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Development of Test Method for Assessment of *In-Vitro* Degradation of Medical Devices

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Abstract: ***The objective of this study is to present test results that have been reported from an in-vitro, real-time degradation study of a circular knitted mesh of a biodegradable medical device. The knitted mesh on the stent eroded up until that point in the trial, which lasted for 6-7 months at 37°±1°C. Hence, in this study, the degradation of a circular knitted mesh formed of 28 micron monofilament Poly D, L-lactide-co-glycolic acid (PLGA) 8523 of biodegradable medical device was evaluated under in-vitro, real-time degradation conditions.***

Keywords: Knitted mesh, biodegradable, *in-vitro* degradation, real time.

1. Introduction

In general, degradation is described as a gradual loss of a material's pertinent features brought on by exposure to environmental factors. One of the largest issues facing medical research today is the deterioration of biomaterials. Both desirable and undesirable deterioration are possible. The safe usage of biocomponents in both scenarios depends on understanding the degradation. The performance of medical devices constructed of polymer is crucially dependent upon the stability of the material.

Surprisingly, the body tissues provide a hostile environment due to a number of characteristics of their composition, such as the existence of enzymes, free radicals, superoxides, and peroxides, all of which might affect the degradation. Biodegradable polymers were initially developed in the 1960's and have gained popularity in the production of medical equipment due to their capacity to safely decay, which reduces the risks associated with prolonged exposure to non-biodegradable materials in the body. These materials are special in that they may be adapted to a particular application or intended use by using a variety of processing techniques to change their physical characteristics and degradation profiles, which result from diverse production processes. Therefore, through customised in-vitro degradation studies, it is essential to capture the material and its physical qualities in a physiologically relevant environment. To understand how the polymer will behave in a setting that is relevant to human physiology, it is necessary to characterize the degradation rates and changes in the material and its physical properties. The construction of the degradation profile is portrayed by the characterization of the molecular weight, strength retention, and mass loss profiles over a pre-determined time period.

The current study's objective is to assess the polymer blend's susceptibility to degradation when submerged in various solutions. The specimens were kept in an orbital motion to replicate the flow of fluids over the 190-195 days that the biodegradation process took place in stable settings at 37 ^{o}C ± 2 ^{o}C with a pH range of 7.4. The objective is to determine how a solution type, specimen form, and material's composition affect the device's ability to degrade biologically. The monitored parameters included changes in the quantity of a solution absorbed by the specimen, morphological changes in the structure and mechanical properties.

2. Material and Methods

Apparatus and Reagents

Fluid bath maintained at 37°C ± 2°C, Inflation device with (Pressure range: 0-30 ATM), and 0.1M Phosphate buffer saline (PBS) of pH 7.4 ± 0.2 are used. The salts used for the preparation of PBS solution were of analytical grade and dried to constant mass. Solution A: 1/10 mol/litre KH_2PO_4, was prepared by dissolving 6.805 gm Potassium dihydrogen phosphate (KH_2PO_4) in 500 ml of distilled water. Solution B: 1/10 mol/litre Na_2HPO_4, was prepared by dissolving 28.392 gm dibasic Sodium hydrogen phosphate anhydrous (Na_2HPO_4) in 2000 ml of distilled water. A total of 2000 ml of buffer solution was prepared by mixing 364 ml of solution A: (18.2% v/v) and 1636 ml of solution B: (81.8% v/v).11.7 gm (0.585% w/v) of Sodium chloride was dissolved in this buffer solution. The pH value of this buffer solution is 7.42.

Test Procedure

The test samples having 3×19 mm size were taken which were visually inspected for damages before initiating with the test procedure. Stylet along with dual layered Protective sheath was removed from the distal tip of the device. An inflation device; fill with about 10 ml of water, is connected to luer hub of the stent system. The stent system was immersed in a fluid bath maintained at 37°C ± 2°C. The fluid bath was filled with 2000 ml of 0.1M Phosphate buffer saline (PBS) having pH 7.4 ± 0.2. So that, the stent is maintained in immersed condition during inflation and deflation. The stent was conditioned for minimum 60 seconds at 37°C ± 2°C. Later it was slowly inflated, by applying pressure using an inflation device, at the rate of 10 seconds/ATM up to 4 ATM. Thereafter, it was further inflated at the rate of 5 seconds/ATM up to its nominal pressure (9 ATM), and held at the same pressure for 30 additional seconds before balloon deflation. The delivery system, along with the stent was then removed from the

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fluid bath. The stent was unmounted from the delivery system, and carefully dried using a paper towel.

