Effect of Solvent Acids on Physical Properties and Cell Attachment to Chitosan Microsphere-based Scaffolds

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Statement of Purpose: Chitosan (CS) materials have shown much potential for bone tissue engineering applications in part due to their biocompatibility, degradability and non-toxic degradation products. CS is a co-polymer of glucosamine and N-acetyl-glucosamine sugars, and the ratio of the sugar monomers is referred to as the degree of deacetylation (DDA). CS is soluble in dilute acids which make processing into fibers, films, sponges and other forms relatively easy[1]. Typically acetic acid is used to dissolve CS since it is a mild acid[1]. Many properties of CS are related to DDA such as mechanical strength and degradation. While CS properties may be controlled in part by DDA, different acid solvents may also be used to manipulate CS physical/mechanical properties. Therefore, the aim of this project was to evaluate the effects of three solvent acids, acetic, lactic and formic on the physical/mechanical properties and cell attachment to CS microsphere-based scaffolds

Methods:

Scaffold Construction: Chitosan microspheres ~ 1mm in diameter were made by dissolving 3.57g of 80% DDA CS (Primex ChitoClearTM, Iceland) into 100ml of 2% acid (lactic, acetic, or formic) and then dripping via a syringe pump into a NaOH-methanol solution [2]. Microspheres were washed with DI water until ~ pH 7, air dried, lightly packed into plastic tubes and then rinsed in respective acids (lactic: 0.5%, acetic: 0.05%, formic: 0.25%) to bond spheres into 12.7mm long 6.4mm diameter scaffolds. Scaffolds were sterilized via ethylene oxide gas. Porosity of the scaffolds was determined based on the Archimedes principle using methanol (n=5/acid). *Degradation and Mechanical testing:* Degradation of CS scaffolds (n=4/acid) was measured by mass loss using 500µg/ml lysozyme solution at 37C at 1,2,3, and 4 week intervals. Mechanical properties of the degraded samples (n=4/acid) were tested in compression using an Instron Electromechanical Test System (Model 4465) with a 5kN load cell. Scaffolds were tested to 50% compressive strain at a crosshead speed of 0.5 mm/min. *Crystallinity:* The crystallinity index (CI) of the chitosan microspheres, made with the different solvent acids, was determined using X-ray diffraction on a Bruker D8 Advance using Cu-Kα radiation at 40 kV and 40mA. The CI (n=1/acid) was calculated using the equation: $CI = ((I_{110} - I_{am})/I_{110}) * 100$, where I_{110} is the major chitosan crystalline peak at $2\theta = 20^{\circ}$ and I_{10} is the amorphous peak at $2\theta = 16^{\circ}$. *Cell Attachment:* Mouse stromal cells (W-20-17) were used to test cell attachment CS scaffolds made using different acid solvents after 1 day. Cells were seeded onto scaffolds of each type (n=5) at a density of 2 X 10^5 cells/scaffold in 1mL of complete growth media (DMEM+1%AB/AM+10%FBS). The seeded scaffolds were then placed in an incubator at 37˚C/5% CO_2. The number of attached cells was estimated using the CellTiter-Glo® bioluminescent assay kit, which estimates cells based on amount of ATP using the luciferin-luciferase reaction. Data are expressed as relative light units.

Results:

Table 1 shows the results of the porosity and crystallinity of the scaffolds. Scaffolds made with acetic acid solvent exhibited greater porosity and higher CI values than scaffolds made with lactic or formic acid.

Table 1: Physiochemical properties of CS scaffolds

Solvent Acid	Porosity	Crystallinity
Acetic Acid	29.74 ± 2.61	98.87
Formic Acid	26.69 ± 2.10	90.22
Lactic Acid	26.22 ± 3.22	86.68

Percent mass loss as an estimator of degradation was minimal over the 4 week period for all scaffold types. However, there was significant decrease in compressive modulus over time for all scaffold types with the scaffold made with formic acid loosing mechanical integrity faster than the other two types (Figure 1). The little change in mass may be due to the high density of the microspheres, but loss in mechanical properties was due mainly to the loss of bonding between microspheres which may have occurred due to the lysozyme action. Cell attachment after 1 day seeding was greatest on the scaffold made with lactic and formic acid solvents than on scaffolds made with acetic acid (Figure 2).

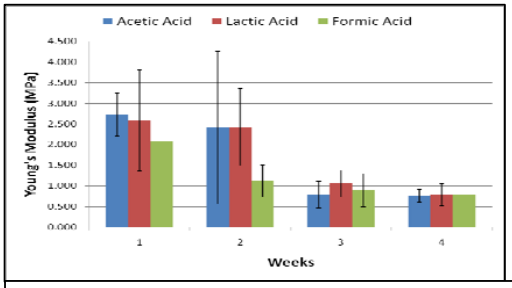

Figure1: Young's modulus of CS scaffolds made with different acids using lysozyme (n=4) over a 4 week degradation study

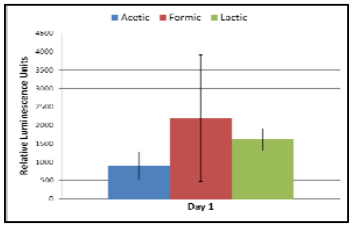

Figure 2: Cell attachment on CS scaffolds made with different acids (n=5).

Conclusion:

Chitosan microsphere based scaffolds made with lactic or formic acid solvents exhibited lower CI and mechanical properties but supported greater cell attachment than scaffolds made with acetic acid solvent. Different acid solvents may be used to modulate chitosan physical and cell properties.

References:

1. Khor E. "Chitin." 1st ed. Elsevier Science Ltd. 2001.
2. Chesnutt B, et al. J Biomed Mater Res-A. 2009; 88(2):491-502.

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