CHAPTER 1

INTRODUCTION

One of the main challenges in the development of new anticancer drugs is the low approval rate (Pagano et al., 2021). Of the hundreds of novel anticancer drugs in development, over 60% do not make it past phase 3 clinical trials, and only >5% are approved by the FDA (Pinto et al., 2020; LeSavage et al., 2021; Chen et al., 2019). The failure of these drugs, often due to the lack of efficacy or safety issues, accounts for 60% and 30%, respectively (Powley et al., 2020). The issue lies in the poor predictive value of the preclinical cancer studies which often show dramatically different results from clinical trials (Long et al., 2021). Existing preclinical models such as *in vitro* models or animal models are able to model certain behaviors of cancer cells in both a 2D and 3D setting to an extent, however *in vitro* models are unable to represent the cellular heterogeneity or complex tissue architecture, while animal models are costly, time-consuming, and require excessive maintenance (Gebeyehu et al., 2021; Pagano et al., 2021; Kaushik et al., 2018). Hence, creating an accurate and practical preclinical model is critical for novel anticancer drug screening.

3D *in vitro* cancer models, such as spheroids, organoids, or cell-seeded scaffolds can emulate the tumor microenvironment (TME), metastasis, invasion, and growth; providing a great model to study anticancer drugs. (Pinto et al., 2020). Scaffold models are one of the 3D *in vitro* models that has recently gained attention since they are able to display the cell-cell interactions and cell-extracellular matrix (ECM) interactions, as well as mimic the TME (Unnikrishnan et al., 2021). A promising candidate for scaffolds for cancer models is hydrogels made from biopolymers & biomaterials, owing to their high biocompatibility and biodegradability. Unfortunately, existing 3D biomaterials that have been previously utilized as 3D scaffolds do not support cell growth relatively expensive, and/or have limited availability (Lim et al., 2020; Axpe & Oyen, 2016; Achilli & Mantovani,

2010; Andersen et al., 2015). There is a need to explore other biomaterials, especially those that are bioactive, accessible, and maintain a stable 3D structure (Gelinsky, 2018).

Keratin has the ability to form porous gels composed of self-assembling fibers due to the crosslinking of disulfide bonds, it is also known to support cell proliferation, migration, and growth (Veiga et al., 2021). It is also a renewable resource that can be directly obtained from human hair, making it a cost-effective choice as a bioink material. Still, it also has poor mechanical properties that need to be improved (Wang et al., 2015). Thus, combining keratin with other biomaterials has been a method highly explored for its biomedical application (Hartrianti et al., 2016). Polymers like pectin show great promise as complementary material due to their ability to form a stable 3D network with the addition of bivalent ions due to the formation of ionic crosslinking, allowing it to form a 3D hydrogel (Neves et al., 2015). While studies have utilized keratin alone and pectin alone, no studies are using a combination of the two (Navarro et al. 2020; Banks et al. 2017). This study explores the combination of keratin and pectin as a 3D cancer model, where it is expected to have stable mechanical properties that can support the growth of various cancer cell lines, HeLa and HT129, in a three-dimensional setting.