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# Profiling In-Vitro Drug Release Kinetics from a Bioresorbable Scaffold Implant to Predict In-Vivo Drug Retention in the Treatment of Peripheral Artery Diseases

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**Abstract:** ***Atherosclerosis-induced peripheral artery disease (PAD) frequently leads to significant arterial blockages, necessitating various treatment approaches such as medical management, endovascular interventions, and surgical bypass. This research article introduces a study on an innovative treatment modality for PAD, i.e. the development of "Sirolimus Eluting Self Expanding Bioresorbable Braided Peripheral Scaffold System" The scaffold comprises a bioresorbable polymer called PLGA, which is coated with an elastomer to facilitate progressive drug release and scaffold biodegradability. Sirolimus, an immunosuppressive drug, is incorporated into the scaffold to mitigate immune reactions during or after implantation. The study primarily focuses on the effective loading and release mechanisms of the drug from the scaffold, as these aspects are pivotal for the success of drug-eluting scaffolds. Through rigorous in-vitro testing, the authors successfully loaded sirolimus onto the scaffold and demonstrated a gradual and sustained release profile over time, which could effectively replicate the in-vivo drug release pattern observed in peripheral arteries. Consequently, the aforementioned "Sirolimus Eluting Self Expanding Bioresorbable Braided Peripheral Scaffold System" holds promising potential in reducing the risk of restenosis in peripheral arteries.***

**Keywords:** Atherosclerosis, peripheral arteries, restenosis, sirolimus, self-expanding, bioresorbable braided scaffold

## 1. Introduction

The present research work discusses the concept of bioresorbable scaffolds used in treating coronary artery disease and peripheral arterial disease. These scaffolds are inserted into blood vessels to expand and relieve blockages, similar to traditional metallic scaffolds or stents. However, bioresorbable scaffolds are made of materials that can dissolve or be absorbed by the body, offering advantages over metallic ones. They have the potential to overcome limitations such as late stent thrombosis and local inflammation caused by foreign bodies. The use of bioresorbable scaffolds composed of poly (lactic-co-glycolic acid) (PLGA) shows promise. These scaffolds degrade over time, leaving a natural artery without foreign material, which is particularly beneficial for younger patients who may require future interventions. Balloon angioplasty, although effective initially, can lead to re-narrowing of the artery over time. Innovations and advancements in scaffold technology, especially drug-eluting scaffolds, are expected to dominate the market. Bioresorbable scaffolds, with their high stability and bioresorbable properties, are projected to experience the fastest growth rate.

The "Sirolimus Eluting Self Expanding Bioresorbable Braided Peripheral Scaffold System" is a medical device designed to treat atherosclerotic diseases in the peripheral arteries. It is used for procedures like Percutaneous Transluminal Angioplasty (PTA) and Scaffolding in vessels with diameters ranging from 5.00 mm to 8.00 mm. Atherosclerosis is a condition where plaque, a sticky substance, builds up in the arteries. Risk factors for atherosclerosis include high cholesterol and triglyceride levels, high blood pressure, smoking, diabetes, obesity, physical inactivity, and saturated fat consumption. To prevent blood clots, the scaffold system is coated with a slow-release drug called sirolimus. Sirolimus would gradually released into the artery wall to prevent inflammation if caused during implantation. Sirolimus also works differently from other immunosuppressant medications by inhibiting T-lymphocyte activation and proliferation caused by antigenic and cytokine stimulation. It also prevents the formation of antibodies.

Drug loading is the process of placing a drug onto a scaffold, while drug release refers to the gradual release of the drug from the scaffold. The drug release profile is crucial for the effectiveness of drug-eluting scaffolds (DES). Sustained release is preferred as it maintains therapeutic drug levels for longer, while burst release can lead to negative effects like blood clots or inflammation. A current research study aims to enhance the drug release profile of a bioresorbable scaffold by coating it with a poly(lactic-co-glycolic acid) (PLGA) polymer and an elastomer, and loading it with the drug sirolimus. The PLGA polymer degrades gradually, resulting in controlled release and a sustained drug release profile. The findings of the pre-clinical study will be disclosed in an upcoming article within a short period of time.

