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Original

A Volume Evaluation of Dental Caries in a Mouse with the Use of the Micro- CT Scan

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Abstract: The purpose of this study is to develop a three-dimensional method of caries evaluation; once a method is established genetic variables can be altered in order to detect the gene(s) involved in the development of dental caries in mice.

As dental caries has a three-dimensional nature to its course, the Micro-CT scan quantifies the volume of caries. Inbred Mice were used of known caries susceptibility –BALB/c mice. Mice were divided into the control and experimental group; later inoculated with Streptococcus mutans . The experimental group was fed a caries promoting diet and later caries volume was evaluated by use of the Micro-CT scan in both groups. There was a clear difference in tooth volume of mice infected for 7 weeks and 9 weeks, the Micro-CT scan indicated this evidently.

Key Words: Micro-CT , Dental imaging, Three-dimensional, Caries volume, Genetic contribution, Inbred mice

Introduction

This study aimed to demonstrate the effectiveness of the Micro-CT scan in carious volume evaluation. Dental caries is the most common bacterial infectious disease occurring in the oral cavity, and probably the most costly, since dental treatment demands high levels of manpower and is time consuming [1)].

It is known a sucrose diet, plaque bacteria, a host and time are all factors which influence the development of dental caries. It is useful to find a host genetic factor and a defence mechanism which influences the development of dental caries. In predicting these factors during the earliest stage of growth appropriate preventative strategies can be sought. Twin's studies have shown that genetic factors also contribute to caries susceptibility [2-4)]. The earliest and most convincing demonstration of a genetic contribution to caries came from breeding experiments in rats [5-11)]. Previous studies have shown that mice models are powerful tools in the study of the genetic contribution to dental caries [12-16)].

Dental caries has a natural three-dimensional nature to its course however when considering caries research, many have been conducted regarding the extent of caries in various conditions in a two-dimensional manner. This conventional method involves in most cases the extraction of the tooth and its destruction in order to obtain thin slice or cut sections to view under a microscope. While obtaining these thin sections of the tooth a part of the caries will be missed due to the nature of the procedure. Therefore a misjudged quantity of caries will be recorded. In this method there is a lack of sensitivity in detecting the small changes, which occur in the hard tissue.

The Micro-CT scan evaluates caries in a non-destructive manner whereby the whole tooth is applied to the Micro-CT scan. A series of cross-sectional images are generated and combined to produce an image of the tooth. A greater accuracy in carious tooth volume can be ascertained. In this study the following variables were applied,

1) A caries promoting diet : Diet 2000, 30% sucrose [17)] .

2) A susceptible host –BALB/c mouse [12)].

3) A cariogenic micro-organism – rodents inoculated with *S. mutans*[18)].

Once this method of quantification of caries is established an *index* can be formulated regarding the three dimensional nature of caries. It is important to quantitate the carious volume in order to detect the gene(s) involved in the development of dental caries in mice.

