Compatibility of Sodium Fluoride Patch as an Innovation Model of Transferring Fluoride in Dental Care: A Quantitative Study Using in Vitro & in Vivo Rabbit Skin

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ABSTRACT

Background: Fluoride reduces dental caries by creating the fluor apatite which is more invulnerable to acid caries attack. However, the conventional fluoride found in toothpaste is seldom to reach the therapeutic achievement level when interacting with water during teeth brushing. As such fluoride in a systemic mode without passing metabolism with safe dose needs developing through transdermal drug delivery. This study aims to investigate the compatibility of Sodium Fluoride (Na F) patch as the transporter of fluoride to prevent caries attack besides the viability of macrophage cell and haemolytic acute to secure safety.

Method: This research applied experimental clinical laboratories with pre and post control group design. The sample of the acute haemolytic test was performed with a physiologic saline solution and oxalate blood. The sample of viability was the suspension of macrophage cell on a trifan blue solution. Furthermore, the irritation skin test was tested on five rabbits attached Na F patch on the left back side while the one as the control group was attached a patch without Na F on the right side.

Results: There were no differences of erythrocyte lysis and macrophage viable between the group of Na F patch and without Na F patch. Skin irritation test was shown on Mann Whitney result the significance of 0.317 indicating no differences of skin reaction between rabbit back which was applied with Na F patch and not attached.

Conclusions: Na F patch has excellent biocompatibility and safe to be used.

Keywords: Na F patch, biocompatibility, haemolytic, irritation, rabbits

INTRODUCTION

Caries is a disease that involves many factors including Streptococcus Mutants (SM) bacteria which if interacted with carbohydrate will cause pH to decrease in the oral environment and increase demineralization process. However, demineralization process will not cause caries if it is balanced with the higher remineralization process such as by adding fluoride (1). Administration of fluoride in small doses and administered over a lifetime as well as regularly is the most appropriate method for increasing tooth resistance against caries attacks (2). In this case, the use of fluoride toothpaste and adding fluoride to drinking water is the most appropriate method.

In Indonesia, the use of toothpaste reaches the public widely as almost all toothpaste on the market contains fluoride. The therapeutic dose of fluoride in saliva is 0.1 ppm (3) but in every time of tooth brushing, fluoride absorbed in the saliva is only 0.02-0.04 ppm which is still lacking in the level of therapeutic achievement. The concentration of fluoride in toothpaste cannot be directly made to a higher dose (maximum 1500 ppm in 1 tube) due to the dangerous effect if fluoride is being ingested (4). Another method is water fluoridation where the fluoride is added to drinking water at a concentration
of 0.7-1 ppm. This model has the advantage of being able to reach a broad layer of society and nature in its application. Many countries have already succeeded in implementation and continue up to this day \(^{(5)}\). However, success in some countries cannot be done in developing countries such as in Indonesia due to the high price and limited expertise\(^{(6)}\).

Due to the limitations of the fluoride supplying model above, it is necessary to consider a fluoride delivery method which can be continuously administered at low doses, safe, convenient and affordable. Current preparations, which may become an alternative is the supply in the form of TDD (transdermal drug delivery) which is the delivery through the skin in the shape of a gel, cream, and patch. The patch model has been widely used and proven effective such as nicotine, contraception, analgesic, and antibiotic patches\(^{(7)}\). However, a patch containing fluoride is still in an infant stage and should be developed as a new model in combatting carries.

Patch could penetrate the skin of a mouse and survive in the blood plasma and tooth denoting an excellent bioavailability\(^{(8)}\). Development of the physical form of the Na F patch has been performed and proved that the combination of PVP: PVA with the ratio 2: 1 is the best polymer based on its physical properties after storage\(^{(9)}\).

