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Chemical characterization and degradation behavior of a polylactide surgical implant for the fixation of osteochondral fragments after activation with ultrasound energy

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ABSTRACT

The purpose of this study was to demonstrate that the ultrasonic energy used to melt the tip of an absorbable polylactide Pin (SonicPin) does not have a significant effect on the chemical characterization and associated degradation properties of the material. The results indicate that the polylactide Pin after ultrasound is chemically equivalent to the SonicPin prior to ultrasound with regard to chemical structure and risk of leachable compounds. The evidence gathered from literature and testing suggests that any potential toxicological risk to the patient is not amplified by the application of ultrasonic energy to melt the tip of the device.

KEYWORDS

SonicPin;
Biocompatibility;
Polylactide;
Osteochondral fragment;
Ultrasound.

INTRODUCTION

Biodegradable implants are increasingly used in the field of operative sports medicine. Today, a variety of implants such as interference screws, staples, sutures, tacks, suture anchors, and devices for meniscal repair are available. These implants consist of different biodegradable polymers that have substantially different raw material characteristics such as in vivo degradation, host-tissue response, and osseous replacement[1].

The clinical suitability of applying resorbable pins with an ultrasound welding procedure has, among other things, already been confirmed in independent in vitro and in experimental studies in animals and they thus represent a further innovative development of conventional surgical techniques. Here, for example, one should refer to the publications of Langhoff et al.[2], Mai et al.[3] and Pilling et al.[4,5], who all base their findings on positive animal studies in sheep, which were carried out with the already cited and directly comparable pins from KLS Martin. In all these cases a biomechanically stable internal fixation, without complications, and resorption of the implant material without artifacts could be demonstrated. Changes in the material caused by sonochemical processes could not be demonstrated.

The SonicPin™ (Stryker, Schoenkirchen, Germany)

is a newly developed absorbable pin which contains of 99.96% Poly (L-lactide-co-D, L-lactide) at a molar ratio of approximately 70:30 (L, lactide:D, L-Iactide). The remaining 0.04% of the SonicPin is comprised of a blue colorant (D&C Blue No.6). The required (bio)mechanical strength (especially the flexural strength and the tensile strength, with ~97 MPa and ~68 MPa, respectively, for sterilized samples) of the polymer during the primary healing phase in situ, of up to 6 weeks, which for example in vitro shows a marked reduction only after about 180 days, is not significantly affected by its bioresorption properties. In these cases the half-life is about 290 days. As a result of hydrolysis, within 480 days the molecular weight or the inherent viscosity falls from about 1.5 to 0.3 dl/g. Claes et al.[6], with the Polypin® from Synthes Co., Bern, Switzerland and Moser et al.[7], with a resorbable plate-and-screw system, both from the same polymer material, arrive at a comparable result.

After implantation into bone, the SonicPin is completely resorbed by the body within 2-3 years by the natural physiological process of hydrolysis, ultimately resulting in the degradation products of H_20 and CO_2. The SonicPin™ is approximately 2.2mm in diameter, and comes in lengths of 22mm and 26mm. The SonicPin is applied with the SonicDriver Handpiece to apply a pulse of ultrasonic energy that melt down 8mm of the SonicPin within 6 seconds as the implant is placed within a pre-drilled hole in bone. The ultrasonic energy causes melting and liquification of the tip of the SonicPin™. The liquefied tip becomes integrated into the surrounding porous structure of the existing bone, resulting in improved implant fixation and minimizing the occurrence of implant loosening. This special mode of application corresponds, in principle, to the ultrasound welding procedure already known from the plastics processing and seaming technology[8,9]. This study provides an equivalency assessment of polylactide resins used to manufacture a bioabsorbable bone implant called the SonicPin. The purpose was to investigate, if the ultrasonic energy used to melt the tip of the SonicPin device does not have a significant effect on the chemical characterization and associated degradation properties of the material of manufacture. Thus, the biocompatibility aspects of the bioabsorbable SonicPin were also expected to remain unchanged.

