough to induce a pro-osteogenic effect, capable of being maintained for a long [30]. According to our data, the ES time application could vary according to MSC choice, in order to enhance the osteogenesis. For tissue engineering, data reported would benefit results, which could be possible the pre-treatment of MSCs ex vivo with ES before applying them to the bone defect.

## Conclusion

ES (direct current) at 10 µ *in vitro* up to 300 s time application showed to be safe (non-cytotoxic) for rat osteoblasts, inguinal and abdominal adipose derived mesenchymal stem cells. The immunophenotypic and multipotentiality characterizations revealed fine isolation of MSCs. Both MSCs sources presented different mineralization response. Inguinal MSCs showed greater mineralization by 300s ES application, while abdominal MSCs showed greater by after 60, under the same experimental conditions. Further investigation is required to fully understand its use in the regenerative medicine, but both of them demonstrated osteogenic potential under electrical stimulation therapy, which could provide an important cellular stimulus for clinical craniofacial defects.

## References

1. Ginebra MP, Montufar EB (2019) Cements as bone repair materials. In: Bone Repair Biomaterials. Elsevier, p. 233-271.
2. Zhang J, Li M, Kang ET, Neoh KG (2016) Electrical stimulation of adipose-derived mesenchymal stem cells in conductive scaffolds and the roles of voltage-gated ion channels. Acta Biomaterialia 32: 46-56.
3. Leppik L, Oliveira KMC, Bhavsar MB, Barker JH (2020) Electrical stimulation in bone tissue engineering treatments. Eur J Trauma Emerg Surg 46(2): 231-244.
4. Nicksic PJ, Donnelly DT, Hesse M, Bedi S, Verma N, et al. (2022) Electronic Bone Growth Stimulators for Augmentation of Osteogenesis in *In Vitro* and *In Vivo* Models: A Narrative Review of Electrical Stimulation Mechanisms and Device Specifications. Front Bioeng Biotechnol 10: 793945.
5. Gao C, Peng S, Feng P, Shuai C (2017) Bone biomaterials and interactions with stem cells. Bone Res 5(1): 17059.
6. Li J, Liu X, Crook JM, Wallace GG (2020) Electrical stimulation-induced osteogenesis of human adipose derived stem cells using a conductive graphene-cellulose scaffold. Materials Science and Engineering: C 107: 110312.
7. Lin H, Sohn J, Shen H, Langhans MT, Tuan RS (2019) Bone marrow mesenchymal stem cells: Aging and tissue engineering applications to enhance bone healing. Biomaterials 203: 96-110.
8. Caetano G, Wang W, Murashima A, Passarini JR, Bagne L, et al. (2019) Tissue Constructs with Human Adipose-Derived Mesenchymal Stem Cells to Treat Bone Defects in Rats. Materials 12(14): 2268.

9. Silva EP, Huang B, Helaehil JV, Nalesso PRL, Bagne L, et al. (2021) *In vivo* study of conductive 3D printed PCL/MWCNTs scaffolds with electrical stimulation for bone tissue engineering. Bio-des Manuf 4(2): 190-202.

10. Mishra R, Raina DB, Pelkonen M, Lidgren L, Tägil M, et al. (2015) Study of *in Vitro* and *in Vivo* Bone Formation in Composite Cryogels and the Influence of Electrical Stimulation. Int J Biol Sci 11(11): 1325-1336.

11. Zhai Q, Dong Z, Wang W, Li B, Jin Y (2019) Dental stem cell and dental tissue regeneration. Front Med 13(2)152-159.

12. Bagne L, Oliveira M, Pereira A, Caetano G, Esquisatto M, et al. (2019) Microcurrent and magnetic electrostimulation: therapies for the improvement of grafts in bone repair. International Journal of Oral and Maxillofacial Surgery 48: 270-271.

