

# Potential Effect of Different Pulp Capping Materials on Human Dental Pulp Stem Cells: A Laboratory Study

Mohamed A. Bedir<sup>1</sup>, Laila Amin<sup>2</sup>, Essam soussa<sup>3</sup>

## Abstract:

**Objective:** This study aimed to evaluate the regenerative capacity of two different pulp capping materials TheraCal LC and Nano hydroxyapatite on stem cells extracted from the human dental pulp as a treatment modality for pulp capping. **Materials and Methods:** Human dental pulp stem cells were obtained and characterized. They were prepared in 12-well plates, cultured in standard culture media, and separated into three groups with 12 each. The first group served as the negative control group and contained only human dental pulp stem cells; the second group contained TheraCal LC, which was incubated with human dental pulp stem cells; and the third group contained nano-hydroxyapatite, which was incubated with human dental pulp stem cells. The 3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide (MTT) test was used to examine the cytotoxicity and proliferation of human dental pulp stem cells. Osteogenic differentiation was determined using alizarin red staining. **Results:** Nano-hydroxyapatite showed significantly higher cell viability at 5 days ( $0.281\pm 0.03$ ) compared to the control group ( $0.262\pm 0.02$ ) while the TheraCal LC group showed less cell viability ( $0.167\pm 0.02$ ). Nano-hydroxyapatite showed the largest calcified nodules and TheraCal LC showed the smallest. **Conclusions:** The application of TheraCal LC and Nano-hydroxyapatite in combination with the stem cells extracted from dental pulp tissues can be considered a suitable treatment modality for capping the pulp.

## Introduction:

A therapeutic procedure used commonly to protect tooth vitality is direct pulp capping (DPC), after its accidental exposure in developing or mature teeth. The biocompatibility and bioactivity of the capping material used are so important, and they influence the success of the DPC operation. DPC ensures the successful removal of irritation and infection from the exposed dental pulp. To preserve the long-term functionality of the tooth, it's critical to keep the pulp healthy, so the pulp must be sealed if it is exposed to the oral cavity. Furthermore, the DPC material should encourage the dentine-pulp complex's healing and regeneration.<sup>1,2</sup>

TheraCal LC is a resin liner, which contains calcium silicate. It was developed as a protective liner under the final restorations in direct and indirect pulp capping procedures. It was described that it has low toxic effects, if it is compared with others, and can enhance additional calcium particles' precipitation compared to other cement materials. TheraCal LC was revealed to be favorable to stimulating the production of secondary dentine bridges. Although TheraCal lacks aesthetic appearance, its physical and chemical characteristics are more appealing, TheraCal LC with poor solubility properties precipitated more calcium ions than either ProRoot MTA or Dycal.<sup>3,4</sup>

Nano-hydroxyapatite (NHA) is calcium phosphate cement that structurally resembles dental hydroxyapatite. It was designed as a bioactive material

in several endodontic procedures, owing to its capacity to produce calcium and phosphate ions on hydration. Additionally, NHA revealed a proper role in mesenchymal stem cell differentiation.<sup>5</sup>

The study of stem cells offers hope for tissue regeneration. Many tissues, including bone marrow, skeletal muscles, skin, and bone, depending on postnatal stem cells. In dental tissues (ex; adult pulp tissue, apical papilla, deciduous dental pulp, and periodontal ligament) the presence of stem cells has been confirmed. In both in-vivo and in-vitro experiments, dental pulp stem cells (DPSCs) have confirmed their ability to self-renew and multi-lineage differentiate into cells that resemble odontoblasts, and to enhance calcified nodule formation. The ability of hDPSCs to restore dental tissues is one of their most notable traits because it is essential in the healing process. As a result, it improves the in vitro biological characteristics of the biomaterials, particularly their capacity to create tertiary dentin.<sup>6-8</sup>

Dental pulp cells multiply and move to the wounded area during pulp healing, then differentiate into cells that resemble odontoblasts, and deposit reparative dentin at the wounded area. These dental pulp cells that are responsible for this regeneration and repair process come from the perivascular zone and from the pulp tissue itself. During the production of tertiary dentin, hDPSCs played a significant role. The pericyte-associated antigen is expressed by STRO1-positive hDPSCs, representing the formation of the perivascular niche of hDPSCs.<sup>9,10</sup>

These cells are more capable of proliferating and resemble fibroblasts when compared to mesenchymal stem cells produced from human bone marrow. hDPSCs exhibit mesenchymal stem cell markers and have the capacity for multi-potential differentiation. It

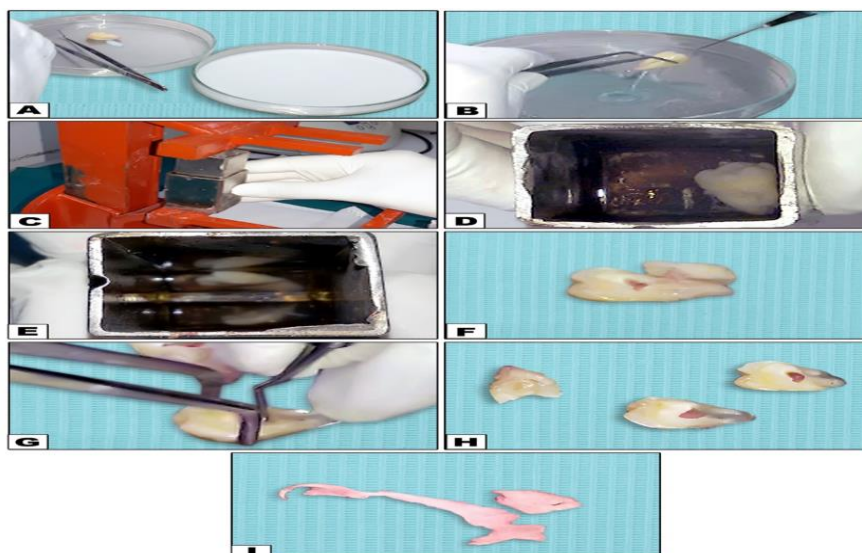
<sup>1</sup> Postgraduate MSc Student, Department of Oral Biology, Mansoura University 35516, Mansoura, Egypt. [mbedir.1189@gmail.com](mailto:mbedir.1189@gmail.com)

<sup>2</sup> Associate Professor, Department of Oral Biology, Mansoura University 35516, Mansoura, Egypt.

