Supplemental Information

# Simulated Gastric Leachate of 3D Printer Metal-Containing Filaments Induces Cytotoxic Effects in Rat and Human Intestinal Models

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Concentration of elements in ICP-MS standard solutions

For inductively couple plasma mass spectrometry (ICP-MS) instrument optimization, we used NexION setup solution (Part No. N8145051) containing 1 ug/L of Be, Ce, Fe, In, LI, Mg, Pb, U 500 mL; and NexION dual detector calibration solution containing 200 ug/L of Al, Ba, Ce, Co, Cu, In, Li, Mg, Mn, N, Pb, Tb, U, Zn purchased from Perkin Elmer (Waltham, MA). A 100 ppm SPEX instrument calibration standard containing the elements Ag, Al, As, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, Mg, Mn, Mo, Na, Ni, P, Pb, Se, Sb, Sn, Sr, Ti, Tl, V, Zn, and a separate internal standard solution with 10 ppm of Bi, Ge, In, Li, Sc, Tb and Y were purchased from Spex CertiPrep (Metuchen, NJ).

Table S1 PerkinElmer ICP-MS operation conditions and acquisition parameters

| **Description** | **Value** |
|---|---|
| Mode | standard |
| Scan mode | Peak Hopping |
| Nebulizer Gas flow | 0.98 |
| Auxiliary Gas flow | 1.2 |
| Plasma gas flow | 18 |
| ICP RF Power | 1600 |
| Sweeps/reading | 20 |
| Readings/replicate | 1 |
| Replicates | 5 |
| Sample flush | 30 sec at 35 rpm |
| Read delay | 10 sec at 20 rpm |
| Wash | 45 sec at 35 rpm |
| Dwell time | 50 milli sec |

Table S2 Linear combination fitting results for Cu XANES spectra shown in Figure 10. R-factor is the fraction of the data not accounted for in the final fit.

| Sample ID | Standard | Contribution % | R-factor |
|---|---|---|---|
| Cu-1: Extruded copper-fill filaments, not extracted | Cu-Metal | 100 ± 2.7 | 0.015 |
| Cu-3: Media filtrate | Cu-Organic | 100 ± 5.4 | 0.006 |
| Cu-4: Extruded copper-fill filament, extracted | Cu-Metal | 100 ± 3.5 | 0.049 |
| Cu-5: Copper-fill extract, residue (filter, 10 KD) | Cu-Organic | 100 ± 3.6 | 0.007 |
| Cu-6: Copper-fill extract, filtrate | Cu-Organic | 100 ± 4.2 | 0.007 |

