Long-term Local and Systemic Safety of Poly(l-lactide-co-epsilon-caprolactone) after Subcutaneous and Intra-articular Implantation in Rats

Yuval Ramot1, Abraham Nyska2, Elana Markovitz3, Assaf Dekel3, Guy Klaiman4, Moran Haim Zada5, Abraham J. Domb5 and Robert R. Maronpot6

Abstract
The use of biodegradable materials is gaining popularity in medicine, especially in orthopedic applications. However, preclinical evaluation of biodegradable materials can be challenging, since they are located in close contact with host tissues and might be implanted for a long period of time. Evaluation of these compounds requires biodegradability and biocompatibility studies and meticulous pathology examination. We describe 2 preclinical studies performed on Sprague-Dawley rats for 52 weeks, to evaluate clinical pathology, biocompatibility, biodegradability, and systemic toxicity after implantation of 2-layered films or saline-inflated balloon-shaped implants of downsized InSpace™ devices (termed “test device”). The test devices are made from a copolymer of poly-L-lactide-co-ε-caprolactone in a 70:30 ratio, identical to the device used in humans, intended for the treatment of rotator cuff tears. Intra-articular film implantation and subcutaneous implantation of the downsized device showed favorable local and systemic tolerability. Although the implanted materials have no inherent toxic or tumorigenic properties, one animal developed a fibrosarcoma at the implantation site, an event that is associated with a rodent-predilection response where solid materials cause mesenchymal neoplasms. This effect is discussed in the context of biodegradable materials along with a detailed description of expected pathology for biodegradable materials in long-term rodent studies.

Keywords
biodegradable materials, safety studies, rotator cuff syndrome, biocompatibility, biodegradability

Introduction
One of the most common musculoskeletal disorders is shoulder pain, which can have a prominent negative effect on quality of life (Whittle and Buchbinder 2015). The rotator cuff (RC) encompasses a group of muscles that engulf the shoulder joint and allows movement and stabilization of this joint (Whittle and Buchbinder 2015). Tears of the RC are the most common cause for shoulder pain (Lorbach et al. 2015), and they often necessitate surgical reconstruction (Yamaguchi et al. 2006). However, the proper way to treat RC tears is still debated, considering the high rate of treatment failure (DeHaan et al. 2012; Duquin, Buyea, and Bisson 2010; Lorbach et al. 2015), and there is growing interest in ways to improve tendon-bone healing (Anz et al. 2014; Beitzel et al. 2013), including use of biodegradable orthopedic devices.

Biodegradable devices are commonly composed of polymeric materials that degrade into products that can be eliminated from the body or degrade into normal metabolites (Nyska et al. 2014). They are especially useful for orthopedic applications taking into consideration their slow degradation, allowing the gradual transfer of load to healing bones and joints (Nyska et al. 2014; Ramot et al. 2015). Indeed, their use has also been advocated for the treatment of the RC syndrome (Zhao et al. 2015; Zhao et al. 2014).

A novel addition to the biodegradable armamentarium for RC syndrome includes a subacromial implantable balloon-shaped spacer (InSpace™ system, Ortho-Space, Caesarea, 1

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Animals, Materials, and Methods

Test Device

The InSpace spacer, supplied by Ortho-Space Ltd., is constructed of medical grade copolymer of PLCL in a 70:30 ratio. Implanted test devices were produced using the manufacturing process and full ethylene oxide sterilization cycles identical to the device as used in humans, but downsized to accommodate animal size. In humans, the device is available in 3 different sizes: small (40 × 50 mm), medium (50 × 60 mm), and large (60 × 70 mm; Savarese and Romeo 2012). For the shoulder implantation (first study), 2-layer films, sized to 5 × 5 mm to accommodate rat shoulder size, were used. This adjusted device size represents similar size and amount of material as being used in human shoulders (50 × 50 mm). For the subcutaneous implantation study (second study), each animal was implanted with 2 test devices downsized to fit the selected rat animal model. Each test device was downsized to 20 × 30 mm and inflated with approximately 2 ml of physiological saline. The relative device material amount and implanted area in the rats was approximately 80 times greater than the relative area and amount of material used in human subjects. The size of the downsized device was selected to provide a device that can be properly inflated and measured and big enough to enable chemical characteristic evaluation, but that can still fit the animal’s size and shape without interfering the animal’s welfare and in accordance with Annexure B of ISO 10993-6 and ISO 10993-11.

Animals and Housing

Male Sprague-Dawley rats, 7 to 8 weeks of age, were obtained from Harlan Laboratories (Rehovot, Israel) and maintained on standard chow (Harlan Teklad diet 201S, Madison, WI). They were allowed free access to drinking water, supplied to each cage via polyethylene bottles with stainless steel sipper tubes. The water was filtered (0.2 μm filter), chlorinated, and acidified (pH 5.0–5.9). During acclimation and throughout the entire study duration, animals were housed within a limited access rodent facility and kept in groups of a maximum of 3 animals/cage, in cages fitted with solid bottoms and filled with wood shavings as bedding material (7093 Harlan Teklad Shredded Aspen, Harlan Laboratories, Madison, WI). The rats were allowed a 5- to 6-day acclimation period to facility conditions (20–24°C, 30–70% relative humidity, and a 12-hr light/dark cycle) prior to inclusion in the study. Animal care and the test device implantation were conducted at a good laboratory practices (GLP)–certified site (Harlan Biotech Israel Ltd., Rehovot, Israel) and after receiving the approval of the National Council for Animal Experimentation.