<sup>3</sup> Professor, Department of Oral Biology, Mansoura University 35516, Mansoura, Egypt.

Received January, 2023; Accepted February, 2023.

DOI: 10.21608/mjd.2023.301973



**Figure 1:** Photographs showing (A) A tooth in sterile dish, (B) Irrigation with Kenara mouth wash, (C) Hand held pulp isolator (HHPI) device, (D) Resting the tooth on its most regular surface in the HHPI, (E, F) Splinting the tooth into 2 parts, (G) Dissection of the pulp by small excavator, (H) Remaining hard dental tissues, and (I) The dissected pulp tissue.

has been shown that hDPSCs can differentiate into cells with ectodermal, mesodermal, and endodermal lineages, so hDPSCs are recommended as an alternating cell source for regeneration processes.<sup>11</sup>

In the current in vitro study, the cellular bioactivity of these pulp capping materials with regard to their influence on the ability of hDPSCs to differentiate into odontogenic tissue was assessed.

### Materials and Methods:

This study was accepted by the ethical committee, Faculty of Dentistry, Mansoura University (Code: A01080920). The Department of Oral Surgery, Faculty of Dentistry, Mansoura University extracted healthy donors' (16–22-year-old) permanent teeth as part of the dental treatment plan (impacted teeth).

After teeth were extracted, normal saline, 70% ethyl alcohol, and phosphate buffer saline were used to irrigate them for 15 seconds (PBS; Gibco). After irrigation, the teeth were kept in good condition in sterile falcon tubes that were filled with pasteurized whole milk. The extracted teeth were then sent to the Nile Experimental Center to be processed under strictly sterile conditions.<sup>12, 13</sup>

**Pulp harvesting, Figure 1:** All the following steps were performed under careful aseptic conditions in the laminar flow hood as the device was set and sterilized and its surfaces were disinfected with 70% ethyl alcohol before the arrival of the extracted teeth by 30 minutes. The teeth in the sterile falcon tube with pasteurized milk were then removed under the hood with sterile tweezers to be transported into a sterile culture dish, Figure 1A. All of the soft tissues that were still connected to the extracted teeth were scraped off

with a curette before being irrigated with chlorhexidine (Kenara mouthwash; Macro Group Pharmaceuticals) and then PBS, Figure 1B.

The Hand Held Pulp Isolator, Figure 1C, a device developed by Prof. Dr. Youssry Mahmoud Elhawary, professor of oral biology, Faculty of Dentistry, Mansoura University, and Ahmed Shetewy, Faculty of Dentistry, Mansoura University, was used to separate the pulp of each tooth. The tooth was placed into the container of the device, resting horizontally on its broadest and regular surface in 70% ethyl alcohol with the blade leaning above it, Figure 1D. Then, the device's container was placed into its place in the device, and rolling was begun, until hearing the sound of the tooth smashing. Using the device handle, the tooth was splatted into two portions. The two portions were then transferred to sterile dishes, Figure 1E and F. The pulp was excavated using the blunt side of a small sterile excavator from the surrounding hard tissue Figure 1G<sup>13</sup>.

**Isolation of hDPSCs:** HDPSCs were isolated from the permanent teeth of healthy patients (teeth are freshly extracted for surgical reasons) and informed consent was attained. According to Gronthos et al.<sup>14</sup> the isolation steps were done in that order.

Each tooth's isolated pulp tissues underwent three phosphate-buffered saline washing (PBS). The samples were kept on a sterile dish and divided into three pieces that were each one millimeter long, with sterilized surgical cutters. Pieces that had been cut apart were treated for two hours at 37°C with a mixture of three mg/mL collagenase type 1 (Sigma-Aldrich, St. Louis, MO). The solution was run through a 70-mm cell strainer (BD Biosciences, Franklin Lakes, NJ) to obtain single-cell suspensions, which were then seeded in

culture dishes with a culture medium comprising ten percent fetal bovine serum (FBS; Gibco, Grand Island, NY), two mmol/L L-glutamine (Gibco), and antibiotics (penicillin one hundred U/mL and streptomycin 100 Life Technologies Corporation, Carlsbad, CA) at 0.25 µg/mL in a humidified atmosphere of five percent carbon dioxide at thirty-seven Celsius, and every three days, the medium was changed.

**Sub-culturing of hDPSCs:** When the first culture of adherent cells reached about 80% confluence, it was washed with a disinfected liquid of trypsin/ethylene-diamine-tetra-acetic acid for 5-10 minutes at 37 degrees Celsius in the incubator using alternating shaking to detach the adherent cells. In subsequent cell cultures, the primary cell culture was reproduced and enlarged. Over the 21 days course, each week the cells were sub-cultured, and with changing the culture media every 3 days. That was performed in a sterilized air laminal flow shelter, and with sterilized tools.

