Guide to scoring of *in vivo* biofilm from SEM images
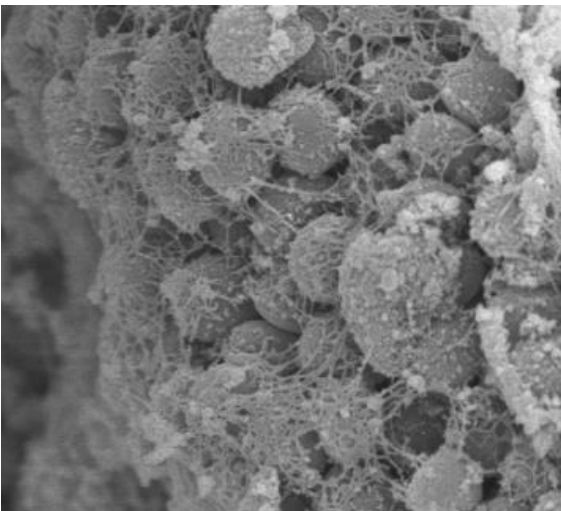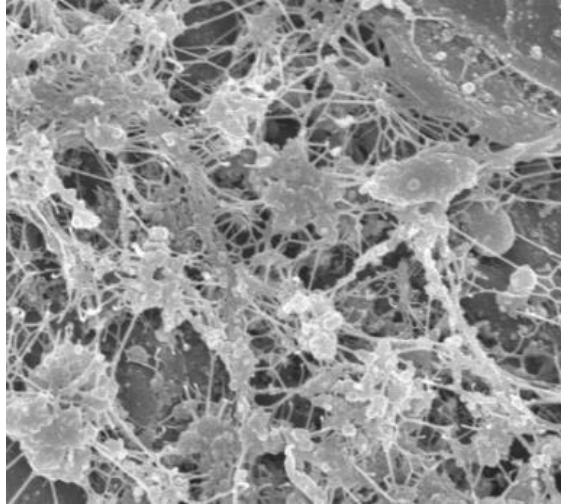
Mayo Clinic Infectious Diseases Research Laboratory

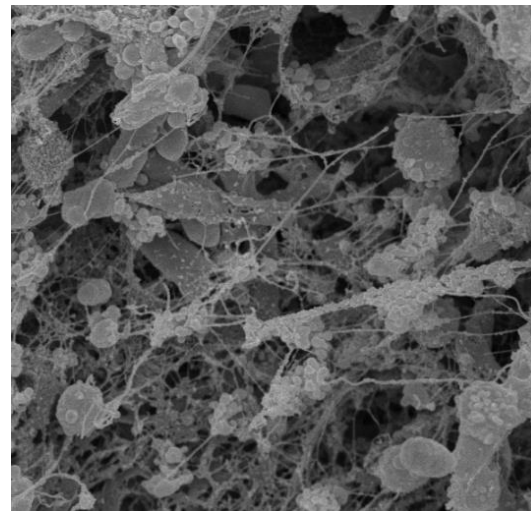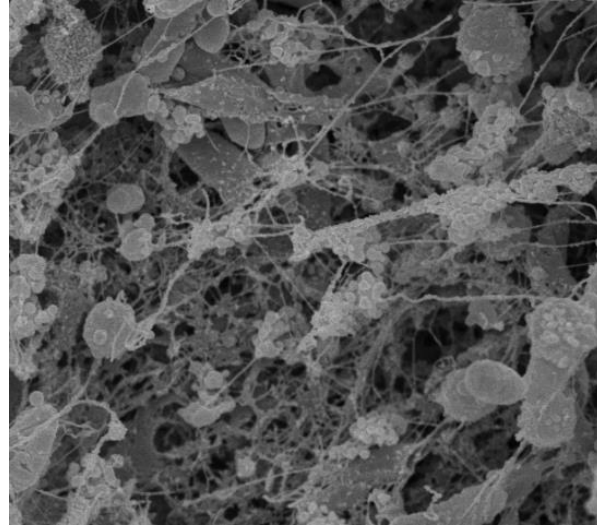# *In vivo* biofilms are scored based on the following criteria:

