

BIOCOMPATIBILITY OF CALCIUM SILICATE-BASED CEMENT INCORPORATED WITH SILVER OR GOLD NANOPARTICLES - AN IN VITRO STUDY

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ABSTRACT

The effectiveness of nano incorporation into materials has brought about a new horizon into dentistry and the society, as the past and current literature so far reports that the perceived benefits outweigh the perceived risks. The objective of this study was to determine the cytotoxicity of calcium silicate-based cement incorporated with either silver or gold nanoparticles on human lymphocytes. The results showed that the incorporation of silver or gold nanoparticles did not affect the overall biocompatibility of calcium silicate-based cement.

Key words: Calcium silicate, cytotoxicity, Mineral Trioxide Aggregate, nanoparticle.

Introduction

The success rate of conventional root canal treatment (RCT) has been reported in previous literature nearing to 95%. However, when the RCT fails, the first choice of treatment is non-surgical retreatment. When non-surgical retreatment is unwise in certain situations, surgical treatment is advised.¹ A retro filling material following the surgical procedure seals the communication gap between the root canal and periapical tissues.² An arduous search for an ideal retro filling material has led to the evolution of calcium silicate-based cement. The development of Mineral Trioxide Aggregate (MTA) has been a boon to endodontics with the ability to fulfill most of the ideal requirements of retro filling material.³ However, its major drawbacks include an increased set time and a limited antibacterial activity. Various combinations of MTA with additives have been tried to improve its properties. Few other retro filling materials have also been formulated to overcome the limitations of MTA.⁴ Available scientific literature on calcium silicate cement have shed light on their advantages and use in various applications in restorative dentistry and endodontics with a yield of good results. Their property of 'bioactivity' by eliciting favorable responses when in contact with periodontal tissues has been evident in various studies.⁵ Although a plethora of calcium silicate cement has been available in the market, the enhancement in their antibacterial property has been of question. The advent of nanotechnology in dentistry has shown to improve the

physicochemical, mechanical, and antibacterial properties of dental materials.⁶ Nanotechnology is a good tool for delivering low molecular weight drugs and macromolecules such as proteins or genes to cells and tissues.⁷ Nanotechnology is an extremely vital space of research in trendy science and technology.⁸ Silver and gold nanoparticles have been particularly used to inhibit bacterial growth in oral and endodontic applications.^{4,9-11} Nanoparticle (NP) delivery systems play important roles in increasing drug stability, prolonging the therapeutic period, and facilitating enteral and/or parenteral administration, which may minimize drug degradation and metabolism¹². Nanoparticles are solid particles of a size of 10-100 nm¹³. However, the biologic response of these nanoparticles with the living dental tissues has to be evaluated.⁶ This study aimed to evaluate the biocompatibility of an experimental calcium silicate-based cement incorporated with either silver or gold nanoparticles.

Materials and Method:

Preparation of the experimental calcium silicate-based powder was done from pure oxides of powder and weighed on an analytical balance and mixed manually in a porcelain mortar until a uniform mixture was obtained. The experimental liquid component consisted of 10% calcium chloride. Synthesis of gold nanoparticles was done using extra pure chloroauric acid (Loba Chemie Pvt Ltd). Characterization of the gold nanoparticles was done using

UV Vis Spectrophotometer (SL 159, ELICO). The size of the silver nanoparticles obtained commercially (SRL Chemicals) was 20nm.

Human lymphocytes were used in this study which was obtained from the American Type Culture Collection (ATCC; Rockville, MD, USA). The cells were grown in Dulbecco modified Eagle medium (DMEM), supplemented with 5% fetal bovine serum, 100U_{mL}⁻¹ penicillin, 100U_{mL}⁻¹ streptomycin, and 2 mmol⁻¹ L-glutamine, at 37°C in a humidified atmosphere of 95% air and 5% CO₂. Cement pellet was prepared by mixing the powder with the liquid.

The human lymphocyte cells were seeded into 96 well plates (2.7x10⁴ cells) and incubated for 24 hours to allow adhesion. Then, 9 pellets of each experimental group were placed into the culture wells. Cells were treated in 3 experimental groups. Group 4 is the control group where the cells were not exposed to any experimental materials.

Group 1: Mineral Trioxide Aggregate (ProRoot MTA, Dentsply) with 1wt% silver nanoparticles + distilled water

Group 2: Experimental cement with 1wt% gold nanoparticles + 10% calcium chloride

Group 3: Mineral Trioxide Aggregate + distilled water

Group 4: no treatment (control group)

The cement pellets were tested at 0 hours for freshly mixed pellets (D0), after 24 hours (D1), 48 hours (D2), and 72 hours (D3) incubation for cytotoxicity by MTT assay. Spectroscopic absorbance (optical density) was measured at 630nm using an ELISA microplate reader. The experiment was repeated in triplicate.