**Characterization of Isolated hDPSCs and Flow cytometry analysis:** The cells were maintained in Dulbecco's Modified Eagle Medium (DMEM), which was supplied by Gibco and contained 10% fetal bovine serum (FBS), 1% l-glutamine, 100 U/ml penicillin, and 100 g/ml streptomycin (DMEM Gibco BRL, CA, USA) (Gibco). The cells were kept at 37 degrees Celsius in a humid atmosphere with 5% CO<sub>2</sub>. The cells used in this study were obtained between the third and fourth passages. The isolated hDPSCs were defined as mesenchymal cells, based on their fusiform-shaped morphology following the recommendations of the International Society for Cellular Therapy.<sup>15</sup>

Flow cytometric analysis was used to look into the immunophenotypic features of the mesenchymal hDPSCs. The levels of expression of hDPSCs markers were measured. Adherent cells were treated at the third passage, trypsinized, and the harvesting of two million DPSCs occurred. The cells were incubated with 10L of conjugated monoclonal antibodies for 45 min at 4°C in the dark with PerCPconjugated anti-CD45 (Immuno Tools, Friesoythe, Germany), FITC conjugated anti-human CD44 (BD Bioscience Pharmingen, NJ, USA), PE-conjugated antihuman CD105 (Immuno Tools), and CD34 (Beckman Coulter, Brea, CA).

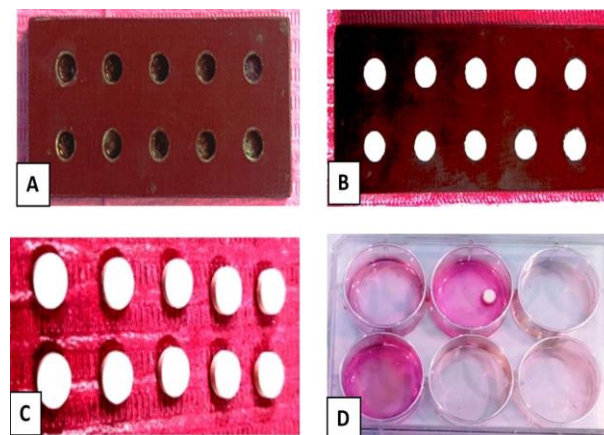
The negative control was the same isotopic species. After 20 minutes of incubation, each tube containing treated monoclonal cells was mixed with two mL of phosphate buffer saline containing two percent fetal bovine serum solution, and then centrifuged for five minutes at 2500 rpm. After discarding the supernatant, the cells had been suspended in five hundred liters of phosphate buffer saline comprising two percent fetal bovine serum. The cells were scanned by a Beckman Coulter Cytomics FC 500 flow cytometer and CXP software version 2.2. Cells were harvested using a trypsin/EDTA solution to produce a single-cell suspension. Gibco's one percent horse serum in sterile phosphate-buffered saline was used to stain the cells

with primary antibodies conjugated with a fluorescent dye (PBS).<sup>16</sup>

### Materials:

TheraCal™ LC was obtained from Bisco Inc., IL, USA. Nano-hydroxyapatite was purchased from (Nano Tech, Giza, Egypt). TheraCal LC and NHA samples had been mixed regarding the manufacturer's orders, to regulate the volume of the material, the freshly prepared materials were applied under sterile conditions in a sterilized Teflon mold (fixed size of holes five mm diameter and four mm depth) with sterile Mylar cover sheets, Figure 2.

Theracal LC was applied to the Teflon mold. It was cured by diode-type light-curing equipment for twenty seconds intervals, but after being protected with a pre-sterilized Mylar cover sheet to inhibit forming an oxygen-inhibiting layer. NHA was synthesized through a wet chemical process between calcium nitrate and ammonium hydroxide ((NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub>). By altering the length of time and temperature at which the grain size of NHA was precipitated at a PH between ten and twelve and room temperature as per the manufacturer's instructions. The set materials were sterilized for three hours under UV light<sup>17</sup> after being put in the sterilized multi-well plate (Nunc; Thermo Fisher Scientific, Waltham, MA) in twelve wells at bottom of a separate well.



**Figure 2:** (A) Photograph showed the fabricated mold with standard sized holes. (B) The prepared pulp capping materials set in the mold. (C) The pulp capping materials after removal from the mold. (D) The DPSC were incubated with pulp capping materials.

**Study design:** 3<sup>rd</sup> passaged hDPSCs were incubated with the tested materials (three thousand cells per well) in a five percent carbon dioxide atmosphere at thirty-seven Celsius for two days. No tested materials were added to twelve wells containing hDPSCs in a tissue culture medium, which act as a negative control group. The study groups were divided into the followings:  
 Group 1: (control negative group) hDPSCs incubated without any biomaterial supplementation.  
 Group 2: hDPSCs incubated with TheraCal LC.  
 Group 3: hDPSCs incubated with Nano-hydroxyapatite.



Media was changed every 3 days with the respective culture media for each plate without removing the biomaterial cylinders from the wells to keep them in contact with the hDPSCs. Morphologic observations were done using an inverted light microscope during all periods and digital micrographs were captured.

