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Real-Time In-Vitro Degradation Study of Bioresorbable Occluders: Implications for Congenital Heart Disease Interventions

Minocha Dr. Pramod Kumar, Kothwala Dr. Deveshkumar Mahendralal, Bhatt Chirag Manshankar, Patel Chirag Jitubhai

Meril Life Sciences Pvt. Ltd., Bilakhia House, Survey No. 135/139, Muktanand Marg, Chala, Vapi - 396 191, Gujarat, India

Abstract

Heart defects are increasingly diagnosed nowadays. There is a variety of heart diseases, each with its own causes, symptoms, and treatments, including congenital heart diseases such as atrial septal defect (ASD) and ventricular septal defect (VSD). The next-generation closure device for interventional treatment of congenital heart disease is considered to be bioresorbable. Bioresorbable occluders are indeed becoming more significant in percutaneous coronary interventions (PCIs) due to their unique advantages. The numerous benefits of this type of occluder make it of interest for commercialization. This study focuses on the in-vitro assessment of a bioresorbable occluder for atrial septal defects (ASDs). The study spanned two years and was conducted under real-time in-vitro degradation conditions. The degradation process included the use of phosphate buffer as an in-vitro degradation solution due to its specific characteristics. The entire in-vitro process was observed, from the sterilization of the phosphate-buffered saline (PBS) to the deployment of the occluder, until the occluder completely dissolved into the surrounding medium. The evaluation of study is based on the visual observation on the specific time durations.

Keywords: Congenital Heart Diseases, Atrial Septal Defect (ASD), Ventricular septal defect (VSD), Bioresorbable occluders (BRO), Phosphate Buffer Saline, In-vitro Degradation Study

Introduction

Heart diseases are on the rise, with many different types being diagnosed over the past few decades. While some heart diseases are treatable with advanced technologies, others remain challenging. These conditions are increasingly prevalent across all age groups. Congenital heart diseases, such as Patent Ductus Arteriosus (PDA), are common in new-borns. Additionally, adults and the elderly are often diagnosed with various heart diseases, including Atrial septal defect (ASD), Ventricular septal defect (VSD), Patent foramen ovale (PFO), and Atrioventricular septal defect (AVSD).

These heart diseases affect different parts of the heart but can be treated with occlusion. Occlusion involves blocking the abnormal blood flow that affects normal heart function using different occluder devices. Two main types of occluders are available: metallic occluders made of biomedical grade metals like nitinol, and occluders made of materials like PLGA, PLLA etc, which reduces the risk of long-term complications and the need for medication.

Several factors influence the choice of occluder. Bioresorbable occluders (BRO) are more biocompatible than metallic alloys and have a lower risk of allergic reactions. Metallic implants, on the other hand, may cause allergic reactions, such as nickel allergies. BRO also have a lower risk of long-term complications, such as erosions and tissue overgrowth, compared to metallic implants, which can lead to complications like thrombosis or erosion, necessitating surgical removal or replacement.

The choice between a bioresorbable and metallic occluder depends on factors such as the patient's age, the type and location of the defect, the need for future interventions, and the physician's preference. In some cases, bioresorbable occluders offer advantages in terms of biocompatibility, reduced long-term complications, and imaging compatibility. This article study focuses on the in-vitro assessment of a Bioresorbable Occluder for atrial septal defect. Three occluders underwent testing with simulated environmental conditions to study its degradation. The biodegradation process was conducted at a stable temperature of 37°C ± 2°C and a pH of 7.4, with the specimens subjected to an orbital motion to simulate fluid flow. For the in-vitro biodegradation study, the occluder was immersed in phosphate buffer with added electrolytes, providing ideal conditions compared to human body fluids. The degradation process lasted approximately 2 years, with initial thickness degradation observed around 9 months. Evaluation by observing visual changes in the occluder documented from the first day to the final day of the study.

Materials and Methods

The in-vitro analysis of the bioresorbable occluder (BRO) involves a prolonged degradation process of an atrial septal defect (ASD) occluder under simulated conditions. This allows for the observation of reaction times and any imperfections in the study, as well as further evaluation of the product for non-clinical and clinical purposes. The in-vitro assessment was conducted in several steps, with careful preparation of sterile conditions for the study. Each step of the biodegradation process is outlined below.