The development of fluoride patch as a new model that will have direct contact with the human body must go through a biocompatibility test. Biocompatibility is the suitability of an ingredient against living tissue when in contact with the material\(^{(10)}\). The patch application is started from the attachment to the skin; fluoride penetrates the skin layer, to the blood vessels, then to the bones and teeth. Based on the fluoride travel route, the biological evaluation must be conducted including irritation reaction and sensitivity to patch material, cytotoxicity test, and haemolysis test. The skin is often irritated because of the direct contact with the environment outside the body like the exposure to chemicals. Fluoride in the form of implants proved to be compatible with tissue based on cytotoxicity and haemolysis test \(^{(11)}\) while its effect on fluoride patch has not been identified. This phenomenon underlies the researcher to examine the effect of Na F patch reaction to blood; viability and macrophage cell phagocytosis activity as well as in vivo application of Na F patch application to skin tissue.

#### MATERIAL AND METHOD

The research design was a quasi-experimental study with post-test only design in the non-equivalent group. Na F piece was made at Health Analyst Laboratory of Health Polytechnic of Semarang and biocompatibility test was conducted in the laboratory of experimental animal Gadjah Mada University Jogjakarta.

Population in acute haemolysis test (in vitro) was the solution of oxalic blood that was used to soak the Na F patch and taken as the sample was 10.2 ml physiological saline solution and blood oxalate. Population in cell viability test (in vitro) was a macrophage cell cultures treated with Na F patch, sample feasibility test in the form of macrophage cell suspension in blue trifan solution with 110-μl volume and skin irritation population test of Na F patch application was five rabbits. Na F patch was attached to left side of the rabbit’s back, and the patch did not contain Na F as the control was attached to the right side of the rabbit’s back.

The procedure of making Na F patch and in vitro test was adopted by previous researchers\(^{(8),(9),(11)}\). Test of rabbit skin tissue response was conducted by firstly adapting the rabbit for a week. Rabbit’s back was shaved with an electric shaver on the left and right side. Treatment and control group were five rabbits patched with Na F patch on left back (treatment), and right patched of non-Na F (control group). The patch was changed every three days. Skin tissue observation was conducted on day 1 and 14 to see an inflammatory reaction. Categories of erythema incidence (state of skin redness tissue) were categorized into 0) no reaction; 1) mild erythema; 3) moderate erythema; 4) severe erythema.

To determine the effect of fluoride patch in the blood, One Way ANOVA was employed. To investigate the effect of Na F patch on the viability of macrophage cells the independent t test was used and to determine the effect of Na F patch towards response of the skin tissue, Mann Whitney test was conducted.

#### RESULTS AND DISCUSSIONS

The differences of the physical appearance of a surface containing Na F patch and the one does not contain Na F is observable as shown in Figure 1. On the skin containing Na F patch is seen coarse grains of Na F powder as the patch is physically easy to be stuck on the skin surface. The optimal Na F patch is a patch of 750 ppm concentration based on research before \(^{(9)}\).
Acute haemolysis test was one of toxicity test aiming to identify the tissue response up to cellular level toward the exposure to an organism or unidentified object, in this case, Na F patch exposure to the blood. Fluoride would absorb into the skin tissue and penetrate the blood vessels. A blood erythrocyte fit test towards fluoride exposure was used to examine the erythrocytes lysis after exposure to fluoride. This study found there was no difference in erythrocytes lysis between red blood cells given Na F patch and the one without Na F patch. This phenomenon proves that fluoride is compatible with erythrocytes (blood vessels). These results were consistent with the research which found suitability in fluoride implant to the blood\(^{11}\).

The percentage of lysis erythrocyte cells after being exposed to Na F patch and non-Na F patch is observable on Table 1. The independent t test results indicated no significant result was showing that there was no difference in the percentage of erythrocyte lysis of blood between the one given Na F patch and the one not provided.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Percentage of lysis erythrocyte cells</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Na F Patch</td>
<td>Non-Na F Patch</td>
</tr>
<tr>
<td>1.</td>
<td>3.023%</td>
<td>2.532%</td>
</tr>
<tr>
<td>2.</td>
<td>3.398%</td>
<td>1.92%</td>
</tr>
<tr>
<td>3.</td>
<td>2.502%</td>
<td>2.98%</td>
</tr>
<tr>
<td>Mean</td>
<td>2.97%</td>
<td>2.48%</td>
</tr>
</tbody>
</table>

Macrophage cells planted in sterile tubes that have been mixed with slices of Na F patch and no- Na F patch were counted after incubation for 24 and 48 hours. Data on the viability of macrophage cells in the group given Na F and not given Na F plaster were presented in Table 2.