Statistical analysis:

The results were evaluated by repeated measures of analysis of variance (ANOVA). Differences were considered significant at $p < 0.05$.

Results:

Group	time	Mean	Std. Dev	Std. Error	95% Confidence Interval	
					Lower Bound	Upper Bound
1.0	0 hour	.212	0.0164	.015	.181	.242
	24 hours	.191	0.0362	.028	.135	.247
	48 hours	.227	0.0346	.017	.193	.261
	72 hours	.226	0.0317	.017	.192	.260
2.0	0 hour	.339	0.0252	.015	.309	.370
	24 hours	.421	0.0782	.028	.365	.477
	48 hours	.272	0.0337	.017	.237	.306
	72 hours	.346	0.0422	.017	.311	.380
3.0	0 hour	.249	0.0546	.015	.218	.280
	24 hours	.241	0.1252	.028	.185	.297
	48 hours	.173	0.0596	.017	.139	.207
	72 hours	.286	0.0559	.017	.252	.321
4.0	0 hour	.391	0.0660	.015	.361	.422
	24 hours	.391	0.0660	.028	.335	.447
	48 hours	.391	0.0660	.017	.357	.426
	72 hours	.391	0.0660	.017	.357	.426

Table 1: Descriptive statistics

Repeated Measures ANOVA

Repeated measures ANOVA suggests that the value of outcome in each group differ significantly over the period ($p < 0.001$).

Mauchly test suggests the violation of the assumption of sphericity ($p < 0.001$), hence we report the conclusions based on Greenhouse-Geisser and Huynh-Feldt correction Both Greenhouse-Geisser and Huynh-Feldt corrections suggest that there is a significant difference between the

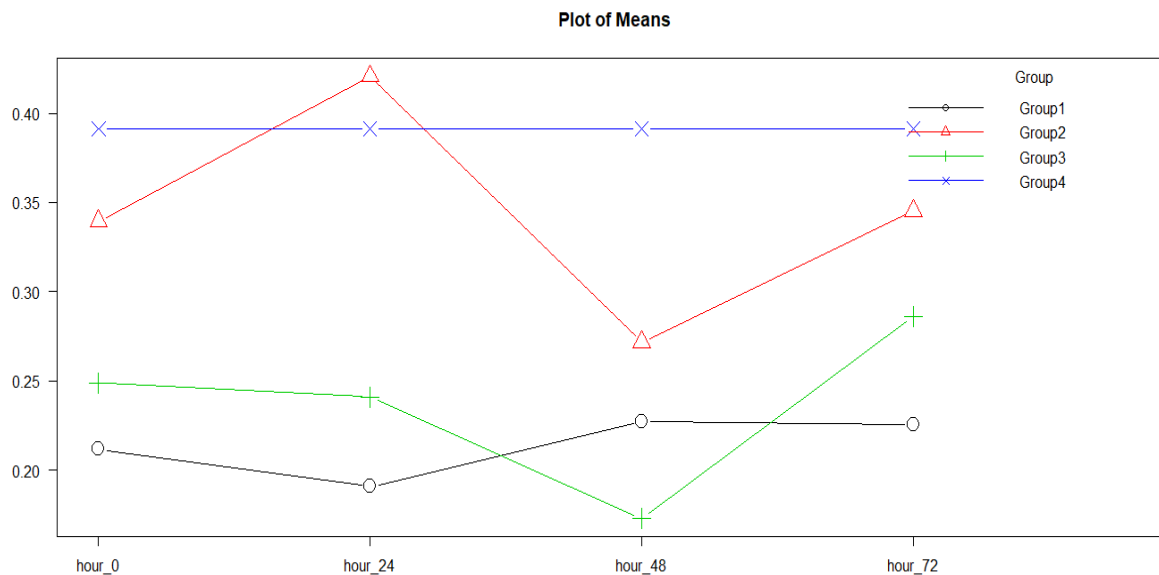
outcomes of the experiment over a while, between the groups ($p < 0.001$).

To identify whether there is a significant difference in the outcome between any two-time points, a paired t-test with Holm's adjustment was performed.

