Artificial blood vessels: a study based on the comparison of synthetic biodegradable polymer scaffolds and autografted scaffolds

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1 | Abstract

The purpose of this project is to explore the ways to improve the performance of small diameter vascular prosthesis. This is done with focus on cellularization of a biodegradable polymer scaffold of small diameter size (MSE, NTU). Cellularization of a biodegradable polymer scaffold has been found to improve the outcome of larger grafts by making them less thromobogenic, thus the method is applied to smaller vessels. In addition to using a biodegradable polymer as a scaffold, autografts (a patient's own vascular tissue) can be used as well (Novahep). As with the polymer based, there is still a challenge in producing smaller diameter grafts. The main reason small diameter graft fail is due to compliance mismatch between the host vasculature and the graft, which is found challenging for many artificial vessel types. In order to mimic the native compliance found in vessels, the material composition of the polymer based graft is adjusted. According to the de-cellularized graft, studies reveal that there are many complications related to developing vessels of small diameter, however the procedure is well suited for larger diameter grafts. According to biocompatibility and sustainability, the two grafts show approximately same performance (if considering that both methods are applied with small diameter vessels), but the morphology and cell attachment is different.

Keywords: Vascular prosthesis/graft, tissue engineering, biodegradable polymer, autograft, cardiovascular diseases, thrombosis, and compliance.
2 | Introduction

2.1 Physiological background

The cardiovascular system allows blood flow to transport nutrients, hormones, heat, waste products, carbon dioxide and oxygen, to and from all tissues in the body. Blood is transported away from the heart through arteries and arterioles, and it is transported to the heart through veins and venules, and the circulation is repeated throughout life [1], see an overview of the blood circulation on Figure 2.1. All vessel types have three layers: tunica adventitia (outer layer), tunica media (middle layer), and tunica intima (inner layer). Tunica intima contains three layers; a layer of endothelial cells, a layer composed of mainly collagen, and a layer composed of loose connective tissue. Tunica media has circumferentially arranged layers of SMCs (smooth muscle cells), and further contains elastin, reticular fibres and proteoglycans. Finally, tunica adventitia is primary composed of collagenous tissue and some elastin fibres. [1].

![Figure 2.1: The blood circulation and the vessel types.](image)

The SMCs located in the tunica media are the vessels contractile element that react on the local blood pressure to assure a homeostatic blood flow. If the SMCs are stimulated, vessel vasoconstriction takes place which leads to a decrease in blood flow and an increase in the blood pressure. On the contrary, vasodilation is a mechanism that takes place when the SMCs relax. This gives rise to a widening of the vessel, and blood flow increase [41].
2.2. Medical rationale

Cardiovascular diseases such as atherosclerosis, hypertension and vascular diseases related to diabetes are main causes of death in the Western world, and every year approximately 17.5% of all deaths are caused by them [2]. The diseases cause vascular damage since they result in lack of oxygen and other nutrients in the local area which leads to cell damage and eventually vascular tissue damage [3].

The most common disease that initiates from birth is atherosclerosis. It is the narrowing and hardening of arteries due to the build-up of plaques, which consist of fatty substances, cholesterol, white blood cells and fibrin [4]. The plaque will cause thrombus formation which exacerbates the situation. Thrombosis can eventually lead to myocardial infarction or embolism at other places. One of the common forms of treatment is the coronary artery bypass graft (CABG) surgery. This form of treatment requires using the patients artery or vein from another area as the graft [5]. However, many patients' preexisting vessels are not available as they most often are damaged due to atherosclerosis or harvested [6]. Thus, there is a need for creating artificial vascular prosthesis in order to replace the native vessels.

2.2.1 Tissue engineering as substitute

The cardiovascular diseases cause vascular damage since they result in lack of oxygen and other nutrients in the local area which leads to cell damage and eventually vascular tissue damage [3]. When the vascular tissue is damaged, the need for a new vessel in the local area is necessary. Vasculogenesis (formation of vessels from stem cells during embryogenesis), angiogenesis (formation of new blood vessels) and arteriogenesis (growth of preexisting vessels) are only possible when there is healthy vascular tissue surrounding the local damaged vessels [7].

Tissue engineering has proved to handle this really well, since the growth of vessels can be extracted in an artificial human environment, by allowing the patients' own stem cells to differentiate into desired tissue. The first tissue engineered vessel was created by Weinberg and Bell in 1986 with the inability of the graft to withstand physiological burst pressures [8]. Over the past decades, many studies were done to enhance the technique to acquire the artificial prosthesis for bypass surgery, which among others includes cell-seeded collagen gels, cell-seeded biodegradable synthetic polymer scaffolds and cellular techniques [4]. In this paper, the focus will be on the biodegradable polymer constructs.