**Viability evaluation (MTT assay):** 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) is a yellow dye used to research and assess the cytotoxicity of the materials. Based on the activity of the mitochondrial succinate dehydrogenase, it provides an indirect way for assessing cell viability and proliferation.<sup>18</sup>

**Alizarin Red Staining:** Alizarin red staining was used to identify matrix mineralization. Six-well plates were seeded with cells ( $5 \times 10^4$ /well) during a 21-day growth period. The cultures were preserved in 95 percent ethanol for 30 minutes at 37°C. The cultures stained with 0.1 percent Alizarin Red Staining (Sigma Aldrich; Merck KGaA) for 30 minutes at 37°C indicated calcium accumulation. To solubilize and release calcium-bound Alizarin Red into the solution to evaluate matrix mineralization, the cells stained with Alizarin Red were treated with 100 mM cetylpyridinium chloride for 1 h at 37°C. A microplate reader equipped with a wavelength of 570 nm was then used to measure the optical density (OD) of the solution after 200  $\mu$ l aliquots were transferred to a 96-well plate (Tanon Science & Technology Co., Ltd., Shanghai, China). Using a Bradford protein assay, the growth of mineralized nodules was calculated as OD/g of total cellular protein. Each experiment was run at least three times in triplicate wells.<sup>19</sup>

**Statistical Analysis:** Data were gathered and examined. One-way ANOVA (analysis of variance) was used to evaluate numerical (parametric) data from more than two different groups to compare the statistical significance of the differences between the groups. For all tests, probability (P) values of 0.05 or less were regarded as statistically significant, and results were given as mean  $\pm$  SD. The SPSS software for Windows, version (20), was used to analyze the data (SPSS Inc., Chicago, Illinois, USA).

The used tests were:

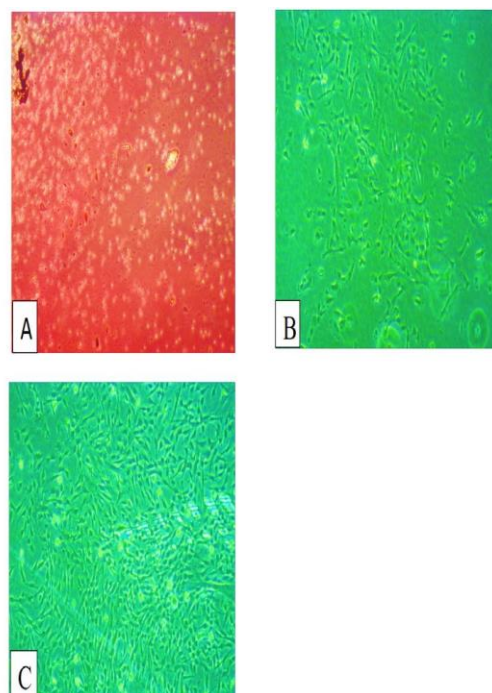
-Student t-test: Was used to compare two groups under study using normally distributed quantitative variables.

-One-way ANOVA with repeated measures: Was used to compare more than two periods or stages for quantitative variables with a normally distributed distribution.

## Results:

**Cell Morphology Characteristics:** After the isolation and culturing procedures using the explant outgrowth technique, at first cells had a rounded floating appearance during the first feeding after 5 days of isolation, Figure 3 A. During the second feeding

procedure after 2 days, cells began to show several morphologies, as some showed a spindle-like appearance while others revealed a fibroblast-like



**Figure 3: Inverted light microscope photographs demonstrating colony formation capacity of DPSCs.**

appearance demonstrating the diversity of pulp cell morphologies, Figure 3 B. After 2 weeks of isolation, the cells started to interlace with each other exhibiting a spindle shape appearance, Figure 3 C.

**Immune-phenotypic Characteristics:** The positive reaction of separated hDPSCs for the cell surface antigens CD105 and CD44 and negative for CD34 and CD45 ensuring their mesenchymal source, which was assessed by the flow cytometric examination of the immune-phenotypic features of the hDPSCs, Figure 4.

## Effect of the tested materials on cell viability

**(MTT assay):** DPSCs were treated with Theracal and NHA for 1, 3, and 5 days then an MTT assay was performed after each period. The absorption rate was then recorded by an ELISA reader. Control group: The highest mean value for cell viability was on day 5 ( $0.262 \pm 0.02$ ), followed by day 3 ( $0.26 \pm 0.01$ ), while the lowest mean value for cell viability was on day 1 ( $0.162 \pm 0.02$ ). The Theracal group: The highest mean value for cell viability was on day 5 ( $0.167 \pm 0.02$ ), followed by day 3 ( $0.163 \pm 0.03$ ), while the lowest mean value for cell viability was on day 1 ( $0.146 \pm 0.04$ ). NHA Group: The highest mean value for cell viability was on day 5 ( $0.281 \pm 0.03$ ), followed by day 3 ( $0.263 \pm 0.02$ ), while the lowest mean value for cell viability was on day 1 ( $0.232 \pm 0.01$ ), Table.

MTT assay revealed on day 1 a significant difference between the Theracal LC group, NHA group, and among the control group, NHA group, and also was a significant difference on days 3 & 5 among the control group, Theracal LC group and between Theracal LC group, NHA group. MTT assay also revealed a non-

