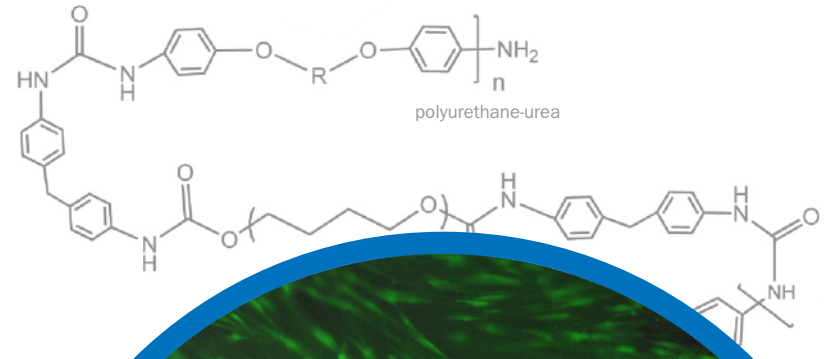
BIOCOMPATIBILITY CHARACTERISTICS OF ARTELON® FLEXBAND® AND FLEXPATCH® SYNTHETIC BIODEGRADABLE IMPLANTS

A TECHNICAL MONOGRAPH

Artelon®



polyurethane-urea

*Mesynchymal stromal cells on Artelon's Dynamic Matrix at 9 days
Bone marrow aspirate concentrate culture with GFP staining

polycaprolactone

FLEXIBLE HEALING

BIOCOMPATIBILITY CHARACTERISTICS OF ARTELON® FLEXBAND® AND FLEXPATCH® SYNTHETIC BIODEGRADABLE IMPLANTS

INTRODUCTION

The Artelon® FlexBand® and FlexPatch® augmentation devices are degradable biomaterial matrices woven from wet-spun fibers of polycaprolactone based-polyurethane urea (PUUR) that have been knitted into textile patches and strips for optimal mechanical properties and ease of use as reinforcement for numerous Orthopaedic soft tissue reconstructive applications (Fig 1). The clinical efficacy of the final product is generated from the combination of the chemical composition, fber spinning, and the textile manufacturing process. As with any implantable matrix, the ultimate performance is vitally dependent on providing the optimal biocompatible chemical configuration for local cells. Historically, polyurethane elastomers have an extensive safety record and have been widely used as biomaterials in long-term medical applications, for example, catheters, vascular prosthesis, and artificial hearts[1-4] due to their adaptable mechanical properties and blood cell compatibility. The PUUR multiblock based copolymer of Artelon's matrix was designed to leverage the well-established biocompatibility of polyurethane biomaterials with the ability to specifically calibrate matrix biodegradation kinetics post-implantation. The final product components have been rigorously evaluated in both *in vitro* and *in vivo* biocompatibility studies to further characterize the scaffold's biological and cellular responsiveness and to ensure patient safety. In addition, Artelon augmentation devices have an extensive clinical history and have been used in Orthopaedics for over two decades as reinforcement devices for soft tissue repair where weaknesses exist during tendon or ligament reconstructive procedures.

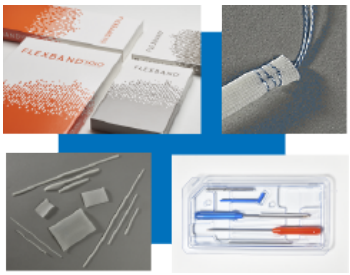


FIGURE 1. Artelon's FLEXBAND, FLEXPATCH, FLEXBAND Plus, and FLEXBAND Multi products.

