EXECUTIVE SUMMARY OF THE THESIS

Acute coating transfer from commercial drug-coating balloons: *in vivo* evidence and controlled *ex vivo* experiments demonstrating the impact of contact pressure on drug-coating delivery

TESI MAGISTRALE IN BIOMEDICAL ENGINEERING – INGEGNERIA BIOMEDICA

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1. Introduction

Cardiovascular diseases are the leading cause of mortality both in Italy and globally. According to data collected in 2019, cardiovascular diseases were responsible for an estimated 18.6 million deaths, with an incidence of 55.5 million cases.[1]. Among cardiovascular disease peripheral artery diseases (PAD) are particularly significant. They regard the total or partial occlusion of one or more vessels due to the formation of a fatty plaque. According to collected data PAD significantly increase the risk of further complications, such as coronary artery disease (45%) and the risk of all-cause mortality (60%)[2]. One of the primary causes of peripheral artery disease (PAD) is atherosclerosis, a pathological condition marked by the thickening and narrowing of arterial walls due to plaque buildup.

1.1 Treatments

There are many treatment options for atherosclerosis, each with its pros and cons. The most relevant are Drug-Eluting Stents (DES) and Drug-Coated Balloons (DCB). DES are medical devices made from biocompatible materials that open within the artery to restore vessel lumen. They can be deployed using angioplasty balloons, which expand and press the stent against the vessel wall, or with shape-memory materials that expand autonomously on-site. DES release drugs to prevent restenosis, a key feature as restenosis, a response to arterial injury from balloon inflation leading to neointimal hyperplasia, can narrow the lumen further and is the primary drawback of stents. The effectiveness of DES is closely linked to

their ability to release the drug in a controlled manner over time, maintaining its concentration within the vessel walls below the toxicity threshold but above the therapeutic threshold. Additionally, the drug release level must remain as low as possible to ensure effective treatment of the tunica intima[3]. Currently, the efficacy of drug release from DES needs to be improved, but in general, stents have shown more promising results compared to Percutaneous Transluminal Angioplasty (PTA) techniques. PTA is a minimally invasive therapy that involves inflating a balloon within the vessel where the plaque is located. This technique has shown good results but has limitations related to the detachment of plaque fragments and the risk of restenosis. Today, the traditional PTA technique has evolved to include DCB, which, like DES, are capable of releasing drugs that prevent restenosis.

DCBs are a promising sustained antirestenotic therapy that facilitate a rapid and uniform drug delivery to the vessel wall, enhancing arterial healing and promoting restoration of normal vessel function. DCBs are a therapy that not need permanent implant so have the potential to improve outcomes in treating atherosclerotic lesions[4].

Currently on the market there are many types of DCBs, each distinguished by different combinations of drugs, polymers and carries. The efficiency of these medical devices relies on the specific properties and interactions of each component. Paclitaxel (PTX) and Sirolimus are the two drugs that obtained the best results and are the most used. The PTX is the drug present on the DCB that we used for the study. It is an anti-neoplastic, cytotoxic drug that inhibits cell division and proliferation, this drug is used in DCB treatment for its physiochemical properties and for its lipophilicity that allows it to penetrate the cell membrane and support the antiproliferative action. Polymers and carriers are essential for drug retention during vascular transit, adhesion to the arterial wall, and effective drug transfer and deposition, while minimizing mechanical trauma. An important aspects of DCBs is the use of excipients that are substances incorporated into the DCBs to enhance drug retention during the transit, facilitate adhesion of the drug the arterial vessel wall and promote drug deposition in the tissue [5].

1.2 Problem identification

DCBs have fallen short of their initial promise, failing to provide consistent and efficient drug delivery. In fact, as demonstrated by *Tzafriri et al.* (2020), only 18% of the balloon coating is transferred during inflation, with significant coating loss observed[6]. The bioavailability of the drug released from DCBs is a critical factor, as it depends on the drug's ability to penetrate the vessel wall and remain effective at the site of action. A key limitation of DCB therapy is the high rate of target vessel revascularization (TVR), which exceeds 30% of cases, often necessitating reintervention. This highlights the need for a deeper understanding of acute coating transfer to the mural endoluminal surface, as this process likely plays a crucial role in determining the treatment's efficacy.

