A Comparison Study among Characterization Methods for the Pore Geometry of Chitosan Scaffolds

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Statement of Purpose: Tissue engineering utilizes porous scaffolds which act as a temporary extracellular matrix (ECM) within which embedded cells are allowed to develop into a functional 3D tissue substitute. It is necessary to characterize the scaffold material to ensure that the cells can infiltrate and attach to it. In additon to the initial porosity, continuing characterization is necessary. Following the incorporation of growth factors or remodeling by the cells, the scaffold undergoes changes which must be monitored. The scaffolds cannot be destroyed or otherwise compromised during the characterization testing thereby permitting cell incorporation and long-term evaluation. There are a variety of known methods which provide infromation on characteristic pore geometry and size including mercury intrusion (MIP) and scanning electron microscopy (SEM). The concern over these testing methods is the destruction of the scaffold in the procedure thus continuing monitoring or evaluation of the scaffold remodeling process cannot be followed. It is, therefore, beneficial to develop a comparable non destructive test (NDT) thereby permitting long-term evaluation of the scaffold both prior to the inclusion of cells and after the cells have been seeded. Electrochemical impedance spectroscopy (EIS) has the potential as a NDT and was therefore compared to the commonly used and proven methods of SEM and MIP.

Materials and Methods: The polysaccharide chitosan forms a porous 3 dimensional matrix and shows a high level of biocompatibility when used as implant material. Scaffolds are fabricated by dissolving 2% wt per vol chitosan powder in 2% acetic acid followed by lyophilization. For the destructive, proven characterization methods, MIP and SEM were performed on dried chitosan scaffold. The SEM images were analyzed using NIH Image J software in order to determine the average pore diameter. During MIP, the applied pressure and the corresponding volume of mercury penetrating into the scaffold were recorded. The MIP software then automatically converts the data into porosity data and mathematically derives the average pore diameter. NDT method of electrochemical impedance spectroscopy (EIS) was run with a constant current of 0.1 mA between two titanium electrodes with the frequency sweep of 10^6 to 10^{-4} Hz. Scaffold samples were run with varying concentrations of phosphate buffered saline (PBS) to allow for testing within a physiological range and 100% PBS was used to evaluate the testing consistency. Pore diameters obtained form SEM and MIP were first compared, followed by comparison on porosity obtained from MIP and EIS. The comparison of the data acquired from each technique allows for the evaluation of the effectiveness of the EIS in relation to the proven pore geometry tests.

Results and Discussion: The SEM image analysis showed an average pore diameter of about 107 μm with very few major deviations. MIP data revealed an average pore diameter of about 82 μm (Table 1). The results suggest that the pore size determined by SEM is comparable to that by MIP. The overall porosity as determined by MIP was about 73% (Table 1).

The EIS produced a Nyquist plot of the real (x-axis) vs. imaginary impedance (y-axis) which showed that the curve was projected to produce an x-intercept at approximately 10600 ohms, which is the bulk resistance of the scaffold. This value was confirmed when the measurement was repeated using a 4-point DC probe configuration. From these data, the bulk porosity of the scaffold was estimated to be 71%, which was very close to that obtained by MIP, suggesting that both techniques are similar in determining porosity. EIS, however, has the added advantage that it can be used to continuously monitor the changes of the scaffold characteristics under aqueous media.

Table 1 Comparison of pore size and porosity of chitosan scaffold using three different methods

Method	Pore Size (μm)	Porosity	Comment
SEM	107.31	---	Do Not Test for Porostity
MIP	82.22	72.88%	Dry Sample
EIS	---	70.99%	Wet Sample

Conclusions: The pore diameter values found by SEM and MIP indicated that both the image based and pressure based analyses provided similar result. In addition, the data provided by the EIS was adequately comparable to the MIP based on the respective porosity values of 71% and 73%. The advantage of the EIS as an NDT is that it can be performed under physiological ionic conditions whereas SEM and MIP require dry samples and vacuum conditions for measurement. These benefits make EIS a viable option for the characterization and long term observation of chitosan tissue scaffolds.

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