Characterization of Sensorized Porous 3D Gelatin/Chitosan Scaffolds Via Bio-impedance Spectroscopy

Muhammad Ahmed Khan, Nicola Francesco Lopomo, Mauro Serpelloni, Emilio Sardini and Luciana Sartore

Abstract Conductive scaffolds are highly used in tissue engineering for bone defect, nerve regeneration, cardiac tissue constructs and many others. Currently, most methods for monitoring cell activities on scaffolds are destructive and invasive such as histological analysis. The research aimed at sensorizing and characterizing a porous gelatin/chitosan scaffold, hence this "Intelligent Scaffold" can behave as a biosensor for evaluating cell behaviour (cell adhesion, proliferation) along with directing cellular growth. Thus, in this research, three-dimensional (3D) gelatin based scaffold has been transformed into conductive scaffold and both the scaffolds are characterized and compared in terms of their electrical conductivity. Carbon black has been used as a doping material to fabricate a Carbon-Gelatin composite conductive scaffold. The scaffolds are prepared by Freeze drying method and carbon black has been homogeneously embedded throughout the gelatin matrix. The scaffold behaviour was characterized by Bio-impedance Spectroscopy method. The preliminary experimental results showed that the conductivity of carbon-gelatin/chitosan scaffold increases around 10 times as compared to simple gelatin scaffold. Thus, these results elucidated the importance of carbon black clustering for development of a conductive network. This shows that carbon black provides conducting path and hence in future, even a small change of cellular activity can be determined by impedance fluctuation within the scaffold.

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1 Introduction

Tissue engineering provides an alternative to conventional and classical transplantation methods, which involves regulation of tissue progression and cell behaviour via designing and development of synthetic extracellular matrix (ECM) that assists tissue regeneration [1, 2]. The fundamental approach used in tissue engineering includes the fabrication of scaffolds, which provide the suitable environment and stimuli for cell growth, differentiation, proliferation and supports functional tissue genesis [3–6].

Many different biopolymers have been engineered and investigated (natural and synthetic) for scaffolds fabrication. Among these, gelatin is widely used because of its unique characteristics including biodegradability, biocompatibility and hydrogel properties [7]. Gelatin is a protein fragment, formed by partial degradation of collagen fiber. It contains free carboxyl groups that can combine with chitosan to form a Gelatin/Chitosan network by means of hydrogen bonding [8, 9]. Gelatin/Chitosan hybrid scaffold has been reported to be useful and effective for skin, nerve, bone, muscles and cartilage tissue engineering [10–14]. Chitosan is a linear polysaccharide and is among the most abundant natural polymers found in nature. It has been widely used in tissue engineering because of its non-toxicity, biodegradability, biocompatibility and anti-bacterial effect [15]. It is produced by alkaline deacetylation of chitin [16–18] and has the capability to interact with adhesion proteins, growth factors and receptors [17, 19].

In addition, along with high strength, high electrical conductivity is also desirable in tissue engineering including neural tissue engineering [20], cardiac tissue engineering [21] and bone engineering [22]. Hence, in order to increase conductivity, carbon-based fillers such as graphene, carbon black, graphene oxide, carbon nanotubes and carbon fibers are widely used [23]. Carbon black fillers are preferred over metal fillers as they don't undergo oxidation, whereas, metal fillers get oxidized and creates an insulation layer on particles surface [24]. Other advantages of hybrid composites made from carbon black fillers include: light weight process capabilities, flexibility, low production costs and absorption of mechanical shock [25].

