Doctoral Thesis

Silicone Elastomers for Artificial Hearts: 3D-Printing, Bioactive Glass and Potential

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Silicone Elastomers for Artificial Hearts:  
3D-Printing, Bioactive Glass and Potential

A thesis submitted to attain the degree of

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(Dr. sc. ETH Zurich)

presented by

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2018
To my Family

Die Technik von heute ist das Brot von morgen –
Die Wissenschaft von heute ist die Technik von morgen.

Richard von Weizsäcker
Acknowledgments

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Zusammenfassung


Das menschliche Herz ist ein weiches Organ, das einen pulsatilen Blutfluss erzeugt. Im Gegensatz dazu bestehen heutige Kunstherzen aber eher aus harten Materialien. Dies hat zur Folge, dass diese Systeme die natürliche Bewegung des menschlichen Herzens unmöglich nachahmen können. Falls man ein künstliches Herz jedoch aus weichen Materialien wie Silikonelastomeren konstruiert, könnte es die Bewegung des natürlichen Herzens besser reproduzieren und somit im Vergleich zu bestehenden Systemen einen natürlicheren Blutfluss generieren. Das Ziel von weniger Nebenwirkungen in der Kunstherztherapie könnte durch die natürlichere, weiche Bewegung eines weichen Kunstherzens erreicht werden.


Bioglass® könnte das ideale Material sein um ursprünglich bioinerte Materialien wie Silikonelastomere zu bioaktiven umzufunktionieren. Dies hätte ein verbessertes Einwachsverhalten und möglicherweise reduzierte Nebenwirkungen bei der Kunstherztherapie zur Folge. Bioglass® ist eine Mischung verschiedener Oxide, die die Wundheilung fördern, eine stabile Verbindung zu Gewebe bilden und das Wachstum von Blutgefäßen unterstützen. Es
könnte daher ein passendes Material sein, um Anwachsen von Gewebe bei der Driveline Hautdurchtrittsstelle zu fördern und somit Infektionsraten zu reduzieren.

In Kapitel 2 wird das Konzept eines komplett weichen künstlichen Herzens (sTAH) eingeführt. Im Gegensatz zu bestehende Systemen, die häufig einen nicht physiologischen, kontinuierlichen Blutfluss erzeugen, soll das sTAH das Form und Funktionsweise des menschlichen Herzens nachahmen. Die Pumpe besteht aus weichen Silikonelastomeren und wird mittels einer 3D-Druck, Spritzgusstechnik hergestellt. Die weichen Materialeigenschaften und der Antrieb mit Druckluft führen dazu, dass sich die gesamte Struktur des sTAH während des Herzschlages bewegt und somit eine vergleichbare Situation zum echten menschlichen Herzen schafft. Die Performance des sTAH wurde auf einer modifizierten Hybrid Mock Circulation validiert. Dabei handelt es sich um einen Testaufbau, der das menschliche kardiovaskuläre System fast perfekt simuliert. Das sTAH pumpt gegen 0 bar Gegendruck ca. 4.5 L/min, während es bei physiologischer Vor- und Nachlast 2.2 L/min erreicht. Der präsentierte Prototyp hat unter realistischen Bedingungen jedoch nur eine kurze Lebensdauer von 3000 Schlägen und ist durch zu geringe Flussraten limitiert. Verbesserungen durch eine optimierte Geometrie und eine bessere Materialwahl erscheinen realistisch.


Kapitel 4 setzt die Diskussion der Bioglass®/Silikonmaterialien aus Kapitel 3 fort indem ihr Einwachsverhalten mittels eines in vivo Chorion-Allantois-Membran (CAM) Assays

In Kapitel 5 werden die Erkenntnisse aus den vorangegangenen Kapiteln final zusammengefasst und mögliche nächste Schritte zur Entwicklung eines weichen Kunstherzens aus Silikonelastomeren genannt.
Summary

This thesis describes possible applications of silicone elastomers for artificial heart replacements. It tackles the importance of the materials and their properties for the introduction of the concept of an entirely soft total artificial heart. Furthermore, this thesis discusses the modification of silicone elastomers with bioactive glass particles in order to improve their tissue integration.

Unlike the human heart, which is a “soft” muscle giving pulsatile flow, state of the art artificial blood pumps are built from rigid materials. Such devices cannot mimic the human heart’s function due to material property constraints. The introduction of softness in artificial hearts could be a first step towards an implant, which provides a human heart-like pumping behavior. More physiologically, softly pumped flow could possibly reduce unwanted side effects of existing devices.

However, silicone elastomers already are important materials in artificial blood pump drivelines, the lead connecting the implanted pump with the external power supply. As silicone does not provide reasonable tissue integration, infections occur very frequently at the skin exit site of the lead. Thus, the modification of such silicone elastomer drivelines could be a suitting approach to improve tissue integration and prevent infections.

In Chapter 1 a general introduction to heart failure, its treatment options using artificial blood pumps and the materials thereof is provided. Additionally, chapter 1 introduces bioactive glass, its properties in contact with hard and soft tissue and current and possible future applications. Artificial blood pumps frequently are implanted, because the number of donor hearts for end-stage heart failure patients remains limited. State of the art artificial blood pumps are primarily made of rigid materials, but some parts are also made of soft elastomers. Materials include polyurethanes, silicone elastomers, titanium alloys and even bioprosthetic materials. However, adverse events still occur very frequently and include thrombi, hemolysis and infections. The application of bioactive glasses could be a suitting procedure to render bioinert materials like silicone elastomers more bioactive, possibly improving tissue integration thereof and reducing infection rates during artificial blood pump therapy. Bioactive glasses are mixtures of different oxides, which promote wound healing, formation of stable interfaces between tissue and implants and they promote angiogenesis. Thus, they could be suitting materials to promote tissue adhesion to the driveline and reduce infection.
In Chapter 2 the concept of an entirely soft total artificial heart (sTAH) is presented. In contrast to existing devices, which often do not provide a physiological, pulsatile blood flow, the sTAH shall mimic the human heart in its form and function very closely. The pump is made of soft silicone elastomers and manufactured by a 3D-printing, lost-wax casting technique. Actuated by pressurized air, the entire structure of the sTAH moves during the beat, giving a similar situation as in the human heart. Validated on a modified Hybrid Mock Circulation, a test setup simulating the human cardiovascular system, the sTAH is able to pump 4.5 L/min against zero pressure and 2.2 L/min under physiological pre- and afterloads. Currently limited by a reduced lifetime of 3000 beats under realistic conditions as well as by insufficient flow, improvements by optimizing the geometry and choice of materials seem feasible.

In Chapter 3 the modification of medical-grade silicone elastomers by blending micro- or nanoparticles into the polymer structure is discussed. Silicone elastomers are important materials in medical implants, because they are regarded biocompatible, but also bioinert. Bioinertness prevents reasonable tissue attachment, because cells cannot adhere well to such materials. As silicones are frequently applied as material of artificial heart drivelines, the limited tissue attachment to silicone results in an entry point for germs, causing infection. In order to improve the cell proliferation and tissue attachment to the polymer, it is of considerable interest, whether the simple incorporation of bioactive glass micro- or nanoparticles can improve the situation. Micro- and nanoparticles of Bioglass 45S5® are blended at specific weight concentrations into medical-grade silicone. Mechanical testing revealed the influence of the particles on the mechanical properties resulting in an increased elastic modulus. A cell study with primary human dermal fibroblasts showed significantly improved cell proliferation on the composites compared to pure silicone.

In Chapter 4 the discussion on Bioglass®/silicone composites of chapter 3 is continued. Composites are assessed in vivo using a chick chorioallantoic membrane (CAM) assay. Ammonium bicarbonate was used to give the materials a porous structure and facilitate tissue ingrowth. Histological cuts were scored with a semi-quantitative tissue integration factor, resulting in particle containing composites having significantly improved tissue integration compared to pure silicone. Also nanocomposites showed better tissue integration than microcomposites. The final statement about the application of Bioglass®-containing silicone at the driveline and a true improvement of the situation will have to be assessed in a more complex and realistic in vivo study. However, the ease of manipulation of the silicone and positive results seem to make an application feasible.
Chapter 5 finally gives a general conclusion of this work and offers an outlook on possible future investigations regarding a soft total artificial heart and the general application of silicone elastomers in artificial blood pumps.
1 Materials in Artificial Hearts
1.1 Advanced Heart Failure and Artificial Blood Pumps

Cardiovascular diseases (CVD) are among the most serious medical conditions and account for more than 4 million annual deaths in Europe alone.\textsuperscript{1} Number of deaths by CVDs even exceeds the corresponding numbers of cancer with relative values of 44% and 23%, respectively (\textbf{Figure 1.1}).\textsuperscript{1} One type of CVD is heart failure (HF), the "complex clinical syndrome that results from any structural or functional impairment of ventricular filling or ejection of blood".\textsuperscript{2} The sheer number of 15 million HF patients in Europe alone (2% of the population) combined with a mortality of 25% in the first year after diagnosis represents a complex clinical challenge.\textsuperscript{3}

\textbf{Figure 1.1} Relative and major causes of death in Europe. Adapted from Townsend et al. (2016)\textsuperscript{1}. Note: No data are available for Andorra. Source: WHO Mortality Database.

Unfortunately, the number of treatment options for HF is limited. Patients suffering from mild-to-moderate or severe HF can be treated with drugs, while options for treatment of end-stage HF remain confined.\textsuperscript{4-6} The gold standard for treating end-stage HF would be the heart transplant.\textsuperscript{6} However, the number of donor hearts remains limited with approximately 5000 heart transplantation procedures worldwide in 2015.\textsuperscript{6-7} In relation to the number of patients, the number of heart transplants almost seems negligible. Alternatives are needed, which led to the development of artificial blood pumps or mechanical circulatory support (MCS) devices. MCS devices can be separated into two main categories: total artificial hearts (TAH) and ventricular assist devices (VAD). A TAH replaces the patient’s native heart, while a VAD merely supports the weakened heart. The comparison is depicted in \textbf{Figure 1.2}, illustrating the placement of a TAH (\textbf{Figure 1.2a}) and a VAD (\textbf{Figure 1.2b}). Most often VADs are implanted on the left ventricle in a left ventricular assist device (LVAD) configuration.\textsuperscript{8} Still, severely ill patients may also require biventricular support, which results in two VADs being implanted, one on
each ventricle (BiVAD). Survival rates of BiVAD patients are significantly lower than patients on LVAD support. As the indications for BiVAD are similar to the ones for a TAH, the treatment with a TAH is a viable treatment alternative for biventricular HF. However, Cheng et al. (2016) have shown that BiVAD support still has a better outcome for the patient than treatment with a TAH, even though not significantly. The question remains, why the treatment with a TAH results in even worse outcome than support with two VADs, which were not even developed for the specific application of BiVAD support.

**Figure 1.2** Comparison of the placement of (a) a total artificial heart (TAH) and (b) a ventricular assist device (VAD) in the human patient. The TAH replaces the native heart, while the VAD supports one ventricle, most often the left one (LVAD). Adapted from Berlin Heart GmbH.

The reasons for the comparably limited success of the TAH are diverse. Only one TAH is approved by the Food and Drug Administration (FDA) in the United States and has the CE-mark in Europe. The SynCardia™ TAH (SynCardia Systems, Inc., Tucson, AZ, USA) is a pneumatically driven, pulsatile pump, which completely replaces the patient’s native ventricles and cardiac valves. It is a descent of the Jarvik-7 heart, which was the first TAH, implanted into a human in 1982. The duration of development and the fact that the implanted part of the SynCardia™ TAH has not seriously been refined since its first implantation in 1982 reflects the drama of TAHs. Long timeframes until first-in-man trials, uncertain outcome, high capital requirements and a very consolidated market make research and development on TAHs unattractive.

In contrast, the situation for VADs fundamentally differs from the one of TAHs. Technically, VADs are smaller pumps, which only support one ventricle of the weakened heart. A VAD does not replace the native human heart, but supports and relieves the demand on the patient’s heart. This has diverse advantages in comparison to the TAH. The surgical procedure
is less invasive and today even possible without the heart-lung apparatus. In comparison to the TAH, there are more types of VADs on the market and also frequently implanted. VADs can primarily be distinguished by their mode of pumping: pulsatile versus continuous. Continuously pumping devices are far more frequently implanted than pulsatile VADs (17,000 implantations of continuous-flow devices between 2006 and 2016 in the US compared to approximately 600 pulsatile flow devices in the same timeframe). The number of TAH implantations was approximately 400. Continuous-flow VADs have various advantages compared to TAHs and pulsatile VADs: improved hemodynamics, end-organ function, quality of life, and functional capacity of patients. They also offer a higher quality of life to the patient by being smaller, quieter and more durable, thus possibly being better suited for long-term support. These advantages and the continuous improvements of VAD systems in most cases allow a long term biventricular support, if indicated. Still, the development of a completely implanted TAH remains a key issue in the research on MCSs, because high shear stresses in VADs frequently result in acquired von Willebrand syndrome, bleeding and other adverse events. Indications for TAH implantations also include aortic regurgitation, cardiac arrhythmias, left ventricular thrombus, aortic prosthesis, acquired ventricular septal defect and irreversible biventricular failure requiring high pump outputs. The following two subchapters will give a short overview on current TAH and VAD systems as well as devices, which are currently under development. A special focus will also be put on the materials used.
1.2 Current Artificial Heart Technology

1.2.1 Total Artificial Hearts

As stated above, the SynCardia™ TAH is the only TAH, which is currently available for implantation in the United States and in Europe.14 This artificial blood pump contains of two ventricles, which can be driven independently with pressurized air. Giving a pulsatile blood flow, the SynCardia™ TAH is able to pump > 9 L/min.15 Its placement in the patient is sketched in Figure 1.2a, while Figure 1.3 gives an overview on the working principle of the SynCardia™ TAH. Each ventricle contains of two housings, a base housing and a dome-like blood chamber housing.14 The base housing is manufactured from IsoPlast™, engineered thermoplastic polyurethane, while the blood chamber housing is manufactured from segmented polyurethane solutions (SPUS), overlaid on a polyester velour (Dacron®) mesh.14 The blood chamber is manufactured from SPUS. The diaphragm, separating the blood from the air is made of four independent SPUS diaphragms to provide added safety.14

![Figure 1.3](image)

**Figure 1.3** Profile of one ventricle of the SynCardia™ TAH and the motion of the diaphragm during the cardiac cycle. (a) corresponds to the fill phase and (b) to the ejection phase. Adapted from Slepian et al. (2013).14

The TAH is powered by pressurized air. Both ventricles have its own source, thus requiring two drivelines entering the patient and connecting the TAH with the controller and pressurized air source. This requirement of two drivelines and the use of pressurized air represent two major drawbacks of the SynCardia™ TAH. First, two drivelines increase the risk of infections, which occurs in 77% of all patients and second, the use of pressurized air requires noisy drivers, which reduce the patients’ quality of life significantly.16
Other TAHs are currently under development or in clinical trials. The French CARMAT® TAH is a biventricular, pulsatile, electrically powered device, but hydraulically actuated pump, which shall mimic the natural heart as implanted in the pericardial sac. The use of electricity resolves one major drawback of the SynCardia™ TAH, because reduced noise improves the quality of life of the patients significantly. The goals of development were reduced rates of infection, thromboembolism and bleeding by reshaping the natural contraction of the human heart. It includes several sensors and a control algorithm, which enables the heart to adapt to the special needs of the patient. This makes it far more advanced than the SynCardia™ TAH. The mode of pumping of the CARMAT® TAH is comparable to SynCardia™ TAH. The blood is moved by a diaphragm, which is deployed back and forth. The diaphragm consists of two layers: the blood-contacting side is a bioprosthetic hybrid membrane, which is made of bovine pericardial tissue, chemically treated by glutaraldehyde to achieve hemocompatibility and potentially reduce the need for anticoagulation. The second, hydraulic fluid contacting side of the diaphragm, is made of polyurethane. The static, blood-contacting surfaces of the ventricles are covered with expanded polytetrafluoroethylene. The device was implanted into four patients, who died within 74, 270, 254 and 20 days. Two were able to be discharged from the hospital. Cause of death were device-related in two cases, respiratory failure and multi-organ-failure. Additional TAHs are under investigation through chronic animal trials like the ReinHeart TAH (Reinheart TAH GmbH, Gütersloh, Germany), the continuous flow Cleveland Clinic CFTAH (Cleveland, OH, USA) or the BiVACOR (BiVACOR Inc., Houston, TX, USA).

