Abstract #503 | Oral

Functional Silk Sericin-Calcium Loaded Hydrogels: Advancing Towards Human Skin Equivalents

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Silk sericin (SS), is a protein traditionally discarded during industrial silk processing, contaminating waste waters, with negative economic and ecological impact to the environment. In recent years there has been a growing interest in the recovery and utilization of SS due to its interesting biological properties. SS-based biomaterial platforms, such as hydrogels, are capable of cell incorporation and maintenance over time, acting as a nutritive natural-based environment for cell proliferation[1]. This opens new avenues to develop more reliable and reproducible *in vitro* models for a better understanding of human skin conditions while minimizing animal studies.

Our team has previously developed an enzymatic crosslinked SS hydrogel using horseradish peroxidase (HRP), to be applied *in situ* for wound healing. This hydrogel promoted cell viability and complete skin regeneration after 21 days when applied in a diabetic wound model[2]. These promising results have motivated the use of this formulation as a platform for cell encapsulation, in an approach to develop a natural-based human skin equivalent (HSE). The incorporation of nanoparticles (NPs) within hydrogels is reported to further enhance the biological behavior of encapsulated cells[3]. In this context, calcium plays an important role in maintaining skin homeostasis and modulating cell proliferation and differentiation[4]. In a recent study, we explored hydroxyapatite (HAp) and HAp/SS NPs as materials to enhance the adhesion and proliferation of human dermal fibroblasts (HDFs), validating the use of this particulate system to support cell growth. The NPs were produced using a continuous manufacturing process in a new modular oscillatory flow plate reactor (MOFPR). The reaction system enables the production of tailored and homogeneous NPs.

In the present work, HDFs and HaCaT were incorporated within a SS/HRP hydrogel to construct a HSE. The system was further optimized with the addition of NPs to the system: a screening was conducted using different HAp and HAp/SS NP concentrations. Our results show that the HAp/SS particles at a low concentration, were associated with the best biological performance (0.05 mg/mL). The co-culture SS system was assembled with a stable silk-fibroin (SF) porous scaffold embedded with human adipose tissue with the addition of neural cells (hiNSCs), as reported by Vidal et al.[5] to develop a full-thickness HSE (**Figure 1**). The sustained viability of the cells in the model over 21 days suggests the formation of a stable and reproducible model representing well some of the characteristics and functionality of native skin (**Figure 2**).

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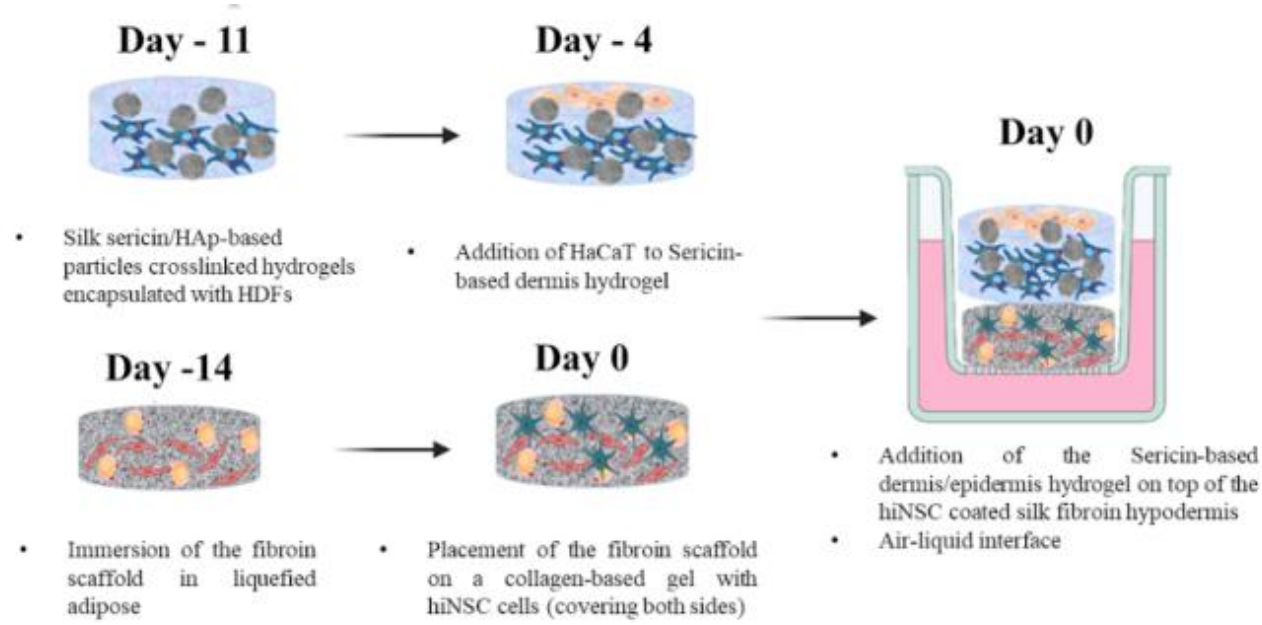

Figure 1.
Schematic representation for the development of the silk-based HSE.

