

Supplementary Materials

Supplementary Material S1: Sample Size Calculation for Animal Study

1. Objective

To determine the minimum number of animals per group required to detect statistically significant differences in caries lesion depth (μm) among three groups (Control, 3M-FRC, 10QM-Resin) in a rat secondary caries model.

2. Pilot Data

Pilot experiment was performed with 2 animals per group. The observed mean (M) and standard deviation (SD) for each group are shown in Table S1.

Table S1. Pilot data summary (caries lesion depth, μm)

Group	Animal 1	Animal 2	Mean (M)	SD (s)
Control	171.03	214.7	192.9	30.9
3M-FRC	126.86	171.13	149.0	31.3
10QM-Resin	81.96	51.48	66.7	21.6

3. Assumptions for Sample Size Calculation

- One-way ANOVA (three groups)
- Significance level $\alpha = 0.05$ (two-tailed)
- Desired power $1 - \beta = 0.95$
- Equal sample size per group
- Common within-group variance σ^2 estimated from pilot data

4. Pooled Variance Estimation

$$\begin{aligned}\sigma^2 &= \frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2 + (n_3 - 1)s_3^2}{(n_1 + n_2 + n_3) - k} \\ &= \frac{(2 - 1) \times 30.9^2 + (2 - 1) \times 31.3^2 + (2 - 1) \times 21.6^2}{(2 + 2 + 2) - 3} \\ &= \frac{954.81 + 979.69 + 466.56}{3} = \frac{2401.06}{3} \approx 800.35 (\sigma \approx 28.3)\end{aligned}$$

5. Sample Size Calculation Using PASS 15.0

We used **PASS 15.0** (Power Analysis and Sample Size, NCSS, LLC, Kaysville, UT, USA) with the **One-Way ANOVA** procedure. The following parameters were entered:

- Number of groups: 3

- Alpha (α): 0.05
- Power: 0.95
- Group means: 192.9, 149.0, 66.7
- Common standard deviation: 28.3 (from pooled estimate)

The calculated result was **4 animals per group** (total 12 animals). The PASS output (screenshot not shown) confirms that a sample size of 4 per group achieves a power > 0.95.

6. Manual Verification Using Non-centrality Parameter Approach

For one-way ANOVA, the non-centrality parameter λ is:

$$\lambda = n \cdot \frac{\sum_{i=1}^k (\mu_i - \bar{\mu})^2}{\sigma^2}$$

where $\bar{\mu} = (192.9 + 149.0 + 66.7)/3 = 136.2$.

$$\sum (\mu_i - \bar{\mu})^2 = (192.9 - 136.2)^2 + (149.0 - 136.2)^2 + (66.7 - 136.2)^2 \approx 8209.0$$

Thus,

$$\frac{\sum (\mu_i - \bar{\mu})^2}{\sigma^2} \approx \frac{8209.0}{800.35} \approx 10.26$$

Solving iteratively for n:

- $n = 3$ gives $\lambda \approx 30.78$, power ≈ 0.93 (below target)
- $n = 4$ gives $\lambda \approx 41.04$, power > 0.99 (exceeds target)

Therefore, the minimum required sample size is **n = 4 per group**, consistent with the PASS result.

7. Final Sample Size

Because the pilot data came from only 2 animals per group (which may underestimate true population variance) and to account for potential animal dropout, we conservatively increased the sample size to **6 animals per group**. This final sample size (n=6 per group, total 18 rats) also aligns with previous rat secondary caries model studies [1].

References

[1] Chen H, Xu M, Zhang B, Yu S, Weir MD, Melo MAS, Masri RM, Tang Y, Xu HHK, Yang D. Novel strategy of *S. mutans* gcrR gene over-expression plus antibacterial dimethylaminohexadecyl methacrylate suppresses biofilm acids and reduces dental caries in rats. Dent Mater. 2024 Oct;40(10):e41-e51.