The characterisation and antimicrobial activity of Ag-nanoparticle-incorporated eggshell membrane for dermal applications

Rosemond A. Mensah[1], Federico Trotta[2], Emily Briggs[1,3,] Nik San Sharifulden[1], Lady V. Barrios Silva[1], Alvena K. Kureshi[4] and David Y. S. Chau[1]

1. Division of Biomaterials and Tissue Engineering, Eastman Dental Institute, University College London, Royal Free Hospital, Rowland Hill Street, London NW3 2PF, UK
2. Metalchemy Limited, 71-75 Shelton Street, London, WC2H 9JQ, UK
3. Department of Materials, Henry Royce Institute, The University of Manchester, Rumford Street, Manchester, M13 9PL, UK
4. Centre for 3D Models of Health and Disease, Division of Surgery and Interventional Science, University College London, Charles Bell House, Foley Street, London, W1W 7TY, UK

***** Correspondence: d.chau@ucl.ac.uk

INTRODUCTION: Current treatments to repair and/or replace skin are severely limited. The egg shell membrane (ESM) is a unique natural material with innate physical and mechanical properties that provides optimal barrier properties and also exhibits biocompatibility/inherent biodegradability. [1, 2]. The ESM composes of three distinct layers: the inner and the outer membranes (fibrous structures) and the limiting membrane (dense structures). The ability to separate the ESM from the eggshell is vital to its application and various methods of extraction have been employed which led to differences in their final composition, structure, biological characteristics and wound healing effects [2]. In this study, ESMs were extracted using different techniques and further enhanced by incorporating with either commercially available silver nanoparticles (AgNP) [Sigma-Aldrich] or, novel AgNP manufactured by means of a unique green patented process [Metalchemy]. These modified samples were thereafter profiled in terms of the physical, biological and antimicrobial characteristics with an ultimate view for dermal applications.

METHODS: ESM samples were prepared using either a manual and/ or an optimised acid decellularization protocol. The ESMs were incubated with AgNPs solution for 20 hours and air-dried at room temperature (~19°C). The deposition of the AgNP on the ESM was confirmed using FT-IR and SEM. Metabolic activity assessment (i.e., MTS assay), LDH-release profiles and Live/Dead staining demonstrated good attachment and spreading to the samples, and high cell viability following 5 days of culture. The chicken embryo chorioallantoic membrane (CAM) assay was also employed to ascertain the angiogenic potential of the AgNP-ESMs. Assessment of the antimicrobial activity of the enhanced AgNP-ESMs were validated using the International Standard ISO 16869:2008 methodology and exploited Cladosporium as the test microorganism ($\geq 5x10^6$ cells/ml).

RESULTS: AgNP-ESMs samples were successfully fabricated and fully characterized. HDFs (PCS-201-012) and BJ (CRL-2522) cells cultured on the AgNP-ESMs samples demonstrated high biocompatibility in terms of high cell attachment, spreading, viability and proliferation rates/characteristics and also allowed the promotion of new blood vessels (i.e., pro-angiogenic) in the CAM assay. The AgNP-ESMs exhibited good antimicrobial activity with the Metalachemy-modified samples demonstrating an exceptionally strong inhibitory effect.

DISCUSSION & CONCLUSIONS: In summary, AgNPs were successfully loaded into the ESMs extracted with different methods. The AgNP-ESMs developed exhibited desirable physical and biological properties for clinical/therapeutic dermal wound dressing and, in context of large-scale commercialization, aspects of "green process technology" and sustainability makes this an exciting opportunity.

REFERENCES

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