1.2.2 Ventricular Assist Devices

The two most frequently implanted VADs are the continuous flow devices HeartWare® HVAD (HVAD, HeartWare Inc., Framingham, MA, USA) and HeartMate™ 3 (HM3, St. Jude Medical, Pleasanton, CA, USA). Both systems are centrifugal pumps, which are implanted at the apex of the patient’s heart. The outflow cannulas are connected to the ascending aorta. In both cases, the continuous blood flow is facilitated by a rotor with speeds of 2400 to 3200 rpm or 3000 to 9000 rpm for the HVAD and HM3, respectively. The more modern HM3 also has a function to give an artificial pulse to avoid blood stasis in the left ventricle. Both devices are small enough to also facilitate BiVAD support. The devices are made of rigid metals without the use of elastomeric or soft materials. In case of the HM3, the primary material of construction is the titanium alloy Ti6Al4V. Figure 1.4 gives a simplified sketch of the working principles of centrifugal VADs.
1.2.3 Adverse Events in Artificial Blood Pump Therapy and Clinical Outcome

The role of missing pulsatility in continuous flow VADs remains a key question in the treatment of HF with such devices. Despite uncertainties regarding long-term effects of missing pulsatility, studies suggest, that continuous flow may be associated with aortic insufficiency, and thus inefficient flow and increased risk of thrombus formation.\textsuperscript{38-41} Pulsatile flow may result in an opening of the aortic valve during support, thus possibly reducing the need for anticoagulation therapy as well as reduced rate of thrombus formation.\textsuperscript{38, 42-43} Main adverse events of continuous flow VADs include neurological events, multiple system organ failure, infection, device malfunction and right heart failure.\textsuperscript{20}

As described earlier, the SynCardia\textsuperscript{TM} TAH is a pulsatile pump. However, main adverse events still occur with this device and include infection (77% of patients) as well as bleeding (62%) and neurological complications (27%).\textsuperscript{16} One major contraindication for the treatment with the SynCardia\textsuperscript{TM} TAH was size restriction in the patient. This problem has been mitigated by supplying a device with 50 cm\textsuperscript{3} ventricles rather than the previous 70 cm\textsuperscript{3} ones.\textsuperscript{44} The mode of pumping of TAHs seems more physiological than in VADs, even though there is no hard data, which demonstrate the superiority of a TAH versus BiVAD support.\textsuperscript{45}
1.3 Materials of Artificial Blood Pumps

1.3.1 Polyurethanes

Polyurethanes are a very important class of polymers for state of the art artificial blood pumps. They are used for blood contacting surfaces in TAHs as well as driveline materials in VADs.\textsuperscript{46} They have excellent properties like high tensile strength, high ultimate elongation, good toughness, abrasion and tear resistance, low-temperature performance and resistance to oil and grease. Their biocompatibility and biostability in combination with the ability to tune the hardness without the use of plasticizers make them a material of choice for medical devices.\textsuperscript{47} The property of tunable hardness is based on the nature of the segmented polyurethane molecule as seen in Figure 1.5. Polyurethanes consist of two segments: the hard one is build up from an diisocyanate and a chain extender diol, while the soft segment is build up from a long chain polyol. The long chain, soft segment is responsible for the flexibility of the polyurethane, while the hard segments form physical cross-links, that provide strength, stiffness and rigidity to the material.\textsuperscript{47-48} The amount, the length as well as the type of long chain polymer can be varied, which gives enough degrees of freedom to optimize polyurethanes for the desired application. As an example, polyether-based polyols are very favorable for medical applications due to their hydrolysis resistance, and thus very favorable biocompatibility.\textsuperscript{47} Also, Bélanger \textit{et al.} (2000) have shown, that poly(ether urethanes) are not hemolytic and therefore have an excellent hemocompatibility compared to e.g. poly(carbonate urethanes).\textsuperscript{49} Also, they showed little cell adhesion capabilities.\textsuperscript{49}

1.3.2 Polytetrafluoroethylene

Polytetrafluoroethylene (PTFE) is an extremely important material in the cardiovascular field. PTFE is a semicrystalline fully fluorinated olefin as seen in Figure 1.6. It has a very low friction coefficient, is chemically inert and biocompatible. Thus, it does not react with surrounding fluids and tissue. PTFE does not show any thromogenic or toxic effects, which makes it a suiting candidate as a material for catheters, other medical tubing as well as large vascular grafts.\textsuperscript{47} However, while applied as vascular grafts, the blood contacting surface of PTFE needs to be modified in order to make it more amenable to cell attachment.\textsuperscript{47, 50-51} Expanded Polytetrafluoroethylene (ePTFE) is a special kind of PTFE with oriented polymer strings. This results in a microporous structure, which may be penetrated by air and moisture, but not by water.\textsuperscript{52}
1.3.3 Titanium Alloy Ti6Al4V

Ti6Al4V is used as housing material in artificial blood pumps and other implants like dental implants, heart valves or pacemakers.\textsuperscript{53} Biocompatible alloys need to minimize the rate of release of corrosion and degradation products and tissue response to them.\textsuperscript{53} Ti6Al4V fulfills this requirement by being highly stable against corrosion and degradation. Also, it is well tolerated by blood, which makes it a suiting material for blood-contacting implants such as cardiovascular devices.\textsuperscript{54}
1.3.4 Bioprosthetic Materials

The French CARMAT® TAH uses bioprosthetic materials for its blood-contacting membrane.26 Such materials are similar to those used in bioprosthetic heart valves and shall reduce the need for anticoagulation.26, 28 Bovine pericardial tissue is processed with glutaraldehyde to achieve high levels of biocompatibility, long-term tolerance and hemocompatibility.28-29, 55 Glutaraldehyde prevents collagen denaturation of the bovine tissue by cross-linking the collagen molecules of the tissue. Also, it reduces an immunological response by cell fixation.28 The main goal of bioprosthetic materials is a reduction of thrombogenic events by reduced adhesion/activation of platelets on blood-contacting surfaces of artificial hearts.27, 55 An image of such a bioprosthetic material can be viewed in Figure 1.7, which shows the blood contacting side of the membrane of the CARMAT® TAH after implantation.

Figure 1.7 Image of the bioprosthetic membrane of the CARMAT® TAH during autopsy. No blood clot formation can be viewed. Reprinted from The Lancet, Vol. 386, Carpentier et al., First clinical use of a bioprosthetic total artificial heart: report of two cases, 1556-1563, Copyright (2018), with permission from Elsevier.
1.3.5 Silicone Elastomers

Similarly to polyurethanes, silicone elastomers are highly relevant materials for medical devices. They are used as material for medical tubing like catheters or post-surgery drains, but also for long-term internal implants like pacemaker leads or VAD drivelines. In contrast to carbon-based polymers with its C-C and C-O bonds, the Si-O and Si-C bonds are very strong and longer than the corresponding C-C and C-O bonds. This enables a free rotation about the chain, and thus high flexibility of the polymer. The basic building block of silicone elastomers is siloxane with the most common silicone polydimethylsiloxane (PDMS) shown in Figure 1.8.

In order to form the elastomer, the silicone polymers have to be cross-linked to form chemical bonds between adjacent chains. For the production of medical-grade silicone elastomers, cross-linking by addition is the method of choice, because the chemical reaction does not have any by-products. Catalyzed by a platinum catalyst, a vinyl endblocked polymer reacts with a Si-H group carried by a functional oligomer as seen in Figure 1.9. The formation of cross-linked siloxane polymer chains forming a network is shown in Figure 1.10.

![Figure 1.8 Structure of siloxane as the main building block of silicone elastomers and the structure of polydimethylsiloxane, the most common silicone.](image1)

![Figure 1.9 Scheme of the cross-linking reaction to form silicone elastomers between a vinyl endblocked siloxane and a Si-H group carried by a functional siloxane (crosslinker). The reaction is catalyzed by a platinum catalyst.](image2)
Figure 1.10 Scheme of the addition cure cross-linking process of vinyl endblocked siloxanes with Si-H groups to form a network of interconnected siloxane polymers.

Silicone elastomers are chemically resistant or fairly resistant to most non-chlorinated solvents. They are chemically inert and biocompatible with very low extractables. Also, when tested for thrombosis, coagulation, platelet activation, hemolysis and complement activation, silicone elastomers do not show any adverse events and are hemocompatible.47
1.4 Adverse Event: Infection

One major adverse event, which affects patients treated with an artificial blood pump is infection. During continuous VAD treatment, it is the second most common complication in the first three months post implantation and becomes the most common adverse event afterwards with rates of 1.64 and 0.55 per patient year, respectively. The risk of infection is specifically enhanced at the driveline exit site. As the device needs to be powered, a connection between the implanted pump and the external power supply is required. This connection, the so called driveline, interrupts the protective barrier of the patient’s skin and gives an entry point for germs. A driveline is a percutaneous implant, which constitutes a foreign object. Body mechanisms aim at extrusion of foreign materials from the epidermis, which can finally lead to loss of the implant. The goal of long-term stability of percutaneous implants and prevented extrusion can only be achieved by bypassing these healing mechanisms. A stable and dynamic epidermal-device interface by formation of a fibrous scar is of great importance to anchor the device as well as to close the entry point for germs. As such implants are designed for prolonged lifetime, bioinert materials, which minimize foreign body reactions are the materials of choice. Driveline materials at the driveline exit site include silicone elastomers and polyurethanes. However, such bioinert materials prevent tissue adhesion to the device, and thus anchoring of the implant within the body as well as preventing a barrier against pathogens. Modification of these bioinert materials to promote tissue adhesion to the implant would be an ideal way to form a long-term stable connection between the tissue and the implant and defend against infections.
1.5 Bioactive Glasses and their Properties in Contact with Soft Tissue

Bioactive glasses (BG) are biomaterials, whose application in contact with rigid tissue like bone and teeth has been studied relentlessly since its invention in 1971.\textsuperscript{61-63} BG has properties like high bioactivity, osteoconduction and osteostimulation.\textsuperscript{63-64} Especially its ability to form a direct and stable bond with bone makes BG an ideal candidate for synthetic bone grafts, scaffolds and generally bone regeneration.\textsuperscript{61} Bone bonding of BG is attributed to its hydroxyapatite (HAp) forming ability when immersed in body fluids.\textsuperscript{63-64} The most famous and most widely studied BG is Bioglass 45S5\textsuperscript{®}, a material with a composition of 45\% SiO\textsubscript{2}, 24.5\% Na\textsubscript{2}O, 24.5\% CaO and 6\% P\textsubscript{2}O\textsubscript{5}.\textsuperscript{65-66} BG 45S5\textsuperscript{®} has approval by the FDA and is clinically used in periodontal diseases and in middle ear surgery.\textsuperscript{67} Despite the primary research focus on BG in contact with hard tissue, it also shows properties, which are crucial in contact with soft tissue and for soft tissue regeneration.\textsuperscript{66}

1.5.1 Manufacturing

BG may be manufactured by two different techniques. Using the melt-quenching approach, the desired amounts of oxides are melted together at temperatures above 1300 °C to obtain the desired BG composition.\textsuperscript{61} In contrast, the sol-gel process is chemistry-based. A solution of compositional precursors undergoes chemical reactions to form a gel. Thermal treatment dries the gel to form the rigid BG.\textsuperscript{68} While melt-derived BGs are dense, sol-gel-derived BGs have inherent nanoporosities, which result in specific surface areas of twice the ones of melt-derived glasses.\textsuperscript{69} In order to obtain an amorphous structure, the gels must not be heated above 600 °C during drying.\textsuperscript{61}

Alternative to the traditional melt-derived process, which usually results in monoliths of not smaller than 10 \(\mu\)m, flame spray synthesis may be used to manufacture melt-derived and amorphous BG nanoparticles (\textbf{Figure 1.11}).\textsuperscript{70} This technique also facilitates doping of the BG nanoparticles with e.g. radio-opaque agents, making them visible in X-ray.\textsuperscript{71} The increased specific surface area of BG nanoparticles increases its potential to form HAp as compared to larger particles. Thus, the transformation process of nano-particulate BG to HAp occurs significantly faster compared to micron-sized BG, which makes it significantly more bioactive.\textsuperscript{67, 72}
Figure 1.11 (a) Transmission electron microscopy image of flame spray synthesized Bioglass 45S5® and (b) scheme of flame spray synthesis process. This figure is reproduced in part from Brunner et al. (2006) with permission of The Royal Society of Chemistry and reprinted with permission from Loher et al. (2005). Copyright 2018 American Chemical Society.

1.5.2 Properties and Possible Applications

Multiple studies have investigated possible applications of BG and the underlying basic processes of BG properties. Very early, it was shown that BG leaches into body fluids, which stimulates osteoblast proliferation. Also, it has antibacterial properties by dissolving into body fluids, increasing the pH and killing microbes. Literature suggests that aside its use in contact with hard tissue, BG has multiple properties, which are highly relevant in contact with soft tissue. In an in vivo setting, Wilson et al. (1981) showed that upon implantation of BG subcutaneously, soft tissue grows and adheres to the implant and even remains adherent under shear stress. Today, this bonding mechanism between BG and tissue is relatively well studied. It is based on dissolution and precipitation reactions at the implant interface upon contact with physiological body fluids. The subsequent specific ion concentrations at the implant interface in combination with an increased pH result in the formation of crystalline HAp (Figure 1.12), which governs protein adsorption and subsequent cell attachment. HAp serves as the bonding interface between the tissue and the implant. A second, intracellular interaction upon BG implantation is an interaction of implant surface protein ligands with cell receptors, determining cellular adhesion, differentiation and proliferation. This mechanism is true in contact with hard tissue, but can also be transferred to the concept of soft tissue regeneration or engineering, making BG an interesting candidate in contact with soft tissue. Requirements for
soft tissue engineering are the rapid formation of an interfacial bond between implant and tissue, a stable bond to prevent micromotion at the interface and resulting inflammatory reactions and a stress transfer gradient across the interface to avoid cell resorption. Existing data suggest that BG fulfills these requirements.

Figure 1.12 Scanning electron microscopy image of hydroxyapatite formed on Bioglass 45S5® nanoparticles, manufactured by flame spray synthesis. Reproduced from Brunner et al. (2006) with permission of The Royal Society of Chemistry.

Additionally, it is established that BG promotes angiogenesis and neovascularization, which is highly relevant for soft tissue regeneration. Angiogenesis describes the formation of new blood vessel, which subsequently supply regenerating tissue with oxygen and nutrients and remove waste products. Angiogenesis is governed by various growth factors, including vascular endothelial growth factor (VEGF). BG has an angiogenic effect by directly stimulating cells to increase the secretion of VEGF, thus supporting the formation of new blood vessels at the implant site.

Results suggest that BG promotes and accelerates wound healing. Ostomel et al. (2006) showed that the clotting time of blood decreases when in direct contact with BG particles, which could be important to stop bleeding. However, this property also impedes the use of BG at blood-contacting surfaces. Additionally, BG ointments accelerate the healing process of full thickness skin wounds in diabetic rats, which is attributed to increased fibroblast proliferation, vascularization and granulation tissue growth. Other studies suggest that BG improves wound healing through its ionic dissolution products, and thus reduces the initial inflammatory reaction, resulting in generally increased wound healing rates.
Summarizing, BG seems to have the required properties to accelerate wound healing by being antibacterial and promoting angiogenesis, but also to facilitate the formation of a stable connection between soft tissue and BG. Modifications of bioinert polymers like silicone elastomers or polyurethanes with BG, in order to promote a stable connection between a soft implant material and the patient’s tissue, could be an interesting concept. One possible applications would be the driveline of artificial hearts in order to possibly reduce infection rates during the treatment with an artificial heart.
1.6 Conclusion

This thesis tackles two separate issues, faced in the treatment with artificial blood pumps. Both topics combine a strong focus on materials choice and materials modification. The first issue tackles the design of TAHs by introducing the novel concept of an entirely soft TAH. As mentioned earlier, currently most implanted artificial blood pumps are VADs with their continuous blood flow and acceleration of the blood using rotors. Even for patients, who suffer from biventricular heart failure, the use of two VADs is often preferred rather than implanting a TAH. However, the long-term effects of missing pulsatility remain unknown. Continuous blood flow is associate with adverse events like aortic insufficiency and thrombus formation. In contrast, the established SynCardia™ TAH has a pulsatile blood flow. However, due to its rigid materials and in comparison to the “soft human heart”, it cannot provide a physiological blood flow. Thus, a novel type of an entirely soft TAH is presented in chapter two. The soft TAH mimics the human heart in its form and function and shall possibly reduce the number of adverse events associated with current, rigid devices. The soft TAH is made of silicone elastomer entirely and manufactured by a 3D-printing, lost-wax casting technique as presented earlier. Driven by pressurized air, the soft TAH provides a pulsatile fluid flow similarly to the SynCardia™ TAH. However, in contrast to the SynCardia™ TAH, the entire structure of the soft TAH is moving during the beat, resulting in a similar pumping behavior as the human heart, and thus possibly giving a more physiological blood flow than current TAHs and VADs.

The second issue tackles the difficulty of bioinertness of silicone elastomers. The driveline is frequently manufactured from silicone elastomers. At the exit site, the Achilles heel of clinical artificial blood pump therapy, infections occur very frequently. Silicone is a bioinert material, which does not support cell attachment and reasonable tissue biointegration. A long-term stable connection between the silicone of the driveline and the tissue of the patient is required to prevent infections. In chapters three and four, the possible application of BG 45S5® to improve the bioactivity of medical-grade silicone elastomers, is discussed. Various studies suggest increased wound healing properties of BG 45S5® as well as antibacterial effects and improved connections between implants and tissues. Different types of BG 45S5® particles were blended into medical-grade silicone elastomers and tested in vitro and in vivo. The results give a first estimation, whether the simple manipulation of silicone elastomers by incorporating BG particles can significantly improve the polymer properties in contact with tissue, while keeping the mechanical integrity of the elastomer.
2 A Soft Total Artificial Heart – First Concept Evaluation on a Hybrid Mock Circulation

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* These two authors contributed equally to the publication.
2.1 Introduction

The number of deaths from cardiovascular diseases keeps on growing, reaching a share of approximately 30% of all deaths worldwide. Heart failure (HF) concerns more than 26 million people worldwide.\textsuperscript{93} Even in developing countries, the number of HF patients is continuously increasing.\textsuperscript{95} The necessity to treat HF and the shortage of donor hearts led to the development of mechanical circulatory support devices, such as ventricular assist devices (VADs) and total artificial hearts (TAHs).

VADs are implantable mechanical pumps that support the heart of HF patients. They can be placed in the left, right or both ventricles but mostly in the left ventricle (LV). Left VAD (LVAD) treatment has dramatically evolved over the last decade, reaching a number of 5,000 devices/year, with 1- and 2-year survival rates equal to 80% and 70%, respectively.\textsuperscript{94} However, adverse events, such as hemolysis and thrombosis as well as infections, still occur and prevent VADs from being established as the gold standard for HF treatment. Prolonged hospital stays resulting from adverse events dramatically increase the cost of the therapy. Therefore, the elimination of these complications needs to be addressed in the future. Furthermore, the quality of life of VAD patients may be significantly improved by minimizing the number of adverse events.

One major adverse event is right ventricular (RV) failure following LVAD implantation. A range of 10% to 50% of all LVAD-patients suffers from postoperative RV failure and when medical therapy does not suffice to support adequately the RV, temporary or permanent mechanical support is required.\textsuperscript{95} In patients with biventricular heart failure primary biventricular VAD (BiVAD) support may be necessary. However, the survival rates of BiVAD support remain significantly lower than the survival rates of patients receiving merely LVAD support.\textsuperscript{11-12}

An alternative treatment option for long-term support of both ventricles is the TAH. A TAH is a single device with two integrated pumps. Today, the SynCardia\textsuperscript{TM} TAH (SynCardia Systems Inc., Tucson, AZ) is the only pulsatile TAH which is approved by the Food and Drug Administration (FDA) in the United States as a bridge to transplantation therapy and has acquired the CE mark in Europe. Thus far, more than 1,400 TAH implantations have been conducted. The 1-year survival rate of TAH patients is approximately 60-79%, but on average still much lower compared to LVAD patients.\textsuperscript{16,94} However, several TAHs are currently under development, aiming at further improving the TAH treatment. The CARMAT\textsuperscript© TAH (CARMAT, Velizy Villacoublay, France) is under clinical investigations, while the ReinHeart TAH (ReinHeart TAH GmbH, Gütersloh, Germany), the continuous flow Cleveland Clinic
CFTAH and the BiVACOR (BiVACOR, Inc., Houston, TX) are under evaluation through chronic animal trials.\textsuperscript{26, 31-33}

All existing VADs and TAHs consist of many mechanical parts, being thrombogenic when in contact with blood. Therefore, in order to prevent thromboembolic complications, both VAD and TAH treatment, require anticoagulation therapy. The risk of thromboembolism and the need for anticoagulation therapy with the inherent risk of bleeding complications are one of the major backdraws of current VAD and TAH systems.\textsuperscript{96}

Recently, the field of soft technologies has driven considerable attention.\textsuperscript{97-99} In this field different completely soft pumps have been presented, which are manufactured by a 3D-printing, lost-wax casting technique.\textsuperscript{89-90} This process allows the low-cost production of pumps of silicone monoblocks with complex chamber design, without any seams and mechanical parts. Schumacher \textit{et al.} (2014) presented a combustion-driven soft pump, which showed human heart-like pumping characteristics.\textsuperscript{89} In the current study, we present a novel concept of a pneumatically-driven TAH, which is made of one silicone elastomer monoblock, and thus is completely soft. This technology aims at replicating biomimetic motion of soft muscular systems using bending, twisting extension and flexion.\textsuperscript{99-100} Thus, the soft TAH (sTAH) should mimic the human heart from a physiological and physical motion point of view, yielding pulsatile blood flow.\textsuperscript{100} The study is structured as follows. First, we explain in detail the design and production process of the sTAH. Then, the evaluation process of the sTAH on a hybrid mock circulation (HMC) is described. Third, the resulting performance of the TAH during pre- and afterload experiments is presented. Finally, the overall performance of the TAH is analyzed and its potential as a future HF treatment option is discussed.

