Chapter 1

Introduction

1.1 Background

The widespread use of cosmetic and dermatological products has increased the importance of ensuring their safety and compatibility with human skin (Couceiro et al., 2025). As they are applied repeatedly to the skin, it is essential to evaluate their potential effects on skin cells. Traditionally, safety and toxicity testing for skin-related products relied heavily on animal models. However, there are limitations in translating animal data to human skin responses, along with the need for more efficient and reproducible testing systems, which have driven a shift towards alternative *in-vitro* methods (Basketter & Gerberick, 2022). In this context, *in-vitro* models have gained popularity for offering a more controlled, reproducible platform for studying skin biology and material interactions. As a result, there is a growing need to develop reliable methods that can better represent human skin physiology, and a way to achieve this is through the development of suitable biomaterials that can effectively support keratinocyte growth and function.

Biomaterials designed for skin-related applications are often used to imitate the properties of the extracellular matrix (ECM), providing structural support and the biochemical environment needed for cell survival and proliferation (González-Díaz & Varghese, 2016). Various natural polymers have been explored for hydrogel development, including polysaccharides and protein-based materials. Among these, gelatin and sodium alginate have presented considerable interest as their properties complement each other. Sodium alginate (SA), a polysaccharide that is naturally derived from brown seaweed, is commonly used due to its ability to undergo ionic crosslinking in the presence of divalent cations, such as Ca^{2+} (Teshima et al., 2026). Ionic crosslinking provides structural stability and mechanical strength and maintains a hydrated environment (Alrata et al., 2025). Additionally, ionic crosslinking was shown to be less toxic to cells compared to covalent chemical crosslinking, such as

glutaraldehyde, and light crosslinking (radiation), such as with UV and gamma rays. Despite that, alginate on its own lacks intrinsic cell adhesion motifs, which may limit direct cell attachment. Therefore, it is often combined with natural polymers that contain cell-binding motifs, such as gelatin, to enhance cell compatibility. Gelatin contains Arg-Gly-Asp (RGD) sequences that promote cell adhesion and cellular interactions (Rotaru-Zavaleanu et al., 2025). However, gelatin on its own exhibits relatively weak mechanical strength that can withstand cell culture conditions, such as warm temperature and high water exposure, thus limiting its usage as a stable scaffold candidate unless a modification or crosslinking is performed. Despite the promising characteristics of gelatin-SA hydrogels, the performance of the resulting scaffold is dependent on its formulation.

Therefore, it is important to evaluate how different gelatin-to-SA ratios of the mixture can influence the biological compatibility and physicomechanical properties of the resulting hydrogels. In this study, HaCaT keratinocytes are used as a model to assess cell-material compatibility on biomaterial-based two-dimensional (2D) coatings. By evaluating cell viability across different formulations, this study aims to identify suitable coating conditions to support keratinocyte growth. Additionally, the physicomechanical characteristics of the hydrogels were assessed through swelling and degradation evaluation. Together, these evaluations serve as a preliminary step towards the selection of an optimal gelatin-sodium alginate hydrogel formulation for future development of more representative and functional *in-vitro* skin models.

1.2 Objective

The objective of this study is to evaluate the cell-material compatibility and physicomechanical characteristics of a gelatin-sodium alginate-based ionically crosslinked mixture using HaCaT keratinocytes. The assessment will be conducted by analyzing cell viability across different formulations as a preliminary step towards developing an *in-vitro* skin model. An additional study

regarding its physico-mechanical characteristics would also be performed using degradation and swelling evaluation.

1.3 Hypothesis

- Cell-Material Compatibility

Null hypothesis (H_0): There is no significant difference in cell viability of HaCaT keratinocytes cultured on 2D hydrogel coatings prepared with different gelatin-SA formulations.

Alternative hypothesis (H_1): There is a significant difference in the viability of HaCaT keratinocytes cultured on 2D hydrogel coatings prepared from different gelatin-SA formulations.

- Physicomechanical Characteristics

Null hypothesis (H_0): There is no significant difference in the physicomechanical properties, including swelling behavior and degradation rate, of different gelatin-SA hydrogel formulations.

Alternative hypothesis (H_1): There is a significant difference in the physicomechanical properties, including swelling behavior and degradation rate, of different gelatin-SA hydrogel formulations.