MATERIALS AND METHODS

Samples of the larger size 26mm SonicPin (before and after ultrasound application) were used for comparative chemical characterization testing. Each sample was subjected to a variety of tests to evaluate the amount and characteristics of extractables using methodology prescribed in the United States Pharmacopeia (USP) for the Chemical Characterization of Materials. Tests were realized at NAMSA, Northwood, OH, USA and financially supported by Stryker Osteosynthesis. Total extractables for Non-Volatile Residue were evaluated by the USP Physicochemical test method using purified water, methanol, and hexane solvents extracted for 24 hours at a minimum of 50°C. Materials that will contact body tissues are typically extracted in both polar and nonpolar environments to capture all the potential chemical compounds that could possibly leach into the patient upon exposure to the device[10]. The USP also describes the use of infrared spectroscopy to identify and fingerprint the extract and the implementation of Liquid chromatography-mass spectrometry for analysis of semi-volatile compounds present. ISO 10993-18 describes the use of chemical characterization to establish equivalence of materials and establishes the precedence for this type of testing. The tests performed on each manufacturing lot were as follows:

1. USP Physicochemical Test - Plastics <661>, Aqueous and Non-Aqueous Extraction in Purified Water (PW), Methanol (MeOH), and Hexane (HX) for Total Non-Volatile Residue
2. USP Physical Test - Chapter <736>, Infrared Analysis using Fourier transform spectroscopy (FTIR) of each extract
3. USP Physical Test - Chapter <851>, Trace Metal Analysis using Inductively Coupled Plasma Spectroscopy and the PW extract
4. USP Physical Test - Chapter <736>, Mass Spectrometry: Gas Chromatography using the PW, MeOH, and HX extracts for analysis of semi-volatile compounds
5. USP Physical Test - Chapter <736>, Mass Spectrometry: High Performance Liquid Chromatography using the PW, MeOH, and HX extracts for analysis of non-volatile organic compounds

To proof biocompatibility it is necessary to obtain infor-

mation to demonstrate the extent to which the constituents of the device material will be available under the actual intended use of the device. This is commonly determined through extraction tests on the device using appropriate extraction conditions (time/temperature/media) to ensure that any potential hazardous substances released during use of the finished device will be released into the extraction media. The extract residue can then be analyzed qualitatively and/or quantitatively to generate data used in the determination of material equivalence and biological safety. Although the SonicPin is absorbed slowly by the physiological processes of the body, it is still considered a permanent implant because the device has direct contact with tissue/bone for a period exceeding 30 days. Therefore, in order to simulate the permanent implantation period, worst-case exhaustive extraction of the SonicPin was carried out using each of the solvents. The exhaustive extraction process involves repeated extraction of the same test article until the amount of residues in a subsequent extraction is less than 10% of that detected in the initial extraction. The Non-Volatile Residue (NVR) is calculated gravimetrically at the end of each extraction by evaporating away the solvent and weighing the residue. The total extractables (NVR) represents all the organic and inorganic (elemental) substances that can migrate from the SonicPin device into the solvent under the controlled temperature and time parameters (70°C when using PW, 50°C used for MeOH and HX). A representative portion of the test material was extracted by exhaustive methods in each of the three solvents at a ratio of 4 grams: 20 ml. The average weight of a 26mm SonicPin is 120mg (0.12 grams). So although the sample quantity extracted in methanol was only 1.4 grams, this weight is equivalent to nearly 12 SonicPin devices, far more than the number of devices that could be expected to be implanted in a single patient.

TABLE 1 : Changes of non-volatile residue before and after ultrasound after exhaustive extraction

Test Article	Extraction Solvent	Change of quantity (before/after ultrasound)	Change of Extraction Volume	Change of Totale Non-Volatile Residue
26mm SonicPin	Purified Water	-2,6%	-2,6%	-15,4%
26mm SonicPin	Methanol	+/-0%	+/-0%	-5,4%
26mm SonicPin	Hexane	-2,6%	-5,5%	-0,0%