\section*{2.2 Experimental}

\subsection*{2.2.1 Design of the soft total artificial heart}

\textbf{Figure 2.1} shows the general design procedure of the sTAH. We used a computer aided design (CAD) file of a real human heart in order to copy its physiological form (\textbf{Figure 2.1a}). As a first step, we used a CAD software (NX8.5, Siemens, Germany) and the respective CAD-file of a healthy human heart. This helped to design the outer form of the sTAH with a similar shape as a real human heart, excluding the aorta, the pulmonary artery, the pulmonary and caval veins (\textbf{Figure 2.1b}). In order to reduce the complexity of the system, we designed the chambers without atriums, thereby creating two ventricles with sizes of 144 cm\textsuperscript{3} and 83 cm\textsuperscript{3} for the LV and RV, respectively. In comparison to the human heart, we placed the inflow to the heart
chambers at the posterior aspect as the artificial heart does not comprise atriums. The diameter of the inflow to the ventricles were chosen as 24 mm. We placed the outflow (diameter 30 mm) from the ventricles in anatomic correct positions to facilitate connection to the aorta and the pulmonary artery (Figure 2.1c). The pneumatically driven expansion chamber (EC) was placed between the ventricles in order to allow simultaneous pumping from both chambers (Figure 2.1d). This addition of another chamber reduced the volumetric size of the right ventricle. Four ports to the EC with diameters of 6 mm were added. These are used for the in- and deflation of the EC (Figure 2.2). Two ports to the EC were placed at the apex, while the two remaining ones were placed at the back of the heart. The EC contained four ports, because the sTAH was initially designed to be run by combustion, which required four ports. Table 2.1 gives the volumetric sizes of the sTAH. Additional detailed sketches of the sTAH, including wall thicknesses and dimensions are available in Appendix A.1.

![Figure 2.1 Illustration of the design procedure for the soft total artificial heart (sTAH). The computer model of a human heart (a) is used to give the outer shape of the sTAH (b). This shape is used as a basis and extended by adding chambers (d) and in- and outflows to and from the chambers, which gives the final form of the sTAH (c).](image)

<table>
<thead>
<tr>
<th>Description*</th>
<th>Volumetric size / cm³</th>
</tr>
</thead>
<tbody>
<tr>
<td>sTAH</td>
<td>679</td>
</tr>
<tr>
<td>LV</td>
<td>144</td>
</tr>
<tr>
<td>RV</td>
<td>83</td>
</tr>
<tr>
<td>EC</td>
<td>70</td>
</tr>
<tr>
<td>sTAH minus chambers (silicone only)</td>
<td>382</td>
</tr>
<tr>
<td>Main Body</td>
<td>592</td>
</tr>
<tr>
<td>Main body minus chambers (silicone only)</td>
<td>294</td>
</tr>
</tbody>
</table>

*LV: Left Ventricle; RV: Right Ventricle; EC: Expansion Chamber.
2.2.2 Production of the soft total artificial heart

The sTAH was prepared by a lost-wax casting method, which had already been presented from our group. A detailed description of the manufacturing process is available in Appendix A.1. Briefly, an injection mold of the designed heart was produced of poly(acrylonitrile-co-butadiene-co-styrene)-plastic (ABS) using 3D-printing. The voids were filled with room temperature vulcanizing (RTV) silicone and cured at room temperature for twelve hours and afterwards at 65 °C in an oven for at least 24 hours. Thereafter, the ABS mold was dissolved in an acetone bath, which gave the sTAH made of one single silicone monoblock. In order to direct the flow, mechanical heart valves (Björk-Shiley type) with a diameter of 23 mm, surrounded by rubber rings were clamped into the in- and outlets of the ventricles using laces. The tensile properties of the used silicone elastomer were determined according to DIN 53504 norm.

Figure 2.2 Representation of the drive of the presented soft total artificial heart (sTAH). (a) Depiction of systole: pressurized air inflates the expansion chamber (EC), thereby displacing the blood and yielding a pulsatile flow towards the arteries. (b) Depiction of diastole: the pressure in the EC is relieved, which causes a pressure drop in the ventricles and finally results in a heart valve-directed refilling of the chambers with blood.

2.2.3 Actuation of the soft total artificial heart

The sTAH is driven pneumatically by inflating and deflating the EC between the two ventricles using pressurized air at 2 bar. Figure 2.2 illustrates this operation-principle. During systole, the EC is pressurized (Figure 2.2a). Thus, the EC expands and thereby presses the blood from both left and right heart chambers towards the aorta and pulmonary artery, respectively. During diastole (Figure 2.2b), the EC relaxes as the pressure is relieved and the heart moves back to its initial shape. The outflow valves close, while the inflow valves open.
and the ventricles are refilled by the preload. In contrast to existing pulsatile TAHs, the presented sTAH expands and shrinks during pumping due to its entire softness. The technical details, including types of valves and controller and in- and deflation times of the pneumatic drive are provided in the Appendix.

2.2.4 Hybrid mock circulation (HMC)

The sTAH was evaluated on a HMC\textsuperscript{101}, which uses a validated numerical model of the human blood circulation.\textsuperscript{102} This HMC has been developed for evaluating LVADs and therefore, had to be modified in order to conduct our experiments with the sTAH. However, the modified HMC is able to test one side of the sTAH at every experiment. Figure 2.3a illustrates the possible placement of the sTAH on a human blood circulation. The RV of the sTAH pumps from the systemic venous system to the pulmonary arterial system, while the LV pumps from the pulmonary venous system to the systemic arterial system. Figure 2.3b shows how the human blood circulation is mimicked on the modified HMC in order to evaluate the sTAH.

The modified HMC can be divided into two parts: the passive and the active part. The passive part was used to simulate constant physiological conditions for the right side of the sTAH. It consists of a bucket and tubes, which are placed such that a hydrostatic pressure difference of 40 mmHg is applied for the RV of the sTAH. The active part consists of the HMC, which was used to simulate varying physiological conditions on the LV of the sTAH.\textsuperscript{101} Specifically, two pressure-controlled reservoirs were used to simulate the pulmonary venous pressure (PVP) and the aortic pressure (AoP). The reference signals for the air pressure controllers (PVP\textsubscript{ref} and AoP\textsubscript{ref}) of the two reservoirs were computed in real-time by a numerical model of the pulmonary and systemic circulations based on the inlet and outlet flows of the LV of the sTAH. These flows are measured by ultrasonic flow probes and recorded at 1 kHz (Figure 2.3). Due to the lack of the interaction of the RV of the sTAH with the numerical model, we had to define the RV inlet and outlet pressures. Therefore, we set and kept constant the central venous pressure (CVP) of the numerical model (CVP\textsubscript{sim}) equal to 7 mmHg, whereas the simulated pulmonary arterial pressure (PAP\textsubscript{sim}) was adjusted by a defined pulmonary vascular resistance of 0.1 mmHg\textcdot s/mL. The schematic of the numerical model is provided in detail in the Appendix.
Figure 2.3 (a) Illustration of the soft total artificial heart (sTAH) on a human circulation. The right ventricle (RV) pumps from the systemic venous system to the pulmonary arterial system, while the left ventricle (LV) pumps from the pulmonary venous system to the systemic arterial system. (b) Illustration of the hybrid mock circulation (HMC) which was used to evaluate the sTAH. In the current setup, the LV of the sTAH is connected to the HMC. The HMC uses a real-time numerical model of the systemic and pulmonary circulations in order to apply the pulmonary venous pressure (PVP) and the aortic pressure (AOP) in the pressure-controlled reservoirs. The RV of the sTAH is connected to a passive hydraulic system that simulates a hydrostatic pressure difference of 40 mmHg.

For the current study, we only present results for the left sTAH. The reasons for not presenting the performance of the right sTAH are explained in the discussion section. We used three sTAH to conduct the experiments.

2.2.5 Experiments

We used the modified HMC to conduct two sets of experiments. The first set included the characterization of the sTAH. For this purpose, the performance of the sTAH with the current actuation system was evaluated while keeping a constant inlet pressure and increasing stepwise the outlet pressure. This experiment was repeated at different heart rates, which varied from 60 bpm to 120 bpm with a stepsize of 10 bpm. The pressure difference – flow (H-Q)
relationship for different pumping rates was derived, where the pressure difference is the
difference between the mean inlet and outlet pressures. Additionally, the diastolic properties of
the right and left sTAH were defined. For this purpose, we stepwise increased the volume of
each chamber separately by 5 mL and measured the generated pressure at each step. The sTAH
was at a non-ejecting mode of operation.

The second set of experiments included pre- and afterload variation experiments. In order
to vary the preload, we increased the PVP from 3 mmHg to 30 mmHg with an increment of
3 mmHg every 10 s, such that we always achieved steady state conditions. At the same time,
the afterload was fixed by keeping the systemic vascular resistance (SVR) equal to
1.11 mmHg·s/mL. For the afterload variation experiment we increased the SVR from
0.2 mmHg·s/mL to 2.1 mmHg·s/mL with an increment of 0.1 mmHg·s/mL again every 10 s.
Similarly, the preload was fixed by setting the PVP equal to 10 mmHg. Appendix A.1 includes
a detailed table (Table A.1.4), which includes the specific values for SVR, PVP, Pulmonary
Vascular Resistance and Central Venous Pressure for each experiment conducted. For all
experiments a glycerol water mixture with a viscosity of 2.8 mPa·s was used to mimic the blood
viscosity.

Durability problems of the material of the sTAH prevented us from completing all
experiments. Therefore, we present results only regarding the H-Q curves, the afterload
variation experiment at 60, 70 and 80 bpm and the preload variation experiment at 60 and
70 bpm.

2.3 Results

The production process yielded an entirely soft artificial heart (except the mechanical
heart valves) with a mass of 390 g and a size of 1.25 times the original heart size by volume,
even though no atriums were included (Figure 2.1a). The volumetric sizes of the sTAH are
summarized in Table 2.1. The elastic modulus $E$ of the silicone was measured as
$E = 2.98 \pm 0.16$ MPa ($n = 5$). Additional geometrical data, including sketches as well as
mechanical properties are available in the Appendix.

2.3.1 Pump performance

Figure 2.4 shows the generated flow from the LV against an increasing pressure
difference for different heart rates with the used actuation system. The flow generally increases
for higher heart rates and smaller pressure differences. At 110 bpm, a maximum flow of
4.5 L/min can be reached at zero pressure. At realistic physiological pressure difference conditions (90 mmHg), the blood flow is limited to approximately 1 L/min. For heart rates larger than 110 bpm, the characteristics of the heart change. In these cases, the blood flow increases with a smaller slope with decreasing pressure difference, compared to heart rates below 110 bpm. This results in lower flows at physiological pressure difference conditions for large heart rates. Similar performance was derived with each of the three tested prototypes. Table 2.2 lists the root-mean-square-error (RMSE) of left heart flow within the same pressure difference between each prototype. The difference is considered negligible. The H-Q curves for each prototype are included in Appendix A.1.

![Figure 2.4](image)

**Figure 2.4** Pressure difference over flow diagram of the left ventricle of the soft total artificial heart (sTAH) for different heart rates.

**Figure 2.5** depicts the relationship between pressure and volume for both sides of the non-ejecting sTAH. For the right sTAH, a pressure of 20 mmHg was achieved at 98 mL, whereas for the left sTAH this pressure was achieved at 147 mL. Both curves present a steep increase in pressure, while the filling volume increases.
Table 2.2 Calculated root-mean-square-error (RMSE) in left heart flow in L/min between all three soft total artificial heart prototypes (sTAH) used for HQ experiments.

<table>
<thead>
<tr>
<th>Operated heartbeat</th>
<th>sTAH₁ vs. sTAH₂</th>
<th>sTAH₁ vs. sTAH₃</th>
<th>sTAH₂ vs. sTAH₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>60 bpm</td>
<td>0.15</td>
<td>0.15</td>
<td>0.07</td>
</tr>
<tr>
<td>70 bpm</td>
<td>0.19</td>
<td>0.11</td>
<td>0.11</td>
</tr>
<tr>
<td>80 bpm</td>
<td>0.25</td>
<td>0.24</td>
<td>0.10</td>
</tr>
<tr>
<td>90 bpm</td>
<td>0.32</td>
<td>0.20</td>
<td>0.15</td>
</tr>
<tr>
<td>100 bpm</td>
<td>0.24</td>
<td>0.25</td>
<td>0.11</td>
</tr>
</tbody>
</table>

![Graph showing diastolic pressure versus volume relationship of the non-ejecting right and left sides of the soft total artificial heart (sTAH).](image)

**Figure 2.5** Diastolic pressure versus volume relationship of the non-ejecting right and left sides of the soft total artificial heart (sTAH).

2.3.2 Use as an artificial heart

**Figure 2.6** shows the measured flow- and pressure signal during an afterload experiment of the LV at an operated heart rate of 80 bpm. At physiological afterload, i.e. SVR equal to 1.11 mmHg·s/mL, the presented sTAH reached a systolic aortic pressure of 71 mmHg, while the diastolic pressure was equal to 36 mmHg. Thus, an aortic pulse pressure of 35 mmHg was recorded. The corresponding peak aortic flow from the LV was 15 L/min, with a left heart flow of 2.2 L/min, which yielded to a stroke volume of 27.5 mL. The backflow reached up to -5 L/min during diastole until the mechanical heart valves closed. The flow remained negative during diastole, due to the characteristics of the mechanical heart valves, which allow backflow to prevent thrombus formation.
Figure 2.6 Beat-by-beat performance of the soft total artificial heart (sTAH), with a heart rate of 80 bpm, when pumping against and systemic vascular resistance of 1.11 mmHg·s/mL. It shows the measured signals of the aortic pressure (AoP), the left heart flow (LHF) and the pulmonary venous pressure (PVP).

Figure 2.7 presents the mean aortic pressure (mAoP), the aortic pulse pressure (AoPP) and the mean left heart flow (mLHF) during all conducted pre- and afterload experiments. In both variations, the mean aortic pressure as well as the mean left heart flow increased with increasing heart rate. For afterload variations, at every fixed heart rate, the mean aortic pressure (mAoP) increased, while the SVR was increasing, whereas the left heart flow gradually decreased. The aortic pulse pressure (AoPP) increased with increasing SVR, but plateaued at approximately 36 mmHg for SVRs larger than 0.6 mmHg·s/mL. Additionally, it seems to be independent of the heart rate, as it plateaued at the similar value for all measured heart rates. In the case of preload variations, the mean aortic pressure (mAoP), and mean left heart flow (mLHF) increased gradually with increasing mean pulmonary venous pressure (mPVP). Again, the mean aortic pressure and the mean left heart flow were larger for larger heart rates, while there was no clear dependence for the aortic pulse pressure. Generally, the influence of the preload variation seems smaller than the afterload variations. Furthermore, the lifetime of the sTAH was limited to approximately 3000 beats, as the membrane between the EC and LV...
ruptured while the sTAH was operated at 80 bpm during the afterload experiment (at SVR equal to 1.7 mmHg·s/mL). Therefore, no further measurements were acquired. This rupture was linear with a size of approximately 2 cm and located at the transition of the internal membrane to the main outer body.

Figure 2.7 Performance results of the left ventricle of the soft total artificial heart (sTAH) during after- and preload variation experiments for different heart rates. The signal of the mean aortic pressure (mAoP), the aortic pulse pressure (AoPP) and the mean left heart flow (mLHF) are depicted as a function of physiological systemic vascular resistances (SVR) during afterload variations and as a function of mean pulmonary venous pressure (mPVP) during preload variations. The experiments were interrupted during afterload variation and 80 bpm at 1.7 mmHg·s/mL due to rupture of the sTAH.

2.4 Discussion

The goal of this study was not to present a new, readily implantable TAH, but rather a new concept for future artificial heart developments. We are at the initial state of research, but think it is valuable for the medical and engineering communities to present the first results. The measurements show that further progress is needed, but there are also some pioneering results. There are two major advantages of the presented concept: first, the low complexity of the heart and its simple production and second, the influence of the heart’s soft material, which results
in a device replicating the biomimetic motion of the human heart. However, the influence of this soft movement on the blood still has to be evaluated and was not part of this study.

The use of widely available and easy to use design software as well as the production using commercially available 3D-printers could allow a personalized and decentralized production in the heart centers and institutes. As the design can be changed very easily, the sTAH could be adapted to specific physiological properties of the individual patient. In comparison, the newly developed CARMAT® TAH is expected to only fit into 65% of all patients.\(^3\) Additionally, the sTAH only comprises of silicone elastomers, which are greatly available in medical grades and can be processed easily without the need of expensive and complex equipment. This could be the first step towards a low cost personalized medicine for implants in artificial blood pump therapy. Still, due to political and regulatory issues, this step into the clinical application is currently unlikely in the foreseeable future. However, 3D-printing technology could enable this possible next step in personalized medicine.

The presented sTAH has a size of 679 cm\(^3\), which is too large for implantation due to its outer shape. Though, we aim for a significant decrease in volume and weight of the implantable part of the sTAH in the future. The goal must be to have the same size as the patient’s diseased heart in order to prevent complications during surgery due to fitting problems. Another shortcoming of our design of the sTAH is a reduced RV volume of 83 cm\(^3\), which was required for the placement of the EC. In this design, this will most likely result in reduced blood flow from the RV to the pulmonary arterial system, and thus yield in reduced blood flow from the pulmonary venous system to the LV. Additionally, the presence of only one EC reduces the possibility to control the LV and RV separately and precisely, if different loads are clinically required. These issues will have to be resolved in a next generation sTAH.

Besides the low complexity of the heart and the placement of the parts which are most likely to fail outside the body (valves, air-pump, controller), an advantage associates to the elastomeric properties of the silicone. The pressurized air causes a movement of the entire heart with every beat, because there are no hard, mechanical parts except the valves. In contrast, every existing LVAD and TAH comprises of at least one rigid, blood contacting surface, which does not move.\(^{25, 31, 45}\)

The entirely soft design of the sTAH allowed us to mimic the physiological movement of the human heart during pumping, and thus to reproduce a physiological blood flow situation. Due to the movement of the entire sTAH structure with every beat, enabled by the softness of the materials, we expect no dead spots of the flow within the sTAH. Dead spots with reduced flow within blood pumps are a major problem as the blood automatically produces thrombin,
once it is in contact with an artificial surface.\textsuperscript{103} However, sufficient blood flow dilutes the thrombin concentration, such that no blood clotting can occur. Thus, due to presumable lack of dead spots, caused by the biomimetic motion of the sTAH, we hope to be able to reduce the amount of required anticoagulation, which is the main reason for bleeding complications and one major cause of death.\textsuperscript{16,103-105} Still, the elastic modulus of the silicone elastomer is greater as compared to the one of the left ventricular muscle (diastolic: 0.007 - 0.11 MPa, systolic: 0.027 – 0.4 MPa\textsuperscript{106}). However, the elastic modulus of the material is much less than the one of currently used rigid systems such as IsoPlast\textsuperscript{®} engineering thermoplastic polyurethane\textsuperscript{14} (elastic modulus IsoPlast\textsuperscript{®}: 1500-17000 MPa\textsuperscript{107-108}), and thus offers a more physiological situation to the human body. Besides the soft movement of the ventricles, the movement of the entire heart during the beats also seems more natural, compared to the hard TAHs and LVADs. One major disadvantage of the presented heart is the need for a percutaneous driveline, which will lead to known disadvantages of the currently used LVADs and TAHs, such as the danger for infections and reduced quality of life for the patients.