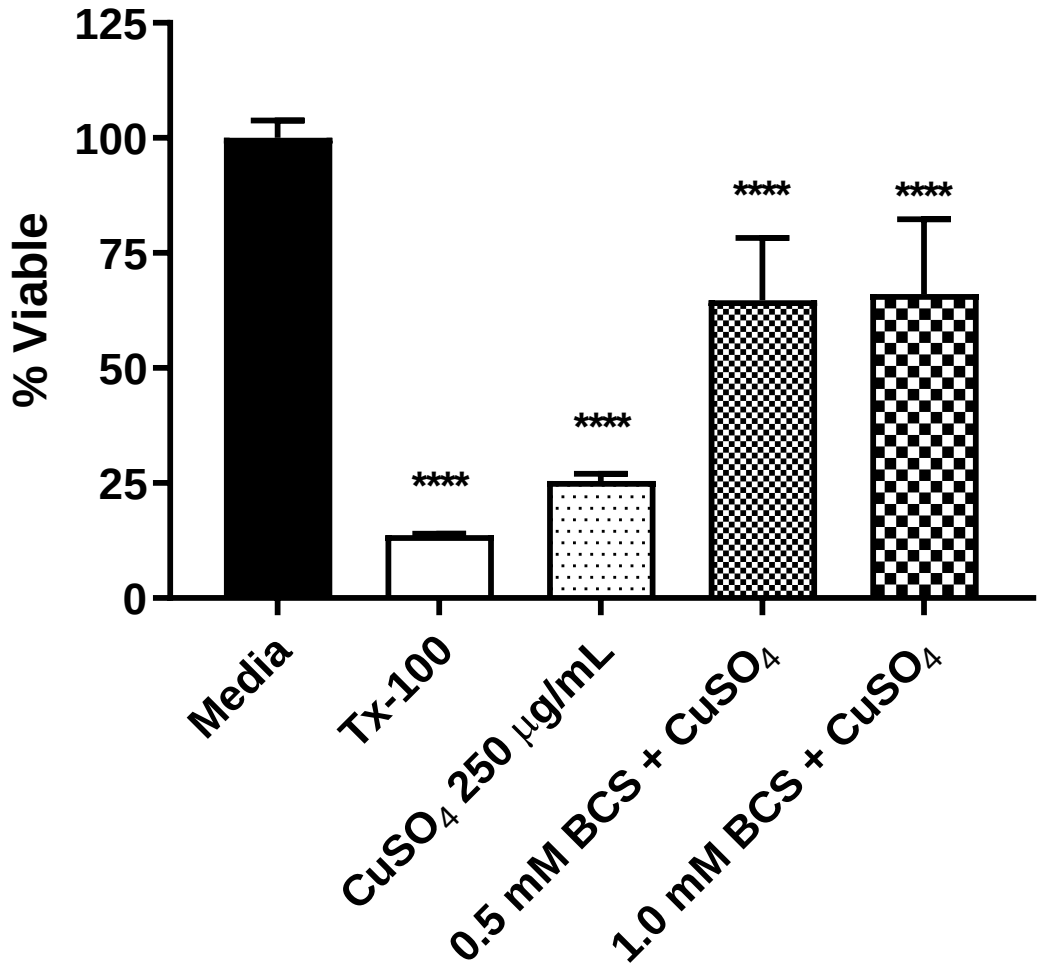


Figure S1 Viability of IEC-6 cells following 4-hr exposure to $CuSO_4$ (250 µg/mL) in the presence and absence of the copper chelator bathocuproine disulfonate (BCS). Viability determined by the MTS assay. Data represents mean ± SD, N=12; ****, significantly different from media, $p < 0.0001$.

