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Summary Report WP1

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Author(s)	Juliana de Souza Nunes (BIOG)
FORCE REPAIR reviewers	Camilo Cortés (VICOM), Damien Dupin (CID)
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R: Document, report (excluding the periodic and final reports)
DEM: Demonstrator, pilot, prototype, plan designs
DEC: Websites, patents filing, press & media actions, videos, etc.

[2] **Dissemination level**: Use one of the following codes (in consistence with the Description of the Action)
PU: Public, fully open, e.g. web
SEN: Sensitive, limited under conditions of the Grant Agreement

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1 Introduction

This deliverable is a summary of work performed in WP1 - Bio-inks formulation, Characterization & Testing. This summary contains the main highlights of findings of WP1, and in compliance with intellectual property protection, no confidential information is given.

2 Summary

The main goal of Work Package 1 (WP1) of the FORCE REPAIR project is the design, formulation, and characterization of bioactive inks intended for the development of smart and multifunctional wound dressings. These dressings are based on a 3D printed scaffold intended to address chronic wound lesions in a personalized manner. They will work by modulating mechanical forces, reducing infections, and promoting skin regeneration. This is achieved by strategically incorporating biomaterial components within the scaffold to achieve several biological responses, simultaneously or in cascade, thereby facilitating wound care management for healthcare professionals and patients. The smart wound dressing will be designed to offer a prolonged regenerative effect over at least 15 days, significantly reducing the current daily frequency of nurse attention to change the dressing, to achieve a healed wound with low probability of reopening. The dressing of the project is based on a unique self-healing hyaluronic hydrogel (HA-Ag-DH) loaded with components that will exert different functions in wounds. To this end, three different bioactive inks were developed in WP1:

- **Antibiotic ink**, containing antibiotic nanocapsules to reduce bacterial infection.
- **Anti-inflammatory ink**, containing anti-inflammatory nanocapsules to reduce inflammation to healthy levels.
- **Pro-regenerative ink**, loaded with elastin-like peptide (ELP), Wharton Gel Complex (WGC) and laminin peptide to relieve skin tension and promote a full tissue regeneration.

To achieve the objectives of FORCE REPAIR, six tasks were defined in WP1:

- **Task 1.1:** Design of ELPs and enzyme-responsive polypeptides (CNRS; M1-M18)
- **Task 1.2**: Formulation and Characterization of stimuli-responsive nanocapsules (CID; M1-M12)
- **Task 1.3:** Formulation & Rheological prop. of the combined bio-inks (BIOG, CID, IDO, UNIPV, HCELL; M1-M30)
- **Task1.4:** Study of the bioadhesive properties (UNIPV, CID, M1-M18)
- **Task 1.5:** Release & biodegradability studies of the combined bio-ink (CNRS, BIOG, CID; M6-M30)
- **Task 1.6:** Preliminary 3D printing of formulated bio-inks (IDO, BIOG, CID, VICOM; M1-M30).

In Task 1.1, ELP was modified with methacrylate moieties to enable the crosslinking to hydrogel matrix. The ELP selected exhibited the desired low critical solution temperature (LCST, 30-32°C) for subsequent UV curing on skin. Task 1.2 focused on the formulation of stimuli-responsive nanocapsules. Parameters such as concentration and molecular weight of the stabilizer, phase used to solubilize the drug, presence of crosslinking points of the stabilizer, and stability under relevant conditions (physiological pH 7.4 and skin pH 5) were investigated with the objective to select the optimal formulation with the highest drug concentration and stability over time. Findings of task 1.2 combined with outputs of WP3 helped to select the best nanoformulations for antibiotic and anti-inflammatory inks.

Task 1.3 aimed to establish the formulation of the three different bioactive inks that met bioprinting requirements (Task 1.6). Initial efforts were centered on the functionalization of hyaluronic acid and on the selection of a printable self-healing hyaluronic hydrogel (HA-Ag-DH). Once this step was

completed, the effect of adding drug-loaded nanocapsules, ELP, WGC and laminin peptide on the rheology and printability of hydrogels were assessed. Results of Task 1.4 demonstrated excellent bioadhesive properties of inks on biological substrates. In Task 1.5, the physicochemical behavior of the dynamic hydrogel matrix was investigated through swelling and biodegradability studies in phosphate-buffered saline (PBS) and PBS containing hyaluronidase at concentrations representative of chronic wounds. Drug release studies from nanocapsules and from final inks were studied by UV-vis spectrophotometry (SOTAX) and high-performance liquid chromatography (HPLC) techniques. Release of the anti-inflammatory agents from nanoemulsions alone showed clear pH-dependent behavior and presented slower kinetics once incorporated into the bio-inks, consistent with expected changes after exposition to physiological conditions and diffusion barriers imposed by hydrogel matrix. Some drawbacks were encountered in quantifying the antibiotic content released due to its low concentration within the polymer matrix. Swelling and biodegradability studies indicate that hydrogels can absorb substantial amounts of medium, which is interesting for exudate management in chronic wounds. In the presence of hyaluronidase, enzymatic degradation was accelerated, with higher enzyme concentrations leading to faster and more extensive mass loss. These results confirm that the hydrogel system is stimulus-responsive, supporting its suitability for wound-triggered degradation and drug release.

3 Conclusion

In summary, WP1 successfully developed hyaluronic acid-based bioactive inks with tunable drug release and enzyme-responsive degradation. The systems exhibit key features required for multifunctional wound dressings, including pH sensitivity, drug release, and biodegradability under wound-relevant conditions. Although the analytical quantification of antibiotics remains challenging, the overall performance of the three bioactive inks is promising. WP1 has established a strong scientific and technological foundation for the development of advanced, stimulus-responsive wound dressings within the FORCE REPAIR project.

4 Next steps

This deliverable completes the activities related to WP1 - Bio-inks formulation, Characterization & Testing. Along with the bIDOstation 3D bioprinter and the bioprinting CAD-CAM software developed in WP2, the bio-inks will be used during *Task 2.5: Testing and validation of the 3D printing protocol*. Finally, the bio-inks will be used in WP5 for the printing of the dressing to be used in WP4 for the in vivo experiments.