In addition to the pure introduction of a new concept for total artificial hearts, a second goal of this study was to evaluate the performance of the sTAH under physiological conditions. For this purpose, we derived the H-Q characteristics for the LV of the sTAH. Additionally, we evaluated the sTAH under various pre- and afterload conditions, when operated at different pumping rates. The pre- and afterload variation experiments provided a broad overview of the pumping performance of the sTAH under possible clinical conditions (Figure 2.7). A simulated representative clinical scenario can be found at the left hand side of Figure 2.7, where the mean PVP (preload) was fixed at 10 mmHg, the SVR was 1.11 mmHg\cdot s/mL and the sTAH pumping rate was 80 bpm. At these conditions the sTAH was able to pump 2.2 L/min, which led to a mean AoP of 48 mmHg and an aortic pulse pressure of 36 mmHg. We admit that the generated left heart flow and in turn the mean AoP are smaller than required for implantation and compared to what other TAHs can achieve. The aortic pulse pressure can be considered physiological and comparable to other TAHs, which are more advanced.\textsuperscript{33, 95} However, this has to be reevaluated with a prototype that achieves a physiological mean AoP as well, in order to prove that a possible elevated pulse pressure is avoided. Furthermore, due to the linear relationship between pressure and flow\textsuperscript{109}, the physiological shape of the signals promises a physiological blood pressure and blood flow, once higher flows can be achieved with a next generation sTAH.
The diastolic properties of the sTAH were defined. Results showed that the sTAH is much stiffer compared to native heart, i.e., the slope is steeper, as the pressure greatly increases after a small change in volume. This is also justified by the elastic modulus of the sTAH.

The performance of the left sTAH against an increasing afterload was presented through H-Q curves, when driven by the used actuation system. At pumping rates larger than 110 bpm, the pumping ability of the sTAH was diminished. We believe that for high heart rates, the need for very rapid in- and deflation of the EC influences the movement of the sTAH negatively and causes this reduction in flow and behavior. However, this performance and the resulted linearity of the H-Q curves can be adjusted by modifying the actuation system and using similar technologies to what is currently in clinical use, e.g. by the Berlin Heart Excor (Berlin Heart GmbH, Berlin, Germany). Thus, the filling and ejection pressures would be defined dynamically to achieve physiological flow conditions.

The experiments were conducted on a modified HMC, which can accurately generate physiological signals. The contour of the recorded signals of the LV flow, the AoP and the PVP resembles the corresponding signals of a human heart. Thus, we assume a physiological blood flow waveform due to the biomimicking motion of the sTAH, despite the lower mean flow and pressure values achieved, as compared to the physiological ones. These physiological signals also prove the physiological performance of our modified hybrid mock circulation. Compared to other MC systems used for evaluating TAHs, our modified MC presents a comparable physiological performance. Furthermore, our modified HMC offers the advantage of implementing easily various physiological conditions, by varying numerical parameters. Therefore, the evaluation of the LV of the sTAH, under various pre- and afterload conditions was feasible by varying the PVP and SVR, respectively. For the RV of the sTAH, a constant strain with a hydrostatic pressure of 40 mmHg was applied. In HF patients, a systolic pulmonary arterial pressure (PAP) of 45-55 mmHg and diastolic PAP of 20-25 mmHg can be observed. The applied elevated constant RV strain was used as a substitute for the neglected dynamic effects.

A limitation of our HMC is that it is designed for testing only one hydraulic device at a time and therefore, we were able to evaluate only one ventricle of the sTAH per experiment. We acknowledge the existence of other MC systems which are able to evaluate both sides of a TAH simultaneously. In our study, we tried to estimate the effect of the RV of the sTAH in both the numerical model and the physical prototype, as described in the “Hybrid mock circulation” section. Of course, the pre- and afterload conditions of the RV would change during
pre- and afterload variations of the LV. However, this interdependence is not considered to have significant influence to our generated results for the LV of the sTAH.

In the current study, no results regarding the performance of the RV of the sTAH are presented. In fact, we were able to switch the connection to the pressure reservoir side of the sTAH, increase the hydrostatic pressure and properly adjust the numerical model. Thus, the RV of the sTAH would be evaluated, while the LV would pump against a hydrostatic pressure. However, this was not feasible due to limited durability of the sTAH (approximately 3000 beats). The sTAH prototypes ruptured at the membrane, which separated the LV from the EC. This seems to be the weak spot of the presented design. Of course, the achieved lifetime differs significantly from the needed 30 to 50 million beats as a first milestone. Still, there is room for vast improvements. Another iteration of the design could remove this weak spot of the membrane separating the LV and the EC, while the use of other, more tear resistant high performance medical grade polymers could give an additional significant reduction of the problem. Alternative polymers are widely available and can be processed similarly. These include elastomeric polyurethanes, which are available in medical grades by multiple suppliers, as well as combinations of polyurethanes with PDMS. Also olefin based elastomers are available in biomedical grades and, e.g. are already used in artificial heart pump diaphragms. Future developments will comprise the lack of mechanical stability of the used silicone material. Still, this issue is a game breaker and needs to be resolved.
3 Bioactive Glass Containing Silicone Composites for Left Ventricular Assist Device Drivelines: Role of Bioglass 45S5® Particle Size on Mechanical Properties and Cytocompatibility

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3.1 Introduction

Bioactive glass (BG) is an amorphous material, which consists of a combination of various oxides. Its classical and best known composition is BG 45S5®, described by Hench et al. in 1971 with a composition of 45 wt% SiO₂, 24.5 wt% Na₂O, 24.5 wt% CaO and 6 wt% P₂O₅. Its properties such as bioactivity, osteoconductivity and osteostimulation make BG a suitable material in contact with hard tissue such as bone and teeth. The bioactive properties of BG result from its reaction with the body fluids to form a direct bond with bone. Also, its leaching in body fluids causes a change in the local ionic environment, which stimulates osteoblast proliferation, increases angiogenesis and acts antibacterial.

Besides the use of BG with hard tissue, an increasing number of studies used BG in contact with soft tissue. Miguez-Pacheco et al. (2015) have presented a thorough literature review on this topic. They reported multiple studies, which showed that “BG can have a stimulatory effect on angiogenesis, which is also applicable to soft tissue engineering.” The applicability of BG for cardiac tissue engineering, wound healing and dressing, nerve regeneration, gastrointestinal regeneration, urinary tract and lung tissue engineering, laryngeal repair and stabilization of percutaneous devices is also reported.

The combination of the aforementioned and well-known properties of BG and the recently reported findings could provide further possible solutions for other medical problems. Issues are e.g. faced in heart surgery and in more detail in heart replacement therapy. An increasing number of left ventricular assist devices (LVADs) are implanted into patients with heart failure every year, reaching 5’000 devices in 2015. LVADs are small blood pumps, which are implanted into the body and support the weakened heart. These implants are either driven electrically or pneumatically, which requires a percutaneous lead, the so called driveline, which connects the power source with the implanted LVAD in the body. The situation is sketched in Figure 3.1. The driveline exit site (DLES) is the place, where the lead exits the patient and is considered as the “Achilles heel” of LVADs. It is most susceptible to infection and constitutes as an entry point for germs. In one of the trials for the HeartMate II, a frequently implanted LVAD, an infection rate of 37% per patient-year was reported.

The LVAD drivelines are approximately 95 cm long and partly covered with a polyethylene terephthalate or polyester velour (Dacron® velour) with a length of approximately 30 cm. The velour is used, because it offers reasonable epidermal biointegration. In the past, the velour was placed at the driveline exit site, with an externalized part of approximately 2 cm (Figure 3.1a), because it was believed to promote tissue ingrowth, and thus optimize the driveline stability at the driveline exit site. However, cardiac surgeons started to put the
smooth silicone or polyurethane surface of the leads itself at the position of the driveline exit site due to seemingly reduced infection rates and faster skin incorporation, thus internalizing the entire velour-covered portion of the driveline (Figure 3.1b).\textsuperscript{,46, 92, 128} The reason for these better patient outcomes are believed to be less dermal inflammation of the polymer-skin interface compared to a velour-skin interface, thus yielding faster incorporation of the skin.\textsuperscript{129} However, infections still occur very frequently.\textsuperscript{57} The main reason for infections with a polymer-skin interface is believed to be trauma at the driveline exit site, disrupting the integrity of the driveline-skin barrier, and thus giving an entry point for germs.\textsuperscript{57} Therefore, a long term stable connection of the skin with the driveline’s material, e.g. silicone, giving mechanical stability between the polymer and skin, is desirable.

![Figure 3.1](image)

**Figure 3.1** Sketch of the surgical technique of implanting the driveline of a left ventricular assist device. In (a) the velour is placed at the driveline exit site yielding a velour-skin interface. (b) shows an implanted driveline with a polymer-skin interface at the driveline exit site. In this case, the velour portion of the driveline is completely internalized inside the patient’s body. With the courtesy of Berlin Heart GmbH.

Ross et al. (2003) tackled a similar problem. They used microscale particles of BG 45S5\textsuperscript{®} to coat a peritoneal dialysis catheter, and studied its influence on tissue ingrowth by implanting the coated silicone catheters subcutaneously in a rat model.\textsuperscript{59, 124} They showed that the BG coated tubes were “palpably fixed to the soft tissue”, while the uncoated control did not result in a stable tissue-silicone interface.\textsuperscript{59} This concept of using bioactive glass on the surface of percutaneous devices could also be applied to percutaneously implanted drivelines of LVADs in order to allow a faster formation and more stable polymer-skin interface, thus giving improved mechanical stability and a long-term stable barrier against pathogens.

To study the influence of BG 45S5\textsuperscript{®} particles on silicone elastomers, different BG particles were incorporated into medical grade silicone. It is of significant interest, whether the
incorporation of these bioactive particles into the silicone elastomer influence their mechanical properties. Further *in vitro* tests using simulated body fluid and cell culture tests with human primary dermal fibroblasts were used as a first assessment, whether the material could be suitable for skin biointegration. We therefore investigated, whether the incorporation of different BG 45S5® particles in medical grade silicone elastomers improves the mechanical properties, the bioactivity and cytocompatibility of silicone elastomers. These experiments provide a first approximation, if BG containing silicone could be used, and if it could improve the polymer-skin interface of percutaneous devices, especially at the driveline exit site of LVAD drivelines.

### 3.2 Experimental

#### 3.2.1 Production of the bioactive glass containing films

Nanoscale bioactive glass particles (nano-BG) of the type 45S5® were produced using flame spray synthesis as described earlier by Brunner *et al.* (2006). Briefly, the corresponding amounts of precursors (based on Si, Na, Ca and P) were mixed and diluted with tetrahydrofuran (THF, inhibitor-free, Sigma-Aldrich, Buchs, Switzerland) at a volumetric ratio of 2:1. The mixture was dispersed in oxygen and ignited in a methane and oxygen flame. The nanoparticles were collected on a filter and sieved subsequently. The commercially available microscale particles were provided by Schott (Schott-BG, bioactive glass 45S5®, SCHOTT, Landshut, Germany) and mo-Science (Mo-Sci-BG, 45S5® Bioactive Powder, Mo-Sci Health Care LLC, Rolla MO, United States). Schott-BG has a primary particle size of 4 µm and Mo-Sci-BG has a primary particle size of ≤ 54 µm, as specified by the suppliers. The silicone elastomer films with the specific BG content (*Table 3.1*) were produced by blending particles into a 2-component addition cure medical grade silicone elastomer (silicone, Silicone Elastomer A-103, Factor II Inc., Lakeside AZ, United States), which is cured by a platinum catalyst. According to the supplier, the platinum catalyst was included in component A. In detail, the corresponding amounts of silicone component A and BG particles were mixed in a dual-axis centrifuge (Speed Mixer DAC 150 FVZ, Hausschild Engineering, Hamm, Germany) for 2 minutes at 3500 rounds per minute (rpm). Afterwards, vacuum was applied to 8 mbar for approximately 5 minutes. For each sample this procedure was repeated 4 times. Afterwards, the corresponding amount of silicone component B was added and again mixed for 2 minutes at 3500 rpm. The uncured BG-silicone mixture was degassed to 8 mbar for approximately 10 minutes. The films were prepared using an automatic film applicator (Elcometer 4340, 120 µm rake, Elcometer
Instruments GmbH, Germany) on aluminium sheets, which had been washed with ethanol (EtOH, puriss. p.a., Sigma Aldrich) before. Subsequently, the films were cured at 150 °C in an oven for 6 hours.

Table 3.1 Silicone composites with different bioactive glass (BG) loadings and types.

<table>
<thead>
<tr>
<th>Particle type</th>
<th>control</th>
<th>nano-BG</th>
<th>Schott-BG</th>
<th>Mo-Sci-BG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary particle diameter [µm]</td>
<td>-</td>
<td>0.02 – 0.06</td>
<td>4</td>
<td>≤ 54</td>
</tr>
<tr>
<td>Concentrations</td>
<td>0 wt%</td>
<td>10 wt%</td>
<td>15 wt%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5 wt%</td>
<td>10 wt%</td>
<td>15 wt%</td>
<td></td>
</tr>
<tr>
<td>b</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

*as specified by supplier or literature; b15 wt% nano-BG could not be manufactured;

3.2.2 Characterization of the materials

3.2.2.1 Characterization of Bioglass 45S5® particles

BG 45S5® particles were analysed by scanning electron microscopy (SEM, FEI NovaNanoSEM450, FEI, Eindhoven, The Netherlands). Prior to SEM, the samples were sputtered with a 5 nm layer of platinum. The particle size distribution (PSD) was measured by analysing the size of at least 300 particles in a random area for each sample. The diameter of the particles was determined using an ellipse to fit the particles’ outlines and taking the average of the major axis and the minor axis as the approximate diameter of each particle.

3.2.2.2 In vitro tests using simulated body fluid and analysis of bioactive glass containing films

The in vitro bioactivity of BG containing films was tested in simulated body fluid (SBF). SBF was prepared according to Kokubo and Takadama with a pH of 7.4. Films were cut (100 x 20 mm) and washed in ethanol, dried in vacuum overnight and weighed ($M_{0, dry}$). Afterwards, each sample was incubated in 45 mL of freshly prepared SBF in a water bath at 36.5 °C for 4 weeks and SBF was replaced once a week. After incubation, the samples were gently dried on paper, weighed ($M_{t, wet}$) and afterwards dried in vacuum for 1 week. Dried samples were weighed ($M_{t, dry}$) to determine the weight loss ($%WL$) and water uptake ($%WA$), which were calculated according to

$$%WL = \left( \frac{M_{0, dry} - M_{t, dry}}{M_{0, dry}} \right) \times 100$$
\[
\%WA = \left( \frac{M_{t,wet} - M_{t, dry}}{M_{t, dry}} \right) \times 100.
\]

The formation of hydroxyapatite (HAp) of the samples was analysed by taking SEM images of the planar section as well as the cross-section of all samples before and after immersion in SBF. The samples for the cross-sectional SEM images were frozen using liquid nitrogen (LN2). Two tweezers, whose tips had also been cooled in LN2, were used to break the film and form a break line. The films were mounted on the SEM sample holders and sputtered as described earlier. Cross-sectional SEM images were taken of this break line. Additionally, X-ray diffraction (XRD, X’Pert PRO-MPD, PANalytical, Almelo, The Netherlands) was used with Ni-filtered Cu Kα radiation (\(\lambda = 0.1541\) nm) from 10-70° in the 2\(\Theta\) scale with a step size of 0.05° at 6 s per step to confirm the presence of HAp.

### 3.2.3 Physical properties analysis

ASTM test method D882 – 12 was used to measure the influence of the different particles and concentrations on the mechanical properties of the silicone. Briefly, BG/silicone composite films were cut into rectangles (100 x 20 mm) and the thickness of the films was measured (Digimatic Outside Micrometer IP65, Mitutoyo, Urdorf, Switzerland) at 3 random locations on each sample to ensure a variation in thickness of less than 10%. The mechanical properties were tested of as-prepared BG-silicone films \((n \geq 4)\) and BG-silicone films, which had been immersed in SBF for 4 weeks \((n \geq 3)\) using a tensile tester (Shimadzu AGS-X, 10 kN load cell, Reinach, Switzerland). The gauge length was 50 mm and the test speed was 500 mm min\(^{-1}\). Engineering stress and engineering strain were measured and a tangent in the linear regime of the stress-strain curve was used to calculate the Young’s modulus. Measurements were conducted until failure of the material and measurements of rupture at the grip were not considered. Static contact angle measurements (NRL C, Ramé-hart Inc., Randolph NJ, United States) were performed in order to determine the hydrophobicity of the as-prepared samples. 20 \(\mu\)L drops of deionized water were added on the surface. Every sample was analysed using three droplets. The left angle of each droplet was measured for 36 s at a rate of 25 images per second, which gave 901 measurements of the static contact angle. The average of these measurements was used to give the static contact angle of each droplet, while the average of the three droplets gave the reported value of each material.
3.2.4 Cell culture study

3.2.4.1 Materials

Normal Human Primary Dermal Fibroblasts from neonatal foreskin were purchased from ATCC (ATCC® PCS-201-010™, Manassas VA, United States). The cells were cultivated in Fibroblast Basal Medium (FBM, Lonza, Walkersville MD, United States) using a low serum Fibroblast Growth Medium Kit (FGM-2 SingleQuot Kit Suppl. & Growth Factors, Lonza) and incubated at 37 °C in humidified air (37 °C, 5% CO₂). Dulbecco’s Phosphate Buffered Saline (DPBS (1X), gibco®, Paisley, United Kingdom) was applied to wash the cells, while Trypsin-EDTA (0.25% Trypsin-EDTA (1X), gibco®) was used for trypsinisation of the cells.

3.2.4.2 Test samples and cell seeding

Uncured BG-silicone was filled into 48 well plates (Nunclon Delta Surface 48 well plate, Thermo Fischer Scientific, Waltham MA, United States), shaken orbital by hand and cured in an oven at 40 °C for 7 days. Afterwards, the cell culture plates were disinfected with UV-C light from four low pressure mercury lamps (253.7 nm, 15 W, HNS 15 ORF, Osram). The UV-C lamps were set at a distance of 50 cm from the sample, resulting in a dose rate of approximately 5.2 W m⁻². The irradiation output was measured by a standard photodiode sensor (PD300-UV, 200-1100 nm, 3 mW 20 pW, OphirPhotonics). Cells (passage #5) were seeded at a concentration of 2,500 cells cm⁻² in 600 µL FBM and incubated at 37 °C and 5% CO₂ for up to 7 days. For every material 12 wells were sampled, 4 for every measuring time. The medium was changed every 48 hours.

3.2.4.3 Relative cell proliferation assay

Relative cell proliferation of human primary dermal fibroblasts on the different materials was measured using a commercial cell viability reagent (PrestoBlue™ Cell Viability Reagent, Invitrogen Ltd., Paisley, United Kingdom), which assesses the cell viability via the metabolic activity of the cells. The reagent was first filtered (Filtropur S 0.2, Sarstedt AG & Co., Nürnberg, Germany). 60 µL of the filtered viability reagent were added to each well containing the different materials. For each measuring time, 4 replicates of every composite were assessed. After addition of the viability reagent, the well plate was gently shaken and incubated at 37 °C in humidified air for 2 hours. Three times 100 µL of each sampled well were added to a 96-well plate (Tissue Culture Test Plate 96F, TPP®, Trasadingen, Switzerland) and analysed using fluorescence (TECAN infinite F200, Tecan Group Ltd., Männedorf,
Switzerland) at an absorbance of 560 nm and emission of 590 nm at 4 different spots in every well. The cell proliferation assay was conducted 1 day, 3 days and 7 days after cell seeding.