The most common biodegradable polymer is the polyglycolic acid (PGA) which degrades
2.2. MEDICAL RATIONALE

through hydrolysis of its ester bond. The rate of degradation could be controlled by
copolymerization ([9]-[10]). Previous work done by Niklason et al. developed a pulsatile
bioreactor to construct a PGA scaffolds seeded with bovine smooth muscle and endothelial
cells ([4],[6]). The resulting vessel was tested and found to withstand the physiological
burst pressure of minimum 2031 mmHg [11]. The patency rate (how long it will maintain
the desired properties) for large diameter (>6mm) vessel often produced promising results
[12] but it is difficult to yield the same results for the small diameter vessels. This is due to
the inner wall becoming thickened by intimal hyperplasia at anastomotic sites - where two
individual small vessels branch and connect (Figure 2.2). The formation of thrombosis
can thereby easily happen due to small diameter vessels that are narrowed ([13]-[14]).

![Figure 2.2: A microvascular blood network showing the small diameter vessels at anas-
tomotic site where there is difficulty in making artificial vessels.](image)

The long term patency of the grafts is influenced by hemodynamics, such as the blood
flow and blood velocity that is affected by the blood pressure. In addition, the vessel
must be compatible with the various blood cells and chemicals in the blood content, so
hemocompatibility is also essential. In furtherance to the blood dynamics, blood vessel
compliance is an important parameter in the long term sustainability of the artificial
blood vessel, and this will be further explained in the following section ([12],[15]). While
most parameters have been discussed in many studies, compliance mismatch is most often
mentioned briefly, however the problem is assessed and studied by an innovative technol-
gy at MSE, NTU, see section 3.2.
2.3. COMPLIANCE

2.3 Compliance

Compliance measures the ability of the graft to return to its original size upon relieving it from expansion of the lumen or the compression of the vascular wall [4]. It is traditionally defined as:

\[ C = \frac{\Delta D}{D P_p} \]  \hspace{1cm} (2.1)

where \( \Delta D \) is the change in internal diameter of the vessel, \( D \) is the internal diameter and \( P_p \) is the pressure of the blood flow [16]. Comparing the differences in compliances between arteries and veins, veins have much higher compliances than arteries due to the thinner vascular walls. This means that veins are a better choice than arteries for CABG.

Investigation done showed that the causes for intimal hyperplasia are mechanical injury and graft-artery compliance mismatch ([17],[18]). Experimental studies done by Abbott WM et al. [19] also suggested that a compliance mismatch will deteriorate the patency rate of the grafts and it could be due to the remodelling of the tissue damaged vessels [17]. Therefore, there is a significant need to match the graft-artery compliance to prolong the patency of the vascular grafts. In most studies, the compliance measured is taken over a short period of time or non-continuously and experiments done 6 days longer will have an error between 10 and 20% [20]. In this paper, we will discuss how compliance tested over a long term will affect the performance of the vascular grafts.
3 | Tissue engineering based on polymers

The biodegradable polymer based artificial vessel can be made with different techniques. This chapter focuses on the techniques used at NTU, MSE. It is important to properly select the biodegradable polymer material, since this must be in compatibility with the native vessels in the human environment - so it must be biocompatible [21]. In addition, the polymer should degrade gradually since it must support the cell attachment and differentiation before it can be inserted, so the polymer choice must be chosen with an appropriate biodegradability rate. The degradation takes approximately 8-10 weeks, which allows the cells to adhere well [5]. The approach that is in focus here is using polymer nano fibers made by melt spinning the polymers. This will give a nano structure that resemble the native extracellular matrix seen in the tissue. Among the polymers that can be used for blood vessels are poly(caproctone) (PCL), poly(L-lactide) (PLA) and poly(glycolide (PGA), and in this case PCL is applied[21], [22].

3.1 Dip-coating

The dip coating technique is a simple approach in getting a biodegradable polymer as a scaffold for the vascular graft [23]. A rod with appropriate diameter (5mm for a small prosthesis) that is desired for the small vessel is dipped in the polymer solution consisting of the PCL in chloroform, which is a quickly evaporating solvent. Usually PCL only undergoes hydrolysis by alkaline treatment, thus the alkaline NaOH is added and the polymer degrades in aqueous solution. After dipping, the rod is set to rest until the polymer is cooled and set. The procedure of coating with the polymer can be repeated until the desired vascular wall thickness is reached.

