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Sensate Scaffolds Coupled to Telemetry Can Monitor *In Vivo* Loading From Within a Joint Over Extended Periods of Time

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Abstract

Polymer scaffolds have been used as a tool to provide growth and integration of engineered tissue substrates to repair damaged tissues in many organ systems including articular cartilage. Previous work has shown that “sensate” scaffolds, with integrated strain gauges have the potential for use as both a delivery vehicle for engineered cartilage as well as a device that can measure real time, *in vivo* joint loading. The purpose of this study was to use an implanted subminiature telemetry system to collect *in vivo* joint loading measurements over an extended period following placement of a “sensate” scaffold. Measurements were collected from seven of nine sensors that were implanted into the stifles of three canines. The limb loading rates and load distribution through gait were dependent on stride time but did not vary with time post op. The peak loads were not dependent on stride time but significantly increased with time post op. This demonstrated that peak loading measured with “sensate” scaffolds can be used to monitor healing. The portability of the “sensate” scaffolds coupled to telemetry systems highlights the potential use of this system in a clinical research setting to gather important information to improve tissue engineering and rehabilitation regimens.

Keywords

scaffolds; gait; tissue engineering; sensor; *in vivo*

Introduction

Polymer scaffolds are widely implemented in a variety of tissue engineering applications designed to repair or replace damaged tissue.¹ Typically, scaffolds are used to provide a sturdy interface between engineered and native tissues surrounding the implant site or are used to guide tissue growth into a specific shape.² Nutrients and growth factors can be incorporated into scaffolds to enhance tissue integration and encourage further growth and development. Osteoinductive materials such as TGF- β and BMP are examples of growth factors that have been used to accelerate bone ingrowth into scaffolds.^{3–8} Scaffolds will be advantageous in musculoskeletal applications because they provide engineered cartilage grown on the scaffold surface with a secure attachment to bone.

Recent work has shown that “sensate” scaffolds (scaffolds with built in sensors) have the potential to serve as both a vehicle for engineered tissues, and as a real time monitoring system of joint loading.^{9,10} Strain gauges incorporated into the scaffold have been calibrated to accurately measure joint loads during bench top testing and to monitor *in vivo* loading patterns.

^{9,10} These “sensate” scaffolds allow direct measurement of loading from within a joint, which can provide a method of assessing healing at the implant site, and the ability to monitor loading during rehabilitation and normal daily activity. These measurements can be used to prevent excessive loading of implanted tissues.

Accurately characterizing the native mechanical environment of musculoskeletal tissues is essential to understanding mechanically driven physiological processes and to developing functional engineered cartilage tissues. Mimicking *in vivo* conditions for *in vitro* experiments has led to increased success rates in tissue engineering,^{11–16} highlighting the value of direct collection of measurements during *in vivo* loading in joints. An accurate characterization of native conditions within joints is expected to provide key insights that will improve the ability to engineer articular cartilage tissue.

In addition to measuring physiologic loading, “sensate” scaffolds may provide a tool to directly measure the loading conditions necessary to study models of osteoarthritis (OA). While mechanical loading can lead to articular cartilage damage and subsequent development of OA,^{17–19} limited direct measurements from within joints have been carried out, and the loads necessary to induce of OA have not been defined. Measurement of pathogenic loading conditions is a major step toward understanding and thereby preventing the onset and continued progression of osteoarthritis (OA). This information can also be used to define effective, fast and safe rehabilitation and exercise regimens for OA patients following surgical placement of tissue-engineered cartilage.

The purpose of this study was to monitor loading *in vivo* from within joints using a recently developed portable subminiature telemetry system coupled to a “sensate” scaffold (Figure 1) designed to support tissue-engineered cartilage. Previously, the accuracy of measurements collected with these “sensate” scaffolds have been characterized in a bench-top model⁹ and *in vivo*.¹⁰ The primary goal of this study was to collect *in vivo* load measurements over an extended period of time to determine whether changes in loading are dependent upon stride time and the length of time following surgical placement of the “sensate” scaffolds. These measurements will also facilitate development of a clinically useful system.