### 3.2.5 Statistical analysis

Results are represented as mean ± standard deviation. The number of samples differed, but was noted, at the presentation of the results, when useful. Statistical significance was analysed using one-way analysis of variance (ANOVA) with a Bonferroni’s post-hoc correction (OriginPro 9.1.0, Origin Lab Corp. Northampton MA, United States). Significance of the results of the experiments was assumed at a $p$ value of < 0.05.

### 3.3 Results

#### 3.3.1 Bioactive glass characterization

The three particle types differed in their shape and primary particle size (Figure 3.2). Nano-BG appeared as spherical particles within large agglomerates, while Schott- and Mo-Sci-BG particles showed a shard-like appearance with larger primary particle sizes and sharp edges. The primary particle size of nano-BG was approximately 40 nm (Figure A.2.9) with agglomerates of 11.85 ± 6.10 µm. Schott- and Mo-Sci-BG particles did not seem agglomerated and had a primary particle size of 3.27 ± 1.79 µm and 10.83 ± 4.08 µm, respectively. Schott-BG showed a lean particle size distribution (Figure A.2.2), while Mo-Sci-BG also consisted of larger single particles of up to 65 µm (Figure 3.2c and A.2.3).

![Scanning electron microscopy images of the different bioactive glass BG 45S5® particles.](image)

*Figure 3.2* Scanning electron microscopy images of the different bioactive glass BG 45S5® particles. 
(a) nanosized bioactive glass (nano-BG), prepared by flame spray synthesis, commercial microparticles by (b) Schott (Schott-BG) and (c) Mo-Sci Corporation (Mo-Sci-BG).
3.3.2 Morphology of nano- and microcomposites

SEM images confirmed the presence of BG particles on the surface of silicone composite films (Figure A.2.10a-c). The large agglomerates of the nano-BG seemed to be broken up into smaller fractions. Especially the very large and shard-like Mo-Sci-particles could be observed. Cross-sectional SEM-images confirmed the evenly dispersed BG particles in the silicone composite films (Figure 3.3 and A.2.8). Agglomerates of nano-BG particles were still present as seen in Figure 3.3, while also smaller, more evenly dispersed nano-BG particles could be observed. Schott-BG and Mo-Sci-BG containing films seemed to have an even dispersion of incorporated particles. More detailed cross-sectional SEM images are available in the Appendix in Figure A.2.8.

![Figure 3.3 Cross-sectional scanning electron microscopy images of as-prepared silicones containing 10 wt% bioactive glass (BG 45S5®) particles. (a) the pure silicone, (b) with nanosized bioactive glass (nano-BG), (c) with microparticles by Schott (Schott-BG) and (d) with microparticles by Mo-Sci-Corporation (Mo-Sci-BG). Figures 3.3e-g show the respective particle containing composite films after four weeks immersed in simulated body fluid.](image)

3.3.3 In vitro bioactivity study

BG containing composites exhibited HAp formation on their surfaces (Figure 3.3e-g, Figure 3.4c, d, Figure A.2.8c, e, g). Schott-BG and Mo-Sci-BG containing films showed crater formation after four weeks in SBF, while HAp formed evenly on the surfaces of nano-BG containing films. Cross-sectional SEM images of the films showed that in nano-BG containing composites HAp was formed more evenly dispersed as compared to microcomposites. For Schott-BG and Mo-Sci-BG containing silicones, the HAp formation was located at the larger
microparticles, which, due to their size, were not as well distributed across the silicone matrix as compared to the nanoparticles.

Figure 3.4 Surface and bulk composite changes after in vitro tests in simulated body fluid (SBF). (a) gives the water uptake (%WA) of the wet films after four weeks in SBF, while (b) shows the respective weight loss (%WL) of the dry films. (c) X-ray diffractogram (XRD) of a nano-BG containing silicone film after four weeks in SBF and its concentration dependence; (d) illustrates the respective dependence of the particle type at a constant concentration of 10 wt% (* 15 wt% composition of nano-BG was not producible).

XRD patterns of as-prepared composites revealed the amorphous nature of pure and BG containing silicone films (Figure 3.4c-d and Figure A.2.4-A.2.6). After immersion in SBF for 4 weeks, the characteristic signals of HAp at $2\theta = 26^\circ$ (0002) and $2\theta = 31.5^\circ$ (112) appeared. All diffractograms of BG containing films showed patterns for HAp and calcium carbonate (calcite) after immersion in SBF. The signals of the HAp for nano-BG containing composites were sharper compared to the microparticles containing silicone composites. The area below the HAp signals increased with increasing particle concentrations in the films and with increasing duration of immersion. Also, depending on the particle type, the integral of the HAp peaks (specifically 112) decreased from nano-BG to Mo-Sci-BG to Schott-BG, indicating the amount of measured HAp.
The water uptake increased with increasing particle concentration (Figure 3.4a). It was the lowest for nano-BG and the highest for Mo-Sci-BG containing silicone films. For larger concentrations, the water uptake seemed to plateau and did not change significantly ($p = 1$) between 10 wt% and 15 wt% Schott-BG and Mo-Sci-BG, respectively. Pure silicone films did not show any swelling behaviour. There was no significant difference in mass loss between pure and nano-BG containing films ($p = 0.68$ for blank vs. 5 wt% nano-BG, $p = 0.82$ for 10 wt% nano-BG, Figure 3.4b). In contrary, the weight of the films containing larger Schott-BG and Mo-Sci-BG particles were significantly larger compared to the blank ($p \leq 2.3 \times 10^{-7}$). These weight gains increased with increasing particle concentrations and plateaued for Schott-BG composites at large concentrations ($p = 1$ for 10 wt% Schott-BG vs. 15 wt% Schott-BG), while it decreased for Mo-Sci-BG composites at 15 wt% compared to 10 wt% Mo-Sci-BG composites ($p = 2 \times 10^{-7}$).

### 3.3.4 Mechanical properties

#### 3.3.4.1 Static contact angle

Pure silicone films showed the highest hydrophobicity and the static contact angle decreased with increasing particle concentration in the composite (Table 3.2). No significant difference between the materials were measured in this experiment ($p \geq 0.28$).

**Table 3.2** Static contact angle [$^\circ$] measurements of bioactive glass (BG) particle containing silicone films with different particle types and concentrations. The measurements were conducted in triplicates.

<table>
<thead>
<tr>
<th>Pure Si</th>
<th>5%</th>
<th>10%</th>
<th>5%</th>
<th>10%</th>
<th>15%</th>
<th>5%</th>
<th>10%</th>
<th>15%</th>
</tr>
</thead>
<tbody>
<tr>
<td>nano-BG</td>
<td>111 ± 3</td>
<td>105 ± 5</td>
<td>107 ± 3</td>
<td>107 ± 1</td>
<td>105 ± 6</td>
<td>105 ± 1</td>
<td>108 ± 2</td>
<td>109 ± 3</td>
</tr>
</tbody>
</table>

#### 3.3.4.2 Elongation at break

The elongation at break of the silicone composites was reduced for composites with incorporated particles (Figure 3.5c). However, a significant reduction of the elongation at break was only measured between pure silicone and 5 wt% nano-BG ($p = 0.03$) and between pure silicone and 10 wt% nano-BG ($p = 0.0003$). The applied *in vitro* conditions did not have an influence on the elongation at break of pure silicone films ($p = 0.91$). In contrary, the elongation at break reduced after immersion in SBF in comparison to as prepared films with increasing BG concentrations and especially for 10 wt% nano-BG containing films with $47 \pm 31\%$. 

Besides a significant reduction of elongation at break of nano-BG containing films (5 wt% nano-BG: \( p = 0.00007 \) and 10 wt% nano-BG: \( p = 0.000002 \)) after immersion in SBF, the reduction was most distinct for films containing large BG concentrations of 15 wt%, with a significant reduction of the elongation at break of the 15 wt% Mo-Sci-BG composite (\( p = 0.001 \)) compared to the as prepared film.

3.3.4.3 Stiffness

The Young’s modulus of particle containing composites before immersion in SBF increased with increasing particle concentrations, while it decreased with increasing particle size (Figure 3.5e). Specifically, nano-BG containing composites were stiffer with increasing particle composition (\( p < 2\times10^{-22} \)), but also Schott-BG composites and Mo-Sci-BG composites were stiffer with increasing particle composition compared to pure silicone. There was no significant difference between Schott-BG and Mo-Sci-BG containing composites at the same compositions (5 wt%: \( p = 0.09 \); 10 wt%: \( p = 0.50 \); 15 wt%: \( p = 0.42 \)). After immersion in SBF, the Young’s modulus increased significantly for all particle-loaded samples compared to the value of the as-prepared composites (\( p < 0.002 \)). The stiffness of pure silicone films did not change significantly after immersion in SBF (\( p = 0.33 \), Figure 3.5f). 5 wt% nano-BG increased by a factor of two, while 10 wt% nano-BG increased by a factor of five. No systematic trend was found regarding a difference of Schott- and Mo-Sci-BG.

3.3.5 Cell culture study

Cell viability of primary human dermal fibroblasts did not differ significantly on BG containing silicone than on pure silicone after 24 hours (\( p = 1 \), Figure 3.6). After three days, the viabilities of the cells on 5 wt% (\( p = 0.02 \)) and 10 wt% (\( p = 0.0004 \)) Schott-BG and 10 wt% Mo-Sci-BG (\( p = 0.04 \)) were significantly larger than on pure silicone on day 3. On day 3, only the viability on 10 wt% Schott-BG was significantly larger compared to other BG containing silicones (5 wt% nano-BG: \( p = 7.3\times10^{-4} \) and 10 wt% nano-BG: \( p = 0.02 \)). After seven days, the viability of the cells on all BG-loaded silicones (\( p \leq 2.8\times10^{-6} \)), except 5 wt% nano-BG (\( p = 1 \)) was significantly larger than on pure silicone on day 7. The viability on pure silicone did neither increase from day 1 to day 3 (\( p = 1 \)), nor from day 3 to day 7 (\( p = 0.11 \)). In general, the cell viability of human primary dermal fibroblasts was the largest on Schott-BG containing silicones, while it was larger on Mo-Sci-BG than on nano-BG/silicone composites.
Figure 3.5 The influence of different bioactive glass (BG 45S5®) particles and particle concentrations in silicone films on the mechanical properties of the composite. (a) gives the tensile strength at break of the as-prepared silicone films as a function of concentration and particle type, while (b) gives the respective values, after immersion in simulated body fluid (SBF). (c) shows the percent elongation at break of the as-prepared films and (d) depicts the value after immersion in SBF for four weeks. (e) and (f) represent the Young’s modulus of the films before and after immersion in SBF (* 15 wt% composition of nano-BG was not producible) (e) also contains typical stress-strain curves of as prepared films with a weight fraction of particles of 5 wt%. The number of samples for the measurements of as-prepared materials was \( n \geq 4 \), while the number of samples for materials, which had been immersed in SBF was \( n \geq 3 \).
Figure 3.6 Cell proliferation of human primary dermal fibroblasts on different bioactive glass (BG 45S5®) containing silicone composites. It shows the dependence of the cell viability on the particle type (nano-BG, Schott-BG and Mo-Sci-BG). The cell viabilities are compared to the one of day 1 on pure silicone. Positive control data are not shown, as it exceeded the viability of the best performing material by approximately 4-fold (*: significant differences for p < 0.05).

3.4 Discussion

The here presented study examined the effect of different BG 45S5® types (nano-BG, Schott-BG and Mo-Sci-BG) on medical grade silicone elastomers for the use at the driveline exit sites of left ventricular assist devices. As this position is specifically susceptible for infection, a stable polymer-skin interface is highly desirable, thus giving a barrier against pathogens. Bioactive glass was chosen in this study, because of its reported wound healing properties and ability to improve the bioactivity of polymers. The experiments explored, whether the simple incorporation of BG into silicone elastomers influences mechanical properties, improves bioactivity of silicone in body fluids and improves the silicone’s cytocompatibility with human dermal cells. The use of different bioactive glasses enabled to study the influence of the particle size on the examined properties.

Incorporation of BG particles into silicone elastomers allowed the modification of mechanical and cytocompatibility properties of the polymer by pure mechanical mixing in an efficient way without the need for additional solvents or additives during production. Immersion of the silicone composites in simulated body fluid proved the HAp forming ability of the materials, and thus its bioactivity. Improved cytocompatibility of primary human dermal fibroblasts with BG-filled silicone was proven. In the context of left ventricular assist device drivelines, the materials are suitable to cover the skin-penetrating driveline at the driveline exit site, improving the bioactivity and cytocompatibility compared to pure silicone.
3.4.1 Cell culture study

The manufacturing process was based on simple mechanical mixing and yielded well-distributed particles within the silicone matrix (Figure 3.3). However, in contrast to the Schott-BG and Mo-Sci-BG microparticles the production of films incorporating nano-BG particles at concentrations larger than 10 wt% was not possible, even at increased curing temperatures. The large surface area of nano-BG compared to the microparticles may result in large agglomerate formation, causing phase separation of the filler and the silicone and finally inhibiting the curing reaction due to the resulting large viscosity. Another explanation could be the inhibition of the platinum catalyst of the silicone elastomer caused by the nanoparticles. This has been reported earlier by Fahrni et al. (2009) in a mixture of iron oxide nanoparticles in polydimethylsiloxane. Schrooten et al. (2004) already reported the use of a BG coating with silicone rubber for percutaneous implants. They used electron beam ablation to coat polydimethylsiloxane with bioactive glass. However, the pure mechanical mixing reported here seems simpler and less technically demanding. As the particles can also be chemically defined prior to mixing into the uncured silicone, it is also possible to produce a more well-defined material, compared to the in situ formation of the BG with electron beam ablation.

3.4.2 Bioactivity

The in vitro study in SBF proved the formation of HAp, and thus the bioactivity of BG containing silicone composites. The formation of HAp was confirmed visually by SEM (Figure 3.3, Figure A.2.8), as well as by its crystal structure observed on XRD patterns with the characteristic signals at $2\Theta = 26^\circ$ (0002) and $2\Theta = 31.5^\circ$ (112) (Figure 3.4c-d, Figure A.2.4-A.2.6). More HAp precipitated on nano-BG than on Schott-BG or Mo-Sci-BG containing silicone materials. This increased potential of nano-BG particles to form HAp was already reported earlier by Mačkovič et al. (2012) and is attributed to the high surface reactivity of the nanoscale particles. Mačkovič et al. (2012) also reported the formation of nanocrystalline HAp on nano-BG compared to BG microparticles. This could not be observed here as the peaks of HAp, formed on all BG containing composites seemed evenly broad, thus allowing no statements regarding HAp crystallite size. The formation of calcite on SBF-immersed Bioglass® was already reported in earlier studies and is attributed to the mechanism of HAp formation in SBF. Larger surface areas of BG favour the release of calcium from BG, which increases the ratio of the calcium to phosphorous ions in solution (Ca/P ratio). This causes the precipitation of calcite at the expense of HAp formation, which takes place in parallel in the first stages of BG reactions in SBF. Swelling of the composites in SBF was
more prolonged for microparticles containing silicones than for nanocomposites. It suggests a reduced shape stability of the possibly implanted devices in the body, when microparticles are used.

### 3.4.3 Cytocompatibility

Human primary dermal fibroblasts were chosen for this study. Besides keratinocytes and dermal microvascular endothelial cells, they serve as a standard cell culture model to evaluate the interface between skin and percutaneous devices. As the goal of this study was to gain a first evaluation, whether BG could serve as a material to improve the cytocompatibility at the skin of silicone elastomers, the study confined itself to the measurement of the fibroblast cell proliferation and the influence of different BG/silicone composites thereof. The results showed that BG containing silicone seems to allow a faster cell proliferation of human dermal fibroblasts than pure medical grade silicone. The slow proliferation of cells on pure silicone is attributed to the silicone elastomers’ inertness, and thus weak protein (Figure A.2.11) and cell attachment, which leads to weak soft tissue integration. The incorporation of BG into the silicone seems to allow a faster cell attachment of human dermal fibroblasts, which is a requirement for the proliferation of this cell type. This faster proliferation of the skin cells on the BG/silicone composites could allow faster wound closure between the implant and skin, thus forming a silicone-skin interface and a barrier against pathogens. Once the dermal cells are able to proliferate on the polymer, faster skin biointegration of percutaneous materials is most likely. Also, the abilities of BG to support rapid wound closure has been shown earlier by Cai et al. (2012), who incorporated BG in an ointment and applied it to full thickness skin wounds in a rabbit model. They observed significantly shorter healing times with BG containing ointments compared to the control. The combination of improved cell proliferation and reduced healing times makes BG a suitable material to improve the cytocompatibility of pure silicone and might therefore form an improved silicone-skin interface. However, the use of such BG containing silicones should not be considered for the use in other silicone elastomer containing implants, such as e.g. breast implants. Here, silicone shell incrustation (calcification) is problematic, leading to stiffening and, in most dramatic cases implant rupture. The use of BG containing silicones with LVADs, would need to be limited to the driveline exit site. The measured cell viability and proliferation on BG containing silicones is limited compared to the surfaces of well plates and cell flasks but the comparison to pure medical grade silicone is promising. In addition, the increased cell viability on BG containing silicones after seven days is indicative that this material allows the formation of a possibly stable connection to dermal
cells. In general, the results allow to make an argument about the dependence of particle concentration of the silicone composite on the cell viability of the human dermal fibroblasts, which increases with particle concentration. Also microcomposites seem to promote cell proliferation better than nanocomposites. The reduced cell viability on the nano-BG containing silicones compared to Schott- and Mo-Sci-BG composites may be attributed to the increased alkalinity, induced by the dissolution of the BG particles. As nano-BG exhibits larger specific surfaces and it increases the pH more than microparticles. The same applies to the viability on day 1. Due to the reaction of the BG with the cell medium, the alkalinity increased in the medium, which supposes a negative impact on the cell proliferation of fibroblasts. Still, also incorporated nano-BG increased the cell viability of cells compared to pure silicone and improved the cytocompatible properties thereof.

3.4.4 Mechanical properties

With exceptions, the results of the evaluation of the tensile strength at break and the percent elongation at break did not have statistical significance. The test method employed considers the tensile properties of thin plastic sheeting with a thickness of less than 1 mm. The test method also regards thin sheeting of elastomeric plastics with a percent elongation of larger than 100%, which justifies the choice of the test method. Despite the lack of significant results, the data still show general trends. Results were compared with the standard theories of ultimate strength and ultimate strain of particle-loaded polymer composites and tensile properties of human skin. The latter is mainly defined by the properties of collagen, whose maximum strain is between 10-20%, while its maximum strength is approximately 70-150 MPa. The tested silicone composites have larger values of ultimate strain, while the tensile strength at break is smaller than the one of collagen. The Young’s modulus of the skin is between 0.42 MPa for young and 0.85 MPa for older humans. Thus, the composites generally show larger elastic moduli, but smaller ultimate strength compared to human skin, when possibly implanted into the body. Under large forces, caused by possible accidents of the patient, the silicon-skin interface or the material could be compromised, depending on the strength of an eventually formed silicone-skin interface.