Degradation Method

The initial values for the tests (proposed during degradation study) were determined before starting the degradation test.

Apparatus and Reagents

Soaking solution (PBS), consisting of KH_2PO_4 and Na_2HPO_4 in double distilled water. The salts used for the preparation of buffer solution were of analytical grade and dried to constant mass.

Solution A 1/15 mol/litre KH_2PO_4, was prepared by dissolving 4.539 gm Potassium dihydrogen phosphate (KH_2PO_4) in 500 ml of double distilled water. Solution B 1/15 mol/litre Na_2HPO_4, was prepared by dissolving 17.801 gm dibasic Sodium hydrogen phosphate anhydrous in 1500 ml of double distilled water. A total of 1500 ml of buffer solution was prepared by mixing 273 ml of Solution A (18.2% v/v) and 1227 ml of Solution B (81.8% v/v). The pH of this buffer solution is 7.38. The buffer solution was filtered through 0.22 µm filter to avoid microbial contamination.

Container

A glass vials, having a capacity of holding 20 ml of soaking solution were used. The glass vials were steam sterilized in an autoclave at 121°C for 20 minutes. A silicon stopper was used to cap the glass vials to avoid loss of soaking solution by evaporation. The containers with test samples were maintained at degradation temperature of 37°C ± 1°C (constant) in hot air oven. And the pH of the soaking solution was measured by using calibrated pH meter at specified test interval. The pH of buffer solution was measured in four different containers at each time intervals. And it was observed that, there was no clouding of the buffer solution occurred during accelerated in-vitro degradation study.

Real-Time Degradation Study

In a glass vial, 10 ml of the soaking solution was taken. After being mounted on a Teflon stylet, the test samples were put in a glass vial, so they would remain completely submerged in the soaking solution. To prevent the soaking solution from evaporating, the vial was sealed with a silicon stopper as shown in the Fig.01. In the manner described above, a total of 02 distinct vials carrying the test samples were created. In a hot air oven with a constant temperature set at 37°C ± 1°C, all test samples were loaded. At regular intervals, the samples were withdrawn from the soaking solution.