1. Extracellular polymeric substance (EPS) integrity/abundance
2. Integrity of microbial cells
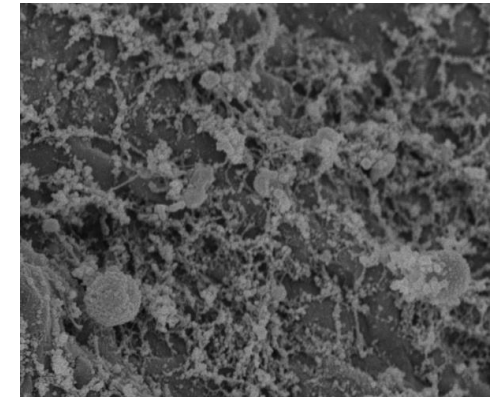3. Microbial abundance

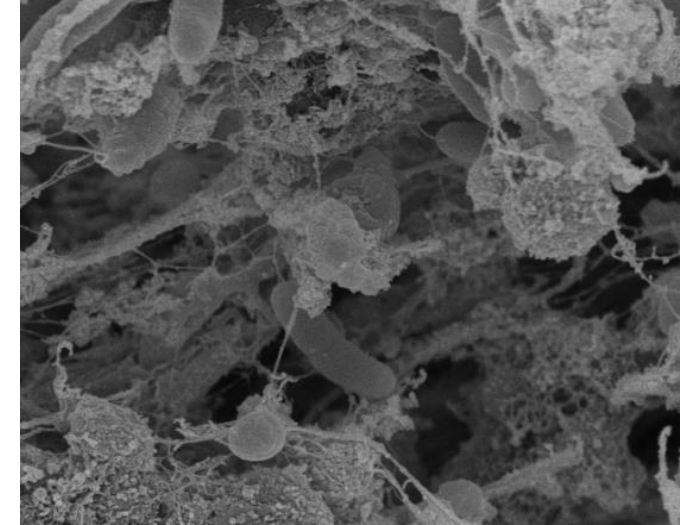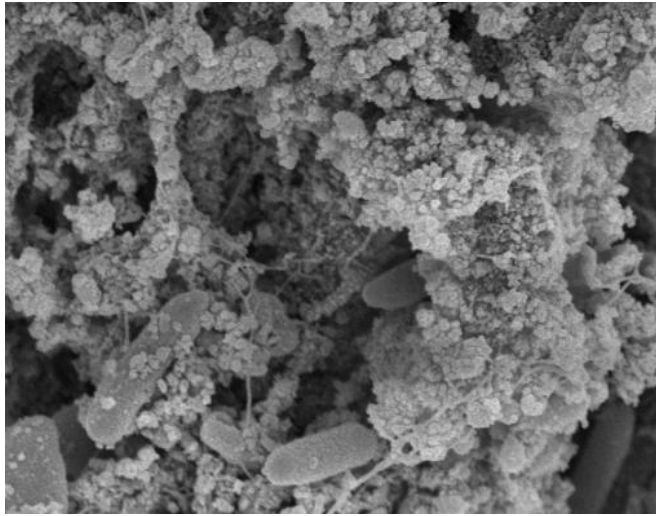# EPS integrity/abundance examples: Stringy EPS
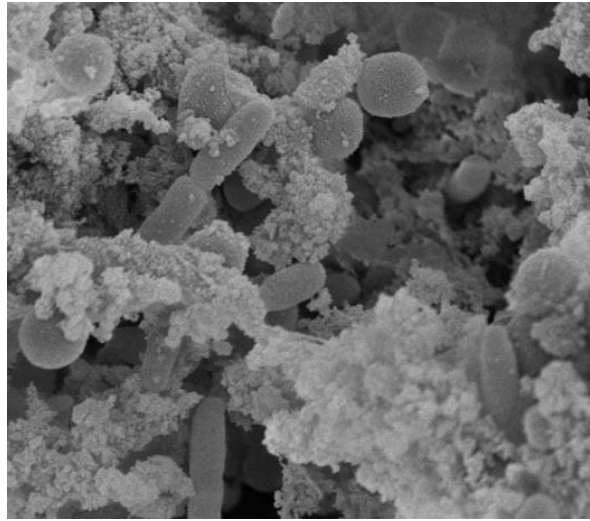
High: 3

