CONTINUOUS PRODUCTION OF HYDROXYAPATITE/SERICIN COMPOSITES AND *IN VITRO* VALIDATION FOR SKIN REGENERATION

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Introduction and Objectives

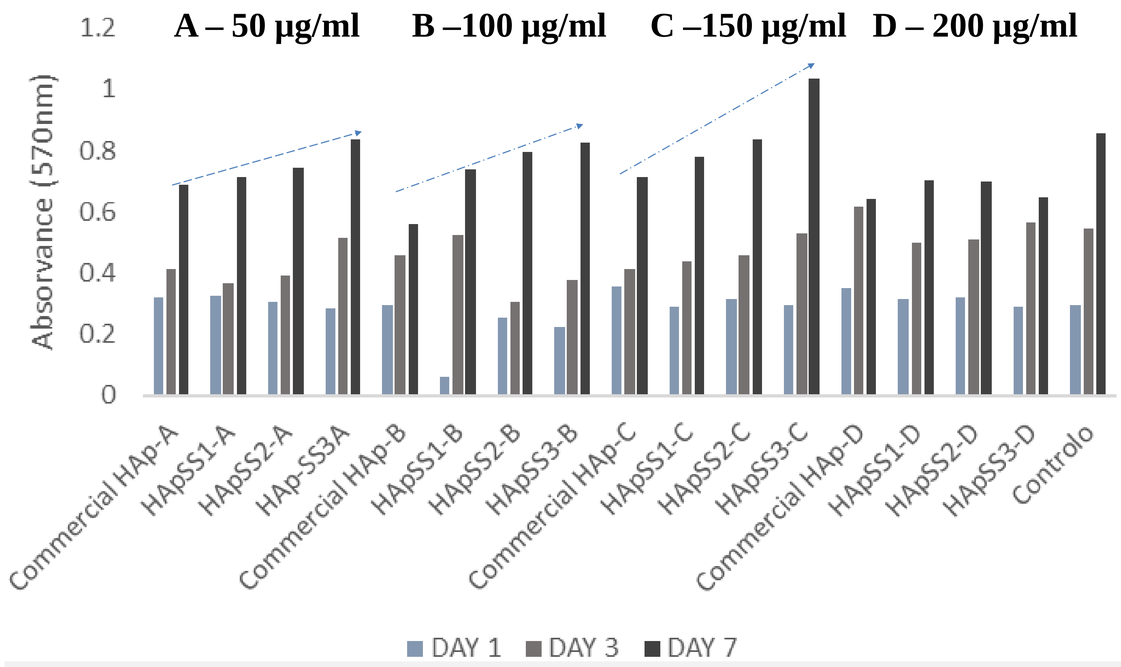
Calcium phosphates (CaPs) are well established materials for bone tissue engineering. However, new possibilities arise for this "old" bioceramic. The latest studies are focusing on reinventing CaPs for skin tissue engineering [1]. Calcium has demonstrated to play an important role in the barrier function repair and skin homeostasis and serves as a modulator in cell proliferation and differentiation.

Recently, we have proposed nano-Hydroxyapatite(HAp) /sericin (SS) composites as suitable candidates for tissue engineering approaches [2]. Silk SS, until recently considered unfit for biomedical use, it is now accepted as a valuable byproduct from the textile industry, able to stimulate collagen production and to elicit an antioxidant, moisturizing and anti-inflammatory effect [3].

The effectiveness of cell attachment on composite particles highly depends on their physicochemical characteristics which in turn is directly depending on the processing strategy. Continuous flow regimes operated through Oscillatory flow reactors (OFRs), improve particle mixing, generating a product with uniform and controlled characteristics.

In the present study HAp/SS composites with different SS concentrations (**HApSS1, HApSS2 and HApSS3: 0.5, 1 and 1.5 g/L**) were produced for the first time using a Modular Oscillatory Flow Plate Reactor (MOFPR). The effects on the physicochemical and biological properties were investigated.

