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# Biofilm growth with starch treatment V.1

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Protocol status: In development

We are still developing and optimizing this protocol

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#### **Abstract**

Protocol to grow mature, mineralised oral biofilm with starches.

Modified from protocol by Sissons et al.:

Sissons, C. H., Cutress, T. W., Hoffman, M. P., & Wakefield, J. S. J. (1991). A Multi-station Dental Plaque Microcosm (Artificial Mouth) for the Study of Plaque Growth, Metabolism, pH, and Mineralization: Journal of Dental Research. https://doi.org/10.1177/00220345910700110301

### Guidelines

The preferred substratum for inoculation is glass or hydroxyapatite. Plastic substrata can be used but are less effective, so surface treatment of the plastic is recommeded (e.g. heated HCl or acetone).

Substrata should be autoclaved prior to use, both before and during the experiment.



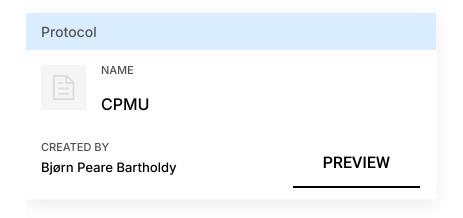
### **Materials**

#### **Solutions**

Artificial saliva



### **CPMU**



20% (v/v) sterile glycerol in dH<sub>2</sub>O

5% (w/v) sucrose in  $dH_2O$ 

0.25% (w/v) potato starch in dH<sub>2</sub>O

0.25% (w/v) wheat starch in  $dH_2O$ 

0.50% (w/v) equal parts wheat (0.25%) + potato (0.25%) in dH<sub>2</sub>O

### **Equipment**

24 deepwell polypropylene plates (w. lid containing pegs suspended from the lid) Shaking incubator



### **Protocol materials**

<b>8</b> S	Sucrose
<b>8</b> S	Sucrose
<b>8</b> S	Sucrose

#### Before start

#### Saliva donor criteria

- Must have no/limited history of dental caries
- Must not have used antibiotics in the past 6 months
- Abstain from oral hygiene 24 hours prior to donation
- Refrain from eating and drinking (except water) 2 hours before donation

For experiments involving starches, donors should avoid eating starch-containing foods on the day of donation. To make this more bearable, saliva donation should take place in the morning before breakfast.



### Saliva collection

- 1 Saliva donors rinse their mouth with water for 30 seconds.
- 2 Stimulate saliva production by chewing tasteless gum or parafilm.
- 3 Collect the saliva by spitting into 50 ml plastic centrifuge tubes.

Note

Make sure donors wear gloves to avoid contamination with non-oral bacteria.

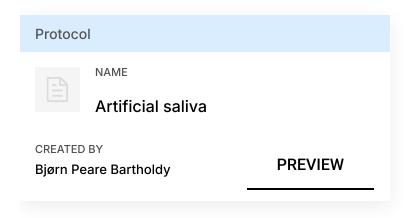
4 Make a 2-fold dilution of saliva in **sterile** [M] 20 % (V/V) glycerol and vortex the solution.

## Day 0: Inoculation and feeding

- 5 Before inoculation, vortex the saliva solution again.
- Pipette the saliva solution into the wells, so approx. 1-2 cm of the substratum is submerged.
- Place the plate in the incubator at 36°C for 4 hours for static inoculation 04:00:00
- After inoculation, transfer the samples to a new plate containing the artificial saliva, and place in a shaking incubator at \$\mathcal{C}\$ 30 rpm, 36°C for \$\mathcal{C}\$ 04:00:00

4h





- 8.1 Add 4 300 mL distilled (or deionized) dH<sub>2</sub>O to a 4 1000 mL beaker, with stirring and heat 🖁 60 °C .
- 8.2 Add:
  - <u>□</u> 2.5 g Mucin from porcine stomach (Type III) Becton Dickinson (BD) Catalog #M1778
  - Trypticase™ Peptone Becton Dickinson (BD) Catalog #211921
  - 🚣 10 g
  - X Oxoid™ Proteose Peptone Becton Dickinson (BD) Catalog #LP0085B

Let the reagents completely dissolve before continuing to the next step

- 8.3 Add:
  - 2.5 g 

    KCl Becton Dickinson (BD)
  - □ 0.35 g 

    NaCl Becton Dickinson (BD)

  - <u></u> ∆ 0.74 g
    - Sodium phosphate dibasic **Becton Dickinson (BD) Catalog #**7558-79-4



- 8.4 Add the remaining 4 700 mL distilled (or deionized) dH<sub>2</sub>O
- 8.5 Adjust to Opt 7 with NaOH Becton Dickinson (BD) and stirring
- 8.6 Transfer to two 1000 ml bottles, so half of each bottle is filled.

