Open Access

SCHOLARENA

Journal of Nanoscience and Nanotechnology Applications

CASE REPORT

Microparticles Containing Strontium Improved Therapeutic Performance In A Model Of Zebrafish Osteoporosis

Letícia Cristina Dias Lima[1], Betânia Mara Alvarenga[1], Juliana Moreira Mendonça Gomes[2], Ives Charlie-Silva[2], Tiago de Andrade Paixão[3], Daniela Chemim Melo[4], Valbert Nascimento Cardoso[3], Simone Odília Antunes Fernandes[3] and José Dias Corrêa Júnior[1*]

[1]Institute of Biological Sciences, Federal University of Minas Gerais, Brazil

[2]University of São Paulo, Brazil

[3]Faculty of Pharmacy, Federal University of Minas Gerais, Brazil

[4]Veterinary School, Federal University of Minas Gerais, Brazil

[*]**Corresponding author:** José Dias Corrêa, PhD, Junior Associate Professor Laboratory for the Study of Chemical-Biological Interaction and Animal Reproduction (LIQBRA) Block O3-174 - Department of Morphology Institute of Biological Sciences Federal University of Minas Gerais Av. Antônio Carlos, 6627 - Pampulha 31 270 901 Belo Horizonte-MG-Brasil Tel #55 31 3409 2792, E-mail: correajr@ufmg.br

Citation: Letícia Cristina Dias Lima, Betânia Mara Alvarenga, Juliana Moreira Mendonça Gomes, Ives Charlie-Silva, Tiago de Andrade Paixão, et al. (2023) Microparticles Containing Strontium Improved Therapeutic Performance In A Model Of Zebrafish Osteoporosis. J Nanosci Nanotechnol Appl 7: 101

Summary Map: This article brings a perspective of microstructured phosphate containing strontium in the treatment of osteoporosis, since there are reports that low concentrations are related to improvements this pathological condition. Microstructured phosphate containing strontium applied in a in single dose in experimental model display effectiveness in the treatment of osteoporosis pathological condition

Abstract

Osteoporosis is an osteometabolic disease that affects more than 200 million individuals worldwide and is considered a public health problem. This study evaluates whether a new approach using formulations containing microstructured strontium can be advantageous in cases of glucocorticoid- induced osteoporosis. Zebrafish (Danio rerio) was tested to detect changes in the osteoporotic picture focusing on mineralization and calcification efficiency. Data were accessed through bone-specific morphometric, staining, cytochemistry, and radiopharmaceutical techniques. Our results show that zebrafish specimens treated with a single dose of Sr-containing phosphate- based microstructure composite showed significant improvement in scale and skeletal mineralization, reaching the same values observed in the control group. Thus, these data reinforce that the cellular pathway induced by low strontium dosage can be explored as a possible therapeutic approach for osteoporosis.

Keywords: Phosphate nanostructured material, Strontium, Scales, 99mTc-MDP, Bone resorption, Bone remodeling, mineralization

Introduction

Osteoporosis is a skeletal disorder that affects more than 10 million Brazilians [1], characterized by impaired bone resistance. It is classified as an of the main public health problems, having as cause disorders that affect bone microarchitecture, which may result in increased porosity, making the bone more susceptible to fractures [2, 3, 4]. In a complement is estimated by [5], an expense for Brazil in 2018 at US$310 million.

Currently, the clinical condition of osteoporosis can be classified according to bone mineral densitometry (BMD) examination, where it is recommended that if the standard deviation varies between -1 and -2.5 the patient exhibits an osteopenia, and when this value is less than -2.5 the patient is osteoporotic [6]. This evaluation is performed by comparing the data obtained by densitometry of standardized bone regions, such as vertebrates, humerus and femur [7,8] of the patient, in relation to an individual control of the same sex and age. Another applied methodology is the Fracture Risk Assessment Tool - FRAX, where the individual anamnesis is performed together with the values of BMD and the existence or absence of fractures [9].

Once identified, the treatment of osteoporosis can be done using anabolic therapies that are premised on the increase of osteoblast activity [10,11,12]and/or anti-resorptive therapies that have as its principle the reduction of osteoclast activity [8,13,14]. Knowing this, and that the strontium presents antiresorptive and anabolic impacts on the bone matrix [15], given its similarity with calcium ion, its use was proposed as a possible therapy. However, its recommended dosage could be dangerous to human health [11,16].