Methods

Scaffold, Strain Gauge, and Transmitter Preparation

Polybutylene terephthalate (PBT) scaffolds were designed using SolidWorks (Concord, MA) and built using a Statasys FDM 1650 modeler (Statasys, Eden Prairie, MN). Each scaffold was designed with a domed top (for the attachment of tissue engineered cartilage), a porous cylindrical section to encourage bone ingrowth, and a rigid exterior surface to mechanically reinforce the scaffold and to provide a surface for strain gauge attachment. Three 1000-ohm FAE-12-100-S6ET single element strain gauges (Micro-Measurements, Raleigh, NC) were attached to the surface along the longitudinal axis of the cylindrical section of the scaffolds with each gauge placed circumferentially and separated by 120° (Figure 2). This strain gauge configuration produced accurate linear elastic load strain measurements during bench top testing over a range of angles that encompass those observed during normal gait^{18,20} and demonstrated consistent results during a pilot *in vivo* study.¹⁰ The gauges were waterproofed with Master Bond (Master Bond, Hackensack, NJ),²¹ coated with a blend of calcium phosphate ceramic (CPC) particles that encourage rapid bone bonding,²² and calibrated using a published procedure⁹ described in the next section. The transmitter was waterproofed using medical grade silicone (Sylgard, Midland, MI). Finally, the transmitter and “sensate” scaffolds were sterilized in ethylene oxide in preparation for surgical placement.

Scaffold Calibration

Scaffolds were loaded in confined compression in Daro foam (Daro Products, Butler, WI) according to a published procedure,⁹ to simulate the mechanical environment of trabecular bone.²³ Testing was performed on a servo-hydraulic materials testing system (MTS Corp., Minneapolis, MI) using a silicone layer to uniformly distribute the load over the scaffold. Scaffolds were loaded at loading rates of 50, 100, 150, and 200 N/s up to the peak load of 150 N. Load vs. strain calibration curves were created for every strain gauge on each scaffold prior to sterilization and were utilized to interpret strains as loads.

Scaffold Placement

NIH guidelines for the care and use of animals were followed throughout this study (NIH publication 82-23, Rev. 1985). Three tall *hound/lab mix* canines weighing between 27 and 32 kg were selected for placement of “sensitive” scaffolds to be used for measuring *in vivo* joint loading in conjunction with the newly developed telemetry system. Scaffolds were implanted into the face of the right medial femoral condyle by an orthopaedic surgeon (JTR) familiar with graft placement procedures using a previously utilized surgical procedure.¹⁰ *In vitro* studies have shown that a trained surgeon following established guidelines for scaffold placement will achieve consistent scaffold orientation within a joint.⁹ The same surgeon that implanted scaffolds into canine knees in previous *in vitro* and *in vivo* studies implanted all the scaffolds used in this study.^{9,10}

Briefly, an incision was made along the medial edge of the patella to expose the joint capsule. The medial femoral condyle was exposed using blunt dissection and two concentric holes were drilled for scaffold implantation. The first 3-mm hole was drilled using a guide drill to allow a wire to pass through the condyle to the mid-diaphysis of the lateral surface of the femur. A second 9-mm diameter hole was reamed using a reamer guided by the guide drill so the scaffold could be press fit into the condyle (Figure 3). The scaffold was implanted so that the dome on the superficial surface of the scaffold was recessed 1 mm below the surrounding articular cartilage. Scaffolds were implanted with the strain gauge axes colinear with the long axis of the femur, which was the loading axis at 30° of stifle flexion (Figure 4). A second incision was made in the flank to implant the digital transmitter (Microstrain, Williston, VT). The transmitter was sutured into place through the silicone waterproofing layer to prevent excessive movement of wires. Wires that exited the mid-diaphysis of the femur, were passed through the quadriceps and connected to the transmitter using a custom made multipin connection (Figure 5). The connection site was waterproofed using Radioque Bone Cement (Stryker, Kalamazoo, MI).

Measurement Collection

Prior to surgery, each test animal was trained to run on a Trotter treadmill (United Medical Company, Millis, MA) at speeds ranging between 2.6 and 9.1 km/hr. The animals were exercised within a week of surgery and returned to a full running regimen on the treadmill after 2 weeks post-op. During post op treadmill running, a power coil was placed on the animal's flank on the skin adjacent to the transmitter location. The coil was energized and measurements were collected by the receiver and recorded on a Macintosh G3 laptop computer (Apple, Cupertino, CA). Data from the telemetry system were collected at a rate of 84.5 Hz for up to 9 s during each collection period so that the data sets would be nearly identical to those obtained with a hardwired system¹⁰ used in earlier experiments. Additionally, video was collected using a digital video camera (Hitachi Ultravision Digital Camera, Tokyo, Japan) to correlate the test animal's gait pattern with stride and stance time measurements.

Gait analysis was also performed using a GAITRite portable walkway system (CIR Systems, Havertown PA). Test animals were walked along a portable force platform at a speed of 8 km/

hr to monitor the symmetry of the test animal's gait and analyze any effects surgery might have had on gait.