## 2. Materials and Method

### 2.1 Scaffold Development

The "Sirolimus Eluting Self Expanding Bioresorbable Braided Peripheral Scaffold System" was developed using braiding technology. A mandrel with a teflon surface and a length of 220 mm was used to braid a 170-micron monofilament of poly (L-lactide co-glycolide) (PLGA) *(Biogeneral, USA)* using a 32-wire braiding machine *(B&B Machines, Ahmedabad, India)* as shown in Figure. 01 A &B

**Figure 1:** (A) Carriers are Mounted onto a Braiding Machine, where the Braiding takes place

(B) Fibers are Spun into Yarn

To create an elastomer coated braided scaffold, the mandrel containing the braided monofilament is subjected to annealing in a vacuum oven for a duration of 18 hours. The braided structure is then manually cut to the required length, keeping in mind the elastomer coating. An elastomer solution is used to coat the braided structure, which is prepared using a solution of polyester PLCL prepolymer *(Nomisma Healthcare, India)* and a cross linker in dichloromethane *(Sigma Aldrich, India)*. This solution is sprayed onto the braided PLGA scaffold structure to form the elastomer coating. Figure.02 illustrates the elastomer coated braided scaffold.

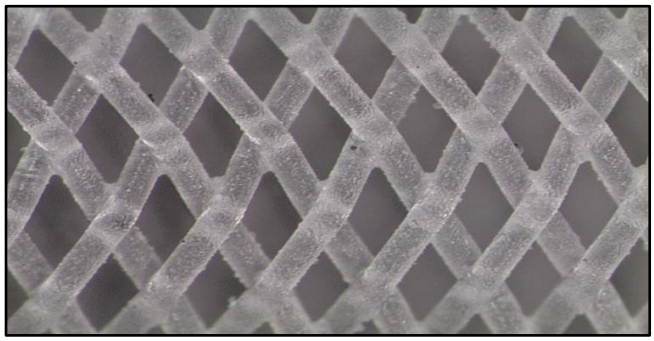


**Figure 2:** Elastomer Coated Braided Scaffold

To enhance the connections between the filaments, the elastomer-coated scaffolds undergo an additional step of vacuum oven treatment. Subsequently, the scaffolds are precisely cut to the required lengths. In order to ensure radio-opacity, markers, as shown in Figure.03 (*Heraeus, Germany*), are affixed to both the top and bottom of the scaffolds.

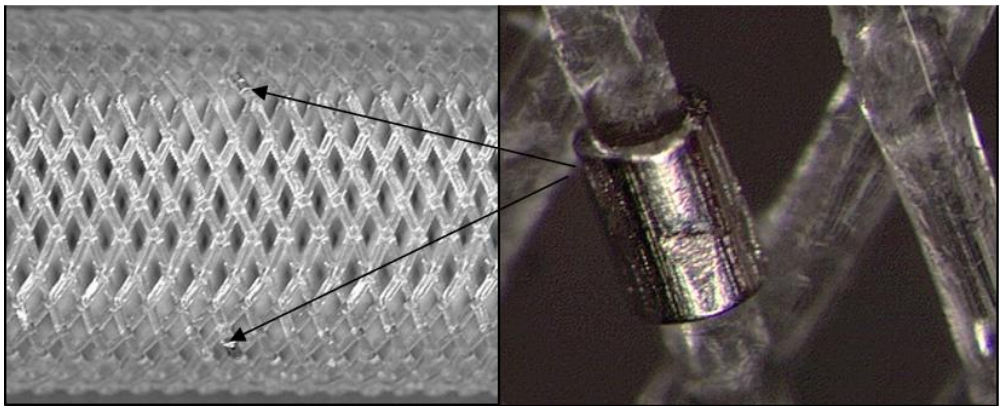


**Figure 3:** Attachment of Radio-Opaque Platinum Markers on the Braided Scaffold

### 2.2 Markers Affixing Process

Radio-opaque markers are attached on braided scaffold in clean room environment. C-shaped Platinum markers are placed at equidistance on both sides of the scaffold with the help of vacuum tweezer. After that, markers are crimped on scaffold with the help of titanium forceps. The marker deposited scaffold is evaluated under stereo-optical microscope for well deposition of markers, braided pattern and dimensional variation.

