NanoSPONGE™ Crosslinked Natural Biopolymers Matrix

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Statement of Purpose: The goal of this work was to produce a crosslinked matrix comprised of two naturally occurring biopolymers, NanoSPONGE™, for use in chronic wound treatment. Key features of chronic wounds are over-production of proteolytic enzymes, fluid exudate and erosion of native tissue. An ideal healing matrix for these wounds will resist enzyme digestion, manage wound fluids and conform to the size and depth of the wound. Inherently, NanoSPONGE™ was designed to retain these features after sterilization. Current commercially available biologically derived dressings do not meet all of these criteria. The work presented here demonstrates the capability to produce a novel matrix that is well-suited to meeting the challenges of the chronic wound environment while taking full advantage of the potential of natural biomaterials.

Methods: The base scaffold used for this preparation was a collagen sponge derived from equine tendons by standard acetic acid extraction and freeze drying. The collagen was covalently crosslinked including the addition of hyaluronic acid, another natural biopolymer. Terminal sterilization was achieved with electron beam radiation to provide a sterility assurance level of 10^{-6} (SAL 10^{-6}). The material was tested for characteristics related to biochemistry, physical properties and biocompatibility. Hydration capacity was tested by measuring the mass of the dry material before a 2 minute soak in normal saline and measuring the hydrated mass after soaking. Mechanical testing for ultimate tensile strength was performed on 1x3 cm pieces. Samples were loaded into the grips of a Chatillon DFE-100 on a manual test stand and tested by pulling at a constant rate of approximately 1 mm per second. Biochemical testing was performed to determine the level of crosslinking imparted on the material. Thermal denaturation temperature (T_d) was measured by differential scanning calorimetry (DSC). DSC scans were taken from 40-120°C at a rate of 20°C/minute on a Mettler Toledo DSC 823^e. Enzyme resistance was determined by exposure to pronase (25 mg/ml, 50°C, 24 hours) with measuring the mass of dry material before and after enzyme exposure. Samples were submitted to the full panel of biocompatibility testing as dictated by ISO 10993. This is the standard testing required by FDA for a topical wound dressing product.

Results: Physical characteristics of the sponge did not change after crosslinking treatments. Tensile strength averaged approximately 6 N. Hydration capacity was greater than 800% change from dry to fully hydrated. T_d for the crosslinked, sterile material was 69.1°C compared to 49.1°C for the non-crosslinked, sterile material. Enzyme resistance results were 79.6% for the crosslinked, sterile material and 0.0% for the non-crosslinked, sterile material. Results of the ISO Biocompatibility panel are shown in the table below. In short-term implant studies (2 week i/m implantation in New Zealand White rabbits) the NanoSPONGE™ material demonstrated ability to persist and act as a frame work to support neo-vascularization, and infiltration of fibroblasts and macrophages. Cellular activity was present throughout the implant without encapsulation indicating histocompatibility of the NanoSPONGE™ material. On the other hand, the control non-crosslinked, sterile collagen sponge was resorbed, confirming its inability to survive the remodeling environment. The infiltrate was predominated by inflammatory cells and minimal amounts of disorganized fibrous tissue appeared replacing the implant area.

Test	Result
Cytotoxicity	Non-cytotoxic
Intracutaneous Reactivity	Non-irritant
Acute Systemic Toxicity	Non-toxic
Sub-acute Toxicity	Non-toxic
Sub-chronic Toxicity	Non-toxic
Hemolysis	Non-hemolytic
Material Mediated Pyrogenicity	Non-pyrogenic
Genotoxicity: Reverse Mutation	Non-mutagenic
Genotoxicity: Mouse Lymphoma	Non-mutagenic
Intramuscular Implant	Non-irritant

Table - Summary of Biocompatibility Results of Crosslinked Collagen/Hyaluronic Acid Material

Conclusions: The challenges of healing a chronic skin wound are myriad. Not only must the ideal dressing be physically compatible in terms of strength, fluid absorption and consistency, but it must also have desirable biochemical features. The ideal material will also be composed of natural biopolymers due to the inherent biocompatibility and presence of such components in normal, healthy tissue. Additionally, the crosslinking in the final, sterile material must be enzyme resistant. In many currently available dressings the crosslinks present do not withstand the degradative enzymes present in a chronic wound. NanoSPONGE™ , a cross-linked collagen and hyaluronic acid matrix developed by Nanotherapeutics not only meets the necessary physical and biochemical criteria, but is terminally sterilized and fully biocompatible. The benefits of using natural biopolymers, collagen and hyaluronic acid, are important to support healthy tissue remodeling as they are naturally present in normal skin tissue.

References:

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