A Bioresorbable Gene-eluting Stent Using Hyperbranched Polymers

Seyed Masih Mousavizadeh[a], Mingzhi Yu[a], Zhonglei He[b], Wenxin Wang[b], Michael Gilchrist[a], Nan Zhang[a]*

School of Mechanical and Materials Engineering, University College Dublin, Ireland
Charles Institute of Dermatology, School of Medicine, University College Dublin, Ireland
*Corresponding author: nan.zhang@ucd.ie , www.nanzhangteam.com

Introduction

Cardiovascular disease (CVD) refers to a group of disorders that affect the heart and blood vessels, posing a significant global health challenge. It encompasses various conditions, including coronary artery disease, heart failure, stroke, and peripheral artery disease, among others. CVD arises from complex interactions between genetic predispositions and lifestyle factors such as smoking, poor diet, physical inactivity, and obesity. The disease is characterized by the gradual accumulation of fatty deposits within the arteries, leading to their narrowing and reduced blood flow to vital organs.

Stenting involves the insertion of a small mesh-like tube, known as a stent, into a narrowed or blocked artery. The stent serves as a scaffold, providing structural support and helping to keep the artery open, restoring proper blood flow to the heart or other vital organs. Despite the initial success of stenting in opening up the blocked artery and improving blood flow, in some cases, the artery can become narrow again over time due to a variety of factors which is called In-stent restenosis (ISR). It can occur as a result of excessive scar tissue formation within the stent, a process known as neointimal hyperplasia. The main issue with the stent is that as long as there is a stent, whether bare metal stent (BMS) or drug-eluting stent (DES), there is the risk of either ISR or thrombosis.

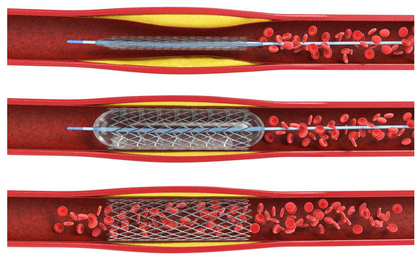

Fig. 1 Schematic of stenting procedure

Bioresorbable platform

A bioresorbable stent, also known as a bioresorbable vascular scaffold (BVS), is a type of stent that is designed to be gradually absorbed and metabolized by the body over time. Bioresorbable stents temporarily (6-12 months) support the treated artery, promoting healing and restoring blood flow. They are commonly used in the treatment of coronary artery disease, where a blockage or narrowing in the coronary arteries restricts blood flow to the heart. These stents provide several potential advantages. Firstly, by gradually resorbing into the body, they eliminate the long-term presence of a permanent metallic implant, which may reduce the risk of long-term complications associated with permanent stents, such as in-stent restenosis or the need for additional interventions. Secondly, the resorption of the stent allows the treated artery to regain its natural function and flexibility, enabling potential future procedures if required.
Magnesium-based alloys have gained considerable attention in the field of bioresorbable stent application due to their biodegradable properties and favorable mechanical characteristics. In this study, we used WE43 Mg-based alloy as it is biocompatible (corrosion products are also biocompatible), and it has good mechanical properties to provide sufficient mechanical support to the treated artery.

The main problem for using Mg-based alloy is that when Mg is degraded, it produces hydrogen (Eq-1). That means we can not have any drug/gene carrier layers on it as it gets peeled off.

Eq-1: $$Mg_{(s)}+2H_2O_{(aq)} \rightleftharpoons Mg(OH)_{2(s)}+H_{2(g)}$$

Therefore, modifications were done to the surface to prepare it as a good platform for maintaining the gene-carrier layer.

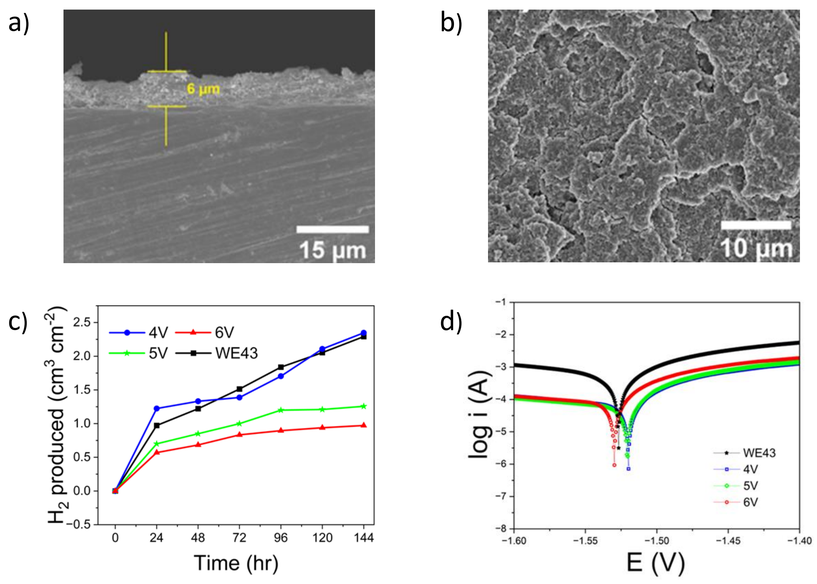

Fig. 2 a) cross-section and b) microstructure of 6V-anodized sample, c) Cumulative hydrogen evolved from the surface of different anodized samples immersed in PBS solution, d) Potentiodynamic polarization curves of WE43 and different anodized samples immersed in PBS solution.