CELL COMPATIBILITY AND INFLAMMATION

Toxicology Studies

Numerous pre-clinical studies have examined the product's biological response at both *in vitro* level and in multiple animal models. The biocompatibility of the matrices was demonstrated by independent, accredited laboratory assessment of cytotoxicity, genotoxicity, and mutagenicity toxicology studies in accordance with ISO-10993 standards.[5] In addition, delayed contact hypersensitivity safety studies were performed and reported both *in vitro* and in the well-validated guinea pig implantation model, with no adverse responses reported.[6]

ANIMAL STUDIES

Standard Biocompatibility Models (Subcutaneous and Intramuscular Implantation)

To compare the biocompatibility of PUUR to materials with well-known inert properties in both human tissue and cells, Titanium (Ti) and tissue culture polystyrene (PS) discs were implanted subcutaneously in the rat. During repair (D21), there was no difference in inflammatory cell number between the groups; however, a relevant shift away from pro-inflammatory cytokine production (TNFα) to an enhancement of anti-inflammatory cell signaling (IL-10) around the PUUR disc was observed. IL-10 is associated with the conversion from the inflammatory phase to the reparative phase of healing, suggestive of an earlier transition in the PUUR group. PUUR biocompatibility was no different than the other groups, as evidenced by lack of necrotic tissue, edema, and capsule thickness formation.[7]

The biological response and systemic toxicity of Artelon was tested by independent lab assessment of New Zealand White (NZW) rabbit intramuscular (IM) model in accordance with ISO 10993-6.[6] Due to the highly vascularized nature of muscle, the tissue is uniquely primed to monitor chronic toxicity to local implants, particularly in comparison to subcutaneous or bone models. Both the 3- and 6-month post-IM implant time points revealed no treatment-related clinical signs nor related systemic changes in hematology, clinical chemistry, and urinalysis. The microscopic evaluation of both Artelon and the test article revealed a normal course of healing in response to implantation with a slight fibrous capsule formation associated with few numbers of inflammatory cells. The Artelon explant exhibited regenerative/degenerative muscle fibers along the implant, consistent with the typical extracellular tissue matrix remodeling phase of healing.

Soft Tissue Analysis

Synovium

The synovial membrane is a highly vascularized tissue and a repository for macrophage-like synoviocytes and, therefore, is naturally designed to swiftly react to any chemical or mechanical stimuli by mounting an inflammatory reactionary response. Artelon matrix was examined by direct suture of the band to rabbit knee synovial membranes and analyzed for any adverse reaction. Despite the close contact between the band and the membrane, no joint reaction or macroscopic inflammation was demonstrated out to 18 months.[6]

Ligament and Tendon

The biocompatibility and efficacy of the material for Anterior Cruciate Ligament (ACL) reconstruction has been demonstrated in both rabbit and minipig repair models. At 6 months, the rabbits revealed no macroscopic pathological reaction, and the intact band was integrated with the tibia and femur. At 24 months, newly formed connective tissue and blood vessels had incorporated with the matrix without the presence of inflammatory cells both externally and within the center of the matrix (FIG 2). This reflects the biocompatibility of the matrix by the host leading to cell migration throughout the matrix and subsequent tissue formation. The histological results were similar in the minipigs and confirmed the observations in the rabbit study with connective tissue ingrowth into the center of the matrix, osseous integration, and complete preservation of the normal morphology of the cartilage in the absence of pathology.

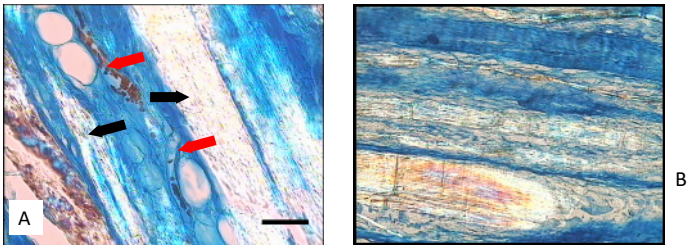


FIGURE 2. Light micrograph of Rabbit (A) and Porcine (B) knee joint ACL implant at 24 months post-implantation. (A) Toluidine blue stain demonstrates integration of Artelon fibers (black arrows) with surrounding connective tissue containing organized vascular channels (red arrows). (B) Type 1 collagen fibers within the connective tissue between the Artelon fibers (white).

