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**Research Report**

# Toxicity test of bioceramic biphasic calcium phospate (BCP) Sr-Ag doping as bone graft in BHK-21 fibroblast cells

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## *ABSTRACT*

***Background:*** *Bone graft is a substitute material that is used to assist reconstruction, stabilize the structure and bonds in bone, stimulate the osteogenesis process and as a healing bone defect. One type of bone graft that has good osteoinductive and bicompatibility is alloplast which is a synthetic calcium phosphate compound. The most frequently used Calcium Phosphate groups are Hydroxyapatite (HA), β-Tricalcium Phosphate (β-TCP), and Biphasic Calcium Phosphate (BCP). In this study the material used was BCP doping Sr2+ and Ag+. Strontium ions (Sr2+) can increase osteoblast activity, reduce osteoclast activity and cytokine production, improve osteointegration, and minimize fractures. Ag+ ion has the ability as an antibacterial agent.* ***Purpose:*** *To explain and prove the toxicity of bioceramic Biphasic Calcium Phosphate (BCP) doped Sr-Ag as bone graft on BHK-21 fibroblast cells.* ***Methods:*** *This type of research is a laboratory experiment with a post-test only control group design. Treatment with Biphasic Calcium Phosphate (BCP) doped Sr-Ag with concentrations of 200 ppm, 180 ppm, 160 ppm, 140 ppm, 120 ppm, 80 ppm, 40 ppm, 20 ppm, 10 ppm, 5 pmm, 2.5 ppm, 1.25 ppm, 0.625 ppm in BHK-21 fibroblast cell culture.* ***Results:*** *The percentage of fibroblast cell life at concentrations of 200 ppm, 180 ppm, 160 ppm, 140 ppm, 120 ppm, 80 ppm, 40 ppm, 20 ppm, 10 ppm, 5 pmm, 2.5 ppm, 1.25 ppm, 0.625 ppm, respectively, the percentage of live cells was 38% 44%, 46%, 50%, 52%, 65%, 69%, 71%, 72%, 75%, 77%, 81%, and 87%. The parameter used in this toxicity test is* $CD_{50}$. ***Conclusion:*** *The results of the toxicity test of bioceramic Biphasic Calcium Phosphate (BCP) doped Sr-Ag as a bone graft showed a toxic and non-toxic effect on BHK-21 fibroblast cells at certain concentrations.*

***Keywords:*** *Toxicity test; Biphasic Calcium Phosphate (BCP) Sr-Ag; Bone graft; BHK-21 fibroblast*

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## INTRODUCTION

Bone grafting is a bone transplant procedure to accelerate wound healing and new bone formation.[1] The purpose of a bone graft is to heal, strengthen, and improve bone function. The properties of bone grafts are osteogenesis, osteoinduction, osteoconduction, biocompatible, and have good mechanical properties.[2] Bone grafts are divided into four types based on the material's source: autograft, allograft, xenograft, and alloplastic.[3] Alloplastic are obtained from synthetic materials, which can be ceramic, hydroxyapatite, tricalcium phosphate, or calcium phosphate.[4] Commonly used calcium phosphate groups are hydroxyapatite (HA), beta-tricalcium phosphate (β-TCP), and biphasic calcium phosphate (BCP).[5]

Biphasic Calcium Phosphate (BCP) is a combination of Beta-tricalcium phosphate (β-TCP) and hydroxyapatite with a ratio of 60:40.[6] The combination of these materials has a ratio of organic mineral matrix close to the ratio of bone, and has good biodegradability and biosorption.[5] BCP optimization is carried out by Sr-Ag doping which will increase osteogenesis and antibacterial abilities. Bioceramic optimization can be done through doping. Doping is done by adding ionic material so that it does not result in a large aggregate.[7] Single-layer dopping uses an ionic material that can increase the ability of osteogenic induction, namely Strontium. Multi-layer doping by adding antibacterial ionic material is needed, especially in cases of infection[8], namely Ag+(Silver).[9]

Mixing more than one material also has the consequence of increasing the risk of toxicity. A study by Basak et al. (2022) stated that BCP concentrations above 80 ppm can cause toxicity effects.[10] In addition, Sr ions have good osteogenesis, can reduce cytokine production and bone resorption, and can stimulate bone formation.[11] However, Sr ions are also known to have a negative impact on normal cells. Excessive doses of Sr ions can cause cell apoptosis through the ERK (Extracellular Signal-Regulated Kinase) pathway.[12] Silver ion (Ag) has the ability as an antibacterial. The ability of silver ions (Ag) as an antibacterial is by


