Incompatibility of Biocompatibility Standards for Biologically-Sourced Biomaterials

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Statement of Purpose: Bringing biologically-sourced biomaterial products ("biologic devices") to market under medical device regulations offers an attractive approach to reducing development costs and timelines. However, medical device regulations typically focus on products constructed of common synthetic polymers, metals, or ceramics, and normative standards or guidance documents often are poorly suited for biologic devices. Biological risk assessment, as codified in the ISO10993 series of biocompatibility standards, is one such area that presents manufacturers of biologics devices with unique challenges. The composition, modes of action, and interactions with patient tissues and organs are often highly complex for biologic devices, and assessing biocompatibility may require substantial deviations from standardized test procedures or wholesale development of custom test methods. Here we present such a custom test method intended meet the requirements of *ISO10993-9: Framework for identification and quantification of potential degradation products* for a biologics devices that in accordance with that standard require *further consideration* outside the scope of available vertical normative standards. Specifically, we developed a collagenase-based, *in vitro* degradation assay to demonstrate that commercially available small intestinal submucosa ECM-based (SIS-ECM) medical devices degrade in predictable patterns congruent with variables in manufacturing and design parameters, e.g. thickness or sterilization method. These data were successfully employed to support biocompatibility assessment and ultimate marketing approval of our biologic devices. **Methods**: In brief, samples of commercially-available, SIS-ECM devices were sub-divided and tared in individual test tubes to determine dry weight before adding 100 units/ml Collagenase I and incubating at 37°C. At predetermined time points, 1 ml of the digest was removed and centrifuged, and the supernatant was tested in a hydroxyproline assay. The remaining digest was centrifuged, and the supernatant discarded before lyophilizing the remaining solids. The percent undigested sample was calculated from the dry weight and undigested weight. Standard curves were made using either hydroxyproline or commercially-available porcine Collagen I. Samples from each product was completely digested in parallel to determine total hydroxyproline and collagen. Percent collagen and hydroxyproline in the test samples were determined and averages were calculated for each time point for n=4 lots. Statistical differences were determined by Student's t-test using a significance level of $p \leq 0.05$. **Results:** First, we explored the effect of an ethylene oxide (EO) sterilization cycle that was not expected to impact the structural integrity of a collagen rich ECM biomaterial. Devices were degraded prior to and after EO sterilization. We found no difference in degradability by weight, and the same null result was seen when the percent of collagen or hydroxyproline remaining was determined. Next, we explored the effect of prolonged storage by analyzing newly-minted devices to those left in controlled storage to their commercial end of shelf-life (range 10-29 months). There was no difference in degradability by weight. However, the effect of shelf-life on the percent of collagen and hydroxyproline remaining showed a significant difference at the 60-minute time point for both. Collagen degradation was complete in both groups at 120 min. We then compared the effect of laminating multiple sheets of ECM by comparing one- and two-layered devices. The increase in the amount of material per unit surface area slowed the rate of degradation, and there were statistical differences at all but the 60-min time point. Approximately 50% of the material was degraded at ~85 min in the 2-layer devices, compared to ~40 min in the 1-layer devices. The 2-layer devices appeared to be completely degraded at 240 min. Normalizing these same data to mass instead of area, we found similar rates of degradation until a maximum amount of component was released. This occurred at about 90 min and 120 min for the 1-layer and 2-layer devices, respectively. Finally, we compared two device types that had been dehydrated by either lyophilization or vacuum pressing. The lyophilized devices degraded slightly faster than the vacuum-pressed devices: Degradability by weight showed a statistical difference at 10 hours between these two groups. Lyophilized devices were ~50% degraded by 3.5 hours and completely degraded by 10 hours, whereas vacuum pressed devices were ~50% degraded by 6.5 hours and completely degraded after 10 and before 24 hours. **Conclusion:** Although the approach we present here may not completely suffice for regulatory expectations of compliance to ISO10993 Part 9, these data will supplement other lines of evidence, such as qualitative and quantitative compositional analysis, that, collectively, may elucidate potential degradation products released from biologic devices. This information should help regulators establish whether such tissue-engineered devices display an acceptable risk profile as they degrade *in vivo*. While our methodology does not capture the complexity of degradation products released from ECM-based medical devices *in vivo* or the mechanistically complex modes of degradation, it does provide a reasonable first-order approximation to aid in mechano-physical risk assessment that a device will behave is accordance with its intended uses and designed process and product parameters.