Rilevanza delle caratteristiche dei materiali utilizzati per il *gel paving* di stent coronarici medicati

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Sommario

Sommario ..... **I**

Indice delle figure ..... **V**

Indice delle tabelle ..... **IX**

Abstract ..... **XI**

Introduzione **1**

1.1 Malattie del sistema cardiovascolare: l'aterosclerosi _____ 2

1.1.1 Patogenesi dell'aterosclerosi 2

1.1.2 Aterogenesi 3

1.1.3 Principali fattori di rischio e prevenzione dell'aterosclerosi 5

1.2 Le terapie chirurgiche per l'aterosclerosi _____ 8

1.2.1 Bypass aortocoronarico (CABG) 8

1.2.2 Interventi di angioplastica percutanea 9

1.3 Gli stent_____ 13

1.3.1 Approccio meccanico 13

1.3.2 Approccio terapeutico 16

1.3.3 Drug-eluiting stent, DES 17

1.3.4 Meccanismi di rilascio nei DES 20

1.3.5 Strategie diverse di coating 23

1.4 Obiettivi del lavoro di tesi _____ 25

Materiali, apparecchiature e metodi **27**

2.1 Materiali 28
2.1.1 Pluronico F-127 28
2.1.2 Alginati 34
2.1.3 α-Tocoferolo 37
2.1.4 Solfato di rame pentaidrato 38
2.1.5 Acqua distillata 38
2.1.6 Stent 38
2.1.7 Solventi e reagenti 39
2.2. Apparecchiature 39
2.2.1 Bagno termostatatato 39
2.2.2 Texture analyzer 40
2.2.3 Dispositivo Arteria Simulata ("Simulated Artery Device", SAD) 41
2.2.4 Spettrofotometro 41
2.2.5 HPLC 42
2.2.6 Centrifuga 43
2.2.7 Altre apparecchiature 43
2.3. Metodi 43
2.3.1 Preparazione delle soluzioni alginato/pluronico per la formulazione del gel paving 44
2.3.2 Preparazione delle soluzioni alginato/pluronico/etanolo 44
2.3.3 Preparazione delle soluzioni alginato/pluronico/molecola modello veicolata da etanolo per la formulazione del gel paving 45
2.3.4 Preparazione della soluzione reticolante a base di solfato di rame 45
2.3.5 Preparazione dei fluidi simulanti il sangue 45
2.3.6 Gel paving in situ dello stent 46
2.3.7 Prove di erosione in SAD 50
2.3.8 Prove di rilascio in SAD 51
2.3.9.a Test effetto co-soluto: preparazione dei film 51
2.3.9.b Test effetto co-soluto: prove meccaniche 52
2.3.10 Analisi spettrofotometrica 53
2.3.11 HPLC 55

Risultati e discussione 57

3.1 Ottimizzazione del Dispositivo Arteria Simulata (SAD) __ 58

3.1.1 Ridimensionamento SAD 58

3.1.2 Conduzione del dispositivo SAD 62

3.1.3 Dissoluzione di stent commerciale 63

3.2 Prove di carico iniziale e di erosione con gel paving non caricato_____ 66

3.3 Analisi delle proprietà dei mezzi simulanti il sangue e degli alginati_____ 68

3.3.1 Mezzo erosivo e forza ionica 68

3.3.2 Viscosità e pesi molecolari alginati 70

3.4 Studio dei fenomeni erosivi e proprietà di rilascio di una molecola modello lipofila da gel paving caricato _____ 73

3.4.1 Effetto del carico di α-tocoferolo sui fenomeni erosivi del gel-paving 73

3.4.2 Rilascio di α-tocoferolo 76

3.5 Determinazione delle costanti di erosione _____ 78

3.5.1 Determinazione delle costanti di erosione del gel paving non caricato e del gel paving caricato con α-tocoferolo 78