Experimental Design—First Study

For the first study, the 30 rats were randomized into 2 groups: the test group consisted of 21 animals, and the control group of 9 animals. Animals were sacrificed at predetermined time points of 12, 26, and 52 weeks postimplantation (Table 1). The animals underwent analgesia (buprenorphine at a dose of 0.05 mg/kg by subcutaneous injection) and anesthesia (isoflurane 2–3% in 100% O2 at the rate of 0.8–1 L/min, administered by face mask). Later, each animal was placed in lateral recumbency, with the right leg upward. The forelimb was positioned in external rotation. A 2-cm skin incision was made over the scapulo-humeral (SH) joint, which was exposed by blunt dissection. The supraspinatus tendon was exposed in its passage under the bony arch consisting of the acromion, coracoid process, and clavicle, and was resected at this location. The subacromial space was debrided in order to enable test device implantation.

In the test group, the test device (i.e., 2 layers of 5 × 5 mm) was implanted in the subacromial space and fixated by single...
suture using appropriately sized nonabsorbable suture material. The musculature and subcutaneous tissues were closed with 3/0 Vicryl sutures. The skin was closed with stainless steel surgical clips. In the control group, an identical procedure was performed, including full exposure of the SH joint, resection of the supraspinatus tendon, and placement of the nonabsorbable anchoring Vicryl suture, without implantation of the test device. At the end of the day following surgery and twice on the following day, animals were administered buprenorphine by subcutaneous injection at a dose of 0.05 mg/kg.

**Experimental Design—Second Study**

For the second study, 15 animals were divided into 4 test groups and were sacrificed at 4, 12, 16 (unscheduled), 26, and 52 weeks postimplantation (Table 2). All test devices were inflated with sterile physiological saline and were sealed before implantation. The amount of saline filled in each test device was between 1.8 and 2.7 ml (average of 2.2 ml), and the average initial device weight was 2.3 g. The animals underwent analgesia (buprenorphine at a dose of 0.05 mg/kg by subcutaneous injection) and anesthesia (isoflurane 2–3% in 100% O₂ at the rate of 0.8–1 L/min. administered by face mask). Later, the animals were placed in ventral recumbency, and a 2- to 3-cm skin incision was made over the dorsal midline in the thoracic area. Subcutaneous tunnels were created from the skin incision in a caudal direction to both sides of the spine overlying the lumbar paravertebral muscles.

<table>
<thead>
<tr>
<th>Number of animals</th>
<th>Test material</th>
<th>Implantation procedure</th>
<th>Sacrifice Time points (weeks postimplantation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>PLCL: downsized</td>
<td>subcutaneous</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>PLCL: InSpace™</td>
<td>implantation in the dorsolumbar area (2 test devices per animal)</td>
<td>12</td>
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<td>implantation in the dorsolumbar area (2 test devices per animal)</td>
<td>26</td>
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<tr>
<td>5</td>
<td>PLCL: InSpace™</td>
<td>implantation in the dorsolumbar area (2 test devices per animal)</td>
<td>52</td>
</tr>
</tbody>
</table>

Note: PLCL = poly-L-lactide-co-ε-caprolactone; RC = rotator cuff.

Two saline-filled, preweighted test devices were implanted in each animal on either side of the spine in the lumbar region, 1 test device on each side of the spine. The test devices were anchored to the underlying muscle using nonabsorbable suture material, which was used to identify the implantation site at test device explantation. At the end of the day following surgery and twice daily for up to 3 days postsurgery, animals were administered buprenorphine by subcutaneous injection at a dose of 0.05 mg/kg.

**Viability and Weight**

For both studies, viability check of all animals was performed at least once daily, and detailed clinical examinations were carried out and recorded once weekly. Body weights were measured prior to implantation and weekly thereafter. The last body weight determination was carried out prior to scheduled termination.

**Hematology and Biochemistry**

For both studies, blood for hematology and biochemistry parameters was collected just prior to euthanasia. Blood samples were obtained by retro-orbital sinus bleeding under CO₂ anesthesia and following an overnight food deprivation. At least 300 μl whole blood was collected into EDTA-coated tubes for hematology, and at least 700 μl whole blood was collected into commercial serum separation gel tubes and centrifuged for separation of serum for biochemistry. The samples were assayed for hematology using the ADVIA 120 Hematology System (Siemens, Erlangen, Germany) and for biochemistry using the ROCHE-HITACHI/MODULAR P-800 & Roche Cobas® 6000 Analyzer.

**Necropsy and Tissue Handling**

In the first study, following animal sacrifice, the implantation sites were examined macroscopically to assess any evident tissue reactions to the implanted test device. In 3 animals sacrificed at the 12-week time point and 3 animals sacrificed at the 26-week time point, the test device was carefully removed from the implantation sites from both shoulders immediately following sacrifice, taking care to cause as little damage to the...
surrounding tissues as possible. The local draining lymph nodes, that is, brachial and axillary lymph nodes, were assessed macroscopically. The test and control sites from all animals and the draining lymph nodes from the animals in the 52-week sacrifice point were resected and preserved in 10% neutral buffered formalin (approximately 4% formaldehyde solution). Tissues were trimmed when necessary and embedded in paraffin. Five step sections approximately 5 microns thick, at 250-μm intervals, were prepared from each test or control site using the suture site as the middle section. Slides were stained with hematoxylin and eosin (H&E).

