

# Development of High Sensitivity, Rapid Detection Kit for Disease-Inducing Oral Bacteria

## Cariogenic bacteria and periodontal bacteria

Oral bacteria (nonpathogenic bacteria (S. salivarius, etc.)

Cariogenic bacteria  
*Streptococcus mutans*



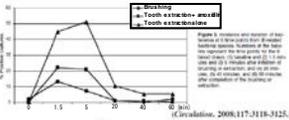
Dental caries

Periodontal bacteria  
*Porphyromonas gingivalis*, etc.



Periodontitis

## Bacteremia Associated With Toothbrushing and Dental Extraction



Brushing, tooth extraction or other procedures may allow oral bacteria to enter the bloodstream leading to transient bacteremia.

## Cardiovascular disease caused by cariogenic bacteria

### Infective endocarditis



A systemic septic disease in which vegetation including accumulation of bacteria forms in the valve, endocardium, or large vessels and which manifests various symptoms including bacteremia, vascular embolization, and cardiac disorders.

Underlying cardiac diseases  
Bacteremia-inducing procedures



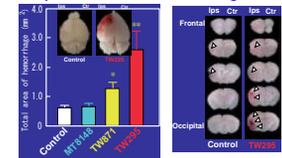
Heart failure  
Bacteremia  
Embolism

## Background

- Investigation of infective endocarditis (in 817 medical institutions)  
Among 697 isolates, major bacteria were: Gram-positive bacteria (93.1%)  
Oral streptococcus (38.6%)  
*Staphylococcus aureus* (20.8%)
- Of 166 cases of infective endocarditis, eleven were reported as having developed cerebral hemorrhage.  
Pruitt AA et al. Medicine 1978;57:329.  
Ben Ismail M et al. Br Heart J 1987;5:872
- The prevalence of highly pathogenic cariogenic bacteria has been found to be high in patients with cerebral hemorrhage by Tanaka et al. (Neurological Surgery, Seirei Hamamatsu General Hospital).

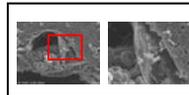
## Effect of Cariogenic Bacteria on Cerebral Hemorrhage

### Study of cerebral hemorrhage in mice



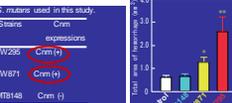
S. mutans, which may cause cerebral hemorrhage, was found.

### In vivo study using SEM



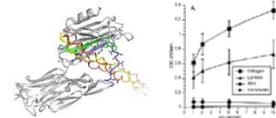
Accumulation of oral bacteria TW295 in injured vessels was also confirmed by electron microscope.

### Characteristics of highly pathogenic cariogenic bacteria



Highly pathogenic cariogenic bacteria with Cnm may aggravate cerebral hemorrhage

### Collagen-binding proteins (Cnm)



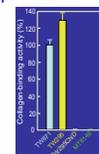
Collagen-binding protein (Cnm), 120kDa  
Collagen-binding proteins (Cnm) of S. mutans bind strongly to the collagen of extracellular matrix. The percentage of patients with Cnm-positive S. mutans in the mouth is about 10 to 20%.

### Study using confocal laser scanning microscope



Red: collagen type IV antibody  
Green: GFP-TW295

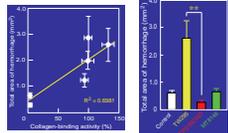
### Accumulation in injured vessels mediated by collagen-binding protein



TW295CND is a modified TW295 in which Cnm is knocked out.

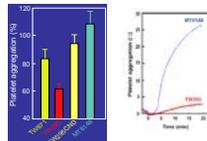
Bacteria adhered to injured vessel with exposed collagen, mediated by Cnm.

### Collagen binding ability and bleeding



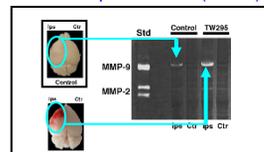
Collagen binding capacity and cerebral hemorrhage were correlated. Hemorrhage was attenuated when Cnm was knocked out.