Medium: 2

Low: 1

EPS integrity/abundance examples: Granulated EPS

High: 3

Medium: 2

Low: 1
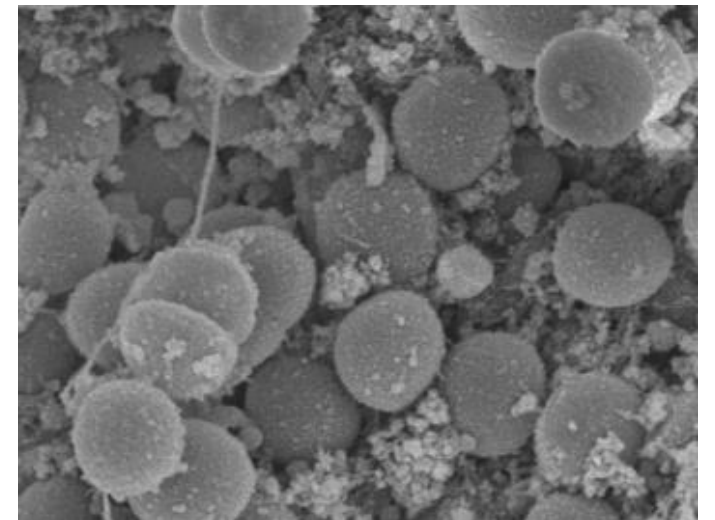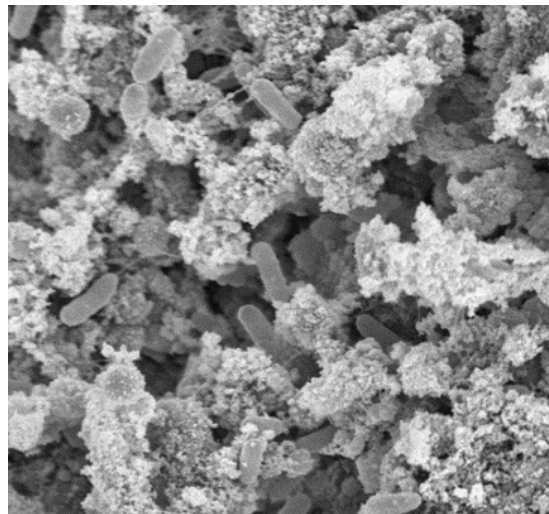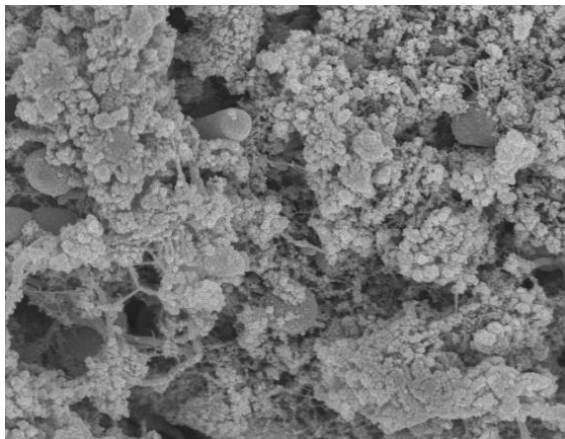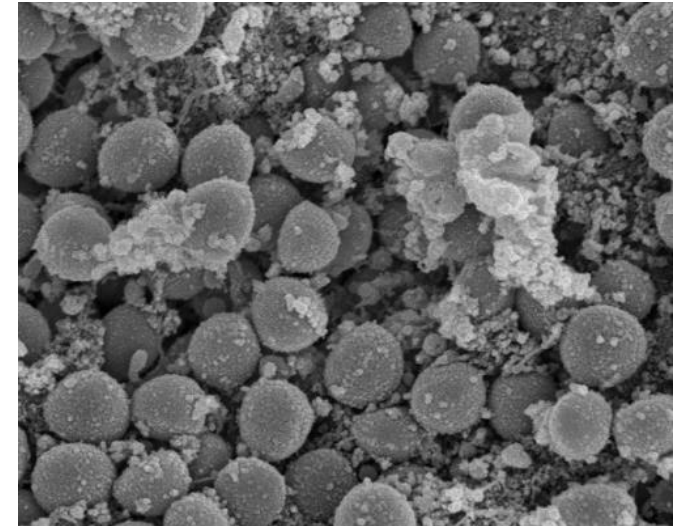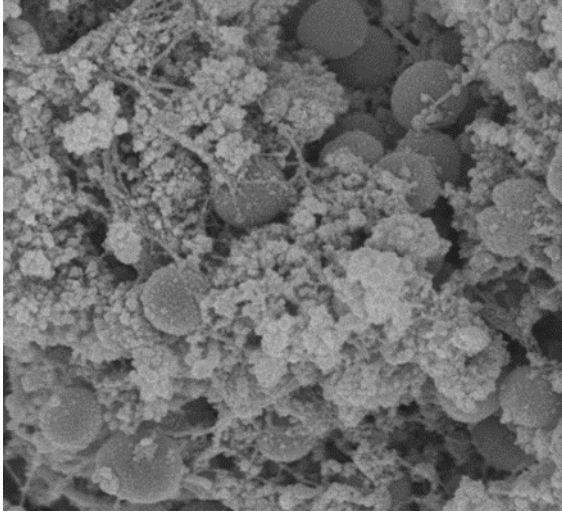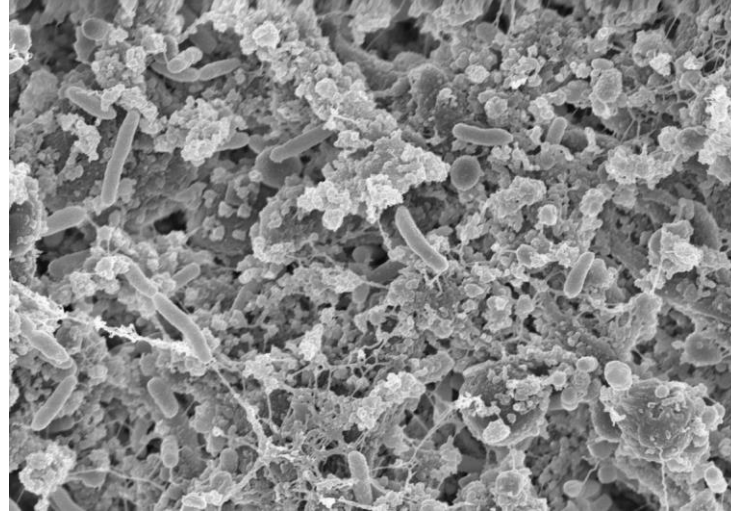
EPS integrity/abundance examples: Combination EPS