Cell viability (MTT assay)

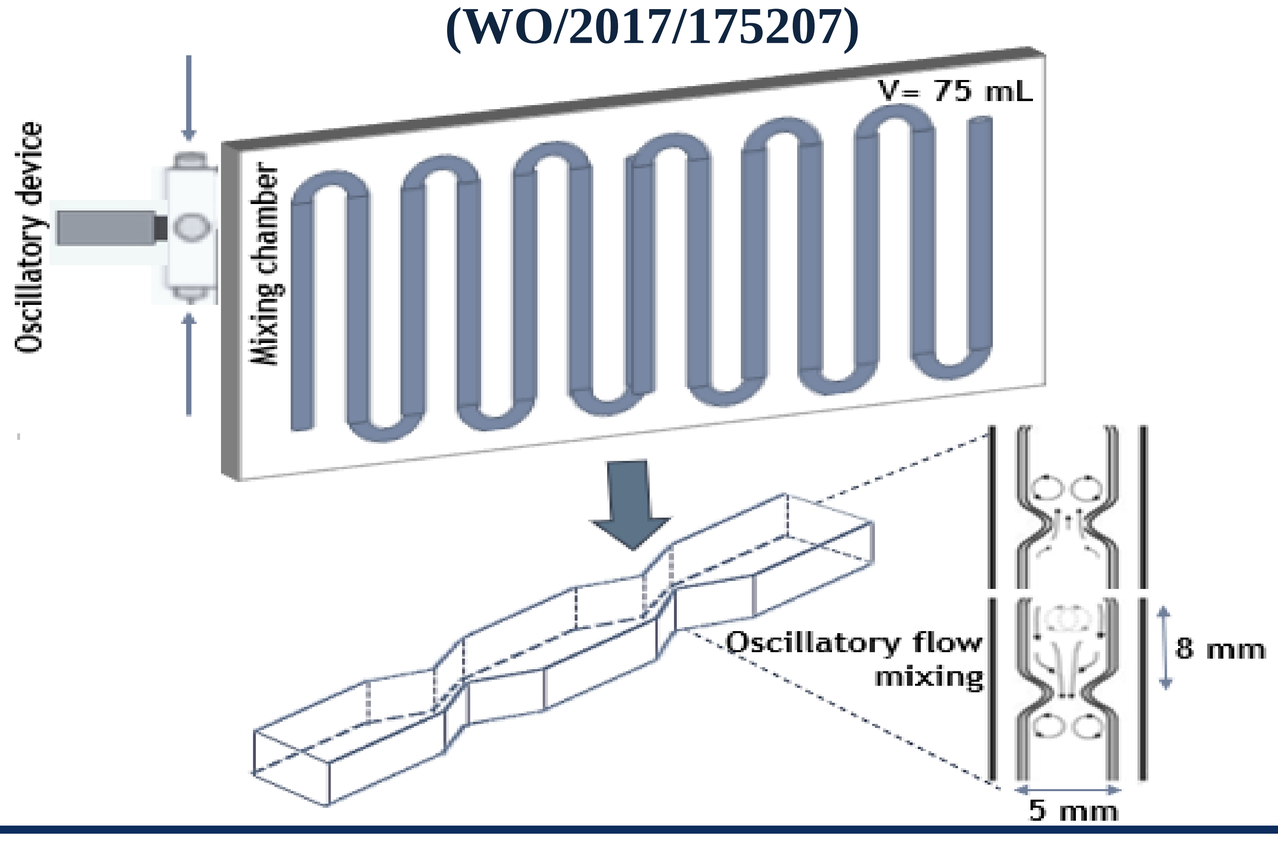


Preliminary *in vitro* results with human dermal fibroblasts (HDFs) demonstrated that after 7 days of culture HAp/SS3 particles promote cell viability. This effect is evident at concentrations of 50-150 µg/ml, being more pronounced at the highest concentration of sericin. In addition, all the conditions studied showed greater viability than commercial HAp, with the exception of the concentration of 200 µg/ml in which all particles generated similar results.

