

Tea Tree Oil loaded Collagen/Hap Aerogel Construct for Chronic Wound Healing

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PORTO

Introduction

Chronic wounds are slow healing skin lesions, often highly exudative, and normally associated with microorganism colonization, which worsens the tissue health and delays the healing even further.

Collagen is the main constituent of the extracellular matrix of several physiologic structures, while calcium is known to be one of its major signal regulators. Regarding skin, calcium regulates collagen deposition, influences fibroblast migration and proliferation, regulates angiogenesis and helps to repair the skin barrier.

Tea Tree Oil (TTO) is a natural extract that possesses antimicrobial, anti-oxidant, and anti-inflammatory properties and has been used in many topical formulations.

In the present work, a novel aerogel formulation has been developed by the combination of collagen (COL), hydroxyapatite (Hap) and TTO to produce scaffolds with interconnected porosity, fluid absorption capacity, and adequate mechanical properties to be used in wound healing.

Methods

Slurries containing collagen and different hydroxyapatite contents were mixed, poured into molds and frozen. Porous aerogel scaffolds were obtained by freeze-drying the slurries.

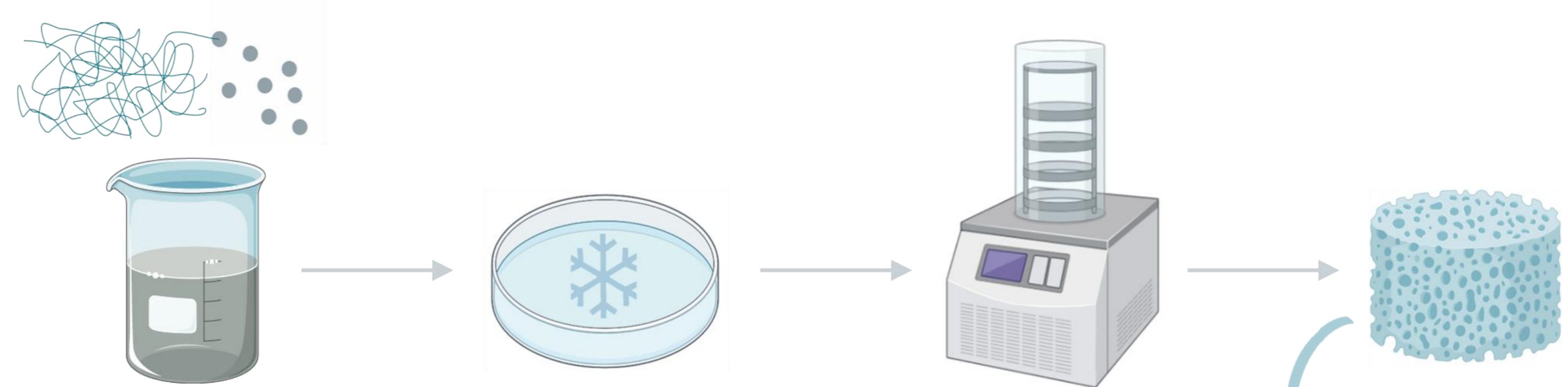


Figure 1. Schematic representation of COL/Hap aerogel scaffolds production method.

- Chemical and mechanical characterization;
- Morphologic characterization;
- Biocompatibility assay;
- Morphologic characterization;
- Microbiological activity.

Results & Discussion

Samples without HAP (**Control**), **0.25Hap**, **0.5Hap**, **0.75Hap** and **1Hap** were chemically characterized by FTIR and their mechanical properties were assessed by a mechanical compression test.

