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Analysis of degradation failure of poly L-lactic acid fixators used in meniscus tears

Menisküs yırtıklarının atroskopik tamirinde kullanılan poli L-laktik asit sabitleyicilerin erimeme nedenlerinin araştırılması

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Amaç: Menisküs yırtıklarının tamirinde kullanılan biyobozunur poli L-laktik asit (PLLA) sabitleyicilerden bazıları erimeyip diz içinde birtakım reaksiyonlara neden olabilmektedir. Bu çalışmada PLLA sabitleyicilerin geç erime nedenleri araştırıldı.

Çalışma planı: Çalışmada hiç kullanılmamış üç adet ve diz artroskopisinden sonra altı ay içinde üç hastada semptomların tekrarlaması nedeniyle çıkarılan üç adet PLLA sabitleyici (BioStinger) Fourier transformed infrared (FTIR) spektrometresi ile incelendi. Okların dış ve iç yüzeyleri taramalı elektron mikroskobu (TEM) ve X-ışını fluoroskopisi (XRF) ile incelenip, yüzeylerin fotoğrafları çekildi. İki gruptan birer sabitleyicinin ^{1}H-nükleer manyetik rezonans (^{1}H-NMR) spektrometresi ile yapısal analizi yapıldı. Ayrıca, birer sabitleyici hidrojen peroksit solüsyonu içinde bekletilerek oksidatif hidroliz süreleri kaydedildi.

Sonuçlar: FTIR analizinde sabitleyiciler kimyasal yapı bakımından farklılık göstermedi. Tüm sabitleyiciler sıcaklık artışı ile bozunuma uğradı; ancak yeni sabitleyiciler akma gösterirken, kullanılmış olanlar akma göstermedi. Taramalı elektron mikroskopisinde yeni sabitleyicilerin iç yüzeylerinin kesiti homojen görünümde idi; kullanılmışlarda ise kristaller gözlendi. XRF'de bu kristallerin potasyum ve sodyum tuzları olduğu görüldü. ^{1}H-NMR ile incelenen örneklerin ikisi de normal yapıda laktik asit polimeri idi. Yeni sabitleyici hidrojen peroksit içinde 10 günde, kullanılmış olan 30 günde eridi.

Çıkarımlar: İki gruptaki sabitleştiriciler arasında, FTIR ve NMR incelemelerinde kimyasal yapı bakımından fark bulunmadı. Kullanılmış sabitleyicilerde oluşan tuzlanma erimeme nedenlerinin en önemlisi olarak kabul edilirken, fiziksel özelliklerindeki değişimlerin de erimeyi geciktirdiği düşünüldü.

Anahtar sözcükler: Emilebilir implant; artroskopi; biyouyumlu materyal/yan etki; menisküs, tibial/cerrahi; polyester/metabolizma; protez ve implant/yan etki.

Objectives: Biodegradable poly L-lactic acid (PLLA) fixators used in the repair of meniscal tears may cause adverse reactions inside the knee due to delayed degradation. This study was designed to determine the reasons for late degradation of PLLA fixators.

Methods: Three unused and three used meniscal PLLA fixators (BioStinger) were analyzed. The latter were removed from three patients due to persisting symptoms within six months after knee arthroscopy. Fourier transform infrared (FTIR) spectroscopy was performed and external and internal surfaces of the samples were examined by scanning electron microscopy (SEM) and X-ray fluoroscopy (XRF). Chemical structural analyses of two samples (one from each group) were made by ^{1}H-nuclear magnetic resonance (^{1}H-NMR) spectroscopy. Degradation times of two samples (one from each group) by oxidative hydrolysis in hydrogen peroxide solution were recorded.

Results: Chemical structure of used and unused fixators did not differ in FTIR analysis. With increasing temperatures, unused and used fixators showed degradation with and without melt flow, respectively. In SEM analysis, inner sections of unused fixators were homogeneous, whereas those of the used ones exhibited crystals which were found to be sodium and potassium chloride salts in XRF analysis. The ^{1}H-NMR spectrum of used and unused samples showed the normal pattern of lactic acid polymer. The unused and used fixators degraded in hydrogen peroxide solution in 10 days and 30 days, respectively.