High: 3

Medium: 2

Low: 1
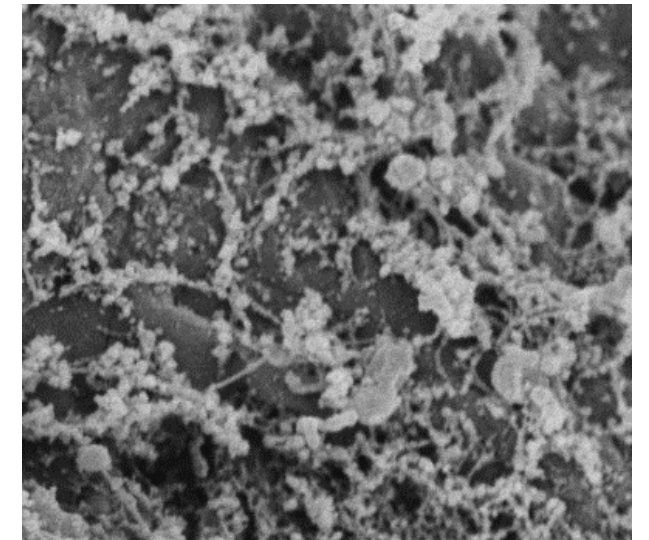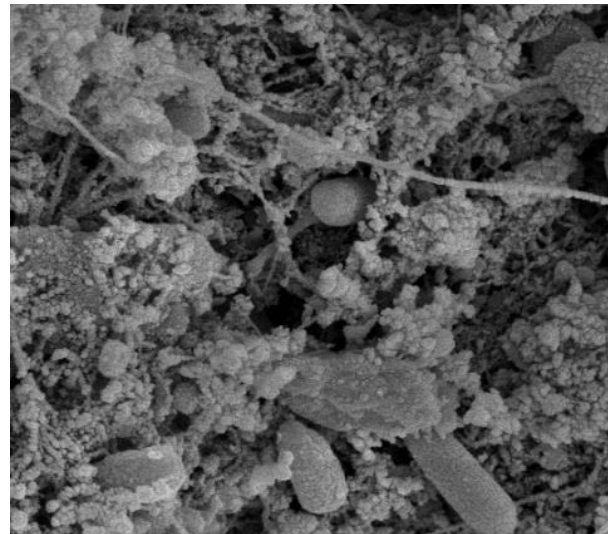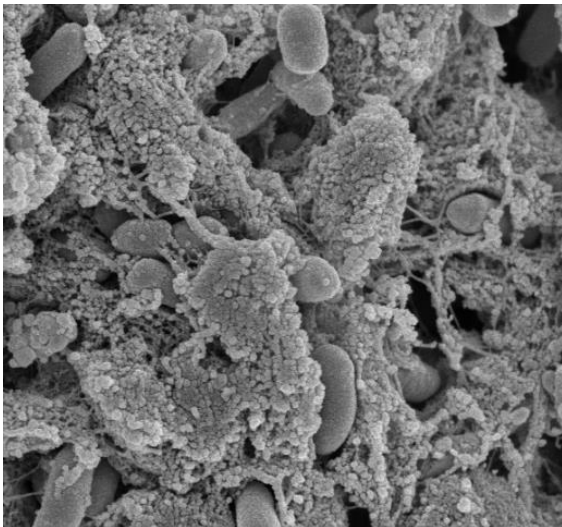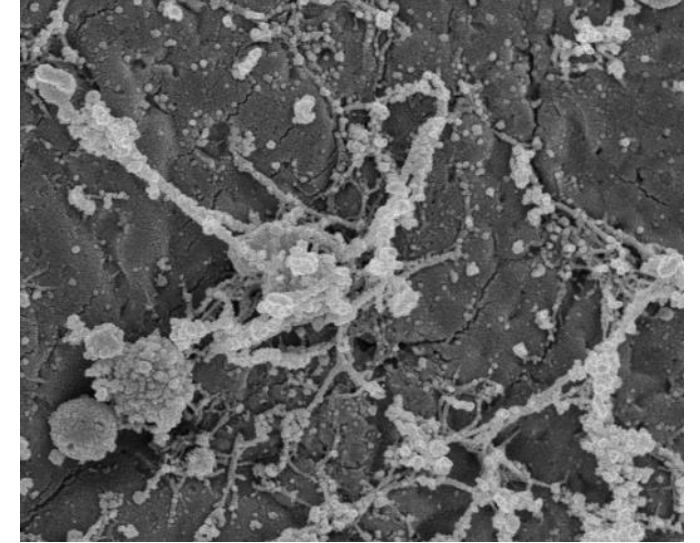
Microbial cell integrity examples
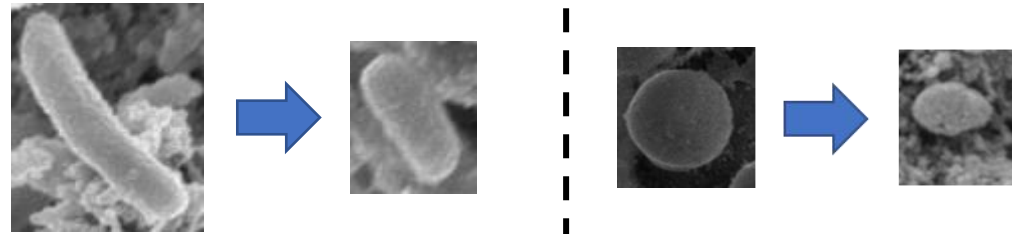
• Shortening • Compacting			**NOT Shortening/Compacting** Appearance of shrinking due to depth of field of view (in comparison to larger-looking cells closer to the surface