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damaging the bacterial cell wall, inhibiting cell metabolism and microbial synthesis. Ag ions can also inhibit the growth of gram-positive bacteria, damage cell membranes, reduce reductase activity, degrade DNA chromosomes, and reduce protein expression.[13] Besides having a positive impact, Ag ions are also known to have a negative impact on normal cells. Excessive doses of Ag ions can cause the release of cytochrome C, which in turn can cause cell apoptosis.[14]

The requirements for materials used in dentistry are that they have good mechanical properties and are non-toxic.[15] For this reason, research is needed to test toxicity as an evaluation of dental materials and as a standard screening procedure.[16] One method to test the toxicity value of a material is to use the MTT (Methylthiazolyldiphenyl-tetrazolium bromide) Assay method.[17] This study conducted a toxicity test of bioceramic Biphasic Calcium Phosphate (BCP) doped Sr-Ag as bone graft on BHK-21 fibroblast cells, which aims to detect whether BCP-Sr-Ag is safe to use as bone graft material.

## MATERIALS AND METHODS

Ethical permission was obtained from the Health Research Ethics Licensing Commission, Faculty of Dentistry, Airlangga University with number 591/HRECC.FODM/VIII/2022 before conducting the research,. This study used an experimental test using BHK-21 fibroblast cells to determine the toxicity of bioceramic Biphasic Calcium Phosphate (BCP) doped Sr-Ag as bone grafts. Comparison of Biphasic Calcium Phosphate (BCP), namely Beta-tricalcium phosphate (β-TCP) and hydroxyapatite with a ratio of 60:40.[6] This combined material has a ratio of organic mineral matrix close to that of bone, and has good biodegradability and bio-absorbability, while Sr ions use three mol% and Ag uses one mol%, the selection of these quantities is based on research by Swe et al. (2020) which states that the use of Sr three mol % and Ag one mol % ions can be used as potential alternative bone substitutes for patients suffering from bone defects.[18]

In this study, the toxicity test was carried out using the MTT (Methyltiazolyldiphenyl-tetrazolium bromide) Assay method, which is the most common method for testing the toxicity value of a material.[17] MTT test (Methyltiazolyldiphenyl-tetrazolium bromide) Assay serves to measure cell cytotoxicity in vitro.[19] The MTT Assay test measures the ability of living cells based on the activity of the cell's mitochondria. The MTT assay method uses a colorimetric test based on the activity of the living cell mitochondrial reductase enzyme, which reduces methyl thiazol tetrazolium (MTT).[20]

The basis of the MTT enzymatic test is to measure living cells based on mitochondrial activity from cell culture. MTT Assay test is generally used to measure living cells quantitatively. MTT Assay test to calculate dehydrogenase cellular activity in converting a yellow water-soluble chemical (MTT) into a water-insoluble formazan blue compound. The enzyme succinate dehydrogenase carries out the breakdown of MTT in the mitochondria of living cells. The absorbance obtained is proportional to the concentration of formazan blue in organic solvents (eg, isopropanol). If the absorbance results are high, more cells are alive (high cell viability).[21]

The color change was calculated using colorimetry and read using a spectrophotometer (ELISA microplate reader). The reading of the results of the ELISA reader is carried out at a wavelength of 540 nm, because it is able to strongly absorb electromagnetic wave radiation at that wavelength,[22] which can then produce an absorbance value (OD), so that the higher the percentage of optical density indicates a cell metabolically active so that it reduces MTT which is also good.[23] The end of the toxicity test is that it can provide information on the percentage of cells that are able to survive.

## RESULTS

Based on the results of observations and readings of the absorbance value of the Biphasic Calcium Phosphate (BCP) toxicity test for Sr-Ag doping on BHK-21 fibroblast cells through an ELISA reader which was divided into several treatment groups, the cell percentage results can be seen in Table 1. From the data of living cell calculation results, a graph can be made as Figure 1.

## DISCUSSION

The results of the Sr-Ag doping BCP toxicity test on BHK-21 fibroblast cells using MTT at a certain concentration showed that the mean lives fibroblast cells were at a concentration of 200 ppm with a percentage of living fibroblast cells of 38%, a concentration of 180 ppm with a 44% of living cells, a concentration of 160 ppm with a 46% of living cells, a concentration of 140 ppm with a 50%

**Table 1.** Live Cell Percentage

| Treatment | OD Value (%) |
|---|---|
| 200 ppm | 38% |
| 180 ppm | 44% |
| 160 ppm | 46% |
| 140 ppm | 50% |
| 120 ppm | 52% |
| 80 ppm | 65% |
| 40 ppm | 69% |
| 20 ppm | 71% |
| 10 ppm | 72% |
| 5 ppm | 75% |
| 2.5 ppm | 77% |
| 1.25 ppm | 81% |
| 0.625 ppm | 87% |
| Media Control | 0% |
| Cell Control | 100% |