Conclusion: Both fixators had the same chemical structure in FTIR and NMR analyses. Formation of salt crystals seemed to be the most important cause of degradation failure, while changes in the physical properties of fixators were thought to be associated with delayed degradation.

Key words: Absorbable implants; arthroscopy; biocompatible materials/adverse effects; menisci, tibial/surgery; polyesters/metabolism; prostheses and implants/adverse effects.

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Although a large majority of the poly L-lactic acid (PLLA) fixators used in arthroscopic intervention for meniscus tears are absorbed through the blood after degradation inside the tissue fluid following the healing of the tear, some resist degradation and absorption. There is literature that reports that such degradation-resistant fixators display certain chemical and mechanical impacts that lead to synovitis in the synovial tissue of the knee and to chondritis of the cartilage.[1-6] In recent years, the physical and mechanical characteristics of PLLA have been studied in depth. It has, however, been accepted that an analysis of polymers together with their environment is a difficult procedure[7,8] and therefore studies that have examined PLLA in its contact with knee joint fluid remain few in number. This study was designed to determine the reasons for late degradation of PLLA fixators. To this end, microanalysis was carried out to compare unused fixators with used fixators which had failed to degrade and had been removed from the knee.

Tools and method

A comparative study was made of a total of six PLLA fixator arrow sutures. Three were unused (Figure 1) and three had been removed within six months following arthroscopy after degradation failure. The used arrows had been removed from the knees of patients who had undergone arthroscopic surgery for a second time due to persistent symptoms. These patients had presented on the average of six months subsequent to the first intervention with persistent complaints of pain suffered while walking, climbing stairs and during sudden movements. Their clinical examinations and magnetic resonance imaging (MRI) had resulted in a diagnosis of torn meniscus. The difference between the diameters of the used and new fixators was assessed with the naked eye. Fourier Transform InfraRed (FTIR) spectrometry (Shimadzu 8303, Shimadzu Corporation, Kyoto, Japan) was performed on all used and unused fixators and analyses were made to determine whether there were any changes in carbon, hydrogen and oxygen bonds. This type of analysis involves the use of distinct infrared bands that reveal qualitative structure; at the same time, the analysis also provides a quantitative assessment based on the intensity and amplitude of peaks.[9] A microphotograph was taken of the internal cross-sectional surfaces of the samples using a JEOL 840A JXA Scanning Electron Microscope (SEM) (Jeol, Tokyo, Japan). The surface and cross-sectional specimens were not coated with gold or osmium during preparation for the scanning. The elementary structure of the crystals forming on the internal surfaces of the used fixators was analyzed by X-ray fluoroscopy (XRF) (JEOL 840A JXA). This method provides information about the elementary structure of the surface and depths of materials by measuring the energy of the electrons emitted by elements interacting with X-rays and surrounding elements. A photon cluster of the same energy can penetrate thousands of angstroms deep into a structure. The surface composition of a solid may be different from its internal composition. Analyses using this method are employed to determine the existence of elements other than hydrogen and helium. The method also provides information about neighboring elements that are bonded to the different elements and their molecular structure. X-ray fluoroscopy is generally used for qualitative rather than quantitative analysis. A Liquid Mercury-VX 400 BB model NMR spectrometer (Bruker AC 200L NMRi Bruker BioSpin GmbH, Rheinstetten, Germany) with an operating frequency of 400 MHz was used for 1H-nuclear magnetic resonance (1H-NMR) analysis of one used and one unused fixator. The specimens were dissolved in deuterated chloroform. 1H-NMR and 13C-NMR analyses make accurate identification of structures possible. Typical chemical bonds are determined; qualitative and quantitative analyses are defined in terms of chemical shift values.[9] The fixators remaining from the other assessment (one used and one unused or new), both of the same dimension and volume, were immersed in 1/10 (gr) hydrogen peroxide solution. Degradation times of the two samples by oxidative hydrolysis were recorded. All of the PLLA fixator arrows were of the same model and made by

