

Pulmonary Toxicity of Single Walled Carbon Nanotubes

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Background

Single Walled Carbon Nanotubes (SWCNT)

- A. Long tube-like configuration of carbon molecules**
- B. Single layer of carbon atoms in a cylindrical arrangement**
- C. Nanotube = 1.5 nm in diameter, up to 1 mm in length**
- D. High tensile strength, high surface area, unique electronic properties, high adsorption capacity**
- E. Used in electronics, structural materials, etc**

Issue

- A. Potentially wide commercial applications**
- B. Little information is available concerning the potential adverse effects of inhalation of SWCNT**

Objective

- A. Characterize the pulmonary responses to SWCNT**
- B. Determine the dose-dependence of the responses**
- C. Determine the duration of responses post-exposure**

Test Material

- A. SWCNT were produced by the high-pressure carbon monoxide process (HiPCO)**
- B. Unpurified SWCNT – contain 30% metal catalyst (ultrafine Fe, or Fe/Ni)**
- C. Purified SWCNT – acid treated; < 0.2 wt% metals**
- D. Suspended material supplied by NASA**

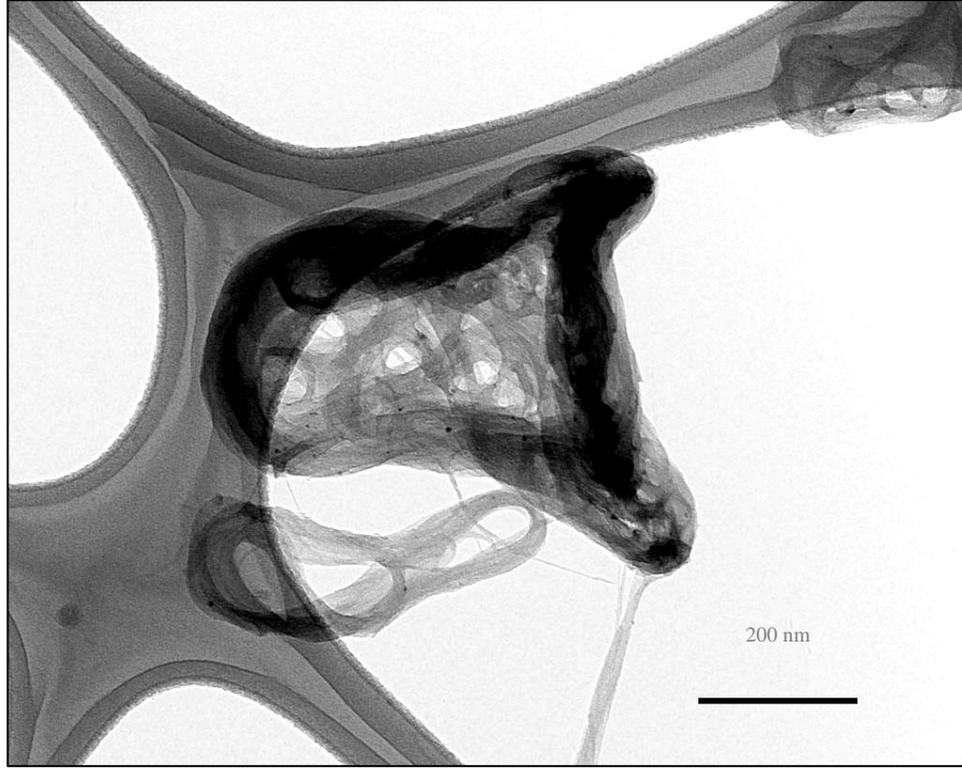
Methods

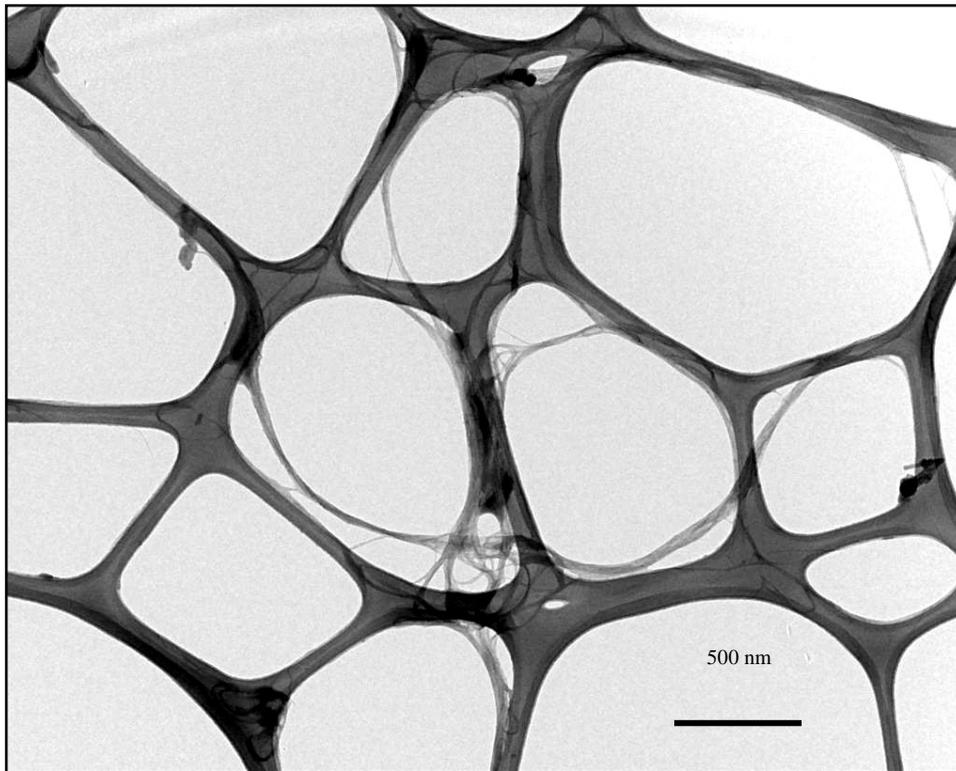
A. Exposure

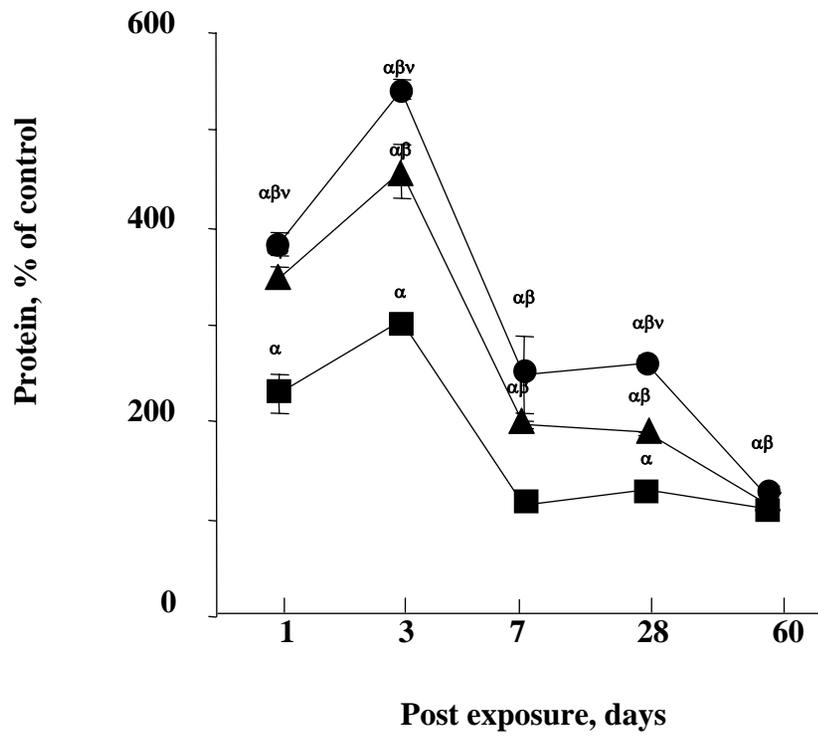
- 1) Pharyngeal aspiration of mice
- 2) PBS or SWCNT (10, 20, or 40 $\mu\text{g}/\text{mouse}$)
- 3) Sacrifice 1 – 60 days post-exposure

