Appendix 4.

Triclosan Efficacy

# IRGACARE® MP (triclosan) in toothpaste

Ciba

# IRGACARE® MP in toothpaste

*Substantivity to dental enamel*

- ✓ Irgacare® MP shows good substantivity to hydroxyapatite which is widely used as a dental enamel model

*Inhibition and killing of oral bacteria*

- ✓ Irgacare® MP inhibits the growth of oral bacteria that are typical early colonizers

*Reduction of dental plaque*

- ✓ Irgacare® MP reduces a set of dental plaque bacteria in a biofilm model

# IRGACARE® MP in toothpaste - Introduction -

*The formation of **dental plaque** :*

**Pellicle formation**
by saliva compounds (mucin, proteins, sugar)

**Early colonizers,** mostly Gram + strains:
*Actinomyces species, Streptococci*

**Late colonizers,** many Gram– species,
playing a central role in periodontal disease:
*Fusobacterium, Porphyromonas, Prevotella,*
*Actinobacillus species* etc.

# IRGACARE® MP in toothpaste - Agar diffusion method -

## Description of the method:

Hydroxyapatite (HAP) discs with a diameter of 1.0cm are stirred for 4hrs in undiluted artificial salive and then air dried

Afterwards the discs are stirred in a 10% toothpaste solution for 30 minutes , rinsed for 15 seconds in sterile water and dried at room temperature

Discs are placed on nutrient agar plates inoculated with oral bacteria species to demonstrate growth inhibition after an incubation period of 48 hrs.

After incubation the inhibition zones are measured and the growth under the disc evaluated

# IRGACARE® MP in toothpaste - substantivity/growth inhibiton -

✓ **Growth inhibition** of oral bacteria:

Inhibition zones of *A. viscosus* and *S. mutans* by IRGACARE®MP dentifrice on HAP discs:

| Product | S. mutans [mm] | A. viscosus [mm] |
|---|---|---|
| Control (placebo) | 0 | 0 |
| Placebo + 0,1% Irgacare MP | 0 | 1 |
| Placebo + 0,3% Irgacare MP | 1-2 | 3 |

# IRGACARE® MP in toothpaste - Bactericidal activity -

## Description of the method:

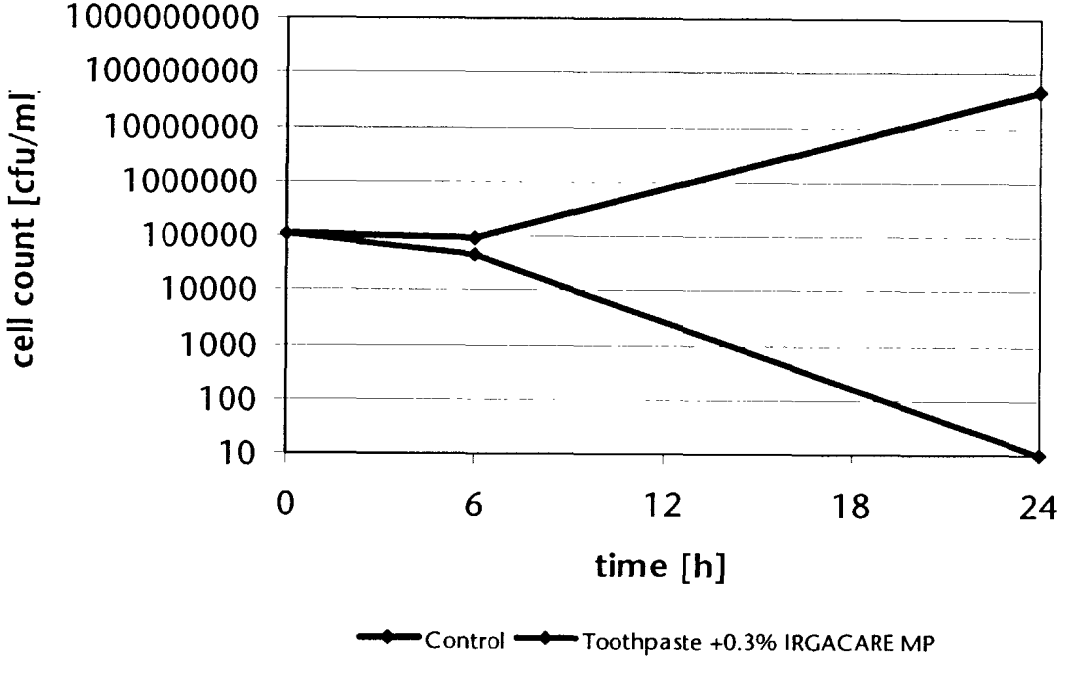
The HAP discs were incubated in artificial saliva for 4 hrs (under stirring), 1x rinsed in 10 mL NaCl, dried over night, and finally incubated in 10 ml of a toothpaste solution containing 500ppm Irgacare MP for30 min.