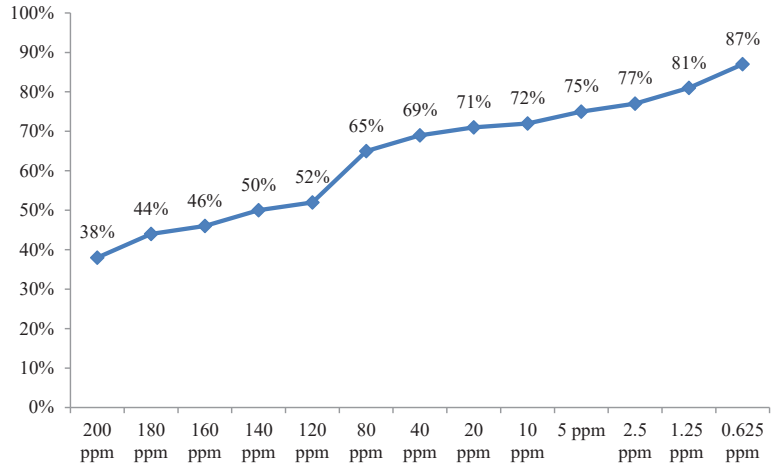


**Figure 1.** Chart Live Cell Percentage

of living cells, a concentration of 120 ppm with a 52% of living cells, a concentration of 80 ppm with a 65% of living cells, a concentration of 40 ppm with 69% of living cells, a concentration of 20 ppm with 71% live cells, 10 ppm concentration with 72% live cells, 5 ppm concentration with 75% live cells, 2.5 ppm concentration with 77% live cells, 1.25 ppm concentration with 81% live cells, concentration 0.625 ppm with 87% live cells, 100% cell control, and 0% media control.

Cell viability is the possibility of cells to live after being exposed to Biphasic Calcium Phosphate (BCP) doping Sr-Ag. From the results obtained, BCP doped Sr-Ag with a concentration of 0.625 ppm produced the highest cell viability, while BCP doped Sr-Ag with a concentration of 200 ppm produced the lowest cell viability. This is also in accordance with the research of Basak et al., (2022) which states that the decrease in cell viability depends on an increase in concentration.[10] This statement is consistent with the theory which states that the toxicity of a substance is directly proportional to exposure. Exposure to a material has a determining factor, namely the concentration of a material. The higher the concentration of a material, the more potential it becomes toxic.[24] In the Sr-Ag doping BCP toxicity test, Sr ions are known to have a negative impact on normal cells. Excessive doses of Sr ions can cause cell apoptosis through the ERK (Extracellular Signal-Regulated Kinase) pathway.[12] Ag ions are also known to have a negative impact on normal cells. Excessive doses of Ag ions can cause the release of cytochrome C which in turn can cause cell apoptosis.[14]

In the study of the toxicity test of Biphasic Calcium Phosphate (BCP) doped Sr-Ag as bone graft on BHK-21 fibroblast cells, the percentage of fibroblast cell life after being treated with Biphasic Calcium Phosphate (BCP) doped Sr-Ag 200 ppm, 180 ppm, 160 ppm, 140 ppm, 120 ppm, 80 ppm, 40 ppm, 20 ppm , 10 ppm, 5 ppm, 2.5 ppm, 1.25 ppm, 0.625 ppm using the MTT Assay method respectively at 38%, 44%, 46%, 50%, 52%, 65%, 69%, 71%, 72%, 75%, 77%, 81%, and 87%. From the results of these percentages it can be seen that the value of the test results is not toxic at concentration of 120 ppm, 80 ppm, 40 ppm, 20 ppm, 10 ppm, 5 ppm, 2.5 ppm, 1.25 ppm, 0.625 ppm because a material can be said to be toxic, if the percentage of living cells after being exposed to the material is less than 50%, and the value of the test results is toxic at concentration of 200 ppm, 180 ppm, 160 ppm, 140 ppm, because the percentage of living cells after being exposed to the material is more than 50%

The results of the toxicity test of Biphasic Calcium Phosphate (BCP) doping Sr-Ag as bone graft showed a non-toxic effect on BHK-21 fibroblast cells at concentrations of 120 ppm, 80 ppm, 40 ppm, 20 ppm, 10 ppm, 5 ppm, 2.5 ppm, 1.25 ppm, 0.625 ppm, and showed a toxic effect on BHK-21 fibroblast cells at concentrations of 200 ppm, 180 ppm, 160 ppm, and 140 ppm.

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