3.6 Studio dell'effetto co-soluto in presenza di solventi per veicolare molecole lipofile_____ 80

3.6.1 Diclorometano 81

3.6.2 Etanolo 81

3.6.3 Analisi meccaniche dei miscele pluronico/alginato/etanolo 83

Conclusioni.....87

4.1 Conclusioni_____ 88

Appendice A.....91

Bibliografia.....95

Indice delle figure

Figura 1 Processo di aterogenesi 5

Figura 2 Evoluzione della formazione di una placca aterosclerotica..... 5

Figura 3 Bypass Aortocoronarico..... 9

Figura 4 Rappresentazione di un intervento di angioplastica coronarica e di un impianto di uno stent 12

Figura 5 Differenti geometrie per stent espandibili con palloncino (a, b, c, d) e stent autoespandibili (e, f, g, h) 14

Figura 6 Rivestimenti di PLLA adibiti al rilascio di farmaco o proteine 15

Figura 7 Schema del meccanismo d'azione sinergico previsto in un DES e aree di competenza..... 16

Figura 8 Confronto tra somministrazione convenzionale di farmaco e sistema di rilascio controllato di farmaco.. 18

Figura 9 Cinetiche di rilascio del farmaco in un dispositivo 19

Figura 10 A sinistra il rivestimento del Cypher stent; a destra il profilo di rilascio in vitro del Sirolimus 22

Figura 11 Schema del Cypher stent per la formulazione di un rilascio lento 22

Figura 12 Profili di rilascio in vitro relativi al Cypher stent..... 22

Figura 13 A sinistra il rivestimento del Taxus Stent; a destra tre differenti profili di rilascio in vitro del Paclitaxel. Con i cerchietti è riportato un rilascio lento, mentre le altre due curve rappresentano un profilo moderato (triangoli) ed un profilo veloce..... 23

Figura 14 Struttura dello stent BiodivYsio..... 24

Figura 15 Sezione della struttura ipotizzata del rivestimento dello stent 25

Figura 16 Struttura di un copolimero pluronico 28

Figura 17 Micella con un farmaco solubilizzato 29

Figura 18 Fasi micellari del pluronico F-127 all'aumentare della temperatura 30

Figura 19 Fiocchi di pluronico F-127..... 30

Figura 20 Rappresentazione della concentrazione critica micellare e di gelazione nei blocchi di copolimeri pluronici 31

Figura 21 Diagramma di fase del Pluronic F127 ricavato da Malmsten e Lindman. I cerchi vuoti indicano la curva di gelificazione, quelli pieni il cloud point 32

Figura 22 Diagramma di fase del Pluronic F127 in presenza di NaCl confrontato con quello in soluzione acquosa semplice 33

Figura 23 Diagramma di fase del Pluronic F127 in presenza di NaSCN confrontato con quello in soluzione acquosa 33

Figura 24 Acido guluronico e acido mannuronico 34

Figura 25 Struttura chimica dell'alginato 34

Figura 26 Composizione percentuale degli alginati in differenti alghe 35

Figura 27 Coefficienti di selettività per due alginati 35

Figura 28 Rappresentazione schematica del processo di gelificazione indotto dallo ione calcio in accordo con la teoria "egg-box". 36

Figura 29 Effetto della concentrazione del catione, rappresentato dalle sfere, sulla struttura del gel: sinistra, modello a basso contenuto di catione; destra, modello ad alto contenuto di catione 37

Figura 30 Molecola dell'alfa-Tocoferolo 37

Figura 31 Stent Cre8™ della CID s.p.a. 38

Figura 32 Bagno termostatato 40

Figura 33 TA.XT Plus Texture Analyzer 41

Figura 34 Schema a blocchi di un HPLC 43

Figura 35 Schematizzazione della ricopertura dello stent con il doppio strato di gel [31] 47

Figura 36 Inserzione dello stent in arteria 47

Figura 37 Sezione del tubo di silicone (a sinistra); disposizione dello stent nel tubo siliconico (a destra) 48