In the second study, all animals were subjected to a full detailed necropy. The following tissues were collected from all sacrificed animals: brain, heart, lungs, liver, and kidneys. These tissues were weighed and preserved in 10% neutral buffered formalin until testing and pathology evaluated. In addition, the tissue surrounding each test device was also preserved in 10% neutral buffered formalin for histopathological examination. Tissues were trimmed when necessary and embedded in paraffin. One section approximately 5 microns thick was prepared from each site. Slides were stained with H&E.

**Histopathology Examination**

For both studies, the histological local response parameters that were assessed and recorded were based on the international standards ISO 10993-6 (2007) and included (but were not limited to) the presence and extent of fibrosis/fibrous capsule; the extent of inflammation based on the number and types of inflammatory cells present; degeneration as determined by changes in tissue morphology and differences in tinctorial staining; presence, extent, and type of necrosis; tissue alterations such as fragmentation and/or presence of debris, fatty infiltration, granuloma formation, and bone formation; presence and form of test device remnants, material fragmentation, and debris; and the nature and extent of tissue ingrowth, if applicable. The scaling of the lesions was based on the semi-quantitative criteria presented by Shackelford et al. (2002), in which grade 1 corresponds to lesion barely noticeable and/or up to 10% of the tissue is affected; grade 2—the lesion is noticeable but is not a prominent feature of the tissue and/or up to 20% of the tissue is affected; grade 3—the lesion is a prominent feature of the tissue and/or up to 40% of the tissue is affected; grade 4—the lesion is an overwhelming feature of the tissue and/or more than 41% of the tissue is affected.

**Test Device Examination**

In the second study, following sacrifice of the animals, the test devices (or their residues) were explanted from their implantation sites, cleaned as much as possible from surrounding tissues and weighed, and then analyzed for degradation by mass loss and molecular weight change. The weight loss was determined by weighing the recovered isolated dry polymer implant residues.

Molecular weight of the implant residues was conducted on gel permeation chromatography (GPC) systems, consisting of Waters 1515 Isocratic high-performance liquid chromatography (HPLC) pump, with L-7490 refractive index detector (RI; Hitachi, Tokyo, Japan) and a Rheodyne (Coatati, CA) injection valve with a 20-μl loop. Samples were eluted with chloroform (HPLC grade) through a linear styragel column THF, HR5E (Waters, MA) at a flow rate of 1 ml/min. The molecular weights were determined relative to polystyrene standards (Polyscience, Warrington, PA), using the Empower computer program.

**Statistical Analysis**

For both studies, results were analyzed using GraphPad Prism (Graphpad Software Inc., CA), using unpaired t-test, Tukey-Kramer multiple comparisons test, and one-way analysis of variance with Bonferroni multiple comparison test.

**Results**

**Survival, Clinical Observations, and Body Weight Gain**

In the first study, 1 control animal was removed prematurely from the study at 12 weeks due to humane reasons. This animal (that had only the sham operation without test device) displayed decreased motor activity and dyspnea as well as a 10% decrease in body weight compared to previous weighing.

There were no other treatment-related effects on body weight, and all remaining animals showed normal body weight gain until sacrifice. No abnormal clinical signs were observed, except for a transient and local crusting on the left cranial shoulder overlying the implantation site in 1 animal from the test device group.

In the second study, 2 animals were removed from the study ahead of their scheduled sacrifice time point. One animal had ulceration of the skin overlying the test devices (both migrated to the right side), from the second week postimplantation and, therefore, was included in the 4-week termination group instead of the originally planned 26-week group. The second animal was removed on week 16 due to protrusion of the left test device plug through the skin. Otherwise, there were no treatment-related effects on body weight, and all animals showed body weight gain at sacrifice. Crusting of the skin overlying the implanted test devices was present at 4 weeks postimplantation in 8 animals and disappeared from all animals except 1 by 8 weeks postimplantation. This observation was considered as procedurally related with no systemic adverse impact.

The presence of solution in the test devices in the second study was evaluated by palpation of the skin or by visual detection at the sacrifice time point. Fluid was palpated in all test devices up to 19 weeks postimplantation, and by approximately 22 weeks postimplantation, fluid was no longer detected by palpation in any of the remaining test devices.

At week 45, a mass on the midline of the back was observed in 1 animal from the 52-week sacrifice group. It grew progressively...
and reached a size of approximately 4 × 4 mm 1 day prior to the 52-week scheduled study termination. None of the other animals had similar observations at any of the sacrifice time points.

**Hematology and Clinical Chemistry**

In the first study, statistical differences were noted in red blood cells, hematocrit, mean corpuscular hemoglobin concentration, glucose, and total protein levels between the control and test groups at 26 weeks postimplantation (Table 3). At 52 weeks postimplantation, there was a statistical significant difference in the white blood cell levels (Table 3). However, all changes were without clinical significance and were within normal reference ranges for hematology and biochemistry parameters in these rats.

