Designing biocompatible nanofibrous mats capable of preventing microbial colonization from resident and nosocomial bacteria for an extended period remains an unmet clinical need. In the present work, we designed antibiotic free durable antimicrobial nanofiber mats by taking advantage of synergistic interactions between polydopamine(pDA) and metal ions with varying degree of antimicrobial properties (Ag\(^{+}\), Mg\(^{2+}\), Ca\(^{2+}\), and Zn\(^{2+}\)). Microscopic analysis showed successful pDA-mediated cross-linking of the gelatin nanofibers, which further improved by the inclusion of Ag\(^{+}\), Mg\(^{2+}\), and Ca\(^{2+}\) ions as supported by mechanical and thermal studies. Spectroscopic results reinforce the presence of strong interactions between pDA and metal ions in the composite nanofibers, leading to generation of robust polymeric nanofibers. We further showed that strong pDA–Ag interactions attenuated the cell cytotoxicity and anticell proliferative properties of silver ions for immortalized keratinocytes and primary human dermal fibroblasts. pDA–Ca\(^{2+}\)/Zn\(^{2+}\) interactions rendered the composite structure sterile against methicillin-resistant Staphylococcus aureus and vancomycin-resistant Enterococcus faecium strains, whereas the silver ion-incorporated composite mats displayed broad spectrum antibacterial activity against both Gram-positive/-negative bacteria and yeast strains. We showed that the strong pDA–Ag interactions help retaining long-term antimicrobial activity of the mats for at least 40 days while attenuating mammalian cell cytotoxicity of silver ions for skin cells. Overall, the results suggest the potential of pDA–metal ion interactions for engineering sterile nanofibrous mats and expanding the antibiotic armamentarium against drug-resistant pathogens.