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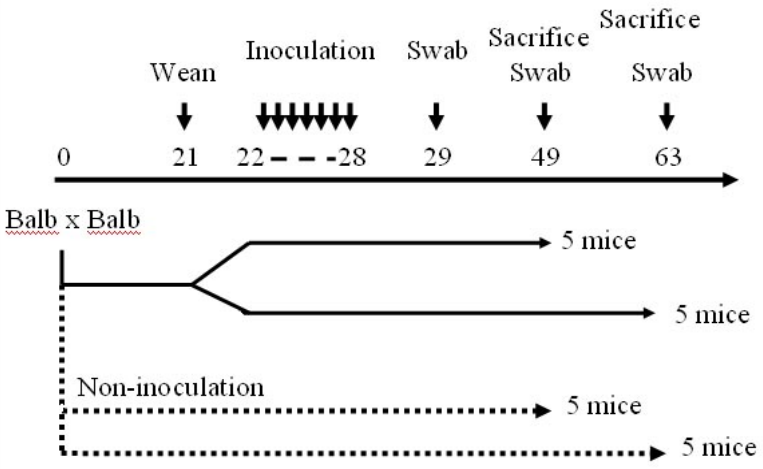
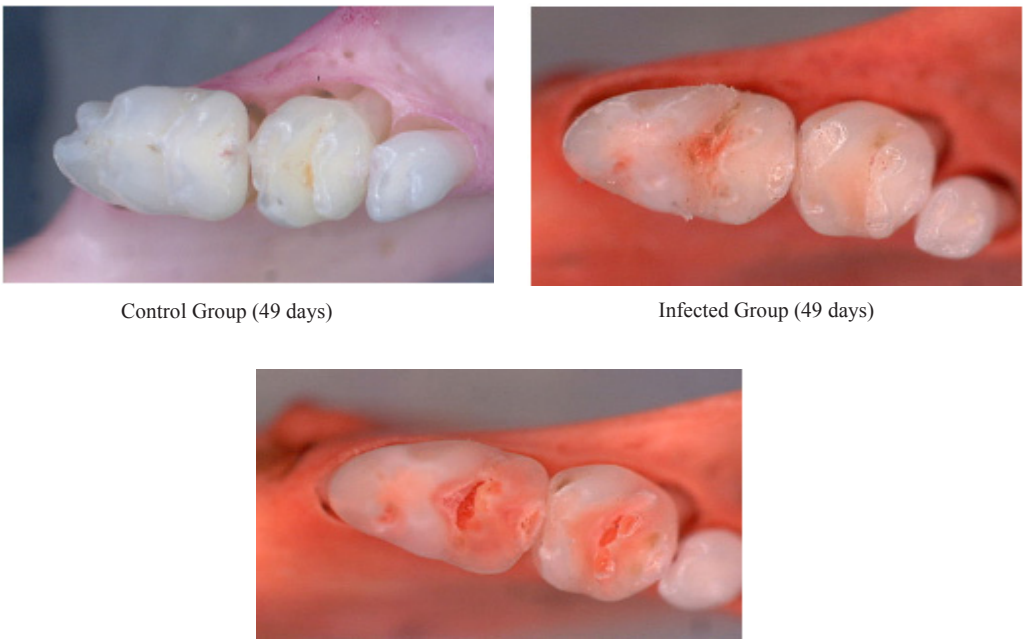


Figure 1: Inoculation schedule



Control Group (49 days)

Infected Group (49 days)

Infected Group (63 days)

Figure 2. Staining of with Arizaline for Caries detection. Notice the vastness of the caries as the length of time of exposure to caries promoting factors increases. A two dimensional caries evaluation will be inaccurate in recording all the caries; hence the Micro-Ct Scan is the most accurate.

Materials and Methods

Sample preparation

Mice were used in this study where by the parameters were controlled. A purebred male and female were obtained of species BALB/cj from the National Institute of Genetic Research and were mated to produce F1 offspring. These particular species are known for their caries susceptibility[12].

Twenty mice were used for this study. These mice were weaned for their first 21 days and subsequently divided into the control and the experimental group each consisting of 10 mice. On day 22, the control group mice began their sucrose free solid diet and the experimental group began their Diet 2000 containing 30% sucrose[19]. From the 22nd day after birth, they were infected for 7 days with *Streptococcus mutans* PS-14 strain (serotype c[26]) by inoculating 1.0 x 10^8 CFU/mouse of a bacterial solution into the oral cavity of the animal. Colonization of the bacteria on the tooth surface was confirmed at days 29, 49 and 63 by placing a sterilized swab into the mouth to collect a sample, which was inoculated onto a Mitis Salivarius agar (Difo, Detroit, Mich., USA) plate supplemented with streptomycin (0.1 mg/ml). On day 49,

Table 1 Tooth volume of non-infected group

Control Group

Sample	7 w (mm^3)	9w (mm^3)
a	0.7702	0.7334
b	0.7502	0.7545
c	0.796	0.7678
d	0.774	0.774
e	0.7824	0.7976
AVE	0.7746	0.7655
SD	0.0168	0.0238

The tooth volume of the non-infected (control) group, at 7 weeks and 9 weeks

Table 2 Tooth volume of infected group

sample	7 w (mm^3)	9 w (mm^3)
a	0.755	0.647
b	0.726	0.686
c	0.698	0.631
d	0.715	0.68
e	0.704	0.65
AVE	0.72	0.659
SD	0.022	0.023

The tooth volume of the infected group, at 7 weeks and 9 weeks.