Finally, the scaffolds are coated with sirolimus, an active therapeutic and antiproliferative drug, combined with a biocompatible bioabsorbable polymer called Polycaprolactone (PCL) (*Corbion, Netherlands*), which acts as a drug reservoir and regulates the rate of drug release. This comprehensive braided scaffold system, depicted in Figure.04, has been designed to maintain the patency of obstructed peripheral vascular arteries.

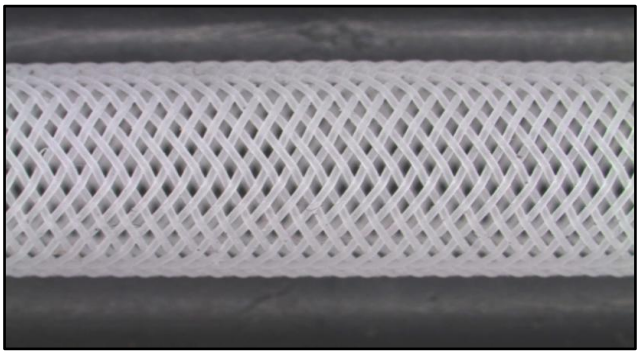


**Figure 4:** Sirolimus Eluting Self Expanding Bioresorbable Braided Peripheral Scaffold System

### 2.3 Advantages of Braided Scaffold over Laser-Cut Scaffold:

Laser-cut stents, due to their limited flexibility, can lead to complications and problems when compared to braided scaffolds. When a laser-cut stent is subjected to external bending forces, the struts of the stent can break after reaching a certain point. However, a braided scaffold can be bent in a similar manner without causing any damage to its wires or filaments, and it can regain its original shape. Additionally, when crimping the stent into the catheter during the stent loading process, there is a risk of breaking struts due to the lack of flexibility and the brittleness of the struts. On the other hand, a braided scaffold can be easily crimped because of its high flexibility property.

### 2.4 Loading of Drug Dosage onto a Scaffold for Enhanced Drug Delivery

The drug coating process on the bioresorbable scaffold was carried out using calibrated drug coating machine *(Vandana Engineerings, India)*, a weighing scale *(Thermolab, India)*, a microscope and the coating solution. Samples were taken and the weight of the each scaffold was measured and recorded. To facilitate the coating, the scaffold was carefully mounted between two collates, and the coating machine was activated. The required amount of drug coating solution was prepared and transferred to the spray gun cup using a measuring cylinder. Once the drug coating solution was completely transferred from the spray gun cup and applied onto the scaffold, allowing the scaffold for the subsequent convection drying with nitrogen gas. The scaffold was then meticulously detached from the collates, and its weight was determined. These procedures were carried out in accordance with the guidelines outlined in ISO 10993-18:2020, which specifically addresses the chemical characterization of materials in the biological evaluation of medical devices. Following the completion of coating process, the scaffold underwent microscopic examination to assess its quality and coating uniformity. The coated scaffold was subsequently stored inside a pre-identified centrifuge tube. All centrifuge tubes containing authorized drug-coated scaffolds were stored in sterile petri dishes and dried in vacuum desiccators. Once the vacuum process was completed, the weight of the scaffold was measured, and its surface was visually examined. The surface of the coated scaffold was found to be smooth and even. Following the coating process, qualitative tests were conducted on the samples.

**2.5 Delivery System Development for Drug Eluting Scaffold:**

The "Sirolimus Eluting Self Expanding Bioresorbable Braided Peripheral Scaffold" incorporates a conventional retractable sheath delivery system, which employs a highly effective "push-pull mechanism" for precise deployment. This system consists of an 8Fr delivery catheter *(Majik Medical)*, two platinum radio-opaque markers *(Heraeus Group)*, and a 0.018" over-the-wire (OTW) compatible guide wire, all working together to ensure accurate and controlled placement of the scaffold.

During the procedure, the retractable sheath allows for smooth advancement and retraction of the scaffold. By applying a controlled push force, the scaffold is accurately delivered to the target peripheral vessel, ensuring optimal positioning for effective treatment. The retractable sheath is designed to securely hold the scaffold during the delivery process, preventing any premature deployment.