3.4.5 Ultimate mechanical properties

The tensile strength at break and percent elongation at break (other than the Young’s modulus, which is measured for small strains) depend on the weakest path throughout the structure, as opposed to the statistically averaged values of the microstructure parameters.
Thus, the tensile strength at break and percent elongation at break are also defined by the size of the largest particles or largest discontinuity in the films, which defines the weakest point of the film (Figure 3.7). The stress-transfer under large strain is specifically weak at these positions, thus compromising the mechanical stability of the entire construct. The incorporation of particles generally decreased the ultimate tensile properties of the composites, which suggests weak particle-matrix interactions.\textsuperscript{145} Before immersion in SBF nano-BG and Schott-BG are well incorporated into the silicone, showing some particle/matrix interaction (Figure 3.3b-c), while in the Mo-Sci-BG films, voids between silicone and particles can be observed (Figure 3.3d). The stress transfer between the silicone and Mo-Sci particles is weak, thus leading to the reduced ultimate tensile properties of Mo-Sci-BG/silicone composites. As the rather large Mo-Sci particles also possess sharp edges due to their shard-like nature, it is possible that these edges cut the silicone under stress and caused a rupture of the film. For the smaller particles of nano-BG and Schott-BG the tensile strength at break is not influenced, even though the Schott-BG particles also show a shard-like morphology (Figure 3.2b). This suggests, that the stress transfer between particles and matrix is better for smaller particles.\textsuperscript{146}

The heavily decreased ultimate properties of the nano-BG composites compared to microparticles incorporating composites after immersion in SBF are probably due to the porosity of the nano-BG agglomerates, which are, besides much smaller aggregates, present in the matrix (Figure 3.3b and Figure A.2.8b). This porosity yields a much larger specific surface area of the nano-BG compared to non-porous particles such as Schott-BG and Mo-Sci-BG, and thus the nano-BG agglomerates have the aforementioned higher potential to form HAp.\textsuperscript{72} This increased formation of HAp of the nano-BG particles in the silicone was also verified by XRD (Figure 3.4). HAp formed on the internal pore walls of these nano-BG agglomerates causing an internal force within the agglomerate, and thus weakening of the structure. Under strain, the agglomerate cracked from the inside, resulting in a large weak spot in the material. Figure 3.3e depicts one of the possible weak spots. These weaknesses could also be observed in light microscopy images (Figure A.2.7b) of the nano-BG silicone films after immersion in SBF. As the ultimate mechanical properties are defined by the weakest path in the polymer, the weaknesses resulted in the destabilization of the entire film. Immersion in SBF also reduced the tensile strength at break of the Schott-BG containing composites, which can be explained by the reduced particle/matrix interactions caused by the formation of HAp on the surface of the particles. The reduced interactions resulted in voids between particles and silicone as seen in the cross-sectional SEM images (Figure 3.3f), thus weakening the stress transfer under strain. Percent elongation at break was highly affected by the immersion in SBF for all particle types.
This is due to the weak force transfer between particles and matrix after immersion in SBF, yielding the maximum stress of the composite at smaller strain.

**Figure 3.7** Tensile strength at break (a), the percent elongation at break (b) and Young’s modulus (c) of silicone films depending on the size of the largest particles or agglomerates, which are incorporated in silicone. In this analysis the mean particle diameter of the three largest particles at the position of rupture were analysed. The films that include particles at a concentration of 10 wt% before and after immersion in simulated body fluid for four weeks and pure silicone were considered.

### 3.4.6 Young’s modulus

The incorporation of particles into a polymer causes a stiffening of the matrix because of the larger modulus of the solid particles.\(^{142}\) Chen et al. (2010) have shown this by incorporating flame spray synthesized nanosized BG particles into poly(glycerol sebacat) (PGS).\(^{118}\) The main difference to this study lies in the hydrophobicity/hydrophilicity of the polymers, and thus the particle/polymer interfacial adhesion. PGS has a similar hydrophilicity as collagen, while silicone is highly hydrophobic.\(^{117, 147-148}\) The results of the BG/silicone composites follow the same trend and coincide with known literature.\(^{142}\) The Young’s modulus is generally not affected by the particle/matrix interactions because for small strains, there is insufficient dilation to cause interface separation.\(^{142}\) Stiffness of silicone increased with addition of BG, indicating that no particle-matrix debonding occurred when samples were subject to tensile loading. The exaltation of the Young’s modulus with increasing particle loading can be explained by the higher modulus of the particles compared to the silicone rubber. As a first approximation of this correlation of modulus and filler volume fraction, the equation of Guth can be used.\(^{149}\)

\[
\frac{E_c}{E_m} = 1 + 2.5V_p + 14.1V_p^2
\]

\(E_c\) and \(E_m\) are the Young’s moduli of the composite and the matrix (pure silicone in this study), respectively. \(V_p\) is the particle volume fraction. Many other and more advanced
equations for the description of the Young’s modulus in relation to the volume fraction of the filler exist.\textsuperscript{142} As indicated by the equation, higher volume fractions of inorganic fillers in the polymer result in a stiffer composite.\textsuperscript{142} This is most probably also the explanation for the increased modulus of BG/silicone composites after immersion in SBF compared to the as-prepared films. As seen in the cross-sectional images of Figure 3.3 and the light microscopy images of Figure A.2.7, the size of the incorporated BG particles is larger, which leads to an increase in volume fraction, and thus causes the increased stiffness of the composites.\textsuperscript{142} At same particle concentrations, the Young’s modulus of Schott- and Mo-Sci-BG differ only slightly, while it is significantly higher for nano-BG ($p < 0.0005$). This increase in Young’s modulus of nano-BG containing polymers compared to microparticles containing polymers was already reported earlier by Misra \textit{et al.} (2008) and is attributed to the true reinforcement achieved using nano-BG.\textsuperscript{141} The finer dispersed nanoparticles form crystalline HAp throughout the silicone matrix, thus causing the stiffening of the composite, while on the microparticles HAp only formers very localized at the particles.

3.4.7 Limitations of the study

The study is limited in several aspects. It cannot definitely predict, whether BG containing silicone are improving the driveline exit site of LVAD drivelines. Cell proliferation measurements of human skin cells (primary dermal fibroblasts) were conducted to assess the cytocompatibility of the material, but do not allow predictions about cell adhesion and long-term skin tissue integration. Mechanical testing showed results with large standard deviations. Some trends are visible, though mainly without statistical significance. Moreover, at increased sample sizes, it is improbable, that standard deviations decrease, as this was tested for 5 wt% Mo-Sci-BG containing silicone with a samples size of 14. However, specifically the incorporation of nanosized particles is difficult at the presented concentrations. The static contact angle measurements also showed large errors and no significant results and trends could be observed. Still, as for the mechanical testing, some minor trends are visible and increased sample sizes could improve the results.

3.5 Conclusion

Incorporation of nano-BG particles into silicone composites showed the highest bioactivity as measured by XRD and least swelling by 50%, but lower mechanical properties with an ultimate tensile strength of only 2 MPa after simulation of the environment in the human
body and lower cytocompatibility. In contrast, micron sized particles were twice more cytocompatible than nanoparticles and had better mechanical properties and easier handling. Choosing the “right” particle type constitutes as a trade-off between different properties and will depend on the specific use. In the case of driveline material for LVAD implantation the use of nanosized BG 45S5® would be more advantageous because of higher bioactivity and less swelling inside the body. In conclusion, this study served as a first evaluation, if BG containing silicone elastomers could be a suitable material for LVAD drivelines. The here presented mechanical properties and cytocompatibility are promising. Whether the materials meet the conditions for long-term implantation as a percutaneous driveline, especially with a focus on mechanical integrity, skin biointegration and reduced infection rates, has to be assessed in an animal model with the final LVAD driveline shape.
4 Modification of Silicone Elastomers with Bioglass 45S5®

Increase *In Vivo* Tissue Biointegration

Manuscript in preparation:


Author contributions: concept/design by NHC, WJS and JB. Experiments planned/conducted by NHC, PW, GMB and JB. Data Analysis/interpretation by NHC and JB. Drafting article by NHC, DM, WJS and JB. Critical revision of article by NHC, KSS, DM, WJS, JB. Approval of article by NHC, KSS, DM, PW, GMB, WJS and JB.
4.1 Introduction

Silicone elastomers are a frequently used material family for the production of medical devices and used in implants such as pacemakers, breast implants and artificial blood pumps. The long-term stability of these implants inside the human body is a crucial factor for their success. Adverse events during the lifetime of implants such as infections or fibrotic reactions are severe clinical problems and affect the quality of life of the patients significantly. Silicones were tested for thrombosis, coagulation, platelet activation, leukocyte activation, hemolysis, and complement activation in a wide range of possible applications and did not show any adverse effects.

However, clinicians from different fields such as cardiac or plastic surgery, are frequently forced to exchange failing implants, which are manufactured of silicone elastomers. The material’s bioinertness results in one major side effect during implantation, causing the failure of the implant: bioinertness does not allow reasonable cell attachment to the material, and thus impedes tissue adhesion and anchorage of the implant. This is a particular problem for breast implants, which are made of silicone. The lack of cell attachment of the breast tissue to the silicone material of the implant and continuous micro-movement at the implant(silicone)-tissue interface frequently triggers constrictive fibrosis. Similarly, percutaneous implants such as ventricular assist device drivelines or catheters are frequently made of silicone. These devices penetrate the skin and, as the skin cannot adhere to the silicone, they continuously disrupt the skin’s protective barrier against pathogens. Due to that, the skin suffers from continued micro-trauma, compromising the integrity of the silicone-skin interface, giving an entry point for germs and causing chronic inflammatory reactions of the body. Biointegration of the driveline into the skin by healing the wound around the device, forming an interface between the material and tissue, and preventing pathogen invasion would be a suitable approach to close the protective barrier and stabilize the implant. These clinical examples demonstrate the desire of clinicians and patients for a long-term stable silicone-tissue connection, which would remove or reduce continuous trauma, mechanical irritation, infections and fibrous encapsulation.

A suitable material to modify silicone material in order to address the aforementioned problems is bioactive glass. This inorganic material can provide silicone with the desired bio-integrative properties. Previously, we have shown that the incorporation of bioactive glass particles into medical-grade silicone improves the cell proliferation of primary human dermal fibroblasts on medical-grade silicone. Bioactive glass is an amorphous material, which is available in various compositions. Its best-known form is Bioglass 45S5® (BG), invented by
Larry Hench in 1971 with a composition of 45 wt% SiO₂, 24.5 wt% Na₂O, 24.5 wt% CaO and 6 wt% P₂O₅. While implanted, BG leaches ions into the body fluids and has a stimulatory effect on angiogenesis and wound healing. Additionally, it possesses antibacterial properties, which renders it advantageous in contact with soft tissue.

The here presented study continuous on our report about BG/silicone composites, which could not show improved tissue adhesion at first but only bioactivity and improved cytocompatibility on smooth samples. Here, we report an in vivo evaluation of non-porous and porous BG/silicone composites using a chick chorioallantoic membrane (CAM) assay. We describe the manufacturing process of porous BG/silicone composites, incorporating either micron- or nanosized BG, an in vitro study in simulated body fluid (SBF) and the influence of the particles and porosity on the mechanical properties of silicone. The in vivo study gives additional information regarding living tissue biointegration of BG-containing silicone foams and justifies the use of BG in medical-grade silicone elastomers.

4.2 Materials and Methods

4.2.1 Manufacturing of Bioglass 45S5® silicone composites

Bioglass 45S5® microparticles (mBG) with a supplier-specified mean particle size of 4.1 µm (d50) were purchased from Schott (mBG, bioactive glass 45S5®, SCHOTT, Landshut, Germany). Bioglass 45S5® nanoparticles (nBG) were manufactured by means of flame spray synthesis as described earlier by Brunner et al. (2006). Briefly, the corresponding amounts of silicone-, sodium-, calcium- and phosphorous precursors were mixed and diluted with tetrahydrofuran (inhibitor-free, Sigma-Aldrich, Buchs, Switzerland) at a volumetric ratio of 2:1. The mixture was dispersed in oxygen and ignited in a methane/oxygen flame. The nanoparticles were collected on a filter and sieved.

Six types of materials were manufactured: pure silicone elastomer and silicone elastomer containing 5 wt% mBG or 5 wt% nBG. All three compositions were manufactured in nonporous as well as in porous form. A medical-grade 2 component platinum addition cure silicone elastomer (silicone, Silicone Elastomer A-103, Factor II Inc., Lakeside AZ, USA) was purchased. The corresponding amount of BG was mixed with component A of the silicone in a dual-axis centrifuge (Speed Mixer DAC 150 FVZ, Hauschild Engineering, Hamm, Germany) at 3500 rounds per minute (rpm) for 2 minutes. Subsequently, ammonium bicarbonate (NH₄HCO₃, BioUltra, ≥ 99.5%, Fluka Analytical, Steinheim, Germany) was added as a porogen at a concentration of 20 wt% with respect to the mass of silicone component A and
mixed for 2 min at 3500 rpm, subsequently. The corresponding amount of silicone component B was added and mixed for 1 min at 3500 rpm. The uncured mixture was transferred into a syringe, degassed in a desiccator at 8 mbar for at least 10 min and transferred to a clean Teflon cylinder (diameter = 30 mm, depth = 40 mm). The silicone was cured in an oven at 200 °C for 2 hours and the porogen evaporated simultaneously.

Additionally, nonporous samples of the same compositions were manufactured. The materials were prepared as described earlier. Briefly, corresponding amounts of silicone component A and BG were mixed in a Speed Mixer and degassed for several times. Subsequently, silicone component B was added, mixed, transferred to a syringe and degassed to 8 mbar for at least 10 mins. Uncured mixtures were poured into a Teflon form, degassed several times and cured at RT overnight to yield a smooth surface. Afterwards, the composites were post-cured for 24 hours at 100 °C.

Thermogravimetric analysis (TG, Linseis TG/STA-PT1600, Selb, Germany) was used to investigate the manufacturing process and assure complete removal of the porogen material. Approximately 25 mg samples were heated at a rate of 10 °C min⁻¹ from RT to 200 °C and kept for 2 hours.

4.2.2 Characterization of materials

4.2.2.1 Particles

nBG, mBG and porogen particles were analyzed by scanning electron microscopy (SEM, FEI NovaNanoSEM450, FEI, The Netherlands) after being sputtered with a 5 nm layer of platinum. A particle size distribution (PSD) of ammonium bicarbonate was measured by taking the average diameter of 298 particles in a specified area. The particle diameter was calculated by fitting the particles’ appearance in the SEM images with an ellipse and taking the average of the major axis and minor axis of each particle as the individual particle diameter.

4.2.2.2 In vitro analysis using simulated body fluid

In vitro bioactivities of porous samples were determined using a SBF assay, prepared according to Kokubo and Takadama with a pH of 7.4. Chemicals with a purity of Ph. Eur. were used for the preparation of SBF. Samples of porous BG/silicone composites were cut with a scalpel yielding cubical shapes of approximately 5 mm x 5 mm x 5 mm (n = 5). Samples were washed in ethanol (EtOH, purists. p.a., Sigma Aldrich), subsequently dried in vacuum overnight at RT and weighted (M₀, dry). Samples (approx. 50 mg) were immersed in 5 mL of fresh SBF and incubated in a water bath at 37 °C for 4 weeks. SBF was exchanged once a week.
weeks, samples were gently dried on paper and weighted \((M_{t\text{, wet}})\). Subsequently, samples were
dried in vacuum for 1 week and weighted \((M_{t\text{, dry}})\). Weight loss \(%WL\) and water uptake \(%WA\)
were calculated with the following formulas:\textsuperscript{131}

\[
%WL = \left( \frac{M_{0\text{, dry}} - M_{t\text{, dry}}}{M_{0\text{, dry}}} \right) \times 100
\]

\[
%WA = \left( \frac{M_{t\text{, wet}} - M_{t\text{, dry}}}{M_{t\text{, dry}}} \right) \times 100.
\]

As prepared and immersed samples were analysed by SEM. Samples were frozen in liquid
nitrogen and broken for cross-sectional observations. Broken samples were mounted on carbon
tape on the sample holder and sputtered with a 5 nm platinum layer. \textit{In vitro} bioactivity of
samples was determined by the formation of hydroxyapatite (HAp), which was identified
visually by SEM.

### 4.2.3 Physical properties of the composites

Porosities of foams were measured by cutting samples to sheets with a thickness of
approximately 10 mm. Circular pieces of foams with a diameter of 12 mm were punched from
the sheets and the thickness of each sample was measured with callipers. Subsequently, samples
were weighted on a balance. Porosities were calculated by comparing the densities of porous
composites with the calculated densities of the non-porous samples \((n = 4)\).

Stiffness of non-porous composites was measured before and after implantation in
chicken embryos to evaluate the change of stiffness under \textit{in vivo} conditions (see below) using
ASTM Norm D412-16. Implanted samples were washed with phosphate buffered saline
solution (PBS without Ca/ Mg, pH 7.2, Kantonsapotheke Zürich). Composites were cut into a
rectangular form (length = 20 mm, width = 6 mm, thickness = 3 mm) with a blade and sterilized
in ethylene oxide. The Young’s modulus was measured using a tensile tester (Shimadzu AGS-
X, 50 N load cell, Reinach, Switzerland). Samples were placed in the sample holder using
pressurized air at 5 bar and stretched at a strain rate of 50 mm min\(^{-1}\). The Young’s modulus was
calculated at low strains of 0.05 in the linear regime of stress-strain curve using a tangent
approximation. The sample sizes for the as prepared and implanted composites were \(n = 5\) and
\(n = 4\), respectively.
4.2.4  **In vivo characterization with a chick chorioallantoic membrane (CAM) assay**

4.2.4.1  **Sample preparation**

Two different types of samples were prepared for the *in vivo* CAM assay. First, non-porous samples were cut to rectangular shapes (20 x 6 x 3 mm³) with a scalpel in order to measure the mechanical properties after implantation. Second, porous samples were prepared by cutting the material to circular pieces with a diameter of 5 mm and a thickness of 3 mm using a scalpel for measurements of tissue biointegration. Samples were washed in EtOH, gently wiped on paper and dried in vacuum overnight, subsequently. Sterilization was performed by ethylene oxide.

4.2.4.2  **CAM assay: implantation of the composites onto the CAM**

Fertilized Lohman white LSL chicken eggs were purchased from Animalco AG, Switzerland, and incubated at 37 °C and 65% relative humidity in an incubator for 3.5 days. For experiments in chicken embryos until embryonic day 14 no IACUC approval is required according to Swiss animal care guidelines (TSchV, Art. 112). Afterwards, using a drill, a window was created in the eggshell after removing 2 mL of albumen. The window was covered with a Petri dish and incubated at 37 °C for another 3.5 days as described earlier. On incubation day (ID) 7, the scaffolds were gently placed on top of the CAM, either without plastic ring for the nonporous samples (*n* = 8 for all groups) or with a plastic ring (diameter = 10 mm) for the porous samples (C: *n* = 5; mBG: *n* = 6; nBG: *n* = 11). Finally, the eggs were incubated for another 7 days, fixated in 4% formalin solution in PBS, and incubated at 4 °C overnight. Then, the scaffolds were excised, embedded in paraffin, cross-sectioned into 5 µm slices and stained with hematoxylin/eosin (H&E).