Table 3 Caries cavity volume of infected group

sample	7 w (mm^3)	9 w (mm^3)
a	0.017	0.125
b	0.046	0.086
c	0.074	0.141
d	0.057	0.092
e	0.067	0.122
AVE	0.052	0.113
SD	0.022	0.023

The carious cavity tooth volume of the infected group at 7 weeks and 9 weeks.

Formula: CV= M ^{7} ***- TV***

M ^{7} = *mean volume of control group at 7 weeks*

TV = *volume of each tooth in the infected group*

CV = *caries volume*

five mice from each group were sacrificed using chloroform. The rest of the mice continued their diet regime and were sacrificed on day 63 in the same manner (Fig. 1).

The mandible of each mouse was dissected; all soft tissues were removed after treatment of the mandible with an autoclave. All specimens were stained with alizarin red in order to detect caries (Fig. 2). The animal use protocol in this study was reviewed and approved by the tsurumi University International Review boad.

\Calculation of the carious score with the micro-CT

Micro- CT images of each mouse jaw were taken in the control and experimented group under the following conditions; Tube

Table 4 Tooth volume (M1+M2) changes(mm^3)

slice number	0-10	0-20	0-30	0-40
7W				
a	0.0155	0.098	0.2503	0.4325
b	0.0007	0.0516	0.1772	0.3378
c	0.0085	0.075	0.2056	0.3912
d	0.0081	0.0593	0.1828	0.3619
e	0.0153	0.0918	0.2416	0.4326
AVE	0.0096	0.0751	0.2115	0.3912
SD	0.0061	0.02	0.0333	0.0422

slice number	0-10	0-20	0-30	0-40
9W				
a	0.0067	0.05	0.1573	0.3151
b	0.005	0.0466	0.162	0.3357
c	0.0062	0.0485	0.1494	0.2932
d	0.0091	0.0649	0.1877	0.3601
e	0.0119	0.0713	0.1842	0.3324
AVE	0.0078	0.0562	0.1681	0.3273
SD	0.0027	0.0111	0.0169	0.0249

Table indicating the increase in tooth volume in the infected groups at 7 weeks and 9 weeks from the cuspal tip towards the cervical region by the usage of the Micro-Ct scan. *These data were later utilised in the reconstruction of the tooth image.*

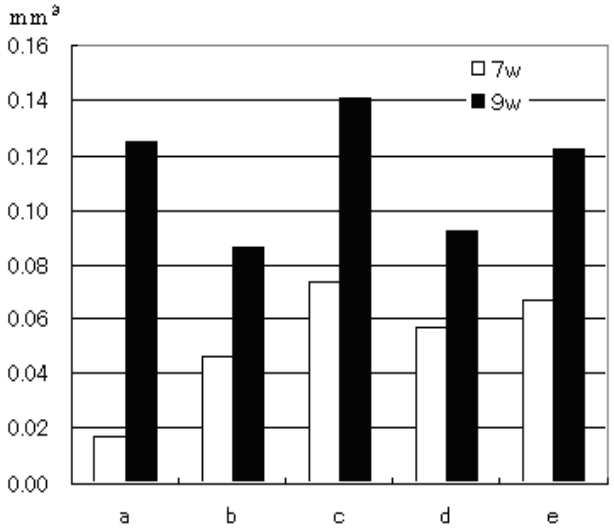


Figure 3.Graph representation of the carious tooth volume of each of the sample mice at 7 weeks and 9 weeks.