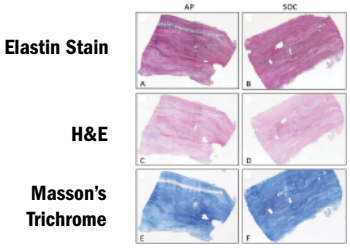


FIGURE 3. Histological analysis of Artelon canine implants (A, C, E) and standard of care (B, D, F) tissue explants. Artelon was well incorporated within the tendon, and there was no evidence of inflammation in all explants.

Gersoff et al., analyzed the healing of an Artelon augmented tendon versus tendon suture repair alone in a validated pre-clinical canine patellar tendon defect model 8. Histological analysis demonstrated there was the same pathological response in the tendon augmented group compared to the animals with suture repair alone, and the graft was well incorporated within the tendon (FIG 3). In parallel, there was an earlier regain of function and range of movement, less postoperative pain, and improved tendon strength in the augmented repair compared to the suture group, reflecting an enhanced and earlier time to healing in the augmented group.

Hard Tissue (Bone)

To assess the biological response of bone tissue to Artelon, implants were performed in NZW rabbits tibial diaphysis and compared with a commercially available polyethylene biomaterial at 6, 12 and 24-wks in accordance with IS 010993-6 by an accredited independent laboratory.[5] A mild inflammatory response was observed in both Artelon and reference implants at the 6-week time point resulting from surgical trauma, which was completely absent at the later time points. No necrosis was noted in either the bone or overlying soft tissue of either test article. Bone ingrowth into and throughout the porous Artelon implant initiated at 6 weeks and progressed through the final time point, demonstrating increased osteogenesis in comparison to the reference control (Fig 4).

HUMAN EVIDENCE

It has been possible to evaluate the response in humans to Artelon by harvesting biopsies from patients at times ranging from 2 to 61 months. Despite the differences between the patient biopsies, e.g. observation times, implantation site, patient diagnosis, age, etc., overall the biopsies exhibited conclusive biocompatibility of the ARTELON material in more than 50,000 human subjects.[5]

Biopsies of four patients that had received Artelon matrix augmentation for an ACL repair were harvested from mid-portion of the implant at 33 to 61 months post-reconstruction for histological analysis. The surrounding tissue tolerated the material well with no signs of chronic inflammation or foreign body reaction and revealed the presence of elongated fibroblasts oriented in parallel with the Artelon material. Immunohistochemistry revealed the prevalence of collagen Type I within the connective tissue with blood vessel integration (Figs 5, 6). At the later time points (39 and 61mos) graft degradation was observed, however, there was no inflammation associated with the material fragments.[5]

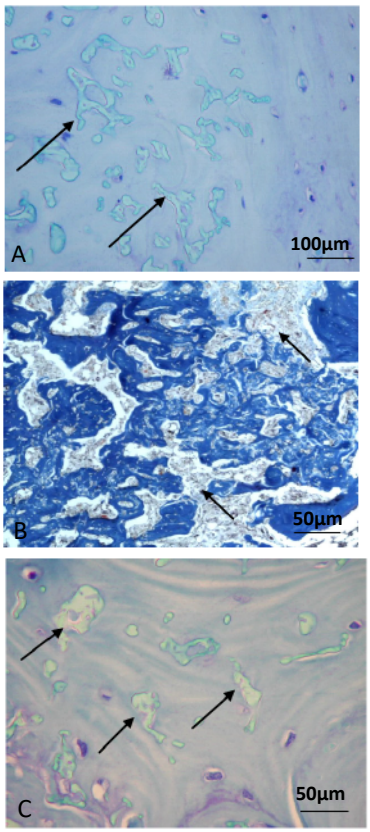


FIGURE 4. Light micrograph of decalcified paraffin sections with Toluidine blue stain demonstrating Artelon scaffold (arrows) integration at 3 (a) and 6 (b,c) within rabbit tibia.