There are some complications related to the dip coating. First of all the composition of the polymer in relation to composition of the contents in the solution (PCL fibers and chloroform) must be taken in consideration, since this may have an effect on the viscosity and thereby the polymer solutions ability to stick towards the rod [22], [24]. If the solution is too viscous, and the rod is dipped fast in and out, the polymer will not have time to settle and stick to the rod. In addition, if the solution is too thin and timing is well, it will still not have settled properly. The chance is that the graft will be inhomogeneous in topology and thereby the lumen is also of nonuniform diameter. Furthermore, the rod must be rotated uniformly during the coating, since the polymer may drip and cause problems with the structure.
3.2. COMPLIANCE TESTING

Figure 3.1: The melt spinning process that creates thin polymer fibers for the biodegradable polymer scaffold [25].

Figure 3.1 is an illustration of the melt spinning process. It is a process where the polymer is melted in order to get small polymer fibers that assure proper cell attachment and alignment. The polymer is melted and the liquid polymer is filtered to thin fiber structures that are cooled and rolled in the package [25]. In contrast to melt spinning, electro spinning that is usually applied in tissue engineering is not used here, since the fibers created with electro spinning are limited in length and since the cells have been observed to attach better to fibers made by melt spinning [21].

Notably, the polymer used here (PCL) is highly hydrophobic which prevents cell attachment. Therefore, the dip coated polymer vessel must be surface modified to accept cell growth by procedures such as plasma treatment. In this content biological polymers as gelatin or collagen are used [22], [24]. Gelatin has arginylglycylaspartic acid (RGD) groups, which aids in the process of cell attachment through integrins. It has been observed that coating with gelatin is more simple due to gelatin being fast water resolvable and it has shown to be less immunological responding than collagen, which is also more expensive [26].

3.2 Compliance testing

An important aspect of having biocompatible artificial vessels is that they must be balanced according to mimicking the physiological compliance in vessels. This is an issue
3.3. CELLULAR SEEDING

in many previous made small diameter grafts, since they do not possess the desired compliance. Therefore the coated vascular graft is tested for appropriate compliance, where it must mimic the native vessels by resisting a natural blood pressure as well as body temperature and blood flow [27]. The vascular graft must have the right wall shear stress and circumferential stress in order to resist the high blood pressure generated by the heart which is what is tested for. Compared to a regular tensile stress test that tests the graft and its ability to withstand stress applied in the longitudinal direction, this test includes radial force which is the stress sensed by the SMC in the native vessel [28]. In furtherance, the vascular graft should resist several cycles of 'heart beat', since they should be sustainable throughout life. This is unfortunately not observed in existing vascular grafts yet, since they generally will have an increased compliance after applying several cycles of load ([29], [30]). Thereby the vessel wall thickness changes and becomes thinner which is undesirable (the integrity of the vessel decreases). The main target in this research is therefore to create a vessel that maintains a homeostatic compliance throughout the applied stress, and this is optimized by controlling the polymer composition and morphology.

3.3 Cellular seeding

After having created the biodegradable polymer scaffold, gelatin is applied as a surface treating coat. This is done by air plasma treating the PCL nanofibers in order to change the chemical backbone chains of the polymer. Hereafter, the gelatin molecules are covalently grafted to the surface by using hydrolytic (water-soluble) carbodiimide as the coupling agent [24]. The graft thereby becomes hydrophil, which is required for cells to adhere, and the cell seeding can thereby begin.

The stem cells are kept at approximately 37.5 degrees C to mimic body temperature until they are mixed with the enzyme trypsin and edta to assure that they do not precipitate and clot together. Hereafter they are cultured in a medium for the vessels. After counting the number of cells and assuring that they are not stuck together in the solution, the vessel scaffolds are added into a medium containing the cells. Approximately 48 hours later, the cells are beginning to adhere to the scaffolds. With fluorescent light it can be possible to see the cell alignment and if the morphology is appropriate for the initial differentiation into SMCs.

Figure 3.1 shows how the stem cells are aligning and adhering. It is seen that the cells have elongated in nucleus structure. The cells can be converted into desired shape by different techniques, and in this case the technique "cell guidance through surface topography"
3.3. CELLULAR SEEDING

![Image of stem cells adhered after 48 hours, coating with a 3% gelatin solution](image)