In the second study, no control group was available for comparison. However, 2 animals had abnormal values compared to the other animals in their groups. One rat that was removed from the study due to skin ulceration had an elevated white blood cell count. The rat with a large subcutaneous mass in the implantation site had increases in creatinine and urea levels along with an elevated white blood cells count due to neutrophilia and anemia characterized by low red blood cells count, low hemoglobin levels, and low hematocrit but without a change to the cell size and hemoglobin content (data not shown). These findings are secondary to the mass on the midline of the back and were not a direct response to the test device.

**Pathology—First Study**

In the first study, macroscopic examination of the tissue surrounding the implantation sites revealed a hematoma-like dark red–colored test device in 1 of the animals at the 12-week interim sacrifice point. No macroscopic abnormalities were found at the 26-week interim sacrifice point. At the 52-week interim sacrifice point, a hardened substance or tissue was observed at the suture site in 6 out of 10 implantation sites. On 3 other cases, a square form (approximately 4 × 5 mm) was identified. No abnormalities were found at the sham-operated animals.

Macroscopic examination of the test device and implantation site at the 12-week interim sacrifice point revealed opaque films measuring about 5 × 5 mm, that were closely adherent to the surrounding tissue in 4 of the 6 devices. One device had curled to a cylinder-like structure and was also closely adherent to the surrounding tissue. One device was observed as a double-layered opaque film measuring about 5 × 5 mm that was easily separated from the surrounding tissue. At the 26-week interim sacrifice point, the test devices were fragmented into small pieces, encapsulated and/or inseparable or adherent to the surrounding tissues.

Histopathologically, no treatment-related changes were seen in the sham-operated animals. In the InSpace-implanted animals, the sites of implantation located at the shoulder were

| Table 3. Hematology and biochemistry findings in rats from the first study after implantation of a two layer films of poly-L-lactide acid and epsilon-caprolactone copolymer in the subacromial joint. |
|-----------------|-----------------|-----------------|-----------------|
|                  | 12 Weeks        | 26 Weeks        | 52 Weeks        |
|                  | Control         | Test device     | Control         | Test device     | Control         | Test device     |
| WBC (10³ cmm)    | 4.78 (4.38)     | 2.21 (2.02)     | 2.11 (1.96)     | 1.07 (0.75)     | 8.4 (7.0)       | 4.17 (3.52)     |
| RBC (10⁶ cmm)    | 9.53 (0.358)    | 9.69 (0.669)    | 9.22 (0.343)    | 9.19 (0.201)    | 9.26 (0.092)    | 9.11 (0.312)    |
| HCT (%)          | 55.0 (0.2)      | 53.8 (3.88)     | 53.2 (2.3)      | 54.2 (0.42)     | 50.5 (1.55)     | 54.2 (0.38)     |
| MCHC (%)         | 30.1 (1.14)     | 30.2 (1.54)     | 30.1 (0.15)     | 30.2 (0.13)     | 30.1 (0.21)     | 30.2 (0.12)     |
| Biochemistry     |                 |                 |                 |                 |                 |                 |
| Glucose (mg/dl)  | 122 (23.0)      | 133 (44.2)      | 107 (7.4)       | 119 (7.0)       | 118 (7.0)       | 127 (7.0)       |
| Total protein (g/dl) | 6.75 (0.236) | 6.86 (0.280) | 7.50 (0.127) | 7.32 (0.127) | 7.42 (0.127) | 7.50 (0.127) |
| Note: HCT = hematocrit; MCHC = mean corpuscular hemoglobin concentration; RBC = red blood cells; WBC = white blood cells. Reference values were obtained from http://en.aml-vet.com/animal-species/rat/hematology and http://en.aml-vet.com/animal-species/rat/chemistry. *p < 0.05; **p < 0.01. unpaired t-test.
identified by the presence of cavity formation surrounded by a mature fibrotic capsule (Table 4). At the 12- and 26-week interim sacrifice points, the fibrotic capsule had a minimal severity grade. At the 52-week sacrifice point, severity ranged from minimal to mild (Table 4). Occasionally, a single layer of macrophages of minimal (12 and 26 weeks) or minimal to mild (52 weeks) severity was present at the interface of the copolymer and the fibrotic capsule. Minimal presence of lymphocytes and rare polymorphonuclear cells were sometimes present within the fibrotic capsule. No evidence for chronic active inflammation, necrosis, chondral damage, articular bone damage, or surrounding muscle degeneration was noted at any time point. Remnants of the implanted device, occasionally seen within the cavity formation, were surrounded by mature fibrotic capsule at all time points.

At the 12-week interim point, the implanted test device was not fragmented; while at the 26-week interim point, the implanted device was fragmented into several pieces in some of the rats. At the 52-week point, the implanted device was fragmented into a few to multiple small pieces in the majority of the implantation sites. When examining the irritation scoring using ISO-10993-6 criteria, a marginal slight irritation (score of 3.0) was confined to the test device at the first time point of 12 weeks versus the respective control sites, while at the 26- and 52-week time points, the test device could be considered as nonirritant (score of <3.0; Supplementary Table 1).