Considering that zebrafish recently have their bone metabolism well characterized and also knowledge improvements on therapeutic efficacy obtained by microstructure phosphate use, in this study we display that the effect of microparticles delivering low strontium content, could be an efficient strategy in the treatment of osteoporosis induced by the use of glucocorticoids in zebrafish animal model [17,18,19].

Materials and Methods

Methodological processing of biological materials analysis

In this study, we used a total n= 40 specimens of wild zebrafish (Danio rerio), weight 0.277 ± 0.05 g and length of 3.7 ± 0.3 cm, divided into 4 aquariums (n = 10 individuals per group, 5 males and 5 females) with 2.1L of water and kept at a temperature of 26 ± 1 °C according by [20]. This experiment has total duration the of 9 days.

The 10 animals of the CT (control group) were not exposed to prednisone and treated. The other (n= 30) were submerged 24 hours before the beginning of the treatments (T-1) the aqueous solution containing 25 μM or 8.96 mg/L of prednisone (prednisolone-21-phosphate, Brainfarma Chemistry and Pharmaceutic Industries S.A), [21], in order to obtain individuals with phenotype similar to osteoporotic clinical condition, (positive control – OPN) to be challenged with treatments with microparticles containing strontium (OPN+NM-Sr) and treatment with strontium ranelate solution (OPN+R-Sr).

517.52 g/mol (C12H6N2O8SSr2 – PROTOS, Les Laboratories Servier Industries).

Twenty-four hours after the induction period (T0), all animals (n= 40) were packed in a semi-static system renewing 1/3 of the water every 24 hours. The treatments in the OPN+NM-Sr and OPN+R-Sr groups were started after 24 hours (T0). For this, the animals were contained with the reduction of water temperature (17 °C) and with the support of a nylon net. The injections were performed in the celomatic cavity using 31-gauge insulin syringes (0.25 mm, BD Ultra-FineTM), containing a volume of 10 μL per dose. The OPN+NM-Sr group received a single dose (T0) intracelomic, from an aqueous suspension containing 10 μg/10 μL obtained from a stock suspension (1 mg/mL of NM-Sr). The OPN+R-Sr group underwent the same containment procedure and received 3 intracoelomic doses of solution containing strontium ranelate, at a concentration of 173 μg/10 μL per dose [22] at T0, T3 and T6.

This experiment was approved by the Ethics Committee for the Use of Animals of the Federal University of Minas Gerais, under protocol 388/2018.

Osteogenic evaluation of Zebrafish scales

At the end of the experiment (T7), about n= 10 scales were removed from the anterior lateral part of each animal, from each experimental group (CT, OPN, OPN+NM-Sr and OPN+R-Sr) with the aid of surgical forceps and maintained in properly identified conical tubes containing aqueous solution of alcohol 70%. After being deposited on histological slides, were submitted to the cytochemical method.

Labelled calcium distribution

To evidence the calcium distribution in the scales, the slides of everyone were cordoned for 10 minutes, using Alizarine red S technique adapted from [23]. After the scales dry (n= 5 scales per animal) on histological slides into an oven at 60 oC for at least 24 hours, these were flushed for 10 minutes in aqueous solution containing 0.1% alizarin red (Sigma, Brazil) (0.02 g in 2 mL of distilled water), followed by 3 washes in distilled water, and subsequent removal of lipid pigments, dripping 20% hydrogen peroxide solution (Reagen, Brazil) and washed in distilled water.

Tartrate Resistant Acid Phosphatase (TRAP)

To evidence the activity of tartrate resistant acid phosphatase in the scales, we submitted the slides obtained as previously described and adapted cytochemical method from[24]. Histological slides (n= 5 scales per animal) were pre-incubated at room temperature in 2 mL of aqueous solution containing 5% sucrose, 20 mM of sodium tartrate (Synth, Brazil) in 0.1 M acetate buffer (Synth, Brazil) pH 5.2 for 15 minutes at room temperature. After this time, they were incubated in a humid chamber for another 1 hour, in a solution containing for every 0.5 mL of distilled water, 1% sodium sulfate (Synth, Brazil), 1 mM of Beta-glycerol phosphate (Sigma, Brazil) and 2 mM of lead nitrate Pb (NO3)2, (Dynamic, Brazil). They were then washed 3 times in distilled water.

