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).

图形用户界面, 日程表

AI 生成的内容可能不正确。

**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.