Preparation of Phosphate Buffer

For the ideal conditions of the in-vitro study, a 0.1 M phosphate buffer saline (PBS) solution at a pH of 7.4 ± 0.2 was utilized. The PBS solution was prepared using analytical grade salts in two separate solutions:

Solution A: A 1/10 mol/lit KH_2PO_4 solution was prepared by dissolving 6.805 gm of Potassium dihydrogen phosphate in 500 ml of distilled water.

Solution B: A 1/10 mol/litre Na_2HPO_4 solution was prepared by dissolving 28.392 gm of dibasic Sodium hydrogen phosphate anhydrous 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). Additionally, 11.7 gm (0.585% w/v) of Sodium chloride was dissolved in this buffer solution. The pH value of this buffer solution was adjusted to 7.42. To avoid microorganism contamination, the buffer solution was filtered through a 0.22 µm filter.

Sample Preparation

The initial step involved verifying the size and lot number of the test sample. Once verified, the occluder and delivery system were inspected for any damage by peeling apart the pouch in which the sample was packed. To load the device into the loader, the luer hub was pushed towards the Y-hub until they were close to each other. The device was then opened up with the help of the delivery sheath, and the loader was pushed over the device. The device was flushed via the side arm, and then attached to the access sheath. The device was advanced into the access sheath by pushing (not rotating) the delivery sheath. The left atrial disc and part of the connecting waist were deployed. The left disc was closed or flattened by pulling back the inner lumen until both discs were close to each other, and then the device was gently pulled. With tension on the delivery sheath, the access sheath was pulled back and the right atrial disc was deployed (after showing the remaining four markers) and closed by pulling back the inner lumen. To lock the device, the delivery sheath was gently pushed while simultaneously pulling out the whole part of the inner lumen from the delivery sheath. After confirming the locking, the hemostasis valve was opened and the delivery sheath was removed from the device. The green thread (detachment thread) was then removed by pulling out one end to detach the occluder. Finally, the occluder was unmounted from the delivery system and carefully dried using a paper towel.

Real Time Degradation

For real-time degradation observation, 100 ml capacity soaking solution glass vials were used. For the elimination of microorganism the glass vials were sterilized by the help of stem sterilizing method, in which vials were autoclave at 121°C for 20 minutes. To seal the vials containing the soaking solution (PBS), pre-sterilized glass stoppers were used. Throughout the study, glass containers were kept at a constant temperature of 37°C ± 1°C using a hot air oven to prevent temperature variations.

For the study performance 50 ml of soaking solution was filled in sterile glass vial. Then test implant was dipped into solution in such a way that it remained fully immersed in soaking solution vial. Once the sample was placed properly the vial was sealed with help of glass stopper to avoid the evaporating loss of soaking solution and maintaining the sterile condition, which illustrated in figure 01. Throughout the process the temperature was maintained at 37°C ± 1°C. At the specific time a calibrated pH meter was used to measure the pH of soaking solution at the specified test interval, also required PBS solution was added to maintain the concentration and add required ml of solution.