Measurement Analysis

Video images were analyzed using QuickTime 6.53 (Apple, Cupertino, CA) and Excel (Microsoft Corp., Seattle, WA) to measure stride time and stance time (time in contact with the ground). They were also used to identify pawstrike, mid-stance, and toe off times to validate time based measurements collected from loading data.

Measurements collected from the “sensate” scaffolds were selected for analysis based on pre-established criteria that included, (1) the identification of three repeatable and distinct peaks during the pawstrike, mid-stance, and toe off phases of gait; (2) two clearly observable valleys (between pawstrike and mid-stance and between mid-stance and toe off); (3) a clear baseline before and after each step; and (4) at least four steps must have met the above established criteria in a single recording session. The measurements collected from the analysis performed on each peak were averaged for the four steps per recording session, and the average was used for statistical analysis (Figure 6).

Each step meeting the above criteria was analyzed and the temporal locations of the pawstrike and toe off peaks, the two valleys, initiation of pawstrike loading, and completion of toe off unloading were identified (Figure 6). Strain measurements were converted to load (Newton) using the calibration curves established for each gauge.⁹ Impulse was calculated (the area underneath the force-time curve) and the distribution of impulse through pawstrike, mid-stance, and toe off through each step was also calculated (Table I). Additionally, maximum loading rates were measured for pawstrike and toe off. All measurements and calculations were recorded as a change in load relative to the baseline value, which was established as the load measured during the swing phase of gait.¹⁰ For statistical analysis all the load and impulse data were normalized to percent body weight to account for the differences in the weights of the test animals.

Prior to performing the statistical analysis, time post-implantation was categorized into three groups: early (2–5 weeks), middle (6–9 weeks), and late (10–14 weeks). Stride time was categorized as either long or short (long > 0.716 s, short ≤ 0.716 s). A multivariate ANOVA using time post-op and stride time as independent variables was used to determine statistical significance, and a Tukey post-hoc test was used to determine which time points were significantly different. The dependent variables analyzed included total impulse, pawstrike impulse, toe off impulse, % pawstrike impulse, % toe off impulse, % mid-stance impulse, pawstrike load rate, toe off load rate, and maximum load. A p value ≤ 0.05 was considered statistically significant. All data were reported as an average ± standard deviation (Table II).

Results

In vivo load measurements were recorded beginning at 2 weeks post op, and continued for up to 14 weeks after scaffold placement. Failure of water proofing of some wire connections as well as mechanical failure of transmitters prevented collection for longer periods in this group of test animals. Waterproofing factors also reduced the data being collected to measurements from seven of the nine strain gauges. During the course of the study 120 data sets were collected from the strain gauges that met the pre-established criteria for analysis. Measurements of stride time made using the digital video camera correlated well with the stride time determined using the sensate scaffold system, as all the measurements collected using the video were within 0.03 s of those collected using the “sensate” scaffold system. Gait analysis using the GAITRite force walkway showed that the ratios between the left hind paw pressure and the right hind paw

pressure for normal non surgically treated limbs were similar to those measured following surgical placement of scaffolds (Table III).

Altering treadmill speed resulted in changes in load distribution throughout gait. Stride time was inversely proportional to the percentage of impulse observed during pawstrike ($p = 0.05$) and proportional to the percentage of impulse occurring during toe off ($p < 0.01$). Additionally, decreasing stride time resulted in a significant increase on maximum pawstrike loading rate ($p < 0.04$). Altering stride time did not have an effect on the maximum load measured during gait ($p = 0.7$).

Maximum load measured from scaffolds increased with time post-implantation ($p = 0.03$) between the early and late time points from 9 to 95 N, which represented an increase from 3% to 38% of the test animal's body weight. In addition, total impulse ($p < 0.02$) and duty factor ($p < 0.02$) increased with time post-implantation (Figure 7).

Discussion

To our knowledge, the measurements collected in this study represent the most comprehensive direct *in vivo* load measurements collected from within a native joint. The results in this study demonstrate that the “sensate” scaffolds provide precise measurements of load and load rate during treadmill running. The accuracy and precision of the temporal data provided by the sensors was high, as the stride time measured via video and that measured from the implanted scaffold were always within 0.03 s. This indicates that the load measurements collected through the telemetry based system provide adequate temporal resolution, which is in agreement with published results comparing the telemetry system to a hard-wired system.^{10,24} In addition, the results demonstrate that these sensors can provide temporal data about gait as early as 2 weeks, which is sensitive to gait speed but not time post-implantation. The changes noted in load rate also confirm the utility of these sensors to accurately monitor joint loading with sufficient temporal resolution, as the sensors measured higher load rates as stride time decreased. This was expected, as loading rates are inversely proportional to stride time.

