**Effect of Ionic Concentration on Chitosan Scaffold Characteristics Measured by Electrochemical Impedance Spectroscopy**

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## Introduction

Current tissue engineering approach utilizes porous scaffolds within which embedded cells are allowed to develop the scaffold into functional 3D tissue substitute. The construction material of the scaffolds needs to be conducive to the incorporation of cells *in vitro* and to maintain the structural integrity of the scaffolds under *in vivo* conditions. It is necessary to characterize the scaffold material under these conditions and to determine the property change of the cell-seeded scaffolds during the remodeling process. It is also important that the scaffolds cannot be destroyed or otherwise compromised during the characterization testing thereby permitting cell incorporation and long-term evaluation. Chitosan is a naturally occurring polysaccharide which is capable of forming a porous matrix that allows cell infiltration and has shown a high level of biocompatibility when used as implant material [1]. In this study, electrochemical impedance spectroscopy (EIS) is used to non-destructively characterize the chitosan scaffold under varying ionic composition, from which the porosity of the chitosan scaffold can be derived [2,3].

## Materials and Methods

Chitosan scaffolds were fabricated by dissolving 4 wt% dry chitosan powder in 2 vol% acetic acid (all from Sigma) heated at 35 ˚C. The chitosan solution was cast into a specimen container and frozen for 24 hours, followed by lyophilization for 24 hours resulting in a foam-like 3D matrix. EIS was performed on the dry sample using titanium wire probes attached to an impedance analyzer (model 1260A; Solartron). A constant current of 0.1 mA was used for the evaluation and scanning was performed over the frequency range of $1x10^{-2}$ to $1x10^{4}$ Hz. The chitosan scaffold was then immersed in deionized water until reaching total saturation of 16 ml, followed by EIS using the same current and frequency range. Then, the scaffold was submerged in solutions of phosphate buffered saline (PBS) with various dilution ratio, 100%, 50%, 29%, and 20% (v/v). Impedance analysis was repeated with identical experimental parameters. The magnitude of the impedance was plotted against the frequency. The effect of ionic composition on the shift of the impedance magnitude was examined. The dielectric permittivity of the chitosan scaffold was obtained by best-fitting the impedance measurement data.

## Results and Discussion

Immediate observation of the wire probe insertion site showed no apparent damage which indicates that the EIS was nondestructive on the scaffold. The impedance analysis of the dry sample resulted in the expected high impedance magnitude over the entire frequency range and multiple runs showed reproducible and comparable results. Saturating the chitosan scaffold with deionized water lowered the impedance. Ionic composition affects the impedance as increasing ionic concentration decreased the impedance values (Fig. 1).

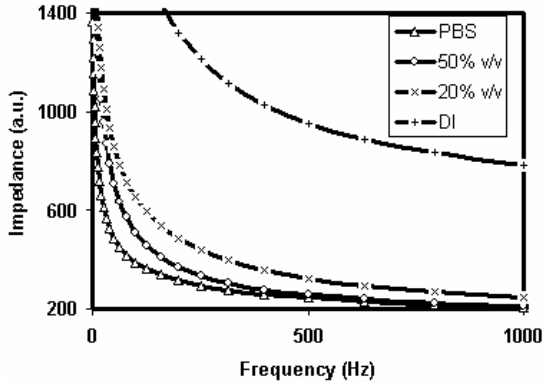


Figure 1. Change of impedance of the chitosan scaffold with respect to frequency under different ionic composition. Δ: undiluted PBS, o: 50% v/v dilution of PBS, x: 20% v/v of PBS, +: deionized water.

Impedance measurement leads to the calculation of complex dielectric permittivity, which in turn is used to derive the porosity. Generally, lowered impedance is associated with increased porosity [2]. Therefore, we expect the porosity of the chitosan scaffolds to be decreased under low ionic concentration. Under such condition, it is possible that the degree of swelling of the chitosan scaffold is higher as compared to that under high ionic concentration, which leads to lowered porosity.

## Conclusion

The data acquired and interpreted from the electrochemical impedance spectroscopy provides an effective non-destructive way of characterizing the physical properties, such as porosity, of the chitosan scaffold *in vitro*. This same method of analysis could be used to characterize the response of the cells incorporated into the scaffold thereby providing an opportunity for non-destructive monitoring of the remodeling of cell-seeded scaffolds into functional tissue substitute.

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## References

1. Li and Zhang, (2005) J. Biomed. Mater. Res. **75A**. 485.
2. Maquet et al., (2000) Langmuir . **16**. 10463.
3. Bagnaninchi et al., (2004) IEEE Trans. Nanobiosci. **3**: 243.