BIOMATERIAL FOR SOFT TISSUE REPLACEMENTS

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Abstract: Typical biomaterials are stiff, difficult to manufacture, and not initially developed for medical implants. A new biomaterial is proposed that is similar to human soft tissue. The biomaterial provides mechanical properties similar to soft tissue in its mechanical and physical properties. Characterization is performed for modulus of elasticity, ultimate strength and wear resistance. The material further exhibits excellent biocompatibility with little toxicity and low inflammation. The material can be molded into a variety of anatomic shapes for use as a cartilage replacement, heart valve, and reconstructive implant for trauma victims. The biomaterial may be suitable for several biodevices of the future aimed at soft-tissue replacements.

1 BACKGROUND

Most of the existing biomaterial technology is limited to materials such as silicones, Teflon®, polyethylene, metal and polyurethanes that do not exhibit the mechanical and physical properties of natural tissue. These materials are stiff, difficult to manufacture, and not initially developed for medical implants. Artificial tissue substitutes have not been found to withstand the rigors of repetitive motion and associated forces of normal life. Cadaver tissue is limited in supply and due to the risk of infection is coming under increased scrutiny by FDA and is not accepted in Europe.

As an example, one of the most successful medical implants is the artificial knee replacement for the treatment of arthritis. Arthritis and joint pain as a result of injury are major medical problems facing the US and patients worldwide. Worldwide, approximately 190 million people suffer from osteoarthritis. This condition affects both men and women, primarily over 40 years of age. The spread of arthritis is also fueled by the rise of sports injuries. Activities enjoyed by many can translate into injury or joint damage that may set up a process of deterioration that can have devastating effects decades later. The number of patients that have arthritis is staggering and growth is expected with baby boomers entering the prime "arthritis years" with prolonged life expectancies. Growth of the world's elderly is expected to increase three times faster than that of the overall population.

Current standard treatment is to surgically implant a total knee replacement (TKR) that is made of metals such as cobalt chromium or titanium. These devices are highly rigid, providing no shock absorption. Further, the metal integrates poorly with bone and the HMWPE caps often create micro-particulate debris with strong inflammation. The invasive surgery not indicated for those under age 60 and usually reserved for end-stage patients. A revision procedure is technically demanding, and amputation may be required.

An alternative biodevice may be a soft tissue replacement. Arthritis stems from damage to cartilage, the soft tissue between the bones. Damaged cartilage leads to grinding of bone on bone and eventual pain and limited joint function. Biodevices that replace the soft tissue would restore diarthroidal joint function much better and protect further damage by a more natural stress distribution.

A similar problem exists for heart valves. Prosthetic heart valves are made from metal and pyrolitic carbon which do not function like native heart valves. The use of hard materials creates high shear zones for hemolysis and platelet activation. Tissue valves are subject to calcification, again a problem of hard tissues not acting like natural soft tissue. An alternative would be to design a biodevice with soft tissue flexibility and endothelial cell

covering to provide a wide-open central flow and low-thrombogenic surface.

Yet another example for the need for soft tissue replacements is a replacement part for reconstructive surgery after traumatic accidents or cancer resection. For many parts of the body, a replacement shape needs to have smooth contours as well as soft tissue compliance to yield a natural shape. The base biomaterial should not have chemical composition that is non-organic, such as silicone, which can induce a hyper-immunogenic response.

Soft tissue replacements should start with a biomaterial that has compliance ranges similar to human soft tissue, be strong and wear resistant, manufactured to personal shapes, and have long-term biocompatibility. Cellular in-growth or preloading of cells can then be performed on this established scaffold. These features are demonstrated in a new biomaterial described in this paper.

2 METHODS

2.1 Biomaterials

Soft tissue-like devices can be made from polymers such as poly vinyl alcohol as thermoset materials. As an example, a PVA cryogel can be made according to the full descriptions in US Patent U.S. Patent Numbers 5,981,826 and 6,231,605. The cryogels are made in a two stage process. In the first stage a mixture of poly (vinyl alcohol) and water is placed in a mold, and repeatedly frozen and thawed, in cycles, until a suitable cryogel is obtained.