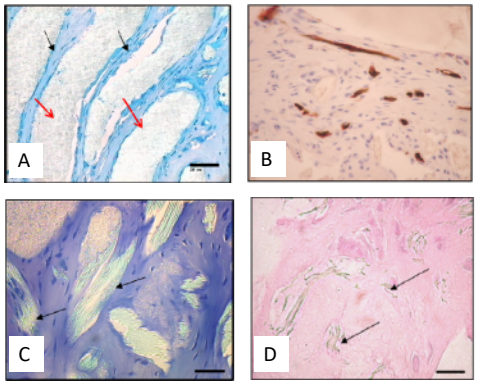


FIGURE 5. Light micrograph of Human ACL biopsies at 33 (A-C) and 61 (D) months post-implantation demonstrating connective tissue incorporation and Artelon Fiber degradation. (A) Toluidine blue stain denoting connective tissue integration (black arrows) with surrounding Artelon Fibers (red arrows). (B) Immunohistochemistry stained with CD34 antibody highlights blood vessel presence within the newly generated connective tissue. Artelon fiber degradation (black arrows) at 33 (C) and 61 (D) months post-implantation. Bar = 25µm

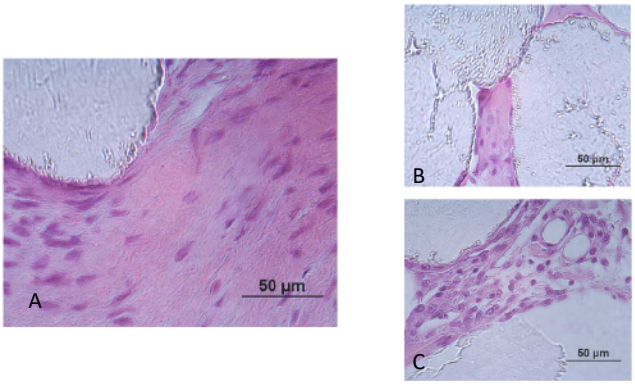


FIGURE 6. Images of H&E stained sections from human biopsies. Ingrowth and close contact between the Artelon fibers and organized connective tissue with blood vessels (C) are observed.

ARTELON MATRIX PROPERTIES

Pore Size

As referenced and evidenced above, the performance of a scaffold is vitally dependent on providing the optimal biocompatible chemical composition for cells. In addition to a biocompatible chemistry, the scaffold should possess biomechanical properties that are relevant to the target tissue, appropriate pore size, distribution, and interconnectivity, and should degrade at a rate that promotes optimal support while encouraging healing.[9]

Pore size and distribution within a scaffold are significant considerations for medical applications as these have significant influence on cell viability, proliferation, penetration into the scaffold, production of tissue-specific matrix and ultimate clinical success.[10] Artelon's multifilament fibers made from wet spinning have a high void content and when processed into woven structures, varying degrees of porosity can be achieved. Artelon matrix has a porosity range of approximately 8um-600um, with the majority of the pores concentrated in the smaller subrange, and a texture capable of accommodating matrix producing cells to form a functional tissue (Fig 7).[11] A study examining the cartilage tissue formation capacity of adult human articular chondrocytes on PUUR scaffolds of multiple pore sizes revealed the scaffold induced cell proliferation, the chondrocytes maintained their chondrogenic phenotype and secreted extensive cartilage specific ECM within the device. The study emphasizes the importance of PUUR pore size for tissue generation, but further demonstrates biocompatibility of the matrix in another important human cell type.[12] In addition, the consistent histological observation of tissue integration into the scaffold reported in both animal studies and human biopsies referenced above provide further support for the exceptional cell affinity (i.e., attachment, proliferation, and differentiation) for the Artelon scaffold.