2 Aim of the work

Numerous studies in the literature have demonstrated that physical parameters can influence the transfer of coating from DCBs to the arterial wall. However, the specific role these factors play in the coating transfer process remains underexplored. This thesis aims to investigate how such factors affect the coating transfer mechanism by employing various research methodologies, with the goal of enhancing the effectiveness of DCB treatment.

In the initial phase, imaging data from the "DECODE" MSCA project, obtained through Optical Coherence Tomography (OCT) and Scanning Electron Microscopy (SEM), were analyzed to evaluate the acute distribution of coating transfer. Quantification techniques were developed and assessed by comparing the results with bulk drug measurements.

The second phase involved conducting bench-top experiments designed to simulate in vivo conditions of DCB angioplasty using a coaxial machine. These experiments were aimed at investigating the correlation between contact pressure and coating transfer in a controlled laboratory setting supported by *in silico* simulations.

3   Material and methods

3.1   SEM and OCT

In the *in vivo* part of our thesis, we analyzed images obtained from an animal study conducted by CBSET, which involved 8 pigs. During the study, two different types of images were collected: SEM and OCT. Our analysis began with the segmentation of the SEM images. We employed a semi-automated segmentation method using ImageJ. Initially, we marked all coating particles present in the artery using a plugin installed in ImageJ, which enabled us to segment the images morphologically. However, we observed that ImageJ introduced numerous artifacts because it did not always accurately distinguish between coating and non-coating areas. Consequently, we decided to manually correct the images using PowerPoint to address these artifacts and to accurately mark all coating particles. Once segmentation was complete, we used a MATLAB script to merge all the images of a single artery, creating a composite map where the coating was highlighted. We then cropped the background and any holes present in the artery images to isolate only the surface of the artery for coating transfer analysis. Using GIMP, we quantified the coating by counting the red pixels and converting this measurement into mm². Additionally, we generated distribution graphs for each artery using MATLAB to illustrate how the coating was distributed. To validate our results, we compared them with HPLC analyses not performed by us. In order to do it we created a graph to interpolate our values for each artery with those obtained by them. In total, 8 arteries were segmented.
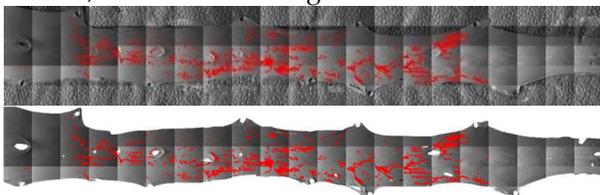

Figure 1 : Shows above the artery 52_3_LSFA before the cropping operation. Under the artery after the cropping treatment

Regarding the OCT analysis, only one artery was segmented. The process began by identifying the lumen of the vessel using SLICER 3D. After this, we divided the lumen into 8 circumferential sectors, with the center located at the midpoint of the lumen. The sectors were numbered from 1 to 8 in a clockwise direction. This division was implemented to assist in the subsequent stages of segmentation and analysis. The segmentation process then commenced. Using PowerPoint, we marked all the particles that exhibited the following characteristics:

- A bump or protrusion on the inner wall of the vessel
- A yellow-lighter stain on the vessel
- A shadowing effect on the vessel

Once all particles with these characteristics were marked, we recorded the sectors containing coating particles in an Excel file. Each image had 8 columns, one for each sector, and we used "yes" and a red color to indicate the presence of coating in the respective sectors. This initial process resulted in a preliminary map.
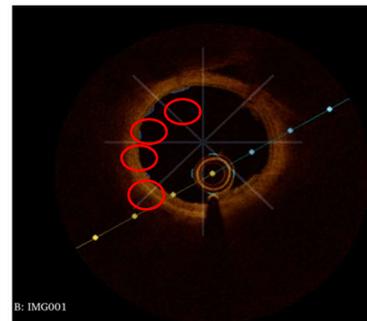

Figure 2 : A segmented OCT image, with the areas identified as coating circled in red.