### Effects on platelet aggregation



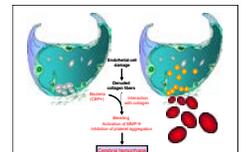
Highly pathogenic cariogenic bacteria with Cnm suppressed platelet aggregation

### Activation of matrix metalloproteinases (MMPs)

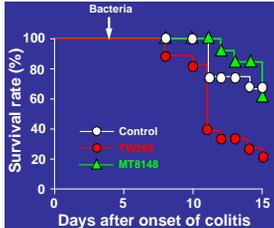


Activation of MMP-9 was observed in the TW295 group.

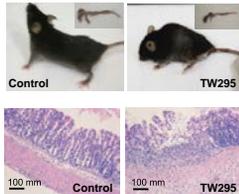
### Cerebral hemorrhage-inducing mechanism of



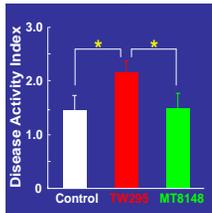
## Effects of Cariogenic Bacteria on Colitis



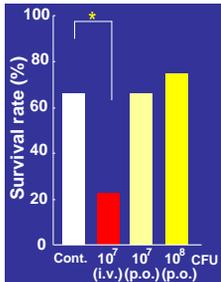
Cariogenic bacteria with Cnm (TW295) increased mortality rate in mice



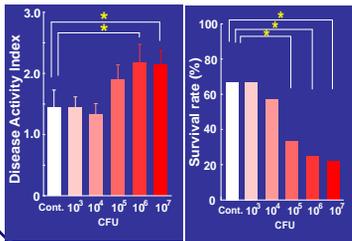
Clinical signs were worsened by TW295 and colonic mucosa was damaged



Disease activity index of inflammatory bowel disease was markedly worsened by TW295.



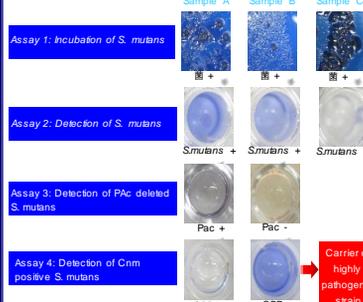
Survival rate was not changed by oral administration, and worsened only when intravenously administered.



Disease activity index of inflammatory bowel disease and survival rate were worsened in a dose-dependent manner by TW295.

## Development and Clinical Study of Detection System for Cariogenic Bacteria

Detection system for highly pathogenic cariogenic bacteria



- Conducted with the approval of the IEC of Seirei Hamamatsu General Hospital
- Conducted at Stroke Care Center, Seirei Hamamatsu General Hospital

• Inpatient treatment in the period February 16, 2010 to February 28, 2011

- Patients 16 to 98 years of age
- 511 patients
- 236 male, 211 female
- MF = 1:0.894

### Analysis result

MF	Age (mean ± SD) (max. - min.)	S. mutans detection rate	Compositive rate (positive/S. mutans)	P-value (vs. healthy subject)	
Healthy subject (N=51)	21:30	64.1 ± 8.8 (36-76)	30/51 (58.8%)	4/30 (13.3%)	-
Cerebral hemorrhage (N=59)	35:24	68.0 ± 12.8 (30-97)	31/59 (52.5%)	13/31 (41.9%)	0.0212
Stroke, cardio-cerebral embolism (N=49)	30:19	76.0 ± 10.0 (55-98)	22/49 (44.9%)	11/22 (50.0%)	0.0057
Stroke, other (N=161)	92:69	72.1 ± 9.8 (46-96)	93/161 (57.8%)	29/93 (31.2%)	NS

Fisher's exact test

It was found that highly pathogenic cariogenic bacteria are likely to be involved not only in oral diseases but also in systemic diseases. Detection of such highly pathogenic oral bacteria is important for risk assessment, prevention, and treatment of systemic diseases. We are aiming at developing a detection kit which will allow for detection of highly pathogenic bacteria rapidly and with high sensitivity. We seek for companies interested in risk assessment, prevention, and treatment of systemic diseases based on oral bacteria.