For **lung cancer tissue dissociation**, digestion time is one of the most critical variables affecting both cell yield and the biological quality of your scRNA-seq data. The consequences of going too long or too short differ, and the trade-offs are particularly important for lung because of its high immune infiltration, mucin content, and heterogeneous matrix composition.

---

1. If digestion time is too long

a. Cell viability loss

- Extended exposure to collagenase, dispase, or proteases will damage cell membranes.
- Epithelial cells, especially tumor epithelial cells, are more fragile and will lyse first, reducing the representation of tumor cells.

b. Transcriptional artifacts

- Prolonged warm digestion (37 °C) induces a **conserved enzymatic dissociation stress program** (FOS, JUN, HSPs, MHC-I upregulation) in many cell types.
- This makes cells from different samples more similar in the "stress" dimension, masking true biological differences.

c. Shift in cell composition

- More resistant populations (e.g., myeloid cells, fibroblasts) become enriched as fragile cells die.
- This biases downstream cell type proportion analysis.

d. Increased debris and ambient RNA

- Dead and lysed cells release RNA into the supernatant, increasing ambient RNA contamination in 10x data.
- Leads to higher background gene counts and "soup" effect in all barcodes.

---

2. If digestion time is too short

a. Low cell yield

- Tissue remains in chunks, trapping cells inside.
- You may get disproportionately high immune cell content (since they are easier

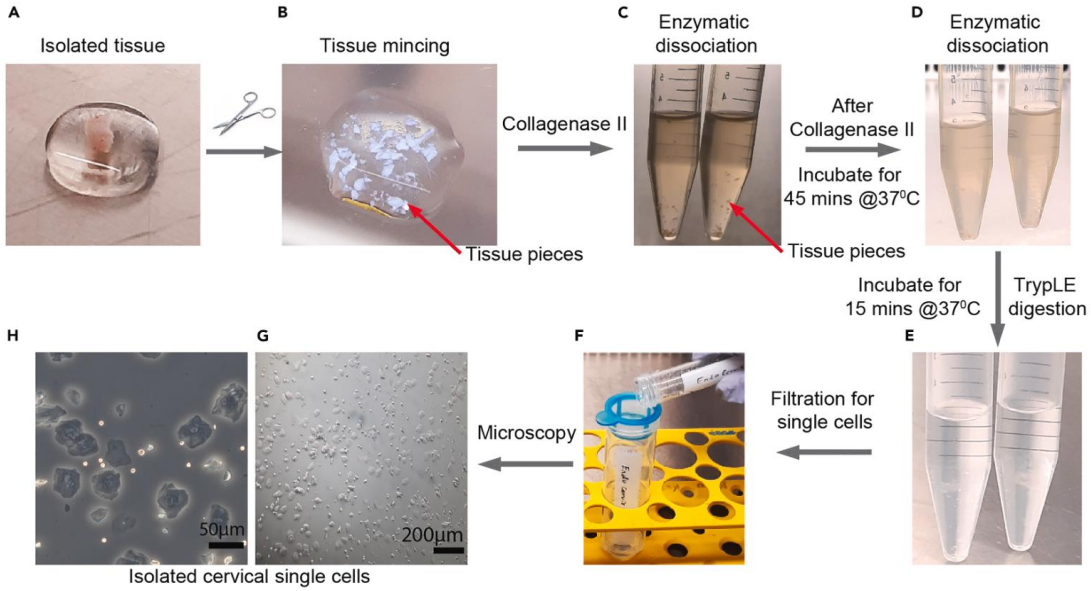
to release) and under-representation of tumor epithelial and stromal cells.

b. Incomplete dissociation → clumps

- Clumps cannot be loaded efficiently into the 10x microfluidics system, causing:
    - Reduced actual cell recovery.
    - Higher multiplet rate if clumps partially enter droplets.

c. Biased composition

- Large stromal and epithelial clusters remain trapped, so the single-cell suspension skews toward smaller, more easily liberated cells (T cells, macrophages).



---

3. Practical recommendations for lung cancer

1. **Monitor dissociation in real time**
   Every 10–15 min, pipette a small aliquot, dilute, and check under a microscope for:
    - Proportion of single cells vs. clumps.
    - Presence of intact epithelial-like cells.
    - Dead cell debris.
2. **Stop at "just enough"**

For lung tumors, 25–45 min at 37 °C with gentleMACS (Miltenyi “medium” program) is typical, but adjust:

- Softer/more necrotic tissue → shorter time.
- Very fibrotic tissue → slightly longer but watch viability.

3. **If aiming to preserve native transcriptomes**
   Use **cold protease** (6 °C) to allow longer digestion without inducing heat-shock transcripts.
   This allows you to extend time for tough tumor matrix while limiting stress artifacts.
4. **Post-dissociation cleanup**
   If you suspect over-digestion or high debris, include a DNase wash and possibly a debris removal step to reduce ambient RNA before 10x.

If you want, I can prepare a **time–outcome matrix** specifically for lung cancer scRNA-seq, showing the trade-offs in yield, viability, cell type bias, and transcriptional artifacts at different digestion durations for both warm and cold protocols. This makes it easier to optimize your digestion window empirically.