HYPOTHESIZING THE USE OF AYURVEDIC PREPARATION (PRAVAL BHASMA) TO REPLACE STANDARD OSTEOGENIC BONE COCKTAIL FOR INDUCING OSTEOGENIC DIFFERENTIATION IN HUMAN DENTAL PULP STEM CELLS (HDPSCS).

Dr Uday Londhe[1*], Dr. Kalyani Bhate[2], Dr Ramesh Bhonde[3], Dr Lakshmi Shetty[4], Avinash Kharat[5], Vd Arati Dubewar[6], Dr Deepak Kulkarni[7]

Article History: Received: 04.05.2023 Revised: 14.06.2023 Accepted: 10.07.2023

Abstract

Introduction: The treatment of bone defects is a major problem in Dentistry. Regeneration of bone can be done using stem cell therapy. Stem cells can be derived from Dental Pulp (hDPSc). hDPSc when exposed to Standard Osteogenic Bone cocktail (Dexamethasone, Ascorbic acid and beta glycerophosphate) differentiate into osteoblast and deposit bone. Except for Ascorbic Acid, Dexamethasone and glycerophosphate have little osteogenic potential.
Hypotheses: Human Dental Pulp Stem Cells (hDPSCs) when preconditioned with Praval Bhasma induce osteogenic potential in Human Dental Pulp Stem Cells (hDPSCs).
Evaluation Of Hypotheses: The Human Dental Pulp Stem Cells (hDPSCs) will be preconditioned using Praval Bhasma as a constituent of Standard Osteogenic Bone Cocktail by replacing one ingredient with Praval Bhasma.The cell culture will be incubated at 37 degrees celcius and evaluated histologically for evaluation of bone deposition.

Keywords: Human Dental Pulp Stem Cells (hDPSCs), osteoblastic differentiation, Standard Osteogenic Cocktail, Praval Bhasma.

[1*]M.D.S Associate Professor, Dept. of Oral & Maxillofacial Surgery, Dr. D. Y. Patil Vidyapeeth. Pimpri Dr D Y Patil Dental College and Hospital, Pimpri, Pune
Orcid no. [1*]http://orcid.org/0000-0002-5569-9379
Email: [1*]uday.londhe@dpu.edu.in
[2]M.D.S Ph.D, Professor, Dept. of Oral & Maxillofacial Surgery, Dr .D. Y. Patil Vidyapeeth. Pimpri Dr D Y Patil Dental College and Hospital, Pimpri,Pune
Orcid no. [2]http://orcid.org/0000-0002-6654-6060
Email: [2]kalash4@rediffmail.com
[3]PhD, Professor, Dr D Y Patil Vidyapeeth, Pimpri Dr D Y Patil Dental College and Hospital, Pimpri,Pune
Email: [3]ramesh.bhonde@dpu.edu.in
Orcid no: [3]http://orcid.org/0000-0001-9955-2056
[4]M.D.S, Professor, Dept. of Oral & Maxillofacial Surgery, Dr .D. Y. Patil Vidyapeeth. Pimpri Dr D Y Patil Dental College and Hospital, Pimpri,Pune
Orcid no: [4]https://orcid.org/ 0000-0003-2814-8026
Email: [4]lakshmi.shetty@dpu.edu.in
[5]MSc Regenrative Medicine Laboratory Dr .D. Y. Patil Vidyapeeth. Pimpri Dr D Y Patil Dental College and Hospital, Pimpri,Pune

Orcid no. [5]http://orcid.org/0000-0002-5647-8340
[6]Dept. of Rasashastra, Dr D Y Patil College of Ayurveda, Dr .D. Y. Patil Vidyapeeth. Pimpri
Orcid no: [6]https://orcid.org/ 0000-0003-3036-7607
Email: [6]aratidubewar@gmail.com
[7]MDS Consultant Oral & Maxillofacial Surgeon,
Email: [7]uday.londhe22@gmail.com

[*]**Corresponding Author:**
Dr Uday Londhe[1*]
M.D.S Associate Professor, Dept. of Oral & Maxillofacial Surgery, Dr.D. Y. Patil Vidyapeeth. Pimpri Dr D Y Patil Dental College and Hospital, Pimpri,Pune
Orcid no: [1*]http://orcid.org/0000-0002-5569-9379
Email: [1*]uday.londhe@dpu.edu

DOI: 10.31838/ecb/2023.12.6.164

1. INTRODUCTION

The treatment of bone defects is a major problem in Oral and Maxillofacial Surgery. Bone defects are caused due to trauma, cancer surgeries, infections or congenital defects. The materials used in bone regeneration should necessarily be safe, biocompatible, biodegradable and should have osteogenic potential. The materials best suited for bony defect reconstruction are autologous in origin, but autologous bone grafts have limitations like donor site pain and morbidity, secondary surgery, bone resorption, osteonecrosis, paresthesia, cutaneous nerve damage, vascular injury, infection, fracture and chronic pain. Allogenic bone and biosynthetic materials are also limited by biocompatibility issues, infection, immune rejection and graft displacement. Stem Cell therapy has recently gained popularity as an option in bone regeneration treatment.