After being ready, the scales of the techniques mentioned above were analyzed in an eclipse E1 optical microscope (Nikon), attached to an imaging system (Zeiss). The scanned images obtained with a 10x lens were calibrated with a micrometric ruler and processed for morphometric analysis, in the form of percent average of the marking area and mean intensity, using the computer programs ImageJ (FIJI), Excel (Microsoft) and Prims5 (GraphPad).

Zebrafish Body Osteogenic Evaluation

In T7 the specimens of each group (CT, OPN, OPN+NM-Sr and OPN+R- Sr) were anesthetized in 2 moments: (i) mild sedation in a solution containing 50 μL of eugenol (Biodynamics, Brazil) in 100 mL of distilled water, adapted from Grush (NOAKES & MOCCIA, 2004), for application of intracoelomic injection containing 10 μL of the radiopharmaceutical ^{99m}Technetium-methylene diphosphonate (^{99m}Tc-MDP) with activity of 0,111 MBq (3 μCi), and (ii) 1 hour after the injection of the ^{99m}Tc-MDP, a new anesthesia was performed in adapted [25] containing 700 μL of eugenol (Biodynamic, Brazil) in 100 mL of distilled water and the animals were taken to the gamma camera (NuclideTM TH 22, Mediso, Hungary) in order to obtain the images. Scintigraphic images were acquired using a 256 × 256 × 16 matrix size with a 20% energy window set at 140 keV for a period of 5 min and an orientation of the animal on a 90º axis. After obtaining the images, the animals were euthanized in a solution also adapted from [25], containing 2 mg of eugenol (Biodynamics, Brazil) in 100 mL of distilled water, for 10 minutes. After confirmation of death, the animals were dissected, separating the head from the body and removing the viscera. These materials were then weighed and placed in appropriate previously tared tubes for radioactivity determination. All tubes were counted in an automated gamma counter (Wizard 1480, PerkinElmer, Finland). Results are expressed as counts per minute (cpm).

Statistical analysis

The recorded data were expressed as mean ± standard mean error. The results obtained were tested by ONE Way: one-way ANOVA, considering statistically different values with p.<0.05.

Results and Discussion

Osteogenic evaluation of Zebrafish scales

Once two staining techniques would be performed with these, the final n of scales obtained (FIG.1) was divided equally into groups.

Test	n of Scales	Groups	
		CT	OPN
% Calcium labelled	30	0.39 ± 0.01	0.36 ± 0.01a
Rate bone resorption %	35	0.15 ± 0.009	0.10 ± 0.003a
TRAP			

[at] significance level p< 0.05 as determined by One Way method: Tukey's multiple comparison test, followed by paired t-test, mean ± standard error.

Figure 1: Determination of the minimum number of Zebrafish scales

As well as the results obtained by [21], our trials also demonstrated that the OPN group presented by the technique of alizarin red S, reduction of mineralized areas, accompanied by the decrease in the intensity of marking in the red channel for calcium, associated with the increase of areas of bone resorption in zebrafish scales, evidenced by TRAP technique (Figure 2A).

A				
Test	Groups			
	CT	OPN	OPN+NM-Sr	OPN+R-Sr
% Calcium labelled	0.64 ± 0.06 a	0.39 ± 0.07 b	0.74 ± 0.04a	0.61 ± 0.06 a.b
Intensity of Red Channel	3802 ± 405.7a	2304 ± 424.3b	4329 ± 244.5a	3574 ± 364.7
U.I.				a,b
Bone resorption % TRAP	0.45 ± 0.03 a	0.66 ± 0.01 b	0.44 ± 0.02 a	0.49 ± 0.02a

[a, b] significance level p < 0.05 as determined by One Way method: Tukey's multiple comparison test, followed by paired t-test, mean ± standard error.