At all time points, no adverse reactions such as mineralization, necrosis, or immune-mediated inflammation were noted, indicating good long-term tolerability of the test compound.

Pathology—Second Study

In 7 animals, the test devices on the left side were displaced to the right side of the animal. It is not uncommon for subcutaneous implanted items in rodents to be displaced from the implantation site, especially considering their body activity and the relative large amount of loose skin in rats.

Macroscopic examination of the test sites at 12 and 26 weeks postimplantation revealed that all test devices were encapsulated by a connective tissue with some yellowish discoloration of varying degrees in the surrounding tissue.

Macroscopic examination of the explanted test device revealed that up to 16 weeks postimplantation, the test devices were intact and were still filled with clear fluid to varying degrees (Figure 1). While inflated, the test devices were not adherent to their surrounding tissue aside from 1 test device in the animal with ulcerated skin. At 26 weeks postimplantation, the test devices disintegrated to multiple small fragments, which were encapsulated by surrounding tissue, and by 52 weeks postimplantation, the test devices were fragmented into very small (<1 mm in size) pieces, embedded in and inseparable from the surrounding tissue.

Histopathologically, at all time points up to 26 weeks, the sites of implantation located subcutaneously at the dorsolumbar area were identified by the presence of a cavity surrounded by a mature fibrotic capsule (Table 5; Figures 2 and 3). Occasionally, a single layer of macrophages with occasional giant cells at the interface of the copolymer and the fibrotic capsule was seen. No evidence of chronic active inflammation or necrosis was present. Minimal presence of lymphocytes and rare polymorphonuclear cells were sometimes present within the fibrotic capsule. Up to the 16-week interim sacrifice point, the test device was not fragmented. At the 26-week interim sacrifice in all but one of the test sites, the implanted device was fragmented into multiple pieces (Figures 4 and 5).

At the 52-week interim sacrifice, in all of the test sites, the implanted device was fragmented into multiple small pieces, each piece surrounded by a fibrotic capsule (Figures 6–9) with occasional macrophages and giant cells at the interface of the fibrous capsule and the fragmented device material (Figure 9). Overall, the local changes indicated favorable host response to the device.

No evidence of systemic toxicity was present in the liver, lungs, kidneys, heart, and brain from all animals, irrespective of time of sacrifice or the presence of local reaction. A small range of spontaneous hepatic and renal age-related background lesions commonly seen in untreated rats of the same age and strain were present.

At the 52-week time point, 1 rat had a subcutaneous mass approximately 3.5 × 4 cm on the midline of the back. The center of the mass was red/pink with firm consistency and its periphery was white and less firm. Histopathological examination of the midline mass revealed the presence of a solid fibrous mass, that was relatively well circumscribed, with irregular areas of necrosis at the center. The histology features of the mass included a storiform growth pattern of uniform plump spindle cells, presence of small rounded cells, some highly pleomorphic spindle cells, and tumor giant cells (Figures 10–12). Occasional mitotic figures were noted. Based on these histological features, the mass was consistent with a pleomorphic fibrosarcoma. Fragments of the test device were embedded within the neoplastic tissue.

Time-related Changes in Test Device Mass and Molecular Weight

In the second study, the explanted test devices were subject to weight loss determination and molecular weight analyses. These analyses were performed on test devices explanted from the animals sacrificed at 4, 12, 16, and 26 weeks, since at 52 weeks postimplantation, the test device was almost fully eliminated, fragmented into very small (<1 mm) fragments that could not be separated from the surrounding tissue for evaluation. While the dry weight of the test device was relatively stable until 16 weeks postimplantation, it was significantly decreased by 26 weeks postimplantation (Figure 13A). Molecular weight of polymers showed gradual decrease with the progression of the study (Figure 13B).

Discussion

Both studies described in this article utilized PLCL, an FDA-approved polymeric material that has been
<table>
<thead>
<tr>
<th></th>
<th>Sham operation (control)</th>
<th>Test device explanted</th>
<th>Test device present</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>−500 −250 Suture site +250 +500</td>
<td>−500 −250 Suture site +250 +500</td>
<td>−500 −250 Suture site +250 +500</td>
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<tr>
<td><strong>Histology section distance from suture line in micrometer</strong></td>
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<td><strong>12-week postimplantation</strong></td>
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<td><strong>26-week postimplantation</strong></td>
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<tr>
<td><strong>52-week postimplantation</strong></td>
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<td></td>
<td></td>
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<tr>
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<td>0.2 (1/6)</td>
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<tr>
<td>Presence of macrophages</td>
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<td>0.0 (0/6)</td>
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<td>0.0 (0/6)</td>
<td>0.0 (0/6)</td>
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<td>the cavity of implantation—device fragmentation)</td>
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</table>

**Note:** NA = not available.

*Five histology sections taken at 250-μm intervals from each test or control site using the suture site as the middle section.

*Mean severity scores of lesions based on the following: 0 = normal, 1 = minimal, 2 = mild, 3 = moderate, and 4 = severe.

*Number of affected sites/number of examined sites.
successfully used in a large number of medical applications (Burks et al. 2006; Jeong et al. 2004; Cho et al. 2008). Therefore, it is known that this copolymer is not pyrogenic, is without in vitro and in vivo genotoxicity, and is free of leachable materials that might be carcinogenic.