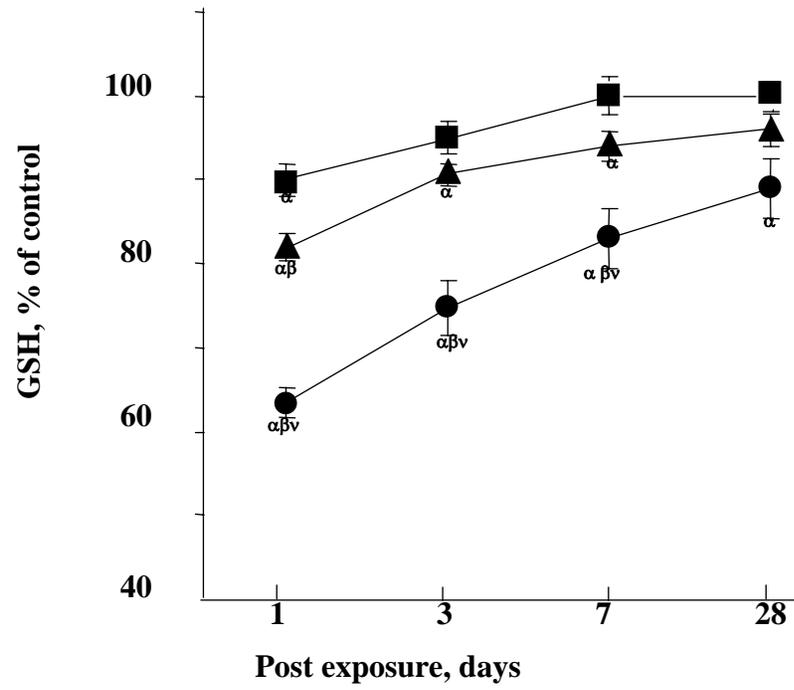
B. Endpoints

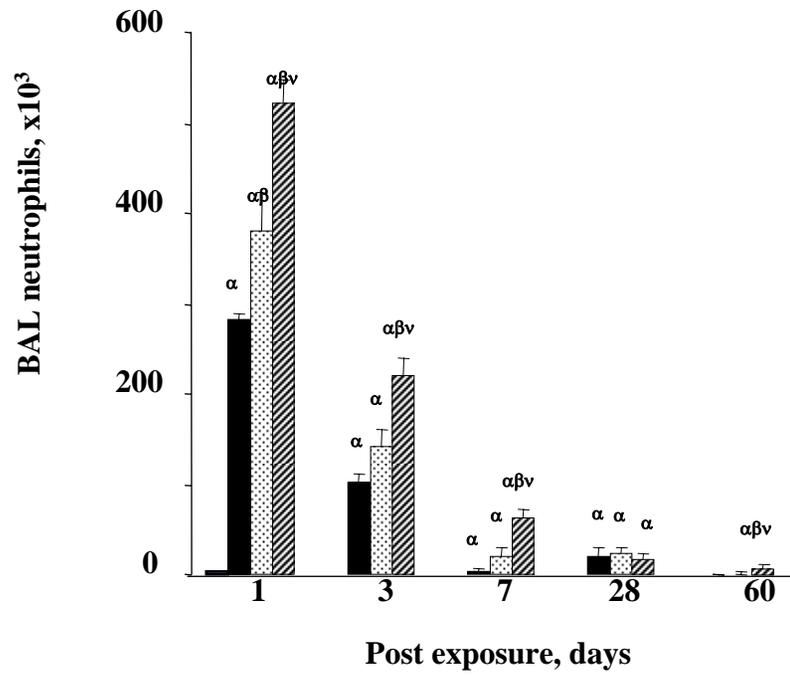
- 1) Damage – BAL protein and LDH
- 2) Inflammation – BAL cells and cytokines
- 3) Oxidant stress – lung GSH
- 4) Histology – particle deposition, inflammation, granulomas, and fibrosis