On the other hand, the groups treated with strontium showed significant results in relation to the OPN group and similar to the CT group, indicating that both (OPN+NM-Sr and OPN+R-Sr) had the bone dynamics influenced. These showed an increase in mineralized areas and intensity of red channel marking for calcium and decrease in bone resorption areas (Figure 2).

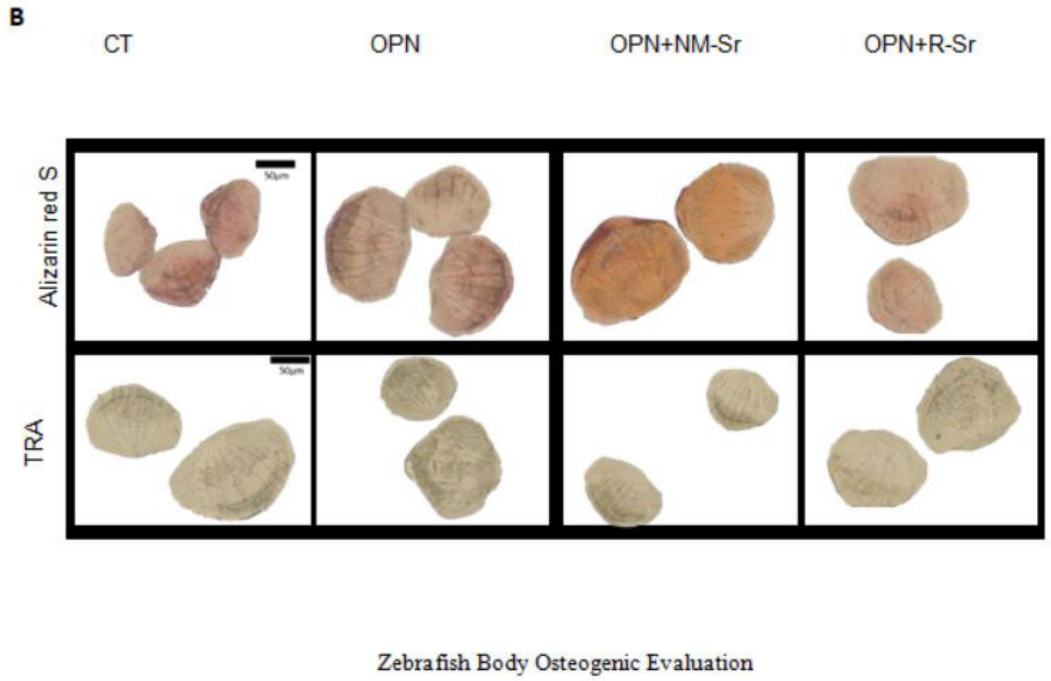

Figure 2: Bone remodeling in scale of Zebrafish scales, 8 days treatment. (A) histochemical quantification of marked scales and (B) image scales in the groups/treatment.

The 99mTc-MDP can be used for the diagnosis and follow-up of osteoporosis [16] due to its interaction with the bone matrix where remodeling is taking place; and as prednisone induces a picture similar to osteoporosis in Zebrafish was is possible to observe a greater uptake in these groups.

The values obtained of 99mTc-MDP show that there is an uptake increased in the cranial region of the OPN and OPN+NM-Sr groups when compared to CT group. On the other hand, the OPN+R-Sr group showed intermediate values when compared to CT group (FIG 3). The increased uptake rate may be related to the propensity to microfractures due to osteoporosis in the skull and vertebral areas of zebrafish, a characteristic similar to that observed in mice and humans [13, 3, 28]. The data showed also significant decreases of the 99mTc-MDP uptake in the body and visceral.