Stem cells are derived from a natural source, which can give stable differentiated cells leading to tissue formation. The human body contains several stem cell sources like bone marrow, umbilical cord, amniotic fluid, adipose tissue, dental tissues, Human Dental Pulp Stem Cells (hDPSCs) and periodontal ligament. When exposed to various preconditioning factors mesenchymal stem cells (MSCs) have the ability to differentiate into a variety of cells like myoblasts, adipocytes, osteoblasts, and chondrocytes. [1,2]

For several experimental studies, including tissue engineering studies, osteogenic differentiation protocols often use the "Standard Osteogenic Cocktail" consisting of dexamethasone (Dex), ascorbic acid (Asc), and β-glycerophosphate (β-Gly).[3] In osteoblastic cell cultures, application of Dexamethasone for a short duration increases bone deposition, but the application of Dexamethasone for a prolonged duration decreases bone deposition. Glycerophosphate has very little osteogenic potential. Ascorbic Acid improves osteoblastic differentiation by causing an increase in collagen accumulation, leading to an increase in Alkaline phosphatase (ALP) expression in a few osteogenic cells. Except for Ascorbic Acid, Dexamethasone and glycerophosphate have little osteogenic potential of their own.[4] Moreover, the time required is 3 weeks to get a maximum of only 30-40% osteogenic differentiation from MSCs. Hence, there is a need to search for newer preconditioning agents having better osteodifferentiation potential on hDPSCs in tissue engineering.

Praval Bhasma is routinely used in Ayurveda for treating osteoporosis.[5,6] Praval bhasma (Coral calx) is a natural source of rich calcium that has long been used as a supplement in the treatment of several bone metabolic disorders linked to calcium deficiency in Indian traditional medicine.[7]
Praval is the calcareous skeleton of marine organisms and it belongs to the phylum

Coelenterate. The skeleton is shaped like minute irregular deposits, which are called spicules containing mainly calcium carbonate. Amorphous calcium carbonate (ACC) forms as a precursor to crystalline carbonated apatite/hydroxyapatite in human bone (HA).[8] The calcium carbonate (CC) skeleton of marine corals is osteoconductive. The alteration of the calcium carbonate (degradation and new crystal formation) is essential for osteoblastic apposition and differentiation of osteoprogenitor cells into osteogenic cells ultimately leading to bone formation.[9] The percentage of Calcium in Praval Bhasma is 38.2 % w/w. The particle size of Praval Bhasma is 20-100nm. This nanosize particle differs in chemical composition and structure, increases the surface area and helps the drugs to reach the target area effectively.[10] Praval Bhasma has never been studied in hDPSCs as a preconditioning agent. Praval Bhasma is economical and easily available. Hence, we hypothesize that Prawal Bhasma can be used as a preconditioning agent to induce Osteogenic differentiation in hDPSCs.

1.1 HYPOTHESIS

Praval Bhasma induces osteogenic differentiation in Human Dental Pulp Stem Cells (hDPSCs).

1.2 EVALUATION OF THE HYPOTHESIS

We propose the hypothesis can be proved by the following method,

The hDPSCs will be preconditioned with Newer Osteogenic Cocktail. A Newer Osteogenic Cocktail will be prepared by replacing Dexamethasone (Dex) in Standard Osteogenic Cocktail with Praval Bhasma. A second Osteogenic Cocktail will be prepared by replacing Ascorbic acid (Asc) in the Standard Osteogenic Cocktail withPraval Bhasma and a third Osteogenic Cocktail will be prepared by replacing β-glycerophosphate(β-Gly) in the Standard Cocktail with Praval Bhasma. The cells will be preconditioned using all the above 3 preconditioning agents for 24-48 hours and thenprocessed for osteogenic differentiation. The cells will then be identified using the microscope. Osteogenic differentiation of hDPSCs with all the preconditioning agents using HandE stain, Alizarin red Stain and Immunohistochemistry.

Autologous DPSCs can be a new tool in bone tissue engineering techniques. hDPSCs therapy is efficient has low morbidity of the donor site and is free from diseases transferred by the transmission of pathogens. The regeneration process is fast. The use of Praval Bhasma may prove to be a better preconditioning agent for bone formation in hDPSCs.