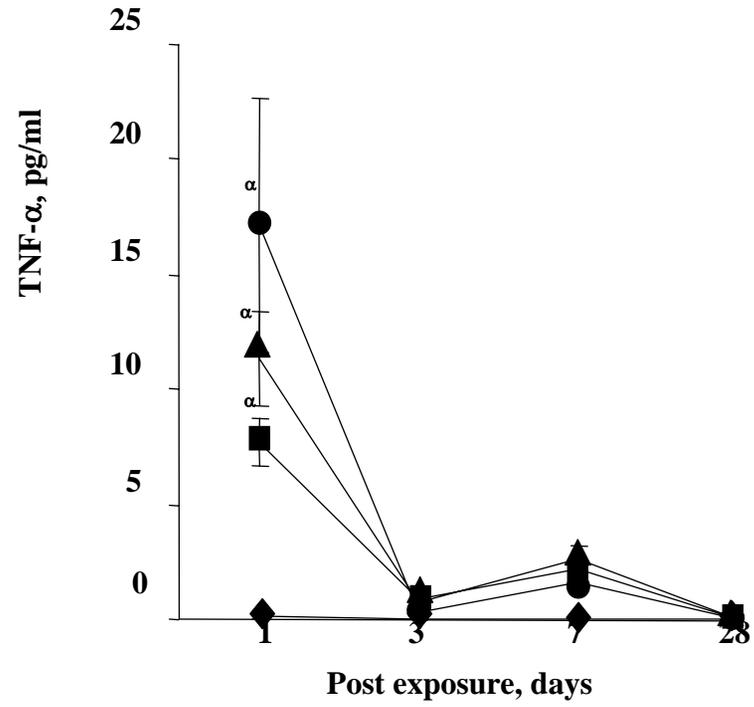


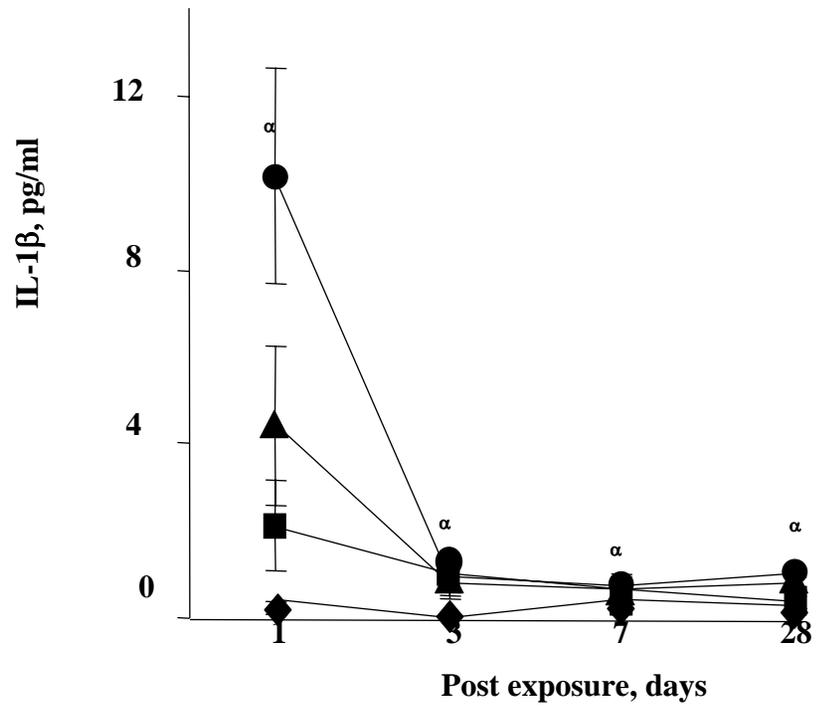


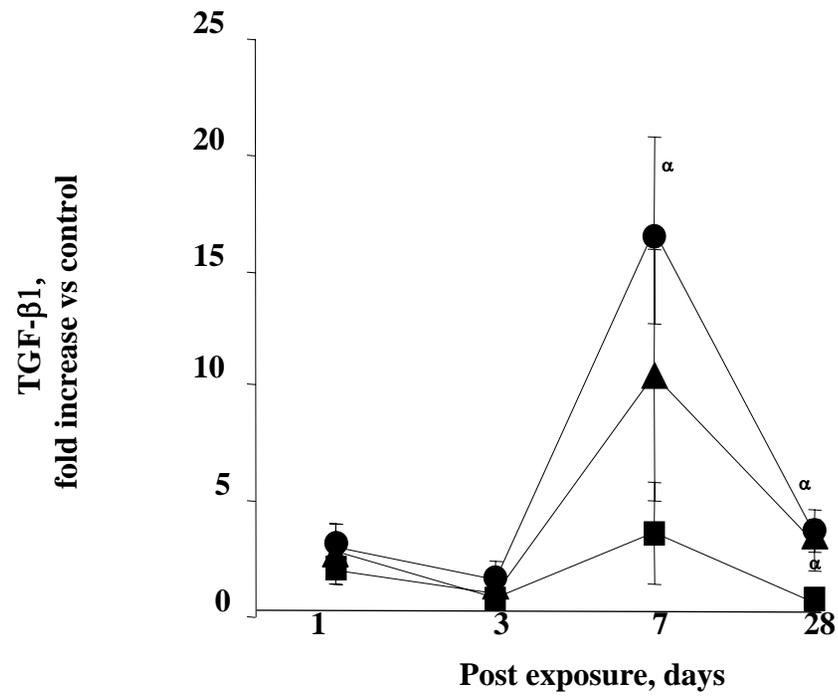






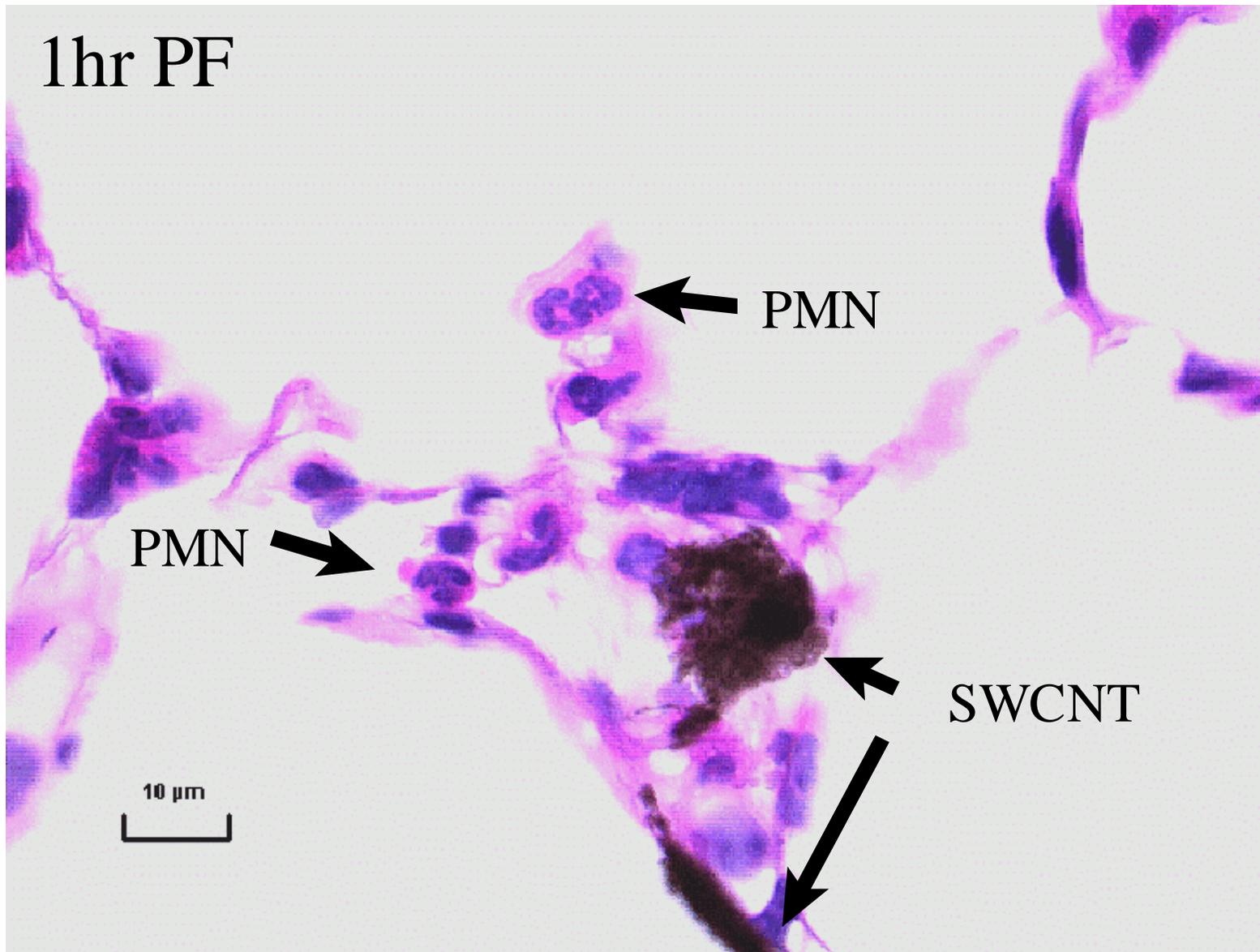




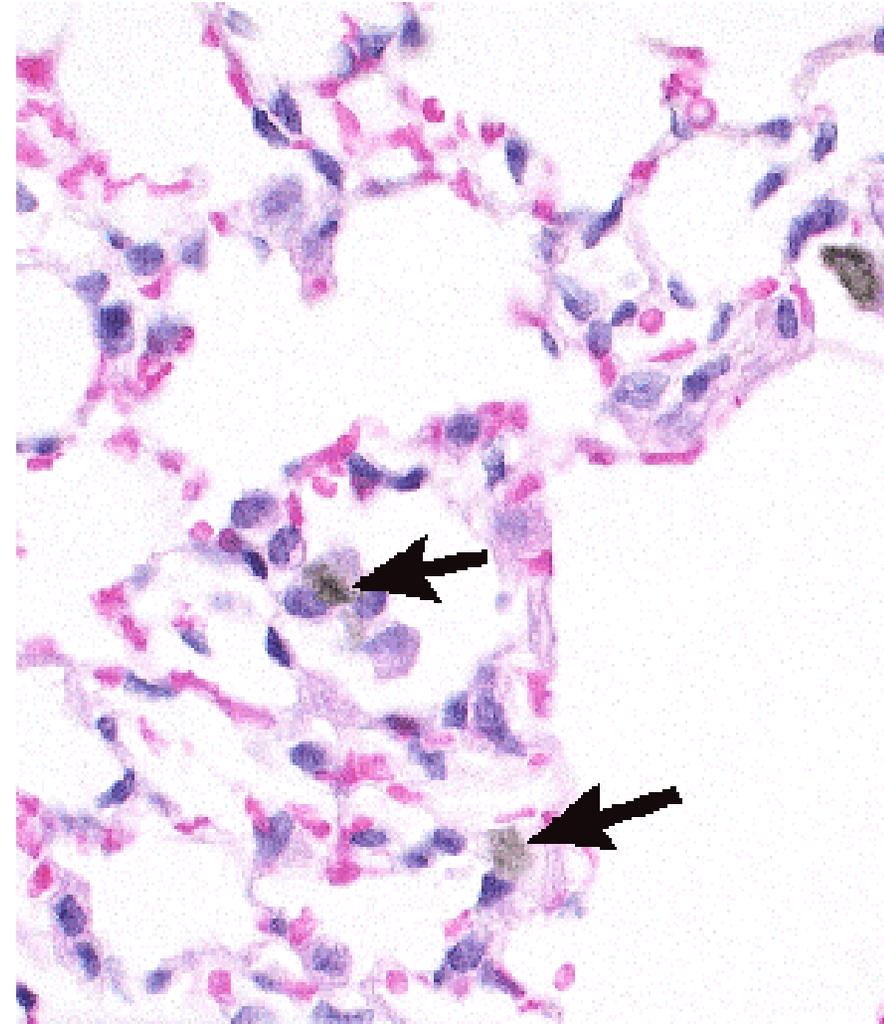
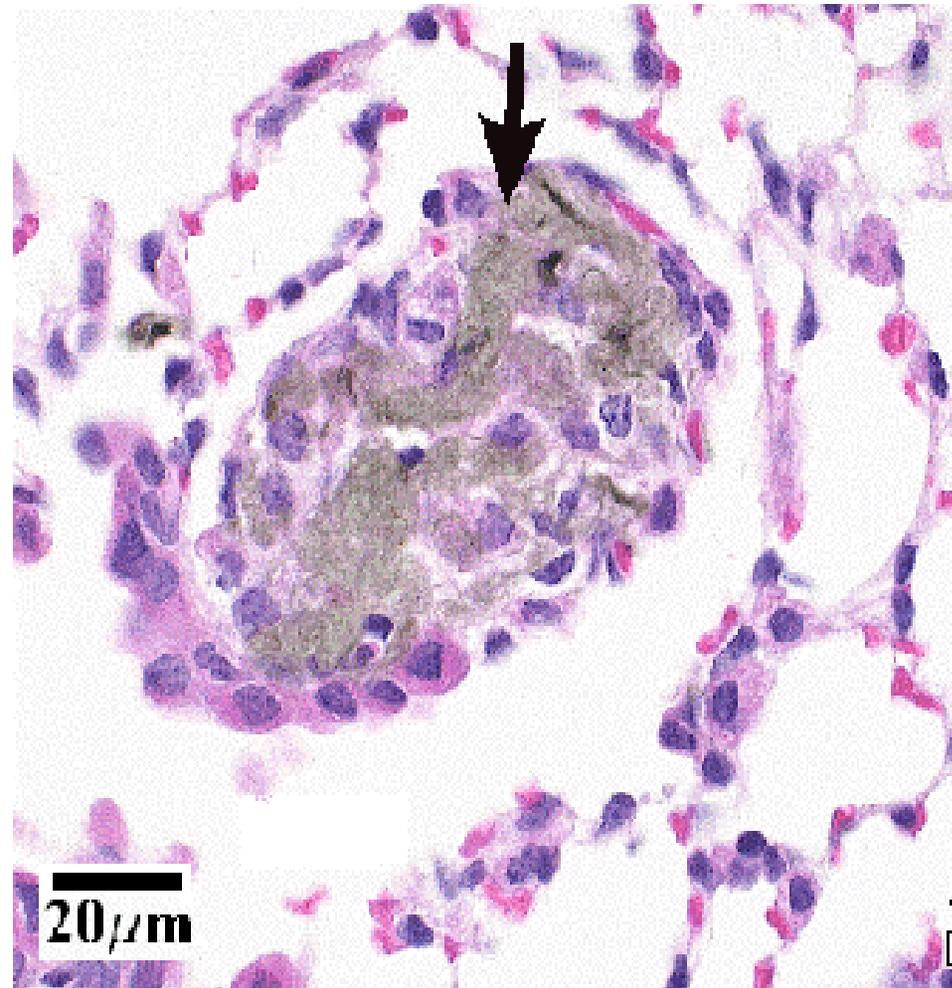