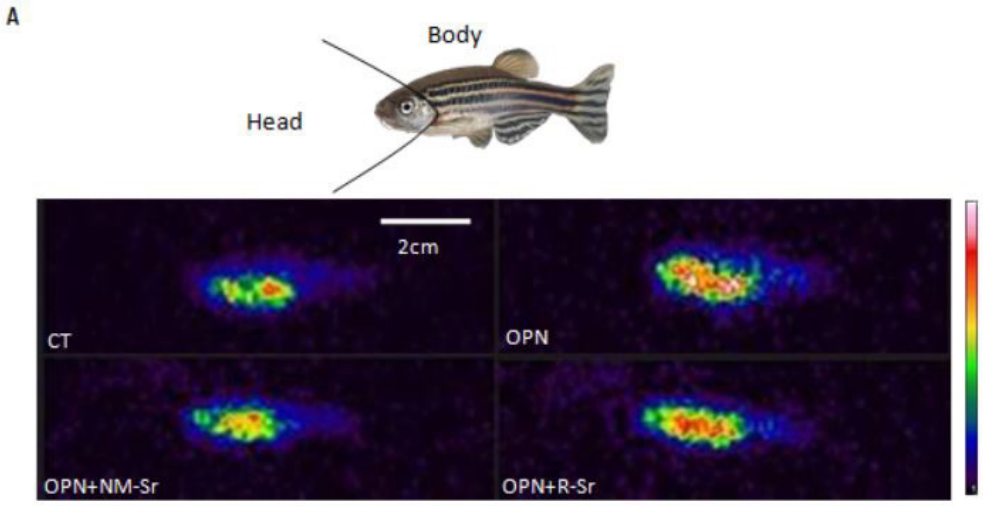

Figure 3: Biodistribution of the ^{99m}Tc-MDP in the skeletal part of Zebrafish in 8 days of treatment. (A) image in the groups, and (B) quantification of the radiation count per minute (cpm) in the groups related to each region investigated.

B				
	Groups			
Test	_			
	CT	OPN	OPN+NM-Sr	OPN+R-Sr
Total Animal cpm				
	1.64 ± 0.01	1.64 ± 0.03	1.61 ± 0.06	1.60 ± 0.04
(LOG)				
Head cpm (LOG)	5.18 ± 0.22a	5.85 ± 0.06b	5.75 ± 0.08b	5.54 ± 0.11a.b
Body cpm (LOG)	5.89 ± 0.08a.b	6.00 ± 0.06a	5.64 ± 0.07b.c	5.58 ± 0.08c
Viscera cpm (LOG)	5.54 ± 0,26a	4.68 ± 0,27b	4.31 ± 0,19b	4.15 ± 0,15b

[a, b, c] significance level $p < 0.05$ as determined by One Way method: Dunnett's multiple comparison test, followed by paired t-test, mean ± standard error, n= 8.

However, in total body radiation, the osteoporotic effect in the OPN, OPN+ NM-Sr and OPN + R-Sr groups is not evident because the data did not show any differences among the groups investigated, although it is observed that the 99mTc-MDP complex has a wide biodistribution, as described by [30].

Although so far there is no evaluation of 99mTc-MDP pathway in fish in the literature, it is possible to observe that the values and biodistribution obtained are similar to the results in mice[31]. This complex can be associated with plasma albumin [32] and mononuclear cells [33] resulting on its gradual transport.

The reduction in the uptake rate may be an indication of the effectiveness of the treatment since it has the ultimate goal of benefiting bone regeneration.

Another factor visualized is the reduction in the uptake rate in the viscera of the OPN, OPN+NM-Sr and OPN+R-Sr groups in relation to CT, since the exposure time to the 99mTc-MDP complex was 1 hour for all the groups. This finding raises the hypothesis that, as the complex does not easily bind to the bone matrix due to the bone tissue being mature [26] in the CT group, the compound tends to move through the bloodstream until its excretion, and this occurs and a period of time longer than 1 hour, with the visceral part being as well irrigated as the other regions of the body, ends up presenting a greater uptake of the complex in the CT group, when compared to the other groups.

Although the OPN+R-Sr group is different from the CT group, it should be remembered that high concentrations of strontium were considered harmful to health [16]. So, the effect observed in the OPN+NM-Sr group indicates the successful on elemental mobilization and targeting mediated biologically. This approach may be more effective in the osteoporosis remission without the compromise and/or unwanted development of properties related to bone metabolism or other systems like cardiovascular. Moreover, due to Sr use in a single low dose, highlights the possible advantages on prospection of bone cells metabolism new therapies aproaches using the Sr pathway knowledge [34].

Conclusion

We demonstrate that the NM-Sr microstructured has a possible therapeutic action in the framework developed in zebrafish, in addition to opening a window, regarding the use of this model for other techniques and analysis, in order to assess the developed osteoporotic condition.