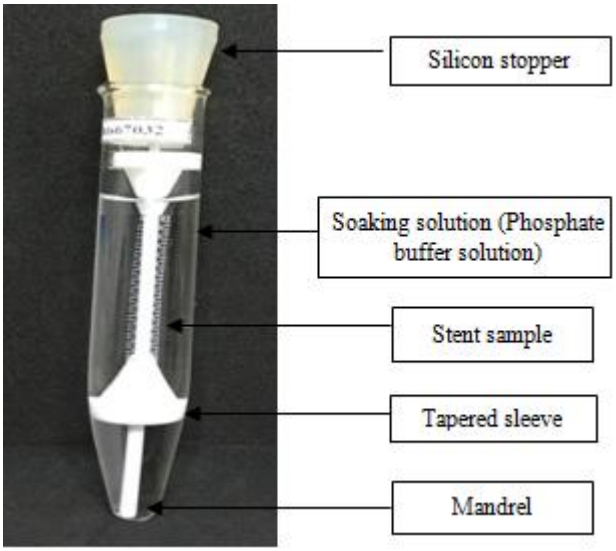


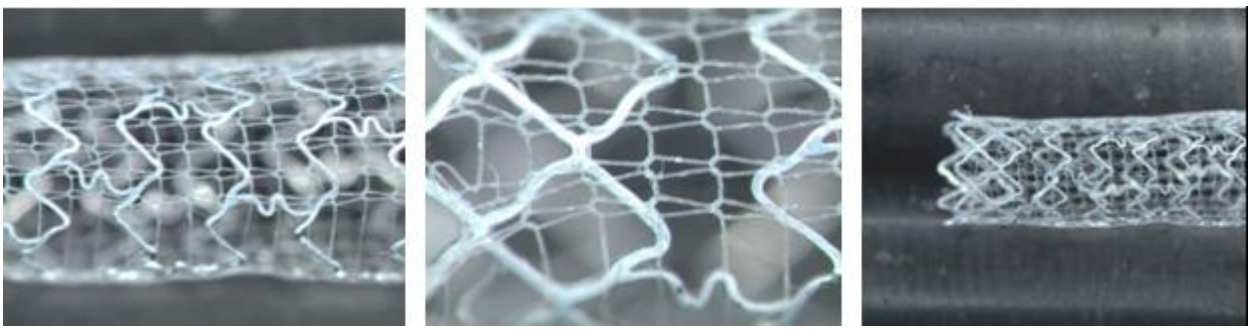
Figure 1: Glass vial sealed with silicon stopper to avoid evaporation of soaking solution

3. Result and Discussion

On a daily basis, samples were taken out of the soaking solution and visually examined using an optical microscope with a 40X magnification to check for stent/mesh integrity. For better visibility, the surface of stent/mesh was dried carefully using a paper towel. The structure and geometry of the circular knitted mesh's aperture, dimensions, and monofilament breaks and cracks were all noted. The real-time degradation study was terminated when the circular knitted mesh had completely decomposed. The visual observation results from the real-time degradation study at successive time intervals are summarized in Table.01 and with respective images in Fig.02, 03 & 04 and 05.

Table 1: Visual observation during Real-Time degradation study

Sr. No.	Real-Time Days	Visual Inspection
1.	Initial day to Day 105	Knitted Mesh as per Specification
2.	Day 153	Knitted mesh structure breaks
3.	Day 167	Bulk Erosion of Knitted mesh
4.	Day 201	Knitted mesh completely solubilized



Observation: Knitted mesh's structure as per specification

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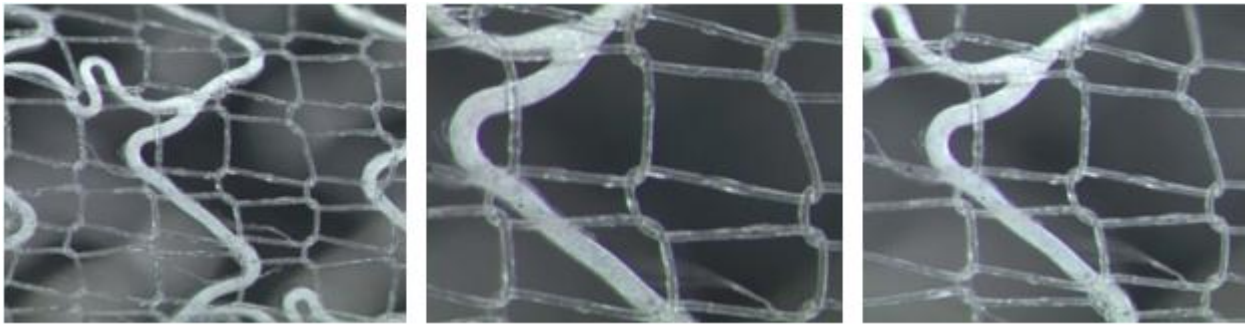
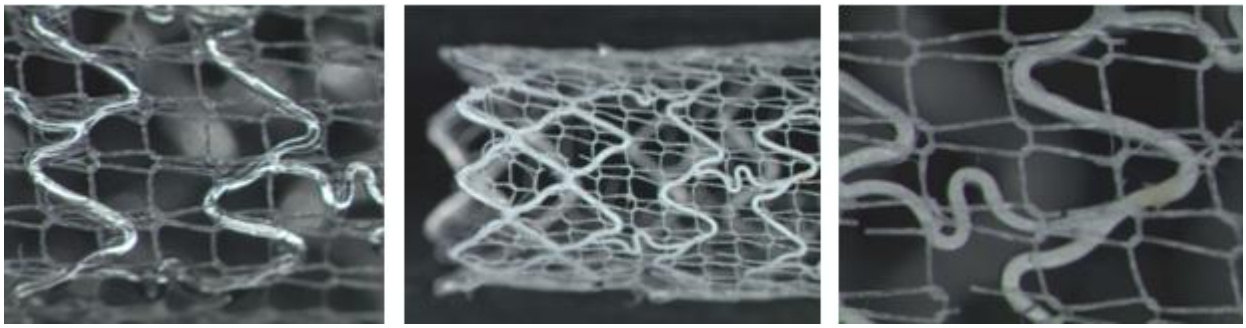
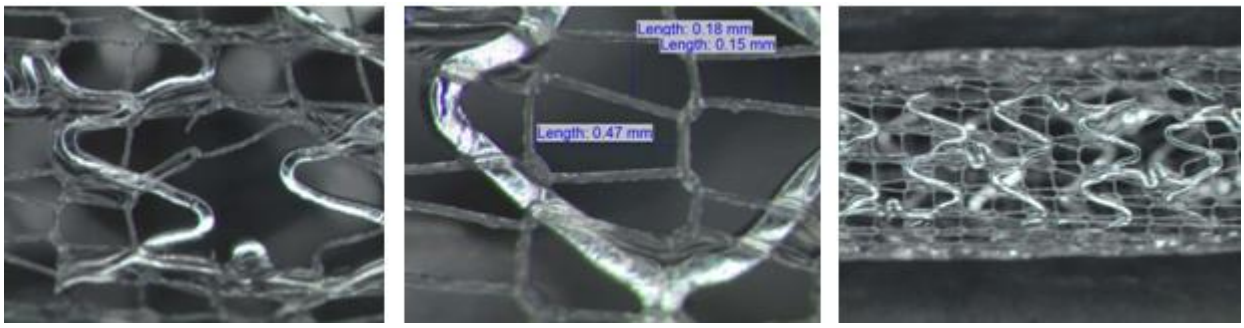


