# Effects of Compound Topical Anesthetic on Oral Soft Tissue

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## INTRODUCTION

Local anesthesia is routinely used in pediatric dentistry to reduce pain and improve patient cooperation during dental procedures. Topical anesthetics are commonly applied prior to local anesthetic injections and may also be used independently for minimally invasive procedures.

Compounded topical anesthetics are pharmacist-prepared formulations that combine multiple anesthetic agents. While these formulations may provide faster and more profound anesthesia compared to single agents, they may also increase the risk of toxicity due to enhanced potency and mucosal absorption.

Because topical anesthetics are applied directly to oral soft tissues, there is potential for cytotoxic effects on gingival fibroblasts. Previous studies have demonstrated that anesthetic concentration and exposure time can negatively affect cell viability and function.

However, there is limited data evaluating the cytotoxic effects of commonly used compounded topical anesthetics on oral soft tissues or comparing these formulations to individual agents.

Therefore, the objective of this study is to evaluate the cytotoxic effects of compounded topical anesthetics on gingival fibroblasts by assessing cell viability, proliferation, and motility, compared to single-agent topical anesthetics.

## METHODS

**Cell Lines:** Gingival fibroblasts were isolated from a periodontally healthy 13 year old patient. Cells were maintained in minimum essential medium *alpha* (MEMα) containing 10% fetal bovine serum and 200 units/ml of penicillin and 200 µg/ml streptomycin.

**Compounded Solutions:** Test solutions were prepared by dissolving lidocaine, tetracaine, prilocaine, phenylephrine, and benzocaine into DMSO to a stock concentration of 10% and further dilutions were made using MEMα containing serum and antibiotics.

| Solution | Components |
|---|---|
| C1 | 20% Lidocaine, 4% Tetracaine, 2% Phenylephrine |
| C2 | 20% Lidocaine, 4% Tetracaine, 0.1% Phenylephrine |
| C3 | 12.5% Lidocaine, 12.5% Tetracaine, 3% Prilocaine, 2% Phenylephrine |
| C4 | 10% Lidocaine, 6% Prilocaine |
| C5 | 20% Benzocaine |
| C6 | 5% Lidocaine |
| C7 | 2.5% Lidocaine, 2.5% Prilocaine |

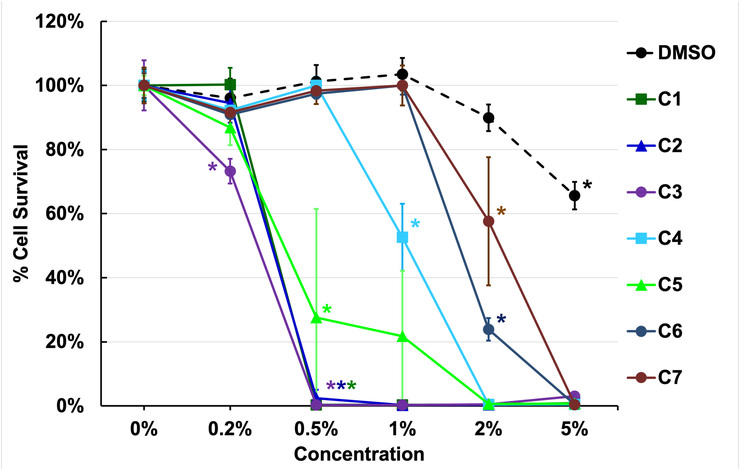
**Calcein staining of live cells:** Cells were allowed to adhere for 24 hours and then exposed to varying concentrations of test solutions. Cell viability was assessed using Calcein AM after 1 hour of incubation. Fluorescence was measured using a BioTek Synergy2 plate reader (excitation ~480 nm, emission ~520 nm). Mean and standard deviation of eight samples were calculated and compared to untreated controls.

**Cell Proliferation Assay:** Cells were plated at 4,000 cells per well and allowed to adhere for 24 hours prior to exposure to compounded anesthetics for up to 24 hours. Cell proliferation was quantified using CyQUANT fluorescent dye. Each condition was tested in eight samples per experiment.

**Cell Motility Assay:** Cells were plated in 12 well plates and allowed to adhere for 24 hours. A standardized scratch wound was created in each well, followed by treatment with compounded anesthetics. Wound closure was measured at 24 hour intervals. For each condition, 25 measurements were averaged and compared to untreated controls. Cells were stained with Calcein AM to aid in visualization and imaged using an inverted microscope.