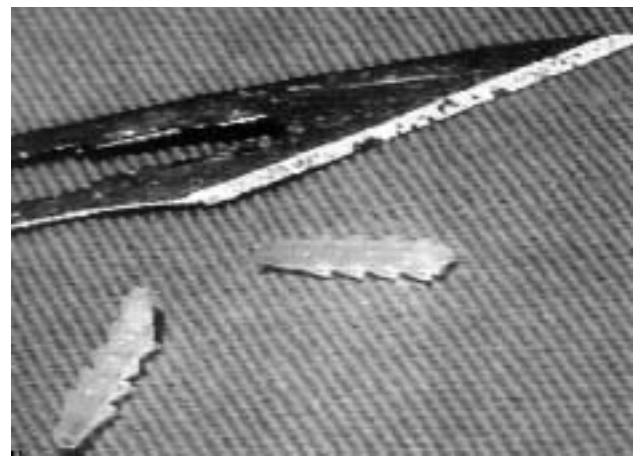

Figure 1. Image of unused fixator.

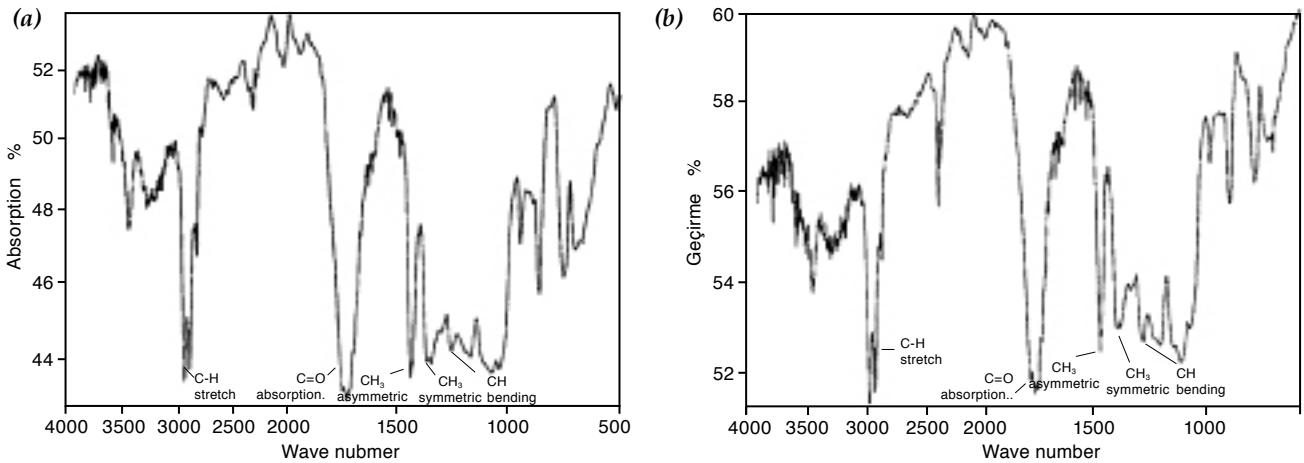

Figure 2. (a) FTIR degradation chart of **(a)** unused and **(b)** used fixators. The C-H stretch peaks at 2950 cm^{-1}, the C=O (carbonil) absorption band at 1758 cm^{-1}, the bands corresponding to the asymmetric and symmetric bending vibrations of methyl groups at 1451 and 1383 cm^{-1}, the vibration peak of methane (-CH) groups at 1270 cm^{-1} are characteristic poly (lactic) acid peaks. The peak observed at 2300 cm^{-1} belongs to CO_2 and derives from the system.

the same company (BioStinger, Linvatec, Largo, FL, USA). An arrow used in one testing process in the study was not reused for another test.

Results

Despite the fact that the used fixators had remained in the knee for a long period of time, it was seen upon removal from the knee joint at the second intervention that no degradation had occurred. An assessment with the naked eye determined that there was however a reduction in the diameters of the removed fixators. The FTIR analysis results on the used and unused fixators have been shown in Figure 2. It was seen in the analyses that there were no differences in the chemical bond structure of the polymers. While the new fixators displayed a melt flow with increasing temperatures, the fixators used in the repair did not. The fixators in both groups were seen to have shown degradation with the rise in temperature. In SEM analysis, inner sections of the unused fixators were homogeneous while those of the used ones were observed to have salt crystals in the shape of white snowflakes (Figure 3). An XRF analysis showed that these salt crystals were mainly composed of sodium, potassium and calcium chloride (Table 1). Both the used and the unused fixators exhibited a normal pattern of lactic acid polymer in 1H-NMR analysis, their profiles showing no difference (Figure 4) It was seen that the unused arrow samples degraded in an average of 10 days in hydrogen peroxide, which is a suitable solvent for polymers. Melting of the unused arrow specimen took 30 days.

