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Review

In vitro and in vivo cell-capture strategies using cardiac stent technology — A review

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ABSTRACT

Stenosis is a symptom of coronary artery disease (CAD), and is caused by narrowing of arteries in the heart. Over the last several decades, medical implants such as cardiac stents have been developed to counter stenosis. Upon implantation of a stent to open up a restricted artery, narrowing of the artery can reoccur (restenosis), due to an immune response launched by the body towards the stent. Currently, restenosis is a major health concern for patients who have undergone heart surgery for coronary artery disease. Recently, there have been new methods developed to combat restenosis, which have shown potential signs of success. One proposed method is the use of stents to capture cells, thereby reducing immune response. This review will explore the different methods for cell capture both in vitro and in vivo. Biological modifications of the stent will be surveyed, as well as the use of surface science to immobilize biological probes. Immobilization of proteins and nucleotides, as well as use of magnetic field are all methods that will be further discussed. Finally, concluding remarks and future prospects will be presented.

Contents

1. Introduction

As the most prevalent type of heart disease, coronary artery disease (CAD) is characterized by a buildup of plaque in the lining of the arteries [1–3]. This effect, known as atherosclerosis, leads to arterial narrowing (stenosis) [1–3]. Cardiovascular disease is the primary cause of mortality across the globe [1–7]. Over the last several decades, a multitude of solutions to combat stenosis have been developed, which includes coronary bypass surgery, balloon angioplasty and more recently, development of coronary stents [1,5]. However due to the poor biocompatibility of bare metal stents (BMS) and subsequent re-narrowing of the artery (restenosis), drug-eluting stents (DES)

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have been considered a more viable option. Restenosis is a result of intimal hyperplasia, thrombus formation and cell injury during percutaneous transluminal coronary angioplasty (PTCA) [8–12]. Drug eluting stents release a pharmaceutical drug such as sirolimus and paclitaxel at a target site, inhibiting proliferation of smooth muscle cells and inhibiting restenosis [12,13]. On the other hand, DES prevent the natural healing process from occurring [5,13]. DES inhibit proliferation of both smooth muscle cells and endothelial cells [12].

Recently, several reports have proposed the recruitment of endothelial cells to the site of injury and re-endothelialization of stents [14–19]. This has been suggested as a way to reduce neointimal proliferation and stent thrombosis [14–19]. Endothelial progenitor cells (EPCs) are circulating cells in the blood with specific cell surface markers (ie. CD144, VEGF, CD133) [12,20–22]. These cells are capable of differentiating into endothelial cells [12,20–22]. Endothelial cells line the blood vessel and are essential for recovery from injury. EPCs are commonly used as "seeding" cells for endothelialization to occur [12]. It has been shown that in patients with CAD, there are a lower number of EPCs, and these cells have limited function [23]. A low number of EPCs and diminished EPC function has both been implicated in the future development of in-stent restenosis and atherosclerosis [24–37]. Thus far, there have been several strategies for capturing endothelial cells both in vitro and in vivo. The major strategies include use of antibodies, aptamers and cell capture using a magnetic field.

In the following sections, we review each of these strategies in further detail as well as the surface modification that needs to take place on the stent surface in order for the cells to be captured.

2. Use of surface chemistry for immobilization of biological probes

Surface chemistry plays an important role in determining the extent to which circulating endothelial cells are captured by the stent. The development and use of self-assembled monolayers (SAMs) has enabled biological probes to be immobilized on the surface of stents. SAMs are immediately formed upon chemisorption of certain organic molecules onto a solid substrate [38]. They consist of a head group, backbone and end group (Fig. 1). It is the head groups that enable binding of biological probes [39]. These molecular assemblies largely govern the extent to which biological probes are immobilized on the stent surface. A low density of molecules adsorbed on the surface suggests that fewer biological probes can be immobilized onto the substrate.

2.1. Current and potential SAMs for immobilizing biomolecules

In the case of stent technology, a variety of molecules have been used to form a stent coating for immobilization of biological probes. Lee et al. [14] reported the use of a polyethylene glycol (PEG) adlayer on stainless steel stents, for immobilization of VE-cadherin antibody. Through a succinylation reaction, the amine groups at the free end of the PEG polymers were converted to carboxylic groups so that antibodies specific to VE-cadherin surface protein could be immobilized through covalent amide bonds [14].

