Gelatin Coupled with Silane as a New Tissue Engineering Scaffold

Bosun, Kown[1*], Zhi Yang[**] Marcel E. Nimni[**], and Bo Han [2]**
* Dept. of Biomedical Engineering, University of Southern California, LA. CA,
** Dept. of Surgery and Biochemistry and Molecular Biology, University of Southern California, L.A. CA,
[1]bkwon@usc.edu and [2]bohan@usc.edu

Introduction: As a biomaterial, gelatin permits cells to attach and proliferate readily. It also provides highly reactive functional sites, such as hydroxyl group and amide groups, to react with coupling agents for better biological and mechanical properties.[1,2,3] Silicone, also as a biomaterial, provides excellent mechanical and physical properties and it is chemically inert. In this study, we modified gelatin through coupling with organosilane.[4,5] The reaction and resulting modified gelatins with silane (MGS) were characterized by various tests, such as FT-IR, TGA and mechanical analyses. Cytotoxicity was evaluated *in vitro*.

Methods:

Gelatin-Silane coupling reaction: Silane (S) (Dow-Corning; z-6040 and z-6011) with concentrations of 5% (v/v) was adjusted to pH 3.8. Gelatin, with concentration of 5% or 10% (w/v) was heated at 60℃ for 10 min followed by adding 0.5g or 1.0g fructose. Silane solution was subsequently mixed to gelatin solution with ratios of 1:1(v:v). The solution was stirred at 65℃ for 10min and then at 100℃ for 10min. After-curing was proceeded at 70℃ for 1 hour. Film was made by casting. Suitable shapes of material were prepared for mechanical, thermal, and FT-IR analyses.

FTIR assay: FT-IR spectra were acquired on *Thermo Nicolet, 370 FT-IR* with the shape of circle of samples (thickness: < 80 um and diameter=15mm).

Mechanical testing: Mechanical properties were measured using MTS 858 Mini Bionix with force transducer (*Interface Company*) at a rate of loading of 5mm/sec. Samples, 10mm in width, and 80~150 µm in thickness, were tested (n=7).

Cell attachment and proliferation: Fifty microliter of MGS were incubated with culture medium (DMEM with 10% FBS) for different time periods (1 to 24 hours). Human Fibroblasts were seeded with the density 20k cells/well in 48 well-plates. After cells attached for 24 hours, eluted medium from MGS gel were added to the cell culture system for another 24 hours. MTT assay was employed for measuring cell proliferation at the end of 24-hour culture period. Fibroblasts were also cultured on the glass surface coated with patterned MGS. After 7 day culture, cell were fixed and stained with Diff-Quick solution (*Baxter*). Fibroblast attachment and growth were evaluated by a microscope (*Alphashot, YS, Nikon,* 100x magnification).

Results/Discussion:

Gelatin-Silane with improved mechanical properties: FTIR showed the characteristic Si-O-Si and Si-O-C stretching bands in the range of 1200~1000, suggesting S was coupled with G in the MGS films. Increased peaks of C-H bending and C-H stretch vibration (900, 2800-3000) confirmed the G-S coupling (Fig1).

Addition of silane as a coupling agent to gelatin provided a significant improvement of the mechanical properties comparing to G film. Mechanical properties can be controlled by the content of water and by the ratio of gelatin and silane (Table1).

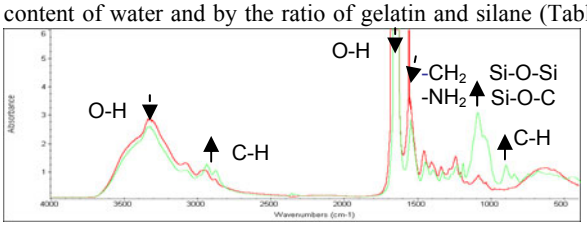

Figure 1. FTIR spectra: G and MGS film with the ratio between gelatin and silane; Red : Pure Gelatin, and Green : G:S=1:1

Table 1. Mechanical properties

Samples	Tensile Strength [MPa]	Elongation at break
Gelatin	18.63±0.37	16.82±6.41
12% H_2O MGS	4.26±1.27	160.54±27.15
5% H_2O MGS	21.79±1.24	121.42±20.94
0.1% H_2O MGS	72.62±10.73	14.8±4.73

Increased structural stability of MGS were confirmed through various tests such as TGA analysis, swellability in water and by enzymatic degradation (Data not shown)

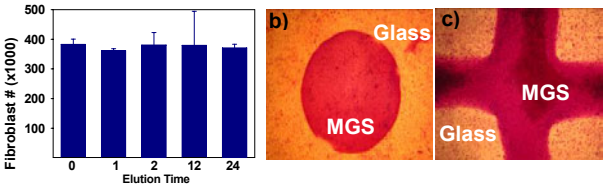

Figure 2. (a). Human Fibroblasts proliferation. (b) and (c). Human Fibroblast attachment and growth on the MGS coated glass for 7 days.

MSG supports cell attachment and proliferation:
Figure 2a showed MGS had no toxicity. Cells were proliferated almost the same to that of control regardless of time intervals. There was no difference in human fibroblasts' attachment and proliferation on the surfaces of MGS or on the glass (figure 2b and 2c). These results suggest MGS film provides a favorable scaffold for cell attachment, colonizing and growth.

Conclusions: Gelatin was successfully coupled with organosilane to produce more stable structure. Cytocompatibility of MGS *in vitro* with better mechanical properties and stability in aqueous condition and in thermal decomposition implies it may be good candidate for tissue engineering scaffolds.

References:

1. Hua A. In vitro Cell. Dev. Biol.-Animal. 2002;38:487-492
2. Krishna B. J. Biomed Mater Res. 2004;68A:210-218
3. Chaobo X. J. Appl Polym Sci. 2002;83:949-955
4. Jie K. J. Polymer. 2006;47:1519-1525
5. Peter E. J. Appl Polym Sci. 2001;82:2016-2026