Antiviral Properties of Self-Disinfecting Impression Materials  P 1728

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OBJECTIVE
To determine the antiviral/antimicrobial activity of self-disinfecting impression materials against Herpes 2(H2) and Human Immunodeficiency Virus-1 (HIV, AIDS) and other organisms isolated from the oral environment.

INTRODUCTION
In recent years, an increased awareness of infectious diseases and their possible transmission within the dental community has led to greater attention to infection control in the operatory and dental laboratories. Numerous methods of dealing with this problem exist, including autoclaving, dry heat, chemical disinfection, physical barriers, etc. However, these treatment modalities are generally more appropriate for the surface of instruments and equipment, rather than the chemical products used in dental procedures.

Dental impressions represent a major avenue through which the spread of infectious organisms can occur from patient to doctor or auxiliary. The primary function of a dental impression material is to make an accurate replica of the oral tissues, and accordingly, they must be elastic, dimensionally accurate and have good surface wetting properties. Application of heat, or contact with a variety of chemicals to achieve sterility or disinfection can cause them to distort, soften, or otherwise defeat their main purpose. This is especially a problem for alginate impressions which are hydrogels of calcium alginate and an inert filler. These compounds can dehydrate rapidly, thus changing dimensions; or if placed in an aqueous sterilizing solution, they attract additional water, thus also changing dimension. As a consequence, normal dental impressions can become contaminated or impregnated with microorganisms, such as bacteria and viruses, from a patient, and these microorganisms can be spread to dental auxiliaries (assistants, technicians) who handle the impressions in the process of making casts, models and prostheses.

It is the purpose of this study to evaluate the effectiveness of incorporating antiviral/antimicrobial agents directly into the impression material, and to study the effect of these agents on the physical properties and biocompatibility of the impression material.

MATERIALS
ALGINATE IMPRESSION MATERIAL

Material A – JELTRATE® PLUS control (no additive)

Material B – JELTRATE® PLUS containing a quaternary ammonium salt additive
Material C - JELTRATE® PLUS containing a nonionic surface active additive

POLYVINYL SILICONE IMPRESSION MATERIAL

Material D - Hydrosil containing a nonionic surface active additive.

METHODS:

A) ANTIVIRAL

I) HERPES 2: ALGINATE IMPRESSION MATERIAL

1. The canister of alginate was tumbled to fluff the powder;
2. Three (3) scoops, leveled with a spatula, were added to a mixing bowl;
3. 57 ml deionized tap water (7 grams powder to 19 ml water ratio) were added to the bowl;
4. The powder moistened with water was thoroughly mixed (to a paste) for 60 seconds;
5. The paste was layered into two (2) sterile 50x9mm Falcon petri dishes, leveled with a spatula, and allowed 5°C solidify.
6. Within 10 minutes, 0.2 ml Herpes 2 virus (10^5 TCID_50 virus/ml) was added to each plate (1 plate inoculated with virus and 1 plate (cytotoxicity control) not inoculated but treated with 2 ml TSB containing 20% serum. A third plate was used as a polystyrene control; 1 ml TSB containing 20% serum aliquoted into plate.
7. Plates were incubated for 10 minutes at 35±1°C.
8. 2 ml diluent (TSB containing 20% serum) was added to plate mold impressions (except polystyrene control plate) and quantitatively transferred and diluted 10 fold in TSB containing 20% serum;
9. A Herpes 2 virus control was also performed. The pool was decimally diluted in TSB (20% serum);
10. 0.1 ml from each dilution inoculated into Hep-2 cells; and
11. Hep-2 cells observed for CPE over a 14 day period.

II) HERPES 2: POLYVINYL SILICONE IMPRESSION MATERIAL

1. 10 grams of each component (maroon-pink paste and gray-white paste) was weighed on a calibrated balance, and immediately mixed with a sterile spatula.
2. Repeat steps 5 - 11 as described in Method Section A-I.