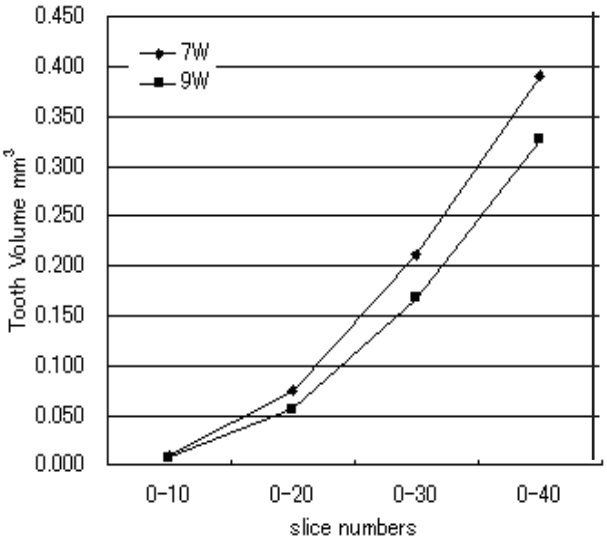


Figure 4.Graph representing the increase in tooth volume from the cuspal tip towards the cervical region. Notice the difference in volumes in those teeth infected for 7 weeks compared to those infected for 9 weeks.

voltage 50kV, Tube current 100mA, slice thickness 13micron meters, and 68 images were collected. The jaws were placed in specially designed moulds which were reproducible for each jaw. After taking the Micro-CT images, three dimensional images were reconstructed from the tip of the molar cusps in a cervical direction, 63 sections were used. A 3D software package (Tri-CT bon, Ratock , Tokyo) was used in the reconstruction and volume calculation of the tooth. The volume of caries was thereby calculated by the subtraction of the experimental carious tooth volume from the control tooth volume. Specimens were stained with alizarin red in order to detect caries (Fig. 2).

Formula

$$CV = M^7 - TV$$

M^7 = mean volume of control group at 7 weeks

TV = volume of each tooth in the infected group

CV = caries volume

The mean of the 9 week tooth volume of the control group was a slightly smaller value than the 7 week tooth volume. There was no statistical difference in tooth volume of the 7 weeks and 9 weeks control group and therefore the mean volume of the 7 week control group tooth volume was used in calculating the caries

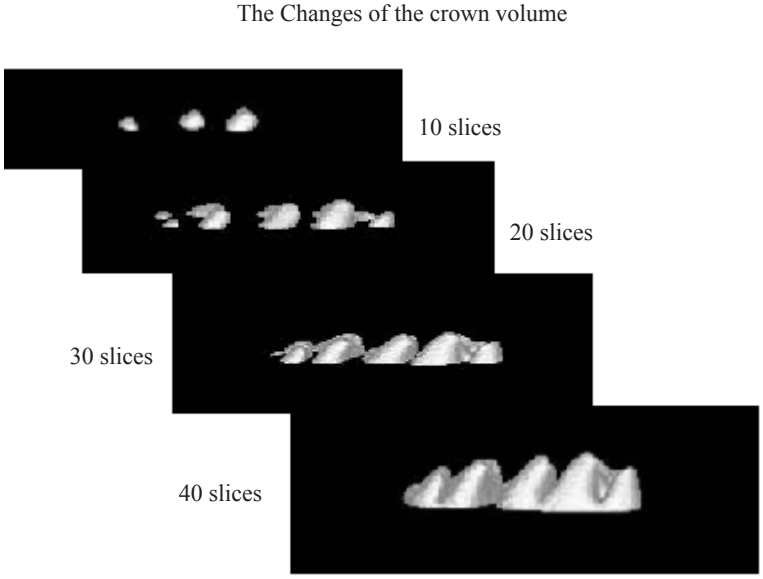


Figure 5. Diagrammatic representation of the reconstruction of the teeth from the cuspal tip towards the cervical region. These can only be recons trued with the use of the Micro-CT scan and a suitable software package. The changes in tooth volume can be observed.

tooth volume. Another reason of using the 7 week tooth volume because the tooth is morphologically more accurate than that of the 9 week tooth.