To aid in visualization and positioning, the system incorporates two platinum radio-opaque markers. These markers are strategically placed to provide clear and reliable radiographic guidance, allowing healthcare professionals to precisely monitor the scaffold's placement within the vessel. The markers enhance visibility during the procedure, ensuring accurate alignment and placement.

Facilitating seamless navigation through the vasculature, the system utilizes a 0.018" over-the-wire (OTW) compatible guide wire. This guide wire acts as a reliable pathway, guiding the delivery catheter and scaffold through the target vessel with ease and precision. Its compatibility with the OTW system further streamlines the procedure, enabling a smooth and controlled delivery process.

**2.5.1 Scaffold Loading**

To facilitate the loading process of the scaffold, a nitinol basket (*Manufactured by Meril Life Sciences Pvt. Ltd)* and a funnel are utilized. The procedure begins by placing the stent into the nitinol basket, as depicted in Figure.05. The nitinol basket is connected to the handle's first switch through a thin tube.

Once the stent is securely placed in the basket, the basket is pushed towards the proximal end of the catheter, as indicated in Figure.06. This movement ensures that the scaffold is precisely loaded within the basket, ready for insertion. The handle's first switch is then moved towards the distal end, allowing the scaffold to be loaded within the catheter.

As the scaffold is partially inserted into the catheter, as shown in Figure.07, the funnel can be carefully pulled back. This action reveals the scaffold loading process and allows for better visibility and control during the procedure. The funnel is gradually retracted, ensuring that the scaffold remains securely positioned within the catheter.

Once the scaffold is fully loaded, as illustrated in Figure.08, the funnel is removed entirely. This step completes the loading process, ensuring that the scaffold is ready for deployment. The scaffold is now positioned within the catheter, securely held and protected until it reaches the desired location for deployment.

This loading technique, utilizing the nitinol basket and funnel, provides healthcare professionals with a controlled and efficient method for accurately loading the scaffold into the catheter. By following these steps, the scaffold can be seamlessly prepared and positioned, and ready for the subsequent stages of the medical procedure.

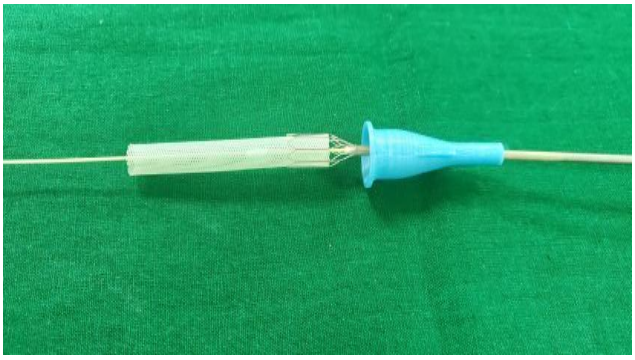


**Figure 5:** The Initial Positioning of the Scaffold

**Figure 6:** The Nitinol Basket is Gently Advanced towards the Proximal End of the Catheter

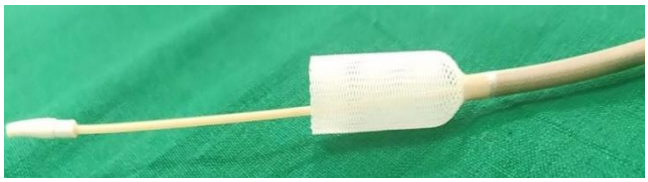


**Figure 7:** The Partially Loaded Scaffold

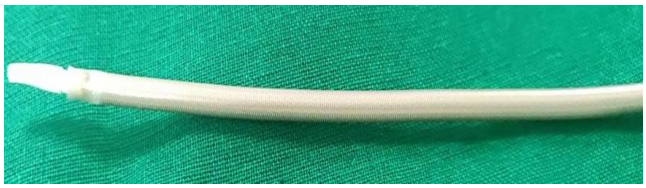


**Figure 8:** The Fully Loaded Scaffold

**2.5.2 Scaffold Delivery Process:**

The delivery process for the "Sirolimus Eluting Self Expanding Bioresorbable Braided Peripheral Scaffold System" is designed to ensure precise placement and deployment of the scaffold at the target site. The procedure typically begins with

the femoral artery access, where an 8Fr retractable sheath delivery system is employed.

