

# Characterization of Natural and Synthetic Polymer Blend Scaffolds for Tissue Engineering

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## Introduction:

Tissue engineering provides an approach for generating functionally replaceable tissue parts. Biodegradable scaffolds are used to support and guide the in-growth of cells. The idea is that the scaffolding material eventually disappears leaving only the necessary healthy tissue in a topologically required form (1,2). While numerous biodegradable polymers have been investigated as candidates for tissue engineering scaffolds, a vast majority do not meet all the required properties. These characteristics include: proper mechanical properties, tunable degradation rates, cyto-compatibility, and the ability to direct cell growth and differentiation.

The typical polymeric candidates for scaffold formation can be separated into two main categories a) natural and b) synthetic. Natural polymers or their derivatives such as chitosan(3), alginates(4), fibrin (5), gelatin (6), collagen and glycosaminoglycans (GAGs) (7) have been used as scaffolding materials due to their inherent bioregulatory activity. On the other hand, synthetic polymers such as Polylactides, polyglycolides and their copolymer poly-lactic-co-glycolic acid (PLGA) (8), polyurethanes (9) polycaprolactone (10) have also been used, due to their easily tailorable degradation rates and mechanical properties. These two main groups of bio-polymers compliment each other in their inherent properties, thus combining natural and synthetic polymers has presented an opportunity to take advantage of each groups desired properties.

We studied the possibility of combining chitosan, a derivative of chitin, which has shown to have favorable biocompatibility and anti-infective properties with PLGA. PLGA has generated immense interest due to its strong approval history and has been investigated for many applications. Currently tissue scaffolds can be synthesized using various fabrication techniques, but most require dissolving the intended polymer in a solution followed by further processing. Since chitosan and PLGA do not share a common solvent, an emulsion system as a possible solution was investigated. In particular, the method of emulsification, stabilizer used, and the type of organic solvent used to dissolve PLGA, was investigated. 3D scaffolds were formed using controlled-rate freezing technique and subsequent lyophilization. Using these scaffolds, the relationship between porosity and concentration of each polymer were investigated. Also the effect of PLGA on the degradation of chitosan was studied. These results show promising potential in tissue engineering.

## Experimental:

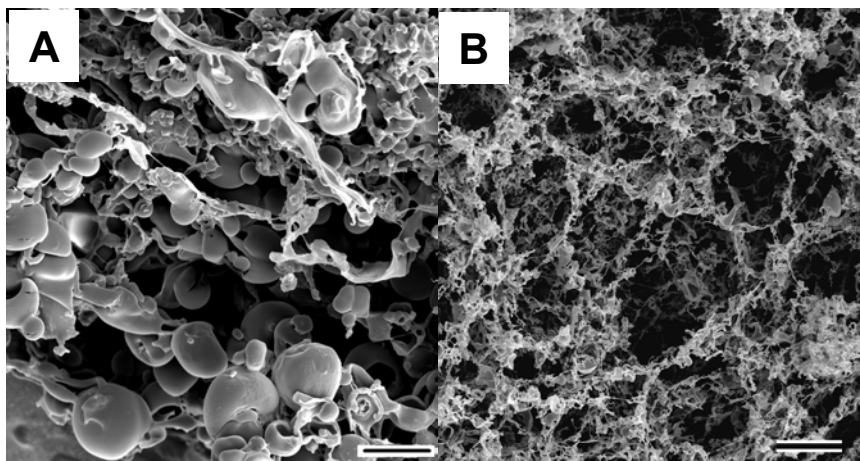
One percent to 0.1% (wt/volume) chitosan solutions were prepared by dissolving in 0.2M acetic acid and mixed with 1 to 10% (wt/volume) 50:50 (glycolide: lactide) PLGA solutions in chloroform, methylene chloride, DMSO, or benzene. Phosphatidylcholine (PTC) was used as emulsifier and was added to the PLGA solution prior to mixing. Scaffolds were prepared by placing said emulsions in 15mm diameter flat bottom vials and freezing at -20 ° C, -78 ° C, or -196 ° C. The frozen samples were lyophilized until dry.

Lyophilized scaffolds were sectioned at various planes and examination under a Scanning Electron Microscope (SEM). Obtained digital micrographs were analyzed for pore area, major axis, minor axis and shape factor using image-analysis software.

Sixty four blend scaffolds using chloroform as the organic solvent were prepared for the degradation study. Half (32) were put in 10 ml of buffer solution and placed in 37°C incubator while the second half were put in 10 ml of buffer solution containing 10 mg/L hen egg white (HEW) lysozyme and placed in similar conditions. Sixty four pure chitosan scaffolds were also prepared to be used as controls and treated to the same conditions as the blended scaffolds. The buffer solution was changed once a week.

#### Results:

First, the amount of PTC was optimized based on the minimum amount required to stabilize the emulsion for 24 to 48 hr. Various quantities of PTC were added to 3:1 solvent ratios of water and chloroform. Stability of the emulsion was observed in all the mixtures with different polymer contents so that the minimum amount of PTC required could be determined. Scaffolds were formed without and with PTC using controlled rate freezing and lyophilization. Micro-architecture analysis using SEM showed globules of PLGA in the chitosan network in the scaffolds formed without the emulsifier (**Figure 1A**). On the other hand, addition of the emulsifier inter-dispersed (**Figure 1B**) the two components uniformly throughout the scaffold and the scaffolds showed a homogenous structure.



**Figure 1.** Panel A. Micrograph of a scaffold in the absence of a stabilizer. Panel B. Micrograph of a scaffold in presence of a stabilizer.

To understand how the MW of PLGA influences the pore morphology, 19kD, 75kD and 160 kD PLGA were blended with 0.3% chitosan. These results showed that the increase in MW of PLGA decreased the pore sizes of the scaffold and the shape of the pores were not affected.

To study the influence of rate of freezing on micro-architecture of the scaffold, 3% 19kD PLGA in chloroform was mixed with 0.3% chitosan and then frozen at various temperatures. These results showed that controlled rate freezing easily regulated the pore size distribution between 2  $\mu\text{m}$  and 300  $\mu\text{m}$ , similar to pure chitosan scaffolds.

Degradation results show that samples containing chitosan/PLGA incubated in the presence of lysozyme suffered structural collapse as compared to the 3 different control samples. The pH measurements indicated that samples containing chitosan and PLGA incubated in the presence of lysozyme show significant reduction in pH as compared to all other conditions.

#### Discussion:

This study evaluated the blending of chitosan with 50:50 PLGA in the emulsification mode using PTC as the stabilizer. An emulsion system was used to homogeneously disperse PLGA within the chitosan scaffold. This was done to prevent chemical changes to chitosan which might affect its desirable properties such as cell attachment and survival. Highly porous three dimensional scaffolds were obtained with no apparent chemical interaction between chitosan and PLGA.

Analysis of the prepared scaffolds showed that as expected the freezing temperature influenced pore size, similar to pure chitosan scaffolds (3), but MW of PLGA as well as the type of organic solvent also influenced the pore morphology. Chitosan degradation seems to be accelerated by the presence of PLGA. This is due to the acidic environment created by the degradation of PLGA which increases lysozymic activity.

This system is not exclusive to chitosan and PLGA combination alone and can be used for any two biopolymers where the solvents are aqueous and organic. In summary, this study shows PLGA can be blended with chitosan and it is beneficial for degrading chitosan faster.

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