Thus, this assay is the first in which single dose of nanostructured phosphated particles are injected in celomatic cavity are successfully associated to histological, histochemical and 99mTc-MDP evaluation are used in zebrafish with an osteoporotic phenotype.

Appreciations

This section should mention any individuals or groups that are not named as authors but who have contributed to the research presented (e.g., in terms of reagents, time, experience) or writing of the manuscript.

Competitive interests

No declared competitive interest.

Financing

This survey has not received any specific concessions from any funding agency in the public, commercial, or non-profit sectors.

References

1. Moraes LFS, Silva EN, Silva DAS, Paula AP (2014). Expenditures on the treatment of osteoporosis in the elderly in Brazil (2008 - 2010): analysis of associated factors. Brazilian Journal of Epidemiology, 17: 3.

2. JAMA (2001). Osteoporosis Prevention. Diagnosis and Therapy, 285: 785 - 795.

3. Emkey GR, Epstein S (2014). Secondary osteoporosis: Pathophysiology & diagnosis. Best Practice & Research Clinical Endocrinology & Metabolism, 28: 911-35.

4. Soriano R, Herrera S, Nogués X, Diez-Perez A (2014). Current and future treatments of secondary osteoporosis. Best Practice & Clinical Research Endocrinology & Metabolism, 28: 885-94.

5. Aziziyeh R, Amin M, Habib M, Perlaza JG, Szafranski K, et al. (2019). The burden of osteoporosis in four Latin American countries: Brazil, Mexico, Colombia, and Argentina. Journal of Medical Economics, 22: 638-44.

6. World Health Organization (WHO) (1994). Assessment Of Fracture Risk And Its Application To Screening For Postmenopausal Osteoporosis. Geneva: World Health Organization, 1-129, Technical Report Series, n. 843.

7. Papaoannou A, Morin S, Cheung AM, Atkinson S, Brown JP, et al. (2010). Clinical practice guidelines for the diagnosis and management of osteoporosis in Canada: summary. Canadian Medical Association Journal, 182, 17: 1864-1873.

8. Cosman F (2019). The evolving role of anabolic therapy in the treatment of osteoporosis. Current Opinion in Rheumatology, 31: 376-80.

9. Eastell R, Rosen JC, Black DM, Cheung AM, Murad MH, et al. (2019). Pharmacological Management of Osteoporosis in Postmenopausal Women: An Endocrine Society* Clinical Practice Guideline. The Journal of Clinical Endocrinology & Metabolism, 104: 1595-622.

10. Cosman F, Nieves JW, Dempster DW (2017). Treatment Sequence Matters: Anabolic and Antiresorptive Therapy for Osteoporosis. Journal of Bone and Mineral Research, 32: 198-202.

11. Hendrych M, Olejnickova V, Novakova M (2016). Calcium versus strontium handling by the heart muscle. General Physiology and Biophysics, 35: 13-23.

12. Russow G, Jahn D, Appelt J, Märdian S, Tsitsilonis S, et al. (2019). Anabolic Therapies in Osteoporosis and Bone Regeneration. International Journal of Molecular Sciences, 20: 1.

13. Nichols DL, Pavlovic A (2018). Chapter 23: Osteoporosis. Physiology of clinical exercise/organization. Jonathan k. Ehrman [et al.]; Scientific review Reury Frank Pereira Bacurau. - 3. ed, São Paulo: Phorte.

14. Cheng C, Wentworth K, Shoback DM (2020). New Frontiers in Osteoporosis Therapy. Annual Review of Medicine, 71: 277-88.

15. Marie PJ, Ammann P, Boivin G, Rey C (2001). Mechanisms of Action and Therapeutic Potential of Strontium in Bone. Calcified Tissue International, 69: 121-9.

16. EMA - European Medicines Agency (Science Medicines Health) (2014). OSSEOR: Strontium ranelate EMA/139812/2014. EMEA/H/C/000561, European Medicines Agency.

17. Paul W, Sharma CP (2012) Synthesis and characterization of alginate coated zinc calcium phosphate nanoparticles for intestinal delivery of insulin. Process Biochemistry, 47: 882-6.

