Incorporation of α-tocopherol in chitosan-based 3D printed scaffolds for tissue engineering

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INTRODUCTION

Skin is a complex organ, providing thermoregulation mechanisms, sensitive capacities and physical protection to the organism1. Deep and wide wounds can impair these functions, thus, the slower would be the repair process, the higher the risk to develop inflammations, infections and scar tissue. Regenerative medicine proposes innovative approaches to enhance wound healing based on the development of chitosan scaffolds characterized by high porosity (simulating ECM and permitting nutrients diffusion) and mechanical properties similar to the autologue tissue2. Furthermore, the 3D printing technique allows both the construction of ad hoc architectures and the distribution of active molecules in the system3. Vitamin E is a polyunsatured fatty acid transported in plasma by a specific transport protein (α-TTP) and it's the most active compound among tocopherols, presenting anti-oxidant and anti-inflammatory activity and playing an important role in keratin turnover and homeostasis in skin tissue4. The targeted result is the development of scaffolds containing a repeatable titer of vitamin E, homogeneously dispersed in the system and capable to enhance cell adhesion and proliferation.

MATERIALS AND METHODS

A 6% w/v chitosan solution was prepared in 2% v/v of acetic acid. Raffinose was blended as hygroscopic thickener agent. α-Tocopherol was added at 1 or 0.1% w/w and homogenized by a rotational dispersion system to obtain an emulsion. The material was then dispensed by the 3D printer nozzle on a freezing surface to maintain the designed framework, successively fixed by gelation in alkaline medium. HPLC stability tests have been conducted dissolving α-tocopherol in methanol and 2% v/v of acetic acid (pH: 4.5) and exposing analogue samples to NH3 vapours (pH: 11). Stability and homogeneity of α-tocopherol in the formulations has been estimated randomly collecting samples just after preparation and till 48 hours. Elastic modulus was obtained by performing traction tests (5 DaN top head; traction speed of 25 mm/min), whereas scaffold moisture content has been determined by gravimetric analysis. In vitro cell cultures highlined an high citocompatibility, cells attached independently the scaffolds seeded with a slight delay of adhesion in the 1% scaffolds, reaching confluence after 28 days.

RESULTS

α-Tocopherol results stable in the acid environment tested with a recovery of 98±5% and in alkaline conditions with a recovery of 99.7%, after 1 hour of exposition to NH3 vapours (time needed for a complete gelation). Sterilization in Ethanol 70 causes a proportional loss of vitamin in function of time (50 ± 1% after 6 hours). Both emulsions result homogeneous and stable within 48 hours. Scaffolds at 1 and 0.1% of vitamin can rehydrate respectively 96±2 and 93±8% starting from 67% of hydration. Calculated elastic modulus of hydrated scaffolds at 24°C is 0.118 and 0.119 MPa. In vitro cell cultures highlined an high citocompatibility, cells attached independently the scaffolds seeded with a slight delay of adhesion in the 1% scaffolds, reaching confluence after 28 days.

CONCLUSIONS

This work demonstrated the possibility to add α-tocopherol homogeneously in the chitosan-based formulation to be printed, in a stable and repeatable way. Chemico-physical parameters are compatible with those reported in literature for what concerns skin tissues, additionally, high biocompatibility highlighted by cells cultures suggests a potential scaffold application in the wound healing field.

REFERENCES

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