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TESTING ALUMINA CERAMIC BEADS AS A BULKING AGENT FOR TREATMENT OF STRESS URINARY INCONTINENCE IN A RAT

Hypothesis / aims of study

Approximately 180,000 surgical procedures are performed to treat stress urinary incontinence (SUI) annually in the US. While endoscopic injection of a bulking agent into the proximal urethra provides a minimally-invasive, effective, and safe alternative to open surgery for those suffering from stress urinary incontinence, the ideal injectable material has yet to be identified. Alumina ceramic has proven to be both biologically-inert and biocompatible in orthopedic hardware. Therefore, this study tests the hypothesis that alumina ceramic microspheres can provide a safe, effective, and durable urethral bulking agent that can effectively treat SUIurination incontinence. In a preliminary study, alumina ceramic beads (ACB) 75-105 µm in diameter were suspended in hyaluronic acid sodium salt (HASS) from Streptococcus equi (Sigma, Aldrich) and injected in the urethra of female Wistar rats. The injected ceramic created a bulge in urethral wall that reduced the diameter of the urethral lumen and showed good volume retention one-month post-injection. The injection of ACB-HA suspension was however problematic and often associated with plugging of the needle. The aim of this study is threefold, to 1) develop the optimal composition of alumina beads, suspended in hyaluronic acid (HA), that could be easily stored, injected via a 22G needle, and resist degradation during sterilization, 2) assess long-term ACB volume retention and its effect on lower urinary tract function, and 3) evaluate ACB integrity and host tissue response prior to and 6 months following injection.

Study design, materials and methods

Preparation of the alumina ceramic beads - hyaluronic acid (ACB-HA) suspension: The ACB (Dakot Milling Media, KwaZulu-Natal, South Africa) used in this study contain micropores, potentially promoting tissue ingrowth, which would serve as an anchoring mechanism. A number of different formulations and varying dilutions (concentrations 1-3%) of HA were tested as well as ACB-HA ratios, with the goal of developing a bulking agent, that even with sterilization, would be stable and easy to inject without plugging the needle. Stability of the ACB-HA solution was assessed by the time it took for the ACB to fall out of solution once mixing had stopped. Ease of injection was determined by the tactile sensation of the investigators.

Bead Injection: Through a lower midline abdominal incision, the rat urethra was identified. ACB, suspended in HA, were injected into the wall of the mid urethra using a 22 G needle, under direct visualization with an operating microscope. The injection concluded with a microphotograph taken of the ACB-HA bleb in the urethral wall.

Baseline Behavioral Voiding Trial: Animals were placed into a metabolic cage with free access to food and water. After a night of acclimation, a 12-hour, overnight, baseline voiding frequency was recorded. Rats were placed in a cage with a wire grid floor, suspended over an analytical balance. Each micturition falls onto the balance causing a change in weight, which is continuously recorded by a software program (Catamount Health Systems, St. Albans, VT). The incremental increase in weight recorded from each micturition was then analyzed to determine flow rate and urinary frequency.

Histological evaluation: Six months post injection, under general anesthesia, the urethra was exposed, and a second microphotograph, using the same magnification as before, was taken of the bleb. The urethra was removed and fixed in Histochoice biological tissue fixative (Amresco, Solon, OH), and processed for hard tissue histology. SEM, as well as light microscopy (Giemsa surface stain) was used to determine bead morphology, host tissue reaction of cross sections, and evidence of tissue in-growth into the ACB micropores.

Results

A total of 15 animals were used in this study. Urethras were excised and histological evaluation was performed in 5 cases, voiding pattern was assessed in a separate group of 10 animas. There was no morbidity in either group. Cross-linked HA (Novoenzymes, Bagsvaerd, Denmark) was chosen as the optimal suspension media, with a minimal and predictable decrease in viscosity after sterilization. A concentration of 2.5% proved to display optimal viscosity, with ACB remaining uniformly suspended 60 minutes from the stop of mixing. A 1:4 ratio of beads to cross-linked HA resulted in an injection without plugging the 22 G needle. Microphotographs of the urethra, taken 6 months post injection were identical to those taken immediately following injection, indicating good volume retention.

