COLLAGEN CONJUGATION PROTOCOL
(most done in tissue culture hood)

Make 0.1N acetic acid in H_2O\textsuperscript{dd} in the fume hood → 285.7µl for 50ml tube (store @ 4°C)
Make 1mg/ml stock in 0.1N ac.ac. in tissue culture hood → mix @ T\textsubscript{room} for 1-3 hrs (store @ 4°C)
Make 50mM sodium acetate buffer, pH 4.5; filter → 0.205g in H_2O\textsuperscript{dd} for 50ml tube (store @ T\textsubscript{room})

Calculate needed final collagen solution volume, \( V = (N \text{ gels}) \times (2\text{ml}) \)
Dilute stock to 0.05mg/ml in s.a. buffer in tissue culture hood → (\( V \times 0.05 \))ml stock for Vml
Add 3.6 mg/ml NaIO\textsubscript{4} crystals → (3.6 × V)mg for Vml; twirl few times gently, don’t shake
Let it sit 30min @ T\textsubscript{room} in tissue culture hood to oxidize collagen

In tissue culture hood add 2ml of oxidized collagen solution per dish
Incubate for 1hr @ T\textsubscript{room} in tissue culture hood (make sure UV is off)
Wash 3 × 10min in PBS (replace PBS under the hood, put on top kim-wipe on outside shaker)
Store collagen-plated gels in PBS @ 4°C

**NOTE:** If plating cells immediately, add cell media instead of PBS and equilibrate ~1 hour in incubator before plating cells.

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