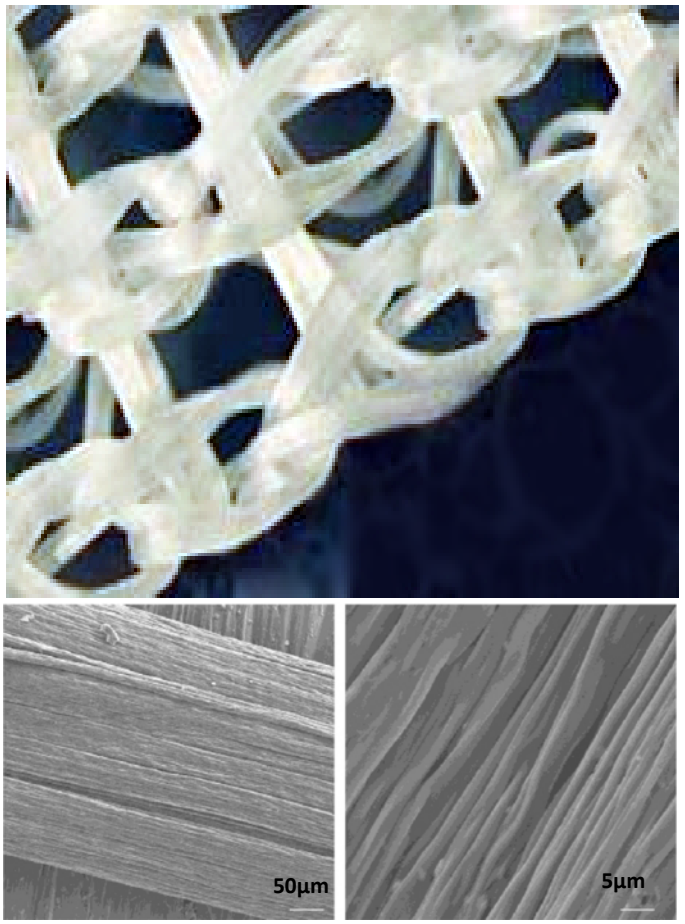


FIGURE 7. SEM images (A,B) demonstrating the nanotexture of the Artelon Fibers. (C) Light micrograph of weave texture of the Artleon matrix

Biomechanical Properties

Historically, soft tissue augmentation devices were designed to have a strong rigid structure and thus, the mechanical properties were not properly matched to the musculoskeletal tissues targeted for reconstruction.[13] This high stiffness profile transfers most of the mechanical load to the augmentation device, which often results in clinical failure due to stress-shielding or device fatigue. In order to prevent the biological breakdown in healing associated with stress shielding, the Artelon device was designed to have an original tensile stiffness measuring at least 50% lower than that of the tissue to be reconstructed.[14] The ranges of elasto-mechanical loading profiles of the Artelon material approximate human ligaments and tendons and yet are more adaptable to deformation than the native tissue.[6] This ensures matrix continuity even if the healing target tissue is overstretched, in which case the damaged target tissue can persist in the healing process while continuing to be supported by the implant. In addition, the generous elastomeric characteristics enable the graft to resist long-term stress relaxation and creep thus, providing the implant with the ability to template the healing tissue to its desired dimension and ensure ultimate functional kinetics.

In addition to the enhanced graft flexibility, the post-implantation endurance profile ensures the graft maintains 90% of its original strength and tensile properties for the first year.[5] This ensures that during the acute phase of healing, when the mechanical properties of the regenerating tissue are compromised, the augmentation matrix will help share loading of the healing tissue, but the tissue itself is still offered adequate mechanical stimuli to induce cells to secrete paracrine factors by the process of mechanotransduction for further cell recruitment, differentiation, and matrix deposition to generate a functional tissue. Simultaneous with maturation of the new tissue, the Artelon device gradually and benignly degrades by hydrolysis.