Figure 3.2: Image captured that shows the stem cells adhered after 48 hours, coating with a 3% gelatin solution

is used for turning the cells into elongated shape in order to mimic SMCs. This is done by adding the polymer fibres into the melt spinning process and during this, long channels are made along the tubes surface wherein the cells can grow in the channels shape (micropatterning to elongate the cells). It must be noted that this step is essential since further differentiation of the cells relies on cell shape and attachment in this stage. Different growth factors (typically TGF - B1 for SMCs) can be added in order to aim the differentiation, and biochemical substances may also be added in order to increase the ability to become SMCs, but they were not added in this case for the vessels. In order to resemble SMCs it is in interest to have the contractile version which remain in place and stabilize the growth of new blood vessels and help them maintain proper blood pressure throughout contraction/dilation [31]. Depending on the stem cells origin, one could also have used other techniques to mimic endothelial cells which are more circular in diffuse in shape.
4 Tissue engineering based on decellularization

4.1 Decellularization

Decellularization is a process that concerns the removal of cellular and nuclear material through physical, chemical, and enzymatic methods. At the same time the aim is also to decrease any side effects concerning the mechanical properties, biological properties and the composition. Furthermore, it is in interest to preserve proteins related to the extracellular matrix to achieve the right stem cell differentiation. There are several different decellularization methods, however not everything will be described in details, since it is not relevant or the focus of this report. The right method of decellularization and its effectiveness depends on the type of tissue and its composition [32]. Often it is necessary to apply different decellularization methods i.e. chemical and physical in order to achieve the wanted result. It has been shown that biological scaffolds based on decellularization have been inserted and used with positive results both in animals and in humans [32].

4.2 Novahep

Novahep, a company located in Sweden, develops individualized blood vessels, which are based on tissue-engineered scaffolds. The scaffolds are decellularized blood vessels, either an artery or a vein depending on the diseased vessel, from a deceased human being[33]. Decellularization is a process that ensures the removal of all cells and DNA, through media and different solutions. Furthermore, Novahep make use of stem cell technology to plant the stem cells on the decellularized scaffold. The stem cells are harvested through a blood sample from the patient instead of the bone marrow. Furthermore, patients suffering with portal vein thrombosis have had a bypass procedure performed. This illustrates that it is possible to apply the individualized vessels by Novahep [34]. In one paper it is stated that the decellularization of femoral vein segments, harvested from cadavers, was performed by using following solution: 1 % Triton, 1 % tri-n-butyl phosphate (TnBP), and 4 mg/L DNase, furthermore 0.02 % sodium azide and 0.18 % EDTA was added in the solution [35]. The paper also states that the decellularization process was a success with the solution mentioned above and hereby also their decellularization and recellularization techniques, due to growth and development of endothelial [35]. In another paper by Olausson et al it is illustrated and stated that VEGFR-2+/CD45+ and VEGFR-2+/CD14+ cells in the patient’s blood are used to ensure repopulation of decellularized
4.2. NOVAHEP

vascular scaffolds [36]. Furthermore, the scaffolds were clinically evaluated and positive results were seen when transplanted into two patients suffering with extra hepatic portal vein obstruction (EHPVO) [36]. Instead of obtaining the stem cells from tissues or bone-marrow Novahep manages to sample them from the blood. This is done by using peripheral whole blood, which contains endothelial cells and their progenitor cells, and then use it for the recellularization of the decellularized vessel [36]. The decellularization process itself often needs several cycles, in this case nine [36] in order to ensure complete removal of cellular and nuclear material. Electron microscopy is used in order to assure lack of nuclei [36]. At the same time Novahep reports that they are able to conserve essential extracellular matrix proteins such as collagen I, collagen IV, fibronectin and growth factors after the decellularization process. Neo-endothelialization and development of smooth muscle cells were demonstrated [36] and concluded that following cell types have an essential role in the development of the wanted cells: VEGFR-2+/CD45+ and VEGFR-2+/CD14+. 
5 | Discussion

In this section synthetic biodegradable polymer scaffolds and biological scaffolds based on decellularization will be compared and the differences and similarities will be discussed.

5.1 Compliance mismatch

The primary issue related to the small diameter vessels that is assessed here is the compliance mismatch. This is a mechanical problem that is very essential in relation to assuring a biocompatible vessel that mimics and sustains as a native vessel. Since the problem is assessed in terms of mechanics, the testing performed is based on the polymer scaffold, and the compliance test seems very promising since its environment mimics the actual human environment in a large sense. However, in terms of a more biological view, there are also other factors influencing the local blood network consisting of the artificial vessel. Chemical substances as NO (nitric oxide) that is present in the vessel wall have proven to have large influence on blood vessel behavior in terms of SMC dilation/contraction [37]. This could have an effect on the compliance as well, since the compliance is an issue related to changes in the diameter (dilation/constriction) of the vessel.

