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A Review on Extracellular Matrix Synthesis Using Collagen Dressings with Plant Extract

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Abstract - *In order to improve wound healing procedures, the goal of this work was to create three-dimensional porous collagen composites (Col) containing polyphenol-rich wormwood extract and to evaluate how they interacted with human skin cells. Scanning electron microscopy was used to examine the ultrastructure of the scaffolds, and biodegradability and the release of bioactive chemicals were examined in a physiological setting. Using two in vitro experimental models, the interactions of composites in direct and indirect contact with human skin cells were assessed. In comparison to Col scaffold, ColWE scaffold had more porosity, a higher degree of swelling, and greater durability against enzymatic degradation. More wormwood extract content in composite scaffolds allowed for more effective regulation of polyphenolic release. Human dermal fibroblast and keratinocyte cell adhesion and proliferation were promoted by the ColWE 0.5 variant. The composite scaffold also promoted the production of skin extracellular matrix components. Our findings showed that ColWE composites with enhanced physical, chemical, and biological characteristics might be utilised in cutting-edge applications for wound healing.*

Key words: Extracellular matrix, Scaffold, Collagen dressing, Plant extract

1. INTRODUCTION

Skin wounds have a lengthy healing process that involves four overlapping stages: inflammation, granulation tissue development, reepithelialization, and extracellular matrix remodelling (ECM). The natural healing process might be hampered by external circumstances like bacterial infections or internal ones like the patient's old age or illness, which can result in chronic wounds [1]. To speed up the healing processes, a variety of passive (gauzes, films, hydrocolloids, hydrogels, and foams) and active (biocomponents loaded in polymeric matrix) dressings were evaluated on various skin wound types [2]. The primary functions of a wound dressing are to preserve moisture while removing excessive quantities of exudate from the injury site, to permit gaseous exchange, and to offer defence against bacterial infection. Moreover, a wound dressing must be simple to remove from the wound, biodegradable, and biocompatible [3,4]. The primary structural element of ECM and the most researched natural polymer for tissue engineering applications is collagen (Col) [5-7]. Because to their high biocompatibility, simple adhesion, capacity for swelling, and protection of the wound bed, collagenic dressings have been used to speed up the healing of skin wounds [8]. Col matrices further offered an ideal three-dimensional (3D) microenvironment for cellular adhesion and proliferation at the sites of lesions, encouraging the creation of granulation tissue, re-epithelialization, and new ECM synthesis as part of the wound healing process. Plant extracts and their physiologically active constituents promoted the healing of skin wounds [9]. Wormwood, or Artemisia absinthium L., has long been used as a febrifuge, antiseptic, antifungal, and antibacterial agent [10,11]. Wormwood polyphenolic extracts also shown antioxidant and free radical scavenging properties, which may improve the healing process of wounds [12-14].

Due to their pharmacological action induced by regulated release of active molecules and longer contact extent with skin lesions, plant extract-polymeric constructions are currently receiving a lot of interest in wound healing applications [15-17]. Recently, sheets made of collagens that were loaded with plant-derived restorative chemicals or whole plant extracts were created for use in skin tissue engineering. As comparison to Col scaffolds [16-18], Col dressings with polyphenols from Hamamelis virginiana had a better ability to inhibit chronic wound enzymes including myeloperoxidase and collagenase. Rats with infected cutaneous wounds were treated with 3D sponges of Col filled with triphala herbal extract, leading to rapid wound closure and tissue regeneration [19,20]. Col-chitosan scaffolds added with Aloe Vera gel displayed better physicochemical and biological characteristics, whereas Col-matrices with Astragalus polysaccharides were produced as potential wound dressings with angiogenic capabilities. The capacity for recruiting, attachment, and proliferation of fibroblasts [21,22].