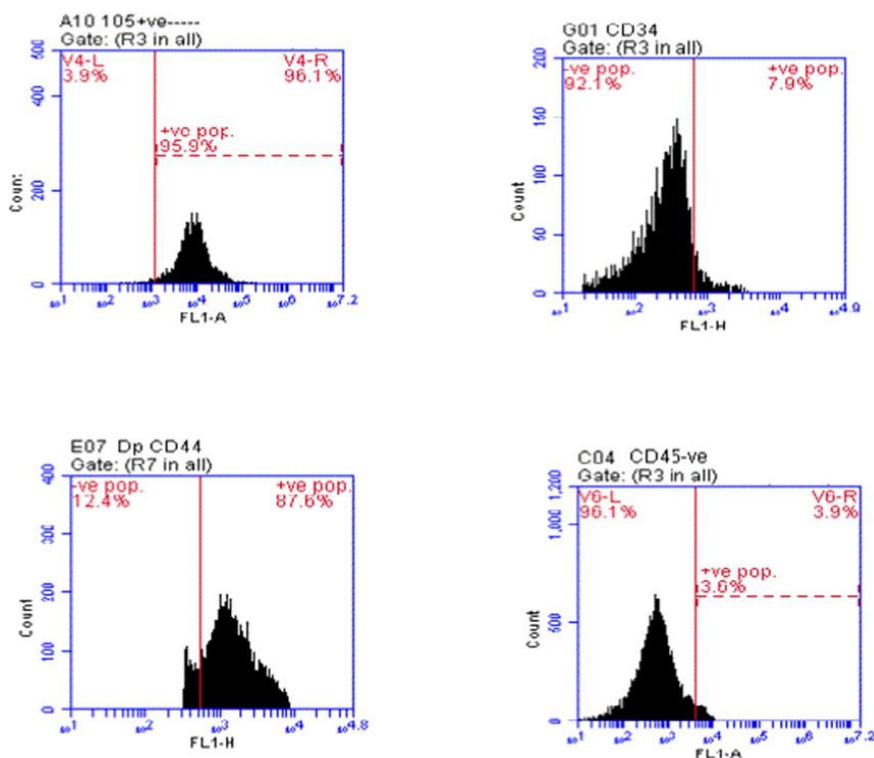


Figure 4: Plots of the flow cytometric analysis of dental pulp stem cells. Histograms of the relative fluorescence height of surface markers against cell count. A Graph representing CD 105 positive flow cytometric results ,B Graph representing CD44 Positive flow cytometric results, C Graph representing CD 34 negative flow cytometric results, D Graph representing CD 45 negative flow cytometric results.

Table. Comparison between studied groups regarding the MTT analysis

	Group (CT) (n=3)	Group (Theracal) (n=3)	Group (NHA) (n=3)	ANOVA test P value	Post hoc LSD test
Day 1					
Mean ± SD	0.162±0.02	0.146±0.04	0.232±0.01	F=10.80 P=0.01*	P1=0.43
Median	0.165	0.142	0.233		P2=0.012*
Min-Max	0.14-0.18	0.11-0.18	0.22-0.24		P3=0.005*
Day 3					
Mean ± SD	0.26±0.01	0.163±0.03	0.263±0.02	F=17.33 P=0.003*	P1=0.002*
Median	0.259	0.174	0.258		P2=0.882
Min-Max	0.24-0.28	0.13-0.18	0.24-0.29		P3=0.002*
Day 5					
Mean ± SD	0.262±0.02	0.167±0.02	0.281±0.03	F=17.84 P=0.003*	P1=0.004*
Median	0.259	0.175	0.287		P2=0.372
Min-Max	0.24-0.28	0.14-0.18	0.25-0.31		P3=0.001*

P1: Comparison between Group (1) & Group (2)  
 P2: Comparison between Group (1) & Group (3)  
 P3: Comparison between Group (2) & Group (3)

F: ANOVA test, \*significant p≤0.05  
 CT: Control  
 NHA: Nano-hydroxyapatite

significant difference between the control group and the TheraCal LC group on day 1 and also between the control group and NHA group on day 3 and day 5, Table.

**Alizarin Red Staining for mineralization:** Nodules of mineralization were easily detectable and stained with Alizarin red stain, whereas the TheraCal group showed red areas of small calcification with scattered minute calcium deposits. The Control group showed larger calcified areas with traces of calcium deposits when compared to the TheraCal group. The NHA group showed the largest calcified areas and calcium deposits, Figure 5.

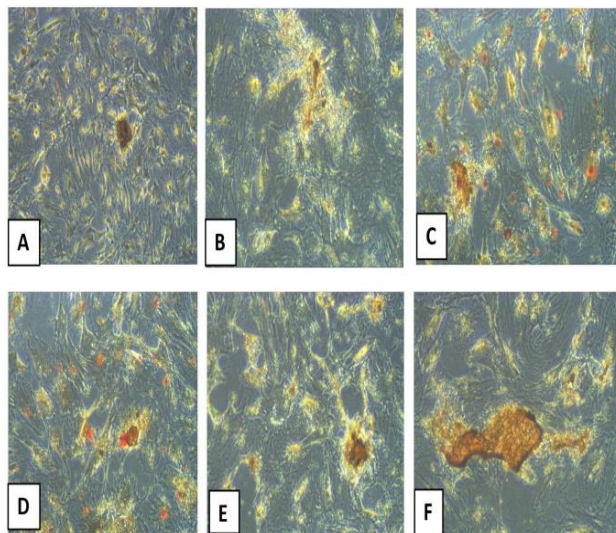


Figure 5: Inverted light microscope photographs showing mineralized nodule formation of DPSCs stained with alizarin red staining (A,B), Treated with TheraCal (C,D) and NHA (E,F).