• Dimpling			**NOT Dimpling/Blistering**
• Blistering			EPS Deposition: Bright, speckled, not a part of the membrane
• Active Lysis			Active lysis is evident when the bacterial membrane breaks down (fractures, holes, fissures)
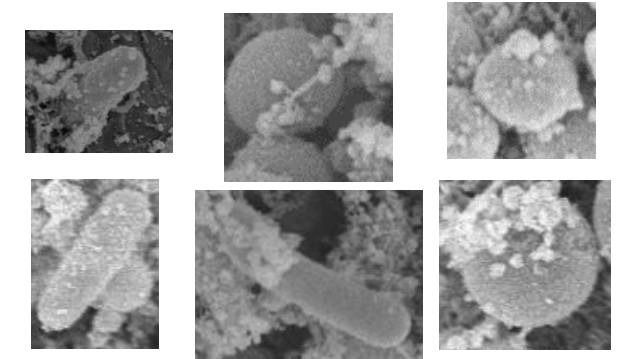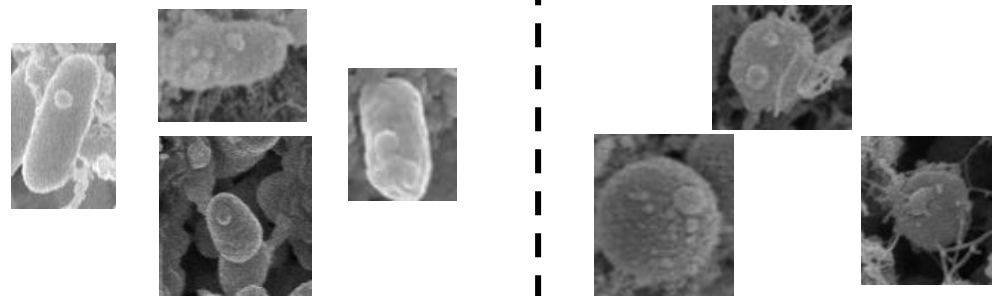
Microbial cell integrity scoring