Afterwards, all treated discs were put in 12 well Nunclon surface titre plates (one disc per well), and 4 ml Caso Broth inoculated with A. viscosus ATCC 43146 (ca.10E5/ml) were added.

The titre plates were incubated at 37°C, samples were taken after 6 and 24 hrs and the colony count was determined.

# IRGACARE® MP in toothpaste - bactericidal activity -

## Bactericidal activity of IRGACARE MP on HAP disc



# IRGACARE® MP in toothpaste - biofilm model -

## *Biofilm model*

- **Description of the method:**

  *Biofilm formation:*

  Hydroxyapatite (HAP) discs as a model for dental substance are pretreated with collected human saliva (*pellicle formation*)

  a biofilm is formed on the discs with a mixed culture of different oral bacteria containing early and late colonizers *(Streptococci, Actinomyces sp., Veillonella & Fusobacterium sp. & al.)*

  *Treatment:*

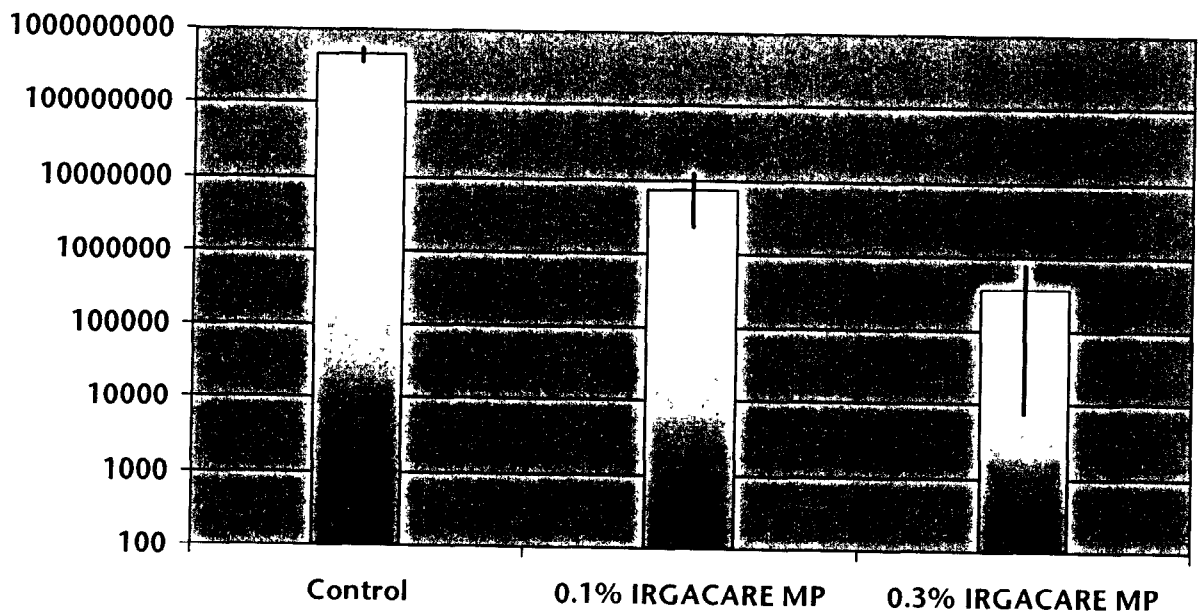
  Discs are dipped into 30% dentifrice suspension for 60 sec. 6 times in 2 days

  Biofilms are harvested and cell count determined is determined

# IRGACARE® MP in toothpaste - biofilm model -

## *Biofilm model*

- **Results:**
  reduction of dental plaque bacteria in a biofilm model on HAP discs (N=2)



# IRGACARE® MP in toothpaste - conclusion -

- **IRGACARE® MP in toothpaste**

  - ✓ shows substantivity to hydroxyapatite as a dental enamel model
  - ✓ shows significant growth inhibition of oral bacteria at 0,3%
  - ✓ significantly reduces the number of plaque bacteria in a biofilm model when used in a concentration of 0,3% Irgacare