Figura 38 Impianto dello stent nel tubo siliconico 48

Figura 39 Immagine di uno stent prima e dopo espansione 48

Figura 40 Sezione del tubo con stent dopo gelificazione (a sinistra, il colore rosa è dovuto ad un colorante aggiunto per meglio evidenziare lo strato di gel adeso) e successiva reticolazione (a destra, la colorazione azzurra è dovuta agli ioni Cu^{2+}). 49

Figura 41 Schematizzazione dei film preparati 53

Figura 42 Condizioni operative impostate per le prove di compressione 53

Figura 43 Cuvetta in quarzo attraversata da un fascio di luce 54

Figura 44 Dispositivo arteria simulata (SAD) 58

Figura 45 A sinistra i componenti; a destra la baionetta assemblata alle estremità alla quale vengono connessi due tubi di diverso diametro 60

Figura 46 Pompa peristaltica 61

Figura 47 Raccordo a Y 61

Figura 48 Schema del fluido in circolazione nella parte fissa dell'impianto 62

Figura 49 Schema del fluido in circolazione nella parte mobile 62

Figura 50 Dissoluzione del principio attivo in arteria simulata 65

Figura 51 Andamento delle assorbanze del sirolimus in SAD 66

Figura 52 Masse residue dopo erosione nelle tre soluzioni simulanti il sangue 69

Figura 53 Andamento della viscosità ridotta in funzione della concentrazione di polimero nella soluzione di NaCl 0.1 M per i due polimeri analizzati 72

Figura 54 Massa residua sullo stent nel tempo 74

Figura 55 Fitting lineare dei dati sperimentali 75

Figura 56Valori assunti dalla retta di fitting fino a completa erosione del gel 75

Figura 57 Massa erosa [mg] e tocoferolo residuo sullo stent [mg] in funzione del tempo. 77

Figura 58 Andamento del tocoferolo rilasciato nel tempo. 77

Figura 59 Calcolo del rilascio completo del tocoferolo in arteria 78

Figura 60 Schematizzazione della ricopertura di gel adesa allo stent 79

Figura 61 Confronto tra l'erosione una miscela di alginato/pluronico e una miscela alginato/pluronico/etanolo. 82

Figura 62 Confronto sul pluronico non gelato 84

Figura 63 Confronto sulle miscele in condizioni di gelazione termica 84

Figura 64 Confronto sui geli in condizioni di gelazione termica e ionotropica 85

Indice delle tabelle

Tabella 1 Principali fattori di rischio per l'aterosclerosi 7

Tabella 2 Principali parametri dei materiali che potrebbero influenzare la risposta biologica nell'organismo [6] 20

Tabella 3 Valori assunti dai numeri di Re e Wo nei vasi sanguigni 59

Tabella 4 Calcolo del carico teorico sullo stent 64

Tabella 5 Confronto tra le masse residue sullo stent dopo erosione dei tre diversi alginati 67

Tabella 6 Caratteristiche dei mezzi di dissoluzione 68

Tabella 7 Carichi teorici e misurati [mg] di tocoferolo su stent 76

Abstract

The stenosis is an effect of progressive development of the atherosclerosis and consists in the occlusion of small and large artery. Stenosis of arteries over all body can induce health problems but coronary arteries occlusion represents an extremely serious health problem because can lead to angina pectoris and heart attack. These latter effects are often lethal and they constitute the first cause of mortality worldwide.

In order to re-vascularize stenotic coronary arteries, since 1979 it has been applied the surgical technique named Percutaneous Transluminal Coronary Angioplasty (PTCA), to remove, by mechanical action, endovascular plaques.