Poly(vinyl alcohol) having an average molecular weight of from about 85,000 to 186,000, degree of polymerization from 2700 to 3500, and saponified in excess of 99% is preferred for creating soft tissue-like mechanical properties. High molecular weight poly (vinyl alcohol) in crystal form is available from the Aldrich Chemical Company. The PVA is then solubilized in aqueous solvent. Isotonic saline (0.9% by weight NaCl, 99.1% water) or an isotonic buffered saline may be substituted for water to prevent osmotic imbalances between the material and surrounding tissues if the cryogel is to be used as a soft tissue replacement.

Once prepared, the mixture can be poured into pre-sterilized molds. The shape and size of the mold may be selected to obtain a cryogel of any desired size and shape. Vascular grafts, for example, can be produced by pouring the poly (vinyl alcohol)/water mixture into an annular mold. The size and dimensions of the mold can be selected based upon the location for the graft in the body, which can be matched to physiological conditions using normal tables incorporating limb girth, activity level, and history of ischemia.

The new biomaterial, commercially available as Salubria® from SaluMedica, LLC, Atlanta, GA is similar to human tissue in its mechanical and physical properties. The base organic polymer is known to be highly biocompatible and hydrophilic (water loving). The hydrogel composition contains water in similar proportions to human tissue. Unlike previous hydrogels, Salubria is wear resistant and strong, withstanding millions of loading cycles; yet it is compliant enough to match normal biological tissue. The material can be molded into exact anatomic configurations and sterilized without significant deterioration.

2.2 Mechanical Characterization

2.2.1 Tensile Testing

Tensile test specimens were cut from sheets of Salubria. They were tested in accordance with ASTM 412 (die size D) in tension to failure using an Instron Model 5543 electro-mechanical load frame pulling at a rate of 20 inches per minute.

2.2.2 Stress-Strain Constitutive Relationship

The stress is a function of the load and the cross-sectional area. However, the cross-sectional area was difficult to measure. But the stretch ratio relates the final and initial area due to the assumption of incompressibility. That means the final area equals the initial area divided by the stretch ratio. Therefore, the ultimate stress calculation is a function of the load at the breaking point of the sample, the stretch ratio and the initial cross-sectional area. The initial cross-sectional area is the product of the initial width of the sample, w_o, and the initial thickness, t_o.

Stretch Ratio: $\quad \lambda = C / C_o \quad$ (1)

Initial Cross-Section Area: $\quad A_o = w_o * t_0 \quad$ (2)

Final Stress: $\quad \sigma_{ult} = \dfrac{F_{ult} * \lambda}{A_o} \quad$ (3)

In order to get an estimation of the pressure in an intact tube the following simplified assumptions were used. It was assumed that a tubular specimen will burst when the circumferential wall stress is

equal to the ultimate stress σ_{ult}. However, when an artery is under physiologic load conditions it is in a state of plane strain and undergoes finite two dimensional stretches.

The stretch ratios are:

$$\lambda_\theta = \frac{r}{R} \qquad (4)$$

$$\lambda_z = \frac{l}{L} \qquad (5)$$

Rewritten to solve for the pressure, P:

$$P = \frac{T\sigma_\theta}{\lambda_\theta^2 \lambda_z R} \qquad (6)$$

This equation can calculate the pressure if we know that data of the initial dimensions, the stress and the stretch ratios. From the equation (6) we can estimate the corresponding pressures.

2.2.3 Unconfined Compression

Cylindrical unconfined compression samples were cast in a custom mold. Samples were tested in unconfined compression on an Instron Model 5543 electro-mechanical load frame and on a DMTA IV dynamic mechanical analyzer. Rates of 1% strain per second and 20 inches per minute were tested.