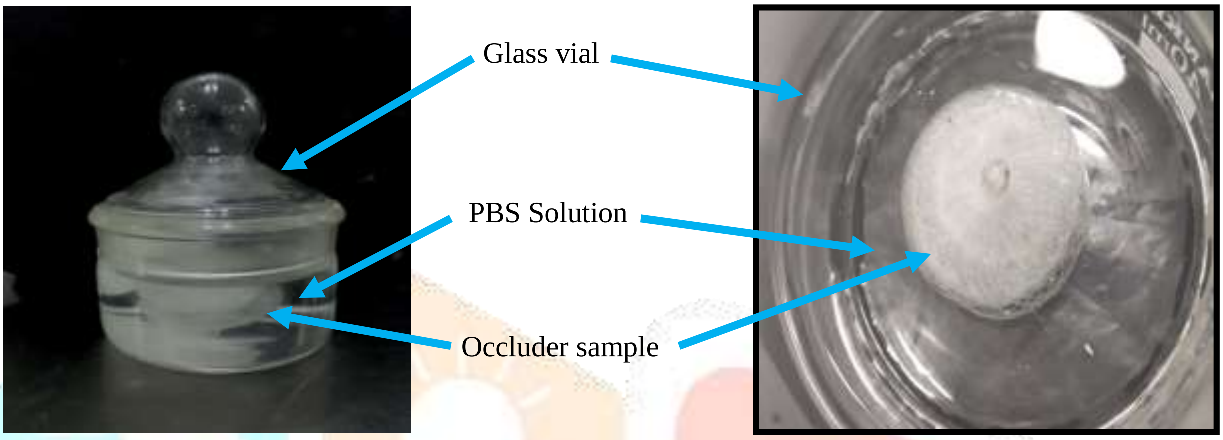


Figure 01 Side veiw and Top veiw of Occluder deeped into PBS Solution

Result and Disccusion

In-vitro degradtion for ASD occluder is achieved by visual observation and continues providing required medium. The samples were taken out of the soaking solution and visually examined under an optical microscope at 40x magnification to assess the integrity of the occluder. To improve visibility, the occluder's surface was gently dried with a paper towel. The examination focused on changes in structure, aperture geometry, dimensions, and the presence of monofilament cracks and breaks. The findings are summarized in Table 1.

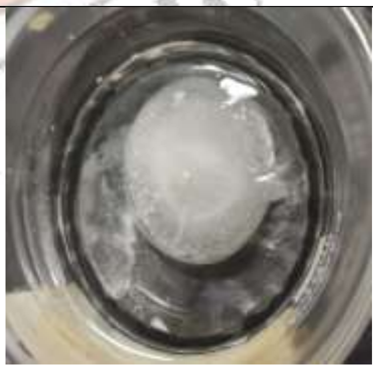
Sr. No.	Real Time IVD Days	Observation
1	Initial	Initially the wet occluders were kept in the study
2	07 days	No degradation observed after 07 days.
3	15 days	No degradation observed after 16 days.
4	30 days	No degradation observed after around 32 days.
5	2 Months	No degradation observed after around 61 days.
6	3 Months	Not observed any type of degradation and filament break after

		around 93 days.
7	6 Months	Not observed any type of degradation and filament break after around 181 days.
8	9 Months	Initial degradation observed; erosion of occluder filament observed and thickness decreased ranging from 170 microns to 155-165 microns.
9	12 Months	Degradation of the occluder filament was observed; multiple occluder filaments breakage was observed.
10	18 Months	Occluders were almost degraded and were kept for further observation till complete degradation.
11	24 Months	The occluder was completely solubilized after around 730 days of incubation at real time condition.

Table 01 : Visual Observation base data of Real time Degradation Study

Real time Degradation

Real-time degradation was observed at specific intervals, showing a gradual progression of positive results. There was no degradation observed during the initial nine months. However, degradation began gradually after this period and continued throughout the remaining time periods, as illustrated in the images below.

Initial Day Observation		
		
Sample 01	Sample 02	Sample 03

Observation: These images of occlude represents the 0 day of study.

06 Months Observation		
		
Sample 01	Sample 02	Sample 03

Observation: Even after the 06 months, degradation was not observed.

09 Months Observation		
		
Sample 01	Sample 02	Sample 03

Observation: After the 09 Months, the occluder showed initial signs of degradation, with the middle broken and detached fabric and frame observed.

12 months Observation		
		
Sample 01	Sample 02	Sample 03

Observation: The 12 month observation depicts, fibre detachment from occluder without impacting fabric.

18 Months Observation		
		
Sample 01	Sample 02	Sample 03

Observation: Into 18th months observation, occluder was almost degraded, where fibres were still visible.

24 Months Observation		
		
Sample 01	Sample 02	Sample 03

Observation: Into 24 months observation, occluder was almost solubilized which indicates complete real time degradation.

Conclusion

The investigation into the degradation of bioresorbable occluders used in treating atrial septal defects, a common congenital heart ailment, yields promising results when simulated in-vivo conditions are considered. Initially, the occluder demonstrates remarkable stability over the first 8 months, indicating its reliability in maintaining structural integrity. However, from the 9th month onward, gradual filament erosion and thickness reduction are observed, suggesting a controlled degradation process. By the 12th month, the occluder undergoes multiple filament degradations, ultimately leading to complete dissolution into the surrounding tissue over a 2-year period. Despite the eventual dissolution, this process is anticipated and aligns with the intended function of bioresorbable occluders. Importantly, throughout this degradation period, we can assure that, the occluder would effectively fulfil its primary purpose by fostering the formation of an epithelial cell layer in-vivo. This outcome underscores the potential of bioresorbable occluders in mitigating risks associated with traditional metallic occluders, such as tissue overgrowth and allergic reactions. Thus, these findings offer a positive outlook for the clinical application of bioresorbable occluders in treating atrial septal defects, paving the way for safer and more effective interventions in congenital heart conditions.

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