To facilitate accurate delivery, an "over the wire" (OTW) technique is utilized. This involves initially placing a guide wire at the desired target site. The guide wire serves as a pathway and reference point for the subsequent delivery of the scaffold system. The guide wire is carefully maneuvered to the target location, guided by fluoroscopy or other imaging techniques such as intravascular ultrasound, optical coherence tomography, angiography etc.

Once the guide wire is properly positioned, the scaffold system is delivered over the guide wire which comprises the self-expanding scaffold, which is housed within a retractable sheath. The sheath is advanced over the guide wire until the scaffold reaches the target site.
At this point, the outer sheath is retracted, allowing the self-expanding scaffold to gradually unfold and conform to the vessel walls. The scaffold's design and properties promote radial force exertion, providing mechanical support to the vessel and aiding in the prevention of restenosis.

Throughout the delivery process, the healthcare professional can utilizes imaging techniques, to monitor and confirm the accurate placement of the scaffold. This ensures that the scaffold is precisely deployed at the intended location, optimizing its therapeutic effects. By employing the retractable sheath delivery system and an OTW technique, the delivery process of the scaffold system offers healthcare professionals a controlled and precise approach to deliver the scaffold at the target site.

## 3. Results and Discussion

To analyze the in-vitro drug release kinetics using HPLC, the following solutions were prepared:

### 3.1 Standard Solution Preparation:

To prepare standard solution, a mobile phase comprising methanol, acetonitrile, and water in the ratio of 65:15:20, respectively. In this solution, 2 mg of the sirolimus drug was gently mixed. To ensure proper dissolution and homogeneity, the mixture was subjected to sonication for five minutes using a sonicator (*Thermolab, India*). Following sonication, the solution was carefully mixed to ensure uniformity and then further diluted with the mobile phase to achieve the appropriate concentration. For subsequent analysis using HPLC, a 2 ml aliquot of this prepared solution was further diluted to a total volume of 20 ml using the mobile phase. This additional dilution step was performed to optimize the concentration range and ensure accurate detection within the HPLC system. To remove any particulate matter or unwanted impurities the mobile phase and sample was passed through a 0.45µm syringe filter before loading into HPLC.

### 3.2 Sample Solution preparation:

To prepare the sample solution for analysis, a single scaffold is carefully placed in a 10 mL volumetric flask and diluted with mobile phase to the desired concentration. The scaffold and mobile phase were mixed thoroughly and the solution was then sonicated for 10 minutes to ensure proper distribution and dissolution of the scaffold in the solvent. After the sonication, the solution was filtered using 0.45µm filter to remove any particles and 20 µl of this solution was injected into the HPLC system for analysis.

### 3.3 HPLC Analysis

The quantification of drug release and drug loading was performed using High Performance Liquid Chromatography (HPLC) (*Shimadzu, Tokyo, Japan*). To analyze the drug content, a C8 column with a particle size of 4µm and a pore size of 80Å was employed. The HPLC system utilized a mobile phase consisting of a mixture of methanol, acetonitrile, and water in a ratio of 65:15:20. This mobile phase composition ensured optimum separation and elution of the drug compound of interest. During analysis, the mobile phase was pumped through the column at a controlled flow rate of 1.0±0.01 ml/min, efficient elution and separation of the drug components. A sample (extracted from scaffold), measuring 20 µl, were injected into the loop of the HPLC system for analysis. The wavelength of 276 nm was selected for detection, as it provided optimal sensitivity and specificity for the drug compound being studied. The HPLC system facilitated the separation and quantification of the drug within a single 15-minute run.