One main problem in the construction of small diameter vessels is formation of thrombosis, which occurs for both artificial vessel types as long as they are of small diameter. Furthermore, stenosis calcium deposition and infections can also occur at when inserting. With the current synthetic biodegradable polymer scaffold produced at NTU, the main issue with the small diameter vessels is the compliance mismatch. From experiments, NTU found that the vessels are stiff when having compliance of 0 to 2%. From 2 to 6%, the vessels are at the optimized compliance level and anything beyond this range would be considered over compliant. NTU calculates the compliance using the outer diameter of the vessels while other studies did it with just one of the parameters. However, not many studies have actually done the compliance testing using both parameters, it could be possible that using both inner and outer diameter could yield more accurate results. This suggests that the present method of comparing compliance can still be improved.

On the other hand, Novahep has not been stating the reason behind the fact that they are currently not able to produce successful small diameter vessels.
5.2 Cell differentiation

The research on biodegradable synthetic polymer grafts has not yet been able to demonstrate which vascular cell differentiation that is wanted, but Novahep has been able to actually control the differentiation and develop the required cells. In addition, an advantage concerning the biological scaffolds is that there is no side effects of immunosuppressive treatments. Novahep has also developed a new process, where stem cells are harvested from the patient’s blood and there is no need to perform a bone-marrow stem cell operation. Thereby, concerning the control and accessibility of the cells, the biological scaffold seems to have a good advantage. Notably, this is for larger diameter vessels, and it may be considered that the cell control is limited in terms of using smaller diameter vessels. This may be due to the fact that the smaller lumen sized vessels have other properties concerning cell layers - the middle layer (that is primary consisting of SMCs, see section 2.1) is much thinner for small diameter vessels. Thereby, the differentiation may differ, and there could arise other problems when applying Novaheps cell control on smaller vessels.

In terms of cell differentiation and control of this, Novaheps method is very promising since they are able to actually test if the cells are efficient in their specific behavior, which is not the primary focus for the biodegradable polymer based one.

5.3 Proposed solution

Novahep has stated that following cell types VEGFR-2+/CD45+ and VEGFR-2+/CD14+ are essential in the differentiation of stem cells into the desired vascular cells. One proposal is to add these cell types to the synthetic biodegradable polymer scaffold in order to ensure that the seeded stem cells develop into smooth muscle cells and endothelial cells. In relation to the vessel morphology, NTU uses melt spinning to have a curvy surface which allows the cells to attach better, and the addition of the mentioned cell types, this cold possibly assure a better outcome in relation to stem cell differentiation. According to the compliance mismatch, many tests are done before and after cell attachment, but until now the small diameter vessel has not passed several cycles. This suggests that the polymer composition could be changed, but in addition the actual test could be performed by including biochemical factors that influence the vessels, in order to mimic native vascularity, and this could possibly also affect the compliance when tested with the cells attached. In terms of testing the scaffold before cell attachment the current compliance test is sufficient, and the polymer composition could be changed, which is the current method. Furthermore, the plasma treatment could possibly be made differently.
5.3. PROPOSED SOLUTION

Studies revealed that Novahep are able to preserve compounds as collagen, and this has been able to provide sustainable cell attachment, which suggests that collagen which is actually present at native vessels, may be suitable instead of using gelatin. It must be noticed that NTU primary works on the compliance problem, and thereby the issue related to cell attachment may be assessed differently.
6 | Conclusion

In the current project, the process and techniques for the construction of synthetic biodegradable polymer has been presented through literature research and laboratory demonstrations. This includes dip-coating, compliance testing and cellular seeding. Furthermore, scaffolds based on decellularization is another option for patients suffering with vascular diseases in the need of artificial vessels. The company Novahep has been working with scaffolds based on decellularization, and have been able to manage the process in a way that ensures essential cell types and that growth factors remain. These cell types and growth factors seem to be essential, since they aid in the correct differentiation of the stem cells i.e. differentiation into endothelial and smooth muscle cells. Our proposal in creating a scaffold would be to add these essential cell types and different growth factors to the synthetic biodegradable polymer, this might guide the stem cells to differentiate as wanted.

The mechanical problem related to compliance mismatch is also crucial in ensuring the long-term patency rate of the prosthesis graft. The current clinical testings showed that most artificial vessels could not obtain a stable compliance after several cycles of loading. The increasing compliance during the test would lead to the vessels become weaker and eventually burst, which is undesirable. Thus, future work could be done in finding the right composition of the polymers to obtain the optimized conditions for the vessels to be able to resist more cycles of loading. The ultimate goal is to create a vascular graft that can be implanted into the human body and remains sustainable throughout life, which may suggest that some of Novaheps techniques can be applied in relation to cell control.
7 Bibliography