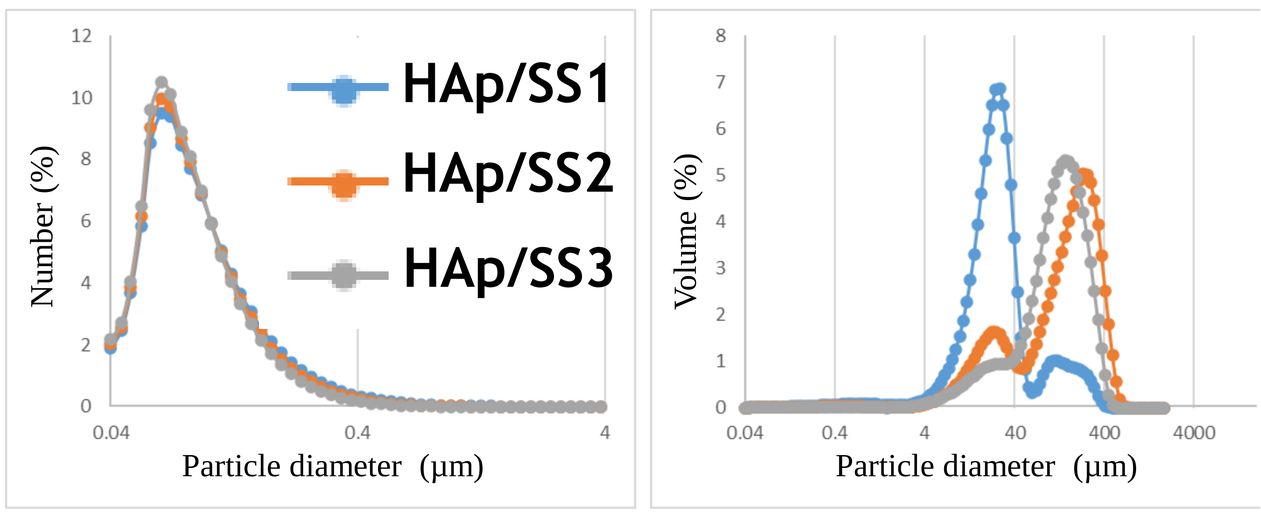
Conclusions

The study carried out constitutes a first validation of the use of HAp/SS nanocomposites for skin tissue engineering application. It was shown that the highest SS concentration (1.5 g/L) resulted in greater cell viability, and that the optimal concentration of particles in contact with HDFs is 150 µg /ml . These particles can be further developed as a topical powder for wound care or used as a cell carrier in 3D constructs.

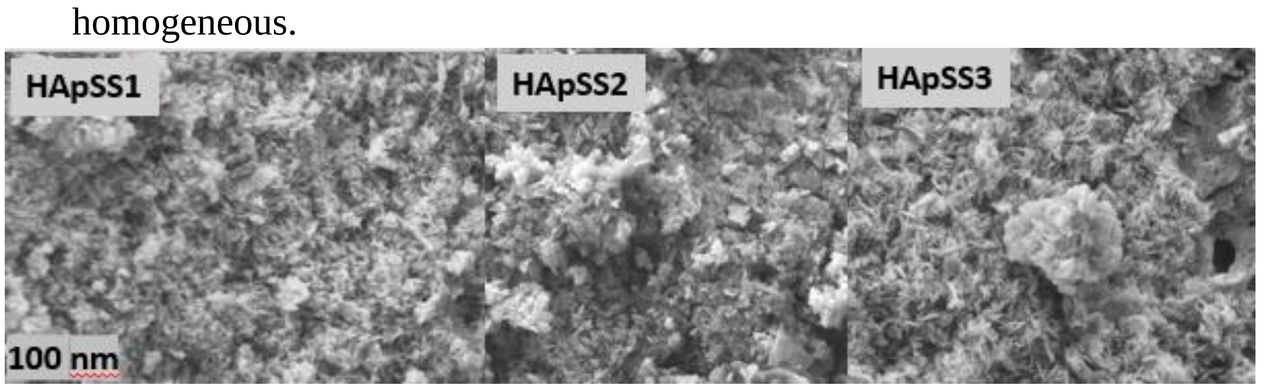
Modular Oscillatory Flow Plate Reactor (WO/2017/175207)



Physicochemical characterization: laser diffraction, HRSEM, FTIR,EDX



- The synthetized particles have a mean size (d_{50}) of ≈ 75 nm.
- The width of the size distribution in number decreases with increasing SS (span of 1.6, 1.4 and 1.0 respectively).
- Particle size distribution in volume evidences the existence of large aggregates, mainly for the highest sericin concentrations (d_{50} of 23.9, 149.0 and 118.1, respectively).
- The observations are in agreement with SEM images, where aggregated rod and plate-like nano-sized particles are visible for all the samples. Moreover, with increasing SS the particles appear to be more homogeneous.



- The Ca/P ratio was determined by EDS, demonstrating that for HAp/SS1 and HAp/SS2 the ratio is close to stoichiometric (1.67).
- On the other hand, HAp/SS3 has a Ca/P ratio of 2.04 characteristic of carbonated HAp.

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