To enhance our analysis and achieve more accurate results, we decided to improve the process. We recorded the coordinates of each coating particles position on the images. Afterward, we converted these coordinates into polar coordinates and generated a new map, which proved to be significantly more accurate than the previous one.

3.2   *Ex vivo* experiments

For the *ex vivo* part of our study, we conducted a stamping experiment using pig arteries and DCB samples. We began by inflating the balloon with a resin that polymerizes and solidifies after mixing its two components. The resin was used to create solid samples for our benchtop experiments. To enhance the contrast between the coating and the resin, we mixed black powder into the resin. The resin was injected into the balloon using a needle, previously sanded, connected to a syringe and inserted between the balloon membrane and the catheter. Parafilm strips were applied to secure

contact. Before starting inflation, we removed the air, and once a vacuum was created, we injected the resin. After 30 minutes, the resin solidified, allowing us to cut the balloon and obtain the samples.

Figure 3 : The balloon obtained after the resin mixed with powder solidify

To ensure regular samples of 1 cm each, we modified a cutting system, previously designed by other students, by adding a wooden block to stop the blade and absorb vibrations, which had previously caused coating detachment and reduced the surface area of the supports on which the balloon rested. The samples were collected using needles and stored away from heat and light. The artery samples were prepared by other researchers. Regarding the experimental setup, we modified the previous design using SpaceClaim. The first change was made to the upper part of the setup, which needed to hold the balloon. Since we also wanted to conduct sliding experiments, we removed part of the structure that was obstructing the motion. For the bottom part, we needed to fix the plate where the artery rested, and we used double-sided tape for this purpose. We then began the experiment. The experiment started with preconditioning of the samples, either through a sliding experiment, reproducing the contact that occurs during the balloon's delivery to the site, or through a longitudinal stamping experiment, simulating possible contact before the delivery phase. After preconditioning, the samples

underwent the stamping experiment. The force applied during the experiment was determined through simulations, which we modified and executed. Specifically, for the sliding experiment, the simulation replicated the device's delivery, collecting data on the extent of the sliding effect. For the stamping experiment, we based the applied pressure on values reported in the literature for real balloon inflation and converted this into newtons using simulations [7]. In conclusion, the pressure applied in the sliding experiments were $F_{p0} = 0$ atm, $F_{pLow} = 0.1$ atm, $F_{pHigh} = 0.5$ atm; while for the stamping experiments were $F_{p1} = 0.05$ atm, $C_{p1} = 0.1$ atm, $C_{p2} = 0.25$ atm and $C_{p3} = 0.5$ atm. Moreover, we used two different time duration for the experiment $t_1 = 60$ seconds and $t_2 = 3$ minutes.

Throughout the experiment, photos and videos were taken, with particular emphasis on images of the balloon's pre and post interaction, which were later segmented. After completing the experiment, we captured images of all the arteries, first using a microscope that allow us to obtain 3D information about the coating, and then, due to the time-consuming nature of the microscope, we switched