4.2.4.3  **Histological analysis**

Integration factors for porous scaffolds were determined based on semi-quantitative scoring of H&E stained sections. Images were taken at 100x magnification using a light microscope (Leica DM 6000 B) equipped with a digital camera. In each histological section, five FOVs (Fields of View) were analysed, resulting in *n* = 15 for groups mBG and nBG and *n* = 30 for Control, respectively. The imprint of the scaffold on the CAM surface with its indentations was scored as a semi-quantitative tissue integration factor: 0 = no integration; 1 = slight integration; 2 = good integration and 3 = complete integration.
4.2.5 Statistical analysis

Results are given as average ± standard deviation. Statistical significance was calculated using a one-way analysis of variance (ANOVA) with a Bonferroni’s post-hoc correction (OriginPro 9.1.0, Origin Lab Corp. Northampton MA, USA) and assumed at a \( p \) value of \( p < 0.05 \).

4.3 Results

4.3.1 Particle characterization

Figure 4.1 shows the differences of BG micro- and nanoparticles. Microparticles had a shard-like appearance and a mean particle size of 4 \( \mu m \) as specified by the supplier. In contrast, nanoparticles had spherical shapes and an approximate primary particle size of 40-100 nm (Figure A.3.1). SEM analysis (Figure 4.1b and A.3.1) revealed fusing of the flame-sprayed primary nanoparticles to larger agglomerates with an average agglomerate size of approximately 12 \( \mu m \)\textsuperscript{70,156}

![Image](a.png) ![Image](b.png)  
![Image](c.png) ![Image](d.png)

Figure 4.1 Scanning electron microscopy (SEM) images of Bioglass 45S5\textsuperscript{®} microparticles (a) and nanoparticles (b). (c) and (d) depict the SEM image and the particle size distribution of ammonium bicarbonate, respectively.

Analysis of the porogen (\( \text{NH}_4\text{HCO}_3 \)) showed particles with an average primary particle size of 60 \( \mu m \) as seen in the particle size distribution of Figure 4.1d. \( \text{NH}_4\text{HCO}_3 \) contained
particles of up to 400 \( \mu \text{m} \) in diameter. SEM images of the NH\(_4\)HCO\(_3\) particles depicted non-spherical, non-agglomerated particles with different forms and softened edges.

4.3.2  Morphology of porous micro- and nanocomposites

The samples (micro- and nanocomposites) were highly porous with measured porosities in the range of 76 to 82%. Pure silicone foam had a porosity of 81.6 ± 1.4% \( (n = 4) \), while the values for micro- and nanocomposites were 77.3 ± 1.4% \( (n = 4) \) and 75.6 ± 1.2% \( (n = 4) \), respectively. The samples had comparable pore sizes of up to 1 mm in diameter and were interconnected.

4.3.2.1  Thermogravimetric analysis

Thermogravimetric analysis revealed complete dissociation of NH\(_4\)HCO\(_3\) under the manufacturing conditions of 200 °C for 2 hours without traces. Complete removal of the porogen from the silicone at the applied curing conditions were shown. Also, pure BG micro- and nanoparticles as well as pure silicone were not affected by the applied temperatures.

4.3.2.2  In vitro bioactivity study of porous samples.

The bioactivity study in SBF showed the formation of HAp on and within the porous composites, incorporating BG. As seen in Figures 4.2e and 4.2f, the HAp is formed evenly on the surface of the foams and within the pores. The presence of HAp after incubation in SBF was proven by its typical crystalline appearance in Figures 4.2h and 4.2i. The foaming procedure of silicone with NH\(_4\)HCO\(_3\) did not have an effect on the bioactivity of the composites.

Comparing the samples before (Figures 4.2a-4.2c) and after incubation in SBF (Figures 4.2d-4.2f) no changes of the general geometry of the pores were noted. The pore structure under simulated implanted conditions remained intact. Only the BG containing foam’s surface structure seemed to be affected by the conditions in the SBF. The results of %WL and %WA are provided in the Appendix A.3 in Figure A.3.2.
Figure 4.2 Scanning electron microscopy (SEM) images of Bioglass®/silicone composites before and after incubation in simulated body fluid (SBF) for 4 weeks. (a), (b) and (c) show pure silicone foam, silicone incorporating 5 wt% micron sized Bioglass® (BG) and silicone incorporating BG nanoparticles (5 wt%) as prepared, while (d), (e) and (f) show the respective samples after incubation in SBF. (g), (h) and (i) are close-up images of the samples after incubation in SBF and show the structure of hydroxyapatite in the case of microcomposites (h) and nanocomposites (i).

4.3.3 Mechanical properties

The stiffness of non-porous composites was affected by the modification with nBG particles. Incorporating solid nanoparticles resulted in significantly stiffer materials compared to pure silicone ($p < 0.05$), while microparticles did not increase the stiffness significantly compared to pure silicone ($p = 1$) (Figure 4.4). After implantation in chicken embryos the elastic modulus of pure silicone did not change compared to as prepared silicone ($p = 1$), while the ones of the micro- and nanocomposites changed significantly to its corresponding as prepared counterparts ($p < 7.8 \times 10^{-4}$). Generally, incorporated BG particles stiffened silicone elastomers significantly in vivo compared to pure silicones ($p < 1.4 \times 10^{-5}$).
4.3.4 *In vivo* CAM assay

Although different degrees of adhesion of the CAM were qualitatively distinguished for non-porous scaffolds, with increasing adhesion in the order of Control < mBG < nBG, semi-quantitative scoring was only performed for porous scaffolds. During histological processing and cutting, materials were separated from the CAM surface, however, it was clearly found that the shape of the scaffold surface was reflected and similar to the shape of the CAM surface – the higher the integration the higher this similarity. Hence, scoring was performed and mBG and nBG were significantly better integrated than pure silicone; with nBG even significantly better than mBG (Figure 4.5).

**Figure 4.3** Thermogravimetric analysis of the manufacturing process.

**Figure 4.4** Influence of in vivo environment in chicken embryos in a chick chorioallantoic membrane (CAM) assay on the mechanical properties of non-porous Bioglass 45S5®/silicone elastomer composites. (a) gives the stress-strain curve in the small strain region and (b) gives the elastic modulus of the materials as prepared (n = 5) and after implantation in chicken embryos for 7 days (post in vivo, n = 4). (control: pure silicone elastomer; mBG: silicone elastomer with 5 wt% Bioglass® (BG) microparticles; nBG: silicone elastomer with 5 wt% BG nanoparticles; *: significant differences for p < 0.05)
Figure 4.5 Results and analysis of the in vivo chick chorioallantoic membrane (CAM) assay of porous and non-porous Bioglass 45S5®/silicone elastomer composites. (a) shows the measured integration factor in porous composites with weight concentrations of 5 wt% microparticles (mBG) and 5 wt% nanoparticles (nBG). (b) shows the samples on the CAM (left: non-porous, right: porous) and (c) defines the integration factor based on histological cuts of the tissue (for control and mBG: n = 15 FOVs; for nBG: n = 30 FOVs). The scores are defined as: 0 = no integration; 1 = slight integration; 2 = good integration and 3 = complete integration. (*: significant differences for p < 0.05; ***: significant differences for p < 0.0001)

4.4 Discussion

We successfully showed that manipulating silicone by blending Bioglass 45S5® particles into silicone and forming a 3D-structure significantly improved tissue integration of the elastomer in an in vivo CAM assay. The simple manufacturing process of blending with BG particles and foaming with ammonium bicarbonate proved to be successful and reliable. Verified by HAp formation in an in vitro SBF assay we illustrated the bioactivity of the materials. A biomechanical analysis showed mechanical integrity of BG/silicone composites with expectable, but little stiffening under implanted in vivo conditions.

In order to address the adverse events of silicone implants, caused by the material’s bioinertness, we decided to investigate the in vivo tissue biointegration of elastomeric silicone composites, which had been modified in their bulk by blending bioactive particles rather than modifying only the composites’ surfaces. Ross et al. (2003) tackled the problem of insufficient tissue adhesion to peritoneal dialysis catheters by coating silicone tubing with melt-derived Bioglass® 45S5 particles with up to 125 μm in diameter and implanting the tubing
subcutaneously in a rat model. The BG coated sections were palpably fixed to the soft tissues as compared to the uncoated silicone, which did not show any adherence to the surrounding tissue. In contrast to a coating, the goal of this study was to investigate, whether blending of BG particles into the material could have similar positive effects of tissue integration in silicone implants. Comparable attempts had been undertaken by Wang et al. (1998), however using rigid high density polyethylene (HDPE) rather than soft silicone rubber. The authors blended Bioglass® microparticles (46 µm) into HDPE and could prove bioactivity in SBF but did neither show an in vitro cell assay on the material nor any in vivo results. In close similarity to the work of Wang et al. (1998), we decided to investigate the potential of blending bioactive micro- and nanoparticles into silicone in order to improve tissue adhesion of this type of soft, elastomeric and medically relevant implant material.

Manufacturing. The manufacturing process of blending BG 45S5® particles into the elastomer prior to cross-linking was easy and versatile. We chose micro- and nanoparticles in order to compare the influence of the particle size, i.e. its surface area on the manufacturing process of the composites, its mechanical properties and its in vivo tissue integration. In contrast to Wang et al. (1998), who investigated particle volume fractions of up to 40%, we limited the weight fraction of the bioactive particles to 5 wt% (2.14 vol%), because we had previously reported, using the same particles, that the maximum amount of nanoparticles to be incorporated into silicone elastomers is limited. This might be due to an incompatibility of silicone with BG, which is particularly enhanced for nanoparticles with a larger surface area as compared to microparticles with comparably small surface to volume ratios. Alternatively, also the nanoparticle-based inhibition of the platinum catalyst for the curing reaction of the silicone seems reasonable. This was reported earlier by Fahrni et al. (2009) for iron oxide nanoparticles in polydimethylsiloxane (PDMS). The incorporation of larger amounts than 10 wt% nBG was not possible and already at this weight fraction, the mechanical integrity of the composites in vitro was significantly compromised. Thus, we decided to limit our study to 5 wt% composite-loading.

The foaming procedure using ammonium bicarbonate in silicone elastomers was adapted from Lin et al. (2002) and Mac Murray et al. (2015). NH₄HCO₃ was chosen, because it decomposes to the gaseous molecules ammonia, carbon dioxide and water above a temperature of approximately 60 °C (Figure 4.3). The gases formed the pores within silicone and were removed completely from the composite foams without residues. A temperature of 200 °C was chosen, because we noted that for lower values, the porogen inhibited curing of the two-component silicone. This coincides with literature, which reports that NH₄HCO₃ inhibits the
platinum catalyst in addition-cured PDMS resins.\textsuperscript{98, 160} We could overcome this problem by applying the comparably high temperature of 200 °C, which resulted in simultaneous curing of silicone and porogen removal. We did not apply a pre-curing reaction at low temperatures before the removal of the porogen at elevated temperatures.

**Composites.** The foaming resulted in an open-pore structure, as already reported by Mac Murray \textit{et al}. (2015), even though it is difficult to verify this with the SEM images presented in \textbf{Figure 4.2}.\textsuperscript{98} However, we could show the interconnectivity of the pores by placing porous samples on absorbent paper and putting an EtOH drop on the samples. After a short period of time the absorbent paper was wetted by the EtOH, having passed through the porous sample, verifying the interconnectivity of the pores. Also, the presence of the BG particles did not seem to have a significant impact during the foaming of the composites, as the 3D-structure of all samples seemed to be comparable (\textbf{Figure 4.2}) as well as the porosity of approximately 80% for each material.

The \textit{in vitro} SBF assay proved the bioactivity of the porous and BG-containing composites, verified visually by HAp formation in the SEM images of the samples after incubation (\textbf{Figure 4.2}). Porous control samples without BG did not show any HAp, which verified the BG inflicted bioactivity of the composites. Also, it proved that using NH\textsubscript{4}HCO\textsubscript{3} as porogen does not affect the bioactivity of the BG, and thus the composites. The presence of HAp, even deeper within the porous structure, could be verified. This served as a first approximation for possible tissue ingrowth into the 3D structure, and thus as an approximation, whether a more stable 3D-fascilitated interface between the composite and the tissue can be achieved.

**Mechanical properties.** Incorporating BG into the non-porous elastomeric polymer resulted in a stiffening of the composites as compared to pure silicone (\textbf{Figure 4.4}), which coincides with theory and previous reports.\textsuperscript{142} Rigid particles have larger moduli than silicone and when blended into the elastomer, increased the moduli thereof. The elastic modulus generally did not depend on particle/matrix interactions, because it was measured in the small strain regime, where no particle/matrix-debonding occurred.\textsuperscript{142} Classical theories on the elastic modulus only give a dependency of the filler volume fraction and not filler diameter.\textsuperscript{142, 149, 161} However, as also shown in our results, a larger modulus was measured with a decreasing filler in primary particle size. This has been reported earlier in multiple studies and is being attributed to a critical particle size.\textsuperscript{142}

After implantation, the particle-loaded composites had significantly increased moduli, which had been reported earlier by us in an \textit{in vitro} assay.\textsuperscript{156} We attributed this to true
reinforcement by nBG. Finer dispersion of the smaller nanoparticles formed crystalline and solid HAp across the bulk of the silicone in vitro, which increased the stiffening of the composites more than for mBG containing composites. HAp could only be formed very localized at the larger BG locations in microcomposites, thus not stiffening the material as much as in nanocomposites.\textsuperscript{141, 156} We assume that this explanation also holds for a biomechanical in vivo assay.

**CAM assay.** The CAM assay is an easy and fast assay often used in the field of tissue engineering and regenerative medicine,\textsuperscript{162} as it allows short-term determination of a biomaterial’s biocompatibility and integration into the chorioallantoic membrane of the chicken embryo.\textsuperscript{163} First, we tested biointegration of non-porous silicone and composites during the 7-day incubation in the CAM assay. Although biointegration was weak for all the three materials tested, there was a qualitative difference between silicone and the composites during removing them from the CAM. While pure silicone rubber could easily be separated from the CAM surface by tweezers (tip width: 1 mm, approximate pulling force: 120 mN), because there was a visible gap between silicone and the CAM, removal mBG and nBG composites was more difficult, caused by the adhesion of the materials’ flat surface to the membrane.

Secondly, we investigated the biointegration of porous silicone and composites. Confirming that pores support biocompatibility and biointegration of many biomaterials\textsuperscript{164}, we found that porous materials were better integrated compared to non-porous materials (data not shown). All materials (silicone, mBG and nBG) were not removable by using tweezers. After formalin fixation, the tissue-material construct was cut out as a whole and processed for histological analysis. Only during cutting with a microtome, the materials were separated from the CAM surface. However, the higher the tissue integration before cutting, the higher the similarity of the imprint on the CAM surface. Hence, we semi-quantitatively scored the integration (Figure 4.5). Obviously, pure silicone was less well integrated than the composites. The presence of BG, either micron-sized or nanosized, improved the quality of biointegration significantly. When mBG was compared to nBG porous composites, there was even a better integration for the nanocomposites as compared to the microcomposites. Such findings stand in accordance with a study by Chan and coworkers where an inert polyetheretherketone (PEEK) was reinforced with HAp nanoparticles and compared to PEEK with HAp microparticles. They reported that nanocomposites showed not only improved mechanics but also an excellent biocompatibility and integration compared to the microcomposites.\textsuperscript{165}

**Limitations of the study.** This study has several limitations. Although the CAM assay fulfills the requirements for an in vivo assay, it only gives information about the materials’
short-term behavior in implanted conditions. It cannot predict the materials’ properties, when it has to fulfill a certain function, in this case the formation of a stable silicone/skin interface to prevent infection. It gives important data on tissue adhesion and biocompatibility but only more complex animal studies will give certainty on the material capacity to fulfill a certain function.

4.5 Conclusion

The incorporation of BG 45S5® into medical-grade silicone elastomer and forming a 3D-structure with a porogen significantly improves the tissue biointegration of the material in vivo. nBG/silicone composites have significantly improved biointegration compared to mBG/silicone composites. Either type of BG (micro or nano) improves the biointegration, compared to pure silicone, significantly. All BG-containing silicones show bioactivity also deeper within the pores. The pure incorporation of BG 45S5®, irrespective of the particle size of the bioactive material and keeping a 2D geometry, does not yield stable tissue adhesion in a 7-day in vivo CAM assay. Whether a 2D material can form a more stable skin-silicone interface with improved healing in a possible application in percutaneous devices remains unknown, however is rather unlikely. The CAM assay showed the requirement for 3D structures to measure short-term improvements in tissue adhesion. The need for a 3D structure complicates the manufacturing procedure, especially when it has to be applied to a specific, existing implant with a required geometry. Still, it remains comparably easy. The mechanical properties prove stable materials within the measured time frame. Summarizing, the manufacturing of the composites by simple blending of bioactive particles and forming a 3D structure in the clinically relevant material silicone is very easy, the in vivo results prove better tissue integration compared to pure silicone and the mechanical integrity remains intact.
5 Conclusion and Outlook

This work presents two concepts of how silicone elastomers can be used for artificial heart developments. First, it introduces the concept of an entirely soft total artificial heart, manufactured by a 3D-printing, lost-wax casting technique and actuated by pressurized air. This technique results in an artificial heart, which is made of one silicone monoblock without any seams, and thus possibly without weak spots. The softness of the materials shall enable a possibly more physiological blood flow situation as compared to existing artificial heart devices. Second, this work introduces the simple manipulation of bioinert medical-grade silicone elastomers with bioactive glass particles. The goal is a bioactive and elastomeric polymer with improved soft tissue biointegration. Stable interfaces of silicone elastomer implants with tissue are important factors to ensure the success of an implant. The simple blending process of bioactive glass particles into medical silicone elastomers could be the suiting process to improve tissue integration of the materials and overcome relevant clinical complications.

It was shown, that silicone elastomers are a suiting material for development of an entirely soft total artificial heart. The softness of such a device as well as its design and manufacturing processes enable several interesting directions for future developments in the field. The combination of modern medical imaging technologies with 3D-printing could enable the production of personalized implants for the first time. Current artificial heart devices have the problem of being too bulky to fit into smaller patients. Medical imaging and design with a CAD program could allow the surgeon herself to design the artificial heart to the specific patient’s needs. Afterwards, 3D-printing allows the manufacturing of this personalized device decentralized in the heart centers. However, due to oversight constraints, this seems highly unlikely at this time. Still, the here presented technologies would facilitate such treatments.

Enabled by the intrinsic softness of the materials, the entire structure of the sTAH was in motion during the beat, similarly to the human heart and in great contrast to existing devices. The measurement on the hybrid mock circulation revealed that the sTAH produced a physiologically shaped aortic pressure waveform and an excellent aortic pulse pressure without specific and complex control of the actuation. However, limitations and unknowns remain. The sTAH had limited blood flow and limited lifetime. Also, the hypothesis of possibly reduced rates of adverse events due to the softness of the pump as well as the more physiological mode of pumping of the sTAH remains unproven. Next steps have to tackle improved lifetime and
flow rates by application of alternative, more tear resistant polymers, optimization of the geometry and optimization of the actuation system. After a soft total artificial heart fulfills the requirements for blood flow and pressures, tests with blood and the influence of softness on hemolysis, while using bioprosthetic rather than mechanical heart valves have to be the next step. The goal of successfully introducing the concept of softness in artificial heart developments was reached. Still, many more steps towards the introduction of such a device into the clinic have to be taken.