13. Fonseca JH, Bagne L, Meneghetti DH, Santos GMT, Esquisatto MAM, et al. (2019) Electrical stimulation: Complementary therapy to improve the performance of grafts in bone defects? J Biomed Mater Res 107(4): 924-932.

14. Zheng F, Wang S, Hou W, Xiao Y, Liu P, et al. (2019) Comparative study of resazurin reduction and MTT assays for cytocompatibility evaluation of nanofibrous materials. Anal Methods 11(4): 483-489.

15. Cai L, Qin X, Xu Z, Song Y, Jiang H, et al. (2019) Comparison of Cytotoxicity Evaluation of Anticancer Drugs between Real-Time Cell Analysis and CCK-8 Method. ACS Omega 4(7): 12036-12042.

16. Griffin M, Bayat A (2011) Electrical stimulation in bone healing: critical analysis by evaluating levels of evidence. Eplasty11: e34.

17. Poltawski L, Watson T (2009) Bioelectricity and microcurrent therapy for tissue healing - a narrative review. Physical Therapy Reviews 14(2): 104-114.

18. Wang Z, Clark CC, Brighton CT (2006) Up-Regulation of Bone Morphogenetic Proteins in Cultured Murine Bone Cells with Use of Specific Electric Fields: The Journal of Bone & Joint Surgery 88(5): 1053-1065.

19. Meng S, Rouabhia M, Zhang Z (2013) Electrical stimulation modulates osteoblast proliferation and bone protein production through heparin-bioactivated conductive scaffolds. Bioelectromagnetics 34(3):189-199.

20. Chen C, Bai X, Ding Y, Lee IS (2019) Electrical stimulation as a novel tool for regulating cell behavior in tissue engineering. Biomater Res 23(1): 25.

21. Mamman HB, Muhammad MAJ, Mohamad NA (2016) Optimization of Electric Field Parameters for HT29 Cell Line towards Wound Healing Application. IJST 9(1): 1-6.

22. Gittens RA, Olivares NR, Tannenbaum R, Boyan BD, Schwartz Z (2011) Electrical Implications of Corrosion for Osseointegration of Titanium Implants. J Dent Res 90(12): 1389-1397.

23. Vadlamani RA, Nie Y, Detwiler DA, Dhanabal A, Kraft AM, et al. (2019) Nanosecond pulsed electric field induced proliferation and differentiation of osteoblasts and myoblasts. J R Soc Interface 16(155): 20190079.

24. Leppik L, Zhihua H, Mobini S, Parameswaran VT, Eischen LM, et al. (2018) Combining electrical stimulation and tissue engineering to treat large bone defects in a rat model. Sci Rep 8(1): 6307.

25. Dominici M, Le Blanc K, Mueller I, Slaper CI, Marini FC, et al. (2006) Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 8(4): 315-317.

26. Taha MF, Hedayati V (2010) Isolation, identification and multipotential differentiation of mouse adipose tissue-derived stem cells. Tissue and Cell 42(4): 211-216.

27. Fu YC, Lin CC, Chang JK, Chen CH, Tai IC, et al. (2014) A Novel Single Pulsed Electromagnetic Field Stimulates Osteogenesis of Bone Marrow Mesenchymal Stem Cells and Bone Repair. Heymann D, editor. PLoS ONE 9(3): e91581.

28. Ross CL, Siriwardane M, Almeida PG, Porada CD, Brink P, et al. (2015) The effect of low-frequency electromagnetic field on human bone marrow stem/progenitor cell differentiation. Stem Cell Research 15(1): 96-108.

29. Mobini S, Leppik L, Parameswaran VT, Barker JH (2017) *In vitro* effect of direct current electrical stimulation on rat mesenchymal stem cells. PeerJ 5: e2821.

30. Eischen LM, Oliveira KMC, Bhavsar MB, Barker JH, Leppik L (2018) Pretreating mesenchymal stem cells with electrical stimulation causes sustained long-lasting pro-osteogenic effects. PeerJ 6: e4959.