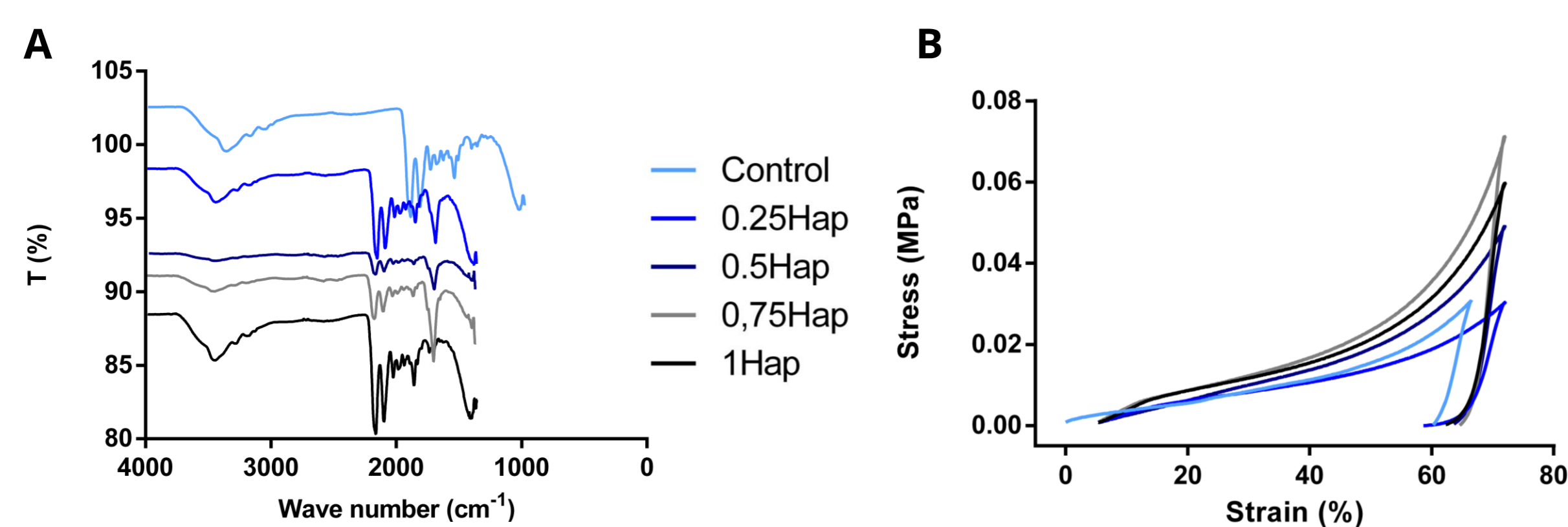


Figure 2. Chemical and mechanical characterization of the five samples presented as: (A) FTIR spectra; (B) stress-strain curve.

To assess scaffolds **biocompatibility** Human Dermal Fibroblasts (HDFs) were seeded directly on top of the materials. At each timepoint, cells were incubated with resazurin.

As depicted in **Figure 3**, the scaffolds were biocompatible and the presence of hydroxyapatite slightly **increased cellular proliferation**.