1.1 Ultrastructure of ColWE scaffolds

ColWE scaffolds had a 3D sponge-like appearance, which is typical of freeze-dried polymeric matrix. Their surface was shown by SEM examinations to have a random microporous structure with tiny holes, comparable to Col scaffold. ColWE scaffolds' transverse cross section revealed a consistent porosity structure, comparable to the Col scaffold. The ColWE scaffolds' whole structure was made up of interconnected pores, whose diameters ranged from 50 to 600 m. The ultrastructure, pore size, and morphology of the composite scaffolds were comparable to those of the Col scaffold, suggesting that before lyophilization, Col gel and WE formed a homogenous combination.

Water crystals sublimated during the freeze-drying process, which led to the formation and shape of pores. In this work, lyophilization was employed to achieve optimal cell type contact and regulated release of bioactive compounds. It has been demonstrated that, due to their 3D porous nature, freeze-dried biomaterials grown with cells assure nutrients diffusion, promote cell proliferation, and produce more ECM than 2D materials or loose hydrogels [23]. Furthermore, by altering the freezing temperature between 80°C and 20°C, the pore size and microstructure of these materials may be further customised [24]. A scaffold's ability to accommodate particular pore diameters favours cell infiltration, adhesion, and proliferation, it has been shown [25].

Skin has the same non-homogeneity in pore size as the composite materials [26]. Micropores improve the mechanics of the scaffold and provide targeted cell migration during tissue regeneration and defect correction, whereas macropores in this complex tissue often serve as spaces for tissue development and vascularization [27]. Contrarily, the lyophilization procedure and vacuum exposure of the upper half, while the lower part was in direct contact with the plastic mould, were responsible for the discrepancies in surface and inside pore morphology [28].

1.2 Physico-chemical characterization of ColWE scaffolds

Water was used as the solvent in a liquid displacement approach to assess the total porosity of the ColWE scaffold variations. Little water molecules dispersed into the scaffolds' various pore diameters before being compelled by pressure to quickly fill the empty space. The mostly hydrophobic Col molecule and the poor water solubility, polyphenol-rich WE inhibited the swelling of the samples during the incubation period. The outcomes are displayed in Table 1. ColWE 0.25 and ColWE 0.5% had a porosity of 80%, whereas ColWE 1 and ColWE 2.5 had a porosity of 70%. Col scaffold that had been freeze-dried had a 97% porosity value (Table 1).As comparison to Col scaffold, incorporation of increasing WE caused a proportionate and substantial ($p<0.05$) reduction in porosity. Nevertheless, ColWE scaffolds with porosity exceeding 70% may be more advantageous for cell colonisation and use in skin tissue engineering [23]. The cross-linking between the chains of Col and the polyphenols and the production of many hydrogen bonds that change the ultra-structure of the porous scaffold may be to blame for the reduction in porosity [27].

Col scaffolds have the capacity to expand, increasing their volume by at least 1500% above that of their starting volume [29]. Composite materials made of Col loaded with increasing amounts of polyphenol-rich WE that had a lot of free hydroxyl groups on the hydrophobic backbone should have better swelling degree and increased hydrophilicity [31].

In this regard, the ColWE composite variations' swelling levels were examined, and the findings are shown in Table 1. Except with ColWE 2.5, the values for ColWE dressings were considerably (p 0.05) higher than those for Col scaffold (Table 1). When the WE concentration increased, the swelling degree decreased from 2342% for ColWE 0.25% to 1936% for ColWE 2.5. This may be as a result of the formation of cross-links between the chains of Col and polyphenols, which would reduce the amount of free amino and hydroxyl side groups with hydrophilic qualities and lower swelling degree. This variance reflected a reduction in porosity. ColWE 0.25, ColWE 0.5, and ColWE 1 versions, however, should be able to help exudate absorption at the wound sites because to their better swelling capabilities. Similar studies have found that adding higher concentrations of vegetal polysaccharides to create a collagenic hybrid material increases the water absorption of Col-polyphenolic biomaterials compared to Col20 and decreases swelling degree [31].

