

[54] **INJECTABLE NON-IMMUNOGENIC
CARTILAGE AND BONE PREPARATION**[75] Inventors: **Anthony Atala**, Weston; **Samy Ashkar**, Boston, both of Mass.[73] Assignee: **Children's Medical Center Corporation**, Boston, Mass.[21] Appl. No.: **286,273**[22] Filed: **Aug. 5, 1994**[51] **Int. Cl.⁶** **A61K 35/32**[52] **U.S. Cl.** **424/548; 424/549; 424/423;**
424/426; 623/11; 623/16[58] **Field of Search** 424/548, 549,
424/423, 426; 623/11, 16[56] **References Cited****U.S. PATENT DOCUMENTS**5,328,695 7/1994 Lucas et al. 424/426
5,336,263 8/1994 Ersek et al. 623/11**FOREIGN PATENT DOCUMENTS**

WO94/21299 9/1994 WIPO .

OTHER PUBLICATIONSAtala, Anthony, et al., "Laparoscopic correction of vesicoureteral reflux" *J. Urol.* 150:748 (1993).

Atala, Anthony, et al., "Endoscopic Treatment of Reflux with Autologous Bladder Muscle Cells," American Academy of Pediatrics meeting held in Dallas, Texas on Oct. 23, 1994, Abstract.

Atala, Anthony, et al., "Injectable Alginate Seeded with Chondrocytes as a Potential Treatment for Vesicoureteral Reflux," Annual Meeting of the Section of Urology, American Academy of Pediatrics, Oct. 10-15, 1992, subsequently published in the *Journal of Urology*, 150:745-7477 (Aug. 1993), Abstract.Atala, Anthony, "Laparoscopic treatment of vesicoureteral reflux" *Dial Ped Urol* 14:212 (1993)*.Atala, Anthony and Anthony J. Casale, "Management of Primary Vesicoureteral Reflux," *Infections in Urology* 39-43 (Mar./Apr. 1990).Atala, Anthony, et al. "Endoscopic treatment of vesicoureteral reflux with a self-detachable balloon system" *J. Urol.* 148:724 (1992).Atala, Anthony, et al., "Sonography with sonicated albumin in the detection of Vesicoureteral reflux", *J. Urol.* 150:756-758 (1993).

BBI, 1985 Report 7062*.

Buckley, J. F., et al., "Endoscopic correction of vesicoureteric reflux with injectable silicone microparticles" *J. Urol.* 149 Abstract 259A (1993).Claes, H., et al., "Pulmonary migration following periurethral polytetrafluoroethylene injection for urinary incontinence" *J. Urol.* 142:821 (1989).Ferro, M. A., et al., "Periurethral granuloma: Unusual complication of Teflon periurethral injection" *Urology* 31:422 (1988).Geiss, S., et al., "Multicenter survey of endoscopic treatment of vesicoureteral reflux in children" *Eur. Urol.* 17:328 (1990).Henly, David R., et al., "Particulate silicone for use in periurethral injections: a study of local tissue effects and a search for migration" *J. Urol.* 147 Abstract 376A (1992).Klagsbrun, Michael, "Large-scale preparation of chondrocytes" *Methods in Enzymology* 58:560 (1979).Leonard, Michael P., et al., "Endoscopic injection of glutaraldehyde cross-linked bovine dermal collagen for correction of vesicoureteral reflux" *J. Urol.* 145:115 (1991).Malizia, Anthony A., et al. "Migration and granulomatous reaction after periurethral injection of polymer (polytetrafluoroethylene)" *JAMA*, 251:3277 (1984).Matouschek, E.: Die Behandlung des vesikorenenal Refluxes durch transueterale Einspritzung von polytetrafluoroethylenepast. *Urologe*, 20:263 (1981)."Medical versus surgical treatment of primary vesicoureteral reflux: a prospective international reflux study in children" Report of the International Reflux Study Committee *J. Urol.* 125:277 (1981).Mittleman, Roger E. and John V. Marraccini, "Pulmonary polytetrafluoroethylene granulomas following periurethral teflon injection for urinary incontinence" *Arch. Path. Lab. Med.* 107:611 (1983).O'Donnell, Barry and P. Puri, "Treatment of vesicoureteric reflux by endoscopic injection of Teflon" *Brit. Med. J.* 289:7 (1984).

Paige, Keith T., et al., "De Novo Cartilage Generation Utilizing Calcium Alginate-Chondrocyte Constructs," 1993 Plastic Surgery Research Council meeting held in Houston, Texas between Apr. 28, 1993, and May 1, 1993, Abstract.

Rames, Ross A. and Ian A. Aaronson, "Migration of polytef paste to the lung and brain following intravesical injection for the correction of reflux" *Ped. Surg. Int.* 6:239 (1991).Vorstman, Bert. et al., "Polytetrafluoroethylene injection for urinary incontinence in children" *J. Urol.* 133:248 (1985).Walker, R. Dixon, et al., "Injectable bioglass as a potential substitute for injectable polytetrafluoroethylene" *J. Urol.* 148:645 (1992).*Contemporary Urology* (Mar. 1993).**Primary Examiner**—David M. Naff**Assistant Examiner**—Jean C. Witz**Attorney, Agent, or Firm**—Arnall Golden & Gregory[57] **ABSTRACT**

Ground bone or cartilage particles are demineralized by extraction with a low ionic strength buffer such as 20 mM HEPES containing a chelating agent and protease inhibitors, then extracted with an acidic solution such as 0.3M citric acid, pH 4.0, containing protease inhibitors. The extracted material generally contains less than 2% by weight phosphate and less than 100 mM calcium. The phosphate content can be further reduced by treatment of the matrix with acid phosphatase, which removes residual organic phosphate. The material is useful in a method of treatment of vesicoureteral reflux and other disorders where a bulking agent is effective in correcting the defect.