In contrast to load rates, peak loads are expected to remain constant as a function of stride time but will change as a function of time post-implantation²⁵ until healing is complete. Previous *in vitro* studies have demonstrated that accurate load measurements can be collected from these sensors,⁹ and *in vivo* studies have demonstrated that accurate load measurements are possible through hardwired sensate PBT scaffolds once secure bone bonding has occurred.^{7,10} Those studies demonstrated that secure bone to implant bonding was necessary for accurate load measurement. The current study shows that the load-transfer to the sensor from the surrounding tissues is a function of the extent of healing. The results of this study are consistent with the previous studies demonstrating that monitoring changes in the peak load could be used to follow healing and will aid in the development of post-surgical rehabilitation regimens. It also indicates that healing must be complete before “sensate” scaffolds can be used to accurately measure normal physiological loads at the cartilage surface during various activities in order to prevent overloading of tissues by active patients.

Although peak loads measured with these scaffolds have been shown to change with bone to scaffold bonding,⁷ pain also influences limb loading.²⁵ Pain is expected to decrease with time post-implantation, and for this reason the animal may spend more time and place a higher load on the limb as pain decreases. Even though the animals did not appear to be in pain or favoring the limb at any time after the first week post op, the animals in this study spent more time on the surgically manipulated limb as the study progressed, as indicated by the increase in duty factor with time post-implantation. This demonstrates that changes in duty factor could be useful as an objective indicator of comfort in animal models. Gait analysis utilizing the

GAITRite portable walkway showed that post op gait patterns were similar to those observed in nonoperated limbs. While more extensive testing using the GAITRite system will be valuable, preliminary results indicate that the test animal was not favoring the surgically treated limb. This implies that the measurements collected from the “sensate” scaffold were not significantly influenced by the surgical intervention itself for the entire duration of the experiment. The potential ability to use cartilage covered “sensate” scaffolds to simultaneously analyze discomfort and load warrants further study as there is currently no objective method to monitor pain in animal models utilized for researching orthopaedic implants aimed at repairing bone and cartilage tissues.

While we were able to record load measurements from the test animals for up to 14 weeks, high noise levels resulting from fluid infiltration and failure of the telemetry system prevented accurate data collection from all the functional sensors for longer time periods. Because of the stringent requirements established for analysis of data prior to the study, only 20% of all measurements were used in the final analysis of loading. Additional development will improve waterproofing techniques and provide a more rugged transmitter design to allow consistent data collection over increasingly longer periods of time.

Overall, the “sensate” scaffold system combined with telemetry to wirelessly transmit load measurements provides a way to monitor *in vivo* joint loading. This study confirmed the temporal accuracy of implanted sensors monitored with telemetry. It has also shown that both stride time and loading rates were dependent upon treadmill speed. The peak load was dependent on time post-implantation, indicating that peak load can be used to monitor healing. Additionally, real time monitoring of load in a clinical setting can ensure that the patient does not place excessive and damaging loads on implanted tissue engineered constructs. While the application of this technology for every patient is currently impractical, improvements in technology that reduce the size of the telemetry to fit within the implant will allow the clinical monitoring of select patients to provide parameters on activities that might damage the tissue-engineered cartilage and to optimize rehabilitation. In addition, measurement of duty factor may provide an indication of joint pain following scaffold placement allowing researchers, utilizing an animal model, to evaluate the effectiveness of analgesics.

The portability of the telemetry system demonstrates the potential for using this technology in a clinical research setting. A similar telemetry based system was used to monitor hydroxyapatite coated strain gauges attached to the vertebra of a patient during spine fusion, where measurements were collected for 7 months following sensor placement.²⁴ The use of telemetry to collect loading measurements from “sensate” scaffolds provides important information to researchers and clinicians that will improve tissue engineering, allow for characterization of the mechanical pathophysiology during the onset and progression of OA, and allow for careful continuous monitoring of the implanted engineered tissue.

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Figure 1.

The “sensate” scaffold and telemetry transmitter system. The external power coil (left) is used to power the transmitter (center) that is directly connected to a “sensate” scaffold (upper right). The entire system is pictured on top of a CD and the scaffold is next to a penny. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Figure 2.

The “sensate” scaffold showing the location of two of the three circumferentially placed strain gauges. The third gauge is not visible because it is located on the opposite scaffold surface. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Figure 3.