Along with tissue regeneration, monitoring of cellular activities on scaffold is also very critical in tissue engineering. Currently, most methods for monitoring cell activities are destructive and invasive such as histological analysis [26]. Thus, there is a need of non-invasive, user-friendly, robust and quick sensing technique that can provide continuous real-time monitoring of cellular activities. Hence, to provide a non-invasive solution, "Confocal imaging microscopy" is used for observing cell culture growth and differentiation with higher cellular resolution. However, this approach has its own limitations and needs optimization for tissue engineering application. In this method, the scaffold has to be sliced in thin pieces of 200–300 mm to allow enough light penetration. In addition, the scaffold material should have low

auto-fluorescence along with optically transparent feature [27]. In order to overcome this limitation, another technique "electrochemical impedance spectroscopy (EIS)" has been introduced that monitors cell activity by correlating biological cellular phenomena with electrical impedance measurements. EIS has been proved as an efficient method for the real-time analysis of biological systems both in vitro [28–30] and in vivo [31]. According to Giaever and Keese [32, 33], as the cells spread and attach on a conductive surface, they change the area available for current flow and cause a fluctuation in the electrical impedance of system. Therefore, the change in impedance characteristics can be correlated with cell spreading, attachment and other cellular activities. This methodology was then used in [34] to measure the epithelial cells proliferation. Moreover, electrical impedance measurement is also used to monitor attachment, morphology and spreading of fibroblasts cells in culture [35].

Thus, in the presented research, 3D Gelatin/Chitosan (G/Ch) scaffolds are doped with carbon black (C), which transforms the simple G/Ch scaffold into conductive sensorized carbon-based composite scaffold (G/Ch/C). The scaffolds are then characterized by bio-impedance monitoring method. In first part of the paper, methodology and materials has been described; which includes scaffold fabrication and experimental setup used to perform scaffold characterization. Whereas, in the later section, results are discussed in terms of bio-impedance measurement and electrical conductivity analysis.

2 Materials and Methods

2.1 Scaffold Preparation

The simple scaffold is mainly composed of gelatin (G), chitosan (Ch) and poly(ethylene glycol) diglycidyl ether (PEG), whereas in carbon based scaffold, conductive carbon black (C) is added as an additional element. In order to prepare scaffold, 5 g of G was dissolved in 50 ml distilled water with gentle magnetic stirring at maintained temperature of 40 °C. Afterwards, 1.4 g of PEG was added which acts as a cross linker and facilitates cell adhesion. Nextly, chitosan solution (2% wt., 30 g) was added into G/PEG mixture. To obtain G/PEG hydrogel, the reaction mixture was gently stirred at 40 °C for 20 min and poured into the glass plate for gel formation. Then the gel was cut into rectangular bar and frozen by dipping into liquid nitrogen bath maintained at a temperature of −196 °C. The frozen samples were freeze-dried for sublimation of ice crystals. Finally, in order to further increase the degree of grafting, the dried Gelatin/Chitosan scaffolds were placed into an oven under vacuum for 2 h at 45 °C. The preparation procedure for carbon black based scaffold (G/Ch/C) is similar to G/Ch scaffold, only with an addition of carbon black. The final composition of prepared scaffolds are listed in Table 1. The structural, mechanical and biological characterization of prepared scaffold has been reported in [36].

Table 1 Scaffold composition

Scaffolds	Composition				Physical structure
	G (%)	PEG (%)	Ch (%)	C (%)	
G/Ch scaffold	72	20	08	–	
G/Ch/C scaffold	62	14	17	07	

2.2 Experimental Setup and Measurement Protocols for Bio-impedance Spectroscopy

The experimental setup used to analyze impedance response of scaffold is shown in Fig. 1. Both G/Ch and G/Ch/C scaffolds were cut into same rectangular shape and size with length, width and thickness of 13 mm, 1.5 mm and 0.8 mm respectively and were made hydrated with distilled water. The impedance analyzer "hp 4194A" is used with its terminals connected with two ends of scaffold. The analyzer is interfaced with LabVIEW software, which controls the analyzer operation and records the measured impedance values. Impedance Measurements are then analyzed on MATLAB, where its magnitude, phase shift and conductivity is evaluated. The parameters set for impedance analyzer operation includes: Frequency Sweep: 10 kHz–3 MHz, Sweep Type: Linear, Sweep Mode: Repeat, Data Averaging: 4, Integration Time: Medium, Total No. of Points: 401 and Sample Rate: 200 ms.