SWCNT Inflammatory Response 1Hour

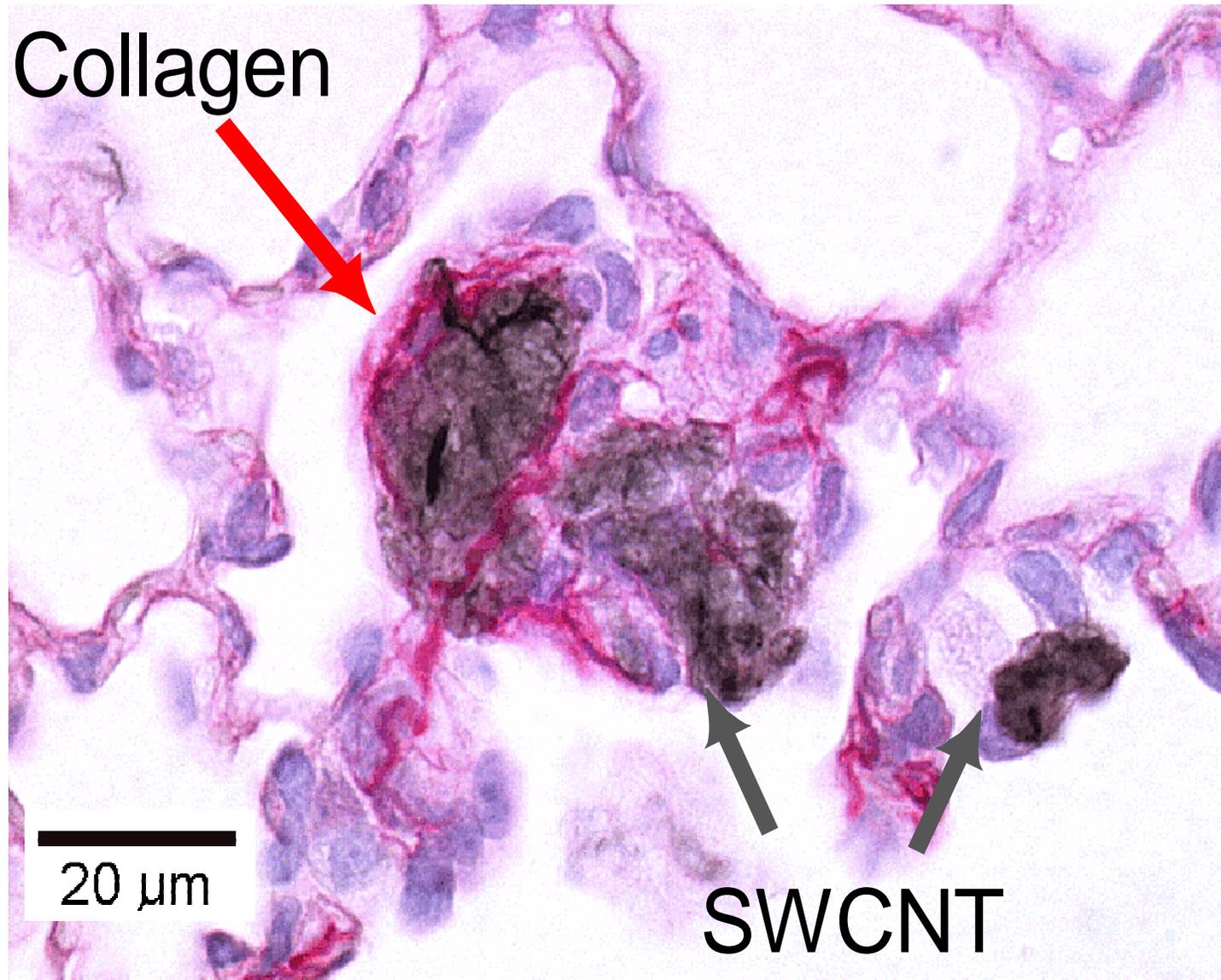
1hr PF



H&E SWCNT 30 days



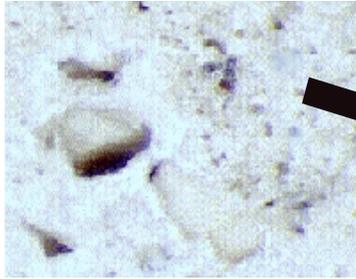
SWCNT Response 7 Days



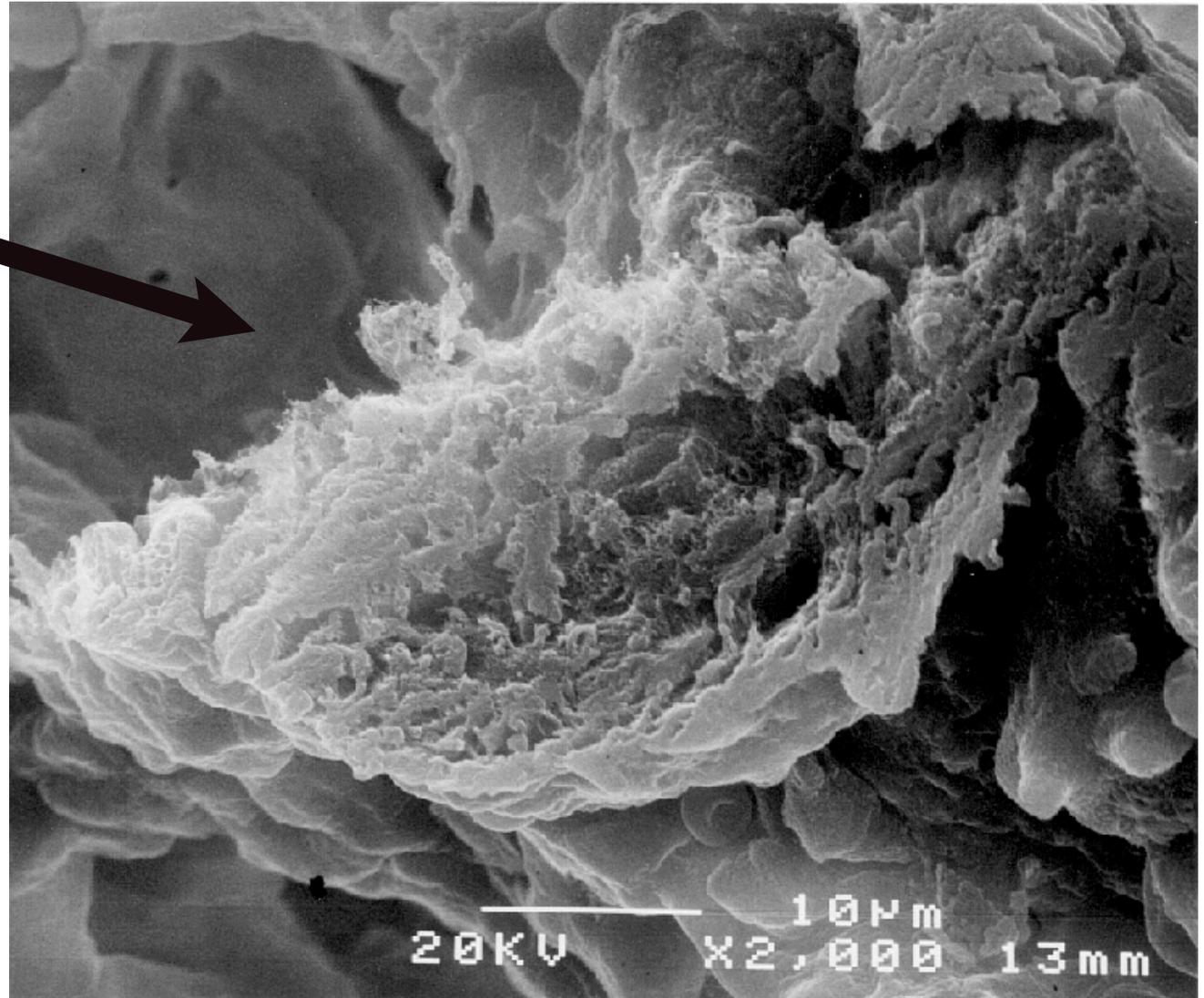
Pharyngeal aspiration of 40 μg SWCNT in C57BL/6 mice.