18. Alvarenga BM, Melo MN, Frézard F, Demicheli CP, Gomes JMM, et al. (2015). Nanoparticle phosphate-based composites as vehicles for antimony delivery to macrophages: possible use in leishmaniasis. Journal of Materials Chemistry B, 3: 9250-9.

19. Tonelli FMP, Lacerda SMN, Paiva NCO, Lemos MS, De Jesus AC, et al. (2016). Efficient and safe gene transfection in fish spermatogonial stem cells using nanomaterials. Royal Society of Chemistry Advances, 6: 52636-41.

20. Charlie-Silva I, Feitosa NM, Fukushima HCS, Borra RC, Foglio MA, et al. (2021). Effects of nanocapsules of poly-ε- caprolactone containing artemisinin on zebrafish early-life stages and adults. Science of The Total Environment, 756: 143851.

21. Vrieze E, Van Kessel MAHJ, Peters HM, Spanings FAT, Flik G, et al. (2014). Prednisolone Induces Osteoporosis-Like Phenotype In Regenerating Zebrafish Scales. Osteoporosis International, 25.

22. Li YF, Luo E, Feng G, Zhu SS, Li JH, Hu J (2009). Systemic treatment with strontium ranelate promotes tibial fracture healing in ovariectomized rats. Osteoporosis International, 21, 11: 1889-1897.

23. Faganello CS, Da Silva VRM, De Andrade MCC, Carissimi AS, Pigatto JAT (2016). Morphology of endotelial cells from different regions of the equine cornea. Rural Science Magazine, Santa Maria, 46: 2223-8.

24. Pasqualetti S, Congiu T, Banfi G, Mariotti M (2015). Alendronate rescued osteoporotic phenotype in a model of glucocorticoid induced osteoporosis in adult zebrafish scale. International Journal of Experimental Pathology, 96: 11-20.

25. Grush J, Noakes DLG, Moccia RD (2004). The Efficacy of Clove Oil As An Anesthetic for the Zebrafish, Danio rerio (Hamilton). Zebrafish, 1: 46-53.

26. IPEN – Nuclear and Energy Research Institute (2015). MDP-TEC. Nuclear and Energy Research Institute, Rev. 00/15.

27. Guarniero R, Oliveira LG (2004). Osteoporosis: update on diagnosis and basic principles for treatment. Brazilian Journal of Orthopaedics, 39: 9.

28. Cesar R, Rollo JMDA, Boffa RS, Fachine LT, Bellani CF, et al. (2013). Evaluation of osteopenia and osteoporosis by 3D microtomography, VI SEB-UFU.

29. SIQUEIRA D (2014). Osteoporosis: an ultrastructural analysis of bone tissue in ovariectomized C57 and ApoEKO mice. Thesis presented to the Graduate Program in Biotechnology of the Federal University of Espírito Santo.

30. Mccracken M, Zinn K, Lemons JE, Thompson JA, Feldman D (2001). Radioimaging of implants in rats using Tc-99m-MDP. Clinical Oral Implants Research, 12: 372-8.

31. Kurniawan A, Mahesa Y, Wibawa THA, Iswahyudi I, Daruwati I, Wangsaatmadja AH (2020). In Vivo biodistribution of 99mTcMDP for early osteoporosis monitoring in ovariectomized BALB/c mice. Indonesian Journal of Nuclear Science and Technology, 21: 1-10.

32. Savelkoul TJF, Van Ginkel JJ, Grouls RJE, Oldenburg SJ, Duursma SA (1985). Protein-binding and urinary excretion of 99mTc (Sn)- MDP and 99mTc-MDP. International Journal of Nuclear Medicine and Biology, 12: 125-31.

33. Suhett GD, De Souza SA, Carvalho AB, De Pinho Rachid R, Da Cunha-E-Silva NL, et al. (2015). 99m-Technetium binding site in bone marrow mononuclear cells. Stem cell research & therapy, 6, 1: 115.

34. Zhang X, Cui J, Cheng L, Lin K (2021). Enhancement Of Osteoporotic Bone Regeneration By Strontium-Substituted 45S5 Bioglass Via Time-Dependent Modulation Of Autophagy And The Akt/Mtor Signaling Pathway. J. Mater. Chem, 9: 3489-501.