The PLCL is a fully biodegradable polymer, degrading into its starting components, lactic acid and caprilic acid. It is later secreted from the body as hydroxy acids or is naturally metabolized into CO₂ and water. According to Woodruff and Hutmacher (2010), PLCL degradation is a 2-step process: first,

**Table 5.** Histopathological findings in rats in the second study after bilateral subcutaneous implantation of a poly-\(\alpha\)-L-lactide and epsilon-caprolactone copolymer (downsized InSpace™ device) in the dorsolumbar area.

<table>
<thead>
<tr>
<th>Sacrifice interval</th>
<th>4 Weeks ((n = 3)^{a})</th>
<th>12 Weeks ((n = 4))</th>
<th>16 Weeks ((n = 2))</th>
<th>26 Weeks ((n = 5))</th>
<th>52 Weeks ((n = 8))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrosis/Fibrous capsule</td>
<td>3.0 (^{b}) (3/3) (^{c})</td>
<td>2.0 (4/4)</td>
<td>2.0 (2/2)</td>
<td>2.0 (5/5)</td>
<td>2.0 (6/8)</td>
</tr>
<tr>
<td>Inflammation within the capsule (lymphocytes)</td>
<td>1.3 (3/3)</td>
<td>0.3 (1/4)</td>
<td>1.5 (2/2)</td>
<td>0.0 (0/5)</td>
<td>0.0 (0/8)</td>
</tr>
<tr>
<td>Histiocytes</td>
<td>1.0 (3/3)</td>
<td>0.0 (0/4)</td>
<td>1.5 (2/2)</td>
<td>0.4 (2/5)</td>
<td>1.0 (6/8)</td>
</tr>
<tr>
<td>Test device debris and fragmentation</td>
<td>0.0 (0/3)</td>
<td>0.0 (0/4)</td>
<td>0.0 (0/2)</td>
<td>1.6 (4/5)</td>
<td>2.0 (8/8)</td>
</tr>
</tbody>
</table>

*Number of sites examined.

**Figure 1.** Macroscopic appearance of a test device (implant) removed from rats at various time points following subcutaneous implantation (second study). (A) Implant removed from a rat sacrificed at week 4 postimplantation. (B) Implant removed from a rat sacrificed at week 12 after implantation. (C) Implant removed from a rat sacrificed at week 16 after implantation. (D) Macroscopic appearance of host fibrotic capsular reaction around the implant fragments (left) and test device fragments (right) extracted from the capsular reaction from a rat sacrificed at 26 weeks postimplantation. The test device in A, B, and C still contains saline and its size is \(20 \times 30\) mm.
Figure 2. Animal sacrificed 4 weeks postimplantation. A central cavity (asterisk) is the site of implantation of a removed downsized test device in the dorsolumbar area. A mature fibrous capsule surrounds the central cavity with minimal sporadic presence of mononuclear cells (arrows) between fibroblasts and collagen deposition. Hematoxylin and eosin (H&E) stain. Figure 3.—Animal sacrificed 12 weeks postimplantation. A mature fibrotic capsule without an accompanying inflammatory reaction in the dorsolumbar area surrounds the cavity (asterisk) from a removed downsized test device. Hematoxylin and eosin (H&E) stain. Figure 4.—Animal sacrificed 26 weeks postimplantation. Site of implantation of the downsized test device in the dorsolumbar area. A large cavity (asterisk) is the original site of implantation. Remaining fragmented pieces of the implanted device (arrows) are each surrounded by separate fibrotic capsules. Hematoxylin and eosin (H&E) stain. Figure 5.—Animal sacrificed 26 weeks postimplantation. This higher magnification of an implantation site contains a fragmented piece of the downsized test device (asterisk) in a cavity surrounded by a mature fibrotic capsule. A discontinuous single layer of macrophages (arrow) and multinucleated giant cells (arrowheads) are present at the interface of the test device location and the fibrotic capsule. Hematoxylin and eosin (H&E) stain. Figure 6.—Animal sacrificed 52 weeks postimplantation. Low magnification view of the site of implantation from the dorsolumbar area showing microscopic fragments of the biodegradable test device encapsulated by mature fibrous connective tissue (arrows). Hematoxylin and eosin (H&E) stain.
Figure 7. Animal sacrificed 52 weeks postimplantation. Higher magnification view of the dorsolumbar site of implantation (shown in Figure 6) with microscopic fragments of test device embedded in a matrix of mature fibrous connective tissue. Hematoxylin and eosin (H&E) stain. Figure 8.—Animal sacrificed 52 weeks postimplantation. Higher magnification of Figure 7 showing fragments of the implanted device surrounded by a mature fibrotic response. Hematoxylin and eosin (H&E) stain. Figure 9.—Animal sacrificed 52 weeks postimplantation. High magnification showing the host reaction to fragments of test device material (asterisks). A discontinuous layer of macrophages (arrow) is at the interface of the fibrotic capsule and test device location. A giant cell (arrowhead) is adjacent to a test device fragment. Hematoxylin and eosin (H&E) stain. Figure 10.—Animal sacrificed 52 weeks postimplantation. Site of implantation with fragments of the implanted test device (asterisks) surrounded by mature connective tissue on the right and fibrosarcoma (arrows) on the left. The fibrosarcoma cells are more densely cellular than the mature connective tissue.
nonenzymatic hydrolytic cleavage of ester groups; and second, when the polymer has a lower molecular weight and is more highly crystalline, it is subject to intracellular degradation by macrophages, giant cells, and fibroblasts.

