Aged Human Dermal Extracellular Matrix as an Innovative Scaffold for Aging on Chip Model

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Abstract

Currently, there is a growing demand for developing innovative biomaterials that represent skin microenvironment more realistically. Here, we present an optimised protocol to extract and characterize an innovative human adult dermal extracellular matrix scaffold to represent the dermal layer in advanced 3D skin models.

Introduction

Dermal extracellular matrix (dECM) is a complex fibrillar meshwork comprising a multitude of components, including collagens, elastic fibres, glycosaminoglycans (GAGs), growth factors, proteoglycans, and glycoproteins. It provides structural support to skin cells and is responsible for the majority of the skin's mechanical properties (Huang et al., 2022).

Conventional and reference *in vitro* skin models fail to adequately represent the complexity of dECM. Most reported full-thickness skin models feature a dermis composed of purified commercial type I collagen hydrogels, overlooking the presence of other less abundant yet equally important components in the dermal microenvironment. Furthermore, the majority of these scaffolds have been observed to exhibit excessive shrinkage as a result of fibroblast contractility and rapid degradation, which has the effect of reducing the lifespan of these models (Risueno et al., 2021).

In order to improve existing commercial biomaterials for use as scaffolds, here we describe an optimized protocol for extracting and characterizing hydrogels obtained from aged human decellularized dECM to generate scaffolds for subsequent use in 3D *in vitro* skin aging models.

Materials and methods

Full-thickness skin samples were harvested from donors aged between 70 and 90 years (CEICA PI22/119). Following and modifying the previously published protocol (Wolf et al., 2012), we extracted and decellularised dECM from the frozen samples. The composition of aged human dECM was then determined by liquid chromatography mass spectrometry (LC-MS) for protein identification and lipids, collagen, sulphated glycosaminoglycans (sGAGs) and elastin were also quantified. The mechanical properties of the hydrogels were determined by rheology and the fibrillar structure was observed by scanning electron microscopy (SEM). Finally, the biocompatibility of the aged human dECM was studied and utilized in the construction of an aging-on-chip model.

Results

Human aged dECM retains several components present in the native dermal microenvironment and reproduces the complex fibrillar network in the formed hydrogels (Figure 1), showing similar mechanical properties. In addition, this biomaterial is biocompatible and is able to control fibroblast proliferation by preventing shrinkage of the material. The described human dECM hydrogels were used to establish a durable microfluidic aging model that more closely mimics the dermal microenvironment (Figure 2).

Conclusions

Innovative aged human dECM hydrogels are a potential biomaterial for use as a scaffold in advanced ageing models on chips. Human dECM hydrogels retain various dermal biomolecules and mechanical behaviour representing the native dermal microenvironment. In addition, adult human dECMs

control fibroblast proliferation and this prevents shrinkage of the hydrogel, extending the lifespan of the model. These findings have significant implications for the establishment of models of ageing, which will allow the generation of more realistic models for drug and cosmetic testing.

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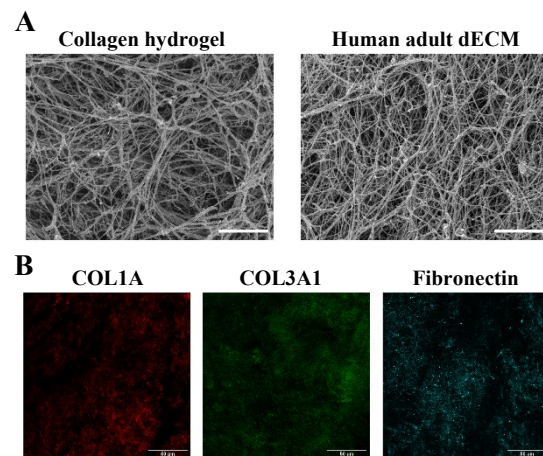
Figure 1. (A) SEM images of collagen type I and human adult dECM hydrogels. **(B)** Immunofluorescence of human adult dECM hydrogels at 4mg/mL stained with antibodies agaist collagen type I, collagen type III and fibronectin. SEM scale: 5µm.
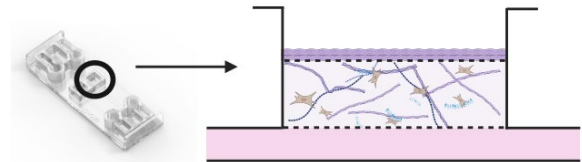
Figure 2. Microfluidic aging model composed by fibroblast embedded within human adult dECM and keratinocytes on top of the hydrogel.