<table>
<thead>
<tr>
<th>Independent t test significance</th>
<th>Viable cell amount</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Na F patch</td>
</tr>
<tr>
<td>16</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>9.3</td>
<td>10</td>
</tr>
</tbody>
</table>

Macrophages were derived from monocytes present in the blood circulation which become mature and differentiated and then migrate to the tissues. Macrophages can be found in large numbers, especially in the connective layer, such as those linked to the gastrointestinal tract, in the lungs (in body fluids and alveoli). They are also found along certain blood vessels in the liver such as Kupffer cells, and to the entire spleen where the damaged blood cells are recycled out of the body.

Macrophages can migrate out of the vascular system by traversing the cell membrane from the capillary vessels and entering the area between the cells being targeted by the pathogen. Neutrophils are the most efficient phagocytes followed by macrophages and can digest significant amounts of bacteria or other cells. Binding of bacterial molecules to macrophage surface receptors triggers the process of swallowing and destruction of bacteria through “respiratory attacks,” leading to the release of reactive oxygen species (ROS). Pathogens also stimulate macrophages to produce chemokine, which calls other phagocyte cells around the infected...
region. In addition to acting as phagocytes, macrophages can also serve as antigen-presenting cells (APC). The role of macrophages as APC appears in effector function but is less significant in lymphocyte activation.

The response test of skin tissue using rabbit is due to the surface of rabbit’s skin most closely look like the human skin. The number of rabbits taken was five. After the rabbit was well adapted to the environment, the fur was shaved in left and right side surfaces with a diameter of approximately 5 cm. The response of rabbit skin surface tissue after application of Na F plaster and controlled plaster is displayed in Table 3.

Table 3: Rabbit skin surface tissue response after Na F patch and control applied

<table>
<thead>
<tr>
<th>Rabbit’s Number</th>
<th>Rabbit’s back tissue response after Na F patch applied</th>
<th>Rabbit’s back tissue response after non-Na F patch applied</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>0 (no reaction)</td>
<td>0 (no reaction)</td>
</tr>
<tr>
<td>2.</td>
<td>0 (no reaction)</td>
<td>0 (no reaction)</td>
</tr>
<tr>
<td>3.</td>
<td>0 (no reaction)</td>
<td>0 (no reaction)</td>
</tr>
<tr>
<td>4.</td>
<td>1 (mild erythema)</td>
<td>0 (no reaction)</td>
</tr>
<tr>
<td>5.</td>
<td>0 (no reaction)</td>
<td>0 (no reaction)</td>
</tr>
</tbody>
</table>

Upon experiment, the surface of rabbit skin on one rabbit had mild erythema characterized by a slightly reddish rabbit skin surface whereas other rabbit skin surfaces were under normal conditions.

The significance value of Mann Whitney test was 0.317 confirming there was no difference in surface tissue response of rabbit skin after application of Na F patch and no Na F piece.

Skin irritation are symptoms of inflammation that occurs in the skin or mucous membranes immediately after prolonged or repeated treatment with chemicals or other materials. Skin irritation caused by a substance can take place in any person resulting from several factors such as the surface state of the skin, the duration of the material in contact with the skin, and the concentration of the material used. Some common symptoms that can occur in the event of irritation are caused by dilation of blood vessels in the affected area indicated with the emergence of redness in the skin area (erythema) \(^{(12)}\). In this study, the state of irritation does not occur, due to levels of fluoride within the safe boundary that contact with the skin surface.

CONCLUSION

Upon completion the testing on rabbits revealed no difference in some blood erythrocytes, the number of viable macrophage cells and rabbit skin surface before and after plastering of Na F and no Na F patch for 14 days. These initial results indicated the feasibility of further testing on the human to use the model as a new means of transporting the fluoride in dental care.

Conflict of Interest: The authors have no conflict of interests related to the performing and reporting of this research.

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Ethical Clearance: Before conduct of the study written permission was obtained from Health Polytechnic Ministry of Health, Semarang, Indonesia.

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