SEM of Granuloma in Alveolar Airspace

Carbon Nanotubes 7 days post aspiration, mouse 1mg/kg

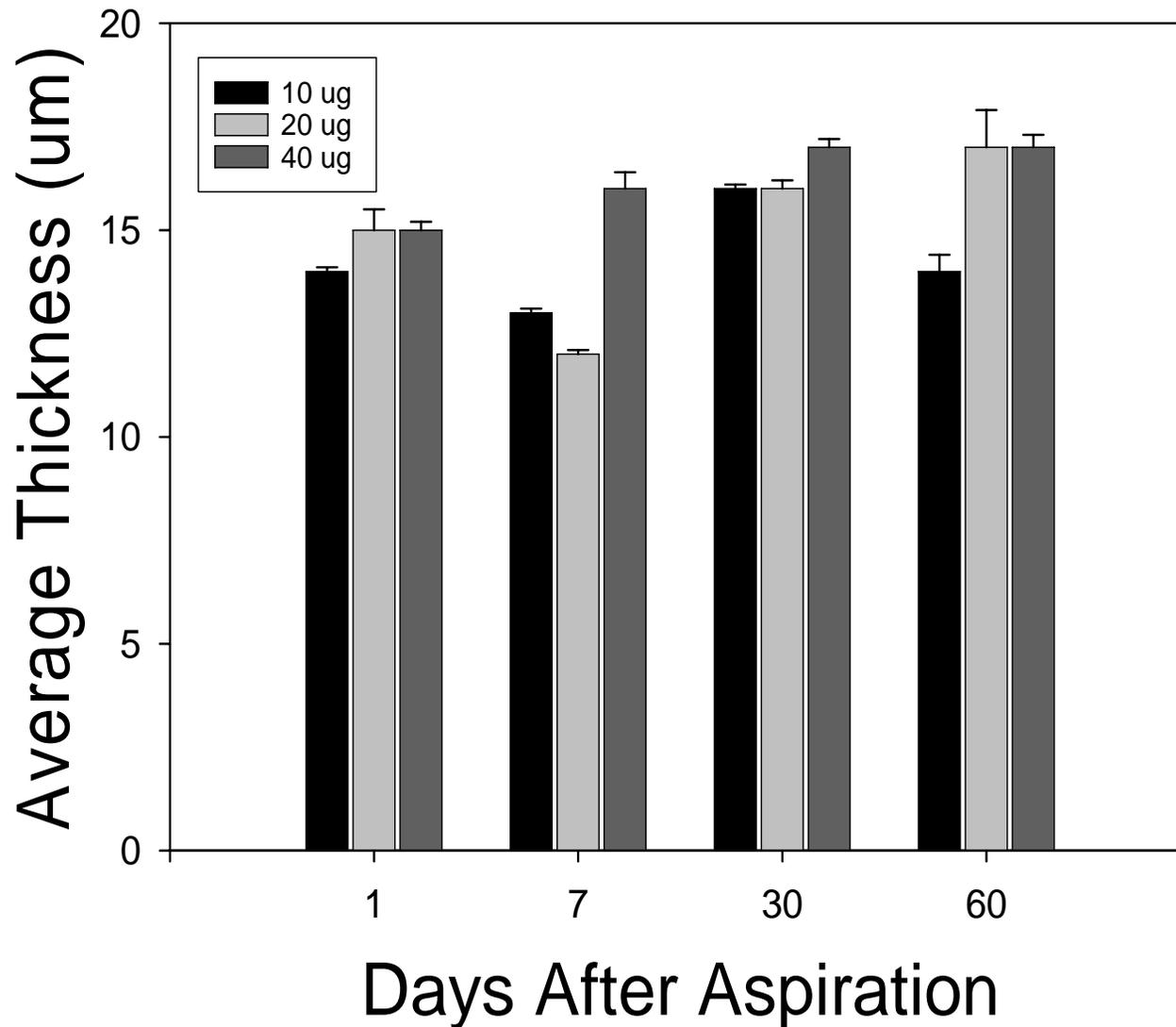


Dissecting microscope

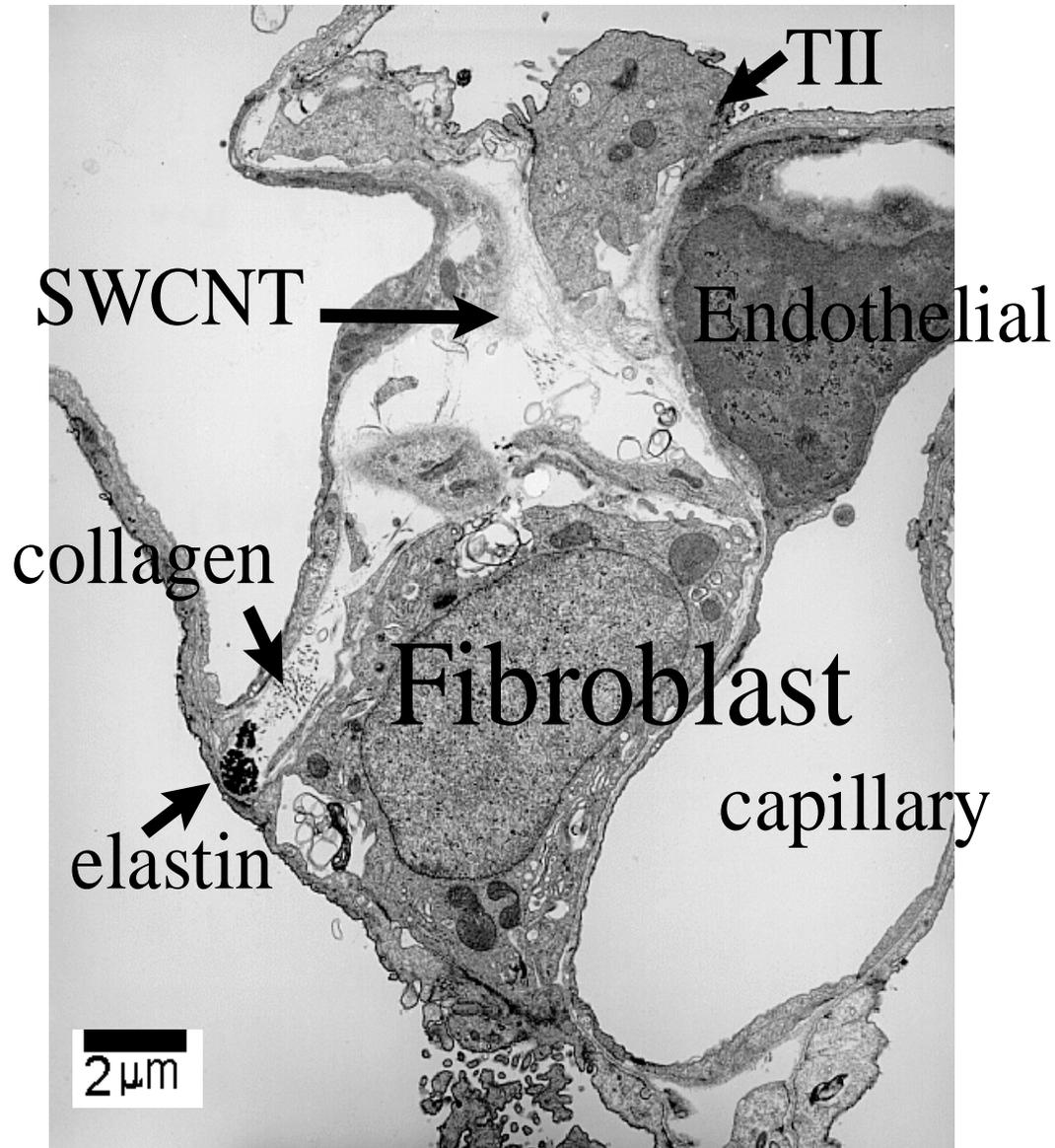


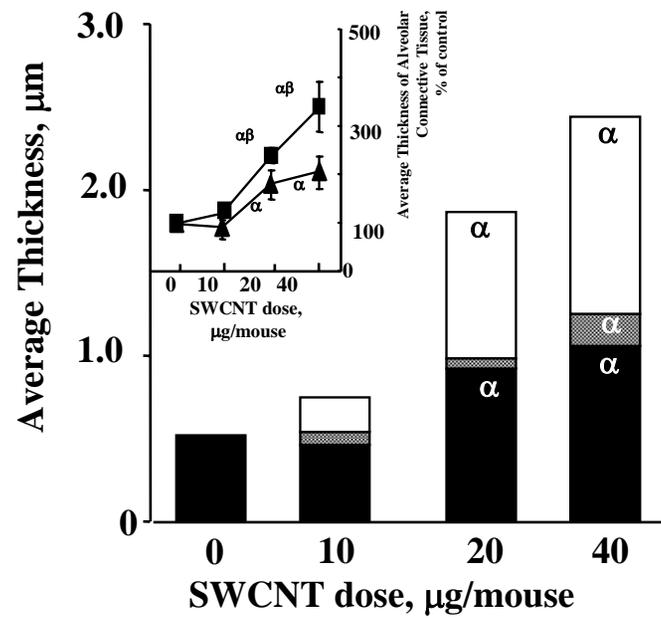
10 μm
20KV X2,000 13mm

Size of SWCNT Deposits

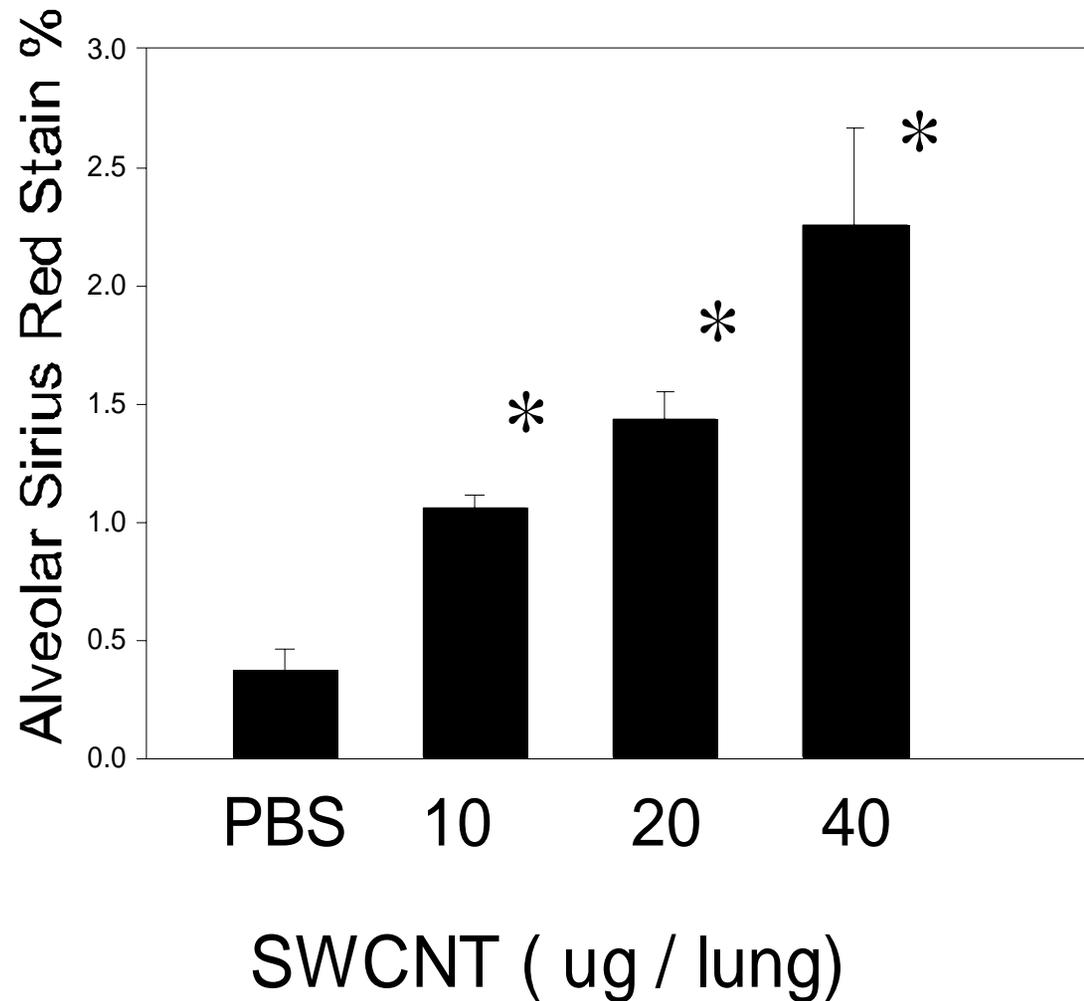


TEM of SWCNT in Interstitium (3 days)

