Crosslinking reaction without MT polymerization. To confirm the effect of MT polymerization during the cross-linking reaction, the cross-linking reactions with tubulins without polymerization were examined. In the procedure to prepare the MT gel, bis-NHS PEG was reacted with tubulin on ice for 4 h. Gelation was confirmed by the tilt-tube method. The samples were incubated in the vials for the sol-gel transitions. Figure S1:A shows the vials inverted for 5 min at 37°C. At a CL ratio (NHS/Tubulin) of 4, the gel was not formed, and the solution remained clear, indicating that the polymerization activity of the reacted tubulins was abolished. This was in contrast to the MT gel prepared by reacting bis-NHS PEG with polymerized MTs (Figure 3B).
To confirm the cross-linking reaction of bis-NHS PEG and tubulins, the products were chilled on ice to depolymerize MTs and analyzed by SDS-PAGE and western blotting in the same way as the MT gel (Figure S1; B–D). The CL ratios (NHS/Tubulin) were 1 and 4.

These results strongly indicate that the NHS reactivity of tubulins and MTs are completely different, and the cross-linking reaction of bis-NHS PEG to tubulins leads to much lower polymerizing ability of PEG-modified tubulins compared to the standard method of MT gel preparation. Thus, the PEG-cross-linking reaction performed in the polymerized MT state was suitable for preparing an MT gel.
**Figure S1** Characterization of PEG crosslinked tubulin by SDS-PAGE and western blotting. The tubulins were cross-linked with bis-NHS-PEG in the unpolymerized state.