The Effect of Chemically Crosslinking SIS-ECM Scaffold Materials on the Macrophage Phenotype

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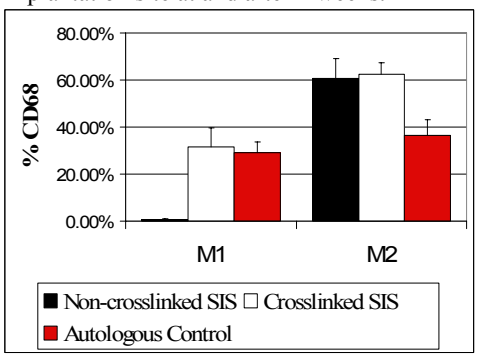
Statement of Purpose: Small intestinal submucosa (SIS) represents a prototype extracellular matrix (ECM) scaffold material that successfully promotes the constructive remodeling of tissues in numerous body systems[1]. The host tissue response to an implanted scaffold material is determined by many factors, one of which is the rate of degradation. Chemical crosslinking of biologic scaffolds such as SIS significantly decelerates the rate of in-vivo degradation and thus leads to distinctly different remodeling outcomes[2]. Mononuclear macrophages are key participants in the host response to an implanted scaffold material. Macrophages are phenotypically characterized as pro-inflammatory (M1) or anti-inflammatory/immunoregulatory (M2)[3]. Macrophage profiling may be useful in evaluating the inflammatory response to biologic scaffolds. The present study determined the M1/M2 profile of the host tissue response to commercially available scaffolds composed of non-crosslinked SIS (Restore™) and carbodiimide-crosslinked SIS (CuffPatch™) in an established model of rat body wall reconstruction. Autologous body wall tissue was used as a control graft material.

Method: Partial thickness 1-cm^2 defects were created in the abdominal wall of Sprague-Dawley rats and repaired with non-crosslinked SIS (Restore™), SIS crosslinked with carbodiimide (CuffPatch™), or autologous excised tissue. The tissue specimens were harvested at 1, 2, 4, and 16 weeks after implantation and fixed in formalin. Serial sections were cut from paraffin-embedded tissue, deparaffinized, and rehydrated prior to immunohistochemical detection of the macrophage phenotype. Antigen retrieval was performed by boiling the slides for 20 minutes in 0.01M citrate buffer. Non-specific antibody binding was blocked by incubation in 2% normal horse serum for 30 minutes at room temperature (RT). The serial sections were then incubated at 4ºC overnight with the following mouse monoclonal antibodies and used at the indicated dilutions: anti-CD68 (pan macrophage marker, 1:50), anti-CD163 (indicative of a M2 profile[2], 1:50), anti-CD80 (indicative of an M1 profile[2], 1:10), and IgG isotype (negative control, 1:10). After primary incubation, endogenous peroxidases were quenched with 3% hydrogen peroxide for 30 minutes at RT. The biotinylated secondary horse anti-mouse antibody (1:50) was applied for 30 minutes at RT. Horseradish peroxidase-conjugated strepavidin detection solution was then applied for 30 minutes at 37ºC. The substrate diaminobenidine (DAB) was applied to each slide and monitored microscopically for proper staining intensity. The slides were then counterstained with Hematoxylin for contrast, dehydrated through a series of alcohols, cleared in xylene, and coverslipped with a toluene-based mounting media. Immunopositive cells were counted in 4-6 site-matched 40X microscope fields using a Nikon e600 microscope in a blinded fashion. The negative control counts were subtracted from the primary antibody groups. The M1 and M2 ratios were calculated by dividing the number of CD80+ or CD163+ cells by the number of CD68+ cells, respectively. The average absolute number of CD68+ cells for each graft type and time point was also compared.

Results/Discussion: The figure below illustrates the M1/M2 ratios for each device at 2 weeks post-implantation (mean ± SEM). The non-crosslinked SIS device showed a predominately M2 response and virtual absence of M1 macrophages throughout the study duration. The crosslinked SIS device showed a progressive dominance of an M1 response over the 16 week study duration, with a complementary decline in the M2 response, and the majority of the macrophages showed an M1 response by 4 weeks. The autologous control showed a duality in the M1 and M2 expression after 1 week. The SIS devices showed a sharp decline in the number of CD68+ cells at 16 weeks, and the autologous control showed a low count of CD68+ cells in the implantation site at and after 2 weeks.



Conclusions: There is a discernible difference in the macrophage response between the non-crosslinked and crosslinked SIS-ECM scaffold materials. The implantation site of the non-crosslinked SIS device (Restore™) showed a prevailing M2 response throughout the remodeling period, and the chemically crosslinked SIS scaffold (CuffPatch™) showed a progressively dominant M1 response. These findings correlate with histological results, in that at 16 weeks post-implantation non-crosslinked SIS scaffolds were replaced with organized muscle and collagenous connective tissue, while crosslinked SIS devices elicited a foreign body reaction that continued for at least 16 weeks. The M1/M2 phenotypic profile of the host tissue response can be used as a predictor of the course of scaffold remodeling and the eventual outcome.

References: 1) Badylak SF. Transpl Immunol. 2004; 12:367-77. 2) Valentin JE. J Bone Joint Surg. in press 2006. 3) Mantovani A. Trends Immunol. 2004;25:677-86.

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