## RESULTS

### Figure 1. Effect of Compound Anesthetic on Cell Survival

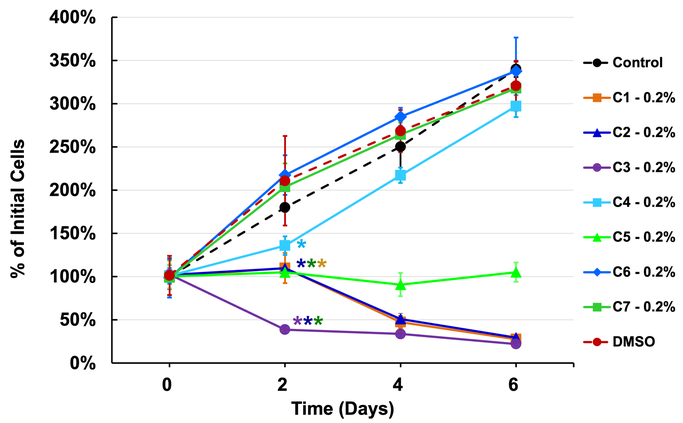



Percent survival of human gingival fibroblasts (GF) after 24-hour exposure to compound anesthetic at various dilutions.

- Significant reductions in cell survival were noted for solutions 1, 2, 3 starting at 0.5% and solutions 4 and 5 at 2%. All samples showed complete cell death at 5%.

**(n=8) (* = P < 0.05)**

### Figure 2: Effect of Compound Anesthetic at 0.2% on Cell Proliferation




Human gingival fibroblasts (GF) proliferation after 24-hour exposure to compound anesthetic at 0.2%.

- Solutions 1, 2, 3 induced cell death at 0.2%. Solution 5 halted cell proliferation at 0.2%. Solutions 4, 6, 7 did not inhibit cell proliferation at 0.2%.

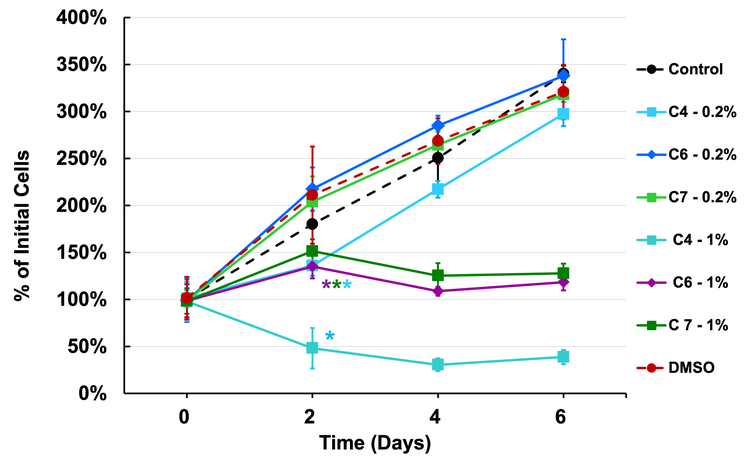
**(n=8) (* = P < 0.05)**

For cell adhesion experiments, cells were allowed to adhere in the presence of test solutions (as above). For proliferation experiments, cells were plated as above at a low density and incubated for 1-7 days. The cells were fluorometrically labeled for quantification as above (n=8).

**Statistical Tests Utilized:**

- Student's T Test
- ANOVA

### Figure 3. Effect of Compound Anesthetic at 0.2% vs 1% on Cell Proliferation

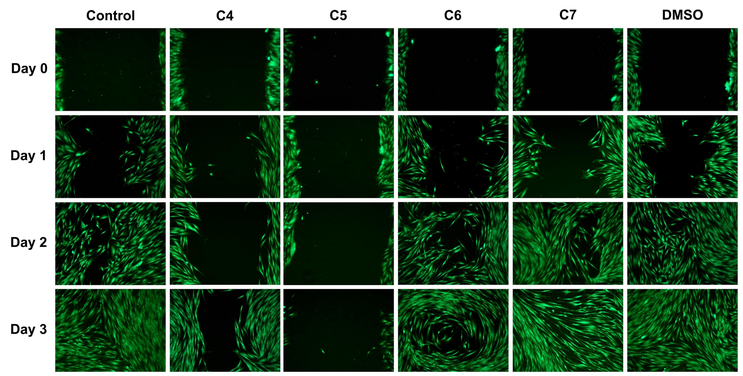



Human gingival fibroblasts (GF) proliferation after 24-hour exposure to compound anesthetic at 0.2% vs 1%.

- Solutions 6 and 7 halted cell proliferation at 1%. Solution 4 induced cell death at 1%.

**(n=8) (* = P < 0.05)**

### Figure 4. Effect of Compound Anesthetic on Cell Migration




Human gingival fibroblasts (GF) morphology after 24-hour exposure to compound anesthetic at 0.2%.

- Solution 4 delayed cell migration and solution 5 halted cell migration.
- Solution 6 and 7 had no effect on cell migration.

## CONCLUSIONS

1. Higher concentration and greater numbers of drugs in combination showed greatest cytotoxicity
2. Benzocaine notably inhibited cell migration and proliferation
3. Low concentration combinations are least disruptive to gingival cell function