With the exception of a single fibrosarcoma, the tissue responses in both rat studies reported here had an expected favorable host response to the test device implants. The test device was identified at all time points up to 52 weeks. The implantation sites were histologically characterized by cavity formation surrounded by a mature fibrotic capsule without evidence of any adverse acute or chronic active inflammation. The intact as well as fragmented device was associated with a single layer of macrophages with occasional giant cells at the interface of the copolymer and the fibrotic capsule with presence of lymphocytes and rare polymorphonuclear cells within the fibrotic capsule. This represents a well-ordered foreign body reaction that would ultimately lead to complete removal of the implanted copolymer material. Copolymer fragmentation was present at 26 weeks with subsequent progression to very small fragments (<1 mm), associated with mass and molecular weight loss of isolated implants in rats at 52 weeks.

In a single animal at the 52-week termination point, a pleomorphic fibrosarcoma was noted at the site of implantation. It was grossly and histologically intimately associated with the implant and was therefore considered related to treatment. However, there is vast literature documenting the association between the long-term close contact between inert materials and the induction of sarcoma in rodents. For example, sarcomas were reported to develop at sites of plastic film implantation (unplasticized vinyl chloride vinyl acetate copolymer of different sizes and shapes; Brand, Buoen, and Brand 1976) or after implantation of radiotelemetric transmitters (Shoieb et al. 2012). A long-term subcutaneous and intramuscular implantation experiment (up to 106 weeks) in 70 rats, using poly-L-lactide (PLLA), the principal (70%) component of the current test device, resulted in the development of 3 cases of fibrosarcoma located close to the implanted material (Pistner et al. 1993). According to the authors, this phenomenon of tumors occurring in rodents following long-term implantation is related to the “Oppenheimer phenomenon” or “solid state” carcinogenesis correlated with the induction of sarcomas in rodent animal models implanted with solid materials (i.e., inert plastics, metals, and other materials), even though the materials themselves have no inherent toxic or tumorigenic properties (Kirkpatrick et al. 2000; Schoen 2013).

A thorough histopathological evaluation was performed on all tissues extracted from the implantation sites in the 2 studies described by us. This investigation included all capsular tissues involving the implanted material. It was confirmed that no foci indicative of mesenchymal cell hyperplasia, suggestive of preneoplasia, were detected. Furthermore, histopathology evaluation of

Figure 13. (A) Dry weight measurements of the explanted test devices. Mean mg ± standard error of the mean (SEM), n = 5 for 4 and 26 weeks, n = 8 for 12 weeks, and n = 2 for 16 weeks. ***p < .001. (B) Molecular weight measurements of the explanted test devices. Mean Daltons ± SEM, n = 6 for 4 weeks, n = 8 for 12 weeks, n = 2 for 16 weeks, n = 11 for 52 weeks. **p < .01, ***p < .001. one-way analysis of variance, Bonferroni’s posttest.

Figure 10.—(Continued) tissue on the right. Hematoxylin and eosin (H&E) stain. Figure 11.—Animal sacrificed 52 weeks postimplantation. Higher magnification of Figure 10. A storiform growth pattern (arrows) is present in the fibrosarcoma adjacent to small device fragments (asterisks). Hematoxylin and eosin (H&E) stain. Figure 12.—Animal sacrificed 52 weeks postimplantation. Same site presented in Figures 10 and 11 showing pleomorphic round and spindle cells (arrows) present in the fibrosarcoma adjacent to a device fragment (asterisk). Hematoxylin and eosin (H&E) stain.
implantation sites from other animal studies with the same device material performed in pigs, dogs, and rabbits (data not shown), with up to 1-year follow-up, indicated the formation of capsular reaction with minimal inflammatory cell infiltration, without any local mesenchymal cell proliferation suggestive of preneoplasia.

The histopathology evaluation also showed that there was a time-related effect on the loss of integrity of the implanted devices, with their being broken into more pieces and surrounded by a relatively more intensive mononuclear cell reaction at the 52-week than at the 26-week time point. The mononuclear cell reaction is the expected body response to the presence of biodegradable material and indicative of progressive absorption of the test device material (PLCL; Nyska et al. 2014).

In this regard, it is relevant to mention the pivotal work of Kirkpatrick et al. (2000), in which histopathological evaluation was performed on rats up to 2 years postimplantation of biomaterials (i.e., polymers, metals, and ceramic). The authors reported an approximately 26% incidence of various types of mesenchymal tumors as well as the presence of a spectrum of changes defined as “focal proliferative lesions through pre-neoplastic proliferation to incipient sarcoma.” The authors concluded that it is unlikely to implicate chemical sarcomagenesis as the prime etiological factor, due to the vastly different structures involved, each having entirely different chemical composition. In addition, it is improbable that leachable residual monomers from the polymer synthesis and/or compounds used in the plasticizing process or other leachables could play a pathogenic role, due to the standard medical grades of the materials used in this investigation.