Degradation Patterns

The selection of the proper graft polymer chemistry (i.e., polyurethane urea) and textile manufacturing method of the Artelon material enables the biodegradation rate to be advantageously adapted to the expected healing rate and to coincide with the increasing biomechanical properties of the tissue to be reconstructed. The favorable high strength, load sharing, and elasticity of Artelon combined with the unique ability to custom design the graft's biomechanical and biodegradation properties to specifically align with different anatomical locations/tissue types provides an advantage over other non-degradable synthetic grafts. Studies analyzing the early in vitro degradation kinetics of Artelon fibers and fiber-based devices demonstrated the original gross mass, stiffness, compressibility, and tensile properties are maintained to a minimum of 90% at one year, and 50% out to three years, again emphasizing the endurance of the matrix through the critical acute healing phase (internal 1015631).[15] The degradation kinetics of Artelon were studied using in vitro accelerated aging and demonstrated the matrix maintained physical properties measured by gross mass, tensile strength, and size exclusion chromatography throughout the first year and then gradually degraded via hydrolysis out to 6 years.[16] The degradation pattern from 21 human biopsies, with up to 5.5 years' implantation paralleled the in vitro data, and shows the non-permanent support matrix of Artelon material gradually degrades via hydrolysis and is secreted over 4 to 6 years as it is replaced by integrating host tissue[6,17] (Fig 8).

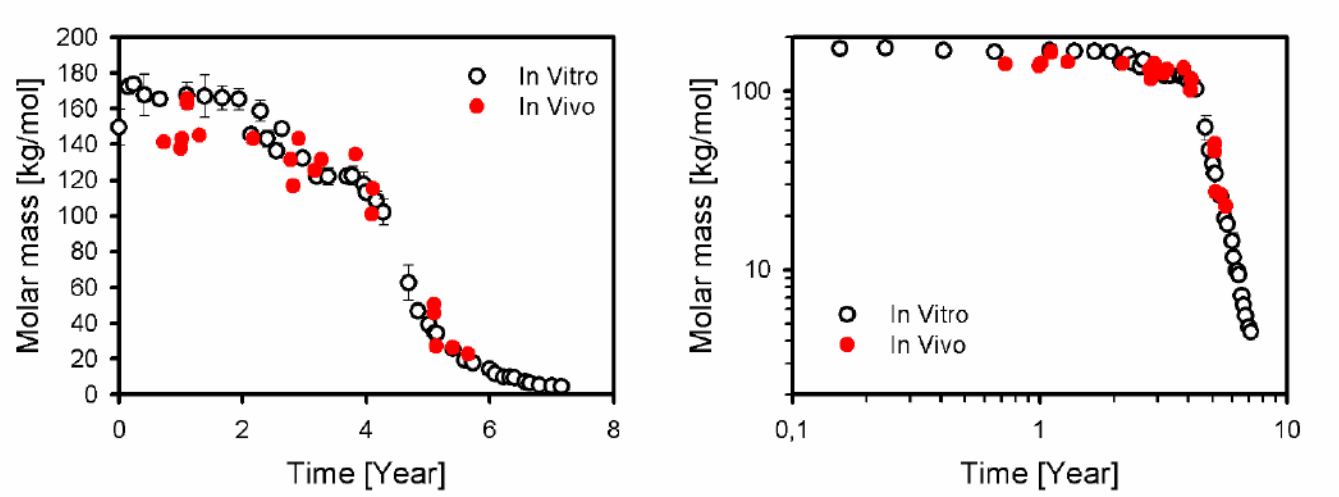


FIGURE 8. Degradation kinetics of in vitro (37 C, pH 7.4) and in vivo (Human biopsy samples) of Artelon's matrix.

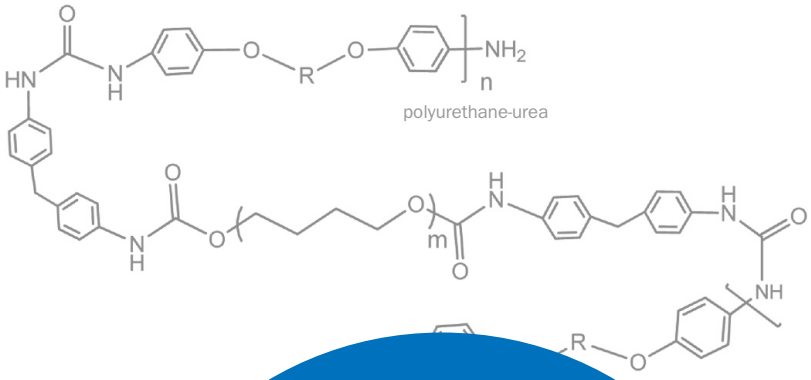
CONCLUSION

The excellent biocompatibility of the Artelon matrix is a complex combination the selected chemical composition and textile manufacturing. In addition, the matrix physical properties of open, interconnected porosity, optimized biomechanics and, degradation kinetics work to stimulate cell attachment and activity. This activity directly promotes local tissue integration and matrix replacement during the reconstructive healing process.