Figure 5 : Above a 3D map representing the coating on the artery, under an artery image collected using microscope with a magnification as 10x
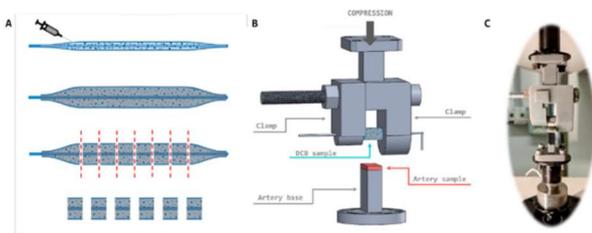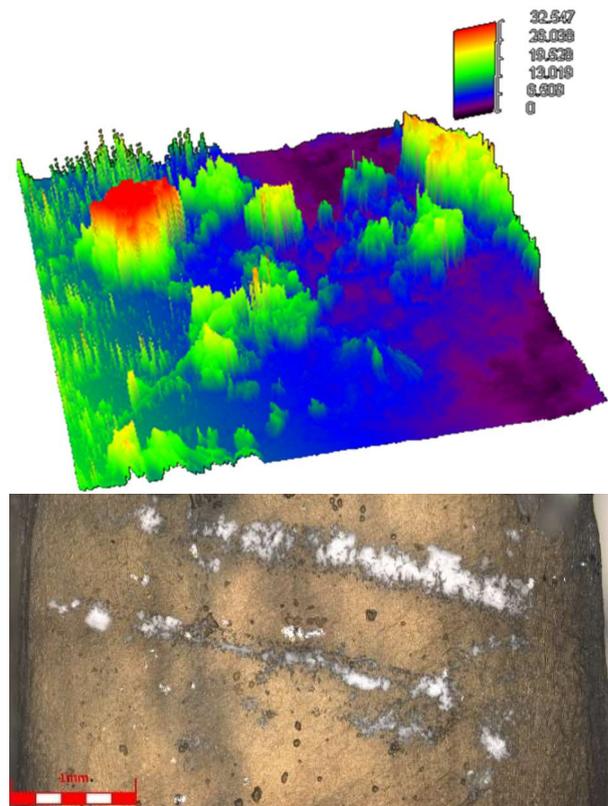

Figure 4 : Experimental procedure for performing the "cylindrical stamping" experiment: A. Steps to obtained DCB samples B. A specialized setup capable of gripping and compressing DCB specimens onto pig arteries is developed. C. The feasibility of the experiment was tested using commercial angioplasty balloons

to a Canon camera, as the results were sufficient for our purposes.

The images were collected and segmented using ImageJ, following the same procedure used for the SEM arteries. By adjusting parameters, we created a mask where all coating particles were highlighted in red.

Once segmentation was complete, we used GIMP to count the red pixels and quantify the coating. After quantification, we created boxplots to observe potential trends. Additionally, we conducted statistical analysis to determine whether differences between the mean values of two experiments, where only one parameter was changed, were significant. The statistical analysis returned a p-value; if it was below 0.05, the difference was considered significant, otherwise not. We used a two-tailed independent samples t-test, as it was the most appropriate for our data. We also attempted to segment the balloon images collected before and after interaction with the artery. The images were segmented using the same method applied to the artery, utilizing ImageJ. As before, we used GIMP to count the red pixels and analyze the collected data. Our focus was on the difference in red pixels between the pre and post interaction images, and we calculated this difference for several balloons.
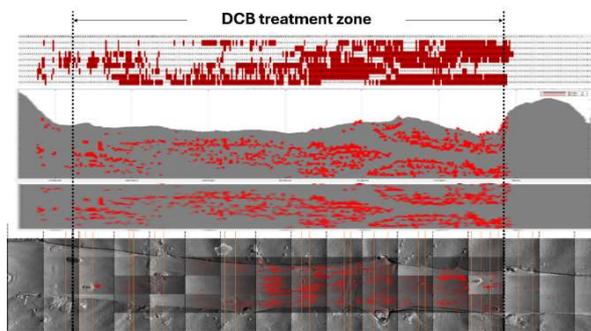

Figure 6 : Shows above the first map obtained form the OCT images, in the middle hte last maps obtained from the OCT. The last image is a SEM artery segmented.