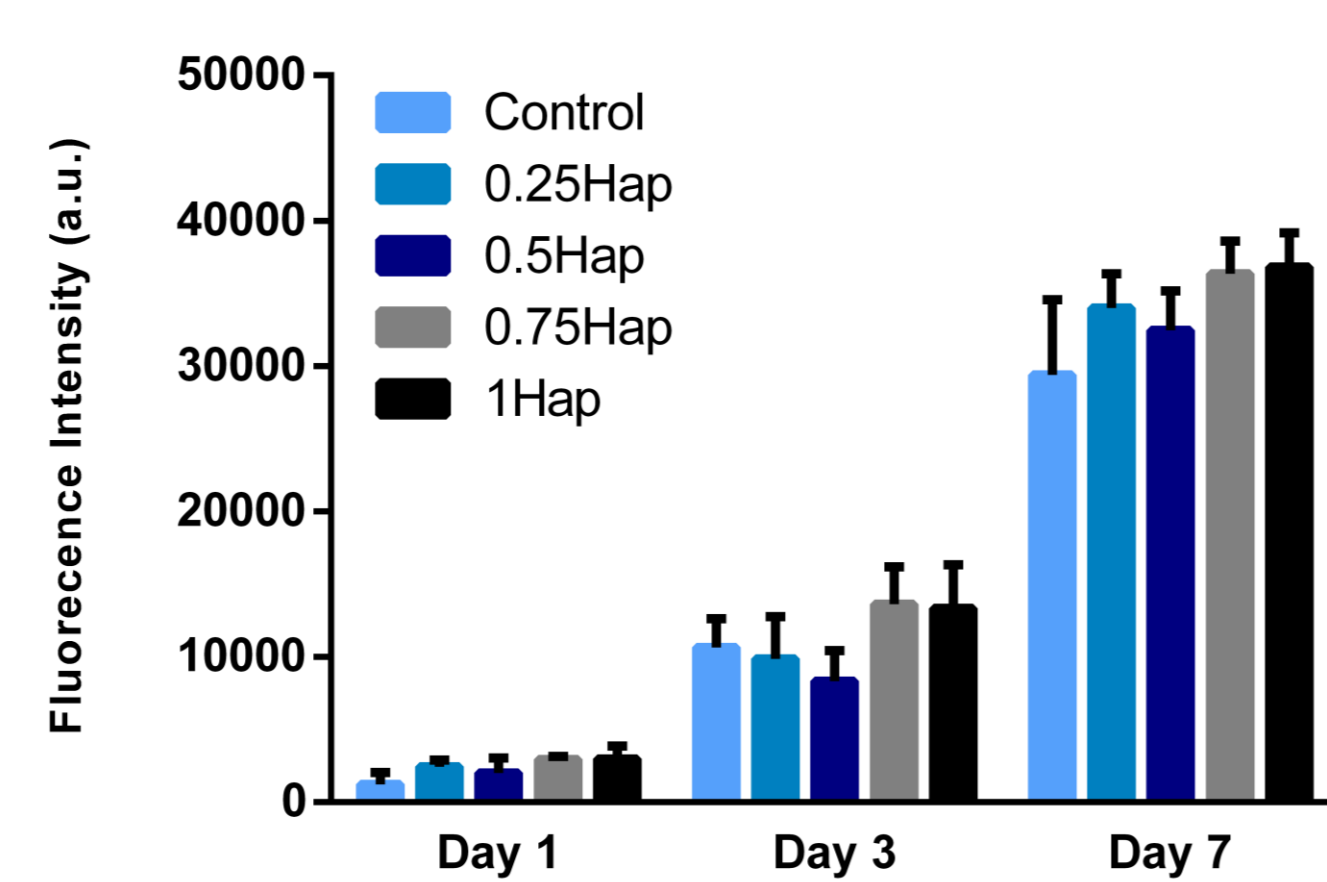


Figure 3. HDFs viability assessed with Resazurin assay at 1, 3 and 7 days of culture. At each timepoint, cells were incubated with resazurin supplemented medium for two hours and the medium fluorescence was measured