PTCA involves three main steps: 1) introduction of a deflated balloon by a dedicated catheter into the coronary narrowing area; 2) in situ balloon inflating, and 3) retrieving the catheter following balloon deflation. To overcome the PTCA related problems of re-occlusion (restenosis) and aneurysm of the treated vessel, the angioplasty has been associated with the implant of a stent, an expandable metal tubular mesh (Bare Metal Stent, BMS), that is placed in the artery lumen at the site of stenosis, improving thus the clinical outcome of patients. The main drawback of this surgical practice is an exuberant proliferation of Vascular Smooth Muscle Cells (VSMCs), particularly in small calibre vessels, that can cause the re-occlusion of the treated vessel (in-stent restenosis). With the aim to overcome the excessive VSMC proliferation observed after BMS implantation, devices able to deliver in-situ anti-proliferative drugs have been developed. These devices are named drug-eluting stents, DES, and can be distinguished by drug loading techniques adopted (on stent metallic surface, in stent polymeric coating).

In this work experimental studies on a novel technique to cover coronary implanted stents by a layer of biocompatible polymers (gel-paving in situ operation), also loaded with a model molecule active, are presented. The overall aim is to produce a new medicated stent for

in-situ pharmacological treatments of coronary pathologies focusing the attention on these two main advantages: reduction of side effects due to local drug administration, custom drug dosages.

In particular, in this thesis to test the gel paving resistance to erosion phenomena when it is exposed to fluid flux, the dedicated device, named Simulated Artery Device, SAD, built up in previous research activities, was optimized to better simulate the fluidodinamic of human bloodstream. Furthermore, studies on materials' physicochemical properties involved in testing activities (alginate solution properties, ionic strength of fluids mimicking blood) were performed.

The hydraulic circuit of SAD was re-built up introducing a short silicon piping to simulate the artery tract in which stent implant (and gel paving) was realized, and analyzing the better setting of peristaltic pump flow rate to pump fluid in the circuit achieving suitable laminar flux. These two actions have allowed to reproduce, in SAD, hemodynamic conditions close to the real ones and, moreover, to improve the hydraulic circuit performance as in vitro apparatus (due the reduced dissolution volume, analytical determinations of drug release have been more reliable).

The effects of different physicochemical characteristics of the polymeric materials, used to prepare the drug gel reservoir, were studied. Gel paving is formed by mixing, in water, two biocompatible hydrogels, pluronic F127 and sodium alginate, in defined composition. At 37°C pluronic F127 in water form a thermoreversible gel, soft gel, while sodium alginate in aqueous solution, after gelation ionotropic by bivalent ions, form a structured gel. The gel-paving is a system designed to double layer: soft gel, intended for contact with the arterial wall, and hard gel, consisting of alginate crosslinked with copper cations, which is intended to be exposed to the blood flow.

In particular, the role of sodium alginate's molecular weight in the formation of gel and in the its resistance to erosion was investigated. It was observed that the higher the molecular weight, the better the arranging of ionotropic gel and thus the resistance to erosion phenomena. It was also studied the effect of properties of the fluids simulating the blood on erosion and drug release from loaded gel-paving. It was seen that ionic strength of dissolution medium can influence the erosion rate of the hard gel: the higher the ionic

concentration (assayed by conductometric measurements) faster the hard gel erosion (assayed by erosion tests in SAD).

Furthermore, it was investigated the release profile of a hydrophobic molecule model, the α-tocopherol, that was loaded in the polymer blend. The most interesting observed results have been: absence of co-solute effect (at the tested concentrations) and resistance increase of gel-paving to erosion phenomena (complete erosion of blank gel paving occurred six hours, while 2% α-tocopherol loaded gel paving eroded in ten hours), presumably for a stabilizing effect dell'α-tocopherol. It is important to note that α-tocopherol determination both in residual gel paving and in simulated blood fluid had been quantitative, thanks to the volume optimization of the hydraulic circuit of SAD.