### Discussion:

To safeguard the dentin-pulp complex and restore tooth integrity, pulp capping materials are now often employed in dentistry. It offers a convenient substitute for root canal therapies. Dental pulp tissue contains hDPSCs, which encourage pulp healing due to its ability to self-renew and differentiate into numerous lineages. hDPSCs multiply, travel to the site of injury and differentiate into odontoblast-like cells that deposit reparative dentine.<sup>20</sup>

A pulp-capping material should have good physical properties, no toxicity to pulp tissue, good sealing ability, suitable setting time, easy handling properties, and antibacterial properties. It should enhance the healing process caused by hDPSCs. Even though many different materials have been created, none of them can satisfy these therapeutic requirements.<sup>21</sup>

The current work aimed to assess the potential roles of both TheraCal LC and NHA (tested materials) on the proliferation and differentiation of hDPSCs into odontogenic cells following their direct contact with the tested materials. The materials employed passed the required safety inspections. The outstanding multi-potential differentiation and proliferation capacity of DPSCs produced from impacted third

molar teeth has been widely explored. Several promising investigations have described the role of human DPSCs in the regeneration of mineralized tissues, highlighting their therapeutic value as a stem cell source.<sup>8</sup> A tissue culture medium was used to keep DPSCs isolated from extracted

human teeth. The morphology of the cells was verified to be spindle-shaped. The surface markers CD105, CD44, CD45, and CD 34 were used to characterize hDPSCs in the current investigation. CD 105 and CD 44 expression was positive in hDPSCs, however, CD45 and CD 34 expression was negative. In 2006, it was revealed by the International Society of Cellular Therapy (ISCT), that the adherence to plastic, expression of cell surface markers such as CD 105 and CD 44, and the absence of expression of CD 45 and CD 34 are identifying criteria for human mesenchymal cells (MSCs).<sup>22</sup>

CD105 was employed as identifying criterion for human MSCs because it has been linked to cell proliferation, differentiation, and migration. CD105+ cells have been demonstrated to be capable of developing in vitro into osteoblastic cells, indicating that CD105+ cells have osteogenic potential. Additionally, CD105+ cells were able to develop into both chondrogenic and adipogenic lineages in vitro, implying yet another MSCs feature. One example of a non-integrin is CD44, which is crucial for different leukocytes to adhere to endothelia and participate in T-lymphocyte activation. Additionally, cells that are mineralizing, such as ameloblasts, odontoblasts, and osteoblasts in calcifying areas, express CD44 abundantly.<sup>23-25</sup>

Additionally, CD 45 and CD 34 were employed as positive hematopoietic stem cell markers but were used as negative hDPSC markers. The CD45 molecule belongs to the protein tyrosine phosphatase family, a group of signaling molecules that regulates biological processes such as cell division, proliferation, and differentiation as well as neoplastic transformation. CD45 expression is necessary for signal transduction through the B cell antigen-receptor complex.<sup>26-28</sup>

The effect of the tested materials on cell viability after its incubation with hDPSCs was assessed by the MTT cell proliferation test. Similar to the MTT experiment, the dehydrogenase enzyme found in the live cells reduces the tetrazolium salt to insoluble formazan crystals, demonstrating mitochondrial activity. Additionally, solubilizing agents are added to the insoluble formazan salt to dissolve it, and the colored result is quantitatively quantified with a multi-plate reader. Different solubilization techniques were employed, including sodium dodecyl sulphate and dimethyl sulfoxide (DMSO) (SDS). Tetrazolium salts can no longer be reduced by dead cells into colored formazan products. As a result, the quantity of live cells in the culture directly correlates with the intensity of the colorful product.<sup>2</sup>



An important cause for concern is how cytotoxic compounds created by pulp-capping materials affect cell viability and apoptosis. The preservation of pulp vitality following restorative intervention is necessary for the pulpal cell populations to survive. Compounds for pulp-capping should either promote cell survival and proliferation or be physiologically inert.<sup>30</sup>

The NHA group had the highest percentage of viable cells in the current investigation, then the control group and then the TheraCal LC group. These findings support the prior finding that NHA had higher levels of odontogenic gene expression than MTA treated with odontogenic media, implying that NHA has a greater odontogenic differentiation potential. The chemical composition and surface topographical differences may account for the higher differentiation potential. NHA has distinct biological and physicochemical properties and shares a composition with calcium and phosphate crystals that make up the hard tissues of teeth.<sup>31</sup>

The biological properties of hydroxyapatite at the nanoscale are superior to those of its bigger, micron-structured counterparts, which are associated with higher surface reactivity. This has been shown to have a favorable impact on stem cell adhesion and differentiation. Also, Zhou et al.<sup>32</sup> reported that NHA increased hDPSCs odontogenic development, which is consistent with our findings. Liu et al.<sup>33</sup> also discovered that hDPSCs seeded on nano-hydroxyapatite/collagen/poly (L-lactide) may differentiate into odontogenic and osteogenic cells, as evidenced by the expression of OCN, COL 1, and ALP.

TheraCal™ LC's cytotoxicity could be attributed to the presence of unpolymerized resin monomers. There should be no unpolymerized monomers when utilizing the correct curing process. Furthermore, the pore volume percentage per total volume of the cured TheraCal™ LC was very low (0.19 0.09 percent). As a result, unpolymerized resin monomers are unlikely to activate cell death. Previous research has shown that when TheraCal™ LC is cured, it releases particular additives such as camphoroquinone and ethyl-4-(dimethylamino) benzoate. Camphoroquinone treatment increased reactive oxygen species generation in human dental pulp fibroblasts. The light-curing ingredients generated by TheraCal™ LC may cause apoptosis by increasing the production of reactive oxygen species (ROS), which causes cell death.<sup>34, 35</sup>

The hDPSCs had successfully differentiated into odontogenic cells, according to the Alizarin red staining data. The formation of calcific nodules was evident in both the TheraCal and NHA groups as well as the control group's favorable colorimetric alterations. When hDPSCs were differentiated into odontoblast and osteoblast using NHA as a scaffold, Liu et al.<sup>33</sup> observed random nodule-shaped islands

after alizarin red staining. NHA possesses biological and physicochemical features that are similar to the natural mineral phase in the tooth hard tissue building unit (calcium and phosphates). The hydration of NHA with physiological fluids produces adequate Ca<sup>2+</sup> concentrations and an alkaline pH (10–12), both of which have been demonstrated to promote cell proliferation and differentiation by generating a strong mineralized interface.<sup>36</sup>

This study's alizarin red stain demonstrated modest calcification with dispersed minute calcium deposits in the TheraCal group, which is consistent with, Sanz et al.<sup>37</sup>. After a culture time with the tested materials, when hDPSCs developed calcified nodules stained with alizarin red stain, Sanz et al. looked at the ability of hDPSCs to mineralize. As anticipated, the mineralization potential of the TheraCal LC-treated groups was much lower than that of the control and other test groups (P.001).