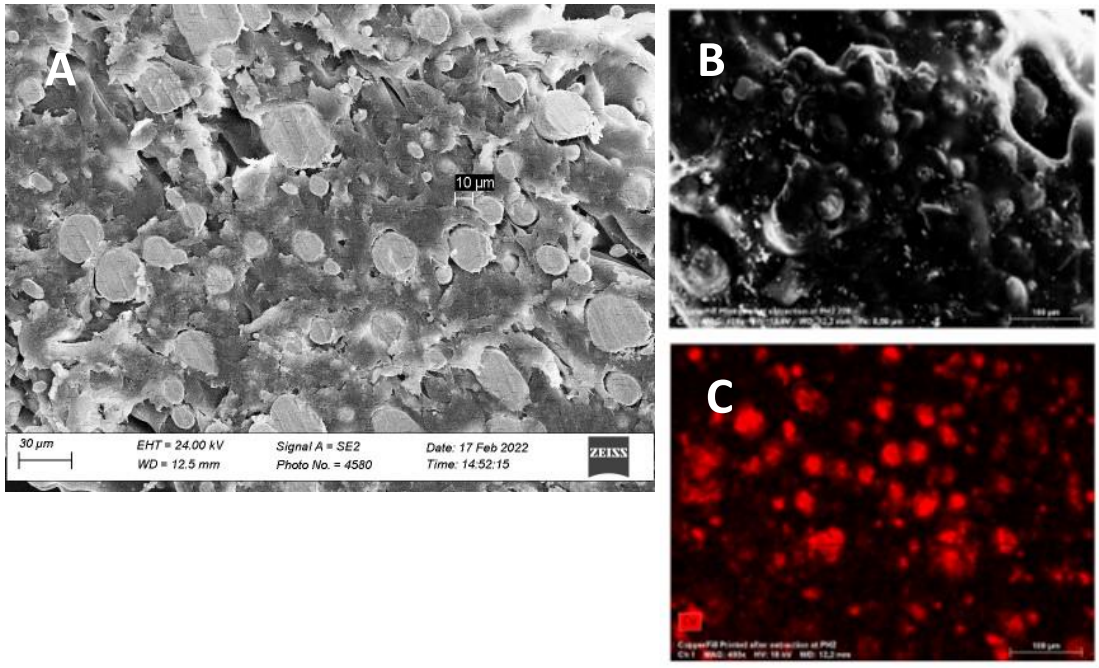


Fig. S2. The SEM-EDS images of extruded copper-fill filament cross-sections before extraction A, and after extraction B, false color EDS shows the presence of copper particles after extraction C. Size bars are 30 µm (A) and 100 µm (B, C).

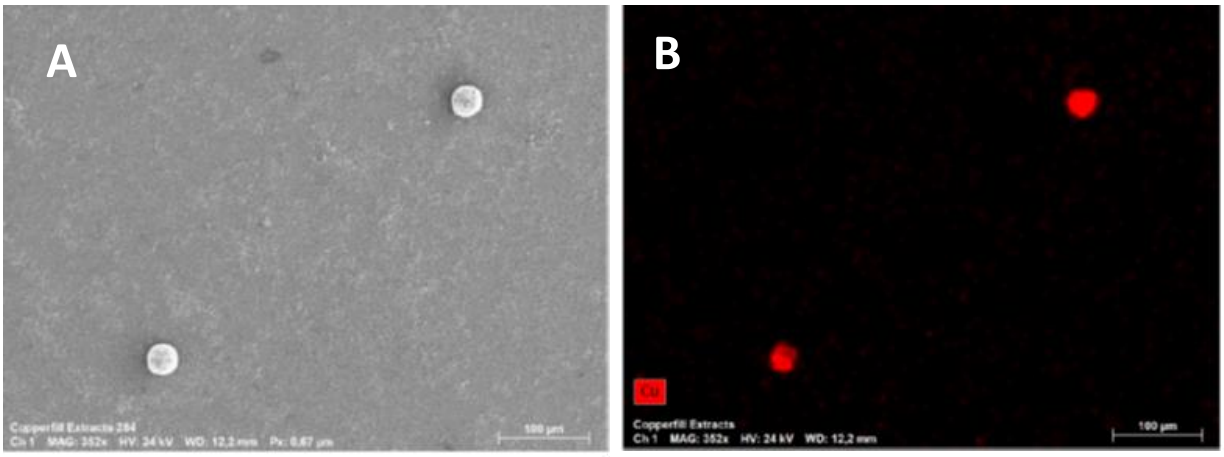


Fig. S3. The SEM-EDS images of the particles that were recovered by centrifugation after gastric leaching of the extruded copper-fill filament. False color EDS mapping shows the presence of copper particles (B). Size bars are 100 μm (A, B).