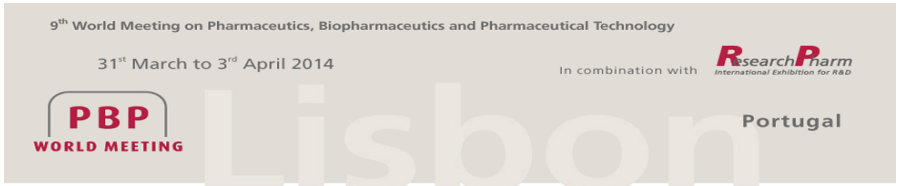
Then a study of possible effects on gelation phenomena due to addition of non-aqueous solvents (to dissolve liposoluble molecules not available in gel formulations) in the pluronic/alginate mixture was performed. It was observed that the addition of ethanol (5% v/v) in the polymer blend does not affect neither thermal gelation nor ionotropic gelation, and that the contact with the blood simulant fluid (an aqueous medium) produces a stiffening effect of the gel paving.

Future research activities could be:

- use of *ex-vivo* animals arteries in the optimized SAD apparatus;
- studies of loading and release of lipophilic active molecules, dissolvable in the mixture pluronic/alginate by ethanol, from gel paving made with coating *in situ*.

Appendice A

Parte dei risultati conseguiti con lo sviluppo del seguente lavoro di tesi saranno presentati a:



Pluronic F127- Alginate blends as gel-paving for coronary drug eluting stent

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INTRODUCTION

Atherosclerosis is a degenerative process of the arteries wall, characterized by the accumulation of fats (lipids) and thickening of the arterial wall, resulting in decreased blood flow, and consequently in the onset of cardiovascular diseases, such as thrombosis or myocardial infarction [1]. Percutaneous transluminal angioplasty (PTA) has been used in the past to treat artery occlusion (stenosis) due to atherosclerotic plaques, by mechanically removing the endovascular plaque. Because of the frequent re-occlusion (restenosis) of the treated vessel, metal stents are usually placed in the artery lumen at the site of stenosis, improving the clinical outcome of patients [2]. The main drawback of this surgical practice is an exuberant proliferation of Vascular Smooth Muscle Cells (VSMCs) that can cause the re-occlusion of the treated vessel (in-stent restenosis) [3]. This problem was overcome by the use of drug eluting stents (DES) which are able to deliver in-situ antiproliferative drugs (. Recently, aqueous mixture of Pluronic F127 and alginate showed a potential application as "paving" for medicated stent (endo-coronary application of a gel layer containing the antiproliferative agent) [4,5]

. The innovative therapeutic procedure provides that, after stent implantation, the PF127/alginate/drugs aqueous solution is injected, by means of a catheter, to the endo-vascular surface achieving gelation of the PF127 due to the body temperature. Subsequently, the inner surface of the gel is rapidly exposed to a bivalent cation solution inducing the formation of a strong alginate gel directly facing the blood. The strong alginate layer is thought to resist to blood flow erosion and to protect, at the same time, the "soft" layer obtained by thermal gelation of PF127

In this work a technique to realize a pluronic-alginate gel covering of stent by simple and repeatable operations at low cost was presented. To test the gel-paving resistance (with and without drug loading) to erosion phenomena when exposed to a fluid mimicking the blood flux, a dedicated device, named Simulated Artery Device, SAD, was built to simulate the human circulatory apparatus.

EXPERIMENTAL

Materials

An aqueous solution of Pluronic F127, PF 127, (18% or 17% w/w, Sigma Aldrich) and Sodium alginate, AL-FMC, (2% or 3% w/w, FMC Biopolymers) was used for gel-paving. PF127 and alginate are biocompatible materials able, respectively, to form reverse thermoresponsive hydrogels (thermal gelation at 37°C) and ionotropic gels (cross-linking by bivalent ions). $CuSO_4 \cdot 5H_2O$ (Sigma Aldrich) was used as cross-linking agent. Commercial stents were used (after expansion they have a length of 13.5 mm and a diameter of 3 mm). To the aim to observe the erosion phenomena of the drug-loaded gel, a model molecules of different solubility, the hydrophilic Vitamin B12 (0.025% w/w) and the hydrophobic α-tocopherol, TC, (1 % w/w) were added to the gel formulation.

