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Development of a hyaluronic acid/plasma-derived hydrogels for the optimization of dermo-epidermal autologous equivalents

Andrés Montero¹, Marta García^{1,2,3}, Diego Velasco^{1,3}, José Luis Jorcano^{1,2}

¹ Department of Bioengineering and Aerospace, Universidad Carlos III de Madrid (UC3M), Leganés (Madrid), Spain

² Regenerative Medicine Unit and Epithelial Biomedicine Division, CIEMAT, Madrid, Spain

³ Instituto de Investigación Sanitaria de la Fundación Jiménez Díaz, Madrid, Spain

anmonter@ing.uc3m.es

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Introduction. Over the past several years, the extensive use of laboratory-grown plasma-based skin equivalents for the treatment of patients with skin injuries has allowed the identification of some practical problems. The main issues comprise the poor mechanical properties and the shrinking during *in vitro* culture of plasma-derived fibrin hydrogels [1]. Furthermore this low stiffness make more difficult surgical handling which might compromise graft integrity before implantation [2]. The combination of plasma-derived fibrin with other natural polymers can be considered for improving hydrogel mechanical properties without compromising biocompatibility. Hyaluronic acid (HA) is a natural polymer present in the extracellular matrix (ECM) of the skin (estimated 0,05-0,3% w/v). For this reason, in this work, commercially available thiolated form of hyaluronic acid crosslinked with poly (ethylene glycol) diacrylate (PEGDA) was incorporated in a well-established protocol in order to improve biological and mechanical properties of plasma dermal equivalents.

Methods. Plasma-derived fibrin hydrogel (without HA) was prepared following the protocol described previously [3]. The protocol was modified to incorporate thiolated HA (0,05-0,1-0,2% w/v) and PEGDA (2:1, 6:1, 10:1 and 14:1 thiol:double bond mole ratio). Gelation times, swelling/deswelling dynamics, the capacity of human fibroblast to contract the hydrogels was studied at days 0, 1, 4, 7, 10 and 15. Structure was studied through SEM visualization and protein release through Bradford assay. Human fibroblast were embedded in the hydrogels and Alamar Blue proliferation assay was performed.

Results. Introduction of HA and PEGDA in fibrin hydrogels increased the gelation time, which showed a growing tendency upon increased HA and PEGDA concentrations. Plasma hydrogels showed contractile behaviors both in the presence and absence of human fibroblast. The combination of HA and fibrin hydrogels gave a satisfactory result delaying contraction of hydrogels and inhibiting protein release. Structural study showed increasing density corroborated visually (SEM). Alamar Blue proliferation assay yielded positive results by increasing overall proliferation rate when HA and PEGDA was incorporated (HA 0,1% 6:1 PEGDA).

Conclusions. In this work we have demonstrated that the incorporation of HA and PEGDA to plasma hydrogels showed superior performances in terms of handling and mechanical properties, contraction with and without cells and human fibroblast proliferation. Further *in vitro* and *in vivo* experiments are required to assess the quality of the engineered skin. However, it appears that HA-plasma dermal equivalents are very promising candidates to enhance both mechanical and biological performance of dermo-epidermal grafts.

References

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