NOVEL BIODEGRADABLE PATCH FOR ATRIAL SEPTAL DEFECT CLOSURE

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ABSTRACT

Since there are still occuring severe complications due to atrial septal defect (ASD) occluder, the development of a suitable ASD device is still in procuring. In the last years researcher focused on biodegradable devices. Accordingly, in this research a degradable occluding patch has been developed, to analyse the ability to solve existing complications. Therefore biodegradable patches were electrospun using medical grade polyurethane (PU) added with bioactive agents, chitosan nanoparticles (ChNP), collagen (Co) and heparin (Hep). The control patch was pure PU. ChNP were added to improve the mechanical properties and bolster the PU.The collagen is expected to provide an extracellular matrix improving cell adhesion and cell growth, serving as a biological sealant of the ASD.

FTIR showed characteristic vibrations of active constituents and changes in the absorbance due to the ingredients. The contact angle analysis demonstrated no significance comparing control and composite patches. The mean values for the PU, PUChCo and coated PuChCo were found as $84.23° \pm 1.06$, $87.62° \pm 3.73$ and $90.42° \pm 1.41$ ($p < 0.05$). Moreover, the structure of the electrospun composite fibres were meticulously displayed through scanning electron microscopy. The decrease in nanofibre diameter (PU: Ø445.7 nm to PUChCo: Ø275.0 nm) between control and composite is due to a change of viscosity of the spinning solution after adding Co. The haemocompatible properties of the patches (PU, PUChCo, PUChCoHep) were inferred through *in vitro* tests, e.g. activated partial thromboplastin time (72.92 s, 70.77 s,103.33 s), prothrombin time (25.73 s, 29.4 s, 35.67 s) and haemolysis assay (3.64 %, 2.39 %, 2.12 %). In conclusion, the developed patch was observed to show desirable properties for an application in an ASD occlusion device.

ABSTRAK

Memandangkan masih berlakunya komplikasi yang teruk disebabkan oleh kecacatan septal atrial (ASD), penciptaan peranti ASD yang sesuai masih dalam pemerhatian. Sejak beberapa tahun kebelakangan ini, penyelidik memberi tumpuan kepada peranti mesra alam. Oleh yang demikian, penyelidikan patch menyekat aliran darah terurai telah dibangunkan bertujuan untuk menganalisis keupayaan penyelesaian komplikasi yang sedia ada. Oleh itu, patch biodegradable telah dielektrospunkan dengan menggunakan gred perubatan poliurethana (PU) ditambah dengan agen bioaktif, nanopartikel chitosan (ChNP), kolagen (Co) dan heparin (Hep). Patch kawalan adalah tulen PU. ChNP telah ditambah untuk meningkatkan sifat-sifat mekanikal dan meningkatkan kolagen PU.Penggunaan kolagen adalah untuk menyediakan matriks extracellular bagi meningkatkan lekatan sel dan pertumbuhan sel and juga berfungsi sebagai sealant biologi ASD. FTIR menunjukkan getaran ciri komponen-komponen aktif dan perubahan berdasarkan kuantiti bahan. Analisis sudut kenalan menunjukkan tiada perubahan ketara berbanding dengan kawalan dan patch komposit. Nilai min bagi PU, PUChCo dan PuChCo bersalut adalah 84.23 1.06, 87.62 3.73 dan 90.42 1.41 (p ¡0.05). Selain itu, struktur gentian komposit elektrospun dapat dilihat melalui mikroskop elektron imbasan. Penurunan diameter nanofiber (PU: 445.7 nm untuk PUChCo: 275.0 nm) antara kawalan dan komposit adalah disebabkan oleh perubahan kelikatan semasa pemintalan dan selepas penambahan Co. Sifat haemokompatible daripada patch (PU, PUChCo, PUChCoHep) adalah melalui dalam vitro ujian, pengakifan separa masa tromboplastin (72.92 s, 70,77 s, 103,33 s), masa prothrombin (25.73 s, 29.4 s, 35.67 s) dan hemolisis assay (3.64%, 2.39%, 2.12%). Kesimpulannya, patch maju telah menunjukkan sifat-sifat yang sesuai bagi penggunaan dalam alat stalemate ASD.