RESULTS

As the table of extracted residues demonstrates, the total extractables (NVR) of the SonicPin material before and after ultrasound melting remained on a similar level. The NVR change after water extraction of the two samples was the highest with a reduction of 15,4%. However the absolute residual amounts were more than 10 fold below the guidelines established by the USP (~ 15 mg) for water extraction of polymer-based devices. In polymeric materials like the crosslinked Polylactide used to manufacture the SonicPin, Methanol and Hexane are typically used to evaluate lipophilic extractable compounds. Here no limits exist for NVR using Methanol or Hexane as the extraction medium. But the total extracted residue obtained from the two different SonicPin devices (before and after ultrasonic application) were nearly equivalent in Methanol and in Hexane. Thus from a qualitative assessment after exhaustive extraction was carried out in 3 different solvents, the ultrasonic energy applied by the SonicFusion handpiece has no significant effect on the degradation properties of the SonicPin and its resistance to physiological fluids encountered in the body. The test extracts will be further evaluated via gas chromatography and mass spectrometry (GC/MS) and liquid chromatography and mass spectrometry (LC/MS) to characterize the configuration and quantities of non-volatile and semi-volatile compounds evident to leach from the bioabsorbable SonicPin material. A portion of each SonicPins extract (water, methanol, and hexane) was dried and the resulting residue analyzed by infrared spectroscopy. This sensitive analytical method is used to identify the major structural components of a given chemical molecule, demonstrated by absorbance peaks of the recorded spectrum. Small differences in the structure and constitution of a molecule can result in significant changes in the distribution and height of absorbance peaks in the fingerprint region of the resultant spectrum[11]. Using this test method, IR (infrared) energy is passed through a thin film of material (or extract) and the amount of energy absorbed at various wavelengths is measured. The re-

sultant infrared scan charts wavelength peaks versus absorption that is characteristic of a chemical structure, typically matched from a spectrum library of known materials to aid the identification process. Based on the FTIR database of infrared spectra of various compounds, the major bands at the wavelengths identified in the majority of the spectrums most closely matched the reference scan for polylactide. Polylactide (PLA) is an aliphatic polyester that makes up the backbone of the RESOMER LR 706S resin used to fabricate the SonicPin, thus this correlation of the spectral scan is expected. Direct comparison of the SonicPin methanol extractions (pre- and post-ultrasonic application) show that the scans were nearly identical, with each spectrum exhibiting peak maxima at approximately 1750, 1200, and 1100 cm^{-1}. The methanol extractions were considered for comparative evaluation since the quantities of non-volatile residues were much higher than the NVRs obtained after extraction in water or hexane. Based on the infrared spectral data, the extracted residues derived from each SonicPin configuration in methanol are considered to be chemically equivalent. No unknown bands of any significance were noted in the spectra of the material samples. This provides additional evidence that no hazardous derivatives or other degradation compounds are readily apparent after processing of the RESOMER LR 706S into SonicPin devices, further limiting the potential for toxicological risk to the patient. A small portion of each purified water extract (from the NVR assay) was analyzed using Inductively Coupled Plasma spectroscopy. A scan for 27 trace metallic elements in the extract was conducted, with an instrument detection limit of 0.05 ppm (μg/ml). The results found trace amounts of Boron, Aluminum, Barium, Calcium, and Phosphorus in the SonicPin water extractions, however no quantity exceeded 2.3 ppm, thus these findings are not significant from a toxicological perspective. Each of these elements is naturally occurring in the human body or widely exposed through daily water and dietary intake. Since the SonicPin is manufactured from the organic polylactide molecule (containing only carbon, oxygen, and hydrogen elements), the presence of trace metallic elements in the water extractions is most likely attributed to leachable substances from the borosilicate glassware used for the test or crosscontamination impurities in the instrumentation from previous test samples.

