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Piezoelectric plastic compressed collagen-mesh scaffold for artificial skin

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Abstract— Our skin, and many other tissue types, display electrical and electromechanical properties, which play an important role in tissue regeneration and healing. This work explored a new route for artificial skin with similar properties and presents an opportunity for further enhancing tissue repair. To this end, the electrical properties and piezoelectricity of a collagen-mesh scaffold are investigated. This method has demonstrable success in the rapid fabrication of 3D tissue scaffold equivalents for a skin substitute. As hydration is known to reduce the piezoelectric response of biological materials, as well as alter the conductance and charge storage capabilities, efforts were made to characterize the scaffold in both the dehydrated and hydrated states. The measured capacitance, at 100Hz frequency, was 37nF and 0.45nF for the hydrated and dehydrated scaffold, respectively. The piezoelectric sensitivity of the dehydrated scaffold was $\sim 17 \text{ mV N}^{-1}$ which is large enough to be detected by cells. The results of this study could open a pathway for advancing human-like sense of touch in eSkin with piezoelectric collagen fibrils.

Keywords— piezoelectric; scaffold; plastic compression; collagen; tissue regeneration; eSkin

I. INTRODUCTION

Various human skin substitutes are available on the market to temporarily or permanently replace damaged skin by providing an adequate environment for the regeneration of skin in cases of chronic wounds and skin burns [1]. In some cases, they act as a biologically active wound dressings, providing growth factors, cytokines and extracellular matrix for host cells while reducing inflammatory response and subsequent scarring [2]. In general, skin substitutes aim to mimic the functional properties of natural skin as much as possible. Currently available tissue-engineered artificial scaffolds for skin substitution only partially address the functional requirements [3]. Most of the skin substitution studies have focused on the biocompatibility and mechanical properties to develop artificial skin closer to the natural skin. But, this also means that these skin tissue-engineered products are unable to restore functions such as touch sensation, temperature sensation, electromechanical properties and thermoregulation etc [4].

The discovery of endogenous electric fields in biological tissues, and the promising effect of electrical stimulation in enhancing the wound healing capabilities of tissue [5, 6], shows the importance of investigating the electrical and electromechanical properties of skin scaffolds. In this regard, piezoelectric scaffolds have been explored using various materials as they can generate an electric field without the need

for an external stimulus [7, 8]. Therefore, piezoelectric scaffolds offer a means of mimicking endogenous electric fields and may provide the necessary stimulation for cell regrowth in order to augment wound healing and tissue repair [9, 10]. In addition, piezoelectricity is thought to contribute to sensory pathways in a number of excitable tissue types, including skin, and nerve. Therefore, harnessing the piezoelectric nature of biomaterials also represents an attractive route for advancing the sensory functionality of artificial skin, bridging the gap between biology and engineering [11].

In view of the evidence, electrical properties ought to be considered as a critical parameter in the design of scaffolds. A huge variety of biomaterials are available for the fabrication of skin substitutes; each have their own advantages and disadvantages. Collagen is an attractive natural biological material [12] as it represents the most-abundant protein within the extracellular matrix (ECM) of skin, and so supports the construction of a more natural new dermis with excellent reepithelialisation. In addition, collagen displays piezoelectric properties due to the displacement of hydrogen bonds within its quasi-hexagonal crystalline structure [13]. Collagen hydrogels exhibit good tuneability, but are limited by mechanical instability due to their high water content [14]. A dense network of biomimetic 3D collagen can be generated by plastic compression [16, 17], which is a technique that employs mechanical loading to expel excess water through capillary fluid flow [15]. In doing so, the mechanical properties of collagen hydrogels are improved. Plastic compression removes the dependence on cells for the generation of extracellular matrix in engineered tissue constructs, and significantly reduces the fabrication time. If required, cells can also be incorporated into the collagen network prior to compression [18] as there are no adverse effects on cell viability or biological function [15, 19].