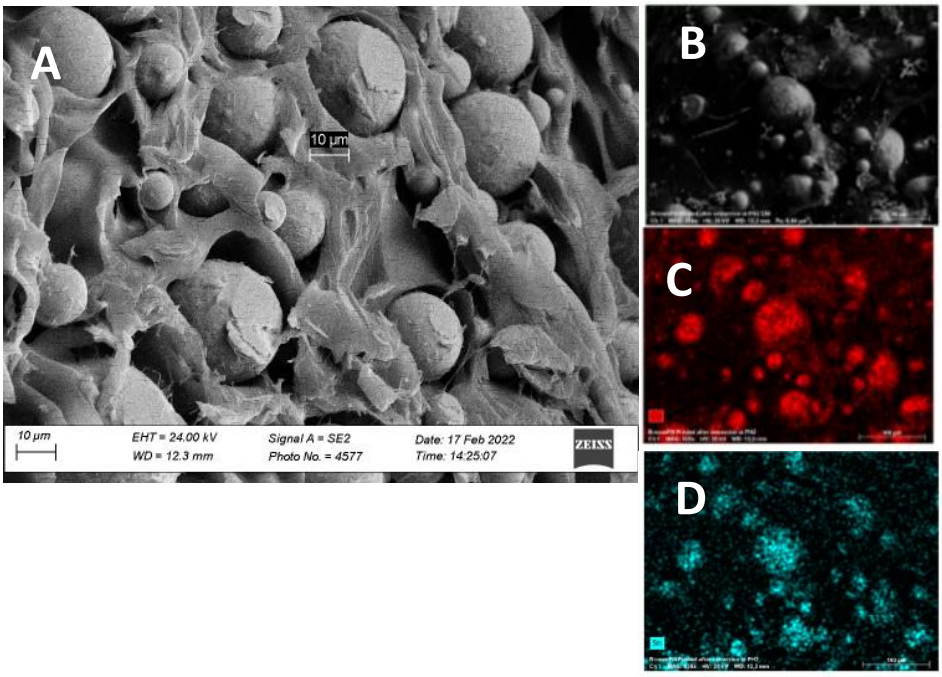


Fig. S4. The SEM-EDS images of extruded bronze-fill filament cross-sections before extraction (A), and after extraction (B), false color EDS shows the presence of copper particle content (C) and tin particle content (D). Size bars are 30 µm (A) and 100 µm (B, C, D).

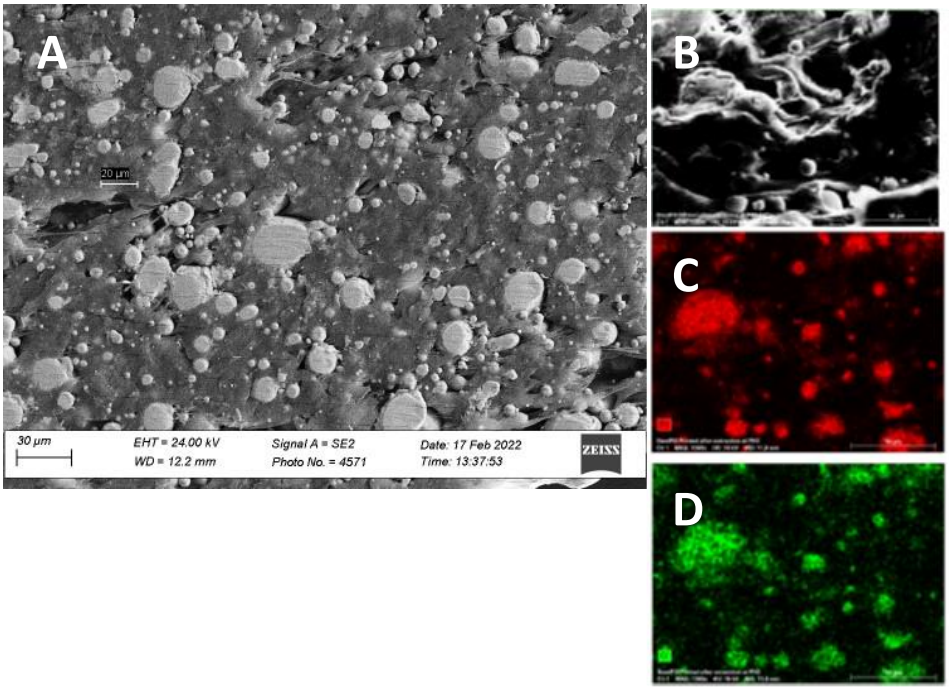


Fig. S5. The SEM-EDS images of extruded steel-fill filament cross-sections before extraction (A), and after extraction (B), false color EDS shows the presence of iron particle content (C) and chromium particle content (D). Size bars are 30 µm (A) and 100 µm (B, C, D).