Pairwise Comparisons						
Measure: MEASURE_1						
(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Pvalue ^b	95% Confidence Interval for Difference ^b	
					Lower Bound	Upper Bound
1.0	2.0	-.130*	.020	< 0.001	-.186	-.075
	3.0	-.023	.020	1.000	-.078	.032
	4.0	-.177*	.020	< 0.001	-.232	-.122
2.0	3.0	.107*	.020	< 0.001	.052	.162
	4.0	-.047	.020	.136	-.102	.008
3.0	4.0	-.154*	.020	< 0.001	-.209	-.099
Based on estimated marginal means						
*. The mean difference is significant at the .05 level.						
b. Adjustment for multiple comparisons: Bonferroni.						

Table 2: Pairwise comparisons

The outcome of the experiment at only 48 hours is significantly different from the outcome at 0, 24, and 72 hours ($p < .05$).



Graph 1: Plot of means for the groups

There is no statistically significant difference between Group 2 and Group 4. Also, there is no statistically significant difference between Group 1 and Group 3.

Group 4 shows consistently higher values and Group 1 shows consistently lower values across all the time points. Group 2 and Group 3 are varying much with time.

Discussion:

The progression of the calcium silicate-based cements in endodontic and restorative applications has been quite remarkable. Their notable features of biocompatibility and bioactivity have made its use in endodontics utmost popular.⁵ Mineral Trioxide Aggregate is the first effectual

calcium silicate-based material that has been introduced in endodontics.¹⁴ Alternative types of cement have been introduced in the market to better the existing cement. One such cement is Biodentine, that claims to have a shorter set time than MTA.¹⁵ The accelerated setting is most important because the risk of partial material loss and interface alteration during the final finishing procedures is minimized.¹⁶ However, the antibacterial and antifungal activities of these materials have been attributed only to their alkaline pH.¹⁷ Attempts have been made to improve the antimicrobial properties of calcium silicate-based cement. Silver nanoparticles addition to MTA has proven to inhibit *E.Faecalis*, *P.aeruginosa*, *S.aureus*, and *C.albicans*,^{3,4} *A.actinomycetemcomitans*, *F.nucleatum*, *P.intermedia*, and *P.gingivalis*¹⁰ in various studies. Recently, the use of gold nanoparticles in endodontic irrigation combined with lasers has been studied to reduce the microbial load inside the root canal.¹¹ Their decreased toxicity combined with a wide array of possible chemical functionalizations has been considered, for utilization in a variety of applications.¹⁸ The cytotoxic reactions of calcium silicate-based cement combined with these nanoparticles need to be evaluated due to the proximity of these materials with the oral and periapical tissues.⁶ The cytotoxicity of the experimental groups was analyzed using the MTT assay. MTT depends upon the capacity of mitochondrial dehydrogenase enzyme to convert the water-soluble tetrazolium salt into dark blue formazan crystals. Cell viability depends on the quantity of formazan that is produced in the reaction.¹⁹ The cell lines that were used in this study were human lymphocytes. The advantage of using these cell lines is that they are karyotypically normal human cells and claim to be standard indicators for any systemic burden by exposure factors.²⁰ Therefore, human lymphocytes were chosen in the present study.

Gold compounds are generally considered safe and have been in routine clinical use for many years, for example, in the treatment of rheumatoid arthritis.²¹ However, once reduced to the nanometer scale, particles are known to undergo profound changes in terms of their biochemical properties which necessitates renewed investigations into their cytotoxic profile.²² The results of our study showed that there was no statistically significant difference between the control group and group 2 (experimental cement with gold nanoparticles) in terms of cytotoxicity, suggesting that the overall biocompatibility of group 2 in the time was better than group 1 (MTA with silver nanoparticles) and group 3 (MTA with distilled water). The cytotoxic behavior of Groups 1 and 3 was similar. This leads to an indication that gold nanoparticles were relatively less cytotoxic when

compared to silver nanoparticles. There are several studies in the literature that have proved the enhanced antimicrobial activity with the addition of silver nanoparticles to dental restorative materials. Most of these studies indicated that the nanosilver incorporated dental materials were relatively biocompatible.^{9, 23, 24} Currently, no studies are comparing the biocompatibility of silver and gold nanoparticles when combined with calcium silicate-based cement. Further studies are being carried out to determine the antibacterial activity.

Conclusion

Within the limitations of this study, it was concluded that the biocompatibility of the experimental calcium silicate-based cement was significantly improved when combined with gold nanoparticles. However, there was no difference between the nanosilver incorporated MTA and the gold standard MTA. Thus, silver and gold nanoparticles could be used as suitable additives to be incorporated into the experimental calcium silicate-based cement to improve the physical and mechanical properties.

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