Autoclave at \$\mathbb{L}\$ 121 °C , \$\overline{Q}\$ 1 Bar for \$\overline{Q}\$ 00:15:00 minutes

### Safety information

Do NOT screw bottle caps on tightly.

Loosely screw the caps on the bottles or cover the tops with foil

- 8.7 Once the solution has cooled, add:
  - Menadione Becton Dickinson (BD) ∆ 1 mg
  - W Urea Becton Dickinson (BD)
- 8.8 Store in fridge at ca. 🖁 4 °C

Occasionally test the pH to ensure it stays around of 7

9 Transfer the samples to a plate containing a 5% (m/v) Sucrose solution for 00:06:00 , then transfer back to the artificial saliva and leave Overnight .

## Day 1-2: Feeding

8h 12m

6m

15m

	10	First thing in the morning, transfer the samples to a new plate containing a 5% (m/v) Sucrose solution for 00:06:00. While in the sucrose solution, add more artificial saliva to the wells on the original plate that have been partially depleted overnight.	6m
	11	After the 6 mins. return the samples to the plate with <b>artificial saliva</b> , and cover up the sucrose plate and leave for 08:00:00 .	8h
	12	After 8 hours, transfer the samples back to the plate with 5% (m/v)  Sucrose Contributed by users solution for 00:06:00 . Transfer back to the artificial saliva and leave Overnight . Dispose of the sucrose.	6m
	Day	3: Inoculation and feeding	8h
	13	Repeat steps from saliva collection and Day 0: Inoculation and feeding.  Expected result	8h
		A layer of clear plaque should be visible on the substrata	
	14	Prepare a new plate with artificial saliva. Transfer the samples from the inoculation plate to the <b>artificial saliva</b> .	
Day 4: Feeding		8h	
1	15	Repeat steps 10 through 12  3 go to step #10	8h
		Note	

Prepare a new plate of artificial saliva every third day throughout the experiment. Every other morning, top up the wells with artificial saliva (so ca. 1-2 mm of the substratum is submerged).



## Day 5: Inoculation

16 Repeat steps 1 through 9

**5** go to step #1

## Day 6-8: Feeding

17 Repeat steps 10 through 12

**5** go to step #10

## Day 9-14: Starch treatment

8h 6m

18 Transfer the samples to a plate with the starch treatment(s) for 00:06:00 at 

6m

### Safety information

If you are using multiple starch treatments within a single plate, take care to avoid crosscontamination.

19 Transfer the samples back to the artificial saliva plate for 6008:00:00 at 

8h

20 Transfer the samples to a plate with the starch treatment(s) for 600006:00 at 

6m

Transfer the samples back to the artificial saliva plate at 30 rpm, 36°C and leave

Overnight

21

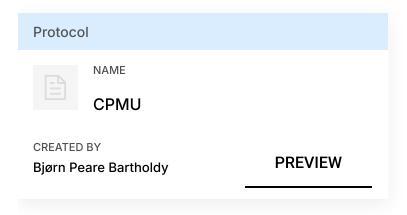
# Day 15-24: Mineralisation

8h 30m

22 Transfer the samples to a plate containing the calcium phosphate monofluorophosphate urea (CPMU) solution for 🚫 00:06:00 at 🗘 60 rpm, 36°C

6m





- 22.1 Add  $\perp$  300 mL distilled (or deionized) dH<sub>2</sub>O to a  $\perp$  1000 mL beaker, with stirring and heat 🖁 60 °C
- 22.2 Add:
  - 🚨 1.55 g X Calcium Chloride Sigma Aldrich
  - ☐ 1.44 g Sodium Phosphate monobasic Sigma Aldrich
  - ☐ 0.72 g Sodium Fluorophosphate Sigma Aldrich Catalog #344443
  - 🚨 0.08 g Magnesium Chloride Sigma Aldrich Catalog #AC223210010
  - 30 g 

    War Urea Sigma Aldrich
- 22.3 Add the remaining 4 700 mL and keep stirring until precipitate has completely dissolved Store in fridge at 4 °C
- 23 Transfer the samples back to the **artificial saliva** for 02:00:00 at 30 rpm, 36°C

Put a lid on the plate with CPMU (or cover with foil) to prevent evaporation.

Repeat step 22 and 23, four more times every two hours.

24 Transfer the samples to a plate with the **starch treatment(s)** for (6) 00:06:00 at 

2h

6m



25 Transfer the samples back to the **artificial saliva plate** at \$\circ{1}{30}\$ rpm, 36°C and leave

Overnight

# **Analysis**

26 Samples should be dried before sampling.

Transfer to a new plate with no liquid and leave in the incubator.

27 Once dried, samples are processed like archaeological samples.