3 Result and Discussion

3.1 Bio-impedance Measurement

The preliminary experimental results (Fig. 2) show that hydrated scaffold (with distilled water) changes its impedance response with respect to time. Initially, at 7 h, scaffold shows more capacitive behaviour which progressively turns into resistive after 72 h. As scaffold responds differently at different time period in terms of impedance fluctuation, therefore, in future this characteristic would be helpful to monitor the cell activities on scaffold. In addition, the results exhibit that by adding carbon black the impedance value of G/Ch/C (Fig. 2b) scaffold decreases as compared to G/Ch scaffold (Fig. 2a) which will also increase the overall conductivity of carbon based scaffold.

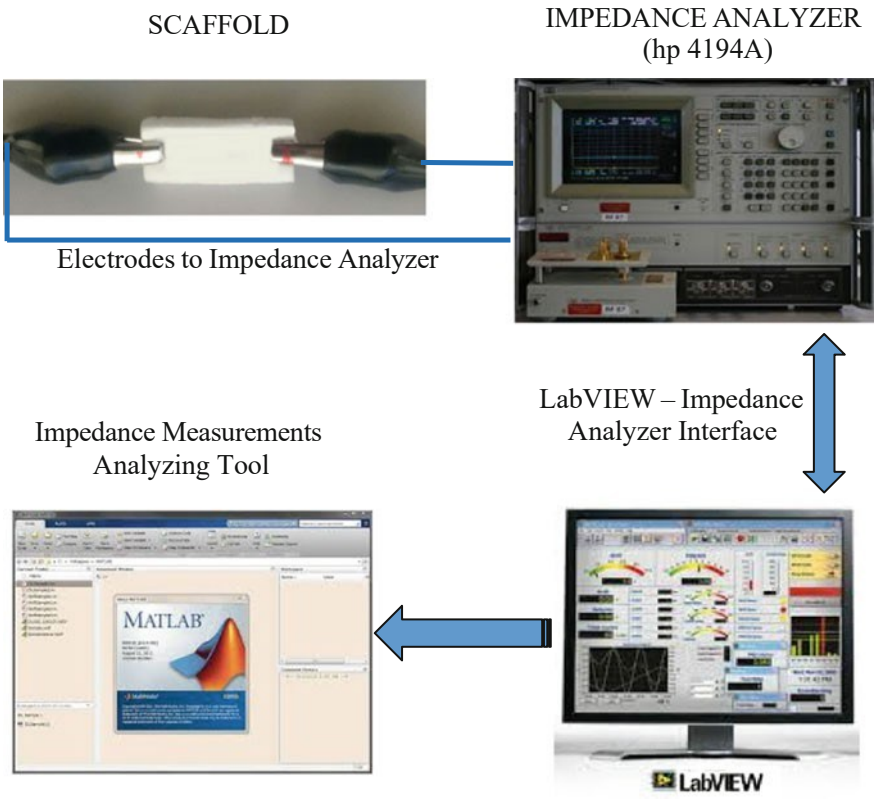

Fig. 1 Schematic representation of experimental setup

3.2 Electrical Conductivity Analysis

From impedance magnitude, resistive values were extracted and it was found that internal resistance within scaffold structure decreases as carbon black was added. This suggests the formation of perfect embedded electric network between carbon black and gelatin matrix. Furthermore, resistive values were used to evaluate the electrical conductivity (σ) of simple Gelatin/Chitosan and carbon-based Gelatin/Chitosan scaffold using "Pouillet's Law Equation" (Eq. 1).

$$\sigma = \frac{L}{R * A} \tag{1}$$

where "σ" is scaffold's electrical conductivity, "L" is the distance between electrodes, "R" is the scaffold's resistance and "A" is cross-sectional area of scaffold. A comparative analysis of the scaffold's conductivity shows that carbon black strongly enhanced the electrical conductivity (around 10 times) as compared to G/Ch scaffold (Table 2).

