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Research Article

EFFECTS OF ODONTOCEM AND MINERAL TRIOXIDE AGGREGATE-ANGELUS TOXICITY ON FIBROBLAST VIABILITY

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ABSTRACT

Objective: Mineral trioxide aggregate (MTA) is proven to be biocompatible and is used for pulp capping treatment. Odontocem, calcium silicate-based cement similar to MTA with the addition of a steroid, has recently been developed and to compare the effects of Odontocem and MTA-Angelus toxicity on fibroblast cell viability.

Methods: Fibroblast cells from embryonated chicken eggs were immersed in Odontocem or MTA-Angelus solution for 24 and 72 h. Cell viability was analyzed by Microculture Tetrazolium (MTT) 3-(4,5-dimethylthiazol-2- yl)2,5-diphenyltetrazolium bromide assay.

Results: After 24 h, the Odontocem and MTA-Angelus groups showed significantly lower viability ($p<0.05$) compared with controls. After 72 h, cell viability was significantly higher in the Odontocem and MTA-Angelus groups. However, there was no significant difference between the Odontocem and MTA-Angelus groups.

Conclusions: Odontocem and MTA-Angelus have low toxicity for dental applications.

Keywords: Fibroblast cells, Toxicity, Odontocem, Mineral Trioxide Aggregate.

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INTRODUCTION

Mineral trioxide aggregate (MTA) is a dental material that was developed at Loma Linda University in 1993 [1]. MTA is composed of calcium oxide and silicon dioxide, which make up 70–95% of its overall composition. The mixture of these components produces tricalcium silicate (Ca_3S), dicalcium silicate, tricalcium aluminate, and tetracalcium aluminoferrite along with aluminum oxide, calcium sulfate, magnesium oxide, potassium sulfate (K_2SO_4), and sodium sulfate. Bismuth oxide is added to give the material radiopaque properties [2].

MTA has good physical properties related to sealing ability and biocompatibility. Its great sealing ability can reduce bacterial infiltration and prevent contamination, whereas its biocompatibility enhances wound healing [3-6]. These properties promote regeneration of tissue that is in contact with MTA. Additional benefits of MTA are that it creates an antibacterial environment due to its high pH, it adapts well to cavity walls to minimize bacterial leakage, and it triggers hard tissue forming cell differentiation and migration. However, MTA does have some drawbacks, including discoloration potential (especially gray MTA), difficult handling characteristics, and a long setting time [7,8]. According to the manufacturer's recommendation, the setting time is 15 min for MTA-Angelus which lacks K_2SO_4/gypsum and 4 h for regular MTA [6].

MTA is used in many clinical applications including pulp capping, perforation repair, root canal sealing, and formation of apical barriers after apical surgery [7]. Min *et al*. investigated human cell response to MTA and concluded that the material is non-toxic. In addition, *in vitro* and *in vivo* animal tests showed normal cell growth, healing at the implant site, and no infection after 12 weeks exposure to MTA [9].

Odontocem, calcium silicate-based cement similar to MTA with the addition of a steroid, has been recently developed and is now widely used. The steroid suppresses inflammation so that patients do not experience pain during the healing process. Although favorable to the patient, suppression of the inflammatory process slows down cell proliferation needed to promote tissue healing, thereby delaying the healing process.

Tissue regeneration can be affected by the toxicity of the induction material. Therefore, all materials used in dentistry must be biocompatible, meaning they are non-toxic, non-irritative, and non-carcinogenic and do not trigger allergic reactions in the host. Compared with MTA, there have been relatively few studies on the toxicity of Odontocem. This study was conducted to fill this research gap.

METHODS

Cell culture is commonly used to assess the toxicity of dental materials on cells. Fibroblasts are the preferred cell type for cell culture due to their great vegetative ability and growth time of 18–24 h [10]. Chicken embryo fibroblasts are ideal for *in vitro* tests for ethical, economic, and practical reasons [11]. Thus, we tested the effects of immersion in Odontocem and MTA-Angelus on the viability of chicken fibroblasts. The sample size was determined using GPower 3.1. For an effect size *f* of 0.3, α err prob. of 0.05, and power (1-β err prob.) of 0.8, the necessary sample size was 84.