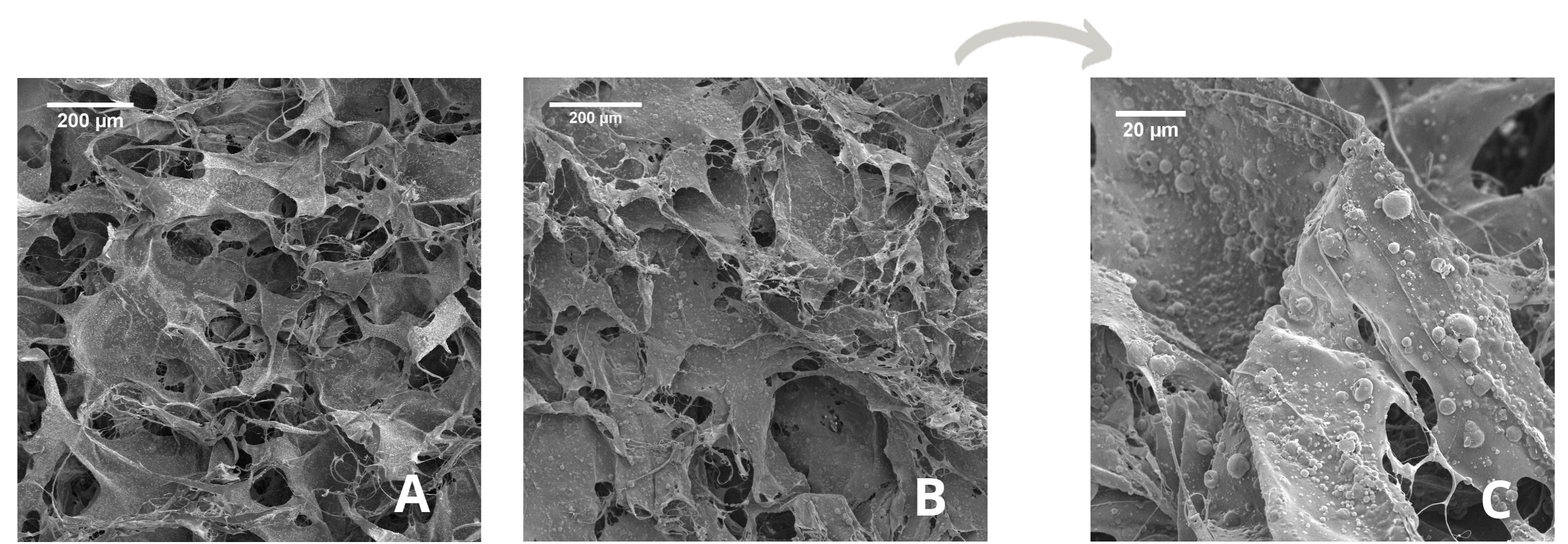


Figure 4. SEM images of samples (A) Control and (B) 1Hap with 200 µm scale and (C) 1Hap with 20 µm scale.

Microbiology tests with TTO emulsions showed that it started exhibiting anti-microbial effects at 1% (bacteriostatic) and had a bactericide effect at 5%. Therefore, Control and 1Hap scaffolds were impregnated with 1%, 2.5% and 5% TTO and tested against different strands.

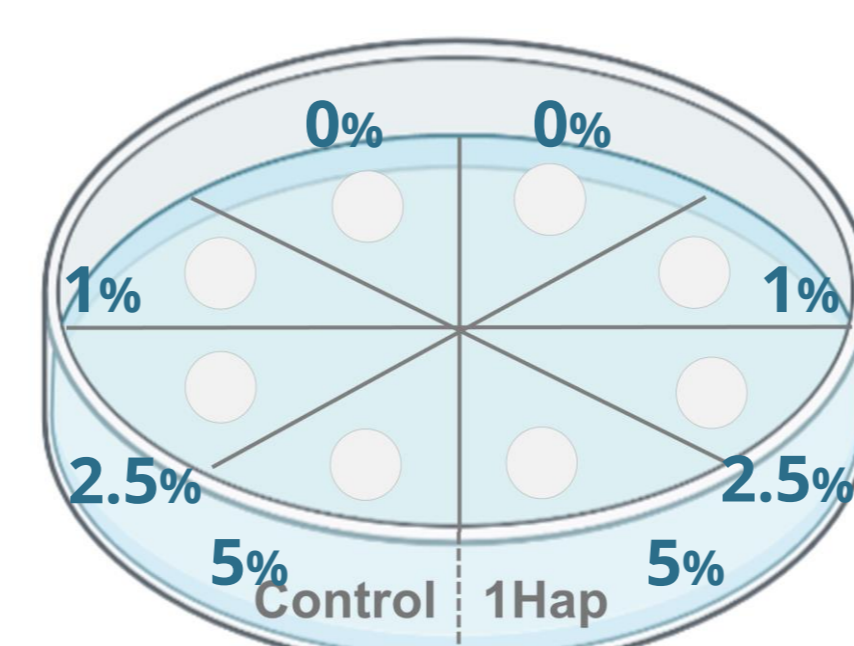


Figure 5. Schematic representation of Mueller-Hinton agar diffusion.