In the present work, plastic compression has been used for the rapid fabrication of acellular ECM equivalents. Mechanical properties of the compressed collagen hydrogels are further enhanced by the incorporation of a surgical mesh: in this case, Vypro (Ethicon). It has been used extensively within clinical practice for the mechanical reinforcement of injured tissue. The mesh allowed for easy manipulation of compressed collagen hydrogels which was important for practical reasons. To date there have been no reported attempt to characterize the electrical properties of plastic compressed collagen scaffolds. In proteins, such as collagen, their electrical behavior differs between the dehydrated and hydrated states; the hydrated state reflects the physiological environment for most. Hydration is known to be

inversely related to the piezoelectric response of collagen [20-23]. The conductance, capacitance and dielectric properties are also affected, as evidenced by huge discrepancies in dielectric constants between dry and hydrated tissue. In the present study, electrical properties were characterized for the scaffold in both the dehydrated and hydrated state. Acellular scaffolds were characterized as cell death would have introduced an additional variable in the comparison of hydrated and dehydrated samples. The present study will aid in understanding the electrical properties of scaffolds produced using this method, and thereby, facilitate future opportunities for augmenting the regenerative process.

This paper is organised as follows: Section II describes the fabrication and characterization of collagen-mesh scaffold. Results are analysed in Section III and finally the key outcomes are summarized in Section IV.

II. FABRICATION AND CHARACTERISATION

A. Preparation of scaffold

The collagen solution was prepared by a neutralisation process [15]. Reagent volumes were guided by the RAFTTM protocol for 3D cell culture. For fabrication of the collagen encapsulated mesh, 4ml of the neutralised collagen solution was dispensed into a 55mm x 18mm x 11mm rectangular mould. A 53mm x 17mm cut rectangular piece of mesh was laid on the surface of the solution. An additional 4ml of the neutralised collagen solution was then dispensed on top. This assembly was covered and transferred to an incubator (37°C, 5% CO₂) for 30 mins to gelate [24]. The collagen solution formed a hydrogel, with a layer of mesh in the middle, and the entire construct could be carefully removed from the mould as shown in Fig. 1a. The hydrogel encapsulated mesh was sandwiched between two layers of electrode fabric (Cu/Ni plated polyester fabric, surface resistivity <0.05Ω) prior to compression (Fig. 1b). A 45mm x 12.5mm piece of fabric was utilised for each electrode layer. Each electrode had a thickness of approximately 0.09mm +/- 12%. The plastic compression process was performed as outlined by Brown et al 2005. The scaffold was allowed to dehydrate under ambient conditions over a 1-hour and 24-hour period to obtain hydrated (H) and dehydrated (D) scaffolds respectively. After 24hrs the collagen scaffolds were dry and brittle. Water loss (%) was determined as weight loss compared to the weight of the gel before compression. Hydration was quantified as the percentage weight of water.

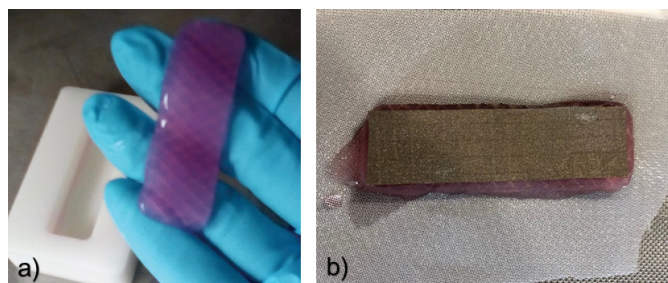


Fig. 1 a) The gelled scaffold with mesh in middle and b) Electrode fabric sandwiches of the scaffold prior to compression.

B. Scaffold characterization

Characterization was performed for both H and D scaffolds under ambient conditions. To measure the piezoelectric response, an electro-mechanical shaker (TIRA model, TV 50018), was used for application of mechanical force at a controlled frequency. The sample was protected by covering with a 100 μm layer of polydimethylsiloxane (PDMS). This construct was then loaded onto a rigid metal plate which held it in place during contact. The force was applied within the range of 1N to 10N at constant frequency (5Hz) and the output voltage generated under applied force displayed on an oscilloscope (Keysight infiniiVision MSO-X 4154A).