Embryos were obtained from 9- to 11-day-old embryonated eggs cleaned with 70% ethanol. The tip of the egg was opened, and the egg membrane was removed using sterile forceps. The embryo was removed from the egg, washed with phosphate buffer saline, and then transferred to a sterile Petri dish. The head, wings, legs, and internal organs were discarded. The remaining parts were chopped into small pieces and incubated in a Petri dish treated with 0.25% trypsin for 5 min at 37°C in an atmosphere of 5% CO_2. The trypsinized embryo was then filtered and centrifuged at 1500 × g for 10 min. The supernatant was discarded, and the cells were resuspended and centrifuged at 1500 × g for 10 min. The cells were suspended again. The cell suspension was dispensed into tissue culture flasks with a complete culture medium (Dulbecco's modification of Eagle medium [DMEM] enriched with 10%

Fetal bovine serum [FBS], 2 mM glutamine, and antibiotics [penicillin and streptomycin]) and incubated at 37°C in an atmosphere of 5% CO_2 and allowed to become confluent. Cells were counted using a hemocytometer. Cells from the second harvest were used in this study.

Odontocem and MTA-Angelus samples were prepared according to the manufacturer's instructions and molded into plastic cylinders measuring 11 mm in diameter and 4 mm in width. After setting, the samples were removed from the mold, immersed in DMEM solution, and incubated at 37°C in an atmosphere of 5% CO_2 for 24 h. The sample and solution were centrifuged for 10 min. The supernatant was then harvested, sterilized, and filtered using a 0.22 μm filter for use in the experiments.

The cells were seeded in 96-well plates at a density of 5000 cells/well and incubated for 24 and 72 h at 37°C in an atmosphere of 5% CO_2 in growth media without FBS. The growth media were then changed to 100 μL of the Odontocem supernatant, MTA-Angelus supernatant, or control (growth medium only). Cell viability was evaluated using the 3-(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide (MTT) assay after 24 and 72 h. The MTT assay is the best test to determine mitochondrial dehydrogenase activity in living cells [12]. Other advantages of this assay include ease, safety, speed, repeatability, and reduced cell death. The absorbance level optical density of each treatment group was measured using microplate reader at 520 nm.

Differences between the groups were evaluated using the Kruskal–Wallis non-parametric test because the homogeneity test showed that at least two groups had significantly different variances but abnormal data distribution. This was followed by *post hoc* Mann–Whitney U-test.

RESULTS

Table 1 presents the viability values after immersion for 24 h. As shown, the MTA-Angelus group (77.57%) had higher viability than the Odontocem group (74.40%). There was an increase in viability after 72 h of immersion for both groups. However, the MTA-Angelus group showed higher viability (105.90%) compared with the Odontocem group (81.67%).

As shown in Table 2, there was a significant difference ($p \leq 0.05$) in cell viability between the control and Odontocem group after 24 and 72 h immersion. The MTA-Angelus group also differed significantly ($p \leq 0.05$) compared with the control after 24 and 72 h. There was no significant difference between cell viability in the Odontocem and MTA-Angelus groups after 24 h, but there was a significant difference after 72 h immersion.

As shown in Table 3, there was a significant difference ($p \leq 0.05$) in viability between 24 and 72 h immersion in the Odontocem group. A similar result was found in the MTA-Angelus group between 24 and 72 h.

Table 1: Mean distribution and standard deviation of cell viability values in the Odontocem and MTA-Angelus groups

Group	n	Mean±SD
Control		
24 h	15	100.0±0.00
72 h	15	100.0±0.00
MTA-Angelus		
24 h	15	77.57±6.36
72 h	15	105.90±8.02
Odontocem		
24 h	15	74.40±2.93
72 h	15	81.67±6.36

SD: Standard deviation. MTA: Mineral trioxide aggregate

Table 2: Significance values of cell viability after immersion in Odontocem or MTA for 24 and 72 h compared with the control group

Control versus odontocem		Control versus MTA-Ang		Odontocem versus MTA-Ang	
24 h	72 h	24 h	72 h	24 h	72 h
0.000*	0.000*	0.000*	0.020*	0.101	0.000*