Results

The volume of each molar tooth of the control groups were recorded at 7 weeks and at 9 weeks. An average hard tissue tooth volume was calculated, the statistical "f test" was used, it was found there was no statistical difference between these results. There was no difference in hard tissue tooth volume in those teeth of 7 weeks compared with at 9 weeks (Table 1).

The greater the length of time the mice were exposed to the carious diet the lower the hard tissue volume of the teeth. The "f test' was conducted, there is a statistical difference. Those mice which were slaughtered at 7 weeks have a greater hard tissue tooth volume to those slaughtered at 9 weeks (Table 2). In each of these groups the hard tissue tooth volume are less than their corresponding control group tooth volume.

The carious tooth volume of each tooth was calculated. The hard tissue volume of those infected teeth was subtracted from the average hard tissue tooth volume of the control group at the corresponding age of mouse. The average carious volume at 7 weeks was 5.2 +/-2.0E-2 and the average carious volume at 9 weeks was 11.3 (Table 3 and Fig 3).

The hard tissue tooth volumes at different slice thickness taken from the cuspal tip towards the cervical area of the molar were calculated by use of the Micro- CT scan, for the seven-week mice and the nine-week mice of the infected groups. Each slice thickness is 13microns, 40 slices were taken in total of each tooth. The hard tissue volumes of the teeth were calculated as a cumulative aggregate after an increase of 10 slices. An average hard tissue volume for each section was calculated, these were used for the reconstruction of the molar tooth by the use of the Micro- CT scan (Table 4 and Figs. 4-5).

Those mice which were slaughtered at 7 weeks have a greater hard tissue tooth volume at corresponding slice thickness to those slaughtered at 9 weeks (Fig. 5).

Disscussion

In clinical studies the direct evidence for a genetic contribution to caries comes from familial studies of normal subjects including twin's studies [2, 20-22]. However, it is impossible to clarify which factors either genetic or environmental affect strongly in the development of dental caries. As controlled factors using human beings are unethical in the study of experimental dental caries, therefore animal models under controlled environmental conditions are essential to investigate the contribution of genetic factors in caries susceptibility[23]. Inbred mice have several advantages in dental and biomedical research compared to other species[14, 18, 14-26]. In order to determine the genetic factors influencing the development of dental caries, a caries scoring method is one of the important elements for a caries-promoting experiment. With regards to the caries scoring, many have been conducted regarding the extent of caries in various conditions in a two-dimensional manner [12,15, 18]. In this method there is a lack of sensitivity in detecting the small changes, which occur in the hard tissue. As previous studies have shown that some candidate genes to caries development are mapped on mouse chromosome[18, 26]. The next challenging frontier is to determine the multiple genes which effect regulation of caries susceptibility. All of the caries evaluations reported previously are insufficient for the genetic analysis, because there is a lack of sensitivity in detecting the small changes in the caries progress. In this study, we demonstrate the effectiveness of the Micro-CT scan in carious volume evaluation (Table 4 and Fig. 4) and established a new caries scoring method with Micro-CT (Table 3 and Fig. 3). The advantages of the Micro- CT include the rapid acquisition of a volume data set in a relatively short period of time, the reconstruction of images at any plane within the helical data set and the possibility of reducing the amount of radiation exposure to specimens when compared to radiographic techniques. Without any additional scanning time, these data can be viewed as conventional transaxial images, as multiplanar reconstruction, or as three-dimensional (3D) reconstructions. Such images can provide accurate images at any arbitrary location within the volume data set. Such images are also essential for the diagnosis of subtle abnormalities, identification of aberrant anatomy, treatment planning, treatment evaluation, and quantification of hard tissue change.

However this non-destructive method has its limitations: it was difficult to determine the boundary between the area of demineralisation and the region of healthy hard tissue in those infected teeth. In order to over come this problem a binary number was decided upon which was applied for each tooth. Based on our results it is strongly suggested that the volume evaluation of dental caries in inbred mice is conducted with the use Micro-CT scan and utilized in genetic analysis.

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