Exploring Monofloral Honey Incorporated to Double-Network Hydrogels for Innovative Cardiac Patch Applications

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Cardiovascular diseases, particularly those affecting the myocardium, remain a leading cause of morbidity and mortality worldwide. Novel approaches to address these issues are essential such as the use of double-network (DN) hydrogels, characterized by a dual brittle/flexible network structure, that have garnered considerable attention in tissue engineering. One of the most crucial factors in this field is achieving the necessary mechanical strength to replicate the mechanical characteristics of native tissue. In this context, DN hydrogels offer the mechanical robustness required for cardiac patches, simulating the native heart tissue's toughness. Another strategy is the use of natural bioactive compounds, such as honey due to antibacterial and angiogenic properties [1]. In particular, monofloral honeys are a well-known compound having nutritional benefits, and other biological activities [2], that could potentially contribute to the functionality of cardiac patches [3]. Moreover, it is known for its water retention capacity, able to improve the hydration and microenvironment within a hydrogel, facilitating a conducive milieu for cell growth and survival. Moreover, monofloral honey's potential antibacterial properties align with the need for maintaining a sterile environment in cardiac regeneration applications. In this work, a chitosan network, providing structural integrity, was mixed with polyvinyl alcohol (PVA), comprising toughness, for developing bioactive DN, [4]. To increase the bioactivity of the DN, an endemic monofloral honey from Chile, was incorporated as it is unique due to its monofloral composition. The rationale for combining DN hydrogels and the endemic honey lies in the potential synergy of their properties for biomedical applications.

Furthermore, to identify the botanical origin of the honey used, we conducted a melissopalynological study. Based on the microscopic analysis of pollen grains present in the sample, we classified the honey as monofloral honey, following the classification standards for Chilean kinds of honey [4]. The antimicrobial activity of the sample against *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella enterica sv. Typhi* was also determined by analyzing the growth inhibition halo, using Penicillin G and Streptomycin antibiotics as controls. According to the laboratory analysis standards, and based on the bacterial inhibition result obtained, the analyzed honey sample is classified as "Active Honey." This classification is used to distinguish honeys that exhibit additional biological activity beyond their basic nutritional characteristics.

This research focused on investigating the impact of varying concentrations of honey (DN C-P-H_0.25 and DN C-P-H_0.50) on the mechanical properties under wet conditions. Figure 1 presents the stress-strain curves from which tensile strength, elastic modulus, and elongation at break were obtained for the hydrogels. As the data demonstrates, an increase in honey concentration within the hydrogels resulted in a reduction in both the elastic modulus and ultimate tensile strength. When considering the influence of stiffness on the DN hydrogels, the incorporation of 0.5% wt. honey led to a significant decrease of approximately 30% in Young's modulus. Conversely, when 0.25% wt. monofloral honey was incorporated, the reduction was more modest, around 8%. This reduction in stiffness may be attributed to honey's capacity to interact with polymer chains due to its hydroxyl groups, indicating a plasticizing effect [5]. Furthermore, this is reflected in the internal porosity of the hydrogels, which decreases from 2 µm to 0.8 µm and 0.6 µm upon incorporating 0.25% and 0.50% by weight of honey, respectively. This results in the formation of a nanoporous internal structure when cross-sectional analyses are conducted using scanning electron microscopy (SEM).

To provide a controlled and precise designed platform, we created 3D-printed scaffold structures from the DN hydrogels (Figure 2.a). This approach allows us to explore the cellular responses to these unique hydrogels under well-defined conditions. In order to evaluate the biocompatibility and cytotoxicity of the DN hydrogels and the effect of the two concentrations of monofloral honey on hydrogel bioactivity, the responses of the human umbilical vein endothelial cells (HUVECs) were measured in an in vitro study using Alamar Blue test on the 1st, 3rd, 7th and 14th days. The results illustrated in Figure 2. revealed that honey promotes cell proliferation during the days of the study. The rapid cell growth on honey-containing hydrogels suggests a beneficial effect, likely associated with the sugar content in the samples. However, over time, we observed a decline in proliferation, which could be due to the gradual dissolution of honey in the cell culture medium. Moreover, no adverse effects on cell viability were observed. Among the prepared hydrogels, C-P 0.5% wt honey exhibited the highest level of biocompatibility. The incorporation of honey into the hydrogel appears to enhance cell

proliferation and viability, potentially by supplying essential nutrients to the cells [6].

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Figures

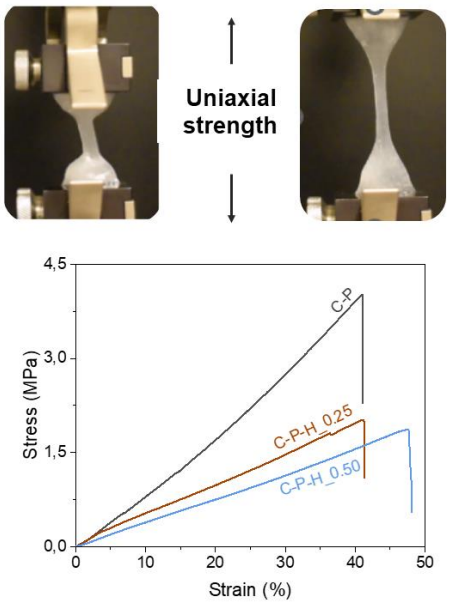

Figure 1. Graph of stress-strain and table of mechanical properties of the DN hydrogel samples.

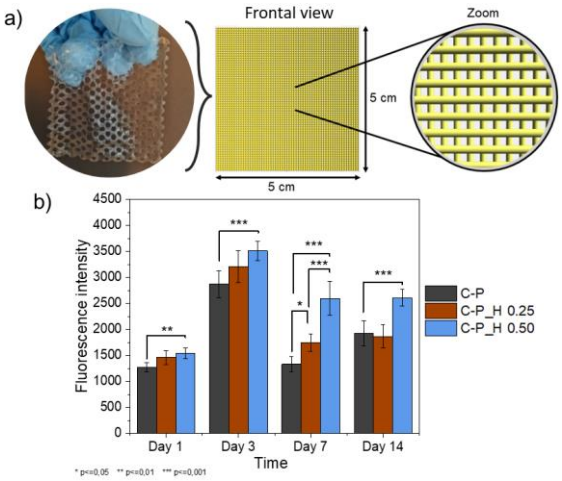

Figure 2. a) 3D-printed scaffolds and b) Cell proliferation analysis of HUVECs seeded on 3d printed scaffolds of DN hydrogel samples.