2.2.4 Ultimate Strength

Ring specimens were pulled in tension until they failed. Ring specimens of Salubria were preconditioned twenty five cycles. Then the specimens were distracted at 0.1mm/s, 1m/s, 10mm/s, and 100mm/s using a MTS 858Mini Bionix Test System. Comparisons are made to normal coronary arteries using identical protocols. The load at failure was recorded as the ultimate load, and the ultimate stress was calculated. Failure of the ring specimen was defined as a complete tear of the ring through the entire wall. The stress was derived based on the assumption of incompressibility and was defined as the ratio of load and cross-sectional area. The stretch ratio was defined as the ratio of the final and initial circumference. The final stress at failure represented the ultimate strength for the tension tests. To determine the final stress, an equation was derived based on the assumption of incompressibility [3] which means that the initial volume V_o and final volume V are equal. In the present experiments the stretch ratio is defined as the

ratio of the final and initial circumference, Equation (1). The ultimate stress, σ_{ult} defined by the load at the breaking point of the sample divided by the final cross-sectional area, was calculated using Equation (3).

2.2.5 Fatigue Resistance

Ring specimens were cycled at different cycles, and then pulled in tension until failure. The frequency of the cyclic tests was set at 2 Hz because this value is close to physiologic frequency of heart beats (~1.2 Hz) and strain rates effects testing showed that there are no significant difference to do cyclic test under 1HZ to 5HZ. The purpose of the cyclic tests was to experimentally determine how the fatigue affects the ultimate strengths of porcine common carotid arteries.

2.2.6 Cyclic Compression

A cyclic compression study was performed to assess the response of Salubria biomaterial cylinders to repetitive compressive loading at physiologic stress of approximately 1.3 MPa. The specimen is loaded for 1 million loading cycles at a rate of approximately 1.5 Hz. Dimensional integrity was measured using an optical comparator and mechanical modulus of elasticity was determined at 20% strain.

2.2.7 Wear Testing

An accelerated wear tester was built to test the wear rate of Salubria biomaterial. Polished stainless steel rollers with a diameter of 1 5/8 inches (chosen to approximate the average radius of the femoral condyles) are rotated so that they slide and roll across the test sample, creating a peak shear load of approximately 90N (0.2 MPa). Separate testing has shown the coefficient of friction for polished stainless steel against Salubria biomaterial to be equivalent to that of porcine femoral condyle with the cartilage surface abraded away to subchondral bone or roughly 4 times that of porcine femoral condyle with intact cartilage surface. The rotating cylinders exert a normal load of approximately 200N on the sample. The wear tester is operated at a rate that subjects the sample to 1000 wear cycles per hour where one cycle is defined as one roller to sample contact. Data has been collected with the sample lubricated and hydrated with water (a worst case scenario since synovial fluid should provide some surface lubrication). Wear rate is measured by

weight loss of the sample over a number of cycles. Salubria biomaterial is tested for >10,000,000 cycles against polished stainless steel rollers.

2.3 Biocompatibility

Biomaterials for use in humans must pass a full complement of biocompatibility tests as specified by ISO and the USFDA. The material was tested for ability to produce cytotoxicity, intracutaneous irritation, sensitization by Kligman maximization, Ames mutagenicity, chromosomal aberration, and chronic toxicity.

2.3.1 Rabbit Osteochondral Defect Model

In addition to the standard biocompatibility testing, the ability of Salubria biomaterial to withstand load or cause local inflammatory responses in a widely used rabbit osteochondral defect model was assessed (e.g., Hanff et al., 1990). A cylindrical plug (3.3 mm diameter, 3 mm depth) was implanted in the right knee of each of sixteen New Zealand white rabbits. An unfilled drill hole was made in the left knee of each animal to serve as an operative control.

After three months of implantation in the patellofemoral groove, eight rabbits were sacrificed for histologic analysis of the implant site and surrounding synovium. The remaining eight animals were sacrificed and the implant assessed for any change in physical properties. In addition, the distant organ sites that are known targets for PVA injected intravenously were assessed histologically for any sign of toxicity due to implantation. Tissues assessed included liver, spleen, kidney, and lymph node.

