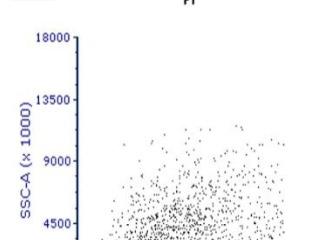
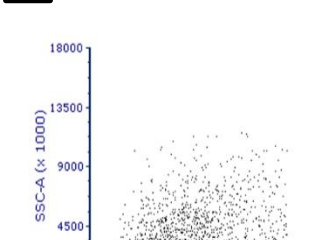
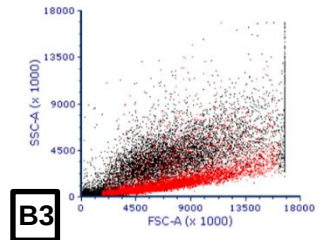
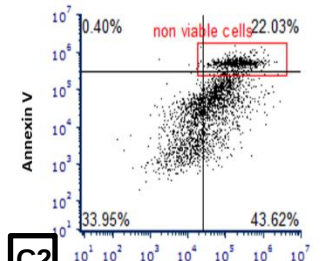
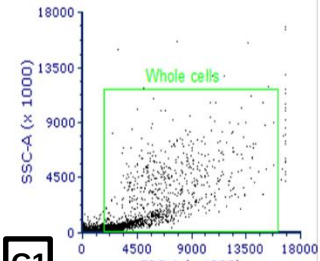
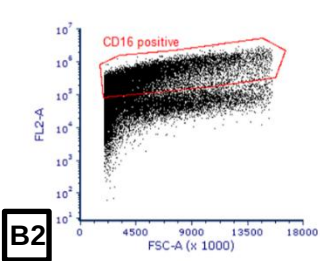
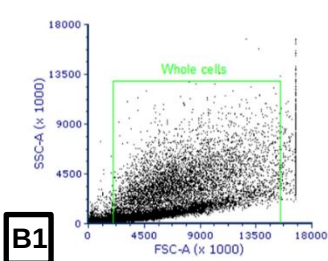
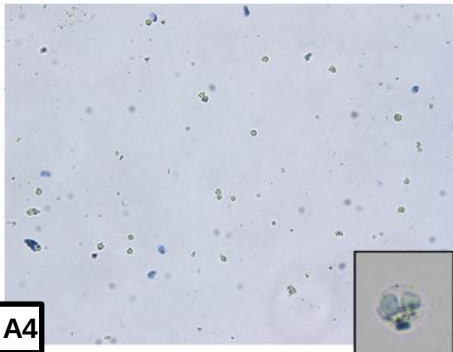
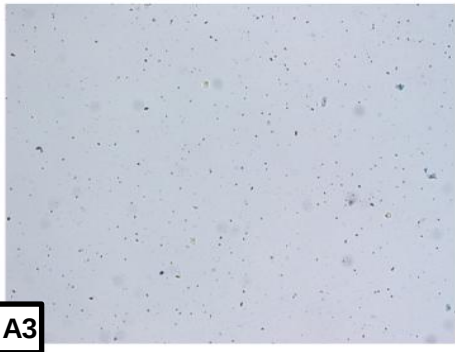
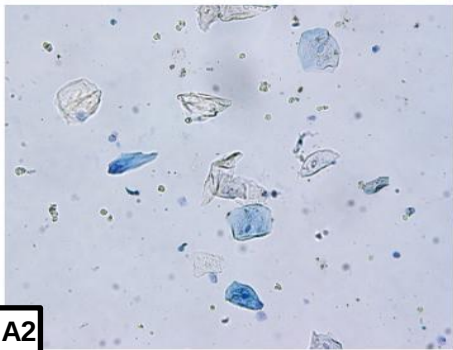
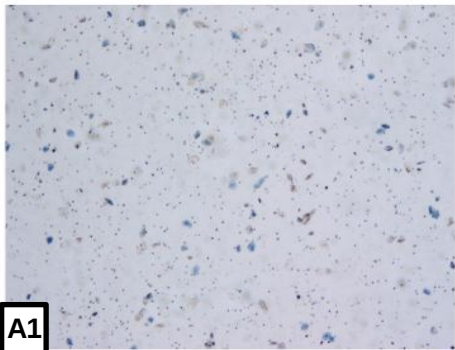


Supplementary Figure 1 - Workflow for oral neutrophil isolation and purity validation.



Legend for supplementary figure 1:

Supplementary Figure 1. Oral neutrophil isolation and characterization.

Participants rinsed with 2×15 mL of Hanks' Balanced Salt Solution (HBSS, containing calcium and magnesium) for 30 seconds each. The pooled suspensions were sequentially filtered through 40- μ m and 10- μ m nylon meshes to remove debris and larger cells. The filtrate was centrifuged at 1250 rpm for 10 minutes at 10 °C, and the resulting pellet was resuspended in HBSS. Cell viability was assessed by Annexin V/propidium iodide (PI) staining, and neutrophil purity was confirmed by CD16 staining and flow cytometry (>95% neutrophils).

(A1–A4) Microscopic images of oral cells. A1 and A2: 10 $\times$ and 20 $\times$ magnification of cells after filtration through a 40- μ m mesh, showing oral neutrophils (small, round cells) alongside epithelial cells (large, amorphous). A3 and A4: 10 $\times$ and 20 $\times$ magnification of cells after sequential 40- μ m and 10- μ m filtration, showing predominantly oral neutrophils (inset) with only rare epithelial cells.

(B1–B3) Flow cytometry gating strategy for neutrophil purity. B1: Forward scatter (FSC) vs. side scatter (SSC) of unstained oral neutrophils after 10- μ m filtration, with gating of intact cells (green), excluding debris and aggregates. B2: FSC vs. CD16 fluorescence (FL2) showing ~60% CD16⁺ cells after applying the intact-cell gate. B3: CD16⁺ cells (red) overlaid on FSC vs. SSC plot, confirming neutrophil population.

(C1–C4) Cell viability by Annexin V/PI staining. C1: FSC vs. SSC of unstained oral neutrophils with intact-cell gating (green). C2: Scatter plot of Annexin V vs. PI staining showing ~22% Annexin V⁺/PI⁺ (non-viable) cells. C3: FSC vs. SSC of Annexin V⁻/PI⁻ viable neutrophils. C4: FSC vs. SSC of Annexin V⁺/PI⁺ non-viable neutrophils.