Objectives. The clinical frame of chronic wounds, including bedsores and diabetes-associated ulcers, is generally characterised by hypoxia, exacerbated inflammation, and impaired skin tissue remodelling. Additionally, it is often worsened by bacterial or fungal infections. To counteract hypoxia, we have recently developed a new platform of oxygen nanocarriers based on chitosan as shell polysaccharide and 2H, 3H-decafluoropentane as core fluorocarbon. So-called oxygen-loaded nanodroplets (OLNDs) proved effective in delivering oxygen to hypoxic tissues either in vitro or in vivo upon ultrasound (US) activation. Intriguingly, chitosan has been reported for antimicrobial and immunological properties. Here, we evaluated OLND potential as adjuvant therapeutics for infected chronic wounds by investigating their antimicrobial activity against methicillin-resistant Staphylococcus aureus (MRSA) or Candida albicans, along with toxicity on human keratinocytes. Furthermore, we studied US abilities to promote OLND transdermal delivery, and whether US might affect OLND antimicrobial properties.

Methods. OLNDs and oxygen-free nanodroplets (OFNDs) were prepared as previously described (Magnetto et al., RSC Adv 2014; 4:38433-41) and further characterised for morphology, size, and zeta potential by transmission electron microscopy (TEM) and light scattering. Nanodroplet cytostatic activity was evaluated through microbiological assays by incubating MRSA or C. albicans (10^6 CFU/ml) with or without 10% v/v OLNDs or OFNDs for 2, 3, 4, and 24 h in the presence or absence of complementary high frequency US (f=2.5 MHz; P=5 W; t=5 min). Nanodroplet toxicity was studied by lactate dehydrogenase and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assays after incubating HaCaT keratinocytes (10^6 cells/ml) with or without 10% v/v OLNDs or OFNDs for 24 h either in normoxic (20% O_2) or hypoxic (1% O_2) conditions. High frequency US ability to trigger OLND trespassing of skin layers was tested in vitro by using a home-made apparatus comprising two sealed cylindrical chambers (donor and recipient, respectively) separated by a pig ear skin layer. OLND and chitosan amounts before and after US administration were evaluated in both chambers by TEM and fluorimetric assay.

Results. OLNDs displayed spherical morphology, 700 nm average diameters, and cationic surfaces. Either OLNDs or OFNDs showed short-term (up to 4 h) antibacterial activity against MRSA and long-term (up to 24 h) antifungal activity against C. albicans, as a likely consequence of the presence of antimicrobial chitosan in the nanodroplet’s shell. No cytotoxicity on HaCaT keratinocytes was observed after OLND administration, whereas OFNDs slightly affected cell viability, thus suggesting a protective role for oxygen on skin cells. Finally, complementary US treatment effectively promoted OLND trespassing through pig skin layers, without altering their antimicrobial properties.

Conclusion. Collectively, these data support a role for US-activated chitosan OLNDs as promising, nonconventional and innovative antimicrobial tools for adjuvant treatment of chronic wounds.