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LIST OF ABBREVIATIONS

AcOH	-	acetic acid
APTT	-	activated partial thromboplastin time
ASD	-	Atrial Septal Defect
ASO	-	Amplatzer Septal Occluder
ASDOS	-	Atrial Septal Defect Occlusion System
Ch	-	chitosan
Co	-	collagen
DMF	-	dimethylformamid
ePTFE	-	expanded polytetrafluoroethylene
FTIR	-	Fourier transform infrared spectroscopy
FUA %	-	dluid uptake rate
Hep	-	heparin
NP	-	nanoparticle
PBS	-	phosphate buffered solution
PLA	-	polylactic acid
PLLA	-	poly-L-lactide
PLGA	-	polylactid-co-Glycolid
PPP	-	platelet poor plasma
PT	-	prothrombin time
PU	-	polyurethane
PVA	-	polyvinyl alcohol
RBC	-	red blood cells
SEM	-	scanning electron microscope
TPP	-	tripolyphosphat
ZnSe	-	zinc selenide

LIST OF SYMBOLS

d	-	collector distance
k	-	wavenumber
W_w	-	weight of the wet sample
W_0	-	weight of the dry sample
NC	-	absorbance of the negative control
HR	-	Hemolysis ratio
P	-	level of significance
PC	-	absorbance of the positive control
TS	-	absorbance of the test sample
U	-	voltage
V	-	volume
$wt/vol\%$	-	weight per volume percentage solution
$wt\%$	-	weight percent
λ	-	wavelength

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CHAPTER 1

INTRODUCTION

1.1 Overview

Congenital heart defects have the largest proportion of organ malformation in neonates. They occur in eight to ten out of every 1,000 children born in the USA. In adulthood even 1 out of 150 is expected to suffer from a congenital heart defect [1, 5]. These heart defects can involve the interior walls of the heart, the valves and the arteries/veins carrying blood to the heart. In general, they have negative effects regarding the normal blood flow through the heart which can lead to severe symptoms. The blood flow might be slowed down, shunt in the wrong direction or blocked completely, which ultimately may lead to death [6, 7]. There are different kinds of congenital heart defects depending on the position where they occur and which anatomical structure they affect, e.g. Pulmonary or aortic stenosis, ventricular septal defect or tetralogy of Fallot. The third most common among these is the atrial septal defect, thus a hole between the left and right atrial chamber. The clinical treatment differs depending on the kind of defect as well as the severity in each special case [8]. The first transcatheter device closure of an ASD was reported by King and Mills in 1976. The transcatheter is nowadays the most common treatment for the ASD defect type 2 called secundum ASD [9, 6].

The major challenge of the transcatheter approach is the biocompatibility of the used implant. The materials need to show high biocompatibility, especially when implanted directly into the blood circuit [10]. Commonly used polymers are mostly bioinert, so they neither evoke a rejection reaction nor show any side effect in favour of the body [11]. In the last years several researches have been conducted to develop not only inert, but biodegradable devices. Consequentially, it is possible to embed drugs in the implants' material. The drugs will be released while the polymer decomposes, so their effect will slowly be applied over a certain time [12, 13].

Among the huge group of biodegradable polymers, which are favourable caused by their mechanical properties and biocompatibility, one possible polymer, which is not only bioinert but biodegradable, is medical grade PU (PU). It degrades without any toxic remaining. Furthermore, solved in dimethylformamid, it is electrospinnable which allows to include bioactive agents or drugs in the produced nanofibres to increase the biocompatibility of the implant directly[14, 15]. Nanofibres characterise themselves by peculiar surface properties, like orientation or fibre diameter, two attributes, which have shown to affect the cellular behaviour of various cells [16, 17]. There are several techniques to obtain nanofibres. The common techniques are melt processing, interfacial polymerization, electrospinning and molecular self-assembly. Electrospinning is one of the facile methods to fabricate continuous nanofibres which provides the possibility to control the fibre diameter, the density of the fibres and the porosity of the membrane [18, 19, 20, 21, 22]. Li et al. used polyaniline with gelatine to electrospun nanofibres. Their research showed persuasive results concerning the growth and proliferation of cardio cells (myoblasts) in rats[21]. The mechanical and electrical properties of the nanofibres and their capability of cell adhesion is depending on the material composition in the electrospinning solution[19]. However, there are numerous researches using the electrospinning method to produce nanofibres for medical uses (carried out). The fabrication of an electrospun bioactive PU patch for the ASD closure will be performed for the first time throughout this research.

1.2 Research background

Although the existing methods for ASD closure show acceptable results, the implant remains as foreign body in the heart and might evoke rejection reactions of the body or other complications. One huge problem is the clotting of the blood and accruing thrombosis. Some other complications are erosion of the surrounding tissue, arrhythmia, fracture of the device arm etc. [23, 24, 25]. Whenever blood interacts with implants the following complications occur:

1. Blood components interaction with surfaces resulting in protein and water adsorption
2. Blood cells interfere with the surface of biomaterial
3. These actions lead to the haemostasis and coagulation [26].

Therefore, a new approach towards biodegradable, bioactive implants may serve as a promising solution for these daunting challenges. The existing patches, which are

already used for the transcatheter implementation, are mostly consisting of a Nitinol frame to obtain the necessary robustness. The frame is filled with a polymer mesh, which varies depending on the used device, e.g. Amplatzer uses polyester. The existing devices evoke some severe complications, e.g. thrombus formation due to the occluder (2.5%) [23], stroke due to late ASO thrombosis as well as recurrent neurologic events (2.6%) [24] and an allergic reaction against nickel (15%) [25].