The surgical procedure used to implant the scaffolds involved: exposing the medial femoral condyle (a), creating a defect in the cartilage with a 9 mm reamer (b), and threading wires from a scaffold through the femoral condyle (c). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Figure 4. Schematic illustration depicting the location within the medial femoral condyle where the “sensate” scaffold was placed.

Figure 5. Radiograph illustrating the location of the scaffold (highlighted in lower right) and telemetry transmitter. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Figure 6.

Representative line graph of loading during treadmill gait depicting the measurements collected from each step. PS, pawstrike; MS, mid-stance; T, toe off; M1, minimum between pawstrike and mid-stance; M2, minimum between mid-stance and toe off; B, baseline during swing phase.

Figure 7.

(a,b) are representative load vs. time graphs from 2–5 weeks time post op; (a) represents a short stride time while (b) represents a long stride time. (c,d) are load vs. time graphs from 10–14 weeks post op; (c) represents a short stride time while (d) represents a long stride time. Note that independent of stride time, measured loads increased during weeks 10–14 compared with weeks 2–5.

Table I
Measurements Derived From Data Collected for Each Step With the Corresponding Description

Measurement	Description
Stance time	Time between beginning of pawstrike loading and end of toe off unloading (time in contact with the ground)
Stride time	Time between pawstrike maximums for consecutive steps
Total impulse	Area beneath load-time graph and above baseline between beginning of pawstrike and end of toe off unloading
Pawstrike impulse	Portion of total impulse between beginning of pawstrike loading and pawstrike to mid-stance minimum
Toe off impulse	Portion of total impulse between mid-stance to toe off minimum and end of toe off unloading
Mid-stance impulse	Portion of total impulse between two defined minimums
Peak loading rate	Maximum slope between any two points on the load-time graph making up the defined peak
Duty factor	Ratio of stance time to stride time indicating the percentage of stride time that the paw was in contact with the ground

Table II

Averages and Standard Deviations

	Short Stride Time			Long Stride Time		
	Early	Middle	Late	Early	Middle	Late
Total impulse	1.661 ± 0.619 [*]	2.482 ± 1.158	4.757 ± 2.779 [*]	2.516 ± 1.301 [*]	3.853 ± 1.588	6.515 ± 4.331 [*]
Strike total impulse [‡]	0.399 ± 0.191 [*]	0.385 ± 0.143	0.725 ± 0.448 [*]	0.267 ± 0.139 [*]	0.449 ± 0.303	0.729 ± 0.349 [*]
Toe total impulse [‡]	0.395 ± 0.238 [*]	0.509 ± 0.247 [†]	1.681 ± 1.121 ^{*,†}	0.992 ± 0.583 [*]	1.42 ± 0.966 [†]	2.573 ± 1.893 ^{*,†}
Max strike load rate [‡]	365.4 ± 226.6	300.7 ± 94	384.8 ± 219	172.1 ± 108.2	213.5 ± 174.8	220.2 ± 77.8
Max toe load rate	121.2 ± 70.3	100.2 ± 30.3	179.4 ± 136.6	98.3 ± 50	79.1 ± 61	156.6 ± 139.4
Peak difference	10.81 ± 4.28 [*]	12.50 ± 4.53	19.43 ± 10.14 [*]	9.53 ± 4.73 [*]	11.79 ± 5.13	18.44 ± 11.69 [*]
Duty factor	0.663 ± 0.031 [*]	0.675 ± 0.062	0.744 ± 0.062	0.69 ± 0.026	0.706 ± 0.03	0.734 ± 0.077 [*]
% Strike impulse [‡]	24.84 ± 10.43	16.81 ± 6.26	14.70 ± 5.73	11.52 ± 5.56	11 ± 5.68	15.11 ± 9.63
% Midstance impulse	52.72 ± 14.05	62.58 ± 7.72	50.58 ± 13.02	49.37 ± 6.69	54.07 ± 10.82	48.07 ± 6.44
% Toe impulse [‡]	22.44 ± 7.90	20.61 ± 6.68	34.71 ± 9.49	39.12 ± 3.51	34.94 ± 9.84	36.82 ± 5.62

^{*} Statistical significance was found between the early and late time points.

[†] Statistical significance was found between the middle and late time points.

[‡] Statistical significance was found between the long and short stride time.

Table III
Averages and Standard Deviations of Pressure Symmetry Ratios Determined Using the GAITRite
Portable Walkway System

	Left hind/right hind	Front/hind
Control limb 1	0.938 ± 0.308	1.687 ± 0.305
Control limb 2	1.158 ± 0.208	1.616 ± 0.727
Experimental limb	1.178 ± 0.179	1.517 ± 0.160