III) HIV-1 (AIDS VIRUS)-ALGINATE-IMPRESSION-MATERIAL

1. The alginate material was prepared as described in Method Section A-I, steps 1 - 4.
2. The paste was layered into two (2) sterile 50x9mm Falcon petri dishes, leveled with a spatula, and allowed to solidify 15-20 min. at 4°C.
3. Within 10 minutes, 0.2 ml HIV-1 virus (10^{3.5} TCID_{50} Virus/ml) was added to each plate (1 plate inoculated with virus and 1 plate (cytotoxicity control) not inoculated - but treated with 5 ml TSB containing 20% serum. A third plate was used as a polystyrene control: 1 ml TSB containing 20% serum aliquoted into plate.

4. Plates were incubated for 10 minutes at 35\pm1^\circ C.

5. 2 ml diluent (TSB containing 20% serum) was added to plate mold impressions (except polystyrene control plate) and quantitatively transferred and diluted 10 fold in TSB containing 20% serum;

6. A HIV-1 virus control was also performed. The pool was decimally diluted in TSB (20% serum);

7. 1.0 ml from each dilution inoculated into H-9 (10 ml) cells; Cells were re-fed every third day with RPMI 1640; and incubated a total of 10 days. At days 8 and 10, 1 ml aliquots were removed from supernatants and frozen in T-25 flasks. HIV activity was measured by an FDA-approved Abbott P24 core antigen capture ELISA kit.

IV) HIV-1 (AD-VIRUS) - POLYVINYL SILOXANE IMPRESSION MATERIAL
1. 10 grams of each component (maroon-pink paste and gray-white paste) was weighed on a calibrated balance, and immediately mixed with a sterile spatula.
2. Repeat steps 2 - 7 as described in Methods Section A-III.

METHODS

B. ANTIMICROBIAL

GENERAL PROCEDURE
1. Swab 0.1 ml of the test culture onto nutrient agar plates.
2. Divide each plate into thirds and place two test samples and one negative control, consisting of 0.1 ml saline, on a 10mm paper disc onto the plate.
3. Incubate plates at 30-35^\circ C for 24 \pm 2 hours.
4. Upon completion of 24 hour incubation, measure any zone of inhibition extending from the edge of the disc.
5. Record results.
6. Remove test sample and control disc and reincubate at 30-35^\circ C for an additional 24 \pm 2 hours.
7. Upon completion of the final incubation period, remove plates from incubator and observe each plate for the following:
   a. Any growth present in area where sample or control was present.
   b. No additional growth.
8. Record results.

INTERPRETATION

A. The test sample is considered bacteriocidal if:
1. A zone of inhibition of growth of the test organism is observed extending from the edge of the test sample and/or no growth is present directly underneath the test sample, and
2. The same conditions apply when the sample has been removed from
the plate and reincubated for an additional 24 hours.

B. The test sample is considered bacteriostatic if:
1. A zone of inhibition of growth of \textit{S. mutans} is observed extending
from the edge of the test sample and/or growth is present directly
underneath the test sample.
2. After sample has been removed from the plate and reincubated for
24 hours, growth is present in areas where it was absent previously.

C. The test sample is considered neither bacteriocidal nor bacteriostatic if
growth is present directly underneath the test sample after 24 hours
incubation.

DISCUSSION:
The results presented in this paper indicate that it is possible to
incorporate an antiviral/antimicrobial agent directly into certain impression
materials without adversely affecting the physical and biological properties
of the material, yet maintain effective kill rates against infectious
organisms.

The incorporation of two different additives (Material B and Material C)
into an alginate impression material resulted in similar activity against
HIV-1 virus and perhaps a slightly more effective kill rate for Material B
against Herpes 2 virus. Contact times of only 10 minutes on the surface of
the alginate was effective in destroying 99 + \% of the infectious virus.
Ten minutes is the amount of time between seating the impression, allowing
the material to set, removal, and immediate pouring of dental stone. In
the case of the polyvinyl siloxane, similar antiviral activity from 10
minute contact was also observed even though the chemistry of the impres-
sion material was quite different.