*p≤0.05: Significance test using Kruskal–Wallis and *post hoc* Mann–Whitney *U*-test. All results were significant except for the difference between the MTA-Angelus and Odontocem groups at 24 h. MTA: Mineral trioxide aggregate

Table 3: Significance values of cell viability after immersion in Odontocem or MTA-Angelus for 24 versus 72 h

	n	Odontocem	MTA-Angelus
24 h versus 72 h	15	0.001*	0.000*

*p≤0.05: Significance test using Kruskal–Wallis and *post hoc* Mann–Whitney *U*-test. There was significant difference in those groups. MTA: Mineral trioxide aggregate

DISCUSSION

This study used an experimental laboratory test to compare fibroblast cell viability after exposure to two bioactive agents, namely, Odontocem and MTA-Angelus. We found that fibroblast viability was lower after 24 h of exposure to Odontocem or MTA-Angelus compared with controls, but higher after 72 h of exposure. The initially lower viability is likely due to the increase in pH after exposure to the test materials. In this case, the pH of MTA reached 10.2 immediately after mixture, increased to 12.5 after 3 h, then gradually lowered to a neutral pH. According to Jafamia (2009), the lower cell viability after 24 h exposure to the high pH of MTA is due to the dehydration process [12].

By contrast, viability was higher in the Odontocem or MTA-Angelus groups compared with controls after 72 h. The significant change between 24 and 72 h of immersion can be explained as follows: Before entering the cell proliferation phase, the wound healing process, which consists of hemostasis, inflammation, proliferation, and remodeling, occurred. Hemostasis starts with vascular constriction, platelet aggregation, degranulation, and fibrin formation. Inflammation is marked by neutrophil infiltration, monocyte differentiation to macrophages, and lymphocyte infiltration [13,14]. In the initial phase of healing (first 24–36 h), neutrophils are recruited to eradicate bacteria or damaged cells by the phagocytosis process. The proliferation phase occurs 2–10 days after injury and is marked by cell proliferation and migration in which fibrin matrix changes by granulation tissue followed by the formation of new vascularization (angiogenesis). Cell proliferation is followed by angiogenesis and collagen and extracellular matrix formation and ends with collagen remodeling, vascular maturation, and regression. The skin wound remodeling phase starts at day 7 and last for about 1 year. At this phase, the extracellular matrix at the wounded area is recreated and scar reorganization starts. Collagen fiber remodeling depends on synthesis and catabolism of collagen. Collagen degradation programmed by various kinds of matrix metalloproteinase enzymes is secreted by macrophages in the inflammation phase, endothelium cells in the proliferation phase, and fibroblast cells in the remodeling phase [13,14].

Increased viability after 72 h of immersion in MTA-Angelus and Odontocem was in accordance with a study by Peng *et al*., who reported significant proliferation in human pulp cells exposed to Ca_3SiO_5 extract solution compared with growth medium from day 3 [15]. Calcium and silicate were also the main components of the materials used in this study [15,16].

The biocompatibility of MTA has been previously assessed using parameters like its impact on the proliferation of various cell types after direct and/or indirect contact. Okiji and Yoshiba found that MTA is

generally less toxic than amalgam, super EBA, and IRM. Cells in contact with MTA appeared to have greater viability and proliferation activity, although freshly mixed MTA had higher toxicity due to its high pH [17].

Holland stated that the action of MTA in enhancing deposition of hard tissue is similar to that of calcium hydroxide. At the beginning, it causes coagulation necrosis if it contacts pulp connective tissue due to its alkaline pH, which is around 10.2 during manipulation and 12.5 after 3 h [18]. MTA also causes a less inflammatory reaction than calcium hydroxide when it contacts connective tissue. Necrotic and granulation zones were created by nearly the same mechanism as calcium hydroxide, but with a lesser inflammatory reaction [11].

Taken together, these findings support the suitability of MTA-Angelus and Odontocem for dental applications.

CONCLUSIONS

Immersion in Odontocem and MTA-Angelus lowered fibroblast viability after 24 h, but increased viability after 72 h. Viability was higher in MTA-Angelus than Odontocem after 72 h.

CONFLICTS OF INTEREST

The author reports no conflicts of interest.

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