In addition to these findings, incidences of discontinuous dentinal bridge development using TheraCal LC direct pulp capping have been observed. During direct pulp capping techniques, non-polymerized resin components (such as HEMA, BisGMA, TEGDMA, and UDMA) may come into contact with pulp tissue. Scattered mineralization could emerge from the non-polymerized monomers that TheraCal LC emits. The possibility of increased microleakage as a result of deprived bridge design is another problem with TheraCal CL.<sup>38</sup>

TheraCal LC is made up of a hydrophobic resin monomer constituent and a neutral or mildly acidic hydrophilic resin monomer constituent. The insufficient polymerized resin monomers may leak in a great amount, causing apoptosis as a result of irreversible impacts on the cell's defense mechanisms and glutathione metabolism, which the cell uses to clean itself. Components of methacrylate resin may injure cells because they change the lipid layers in the cell membrane, which increases permeability. Cell metabolism and protein expression are decreased as a result of TheraCal LC's direct interaction with pulp cells, so further studies using in vivo animal models should be performed to confirm the TheraCal LC's possibly harmful biological effects on hDPSCs.<sup>34</sup>

### Conclusion:

Nano-hydroxyapatite improved the DPSCs' odontogenic differentiation in the presence of normal culture media.

It was also obvious that nano-hydroxyapatite has a stronger odontogenic differentiation capability than TheraCal, as well as a greater rise in mineralization nodule formation.

These findings could be relevant in future studies to increase DPSC odontogenic differentiation and in the development of dentin regenerative therapies.

## References

- Torabinejad M, Abu-Tahun I. Management of teeth with necrotic pulps and open apices. *Endod topics*. 2010;23(1):105-130.
- Dominguez MS, Witherspoon DE, Gutmann JL, Opperman LA. Histological and scanning electron microscopy assessment of various vital pulp-therapy materials. *J Endod*. 2003;29(5):324-333.
- Qureshi A, E S, Nandakumar, Pratap Kumar, Sambashivarao. Recent advances in pulp capping materials: an overview. *J Clin Diagn Res*. 2014;8(1):316-321.
- Kuru S, Sepet E, İrez T, Aktaş E, Yazır Y, Duruksu G, et al. Effects of different pulp-capping materials on cell death signaling pathways of lipoteichoic acid-stimulated human dental pulp stem cells. *Odontology*. 2021;109(2):547-559.
- Talal A, McKay IJ, Tanner KE, Hughes FJ. Effects of hydroxyapatite and PDGF concentrations on osteoblast growth in a nanohydroxyapatite-poly lactic acid composite for guided tissue regeneration. *J Mater Sci Mater Med*. 2013;24(9):2211-2221.
- Huang AH, Chen YK, Chan AW, Shieh TY, Lin LM. Isolation and characterization of human dental pulp stem/stromal cells from nonextracted crown-fractured teeth requiring root canal therapy. *J Endod*. 2009;35(5):673-681.
- Volponi AA, Gentleman E, Fatscher R, Pang YW, Gentleman MM, Sharpe PT. Composition of Mineral Produced by Dental Mesenchymal Stem Cells. *J Dent Res*. 2015;94(11):1568-1574.
- Winning L, El Karim IA, Lundy FT. A Comparative Analysis of the Osteogenic Potential of Dental Mesenchymal Stem Cells. *Stem Cells Dev*. 2019;28(15):1050-1058.
- Goldberg M. Pulp healing and regeneration: more questions than answers. *Adv Dent Res*. 2011;23(3):270-274.
- Osathanon T, Sawangmake C, Nowwarote N, Pavasant P. Neurogenic differentiation of human dental pulp stem cells using different induction protocols.
- Sawangmake C, Nowwarote N, Pavasant P, Chansiripornchai P, Osathanon T. A feasibility study of an in vitro differentiation potential toward insulin-producing cells by dental tissue-derived mesenchymal stem cells. *Biochem Biophys Res Commun*. 2014;452(3):581-587.
- Adan A, Kiraz Y, Baran Y. Cell Proliferation and Cytotoxicity Assays. *Curr Pharm Biotechnol*. 2016;17(14):1213-1221.
- Saeed MA, El-Rahman MA, Helal ME, Zaher AR, Grawish ME. Efficacy of Human Platelet Rich Fibrin Exudate vs Fetal Bovine Serum on Proliferation and Differentiation of Dental Pulp Stem Cells. *Int J Stem Cells*. 2017;10(1):38-47.
- Gronthos S, Mankani M, Brahimi J, Robey PG, Shi S. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. *Proc Natl Acad Sci U S A*. 2000;97(25):13625-13630.
- Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy*. 2006;8(4):315-317.
- Malekfar A, Valli KS, Kanafi MM, Bhonde RR. Isolation and Characterization of Human Dental Pulp Stem Cells from Cryopreserved Pulp Tissues Obtained from Teeth with Irreversible Pulpitis. *J Endod*. 2016;42(1):76-81.
- Seo MS, Hwang KG, Lee J, Kim H, Baek SH. The effect of mineral trioxide aggregate on odontogenic differentiation in dental pulp stem cells. *J Endod*. 2013;39(2):242-348.
- Tabari K, Hosseinpour S, Parashos P, Kardouni Khozestani P, Rahimi HM. Cytotoxicity of Selected Nanoparticles on Human Dental Pulp Stem Cells. *Iran Endod J*. 2017;12(2):137-142.
- Apel C, Buttler P, Salber J, Dhanasingh A, Neuss S. Differential mineralization of human dental pulp stem cells on diverse polymers. *Biomed Tech (Berl)*. 2018;63(3):261-269.
- Komabayashi T, Zhu Q, Eberhart R, Imai Y. Current status of direct pulp-capping materials for permanent teeth. *Dent Mater J*. 2016;35(1):1-12.
- Kawashima N. Characterisation of dental pulp stem cells: a new horizon for tissue regeneration? *Arch Oral Biol*. 2012;57(11):1439-1458.
- Shi Y, Wang Y, Li Q, Liu K, Hou J, Shao C, et al. Immunoregulatory mechanisms of mesenchymal stem and stromal cells in inflammatory diseases. *Nat Rev Nephrol*. 2018;14(8):493-507.
- Mitchell JB, McIntosh K, Zvonick S, Garrett S, Floyd ZE, Kloster A, et al. Immunophenotype of human adipose-derived cells: temporal changes in stromal-associated and stem cell-associated markers. *Stem Cells*. 2006;24(2):376-385.
- De Schauwer C, Meyer E, Van de Walle GR, Van Soom A. Markers of stemness in equine mesenchymal stem cells: a plea for uniformity. *Theriogenology*. 2011;75(8):1431-1443.
- Umemura N, Ohkoshi E, Tajima M, Kikuchi H, Katayama T, Sakagami H. Hyaluronan induces odontoblastic differentiation of dental pulp stem cells via CD44. *Stem Cell Res Ther*. 2016;7(1):135.
- Brown VK, Ogle EW, Burkhardt AL, Rowley RB, Bolen JB, Justement LB. Multiple components of the B cell antigen receptor complex associate with the protein tyrosine phosphatase, CD45. *J Biol Chem*. 1994;269(25):17238-17244.
- Rege TA, Hagood JS. Thy-1 as a regulator of cell-cell and cell-matrix interactions in axon regeneration, apoptosis, adhesion, migration, cancer, and fibrosis. *FASEB J*. 2006;20(8):1045-1054.
- Vašiček J, Shehata M, Schnabl S, Hilgarth M, Hubmann R, Jäger U, et al. Critical assessment of the efficiency of CD34 and CD133 antibodies for