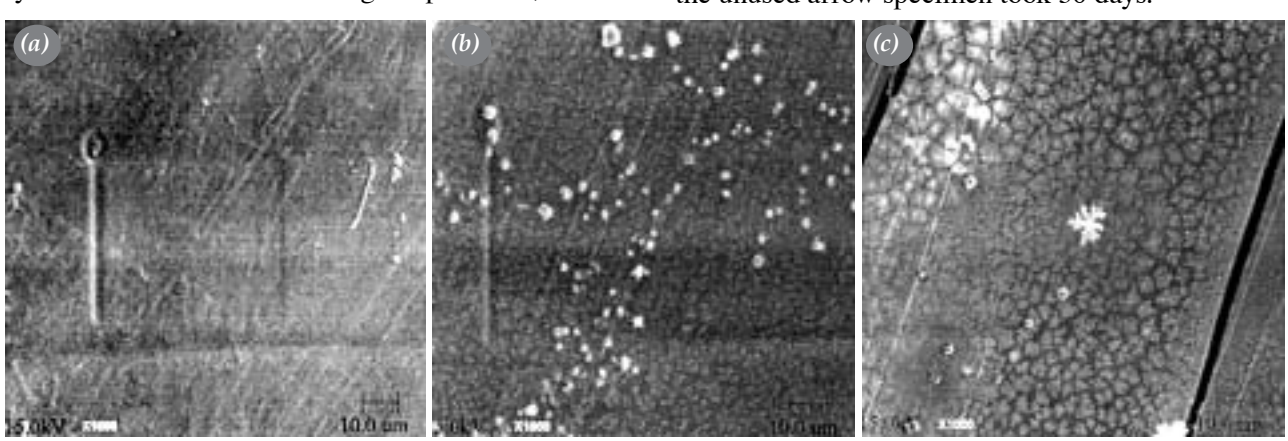

Figure 3. SEM image of fixator inner sections. **(a)** Image of homogeneous surface of unused new fixator. **(b, c)** Microscopic image of crystals of inner sections of used fixator spread out in snowflake form.

Table 1. X-Ray fluoroscopy analysis of crystals on inner sections of used fixator

Element	Density (c/s)	% Weightk
Sodium	1.17	51.150
Chloride	1.18	31.547
Potassium	0.51	17.303
Total		100.0

Discussion

The study showed that diameters of the PLLA fixators that had not degraded in tissue were reduced due to surface melting. It has been reported that such fixators break down inside the tissue but that these pieces can remain within the tissue for up to three years.[10] Degradation of poly L-lactic acid is affected by the pH of its fluid environment[11], the tissue it is inside of[12], porosity and more basically from the physical characteristics of the implant itself (crystallization, molecular weight, internal viscosity and melting temperature). poly(lactic) acid. Biodegradation of polymers takes place through hydrolytic scission brought about by body fluids and to a lesser degree, through the effects of the enzyme chymotrypsin.[13] It has been reported that some polymers degrade on the average in two-and-a-half years when used by themselves.[12,14] PLLA separates into pieces of low molecular weight in the body and finally transforms into lactic acid. The lactic acid is then converted into carbon dioxide and water as part of the carbohydrate cycle.[15,16] An important characteristic of poly(L)-lactic acid is that there is melt flow under the effect of heat; its melting point is 1450C. Some sources state that this melting point is 1850C.[17] Poly(L)-lactic acid is nonresistant to hydrolytic action and cannot endure humid heat.[17] The longer the chains in the polymer structure and the more the branches in the chain, the more difficult it will be for PLLA to melt.[9] While the new fixators in our study melted at a temperature of 100-1200C, the ones used in the repair did not soften at the same degree of heat. Theoretically, the first reason that the fixators used in the repair did not display a melt flow may be the increase in the structure's crystallization, the alignment of the mer-chains and because of this, the shift of the melting point to higher temperatures. A second reason could be the comb-shaped branching after the partial degradation of the chains in the PLLA structure, the lengthening of these chains and the formation of grafts, resulting in a breakdown of the linear structure. It has been asserted that if cross-links have been formed from covalent bonds between the chains of a polymeric structure, the structure may fail to degrade in any solvent or at any temperature.[18] Establishing the existence of salt crystals through X-ray fluoroscopy indicates that polyelectrolyte crystals have formed in the polymer and that the polymer has further strengthened through hydrolysis in water. Degradation is the phenomenon whereby the molecular chains move with increasing speed at rising temperatures and as a result, weak bonds begin to break.[19] Degradation is the opposite of polymerization and signifies breakdown. It was found in our study that both new and used fixators degraded under rising temperatures. Crystallization increases during the time an implant remains in the body, leading to an increase in melting temperatures.[11] On the other hand, partial degradation of the fixators inside the meniscus leads to reductions in molecular weight.[10] The polymer is hydrolyzed in the knee fluid inside the meniscus. The solvent is the synovial fluid. The enzyme chymotrypsin has an additional degrading effect. It can be said that the polymer is adversely affected by conditions in the knee joint (synovial fluid and tissue, damaged cartilege, joint space irregularity, movement of the joint and temperature of 370C).