REFERENCES

1. Boretos, J. W., Pierce, W. S., Baier, R. E., Leroy, A. F. & Donachy, H. J. Surface and bulk characteristics of a polyether urethane for artificial hearts. Journal of Biomedical Materials Research 9, 327–340 (1975).
2. DREYER, B., AKUTSU, T. & KOLFF, W. J. Testing of artificial heart valves. Journal of applied physiology 14, 475–478 (1959).
3. Bruck, S. D. & Mueller, E. P. Materials and biological aspects of synthetic polymer in controlled drug release systems: problems and challenges. Critical Reviews in Therapeutic Drug Carrier Systems vol. 5 171–188 (1988).
4. Zdrahala, R. J. & Zdrahala, I. J. Biomedical applications of polyurethanes: A review of past promises, present realities, and a vibrant future. Journal of Biomaterials Applications 14, 67–90 (1999).
5. Title: Biological Assessment and GAP Analysis According to ISO 10993-1.
6. Liljensten, E. et al. Studies of polyurethane urea bands for ACL reconstruction.
7. Gretzer, C., Emanuelsson, L., Liljensten, E. & Thomsen, P. The inflammatory cell influx and cytokines changes during transition from acute inflammation to fibrous repair around implanted materials. Journal of Biomaterials Science, Polymer Edition 17, 669–687 (2006).
8. Gersoff, W. K. et al. Evaluation of a Novel Degradable Synthetic Biomaterial Patch for Augmentation of Tendon Healing in a Large Animal Model. Journal of Knee Surgery 32, 434–440 (2019).
9. Marberry, T. A. A Synthetic Reinforcement Patch in Repair of Challenging Two-Tendon Rotator Cuff Tears. Shoulder and Elbow 5, 24–29 (2013).
10 Caliari, S. R. & Harley, B. A. C. The effect of anisotropic collagen-GAG scaffolds and growth factor supplementation on tendon cell recruitment, alignment, and metabolic activity. Biomaterials 32, 5330–5340 (2011).
11. Gisselfält, K., Edberg, B. & Flodin, P. Synthesis and properties of degradable poly(urethane urea)s to be used for ligament reconstructions. Biomacromolecules 3, 951–958 (2002).
12. Stenhamre, H., Nannmark, U., Lindahl, A., Gatenholm, P. & Brittberg, M. Influence of pore size on the redifferentiation potential of human articular chondrocytes in poly(urethane urea) scaffolds. Journal of Tissue Engineering and Regenerative Medicine 5, 578–588 (2011).
13. Noyes, F. R., Huser, L. E., Jurgensmeier, D., Walsh, J. & Levy, M. S. Is an Anterolateral Ligament Reconstruction Required in ACL-Reconstructed Knees with Associated Injury to the Anterolateral Structures? A Robotic Analysis of Rotational Knee Stability. American Journal of Sports Medicine 45, 1018–1027 (2017).
14. Data on File. 3000019 Knit Design Reverification Report.
15. Data on File. Report 1015631
16. Data on File. 3000105_A_Biostability Degradation.
17 Data on File. Report 3000054

Artelon®

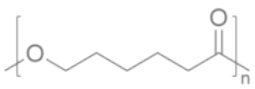


polyurethane-urea

International Life Sciences, dba Artelon®
2150 Northwest Parkway SE, Suite G
Marietta, GA 30067
Ph. 800.610.3446

Email: customerservice@artelon.com

www.Artelon.com



polycaprolactone

4000047 Rev. B