- enrichment of rabbit hematopoietic stem cells. *Biotechnol Prog.* 2018;34(5):1278-1289.
29. Belkacemi L, Jelks A, Chen CH, Ross MG, Desai M. Altered placental development in undernourished rats: role of maternal glucocorticoids. *Reprod Biol Endocrinol.* 2011;(9):105.
  30. Modena KC, Casas-Apayco LC, Atta MT, Costa CA, Hebling J, Sipert CR, et al. Cytotoxicity and biocompatibility of direct and indirect pulp capping materials. *J Appl Oral Sci.* 2009;17(6):544-554.
  31. Hanafy AK, Shinaishin SF, Eldeen GN, Aly RM. Nano Hydroxyapatite & Mineral Trioxide Aggregate Efficiently Promote Odontogenic Differentiation of Dental Pulp Stem Cells. *Open Access Maced J Med Sci.* 2018;6(9):1727-1731.
  32. Zhou GS, Su ZY, Cai YR, Liu YK, Dai LC, Tang RK, et al. Different effects of nanophase and conventional hydroxyapatite thin films on attachment, proliferation and osteogenic differentiation of bone marrow derived mesenchymal stem cells. *Biomed Mater Eng.* 2007;17(6):387-395.
  33. Liu HC, E LL, Wang DS, Su F, Wu X, Shi ZP, et al. Reconstruction of alveolar bone defects using bone morphogenetic protein 2 mediated rabbit dental pulp stem cells seeded on nano-hydroxyapatite/collagen/poly(L-lactide). *Tissue Eng Part A.* 2011;17(19-20):2417-2433.
  34. Nilsen BW, Jensen E, Örtengren U, Michelsen VB. Analysis of organic components in resin-modified pulp capping materials: critical considerations. *Eur J Oral Sci.* 2017;125(3):183-194.
  35. Wang L, Nan X, Hou J, Xia Y, Guo Y, Meng K, et al. Preparation and biological properties of silk fibroin/nano-hydroxyapatite/hyaluronic acid composite scaffold. *Biomed Mater.* 2021;16(4).
  36. Bakhtiar H, Nekoofar MH, Aminishakib P, Abedi F, Naghi Moosavi F, Esnaashari E, et al. Human Pulp Responses to Partial Pulpotomy Treatment with TheraCal as Compared with Biodentine and ProRoot MTA: A Clinical Trial. *J Endod.* 2017;43(11):1786-1791.
  37. Sanz JL, Soler-Doria A, López-García S, García-Bernal D, Rodríguez-Lozano FJ, Lozano A, et al. Comparative Biological Properties and Mineralization Potential of Endodontic Materials for Vital Pulp Therapy: TheraCal PT, TheraCal LC, and Biodentine on Human Dental Pulp Stem Cells. *J Endod.* 2021;47(12):1896-1906.
  38. Bortoluzzi EA, Niu LN, Palani CD, El-Awady AR, Hammond BD, Pei DD, et al. Cytotoxicity and osteogenic potential of silicate calcium cements as potential protective materials for pulpal revascularization. *Dent Mater.* 2015;31(12):1510-1522.