Cold atmospheric plasma (CAP) has recently emerged as possible treatment for opportunistic infections and cancer. CAP is composed of gas ions, superheated electrons, electric fields and reactive oxygen and nitrogen species (RONS). It can be directly applied to biological tissue because heat generation is low and has further the advantage that its reactive cocktail can be modulated by changing the composition of the feeding gas. CAP has shown to damage cell membranes with some selectivity towards bacterial membranes but its mechanism remains unclear, preventing further development of the technology. We found that CAP was able to reduce membrane packing and permeabilize bacteria after a couple of seconds. Interestingly, the intensity of these effects proceeded upon concentric ring patterns emanating from the center of the CAP treatment spot. Mass spectrometric imaging revealed that these pattern were similar to the formation of certain oxidized lipids that could be correlated to permeabilization and the reduction of packing. The formation of certain RONS by CAP also followed these spatial patterns and we can therefore conclude that the effect on bacterial membranes depends at least partly on these specific RONS.