- Compacting, dimpling, blistering, lysis evident in:
  - **High (3)**: ≥25% of cells
  - **Medium (2)**: 10-25% of cells
  - **Low: (1)**: ≤10% of cells

Microbial abundance (EPS abundance must be scored first)

- **High (3)**: ≥75 cells/field of view (10,000X mag) in *low-density EPS* <u>**or**</u> cells <u>visibly prevalent throughout</u> *high-density EPS*

- **Medium (2)**: 25-75 cells/field of view (10,000X mag) in *low density EPS* <u>**or**</u> cells prevalently associated with *medium-density EPS* <u>**or**</u> cells sparsely associated with *high-density EPS*

- **Low (1)**: ≤25 cells/field of view (10,000X mag) in *low-density EPS* <u>**or**</u> cells sparsely associated with *medium- or high-density EPS*

Example scoring sheet (General impressions from 3-4 fields of view per sample)
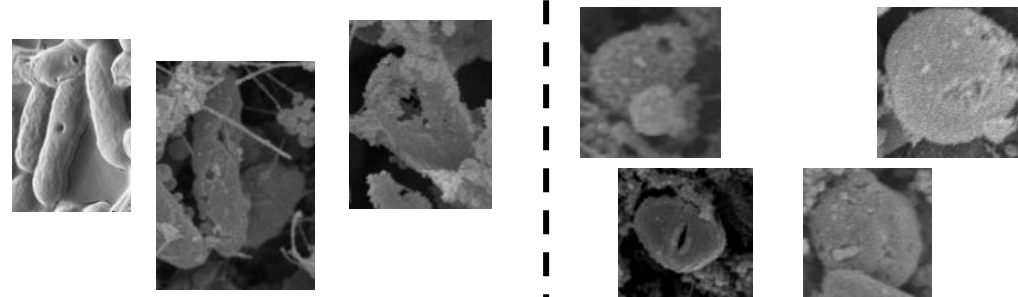
Sample	EPS integrity/abundance	Microbial cell integrity	Microbial abundance
S1	3	3	3
S2	2	3	2
S3	1	2	1
S4	2	1	3
S5	1	1	2
S6	2	1	3
S7	2	2	1