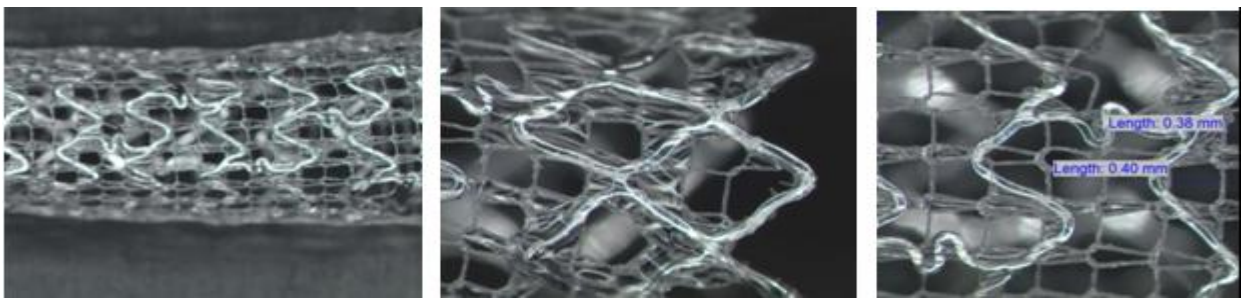
Observation: Knitted mesh's structure as per specification
Figure 2: The microscopic images of the test samples from an initial day to 105 days



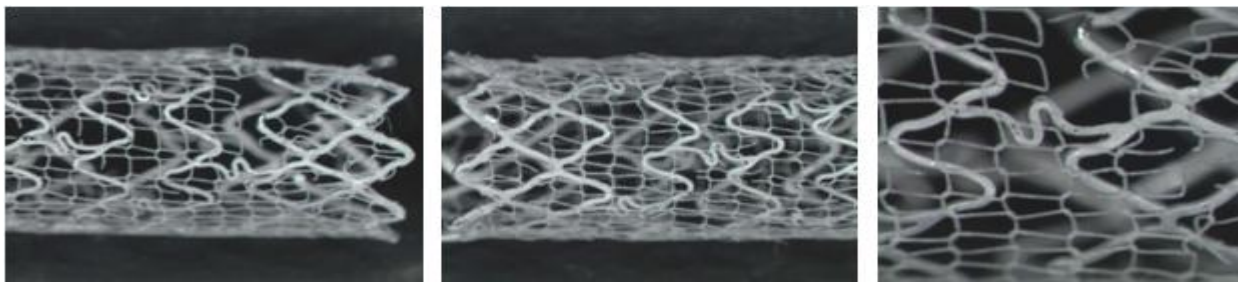
Observation: Knitted mesh's structure breaks at 153 days