TABLE 2 : ICP spectroscopy results

Test Article	Extraction Solvent	Elements Detected	Concentration (ppm)
26mm SonicPin (control)	Purified Water	Boron	2.3
		Phosphorus	1.8
26mm SonicPin (post-ultrasonic application)	Purified Water	Boron	0.51
		Aluminum	0.24
		Barium	0.36
		Calcium	0.36

Materials that come into contact with tissue/bone, like the RESOMER LR 706 S used to manufacture the SonicPin implant, fall into the critical category requiring analytical methods to identify and quantify any significant chemical compounds extracted in solvents intended to mimic typical bodily fluids. Chromatography coupled with mass spectroscopy produces qualitative, fingerprint-like information that can be characterized down to specific molecular structures and quantities based on appropriate standards. A small portion of each extract from the two SonicPin configurations under study was

TABLE 3 : GC/MS analysis of the SonicPin test extracts

Test Article	Extract	Tentatively Identified Compound	Molecular Weight	Estimated Concentration
26mm SonicPin (control)	H_2O	No semi-volatile compounds found	N/A	N/A
		2-methypropyl-hydrazine	88	10.56 ppm
26mm SonicPin (control)	MeOH	3,6-dimethyl-1,4-dioxane-2,5-dione	144	5.54 ppm
		Various organic branches of compounds	N/A	4.05-15.67 ppm
26mm SonicPin (control)	Hexane	No semi-volatile compounds found	N/A	N/A
26mm SonicPin after ultrasound	H_2O	No semi-volatile compounds found	N/A	N/A
		2-methypropyl-hydrazine	88	12.21 ppm
26mm SonicPin after ultrasound	MeOH	3,6-dimethyl-1,4-dioxane-2,5-dione	144	4.15 ppm
		Various organic branches of compounds	N/A	3.74-17.80 ppm
26mm SonicPin after ultrasound	Hexane	No semi-volatile compounds found	N/A	N/A

analyzed in triplicate for the presence of semi-volatile organic compounds using GC/MS instrumentation. A preparation of phenanthrene was analyzed similarly at three concentrations of 2, 25 and 50 ppm to establish a concentration curve for reference against any unknown identified compounds.

As the results in the preceding table indicate, the quantities and types of semi-volatile compounds (if any) identified by GC/MS were essentially equivalent between the SonicPin extract samples tested before/after application of ultrasonic energy from the SonicFusion handpiece. The GC/MS analysis detected no semi-volatile compounds in the purified water or hexane extracts with a concentration greater than the minimum detection limit of 2 ppm. Evaluation of the methanol extract however produced eight (8) compounds or functional organic branches, with an average estimated concentration ranging from 4 - 18 ppm. The compounds identified in the methanol extractions of the SonicPin appear to be side branches or other fragments which have broken from the original polylactide chain that makes up the RESOMER material. This degradation of the polymer into smaller fragments is similar to the normal slow hydrolysis of the polylactide that occurs in the body as the SonicPin implant device is absorbed by the bony tissues.

The 3,6-dimethyl-l,4-dioxane-2,5-dione compound ($C_6H_8O_4$, m.w. of 144) identified by GC/MS is simply the formal chemical name for the D, L-Iactide molecule. Recall that the RESOMER LR 706 S polymer is essentially a long chain of repeating lactide molecules. Therefore, it is certainly plausible that extraction in an aggressive solvent like methanol would cause one of these lactide molecules to separate and be identified as a degradation compound. The 2-methylpropyl-hydrazine compound ($C_4H_{12}N_2$, m.w. of 88) identified by the GC/MS software is likely inaccurate, since there are no nitrogen atoms available in the polylactide RESOMER material or in methanol to produce this particular compound. The compound is more likely a lactide fragment ($C_3H_4O_3$, m.w. of 88) that has been separated from the polylactide chain, similar to the other unidentified organic compounds. Thus, there is no toxicological concern for small organic compounds comprised of oxygen and carbon atoms since they are efficiently removed by the body through normal physiological processes.

The PW, MeOH, and HX extractions were next characterized by high-performance liquid chromatography (HPLC). HPLC is a very sensitive analytical method used to separate and quantitate non-volatile or thermally fragile compounds. Liquid chromatography can typically capture more than 80% of all known organic compounds that could elute from polymeric materials. Stock solutions of three standards (Tinuvin P, BHT, and Irganox 1010) were prepared and diluted to concentrations of 1, 5, and 10 ppm to establish a concentration curve for reference against any unknown identified compounds.