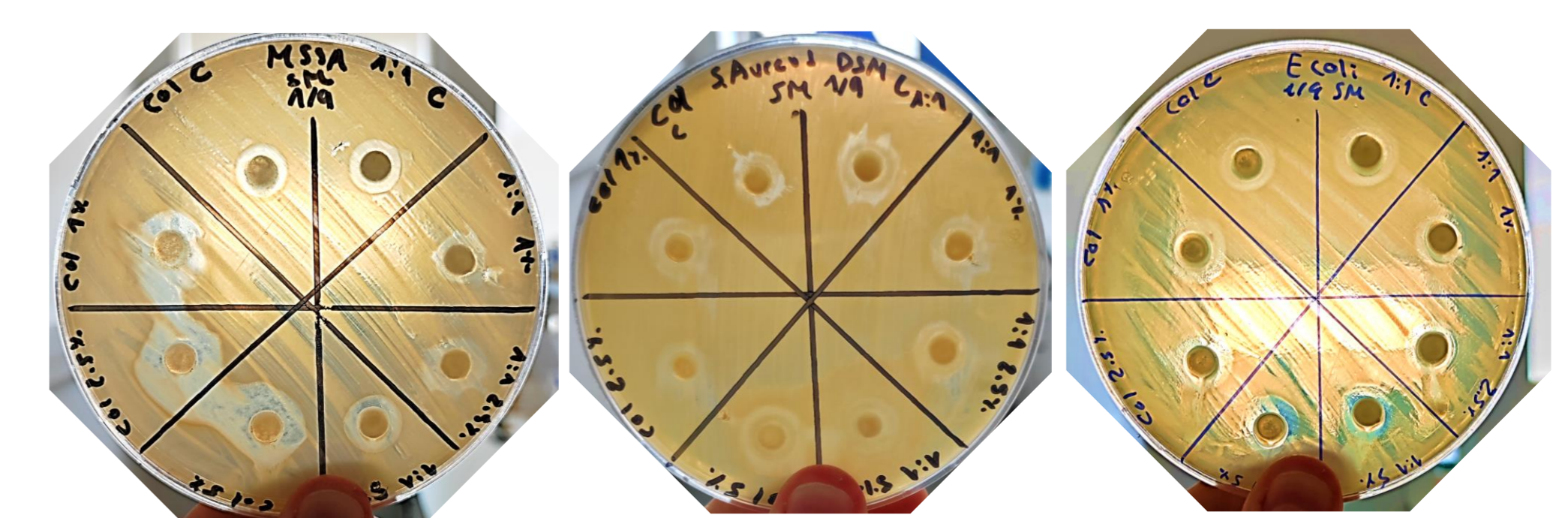


Figure 6. Results of the Mueller-Hinton agar diffusion against: (A) *S.Aureus* (MSSA), Gram+; (B) *S.Aureus* DSM, Gram+ (DSM) and (C) *E.Coli*, Gram -.

No bactericide effects were shown in this test. At 1%, TTO was **bacteriostatic** for the *S.aureus* MSSA strand and for DSM at 5%, in the Control samples.

In the samples containing Hap and for all samples tested with *E.coli*, no differences were found when compared with the control (without TTO).

Conclusions

This preliminary study demonstrates that the obtained hybrid material can offer a complete and promising approach for the healing and regeneration of chronic wounds, while preventing infection.

We were able to confirm that the collagen/hydroxyapatite aerogels are biocompatible and that the presence of Hap as calcium source, promotes fibroblast proliferation. Further test should be made to optimize the impregnation of TTO onto the scaffolds and consequently, studies will be conducted regarding the antimicrobial activity and cell behavior of the final materials.

Acknowledgements

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