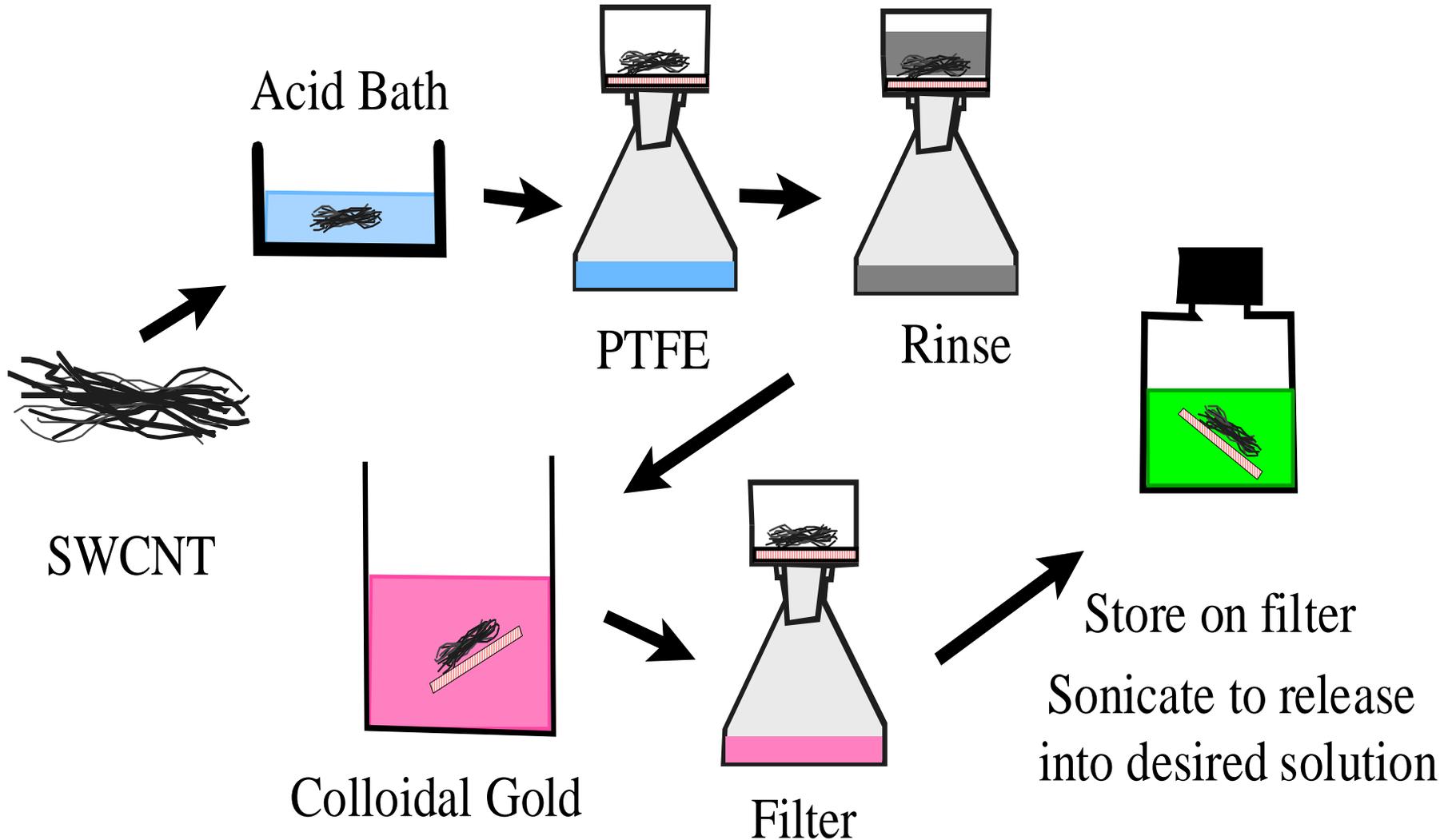




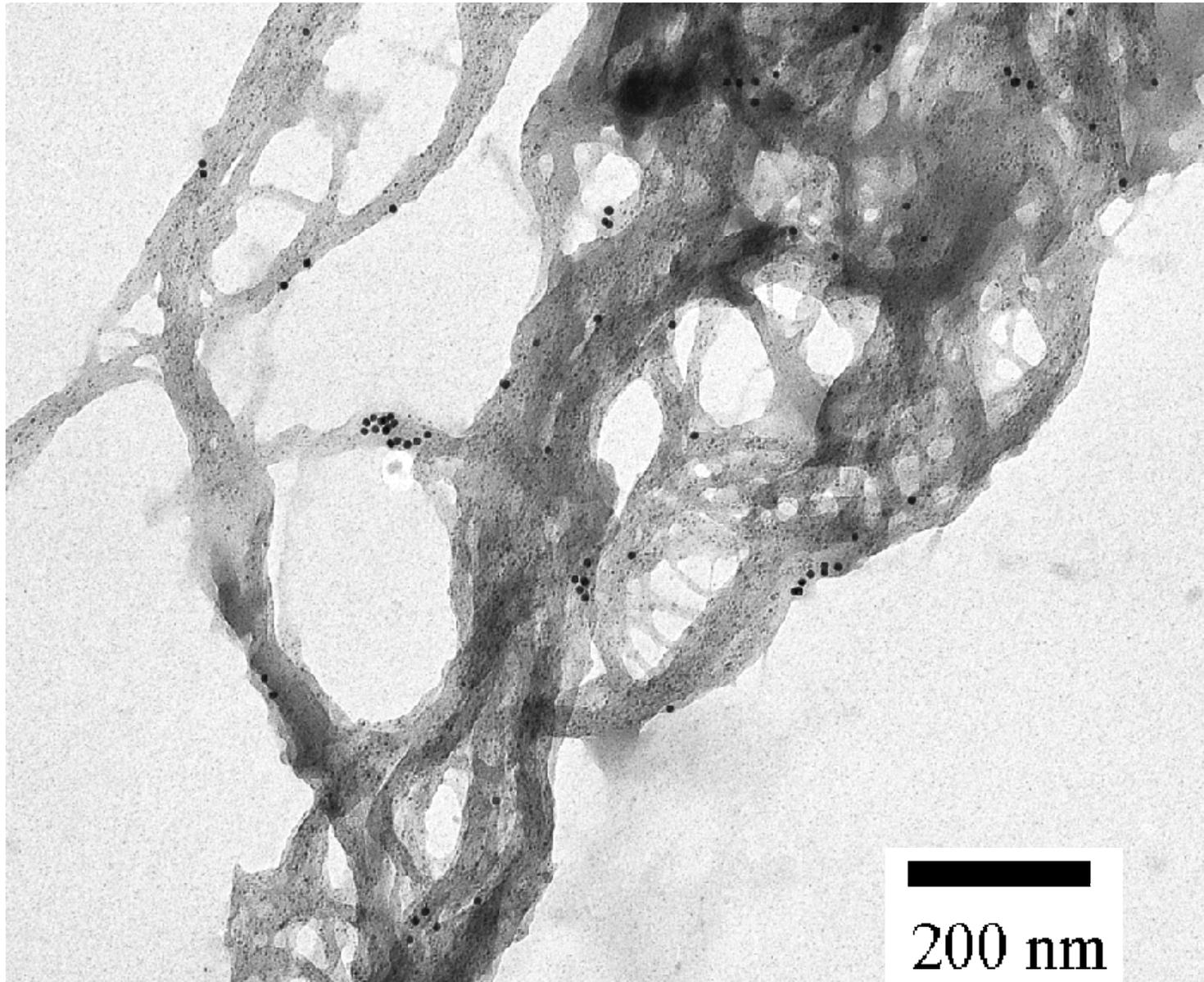
Connective Tissue Response in Alveolar Region (areas outside those containing SWCNT aggregates, 60 days)