4   Results

4.1   SEM and OCT analysis

The results from the SEM mapping required validation, which was achieved by comparing our findings with the gold standard HPLC analysis conducted by another researcher affiliated with the DECODE network. This comparison confirmed the accuracy of our segmentation approach, as we compared the area of segmented coating (mm²) from SEM maps with the quantity of drug (ng) quantified by HPLC. A statistically significant correlation was observed between these two measurements, as demonstrated by the linear interpolation of the data points. To verify this, we performed a Pearson correlation test, which yielded a correlation coefficient of 0.7845 with a p-value of 0.0212, allowing us to confidently assert that a correlation exists. With regard to the OCT images, a pattern similar to that identified in the SEM analysis was observed: a higher concentration of coating was found in the central region of the artery, with progressively less coating in the distal part. However, in the OCT images, a greater amount of coating was observed in the initial segment compared to what was seen in the SEM analysis. When comparing the OCT and SEM results, we found that the distribution patterns were generally consistent. However, OCT showed a greater amount of coating, which could be attributed to the timing of image acquisition. OCT images were taken immediately after balloon inflation, whereas SEM images were captured after a delay, during which some coating particles might have been washed away.

Additionally, OCT images were acquired at intervals of 0.5 mm, and when coating was detected in a single image, its length was approximated to 0.5 mm. It is important to note that only one artery was segmented using OCT.

4.2   Benchtop experiment

For the benchtop experiment results, we conducted a statistical analysis to compare our findings with those obtained from HPLC performed by other researchers from a collaborating group within the DECODE network. The results can be divided into two categories: one from the vertical stamping experiments and the other from the longitudinal stamping experiments.

In the vertical stamping experiments, as we can see in *figure 8*, we analyzed the mean values associated with different contact pressures and found two statistically significant differences: between Cp1 and Cp2, and between Cp1 and Cp3. We also conducted a time-based comparison for vertical stamping, but the data showed no statistically significant differences between the coating transferred after t1 and t2.

In the longitudinal stamping experiments, we focused on samples subjected to longitudinal stamping under four distinct pressure conditions. Significant differences were observed between Cp1 and Fp1, Cp2 and Fp1, and Cp3 and Fp1. Additionally, a comparison between longitudinal and vertical stamping revealed that a larger interaction surface area resulted in an increased transfer of coating.

As our last analysis we compared coating transfer between the adventitia and intima tissue but found no statistically significant difference in these results. The results obtained from the balloon analysis did not yield any significant findings, as the difference in red pixels between pre and post interaction did not correlate with the number of pixels observed on the artery.

5   Conclusion

Regarding the results obtained from the *in vivo* study, we can suggest that the OCT maps we created may serve as a foundation for future analysis methods. In fact, OCT has several advantages over SEM. OCT images are acquired in a shorter time frame, around 1-2 minutes after the treatment, while SEM images are collected after the animals have been euthanized, which means OCT can provide information closer to the time of balloon application. Furthermore, since OCT images can be collected while the animal is still alive, they could potentially be used in human trials, whereas SEM requires the animal to be sacrificed, limiting its use to animal studies.

As for the SEM maps, we can confirm a good correlation between our segmentation and the results obtained from HPLC, which validates our
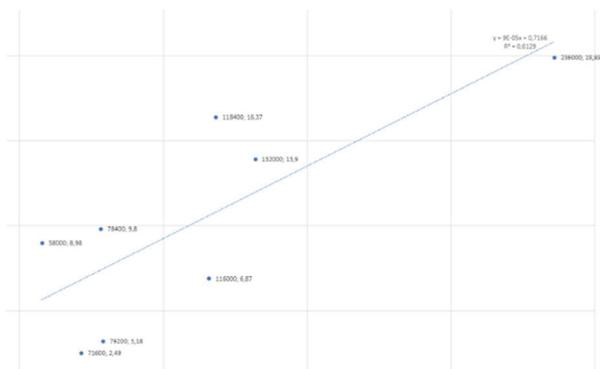
findings as confirmed by the result of the performed correlation test. While discussing the results, we must also acknowledge the limitations of our study. The segmentation process could be improved by adopting an automated method, reducing human error in both OCT and SEM segmentation. Another limitation of the OCT analysis is that we segmented only one artery, so a larger dataset would be necessary to achieve more robust results.

