4.1 Final publishable summary report

The Bacteriosafe project developed responsive theranostic nanocapsules and nanoparticles (NC/NPs) and integrated them into sophisticated engineering processes for the manufacture of biologically active devices to revolutionise burn treatment. The program objective was to develop four main types of NC/NPs which were at TRL 2 and below at the beginning of the project. Over the course of the project materials were evaluated and optimized up to TRL 5-6 with the most promising materials completing prototype demonstration in a relevant environment. All engineering processes were set up and evaluated up to TRL 6 providing results from laboratory testing of a prototype system that is near the desired configuration in terms of performance and volume. The materials included phospholipid – fatty acid vesicles, amphiphilic block copolymer systems, mini-emulsion polymerised nanocapsules and hybrid (theranostic) nanocapsules. In order to reach the goal of materials at TRL 6 over 300 formulations of materials went through a rigorous selection process and only the most promising were carried forward for further development and implementation into engineering processes for wound dressing development.

In order to bring the nanocapsules onto the modified non-woven, we have investigated different processes including: (i) activation of the non woven materials using plasma assisted surface modification (reaching TRL 6), (ii) the deposition of "adhesive" thin films followed by electrostatic immobilisation of NC/NPs, which reached TRL 4-5, (ii) spraying of droplets containing the nanocapsules onto the textile reaching TRL 6 , (iii) ink jet printing the NC/NPs onto a continuous non woven fabric, which reached TRL 6 and (iv) immobilization of the nanocapsules within surface attached hydrogel materials which also reached TRL 6. Several optimized NC/NPs systems that have been validated in simulated wound environment (TRL 6) and selected engineering solutions for the manufacture of prototypes (TRL 6) are available.

The biological performance of the nanocapsules, both in suspension and immobilized on the surface of non-wovens have been evaluated by the biology-clinical team of the project reaching TRL 5 with functionality tests in simulated environment with selected NC/NP formulations. This has been done *in vitro* as well as with some mimics *in vivo* studies using cell tissue cultures. Selected systems are now entering pre-clinical trials at TRL6. Characterization, evaluation and selection of the most promising have been based on stability, ease of synthesis, processability and suitability for upscaling, bio-functionality and cytocompatibility. The microbiological and cell biological investigations form the basis of decisions taken during validation of the different materials. Selected microbiological insights and tests include (i) Minimum Inhibitory Concentrations of different antiseptics (2) hyaluronidase activity of clinical isolates of *S. aureus* strains, determination of hyaluronidase activity compared to a hyaluronidase standard, and (3) tests to detect the cleavage of a fluorescence labeled peptide sequence integrated in a capsule shell. Toxicity tests investigated the effect on inflammatory marker expression in endothelial cells and determined endotoxin residues. In addition and to enable knowledge based decisions within the program bacterial virulence factors, molecular genetics and quorum sensing mechanisms were studied both qualitatively and quantitatively. For all processes and procedures the consortium has established Standard Operating Conditions (SOPs) to ensure reproducibility and consistency. The final prototype dressing prepared at TRL 6, using a dip coated approach, already shows the potential of a TRL 6 wound dressing that will release a fluorescent indicator and antimicrobial components in response to interaction with bacterial cytolytic virulence factors.