TABLE 4 : Changes before and after ultrasound application

Extract	Retention Time	Estimated Concentration change	Molecular Ions
H_2O	1.3 min	-22%	82, 144, 179, 197, 216, 328
	1.6 min	-17%	Not detected
Methanol	1.4 min.	-23%	99, 104, 145, 164, 193, 265, 323
Hexane	N/A	N/A	No Compounts

The LCIMS analysis did not detect any significant levels of BHT, Tinuvin P or Irganox 1010 greater than 2 ppm in any of the SonicPin extracts. Although a few additional peaks were observed in the chromatograms of the purified water extracts, no compounds of any significance were detected (maximum of 3.6 ppm) so that the changes before/after ultrasound also do not contain informative value. Judging by the molecular ion data, the compound observed at 1.3 minutes in the water extract is likely just fragments of the polylactide material that is used to manufacture the SonicPin. Likewise, the hexane test extracts of both SonicPin configurations produced no significant degradation compounds, which correlates well to the NVR data for hexane. The spectrometric data for the methanol extracts also produced similar data between the two samples. A compound of moderate concentration was observed at a retention time of 1.4 minutes in each sample extract. Although the compound cannot be readily identified solely from the molecular ion data, the analytical test methods previously discussed (NVR and GC/MS) have confirmed that the RESOMER polylactide is slightly soluble in methanol and produces residual quantities of

lactide compounds and related fragments. Therefore, based on the combined spectroscopic data, the compound or compounds at 1.4 minutes in the methanol extracts are likely just inert polylactide degradation compounds. Regardless of the actual chemical structure of these compounds, the low concentrations noted in the chromatogram (1.5 - 15.1 ppm), along with the likely compositional makeup of the compounds (oxygen, carbon, and hydrogen are elements indigenous to the human body), result in a minimal to non-existent likelihood that the non-volatile molecules identified in the SonicPin extracts could elicit any sort of toxic effect to prospective patients during use. The degradation compounds detected in each extract solvent were consistent between the pre- and post-ultrasonic test samples, based on the retention times and molecular ion data. This indicates that the application of ultrasonic energy from the SonicFusion handpiece to the SonicPin does not alter the anticipated degradation characteristics of the RESOMER LR 706S polylactide material and therefore has no effect on the bioabsorbable properties of the device after implantation.

DISCUSSION

According to ISO-14971 consensus standard, in order to estimate toxicological risks associated with a medical device or its materials of manufacture, four major factors should be taken into account[12].

- The physical and chemical characteristics of the device materials
- Historical clinical use or human exposure data involving the device materials
- Biological safety data or toxicology on the device or component materials
- Integrity and diligence of test methods conducted on device materials

Evaluation of the chemical nature of the materials can take the form of experimental data and/or information on the chemical inertness of the materials involved. Information characterizing the chemical identity and biological response of materials is useful in assessing a medical device for its intended use in the patient. Factors which affect the biocompatibility of the materials include the identity, concentration, availability and toxicity of all constituents such as additives or processing fluids. Evaluation of substances that are determined to leach from device materials in test extracts can provide valuable information about the amount and classification of the chemicals that could potentially be exposed to a patient from a permanent implant. Chemical characterization establishes a baseline fingerprint of the material in simulated *in vivo* conditions and is often an excellent forecasting tool for device biocompatibility. The amount of data required on the device materials and the depth of investigation is dependent upon the intended use of the device and duration of patient contact. The expected long-term exposure (approximately 2-3 years) of the Stryker Osteosynthesis SonicPin to the bone and surrounding tissue is a significant factor when assessing the overall toxicological risk of the device. A review of ISO 10993-1 outlined the biocompatibility test requirements for the SonicPin device and the principles governing the biological evaluation of medical devices, in particular the non-clinical testing. The slow degradation of the implant categorizes the device as a permanent tissue/bone contacting device. Based on these criteria of expected use and guidance provided by ISO 10993-1, the following biocompatibility testing is recommended: Cytotoxicity, intracutaneous irritation, sensitization, implantation effects, acute/subchronic/chronic systemic toxicity, and genotoxicity. However, additional guidance in the ISO 10993-1 standard allows for the principles of risk assessment to be applied to medical devices in lieu of biocompatibility testing. Clause 3.5 of this ISO standard states:

> *All potential biological hazards should be considered for every material and final product, but this does not imply that testing for all potential hazards will be necessary or practical.* Clause 6 of ISO 10993-1 specifies: *The evaluation may include both a study of relevant experience and actual testing. This evaluation may result in the conclusion that no testing is needed if the material has demonstrable history of (clinical) use in a specified role that is equivalent to that of the device proposed.*

The biocompatibility of the SonicPin has been assessed internally by Stryker Osteosynthesis using a risk assessment methodology that evaluated the biological safety of the component materials (RESOMER LR 706S Poly(L-lactide-co-D, L-Iactide) 70:30 and D&C

Blue No.6 color additive - Indigo Blue). As previously discussed, the SonicPin consists of about 99.96% polylactide resin supplied by Boehringer Ingelheim under the tradename RESOMER® LR 706S. Boehringer Ingelheim has performed extensive pre-clinical biocompatibility testing that meets the rigorous requirements of ISO 10993-1. The remaining 0.04% of the SonicPin is comprised of a blue colorant, D&C Blue No. 6. This color additive is cleared under 21 CFR 74 subpart D, sec. 3106 for use as an additive in absorbable medical devices like the SonicPin up to an amount of 0.25% (w/w). Stryker Osteosynthesis has also determined that compounding the SonicPin with D&C blue color additives has no impact on the biocompatibility or mechanical integrity of the finished device. The SonicPin was tested for cytotoxicity according to the elution method of ISO 10993-5 and showed no evidence of cell lysis or cellular toxicity (grade 0). An implantation study in rabbits evaluated the histological local tissue reaction of SonicPins implanted using the SonicFusion ultrasound insertion technique which effectively melts the tip of the device during implantation. Twenty-eight (28) animals were employed, with the sites examined microscopically for signs of inflammation or other adverse tissue reaction after 5 days and 4 weeks. The study concluded that the SonicFusion technique used to implant the resorbable pins into the rabbit bone demonstrated an acceptable localized tissue reaction after 5 days and 4 weeks. The ultrasonic energy applied to the SonicPin to melt the tip of the device during the surgical procedure had no adverse effect on the bone or tissue at the implantation site[13]. The SonicPin would be expected to be clinically safe and provide benefit for the treatment and fixation of bone fractures, osteotomies, or bone grafts.

CONCLUSION

The leachable data after extraction of the SonicPin (before and after ultrasonic application) in 3 different solvents revealed changes of the total extractables (NR) of -15,4% in purified water, - 5,4% in Methanol and no measurable change in Hexane extraction solvent. However, the residue quantities were still below the current USP limit for polymeric materials. Further analysis of the SonicPin extracts by IR, GC/MS, and LC/MS also found very similar leachable substances from the two SonicPin test samples. The infrared spectra identified only polylactide in those extracts that could be analyzed by the instrument, which is consistent with the RESOMER LR 706S polymer used to fabricate the SonicPin. The only degradation compounds identified by GC/MS and LC/MS in the test extracts were polylactide or chemical fragments of polylactide. The degradation compounds detected in each extract solvent were consistent between the pre- and post-ultrasonic test samples, based on the retention times and molecular weight data. There was no indication of the blue colorant or any other foreign substance leaching from the SonicPin samples in any of the test extracts. The analytical data reviewed demonstrate with assurance that the ultrasonic energy used to melt the tip of the SonicPin during the surgical procedure does not alter the anticipated degradation characteristics of the RESOMER LR 706S polylactide material and therefore has no effect on the bioabsorbable properties of the device after implantation. The polylactide material evaluated in this study is essentially unchanged after the application of ultrasonic energy and would be expected to elicit no adverse toxicological effects given the intended use of the SonicPin implant device. The evidence gathered from literature and testing suggests that any potential toxicological risk to the patient is not amplified by the application of ultrasonic energy to melt the tip of the device. This method of risk assessment therefore provides predictive evidence of material safety given the intended use of the SonicPin implant and associated SonicFusion technology.

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