2.3.2 Particulate Inflammation

Salubria biomaterial was tested for particulate toxicity or inflammatory reaction in the joint. The study design was based on a study conducted by Oka et al. (2000) on a similar PVA-based biomaterial in comparison to UHMWPE. Particulate sizes for the study varied from approximately 1 micron to 1000 microns. The total volume of particulate injected over the 2 divided doses was designed to represent full-thickness wear of 10 x 10 mm diameter cartilage implants.

2.3.3 Ovine Knee Inflammation Model

In vivo testing was performed using a meniscus shaped device made of Salubria and implanted into the sheep knee joint. Devices were removed at 2 week, 3 week, 2 month, 4 month and 1 yr. intervals. Animals were fully load-bearing on the day of operation and after. Full range of motion with no disability was observed. Gross examination of surrounding tissues and histology of target end organs (liver, kidney, spleen, and lymph nodes) were evaluated for acute or chronic toxicity.
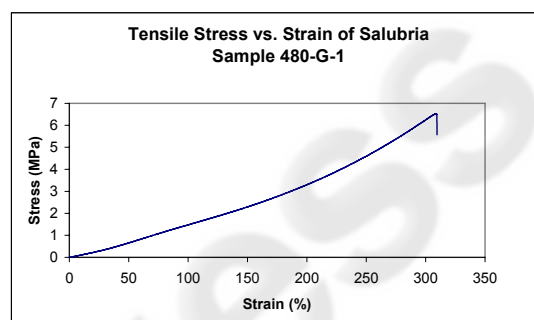
3 RESULTS

3.1 Tensile Testing

Figure 1: Representative Tension Curve.

Plots of stress versus strain in tension (figure 1) show a non-linear response. Due to the non-linearity of the loading curve, tangent modulus values at a defined percent strain are used to characterize the material stiffness. Tangent modulus ranges from 1.2-1.6 MPa. Ultimate tensile strength is 8-10 MPa. The stress-strain curve exhibits a non-linear elastic behavior similar to natural soft tissue.

3.2 Unconfined Compression

Figure 2 is a representative curve of stress versus strain in compression. Compression loading curves show a non-linearity suggesting that Salubria is a viscoelastic material similar to cartilage. Tangent modulus values in compression range from 0.1 to 7 MPa. Plastic compressive failure occurs at or above 65% strain.

3.3 Creep and Creep Recovery

Creep and creep recovery experiments were performed to assess the performance of Salubria biomaterial under long-term static loading at physiologic loads of up to 480 N-force. High loads were applied for 24 hours creating deformation of

50% of the initial height. Initial loading demonstrates a biphasic visco-elastic behavior. After 24 hours of recovery in saline, sample height had returned to within 5% of the original height. The compressive modulus of the material before the test and after creep recovery was unchanged.

3.4 Wear Testing

Results for one formulation of Salubria biomaterial tested for >2,000,000 cycles against polished stainless steel rollers demonstrate minimal wear.

3.5 Cyclic Compression

Repetitive compressive loading at physiologic stress of approximately 1.3 MPa was imposed. After 1 million loading cycles at a rate of approximately 1.5 Hz, there was minimal change (<5%) in sample dimensions and no change in modulus of elasticity at 20% strain.

3.6 Ultimate Strength

Sixty-four specimens were pulled at four different stain rates. Ultimate stress increased as a weak function of increasing strain rates. The ultimate stress at 100 mm/s was 4.54 MPa, greater than the 3.26 MPa at 0.1 mm/s. The differences between 0.1mm/s and 100 mm/s was highly significant with $p<0.001$. The differences between 0.1 mm/s and 10 mm/s gave $p=0.013$; and 1 mm/s to 100 mm/s was $p=0.018$. The difference between 1 mm/s and 10 mm/s was not statistical significance. Strain rates between 1 and 100 mm/s correspond to a cyclic frequency of 0.5 Hz to 5 Hz for fatigue testing.
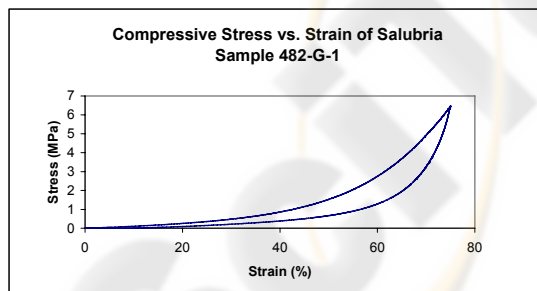

Figure 2: Representative Compression Curve.