Observation: Knitted mesh's structure breaks at 153 days
Figure 3: The microscopic images of the test samples at 153 days



Observation: Knitted mesh eroded in bulk at 167 days



Observation: Knitted mesh eroded in bulk at 167 days
Figure 4: The microscopic images of the test samples at 167 days

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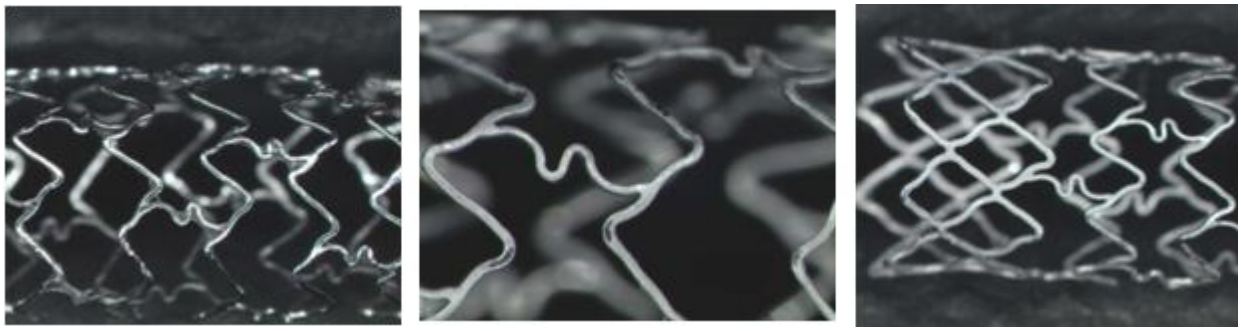
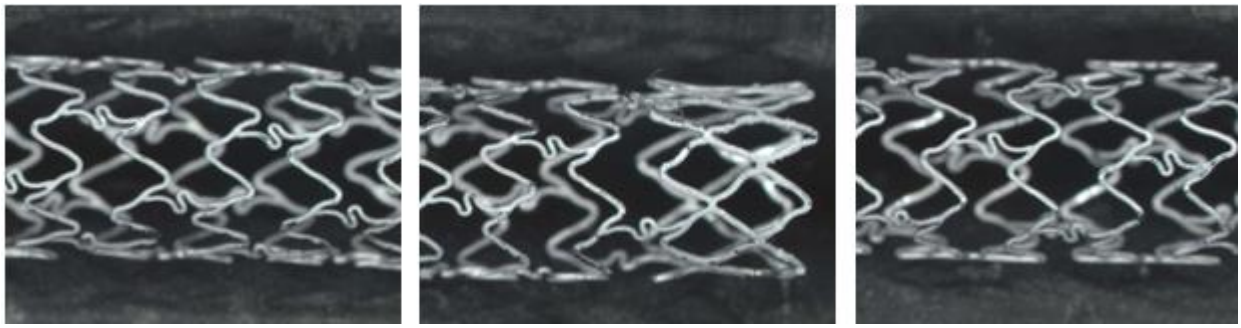


Observation: Knitted mesh complete solubilized at 201 days



Observation: Knitted mesh complete solubilized at 201 days

Figure 5: The microscopic images of the test samples at 201 days

4. Conclusion

Based on real-time degradation research data of medical devices. It has been concluded that, there is no impact of circular knitted mesh on the pH of the soaking solution. Circular knitted mesh's structure and geometry remained unchanged, and until 105 days into a real-time deterioration investigation, no breakages, cracks, or aperture damages were noted. An apertures of knitted mesh structures were broken at some locations at 153 days of real time degradation study. By 167 days of incubation, under real-time conditions, the knitted mesh collapsed/erroded as a result of aperture fracture and breakages. By 201 days of incubation under real-time conditions, the knitted mesh had fully solubilized.

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