Collagenic wound dressings are susceptible to enzymatic breakdown in vivo. The biodegradability test in this study was conducted using collagenase type IA, which is only capable of cleaving the -X-Gly-Pro sequence from connective tissue components. According to the findings, ColWE scaffolds deteriorated to a lesser amount than Col matrix (35%–86%). (Table 1). Also, increased WE concentrations led to improved scaffold resilience against collagenase assault. ColWE 0.25 was therefore 86% degraded, compared to ColWE 1 scaffold's 50% degradation and ColWE 2.5's 35% degradation (Table 1).This may be explained by the polyphenolic extract's high concentration of hydroxyl and carboxyl groups, which favours many hydrogen bonds with proteins, particularly Col [33]. Similar outcomes were previously attained with Col sponges loaded with Hamamelis virginiana polyphenols, which demonstrated enhanced stability towards chronic wound enzymes [19].

1.3 In vitro release of biologically active compounds

PBS pH 7.4 at 37°C was used to study the polyphenolics release from ColWE porous scaffolds in circumstances that mimicked an in vivo environment. The outcomes are displayed. The results showed that within the first 8 hours of incubation, significant levels of polyphenolics were gradually released. Between 8 and 24 h of incubation, the slope of the release profile reduced, and additional incubation led to a plateau in the release of polyphenolics. At the conclusion of the incubation period, the percentage of released polyphenolic compounds for ColWE 0.5 and ColWE 0.25, respectively, achieved high levels of 45.42% and 76.43%. Lower percentages of phenolics (16.13% and 8.85%, respectively) were released in the physiologic environment by ColWE 1 and ColWE 2.5 scaf-folds. The biodegradability of the scaffolds may be to blame for the results, since slower degrading variations released a lesser percentage of bioactive chemicals. The actual levels of phenolics produced in the physiologic environment, however, were contained within a small range. The components that were not involved in physico-chemical interactions with Col molecules presumably accounted for this commonality in released quantities [29].

Table 1.

Sample	Porosity (%)	Swelling degree (%)	Biodegradability (%)
ColWE 0.25	78.90 ± 4.83*	2342 ± 14.42*	86.83 ± 3.22*
ColWE 0.5	80.08 ± 3.19*	2240 ± 15.71*	63.54 ± 4.24*
ColWE 1	71.57 ± 3.24*	2146 ± 15.09*	49.53 ± 4.08*
ColWE 2.5	69.80 ± 3.95*	1936 ± 10.14*	35.61 ± 3.20*
Col	97.06 ± 2.54	1977 ± 14.93	100 ± 4.57

1.4 ColWE scaffold interaction with human skin cells

i. ColWE effect on cell proliferation and viability

In accordance with SR EN ISO 10993-5/2009, a preliminary cytotoxicity test of ColWE variations was carried out in a culture of fibroblasts (L929 clone cell line). After 24 hours of culture, the findings showed that ColWE 0.25 and ColWE 0.5 had good biocompatibility (>80% cell viability), whereas ColWE 1 had moderate cytotoxicity (>70% cell viability), and ColWE 2.5 variant had low cytotoxicity (30% cell viability). Hence, only ColWE 0.25, ColWE 0.5, and ColWE 1 were chosen for additional cell culture research. The high phenolics amount released into the cell culture medium and inadequate Col quantity to protect skin cells may be the cause of ColWE 2.5's cytotoxicity. Moreover, an abrupt rise in the release profile may be related to an increase in the surface-to-volume ratio of the sample utilised in cell culture experiments [33].

In order to assess cell proliferation, fibroblasts and keratinocytes, two kinds of human skin cells, were cultivated in close proximity to ColWE scaffolds. The findings indicated that during the course of the whole culture time, the number of fibroblast cells steadily grew. The cell quantity was considerably (p 0.05) larger in ColWE 0.25 and ColWE 0.5 variants, during each time of culture, and the cells proliferated to a greater degree in 3D ColWE scaffolds than on plastic (control). At 7 days of incubation, ColWE 1 scaffold permitted cell proliferation to a similar level as Col scaffold, but greater than the 2D control group.