Electrical impedance spectroscopy (EIS) was used to investigate the dielectric properties and the electrochemical processes occurring in the scaffold as reflected by a change in impedance during dehydrated and hydrated conditions. The Keysight E4980AL precision LCR meter measured the dielectric properties, capacitance and impedance for the frequency range 20Hz-1MHz.

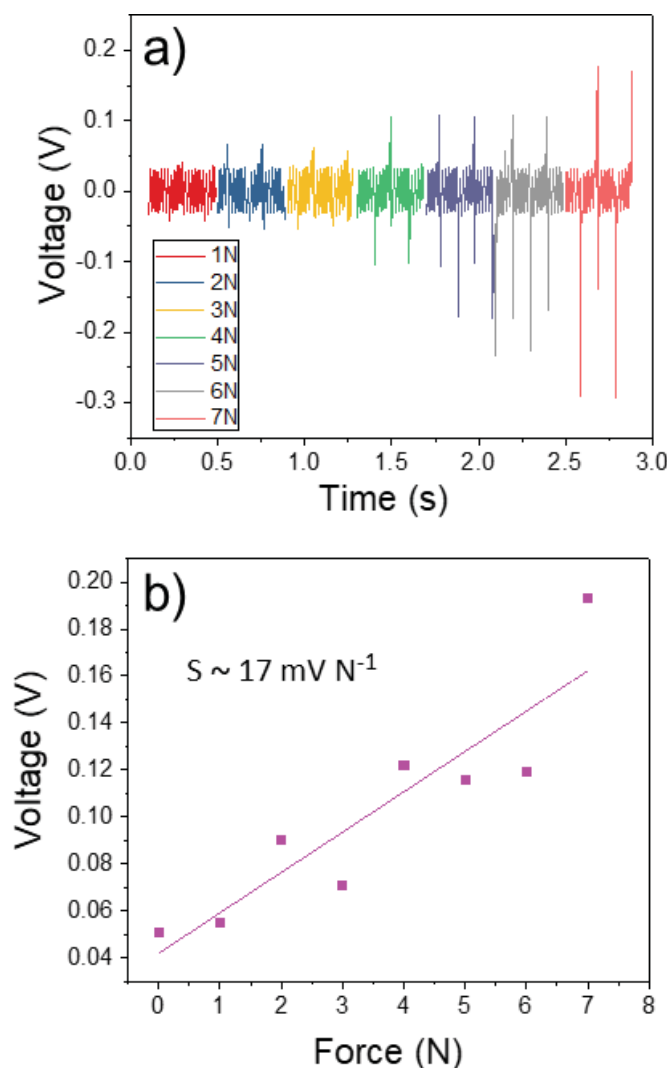


Fig. 2 a) Output voltages of the collagen scaffold under the 1-7 N applied force b) Piezoelectric sensitivity as a function of applied force.

III. RESULTS AND DISCUSSION

A. Piezoelectric characteristics of scaffold

One hour after the scaffold was obtained, its water content was 75.7%. After 24 hours the scaffold was completely dehydrated as evidenced by its dry brittle nature. The electrical properties of both scaffolds were compared. Results confirmed a direct piezoelectric response from the dehydrated scaffold. Due to the electrode sandwich arrangement, the measured voltage reflected polarization generated in the same direction as the applied force. At 1N, the piezoelectric signal was barely distinguishable above the noise, as seen in Fig. 2a. However, as the force increases to 7N, the peak to peak output voltage increases to ~ 400 mV. The voltage sensitivity at 5Hz for the D scaffold was $17\text{mV N}^{-1} \pm 3\text{mV}$ (Fig. 2b), large enough to be detected by cells [25]. However, cells will not survive a dry environment, therefore, the voltage sensitivity of the H scaffold is of greater interest. Crude observation indicated the presence of a direct piezoelectric response, but this could not be reliably reproduced for data collection. In the presence of moisture, adhesion at the electrode-scaffold interface appeared to be disrupted upon application of force. A reduction in piezoelectric response would be expected in the H scaffold due to the charge shielding effect of polar water molecules.