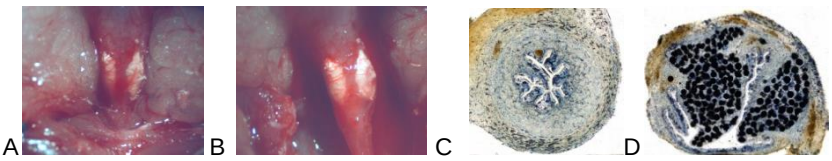

Figure 1: (A) Microphotographs of the bulking agent retention at the time of injection, (B) Six months post injection. (C) Giemsa surface stain – cross-section of intact urethra. (D) Urethra 6 months following injection of ACB-HA, beads remained in the suburothelium and within the urethral smooth muscle.

[illegible] 20.7±3.03 voids/12 hours) and mean urinary flow rate (0.099±0.027, 0.078±0.008, 0.078±0.009 ml/min) at 1 and 3 months post injection compared to baseline. SEM indicated that the porous ACB were unstable in vivo, which resulted in disintegration into smaller particulates inside the rat urethra. Fibrous encapsulation of the beads was evident; however the micropores in the beads were too small to allow for tissue ingrowth. The release of particulate matter from the beads elicited a local inflammatory response.

A B C D

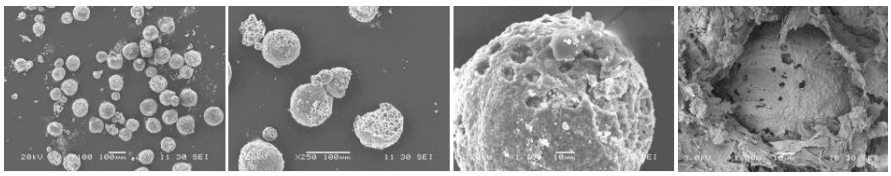

Figure 2: (A-C) Beads harvested from a rat urethra 6 months following injection. Note: significant degradation of beads. (D) Fibrous encapsulation. SEM image magnification (A) 100x, (B) 250x, (C) 1100x, (D) 1000x (right).

Interpretation of results

Clinical data on the variability of outcomes following UBT exists, but there is a paucity of data regarding the exact response of host tissue to the foreign material injected. Host tissue response to foreign materials has been documented as the main reason for complications associated with polypropylene mesh used in the treatment of vaginal prolapse and SUI. Degradation of the material activates the immune system leading to chronic inflammation. These findings highlight the necessity for meticulous pre-clinical testing of implantable materials. This study used a readily available and economical rat animal model to address the critical aspects needing additional research prior to clinical trials. We identified the optimal suspension media, which can uniformly suspend ACB up to 105µm in diameter. This size and type of bulking material has a low propensity for migration, and when injected into the rat urethra, shows good volume retention. However, the alumina beads used in this study lacked uniform size, were unstable in vivo, and did not promote tissue ingrowth. Release of particulate matter from the beads elicited a local inflammatory response and fibrous encapsulation was evident.

Concluding message

ACB has all of the theoretic properties for an ideal injectable and had good volume retention, but was unexpectantly found to encapsulate, degrade and incite an ongoing inflammatory response, rendering it unsuitable as a bulking agent in its present form. Further research is needed to identify ceramic beads which are uniform in size, biologically inert, and resistant to degradation and fragmentation. Migration of ACB into lymph nodes, liver, spleen and lungs also needs to be examined, as well as metabolic and physiologic function of the liver and kidney to exclude any potential affect the beads and their components may have on the function of these organs. All these properties could be tested in the rat model described in this study.

Disclosures

Funding: Vermont EPSCoR and AugmenTrx, LLC **Clinical Trial:** No **Subjects:** ANIMAL **Species:** "Rat" **Ethics Committee:** University of Vermont Institutional Animal Care and Use Committee