Furthermore, Li et al. [40] was able to immobilize orientated CD34 antibody probes on the surface of titanium stents using avidin and biotinylated protein A. Initially, the titanium stents were incubated overnight in 1 mg/mL avidin in 0.9% NaCl solution, followed by immersion of samples in 0.1 mg/mL biotinylated protein A in 0.9% NaCl solution. The samples were treated with bovine serum albumin (BSA) prior to binding protein A and antibodies, in order to prevent non-specific adsorption (NSA) on the stent surface. Immobilization of avidin, protein A, and antibodies were verified through immunofluorescent staining. ELISA tests were also used to quantify the amount of CD34 that was immobilized. The mass density of the antibody that was bound specifically to the surface was 168 ng/cm^2 [40].

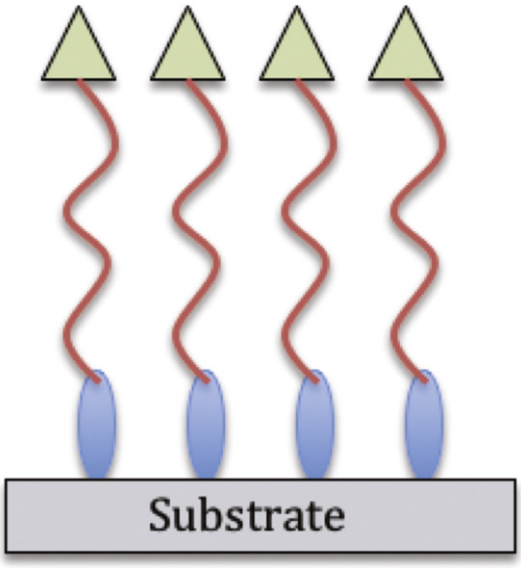

Fig. 1. Schematic of a generic SAM structure. The tail function is represented by the blue circle, the backbone is represented by the red line and the green triangle represents the head group.

Blaszykowski et al. [39] have proposed the use of a new alkyltrichlorosilane surface modifier, for the immobilization of biological probes on substrates. This has the potential to be used as a coating on cardiac stents. Benzothiosulfonate (BTS) has a functionalizable head group that can be used to covalently immobilize biological probes such as antibodies (Fig. 2). In order for the SAM to be formed using this compound, it is imperative that there are distal hydroxyl groups on the surface of the substrate. This is so that the reactive trichlorosilyl tail functions (Cl_3Si–) can form a polysiloxane network and coat the surface of the substrate [39].

2.2. Preventing non-specific adsorption (NSA)

As most biomedical substrates are exposed to serum, NSA is always an issue. This is especially a problem when a stent is implanted into an artery. Sheikh et al. [41] recently observed a novel trichlorosilane molecule (MEG-TFA), has the capacity to reduce NSA on a substrate, upon exposure to undiluted serum (Fig. 3). It contains an internal, single oxygen atom and can be distributed within a SAM. Apart from preventing NSA, it behaves as a 'spacer', allowing the linking molecules of the biological probe to be spaced out. This enhances immobilization of the biological probe onto the surface [41].

The trifluoroacetyl (TFA) group can be cleaved simply by soaking the substrate in a solution of 1/1 (*v*/*v*) methanol and Milli-Q water overnight [41]. This molecule was coated on quartz substrate and undiluted goat serum was passed over it. Using an electromagnetic piezoelectric acoustic sensor (EMPAS), significant differences in resonant frequency between bare quartz, and quartz coated with MEG-OH was identified [41]. This is a novel finding that could very well be used to combat NSA on stents, thereby improving biocompatibility.

3. Cell capture using antibodies

3.1. General considerations of antibody immobilization

Antibodies have been widely applied in biochemical research due to their high specificity and affinity towards antigens [12]. In particular, fragment-antigen-binding (Fab) units of an antibody enable the capture of cells by the stent [33]. In using whole antibody, it is possible for the antibody to become disorientated, when it is immobilized on the