Methods

The stent was introduced in a silicone tube mimicking a human artery (internal diameter 3 mm). Then, the PF127/alginate/water (and eventually the loaded molecule) solution was injected in the silicone tube by a smaller tube mimicking the catheter. The solution was subjected to thermal gelation by putting the system tube/stent/solution in a thermostatic bath for 3 min and then to ionotropic gelation by inserting the bivalent cation solution in the silicone tube for 3 min. In this way, the stent was covered by a double polymeric layer: the *hard gel* in contact with the fluid mimicking the blood and the *soft gel* close to the catheter wall.

Simulated Artery Device (SAD)

The Simulated Artery Device is a hydraulic circuit consisting of a fixed section, made of a framework of tubes, and a mobile part, made essentially of a silicone tube (mimicking a coronary artery), which is extractable for plant and stent coating operations. The fluid simulating the blood (pH 7.4, 37°C) is circulated by a peristaltic pump. After the stent coating, the silicon tube is connected to the fixed section and the buffer solution starts to circulate. At determinate intervals, the buffer circulation is stopped, the mobile part is disconnected from the circuit, and the stent is extracted and weighed to evaluate the eroded mass.

RESULTS

A comparison of the paving erosion profiles between two different formulations was done (Figure 1). A large amount of alginate caused a decrease in erosion times from about 11 days to 6 days, thus the concentration of 2% was preferred.

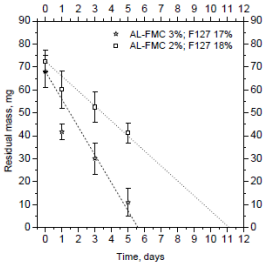

Figure 1. Comparison of residual masses of the *soft/hard* gel between two different gel formulations (AL-FMC 2% and 3%)

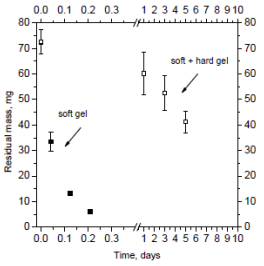

Figure 2. Residual masses profiles of *soft* and *soft / hard* gel (AL-FMC 2%, F127 18%).

In Figure 2 it is worth to note that the in-situ coating technique allows a large amount of gel around the stent of about 75 mg, that keeps high amounts of drug. In absence of alginate cross-linking (only *soft gel*) the gel is washed away in a few hours. Instead, in presence of a cross-linked alginate layer (*hard gel*), the erosive action is much slower. In particular, the gel-paving requires over 10 days to be completely eroded (Figure 2). Figure 3 e shows that the addition of B12 (0.025% w/w) weakens the coating structure provoking a complete erosion in about 6 hours. Instead the presence of TC (1% w/w) affects much less the erosion: time intervals are comparable to those of the gel alone (drug free gel).

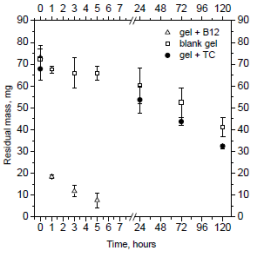

Figure 3. Effect of two molecules (vitamin B12 and α-tocopherol) on gel-paving (*soft/hard* gel) erosion profiles (AL-FMC 2%, F127 18%)

The obtained results showed that the gel-paving was completely eroded in a time of the same order of magnitude of the physiological period required to restore the coronary lesion (subsequent to the atheroma removal) and of a pharmacological therapy to inhibit the in-stent-restenosis pathology.

CONCLUSION

The developed technique of gel-paving allows to obtain the *in-situ* coating of coronary stents with sufficient amounts of *soft gel* in order to load drugs, and *hard gel* able to avoid the washout. The device produced can be a useful tool for conducting reproducible *in-vitro* tests to investigate erosion resistances of pluronic/alginate blends to be used as gel paving in coronary eluting stent.

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