The antimicrobial activity of the two alginate materials was also
examined and found to produce differing results. In the case of Material B,
good activity against all organisms tested was observed at all time periods,
with the exception of \textit{P. aeruginosa}. This organism is more difficult to kill
and required longer time periods (2 hours) to effectively destroy the
microbe. However, with Material C, no activity at any time period for any of
the organisms tested was observed. This was somewhat surprising in view of
the results with antiviral testing.

In light of the above, blends of the additive from Materials B and C were
prepared and retested against \textit{S. aureus} and the more resistant \textit{P. aeruginosa}.
It was interesting to note that the activity of the blends against \textit{S. aureus}
increased even at very low levels of Material B in Material C. However, it
took considerably more of Material B, in Material C to effect a change in
activity against \textit{P. aeruginosa}.

In order to assure that the addition of these antiviral/antimicrobial
ingredients did not alter the other desirable aspects of the impression
material, such as physical properties or biocompatibility, other testing was
completed. As indicated in Table 3, the biocompatibility of the materials were
not compromised and the physical property testing met and exceeded ANSI/ADA
specifications.
CONCLUSIONS:

While various methods of disinfecting impression materials exist, there are limitations to these procedures based upon the chemical nature of the impressions. The materials presented here, provide an effective way to obtain antiviral and antimicrobial activity without jeopardizing the physical integrity of the specimens. As a result of this work, several patents are pending in the United States and other countries.
REFERENCES:


<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>H2</td>
<td>Herpes viridae</td>
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<tr>
<td>HIV-1</td>
<td>Retroviridae (HTLV-III) (HIV-1)</td>
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<td>Human immunodeficiency virus - 1</td>
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<td>TSB</td>
<td>Trypticase soy broth</td>
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<tr>
<td>Hep-2</td>
<td>Human epithelioma lines</td>
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<tr>
<td>H-9</td>
<td>Human T-cell line</td>
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<tr>
<td>CPE</td>
<td>Cytopathic effect</td>
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<tr>
<td>RPMI</td>
<td>Growth media with antibiotics</td>
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<tr>
<td>TCID&lt;sub&gt;50&lt;/sub&gt;</td>
<td>Tissue Culture Infectious Dose 50%</td>
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<tr>
<td>Log Reduction</td>
<td>Material</td>
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<td>---------------</td>
<td>----------</td>
</tr>
<tr>
<td>4.0</td>
<td>B (control)</td>
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<tr>
<td>4.5</td>
<td>C</td>
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<tr>
<td>6.5</td>
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<td>-99.999</td>
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**TABLE 1**

Antiviral Activity - 10 Minute Contact
<table>
<thead>
<tr>
<th>Material B</th>
<th>30MIN</th>
<th>1HR</th>
<th>2HR</th>
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<tr>
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**Antimicrobial Activity – Zone of Inhibition (mm²)**

**Table 2**
<table>
<thead>
<tr>
<th><strong>ANB/ADA SPECIFICATION</strong></th>
<th><strong>19-NON-AQUEOUS ELASTOMERIC IMPRESSION MATERIALS</strong></th>
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<tr>
<td><strong>PHYSICAL PROPERTY TESTING</strong></td>
<td><strong>ALL MATERIALS PASSED THE FOLLOWING TESTS:</strong></td>
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<tr>
<td><strong>ACUTE ORAL TOXICITY (&gt;1G/KG BODY WEIGHT)</strong></td>
<td><strong>PRIMAR Y DERMAL (SKIN) IRRITATION—ANIMALS</strong></td>
</tr>
<tr>
<td><strong>MUCOUS MEMBRANE IRRITATION—ANIMALS</strong></td>
<td><strong>PRIMAR Y DERMAL (SKIN) IRRITATION—HUMANS</strong></td>
</tr>
<tr>
<td><strong>AMES MUTAGENICITY TEST</strong></td>
<td><strong>BIOLOGICAL TESTING</strong></td>
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**TABLE 3**