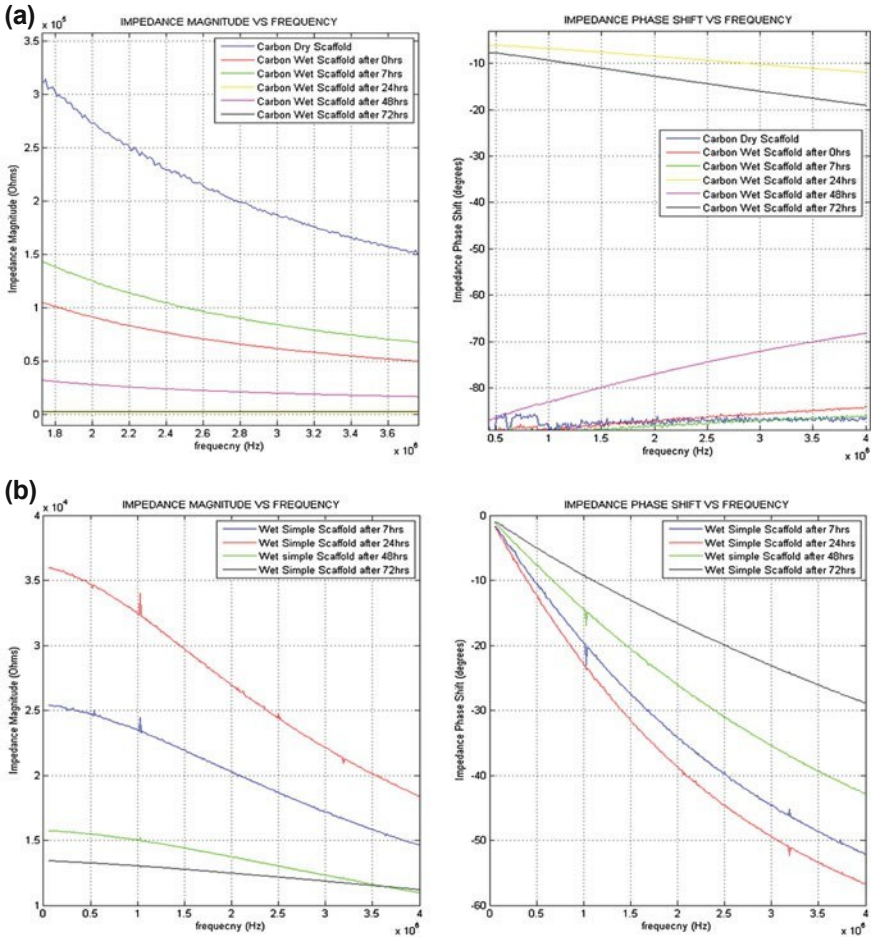

Fig. 2 Impedance Measurement of: a wet G/Ch scaffold at different hours b wet G/Ch/C scaffold at different hours

4 Conclusion

In this research G/Ch has been sensorized and transformed into a conductive G/Ch/C scaffold. As gelatin based scaffold was not highly conductive, therefore, using a suitable conductive filler was necessary to increase its conductivity. The scaffolds are electrically characterized via bio-impedance spectroscopy method and experimental result shows that carbon black decreases the internal resistance and increases the conductivity of scaffold approximately 10 times which shows the development of conductive network throughout the scaffold. Hence, the obtained results are quite promising and it is expected that in future, cell activities could be monitored

Table 2 Scaffold conductivities at different observation hours

Observation hours (h)	Hydrated G/Ch scaffold		Hydrated G/Ch/C scaffold	
	Conductivity (minimum) (S/m)	Conductivity (maximum) (S/m)	Conductivity (minimum) (S/m)	Conductivity (maximum) (S/m)
07	0.022	0.064	0.24	0.51
24	0.016	0.057	0.13	0.19
48	0.036	0.072	0.31	0.54
72	0.043	0.059	0.70	0.74

non-invasively via impedance measurement technique. Thus, future analyses will include the seeding of different cellular types in order to identify specific impedance variations due to cell activities.

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