3.7 Biocompatibility

The following table outlines the results of standard biocompatibility testing performed on Salubria biomaterial, in accordance with ISO 10993-1 and FDA Blue Book Memorandum #G95-1.

3.8 Animal Testing

After three months of implantation in the patellofemoral groove, eight rabbits were sacrificed for histologic analysis of the implant site and surrounding synovium. Tissues assessed included liver, spleen, kidney, and lymph node. The Salubria biomaterial was well-tolerated with subchondral bone formation surrounding the implant, no fibrous tissue layer or inflammatory response, no implant failures or evidence of wear debris formation, no osteolysis, and no toxic effects on the implant site or distant organ tissues.

At time of explantation, the samples were essentially unchanged (see Fig. 3). Based on histologic examination in comparison to the operative control, there was no evidence of inflammatory reaction in either the surrounding cartilage/bone (see Fig. 4) or in the synovium. In fact, a layer of normal hyaline cartilage partially covered the implant surface. The cartilage surface of the patella also showed no changes in the area articulating against the Salubria implant. There was no sign of toxicity on histologic examination of the distant organ sites.

Figure 3: The left-hand knee shows a Salubria implant in the patellofemoral joint of a rabbit knee after 3 months implantation. The right-hand knee is an operative control.

On excision for mechanical testing, the sample edges firmly adhere to the surrounding bone. The indentation force (i.e., the force required to cause a certain amount of sample deformation) of the implant is unchanged from a non-implanted control. Comparison material characterization testing showed that the implanted samples were not different from non-implanted controls.

Table 1: Biocompatibility Testing.

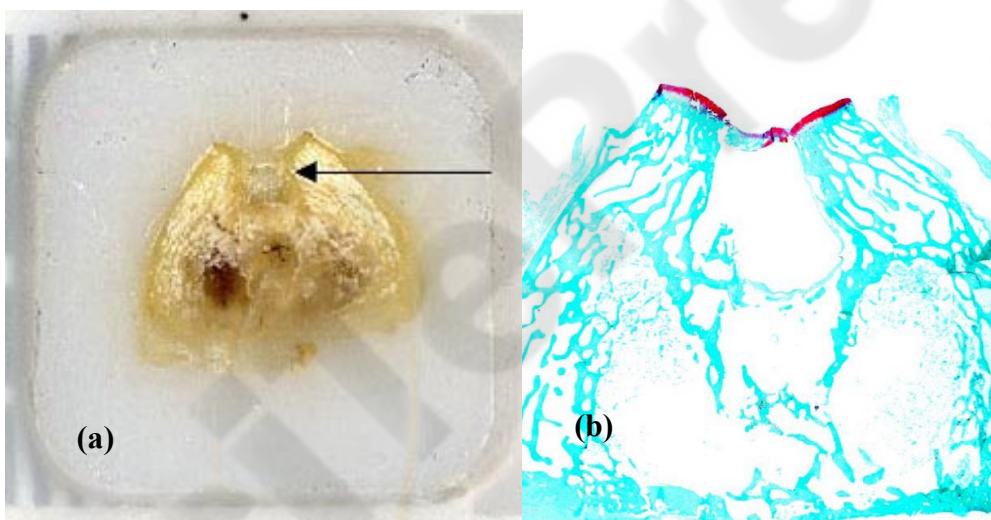| ISO 10993-1 Recommended Testing Requirement	Test Performed	Test Results
Cytotoxicity, ISO 10993-5	ISO MEM Elution L929 cells, GLP.	**Pass**
	Direct Contact Neurotoxicity	**Pass**
Sensitization and Irritation, ISO 10993-10	Kligman Maximization Method	**Pass**
	Primary Vaginal Test: Repeat Exposure	**Pass**
Sub-acute and Sub-Chronic Toxicity, ISO 10993-6	Sub-acute and sub-chronic toxicity	**Pass**
Genotoxicity, ISO 10993-3	Ames Mutagenicity: Dimethyl Sulfoxide Extract, 0.9% Sodium Chloride Extract	**Pass**
	Chromosomal Abberation	**Pass**
Implantation, ISO 10993-6 and 10993-11	Subacute or site Specific Implantation with chronic Toxicity	**Pass**
	Biocompatibility study in Rabbits following Intra-articular injections.	**Pass**
	Rabbit Trochlear Osteochondral Defect	**Pass**