## 4. Statistical Analysis

The experiments were conducted in three replicates under similar condition

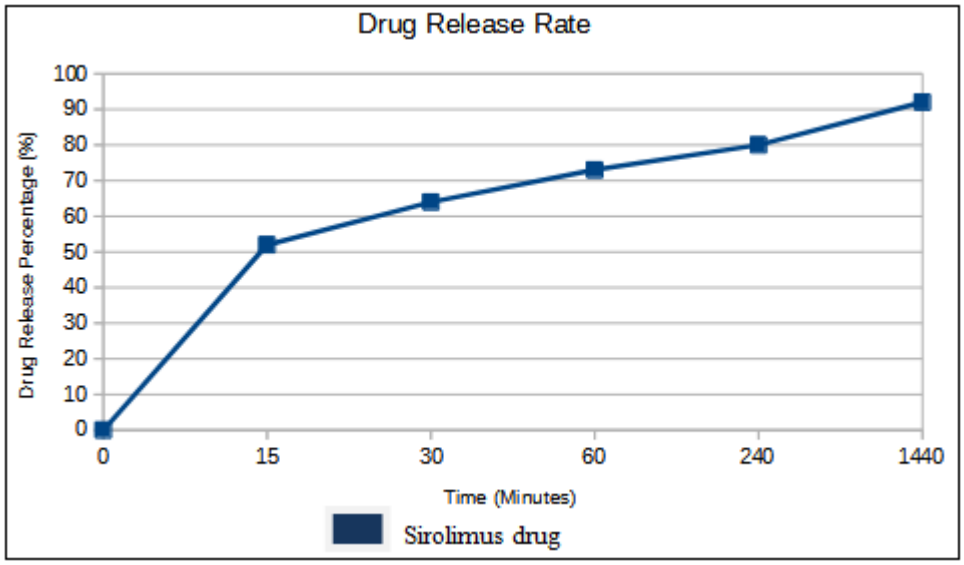
### 4.1 Estimation of Drug Release from the Bioresorbable Scaffold:

Graph.01 illustrates the release profile of sirolimus from a bioresorbable scaffold which was concluded on the basis of HPLC test. The process of incorporating the drug into a polymer matrix or capsule is called drug loading, while the reverse process of liberating drug molecules from the solid phase for absorption and pharmacological action is known as drug release. The drug release rate was evaluated using HPLC. The release of the drug from the scaffold is regulated by an elastomer coating. The proportion of drug released varies over time. Within the first 15 minutes, 52% of the total loaded drug was released, followed by 67% within 30 minutes, 72% within 60 minutes, 83% within 240 minutes, and 91% within 1440 minutes (24 hours). The remaining 9% of the drug is expected to be released over the next 60 days.

Hence, the HPLC analysis of the "Sirolimus Eluting Self Expanding Bioresorbable Braided Peripheral Scaffold System"

reveals that when it would be implanted in human peripheral arteries, the release pattern of the sirolimus drug would closely resembles that observed during the HPLC analysis conducted at normal body temperature. The study predicts that within one day (24 hours) of implantation, approximately 91% of the loaded drug will be released from the scaffold, while the remaining amount will be gradually released over a period of 60 days (2 months). This suggests that the scaffold system has the potential to reduce inflammation within 24 hours, as a significant portion of the drug is released during this time frame. Moreover, it is anticipated that after the complete release of the drug (100%) in 2 months, patients would achieve full recovery from atherosclerotic diseases.

The graph accompanying the statistical data depicts the drug release rate assay and its corresponding duration. This information provides insights into the kinetics of drug release from the scaffold system, indicating a controlled and time-dependent release pattern. The findings suggest that the majority of the drug is released within the initial hours, ensuring an immediate therapeutic response, while a smaller portion is released gradually over an extended period to maintain sustained efficacy. This data is crucial for understanding the behavior of the sirolimus loaded scaffold and its potential clinical applications.




**Graph 1:** The Sirolimus Drug Release Kinetics

## 5. Conclusion

In conclusion, this finding highlights the promising therapeutic potential of the “Sirolimus Eluting Self Expanding Bioresorbable Braided Peripheral Scaffold System” in treating peripheral artery conditions. By providing controlled and sustained release of sirolimus, the scaffold system aims to deliver the drug in a manner that mimics the natural release pattern observed in the body. The rapid initial release of 91% within one day ensures a swift response to inflammation, while the subsequent gradual release over the course of two months ensures prolonged therapeutic effects. This approach holds great promise for patients suffering from atherosclerosis, as it offers the potential for significant relief and full recovery. We have commenced a pre-clinical study, and we anticipate unveiling the results in our forthcoming research article.

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