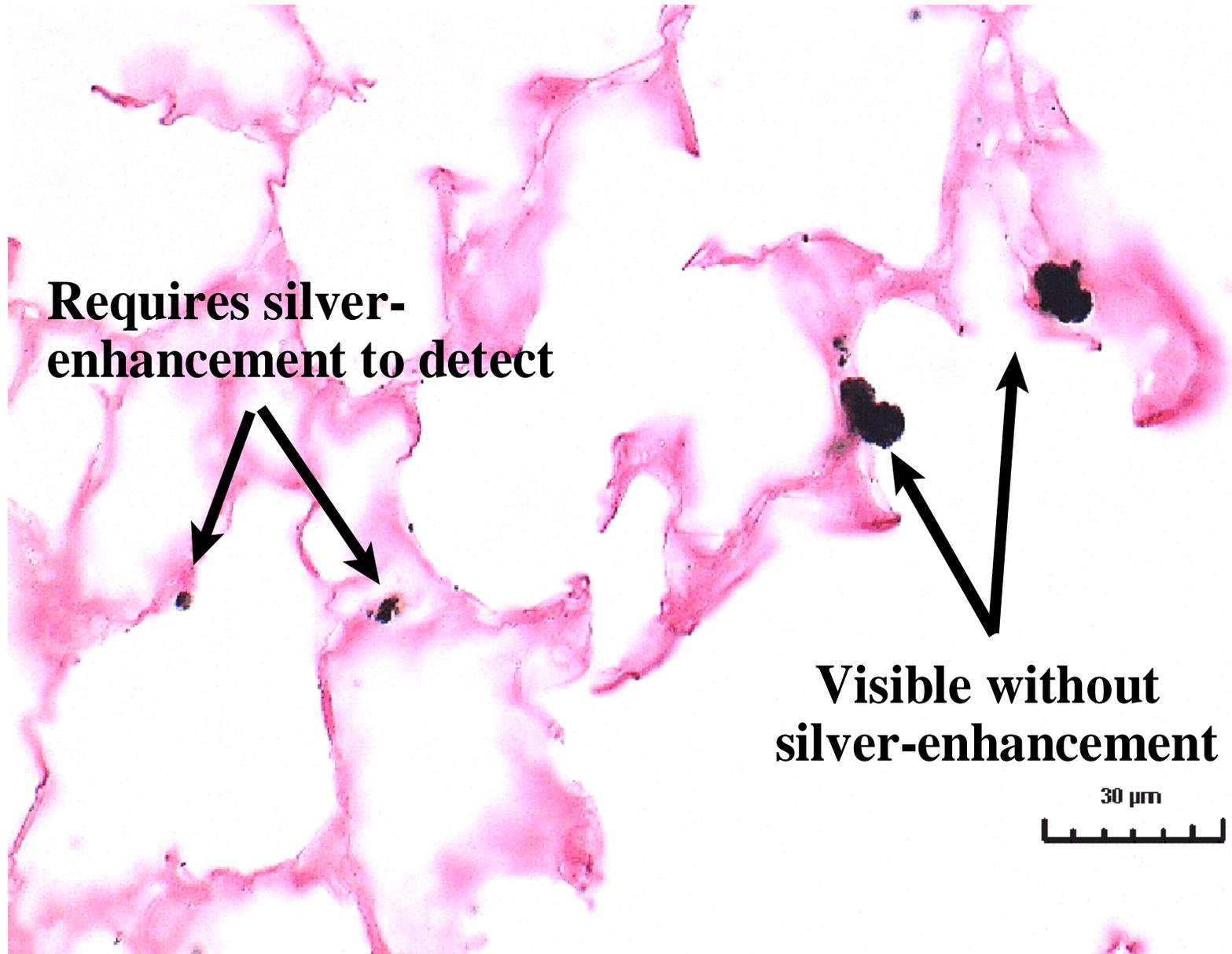
Preparation of Au-SWCNT



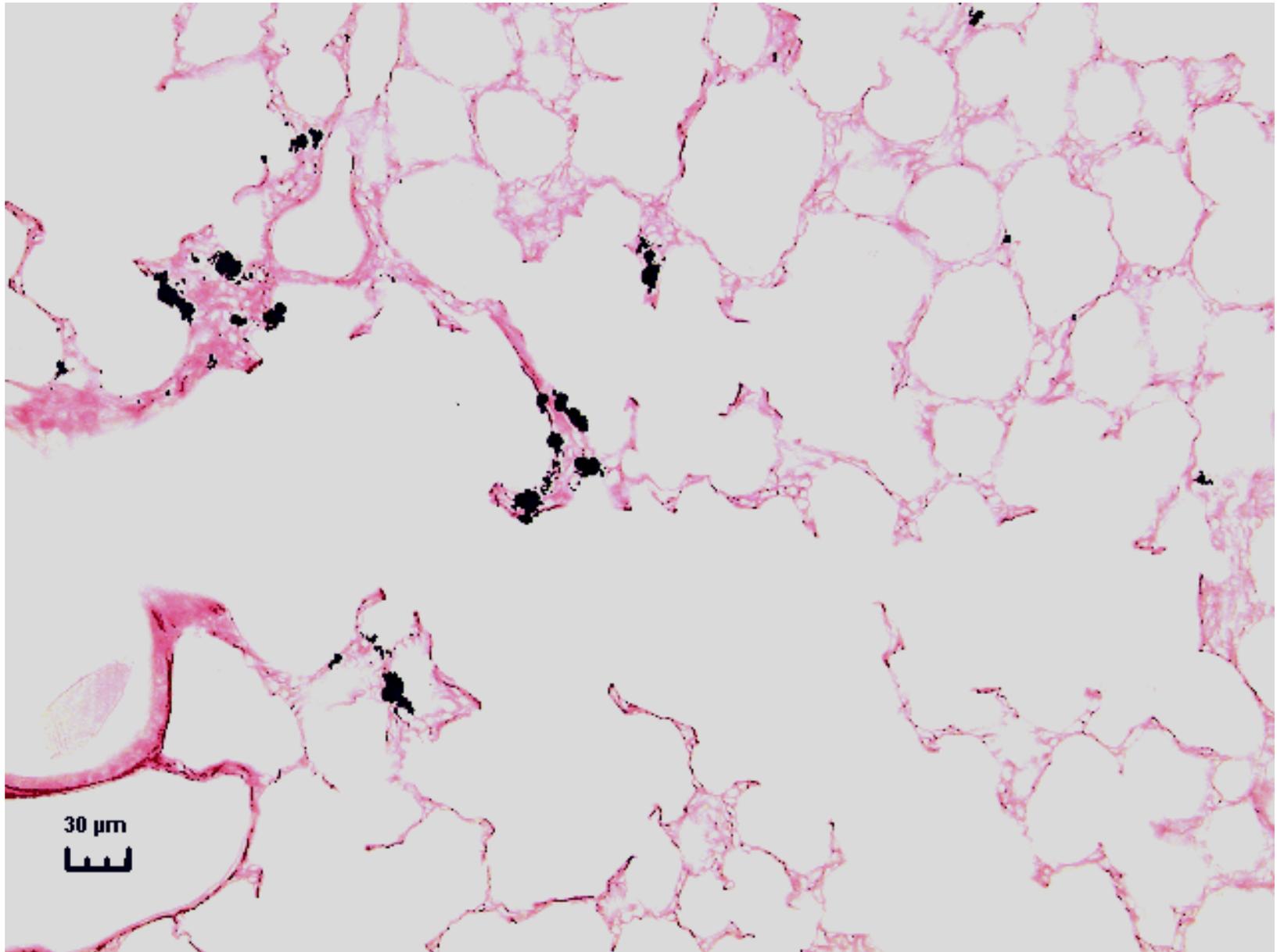
10nm Colloidal Gold-Labeled SWCNT



Detection of Sub-Micron SWCNT by Silver Enhancement

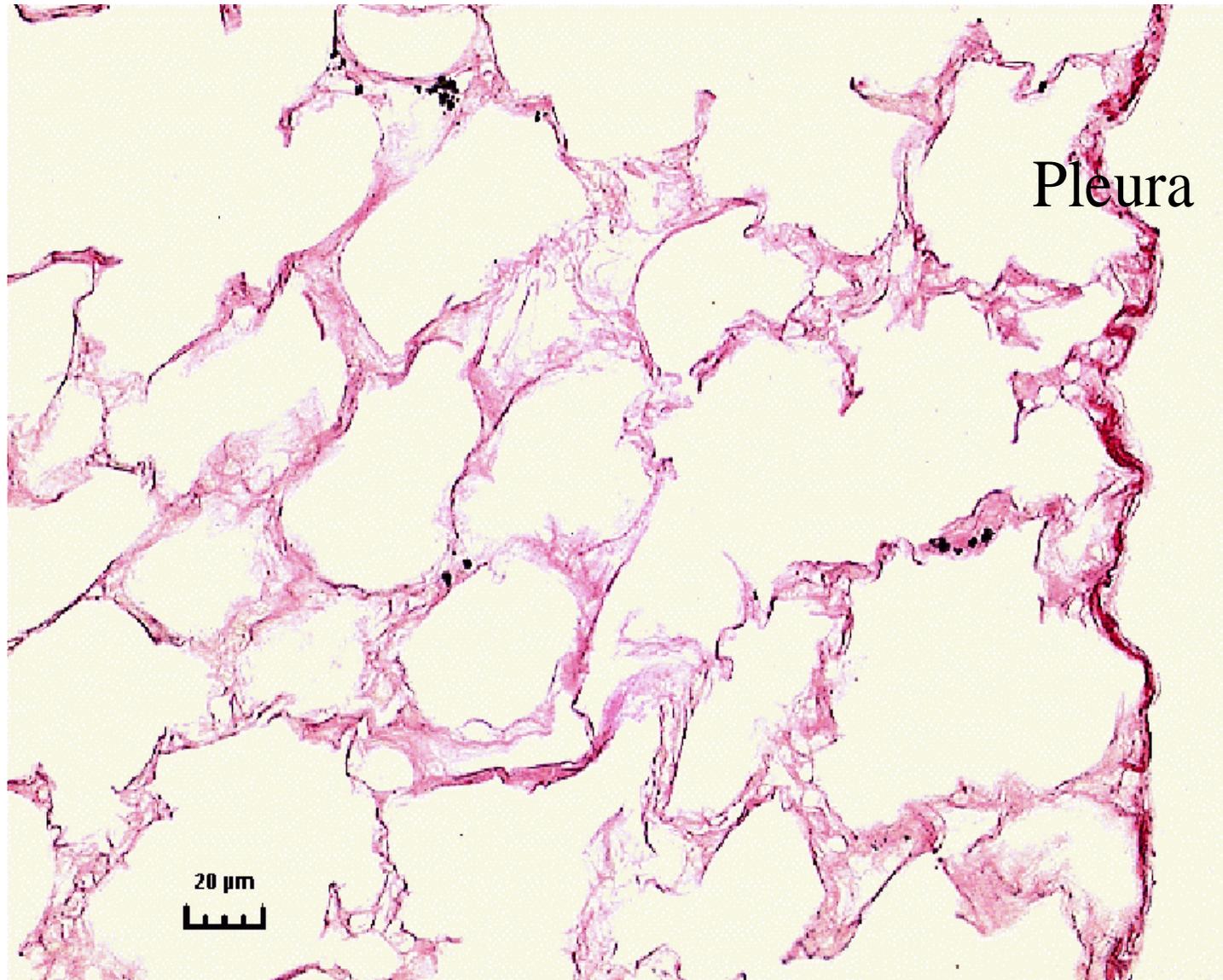


Proximal Alveolar Region SWCNT Day 3



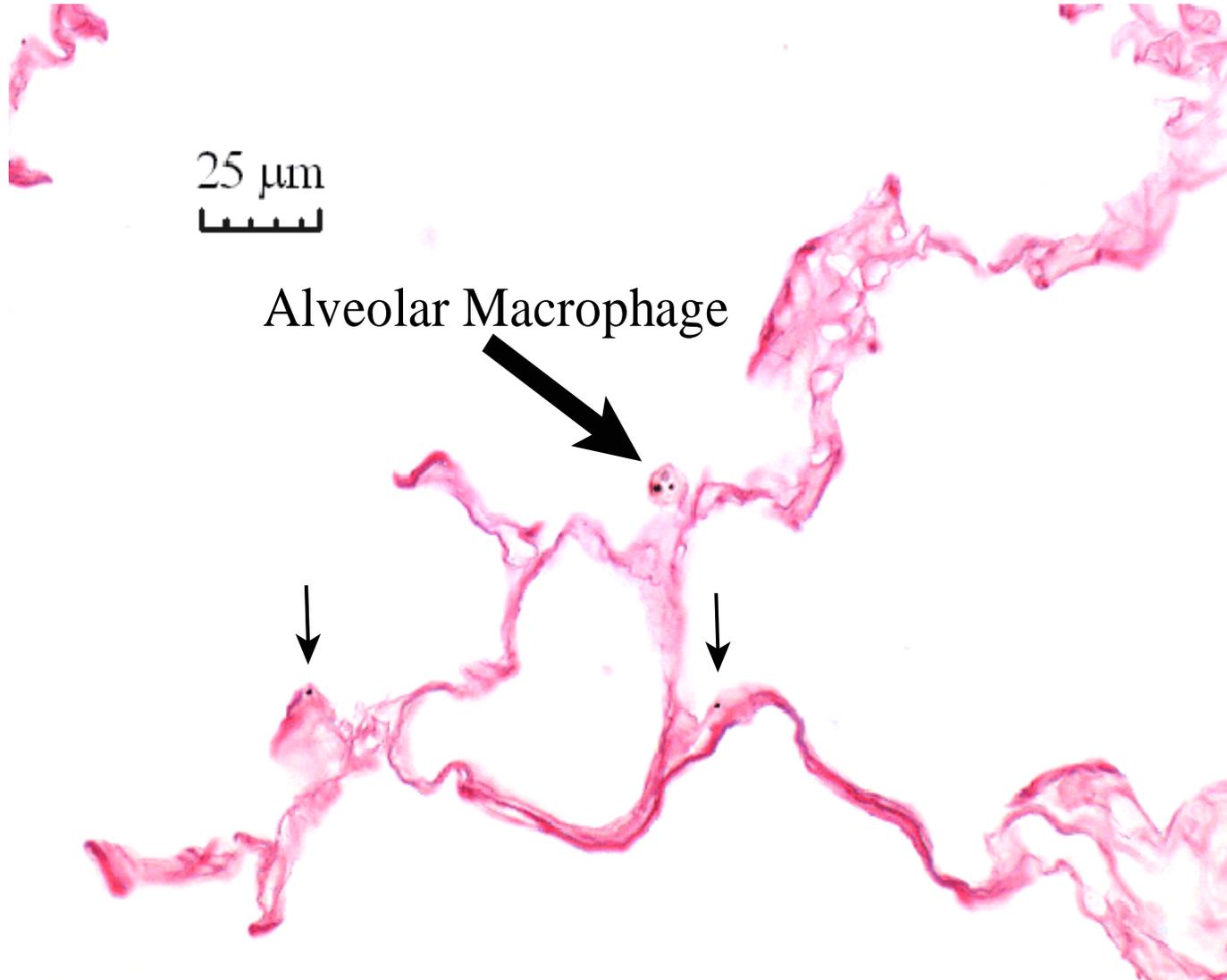
Silver-enhanced gold-labeled SWCNT, 40 ug aspiraton, perfusion fixed

Pleural AU-SWCNT 1 day



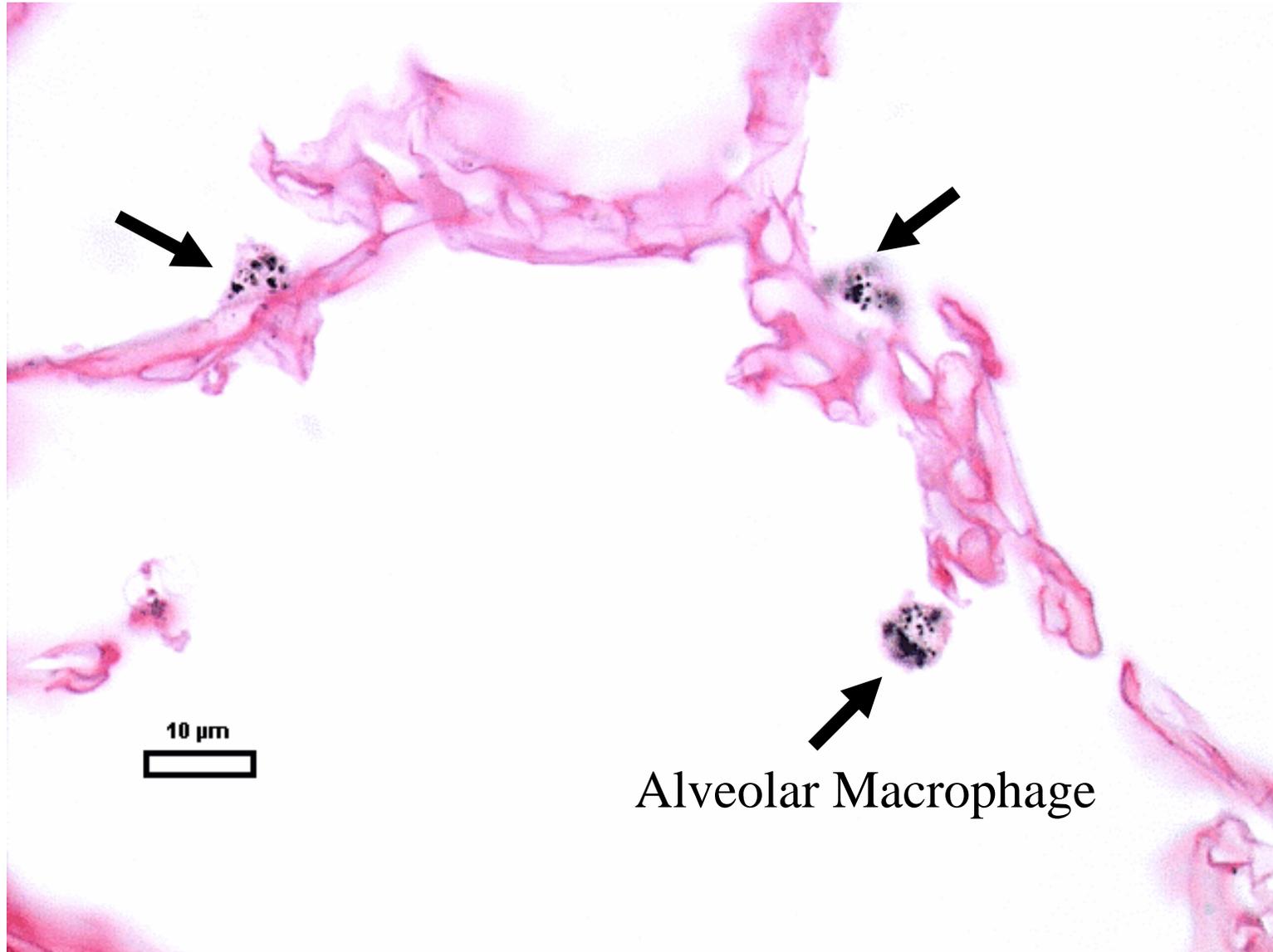
Silver Enhanced Dispersed Au-SWCNT

1 day post aspiration, 10 ug, perfusion fixed mouse



Silver Enhanced 10nm Gold (no SWCNT)

(1 day post aspiration, perfusion fixed mouse)



Summary

- A. Nebulized SWCNT dispersed as aggregates and nanotubes**
- B. Aspiration causes transient oxidant stress, damage and inflammation, peaking by 7 days post-exposure**
- C. Histology visualizes aggregates in the terminal bronchials and proximal alveoli with no visible material in distal alveoli**
- D. Size of aggregates doesn't change with time**
- E. Rapid fibrosis – begins in 7 days and progresses through 60 day post-exposure**
 - 1) Fibrosis in granulomatous lesions containing aggregates**
 - 2) Diffuse interstitial fibrosis in distal alveolar walls with no visible SWCNT**
- F. Used silver enhancement of gold-labeled SWCNT**
 - 1) See aggregates in proximal alveoli and terminal bronchials**
 - 2) See nanoropes in walls of distal alveoli**

Conclusions

- A. See granulomatous lesions at deposition sites of aggregates.**
- B. See interstitial fibrosis in sites of deposition of nanoropes.**

Organ Gold Content After Gold-Labeled SWCNT Aspiration