Focusing on the role of contact pressure in coating transfer, our results indicate that higher contact pressure leads to increased coating transfer. The box plot analysis revealed a change in the slope of the transfer curve, with an initial steeper slope followed by a more gradual one. This suggests that the effect of increasing contact pressure progressively diminishes, eventually reaching a plateau. Overall, our findings demonstrate a direct correlation between increased contact pressure and higher levels of coating transfer. A current limitation of this approach is the reliance on manual segmentation, which can introduce human error. Another limitation is that we did not consider blood interaction during our experiments, this aspect needs to be integrated in the future.
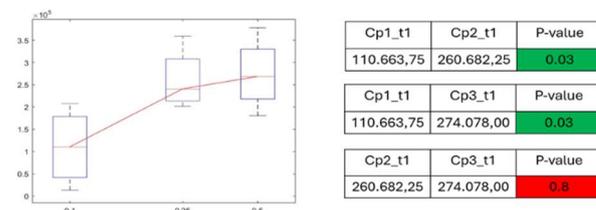

Figure 8 : The boxplot obtained from the data of the experiment on the left, on the right the p-value calculated from the mean value of the experiment

In the future, this study could be useful in inspiring further research on OCT segmentation as an alternative to SEM. The dataset obtained here could also be used to develop an automated segmentation method.

Regarding *ex vivo* part, our 2D segmentation method could potentially replace HPLC for coating transfer analysis, offering the advantage of preserving the integrity of the samples without destructive testing. An important improvement would be the integration of artificial intelligence to automate the segmentation process, enhancing both accuracy and efficiency, our dataset can be used to train the AI to perform the segmentation.

Figure 7 : The graph was on the x-axis we have the amount of coating (ng) on the y-axis we have the number of pixels (mm^2)

6 References

[1] M. della Salute, '29 settembre, World Heart Day'. Accessed: May 24, 2024. [Online]. Available: https://www.salute.gov.it/portale/alleanzaCardioCerebrovascolari/dettaglioNotizieAlleanzaCardioCerebrovascolari.jsp?lingua=italiano&menu=notizie&p=dalministero&id=6353

[2] G. R. Shamaki, F. Markson, D. Soji-Ayoade, C. C. Agwuegbo, M. O. Bamgbose, and B.-M. Tamunoinemi, 'Peripheral Artery Disease: A Comprehensive Updated Review', *Curr. Probl. Cardiol.*, vol. 47, no. 11, p. 101082, Nov. 2022, doi: 10.1016/j.cpcardiol.2021.101082.

[3] F. Bozsak *et al.*, 'Optimization of Drug Delivery by Drug-Eluting Stents', *PLOS ONE*, vol. 10, no. 6, p. e0130182, Jun. 2015, doi: 10.1371/journal.pone.0130182.

[4] H. Ang, T. R. Koppara, S. Cassese, J. Ng, M. Joner, and N. Foin, 'Drug-coated balloons: Technical and clinical progress', *Vasc. Med.*, vol. 25, no. 6, pp. 577–587, Dec. 2020, doi: 10.1177/1358863X20927791.

[5] C. Yerasi *et al.*, 'Drug-Coated Balloon for De Novo Coronary Artery Disease', *J. Am. Coll. Cardiol.*, vol. 75, no. 9, pp. 1061–1073, Mar. 2020, doi: 10.1016/j.jacc.2019.12.046.

[6] A. R. Tzafriri *et al.*, 'Balloon-based drug coating delivery to the artery wall is dictated by coating micro-morphology and angioplasty pressure gradients', *Biomaterials*, vol. 260, p. 120337, Nov. 2020, doi: 10.1016/j.biomaterials.2020.120337.

[7] E. Stratakos *et al.*, 'A Hybrid In Silico & In Vitro Approach To Study Coating Transfer In Drug-Coated Balloon Angioplasty', *Procedia Struct. Integr.*, vol. 49, pp. 30–36, 2023, doi: 10.1016/j.prostr.2023.10.006.