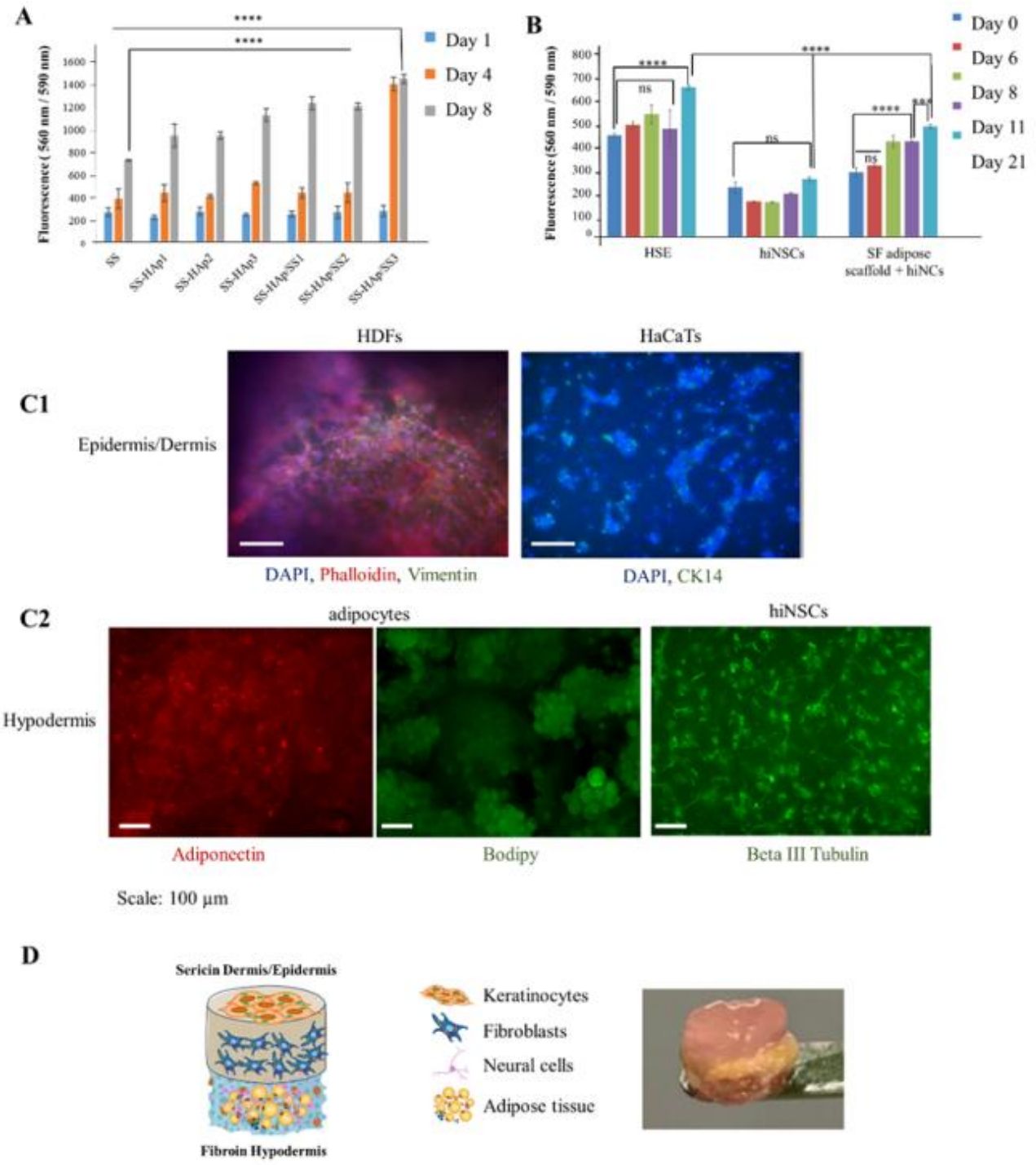

Figure 2.
A) AlamarBlue cell metabolic activity for SS, SS/HAp, and SS/HAp/SS nanoparticles (using different concentrations 1(1), 0.5(2) and 0.05(3) mg/mL) and significant statistical differences registered after 8 days of cell culture (n=8); **B**) cell metabolic activity of the silk-based HSE and controls (hiNSCs and SF adipose tissue with hiNSCs); **C)** Immunostaining of silk-epidermis (HDFs) after 14 days of culture; silk dermis (HaCaTs) after 4 days of culture (C1) and silk HSE after 21 days after model assembly (C2), and (D) macroscopic image of the silk-based HSE.