Figure 4: (a) This digital scan of a paraffin tissue block containing a Salubria implant demonstrates that the implant remains in place over 3 months of implantation in the rabbit patellofemoral groove. (b) Hematoxylin and eosin stain of a section from the tissue block in (a) showing the implant site – the implant has been removed during the staining process. There is no evidence of inflammatory reaction; the surrounding cartilage and bone are normal in histologic appearance.

Salubria biomaterial was tested for particulate toxicity or inflammatory reaction in the joint. Salubria particulates were biologically well-tolerated. The biomaterial particulate was deposited on the superficial synovium with minimal inflammatory reaction. There was no evidence of migration from the joint space or toxicity in the knee or at distant organ sites. There was no evidence of third body wear or osteolysis.

For the goat study, the native articular cartilage surfaces were protected in the test group compared to extensive damage from the control meniscectomy group. No local inflammation was noted on MRI or histology. No distant organ inflammation was seen

in these large animals, confirming the observations in the rabbits.

4   DISCUSSION

The biomaterial described here exhibits the requisite characteristics for soft tissue replacements. For knee cartilage, the material has non-linear viscoelastic properties similar to native tissue. The strength and fatigue properties exceed the requirements for a fully loaded knee articular joint (Stammen, 2001). For heart valves, the material must be moldable to complex anatomies and exhibit low thrombogenicity. For reconstructive anatomic parts, the biomaterial should be easily molded to custom shapes and have low inflammation potential. The biomaterial presented here exhibits these properties and opens the potential for soft tissue replacements that more closely match the anatomic and physiologic requirements.

Although ring specimens and dumbbell shape specimens are both one-dimensional tests, ring specimens were used because they provide a good gripping connection. Ring samples can relieve the experimental error comes from the inappropriate clamping dumbbell specimens which can cause the specimens to slide or break in the neighborhood of the clamp. There may be damage from preparing uniaxial dumbbell shaped strips. Dumbbell strips would also be difficult to obtain because of the small diameter of the tubular samples.

The biocompatibility testing for Salubria reflects previous carcinogenicity testing on other PVA-based biomaterials. PVA hydrogels in the literature are non-carcinogenic with rates of tumorigenicity similar to the well-accepted medical-grade materials, silicone and polyethylene. Nakamura (2000) reports on a 2-year carcinogenicity study conducted on a PVA-based biomaterial subcutaneously implanted in rats. Pre-clinical investigation of other PVA-based hydrogels and Salubria biomaterial demonstrates that these materials are biocompatible in the joint space (Oka et al., 1990). The rabbit is the most commonly published cartilage repair model with study lengths varying from 3 months to 1 year, with little difference in results at 3 months from those at 1 year. These studies indicate that 3 months is sufficient to assess biocompatibility and early treatment failure in the rabbit model. These results are further confirmed by clinical results on SaluCartilage^{TM}.

Based on this study, Salubria soft tissue biomaterial has been shown to be biocompatible with long-term implantation. There is no evidence of inflammatory response or local or distant toxicity. Furthermore, the biomaterial has stable, durable physical properties over the period of implantation in joints and would be suitable for use as structure deceives such as a cartilage replacement. The biomaterial presented here opens the potential for soft tissue replacements that more closely match the anatomic and physiologic requirements for human biodevices.

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