No changes were found between the tested groups after two days of culture for HaCaT keratinocyte cells. The results revealed that ColWE 0.5 (46.9 104 cells) had seen an extensive proliferation of keratinocytes after 5 days of incubation. ColWE 0.25 and ColWE 1 encouraged cell development to a greater extent than Col scaffold (28.5 104 cells) and cells grown on plastic (18.2 104 cells, respectively) (37.2 104 and 34.6 104 cells, respectively) (control). Following 7 days of culture, there were also noticeably more keratinocyte cells than in the Col scaffold (p 0.05). Nevertheless, it fell in all examined groups, including control, most likely as a result of cell overpopulation, which quickly ate up the nutrients available.

Their studies showed that, in comparison to Col scaffold, some ColWE composite versions, such as ColWE 0.25 and ColWE 0.5, were more effective as 3D scaffolds for skin cells, particularly for keratinocytes. Only in the HaCaT keratinocyte cell culture did ColWE 1 dressing outperform Col scaffold. ColWE composites may have a stimulating impact because of their porous design, which made it possible for nutrients from the culture medium to be efficiently absorbed. Moreover, the growth of both

types of human cells was aided by small quantities of WE included in composites and phenolics discharged into the biological medium. Earlier research demonstrated that the polyphenolics found in the Artemisia genus might promote the growth of skin cells [34]. On the other hand, Col, which has a distinct triple helical structure, is known to encourage cutaneous fibroblast cell adhesion and proliferation when prepared as 3D scaffolds [35]. There were no reports of WE-loaded Col cloths interacting with skin cells.

The significant number of live cells that colonised ColWE scaffolds without cytotoxicity was confirmed by fluorescence microscopy measurements. Keratinocytes formed isolated colonies on the surface of scaffolds, whereas dermal fibroblasts moved throughout the whole construction and infiltrated it.

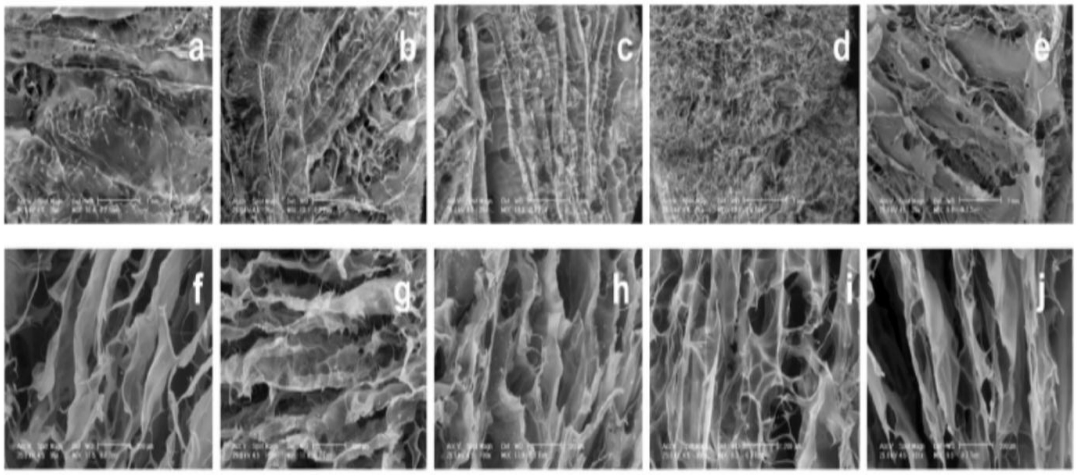

Figure 1. Scanning electron micrographs showing the (a–e) surface and (f–j) transversal cross sections of composite scaffolds: (a, f) ColWE 0.25, (b, g) ColWE0.5, (c, h) ColWE 1,(d, i)ColWE 2.5, and (e, j) Col. (a–e) Scale bar = 1 mm. (f–j) Scale bar = 200 μm [39].