B. Electrical Impedance Spectroscopy

Capacitance was calculated from impedance data. At 100Hz, capacitance was 37nF for the H scaffold compared to 0.45nF for D (Fig. 3). In both scaffolds an electric field was generated, and charge stored. In D, capacitance primarily reflects dipole moments in the collagen and mesh material. In H there is additional charge storage from the ionic content of the solution. Sorption of ions at the electrode surface contributes to the development of double layer capacitance. As a result, the electric field capacitance for the H scaffold is much greater. The presence of water molecules and free ions accounts for an increase in conductance seen in the H scaffold. At 100Hz the conductance for the H scaffold is $6\mu\text{S}$ vs a negligible value for the D scaffold. At high frequencies, conductance appeared similar. This may be a consequence of the electron hopping in the dehydrated scaffold when high frequency AC is applied.

The Nyquist plot for the D scaffold is shown in Figure 4. a. Voltage input had little influence on the properties measured using EIS. The contact resistance was observed as 900Ω . This high value could be due to the poor adhesion contact between the conductive electrode fabric and the scaffold material. The bulk charge transfer resistance (RCT) was found to be $3\text{M}\Omega$. The presence of a Warburg element demonstrates that ionic

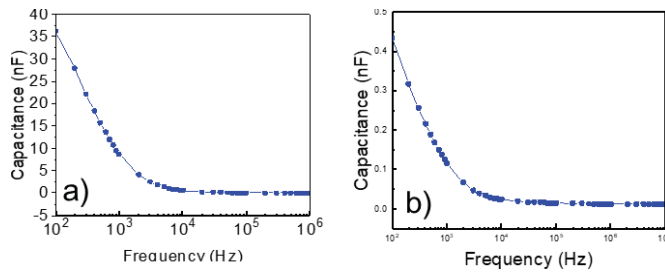


Fig. 3. Capacitance for a) hydrated and b) dehydrated scaffold.

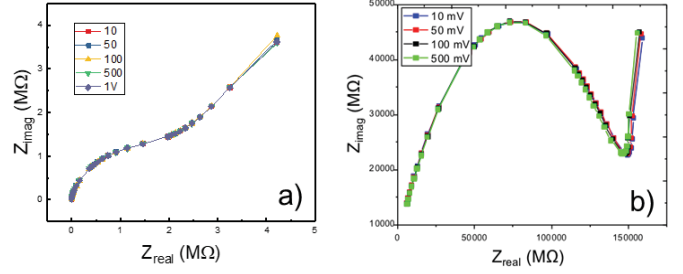


Fig. 4. Nyquist plots for a) dehydrated and b) hydrated scaffold.

diffusion predominates at low frequencies. The incomplete semi-circle is a consequence of Maxwell-Wagner polarization and bulk material polarization occurring within a similar time constant. Analysis of the Nyquist plot for the H scaffold (Fig. 4b) reveals the solution resistance (R_s) is high, with a value of $\sim 6\text{K}\Omega$. Here, the RCT is $0.135\text{M}\Omega$: much smaller than the D sample. A second time constant is visible reflecting the presence of an electric double layer as a result of mobile water ions.

IV. CONCLUSIONS

In summary, this paper confirms the presence of piezoelectricity in dehydrated, plastic compressed, collagen-encapsulated Vypro mesh. The measured voltage sensitivity appears good enough to be detected by the cells. Characterisation of voltage sensitivity in the hydrated state is a priority. The conductance and charge storage capabilities of the scaffold are greatly improved in the hydrated state. Future work will also endeavour to determine the orientation of collagen fibrils to facilitate efforts to optimise the generated piezoelectric response. In the present study, orientation of collagen fibrils was random. As piezoelectric polarization of collagen occurs along the long axis of fibrils, the aim of future work will be to characterise and if necessary, direct, the orientation of fibrils with respect to the expected direction of deformation to obtain the maximum piezoelectric response.

The demonstration of conductance, charge storage, and piezoelectricity in a biologically compatible material with potential suitability for use as a component in artificial skin matrices demonstrates the possibility of generating a novel bioelectrical product which might enhance sensibility. The material is potentially applicable to the engineered repair of other tissues, if electrical activation were to prove beneficial to the cellular behaviours necessary for their healing

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