Table. 1 The fitting results of polarization curves obtained from WE43 and al-treated samples in PBS solution.

Sample	E_{corr} (mV)	I_{corr} (µA)	β_a (mV)	β_c (mV)
4V	-1.52	56.19	40.9	188.5
5V	-1.52	55.44	39.0	260.3
6V	-1.53	71.70	39.8	292.5
WE43	-1.53	131.17	84.8	214.3

Table. 2 Roughness parameters measured for anodized samples.

Sample	R_a(µm)	R_p(µm)	R_v(µm)	R_z(µm)
4V	0.4 ± 0.1	1.6 ± 0.2	2.6 ± 0.2	3.5 ± 0.4
5V	0.7 ± 0.1	3.6 ± 0.2	2.6 ± 0.2	4.7 ± 0.4
6V	1.7 ± 0.1	8.8 ± 0.2	7.2 ± 0.2	14.2 ± 0.4

Thus, anodization is advantageous as it presents the following:

- Lower H_2 formation
- Less degradation
- Higher roughness and adhesion
- Higher wettability

Stearic acid was coated on the anodized layer using dip-coating (DC) and ultrasonic atomization spray coating (UASC) to decrease the wettability, which is not desired as water uptake means more degradation. The wettability was significantly decreased using both methods.
Modifying the surface of WE43 with anodization and stearic acid, there was less hydrogen formation and degradation, and the roughness and contact angle were increased. Thus, the surface is ready for maintaining the gene-carrier layer.

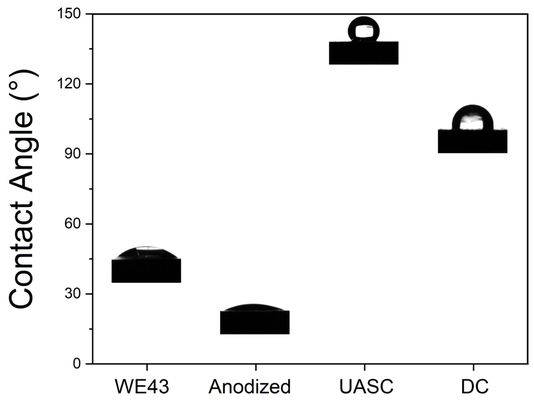

Fig. 3 The contact angle for WE43, anodized, spray-coated, and dip-coated samples.

Polymer nanoparticles synthesis using microfluidic chip

In clinical trial, arterial restenosis has been a major drawback of coronary angioplasty because of injury caused by the stent and transluminal coronary revascularization[1]. How to reduce arterial restenosis after surgery is a big challenge. Researchers used lots of methods to solve this problem: such as using PDGF inhibitors, S-nitroglutathione, add layers and VEFG gene to protect stent[2-6]. We are preparing to add a PCL layer and polymer nanoparticles (with VEFG pDNA) to avoid arterial restenosis (Fig. 3a).
A mixing microchannel are designed (Fig 3b) to synthesis polymer nanoparticles. There are three wells for the microchannel, the left well is inlet 1 to add working solution with polymer, the middle well is inlet to add working solution with pDNA, and the right well is outlet to collect polymer nanoparticles. Through photolithography, PDMS chips were manufactured. 25mM NaOAc buffer with HPAE polymer and 25mM NaOAc buffer with pDNA are pushed into two inlets through injection pumps. After two solutions mix with each other in mixing channel, polymer nanoparticles can be synthesized. The total flow rate is 50 ul/min, 100 ul/min, 200 ul/min, 300 ul/min, and the flow rate ratio between well 1 and well 2 is 1:1. Size and PDI are characterized by DLS, the results are shown in Fig. 3c.