The second topic of improved tissue integration of silicone elastomers by blending bioactive glass particles into the polymer seems more applicable. It was shown that BG 45S5® micro-, as well as nanoparticles significantly improve the in vitro cell proliferation of human primary dermal fibroblasts and the in vivo tissue integration after seven days. The incorporation of rigid particles into the soft silicone matrix did have expectable effects of increased elastic modulus. Also, it was shown, that due to the bioactive particles the mechanical properties of silicone elastomers changed significantly under in vitro conditions. Silicone is a highly hydrophobic material inaccessible for water. As mechanical properties are bulk properties, the incorporation of BG seems to give access of aqueous solutions to bulk silicone, thereby possibly compromising the mechanical integrity of the polymer. Thus, it remains unknown, how the material properties are affected by the conditions in the patient long-term. Still, the ease of manipulation and positive in vitro and in vivo results are promising.

Summarizing, the manipulation by simply blending particles into silicone elastomers, and thus changing the material properties significantly is an easy and practical procedure. The cost of such manipulations are low, especially as both materials have approval by the authorities. The manipulation of silicone elastomers with bioactive glass particles at the driveline of artificial hearts could be a suiting step towards improved connection to the tissue and possibly reduced infection rates. Proven properties of BG in literature such as antibacterial effects and angiogenesis stir up hope, that BG accelerates healing rates at the driveline exit site of artificial blood pumps and forms stable interfaces between the composites and the tissue. Further experiments are needed to show, whether bioactive glasses can have such a positive effect for the specific application in the field.
Appendix
A.1 Supplementary Information Chapter 2

Production of the soft artificial heart

The general production procedure of the artificial hearts was according to previously published work. The mould of the artificial heart was designed using a computer aided design software (NX 8.5, Siemens, Germany) and 3D-printed in commercially available 3D-printers (HP Designjet 3D, Hewlett-Packard or uPrint SE Plus, Stratsys, both United States). The models were built of acrylonitrile butadiene styrene (ABS) with a support structure of polylactic acid (PLA). After the production of the mould, the PLA-support was dissolved in an alkaline bath and the resulting ABS mould was washed with water and dried in an airstream. The voids of the mould were filled with a mixture of room temperature vulcanizing (RTV) silicones (50 wt% RTV 23 and 50 wt% RTV 240, both Neukasil, Altropol, Germany). The corresponding amounts of monomer and cross-linker for both silicone types (RTV 23: 10 parts monomer, 3 parts cross-linker, RTV 240: 10 parts monomer, 1 part cross-linker) were weighted and mixed by hand. Before the silicone was filled into the voids, the mixture was degassed in a vacuum at 1 mbar for 15 minutes. The silicone was first cured overnight at room temperature and then in an oven at 65 °C for 24 hours. After cooling down, the ABS mould was dissolved in an acetone bath until the whole ABS structure was removed. The resulting artificial heart made of a silicone monoblock was again dried in an oven at 65 °C for at least 24 hours. Mechanical heart valves with a diameter of 23 mm (Björk-Shiley type), surrounded by a ring of rubber, were placed in the in- and outlets to the ventricles and fixed by clamping with laces from the outside.

Geometry of the soft artificial heart

The approximated size of the sTAH and its chambers are depicted in Figures A.1.1-A.1.3 and Table A.1.1. Figure A.1.1 gives the sTAH as designed, while Figure A.1.2 shows the geometry of the main body, which was designed using the form of the real human heart. Figure A.1.3 gives the geometries of the chambers (left ventricle, right ventricle and expansion chamber). Table A1.1 summarizes the volumetric data of the sTAH and its chambers, while Table A.1.2 gives the minimum wall thicknesses between the chambers and the outer surface of the sTAH.
Figure A.1.1 Sketch of the soft total artificial heart with its outer dimensions in millimeters. c) depicts the flow direction to and from the right ventricle (RV) and left ventricle (LV).

Figure A.1.2 Sketch of the main body of the soft total artificial heart with dimensions in millimeters.
Figure A.1.3 Sketch of the chambers of the soft total artificial heart with its outer dimensions in millimeters. LV, RV and EC are abbreviations for left ventricle, right ventricle and expansion chamber.

Table A.1.1 Summary of the minimum wall thicknesses of the soft total artificial heart.

<table>
<thead>
<tr>
<th>Wall Thickness / mm</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Outside – LV</td>
<td>3.6</td>
</tr>
<tr>
<td>LV – EC</td>
<td>2.3</td>
</tr>
<tr>
<td>EC – RV</td>
<td>2.8</td>
</tr>
<tr>
<td>RV – Outside</td>
<td>3.6</td>
</tr>
<tr>
<td>EC – Outside</td>
<td>4.1</td>
</tr>
</tbody>
</table>

Measurement of the tensile properties

The tensile properties of the silicone mixture were measured according to DIN 53504 norm. The sample size was \( n = 5 \). A Shimadzu Universal Testing Instrument AGS-X with a 10 kN load cell was used. The samples were produced by filling the degassed uncured silicone mixture into a mould and cured at 65 °C. The thickness of the samples were measured at three positions of the samples. The tensile tests were conducted with a pre-stress of 0.01 MPa and with a speed of 200 mm min\(^{-1}\). The shore-A value of the material was measured three times using a Shore A Meter (LX-A, HANDPI). Figure A.1.4 shows an exemplary stress-strain curve of the used silicone.
Table A.1.2 Summary of the mechanical properties of the silicone elastomer mixture, which the sTAH is made of.

<table>
<thead>
<tr>
<th>Mechanical Properties</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shore A Hardness</td>
<td>28.0 ± 1.2 (n = 3)</td>
</tr>
<tr>
<td>Tensile strength at break / MPa</td>
<td>1.82 ± 0.35 (n = 5)</td>
</tr>
<tr>
<td>Percent elongation at break / %</td>
<td>390 ± 60 (n = 5)</td>
</tr>
<tr>
<td>Elastic modulus / MPa</td>
<td>2.98 ± 0.16 (n = 5)</td>
</tr>
</tbody>
</table>

Figure A.1.4 Typical stress-strain curve of the used silicone elastomer.

Actuation of the soft artificial heart

The pulsatile flow of the artificial heart was created by in- and deflating the expansion chamber between the two ventricles using pulses of pressurized air with the desired rate. A constant pressure of 2 bar was available from the house line. Three valves (VX245JEA, SMC, Japan) were used to control the pulses, one to control the inflow of the air (inflation) and two for the outflow (deflation). An illustration is given in Figure A.1.5. The valves were controlled by a programmable logic controller (PLC). Table A.1.3 gives the durations the valves are opened by the program. The durations were chosen in a way that first, the desired heart rate was reached, second, the silicone did not rupture after one beat and third, the EC was able to relax/shrink to its initial state, before being inflated again. During inflation, valve 1 was opened for $t_{\text{inflate}}$, while valves 2 and 3 were closed. Once $t_{\text{inflate}}$ had passed, valve 1 was closed and valves 2 and 3 were opened for $t_{\text{deflate}}$. Afterwards, the cycle started again.
Figure A.1.5 Scheme of the pneumatic actuation of the sTAH.

Table A.1.3 Summary of the inflation and deflation times for different physiological heart rates. The times also correspond to the opening and closing times of the expansion chamber-controlling valves.

<table>
<thead>
<tr>
<th>Heart rate / bpm</th>
<th>( t_{\text{inflate}} / \text{s} )</th>
<th>( t_{\text{deflate}} / \text{s} )</th>
</tr>
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<tbody>
<tr>
<td>60</td>
<td>0.20</td>
<td>0.80</td>
</tr>
<tr>
<td>70</td>
<td>0.19</td>
<td>0.67</td>
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<tr>
<td>80</td>
<td>0.18</td>
<td>0.57</td>
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<tr>
<td>90</td>
<td>0.16</td>
<td>0.50</td>
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<tr>
<td>100</td>
<td>0.16</td>
<td>0.44</td>
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<tr>
<td>110</td>
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<td>0.39</td>
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<tr>
<td>120</td>
<td>0.14</td>
<td>0.36</td>
</tr>
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<td>130</td>
<td>0.13</td>
<td>0.33</td>
</tr>
<tr>
<td>140</td>
<td>0.12</td>
<td>0.31</td>
</tr>
<tr>
<td>150</td>
<td>0.11</td>
<td>0.29</td>
</tr>
</tbody>
</table>

Production of the blood-mimicking fluid

We used a mixture of 36.5 wt% glycerol (Glycerol, ReagentPlus® ≥ 99.0% (GC), St. Louis MO, United States). The temperature was 22 °C, which led to a viscosity 2.8 mPa s and approximately 32% HCT. The desired ratio was calculated according to formulas given by Cheng et al. (2008). The resulted viscosity was validated through a viscometer.
Description of the numerical model of the human blood circulation of the modified hybrid mock circulation

**Figure A.1.6** Electric analogue of the numerical model of the human blood circulation used for the evaluation of the soft total artificial heart (sTAH) on the modified hybrid mock circulation. The sTAH block is highlighted in grey in the figure to indicate where the interface is implemented.

The PVP and AoP where computed and used for adjusting the pressure of the pressure reservoirs depicted in **Figure 2.3**. The sTAH left side block represents the left side of the physical prototype we were evaluating. As the physical sTAH right side was not interacting with the numerical model, we had to manually define the corresponding numerical inlet and outlet pressures of the right side, i.e. the central venous pressure (CVP) and the pulmonary arterial pressure (PAP). Therefore, we set and kept constant the CVP of the numerical model (CVP$\text{sim}$, **Figure 2.3**) and equal to 7 mmHg. The numerical PAP (PAP$\text{sim}$, **Figure 2.3**) was adjusted by keeping constant the pulmonary vascular resistance at 0.1 mmHg·s/mL. A description and validation of the model, as well as all the values for the additional variables presented in **Figure A.1.6** (i.e. the R, L, C elements of the systemic and pulmonary circulation) can be found in Ochsner et al. (2013).
Figure A.1.7 HQ curves with three different prototypes of the soft total artificial heart.

Figure A.1.8 Preload variation experiment with two different prototypes of the sTAH. The signals of the pulmonary mean pressure (PVP), the aortic pressure (AoP) and the left heart flow (Q), as well as their mean values, are depicted.
Full list of experiments

Table A.1.4 *Full list of experiments conducted with three soft total artificial hearts until rupture.*

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Heart prototype</th>
<th>Description</th>
<th>Heart rate / bpm</th>
<th>Systemic Vascular Resistance (SVR) / mmHg·s/mL</th>
<th>Pulmonary Venous Pressure / mmHg</th>
<th>Pulmonary Vascular Resistance (PVR) / mmHg·s/mL</th>
<th>Central Venous Pressure / mmHg</th>
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<tr>
<td>Experiment</td>
<td>Heart prototype</td>
<td>Heart rate / bpm</td>
<td>Description</td>
<td>Systemic Vascular Resistance (SVR) / mmHg*s/mL</td>
<td>Pulmonary Venous Pressure / mmHg</td>
<td>Pulmonary Vascular Resistance (PVR) / mmHg*s/mL</td>
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</tbody>
</table>

*LV: Left Ventricle; RV: Right Ventricle; stat.: static; AV: afterload variation, PV: preload variation.
A.2 Supplementary Information Chapter 3

Additional Materials and Methods

Light microscopy analysis of the composite films

Particle sizes in the films were investigated by light microscopy (Zeiss Axio Imager.M2m, 100x magnification, bright field mode, Carl Zeiss AG, Feldbach, Switzerland). Rectangles of 5 x 5 mm were cut at the position of rupture of the tested films with a particle concentration of 10 wt%. These rectangles were washed with ethanol and gently wiped in order to remove possible dirt or loosely attached particles on the surface. Subsequently, the samples were dried and examined. The area with the largest particles in this rectangle was chosen and the size of the particles measured using an ellipse as described above. The diameters of the 50 largest particles in the area were considered. Every composite material was measured in triplicates.

Protein adsorption assay (PAA)

A protein adsorption assay was adapted from Wei et al. (2004). A stock solution of 1.25% Fetal Bovine Serum (FBS, gibco®, Paisley, United Kingdom) in phosphate buffered saline (PBS, PBS pH 7.4 (1X), gibco®) was prepared and stored at 8 °C. Samples of the different materials with a diameter of 10 mm were punched and placed in a 1.5 mL Eppendorf tube. 1 mL of ethanol was added and shaken at 1050 rpm for 30 minutes in a thermomixer (ThermoMixer F1.5, Vardaux-Eppendorf AG, Basel, Switzerland) at room temperature. After removal of the ethanol, 1 mL of pure PBS was added and the samples were shaken for 24 hours at 1050 rpm. Subsequently, PBS was removed and replaced by 0.5 mL of 1.25% FBS in PBS and incubated at 37 °C. Protein adsorption was analysed using a commercially available Protein Assay Kit (Pierce™ BCA Protein Assay Kit, Thermo Scientific, Rockford IL, United States). Every composite and every sample tube was tested in triplicates.
Additional Results

Particle size distributions of BG particles

Figure A.2.1 Agglomerate size distribution (PSD) of the nano-particulate Bioglass 45S5®, which was produced by flame-spray synthesis. The PSD was fitted using a non-weighted non-linear Lorentz-fit.

Figure A.2.2 Particle Size Distribution (PSD) of the primary particles of Bioglass 45S5® provided by Schott (Schott-BG). The PSD was fitted using a non-weighted non-linear Lorentz-fit.
Figure A.2.3 Particle Size Distribution (PSD) of the primary particles of Bioglass 45S5® provided by mo-Science (Mo-Sci-BG). The PSD was fitted using a non-weighted non-linear Lorentz-fit.

X-ray diffractograms

Figure A.2.4 X-ray diffractogram of Bioglass BG 45S5® containing silicone elastomer as a function for different immersion times in simulated body fluid. The bioactive glass was produced by flame spray synthesis.
Figure A.2.5 X-ray diffractogram of bioactive glass (BG 45S5®) supplied by Schott (Schott-BG) in silicone elastomer at different weight percentages after immersion in simulated body fluid for four weeks.

Figure A.2.6 X-ray diffractogram of bioactive glass (BG 45S5®) supplied by mo-Science Inc. (Mo-Sci-BG) in silicone elastomer at different weight percentages after immersion in simulated body fluid for four weeks.
Light microscopy images

Figure A.2.7 Light microscopic images of the Bioglass (BG 45S5®) containing silicone composites before (a, c and e) and after (b, d and f) immersion in simulated body fluid. (a) and (b) show the nanosized BG, (c) and (d) show micronized BG by Schott (Schott-BG) and (e) and (f) give micronsized BG by Mo-Science (Mo-Sci-BG).
Scanning electron microscopy images

Figure A.2.8 Detailed cross-sectional scanning electron microscopy images of as-prepared silicones containing 10 wt% bioactive glass (BG 45S5®) particles. (a) of pure silicone, (b) with nanosized bioactive glass (nano-BG), (d) with microparticles by Schott (Schott-BG) and (f) with microparticles by mo-Sci-Corporation (Mo-Sci-BG). Figures A.2.8c, A.2.8e and A.2.8g show the respective composite films after four weeks immersed in simulated body fluid.
Figure A.2.9 Scanning electron microscopy images of the agglomerated primary particles of nanosized bioactive glass (nano-BG) of the type BG 45S5® produced by flame spray synthesis.

Figure A.2.10 Planar section scanning electron microscopy images of as-prepared silicones containing 10 wt% bioactive glass (BG 45S5®) particles. (a) with nanosized bioactive glass (nano-BG), (b) with microparticles by Schott (Schott-BG) and (c) with microparticles by mo-Sci-Corporation (Mo-Sci-BG). Figure A.3.10d-f show the respective composite films after four weeks immersed in simulated body fluid.
Protein adsorption assay

![Graph showing protein adsorption study on different bioactive glass containing silicone composites as a function of particle mass concentration and particle type (*: 15 wt% composition of nano-BG was not producible).]

Figure A.2.11 Protein adsorption study on different bioactive glass containing silicone composites as a function of particle mass concentration and particle type (*: 15 wt% composition of nano-BG was not producible).
A.3 Supplementary Information Chapter 4

Scanning electron microscopy

Figure A.3.1 Scanning electron microscopy image of nanosized Bioglass 45S5® particles.

Weight loss and water uptake

Figure A.3.2 Depiction of the weight loss (a) and water uptake (b) of the porous pure silicone (control), porous silicone, containing 5 wt% Bioglass 45S5® microparticles (mBG) and porous silicone, containing 5 wt% Bioglass 45S5® nanoparticles (nBG). (*: significant differences for p < 0.05)
References


Curriculum Vitae

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Citizen of Germany and Canada
Languages: German (native), English (fluent), French (basic)
Education

08/2015 – current  **PhD studies** at the Department of Chemistry and Applied Biosciences, Institute of Chemical- and Bioengineering, Functional Materials Laboratory, ETH Zürich, Zürich, Switzerland
Advisor: Prof. Dr. Wendelin J. Stark
Title: *Silicone Elastomers for Artificial Hearts: 3D printing, Bioactive Glass and Potential*

09/2013 – 09/2015  **MSc studies** in Chemical and Bioengineering, Department of Chemistry and Applied Biosciences, ETH Zürich, Zürich, Switzerland.
Master thesis: *Development of a sutureless anastomosis device for left ventricular assist device implantation*, Prof. Dr. Wendelin J. Stark, Institute for Chemical and Bioengineering, ETH Zürich.
Research project: *The effect of polyol sugars on the stability of monoclonal antibodies*, Prof. Dr. Massimo Morbidelli, Institute for Chemical and Bioengineering, ETH Zürich.

09/2009 - 02/2014  **BSc studies** in Chemical and Bioengineering, Department of Chemistry and Applied Biosciences, ETH Zürich, Zürich, Switzerland.

09/2011 – 03/2012  Erasmus exchange studies at Imperial College London, London, United Kingdom.

08/2006 – 08/2009  **High-school** (Swiss Matura), Mathematisch-Naturwissenschaftliches Gymnasium Rämibühl, Zürich, Switzerland.
Core subjects: Biology and Chemistry

Until 08/2006  Humboldt Gymnasium Vaterstetten, Vaterstetten, Germany.
Refereed Journal Articles


Honors and Awards

09/2011 – 03/2012 Erasmus Scholarship.


06/2018 Lindau Nobel Laureate Meeting 2018.
Conference Presentations and Proceedings


Student supervision


Teaching experience

09/2015 – 12/2017 Course assistant, laboratory course ‘*Chemical Engineering Laboratory I*’, Institute for Chemical- and Bioengineering, ETH Zürich.

09/2015 – 02/2018 Lecture assistant, ‘*Process control*’, Institute for Chemical- and Bioengineering, ETH Zürich.

02/2014 – 05/2014 Course assistant, laboratory course ‘*Basic chemistry for HEST*’, Institute for Chemical- and Bioengineering, ETH Zürich.

Professional experience

09/2014 – 12/2014 Internship at Functional Materials Laboratory, ETH Zürich.

02/2013 – 06/2013 Internship at Functional Materials Laboratory, ETH Zürich.

04/2012 – 08/2012 Internship at Bayer AG, Operational Excellence Consulting (BTS)