ii. ColWE effect on ECM synthesis by skin cells

Once dermal fibroblasts were grown in the presence of composite scaffolds, the total amount of collagens secreted in the culture medium was first calculated. In comparison to control cell culture, the results showed that ColWE 0.25 and ColWE 0.5 increased collagen synthesis by thrice and fourfold, respectively. Moreover, the results differed considerably (p 0.05) with those recorded for Col scaffold. Compared to control cells, fibroblast cells produced more collagen when exposed to ColWE 1 and Col scaffolds, while the effects were modest. Similar outcomes were obtained when HaCaT keratinocytes were cultured on ColWE scaffolds to produce collagen.

All of these findings showed that, due to specific WE concentrations; ColWE scaffolds stimulated the manufacture of collagen in human skin cells. Earlier research shown the ability of flavonoid glycosides from the Artemisia genus to increase the synthesis of Col in human skin fibroblast cultures, including isoquercitrin, quercetin-3-O-d-glucoside, quercetin-3-O-rhamnoglucoside, and isorhamnetin-3-glucoside [36-38]. Dermal fibroblasts cultured in the presence of ColWE scaffolds produced fibronectin, another important component of the ECM. ColWE 0.5 and ColWE 0.25 versions considerably (p 0.05) boosted its production compared to Col scaffold and the control group.

ColWE 1 scaffold-cultivated fibroblasts produced almost the same amount of fibronectin as the Col scaffold group did. ColWE 0.5 scaffold considerably (p 0.05) increased the amount of fibronectin that was generated in HaCaT keratinocyte cells compared to the Col scaffold group and control cells. ColWE 0.5 scaffold was shown by cell culture findings to be ideal for skin cells' proliferation and metabolism, increasing the synthesis of ECM [39].

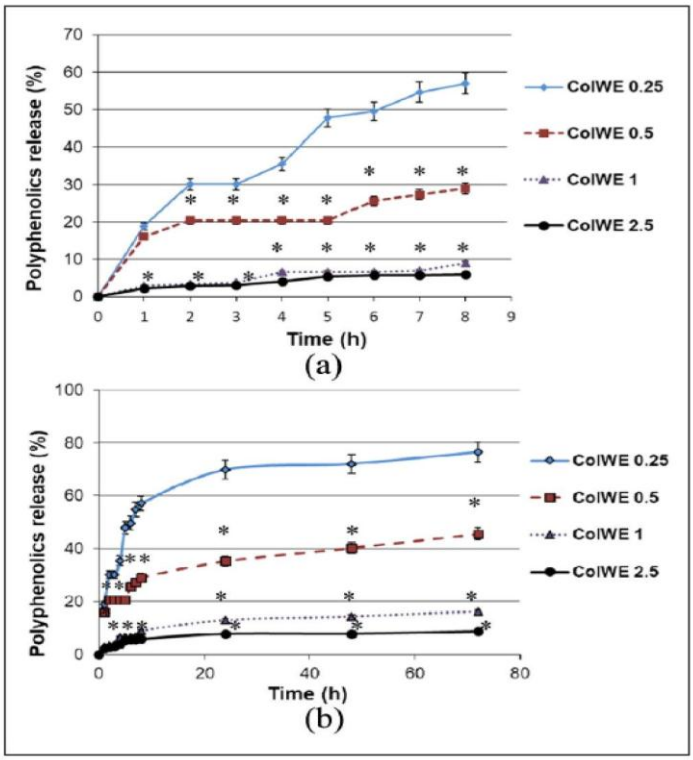

Figure 2. Polyphenolics release profile for composite scaffolds, after (a) 8 h and (b) 72 h of incubation in biomimetic conditions (saline buffer, pH 7.4, 37°C). Total quantity of plant extract in each sample was considered 100%. The results were expressed as mean ± SD (n = 3). *p < 0.05, compared to composite scaffold ColWE0.25 [39].

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