1.3 Problem statement

The existing ASD occluding devices still show drawbacks regarding several complications after deployment of the implant. Although the existing researches show acceptable results, the implant remains as foreign body in the heart and evokes adverse reactions of the body or other complications. One huge problem is the blood clotting, embolization and accruing thrombosis. Other complications, as mentioned above are erosion of the surrounding tissue, arrhythmia, fracture of the device arm, or toxicity of the used materials [23, 24, 25]. Since the majority of these complications are due to the material there is still a research on the market for an ASD occluder showing less drawbacks. Compared to the existing methods, this research uses a degradable polymer patch to realise a complete decomposition of the implant. The bioactive agents ensure, in the case of chitosan, the stability of the patch and an improvement of the tensile strength of the patch as well as it is favourable for the adhesion of endothelial cells. The heparin might improve the complication evoked by the existing devices, which are the blood clotting and thrombus formation. As heparin is used for urgent anti-coagulation, it may have the same effect as it is deployed slowly over the degradation of the patch. Once the hole is covered by a new, regenerated tissue, the device is no longer needed; thus, it is ideal if the device is fully absorbed by the body when the healing is completed. Furthermore, the ease of use of electrospinning of PU, the low costs as well as the easy available chitosan, collagen and heparin are in favour for the development. Hence, it is a reasonable approach for replacing the existing materials involved in ASD closure.
Statements:

1. Continuous search on the market for new bioactive and biodegradable patches to improve the ASD closure device.
2. Developed patch should possess desirable physico-chemical properties to attain the use in an ASD closure device.

3. The patch used in an ASD device needs to show good blood compatibility.
To solve this issue, several ASD occluding devices have been developed and explored but the research for an universal ASD occluding device, which does not evoke any adverse reactions or long-term complications, is still carried out.

1.4 Objectives of the Study

This research is to propose the development of a novel biodegradable, bioactive patch for congenital atrial septal defect closure. The following are the objectives of the study:

1. To fabricate an electrospun PU composite patch comprising chitosan and collagen coated with heparin (PU-Ch-Co-Hp)
2. To characterize the physico-chemical properties of the developed patch
3. To investigate and compare the *in vitro* blood compatibility of the PU composite patch with PU

1.5 Scope of work

First, the concentration of the PU, the bioactive substances like chitosan and collagen have to be optimised. Further, suitable solvents have to be identified in order to make a homogeneous solution for spinning. In addition, the parameters concerning the spinning such as the applied voltage, delivery rate, target volume as well as the distance between the target and collector have to be optimised. Subsequently, the patches are manufactured and dried for further use. The heparin coating is done at last to obtain a complete composite PU patch.
The second and third steps involve the analyses of the properties of the developed patch. The second part includes the surface characterization of the patch with various physico-chemical analyses and the biodegradability test. The physico-chemical characterisation comprises the measurement of the contact angle, functional group analysis using Fourier transform infrared spectroscopy, surface characterization using the scanning electron microscopy. The weight loss of the patches will be measured in simulated *in vitro* conditions in a defined time span to analyse the degradation rate of the material. In the third part, the blood compatibility will be studied to investigate whether there are any adverse reactions to the composite patch. The following *in vitro*

tests namely activated partial thromboplastin time (APTT), prothrombin time (PT), haemolysis ratio and platelet deposition studies will be performed. By assessing APTT and PT, it the efficiency of the chitosan-collagen and heparin coated PU patches against blood coagulation can be determined. Haemolysis ratio is the measurement of damage incurred to the red blood cells when they come in contact with the PU-composite. The platelet deposition studies will qualify the number of platelets adhered to the surface of the PU-ChNP-Co-Hp patch once the blood interacts with it.

1.6 Significance of the study

One out of a hundred newborn children is affected of a congenital heart defect. In adulthood the number of untreated defects is only little less. There are different kinds of congenital heart defects. One of the most common defects is the ASD. There are several complications linked to an untreated ASD like right heart enlargement, arrhythmia, stroke, hypertension in the pulmonary arteries [9]. The two common ways to treat an ASD are the surgical and the transcatheter occlusion. The transcatheter closure is less invasive and has a shorter time of convalescence [27]. However, there are several complications linked to the existing closure devices, e.g. thrombosis formation, tissue erosion and adverse reactions, which indicates the necessity of further development of the existing devices [23, 24, 25]. Another important point is the reduction of device costs, as well as the long-term treatment costs of post surgery complications, when the above mentioned device failures occur. The new developed patch made of PU-ChNp-Co-Heparin might lead to a better biocompatibility compared to the PU patch. In a broader view it could result in a new bioresorbable product, solving evoked complications by the existing ASD closure devices

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