On the other hand, there are several studies reporting the clinical and histopathology safety aspects of PLCL PLLA. None of these studies identified local preneoplastic or neoplastic reactions at the sites of implantation in humans. Pistor et al. (1993) reported that the only local long-term (i.e., 2 to 3 years of follow-up) reaction observed in humans implanted with crystalline block polylactide consisted of an “intense resorptive histiocytic reaction,” but without sarcoma development. McCarty et al. (2013) reported on a large series of patients with complications observed following utilization of PLLA implants to treat either labral or RC pathology. The follow-up time was from 2 to 106 months after implantation. Lesions included giant cell reaction in 84%, presence of polarizing crystalline material in 100%, papillary synovitis in 79%, and arthroscopically documented outerbridge grade III or IV chondral damage in 70%. No local tumor induction was noted.

Freehill et al. (2003) conducted a retrospective cohort study to investigate the complications related to the use of PLLA implants for arthroscopic shoulder stabilization procedures. The follow-up period ranged from 8 to 33 months. Synovial biopsies showed giant cells and histiocytes typical of a foreign-body reaction. Typically, also in this study, the crystalline structures, remnants of the implanted material, were surrounded by a granulomatous reaction. No carcinogenic findings were mentioned or noted in this study.

The exact pathogenesis of local foreign body carcinogenesis is still not entirely clear. A potential mechanism could involve long-term local minor inflammation. It is postulated that local inflammation, even transient, could lead to the release of growth factors and reactive oxygen species from the inflammatory cells, followed by expansion of stem cell pools and by the transient activation of the Hh and Wnt signaling pathways. This activation increases the likelihood of accumulation of multiple additional mutations, and eventually to tumor formation (Gottschling et al. 2001; Shoieb et al. 2012; Upham and Wagner 2001). According to Amini, Wallace, and Nukavarapu (2011), there is increasing evidence that the cause of the sarcoma formation observed at the site of implantation of biodegradable polymers is not due to the chemical contents but rather due to the implant’s form and size, length of the implantation, and degree of inflammation.

The fact that local carcinogenesis was detected in only a single rat implanted with the test device, without the presence of any preneoplastic changes (i.e., hyperplasia) in other animals from these experiments, as well as the fact that the manufacturing process is free of harmful reagents that may be carcino- genic, it is reasonable to suggest that a nongenotoxic mechanism was involved in the pathogenesis of the fibrosarcoma, and eventually, a threshold for human risk may be suggested. In this regard, Grasso, Sharratt, and Cohen (1991) extensively reviewed the potential link between sustained tissue damage induced by nongenotoxic test materials/agents in rodents, as a predisposing factor to tumor development. Their analysis highlights the fact that at various sites (i.e., connective tissue, liver, bladder, and forestomach), there is a threshold dose, below which neither sustained tissue damage nor tumor induction occurs, but above this level both effects are manifested.

In general, rodents are much more prone than humans to develop tumors of mesodermal origin, such as sarcomas, in reaction to foreign body implantation. This might stem from the fact that murine pericytes, the cells that serve as the origin for the foreign body sarcomas, have an exceptionally labile genome (Brand 1987). Therefore, when these cells are forced into proliferation, they are much more likely to develop spontaneous genome errors.

Conclusions
With respect to the tissue response in the 2 rat studies, it can be concluded that the PLCL test compound did not have irritant properties upon long-term exposure and was favorably tolerated without foreign body granulomatous reaction, mineralization, necrosis, or immune-mediated reactions indicative of an adverse effect (Northup 1999; Nyska et al. 2014; Onuki et al. 2008; Ramot et al. 2015; Schuh 2008). There was a well-ordered host response to the implant material with temporal degradation of the copolymer over the 52-week follow-up period.

Given the lack of significant local irritation/inflammation and lack of preneoplastic change in any of the rats implanted with the test device for 1 year, we conclude that the single case of fibrosarcoma reflects the known species-specific rodent
predilection to develop rare mesenchymal neoplasms at implantation sites (Greaves et al. 2013; Schoen 2013). We conclude that the risk of implantation-associated cancer in humans implanted with the InSpace device, which is composed of the biodegradable PLLA acid and epsilon-caprolactone copolymer, is negligible.

**Author Contribution**

Authors contributed to conception or design (AN, EM, AD); data acquisition, analysis, or interpretation (YR, AN, EM, AD, AK, MZ, AJD, RM); drafting the manuscript (YR, AN, EM, RM); and critically revising the manuscript (YR, AN, EM, AD, AK, MZ, AJD, RM). All authors gave final approval and agreed to be accountable for all aspects of work in ensuring that questions relating to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

**Declaration of Conflicting Interests**

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: Abraham Nyska and Robert R. Maronpot serve as consultants for Ortho-Space Ltd. Yuval Ramot has received an honorarium from Ortho-Space Ltd. Elana Markovitz and Assaf Dekel are Ortho-Space’s Directors. Abraham J. Domb is an inventor of the biodegradable balloon technology and has been associated with Ortho-Space. His contribution to this research was not supported and was for scientific interest only.

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**Supplemental Material**

The online data supplements are available at http://tpx.sagepub.com/supplemental.

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