Fig – 1 - Human Dental Pulp Stem Cells (hDPSCs)

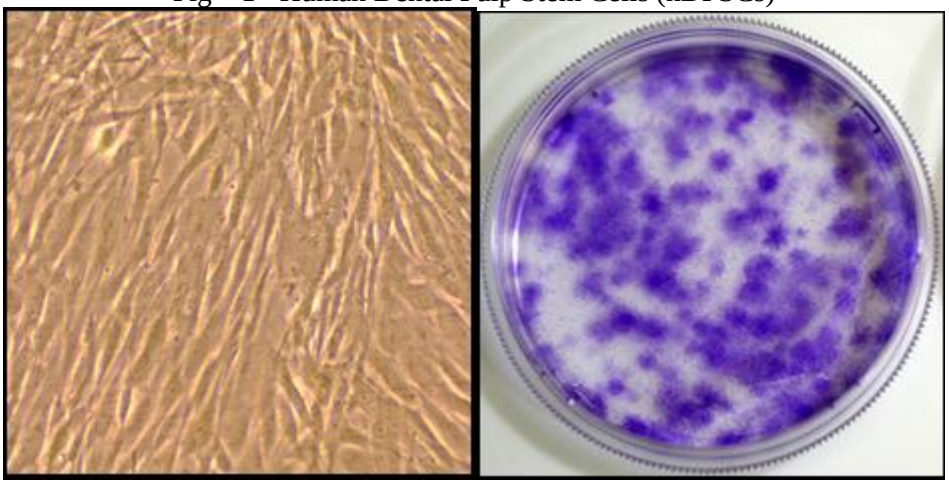

Fig – 2 – Praval.

Declaration of Interests: None

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☒The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Consent Statement/Ethical Approval: Not Required

☐ Please add the consent statement/ethical approval for the paper if Laboratory experiments including animals/Human studies was performed. 'If your study does not require it, then please state this: Consent statement/Ethical approval: Not required'.

Please check this box to confirm that all co-authors have read and approved the version of the manuscript that is submitted. Signatures are not required.

Funding

☒Please confirm that you have mentioned all organizations that funded your research including grant numbers where appropriate.

2. REFERENCES

1. Belmonte GC, Holgado LA, de Labio RW,et al. (2016) The Potential of Human Dental Pulp Stem Cell from Deciduous Teeth in Bone Regeneration: An Experimental Study in Rabbits. Int J Oral Dent Health 2:032. 10.23937/2469-5734/1510032.
2. Uday Suresh Londhe, Arati P. Dubewar, Kalyani Bhate, Ramesh Bhonde,Lakshmi Shetty, Deepak G. Kulkarni. Role of Praval Bhasma in Inducing Osteogenic Potential in Human Dental Pulp Stem Cells. J Dental Res Rev, Volume 7, Supplement 1,2020,S91-S94.
3. 3.Fabian Langenbach and Jörg Handschel . Effects of dexamethasone, ascorbic acid and β-glycerophosphate on the osteogenic differentiation of stem cells in vitr. Stem Cell Res Ther. 2013; 4(5): 117. Published online 2013 Sep 30. doi: 10.1186/scrt328
4. Jun-Beom Park, The Effects of Dexamethasone, Ascorbic Acid, and b-Glycerophosphate on Osteoblastic Differentiation by Regulating Estrogen Receptor and Osteopontin Expression. J Surg Res 173, 99–104 (2012) doi:10.1016/j.jss.2010.09.010
5. Suman lata and Rudrambika S Biradar. Physio-chemical analysis of Praval Bhasam - Prepared by using Praval Mool as raw material. International Journal of Ayurvedic and Herbal Medicine 2015; 5(4): 1954–1963.
6. V. Nageswar Rao and S.K Dixit. Standardisation of Pravala Bhasma. Ancient Science of Life vol. no 17(3), January 1998 pages 203 – 206.
7. Prabhakara N. Reddya,∗, M. Lakshmana b, U. Venkatesh Udupac, Effect of *Praval bhasma* (*Coral calx*), a natural source of rich calcium on bone mineralization in rats. Pharmacol Res *48 (2003) 593–599)*
8. Emad Tolba, Werner E.G. Müller,a Bothaina M. Abd El-Hady, et al. High

biocompatibility and improved osteogenic potential of amorphous calcium carbonate/vaterite. J. Mater. Chem. B, 2016,4, 376-386**.**

9. Hajime Ohgushi,* Motoaki Okumura, Takafumi Yoshikawa, Keisuke Inoue, Norio Senpuku, and Susumu Tamai. Bone formation process in porous calcium carbonate and hydroxyapatite. J Biomed Mater Res, Vol. 26, 885-895 (1992).
10. Sanjoy Kumar Pal. The Ayurvedic Bhasma: The Ancient Science of Nanomedicine. Recent Patents on Nanomedicine, 2015, 5, 12-18 1877-9131/15.