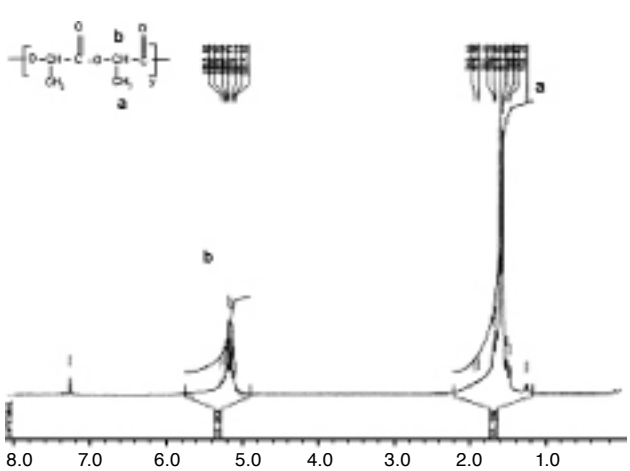

Figure 4. Nuclear magnetic resonance analysis of used fixators. Shifts were seen in **(a)** the methane (CH) protons of the lactic acid units at 5.2 ppm and **(b)** in the metyl (CH3) protons of the lactic acid units at 1.7 ppm. There was a shift in d-chloroform at 7.2 ppm. This analysis shows that the structure is a homopolymer related to poly(lactic) acid.

Reduction in the molecular weight of the polymer occurs with the polymer's subcutaneous implant, when it encounters extracellular fluids and mostly

when the polymer is implanted into the medullary cavity.[17] It has been stated that when poly L-lactic acid is implanted, along with the fibrovascular growth in the tissue in 6-8 weeks, there is also a formation of multinuclear giant cells and that the implant completes changing places with the debris and surrounding giant cells by the 20-24th week. It is further asserted that it is not suitable as an orbital implant.[19] It is significant that fixators that do not undergo degradation within the knee do melt in extracellular environments in hydrogen peroxide. We think that the reason they do not melt inside the tissue is related to the acidic or basic nature of the synovial fluid, the shape of the meniscus vessels and to joint movement.

A rise in the body's lactic acid level will retard degradation and melting of PLLA. The increase in acid may also be the cause of synovial tissue reactions.[7,20] The acid level of lactic acid in the synovial tissue increases during arthroscopic surgery performed with a tourniquet.[20] In-vitro microdialysis studies carried out after arthroscopy to check post-operative physiological changes have shown that more lactate is found in the synovial tissue compared to the subcutaneous fat tissue and that its level rises after surgery.[20] It is not certain whether these changes occur in the meniscus cartilage. The increase of lactate within the joint fluid may hinder the degradation and absorption of the arrow suture due to saturation. In conclusion, no difference was found between used and new fixators in terms of chemical structure in FTIR and NMR analyses. SEM analysis of used fixators and later the results of XRF studies pointed to the formation of salt crystals in these polymers. Since this formation created and increased polyelectrolyte crystallization, this seems to be the most important cause of degradation failure. Another important reason for retarded degradation appears to be cross-linking, comb-shaped branching and irregular polymers.

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