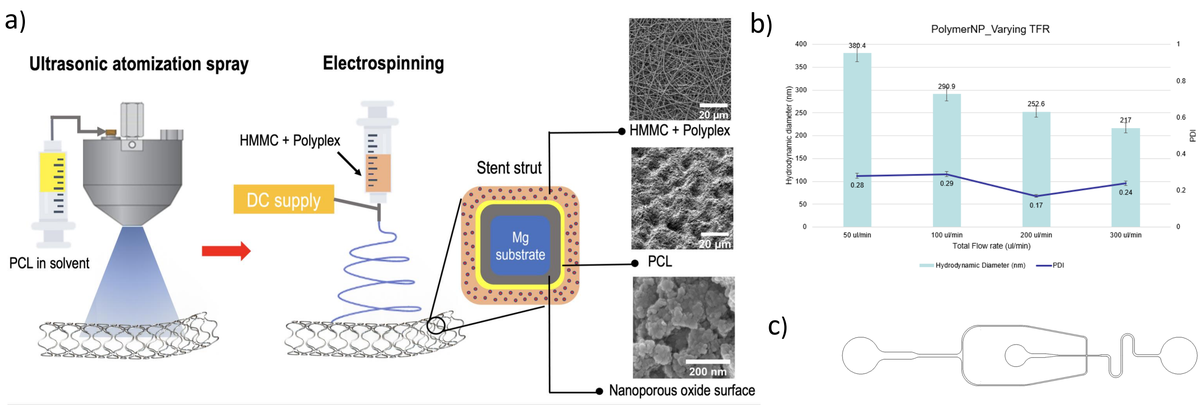

Fig. 3 a) Schematic of multi-layered coating on Mg/Zn metallic core, b) Size and PDI results of HPAE polymer nanoparticles. and c) microchannel,

Transfection of polymer nanoparticles with GFP pDNA

The work flow shown in Fig. 4 is how to do transfection experiment. At day 1, 25k cells/well are added to 96 well plate. After incubate 24 hours at incubator, different polymer nanoparticles solutions are added to 96 well plate. At day 4, transfection results are characterized by fluorescence microscopy. The transfection efficacy results are shown in Fig. 5. These results demonstrate no significant difference between the groups, all of which show good transfection performance.

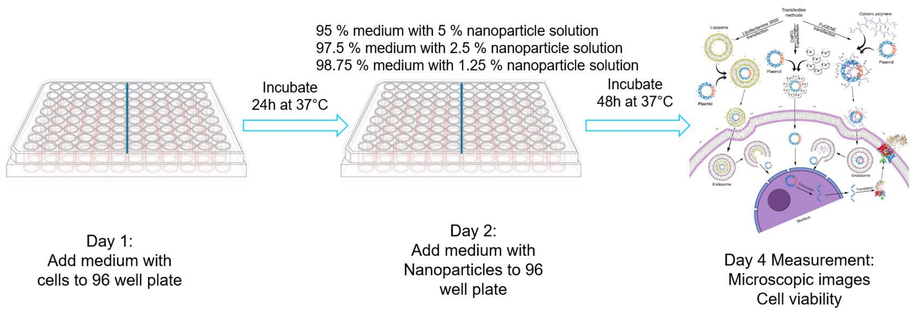

Fig. 4 Workflow of transfection experiments.

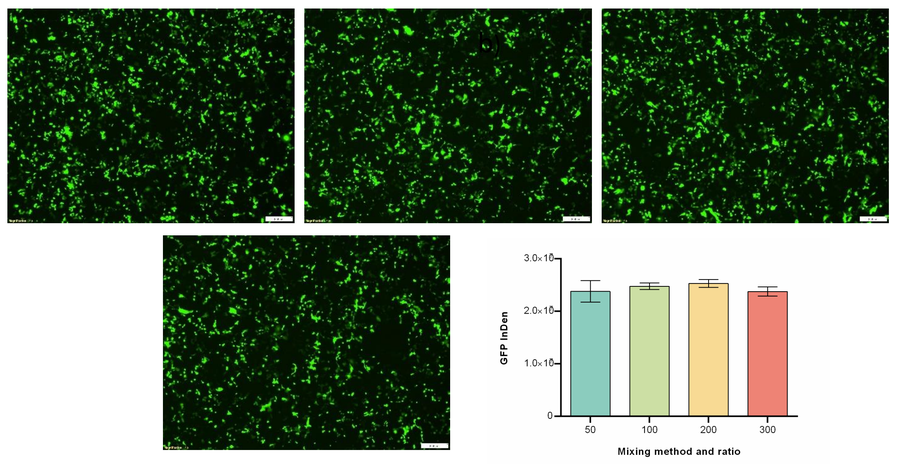

Fig. 4 The transfection efficacy: (a) Microscopic images of HEK293 cells 48 h post transfection; (b) Green fluorescent protein (GFP) expression in HEK293 cells.

Conclusion

In this work, a WE43 Mg-based surface were anodized. After anodization, the surface properties of WE43 Mg-based surface were enhanced. What's more, HPAE polymer nanoparticles are synthesized by microfluidic chips, the size of polymer nanoparticles were from 217 nm to 380 nm. With the increase of total flow rate, the size of polymer nanoparticles increased. HPAE polymer nanoparticles were used to test transfection efficacy. There were no significant difference between the groups.
For next step, we are preparing to add PCL layer to WE43 Mg-based surface. And then, we will add HPAE polymer nanoparticles to PCL layer. Cell viability, confocal microscopy technology will be used to test the efficacy results.

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