ADVANCES IN DNA-BASED FUNCTIONAL MATERIALS AND PERFORMANCE IMPROVEMENTS OF SOFT PUMPS

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To my family

We have two lives. The second begins when we realize we have only one.

Confucius
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Zusammenfassung


Der Mangel an weichen und zugleich robusten Maschinen erfordert die Erforschung von industriellen Fertigungsverfahren, zur Herstellung von leistungsstarken und beständigen weichen Pumpen. In diesem Zusammenhang werden zunächst zwei komplexe Formgebungsverfahren vorgestellt, welche die Production langlebiger weicher Pumpen für den Einsatz als Kunstherz ermöglichen.


Kapitel 3 erklärt die Herstellung einer weichen Pumpe für deren Einsatz als “soft total artificial heart” (sTAH), durch ein industrielles Spritzgussverfahren. Durch den Einsatz von hochtemperaturvernetzendem Silikon erzielte das sTAH ein Herzzeitvolumen von 6,4 L/min gegenüber physiologischer Auslassdrücke auf einer “hybrid mock circulation”. Dieser sTAH-Prototyp pumpte 180’000 Zyklen lang kontinuierlich und ist ein weiterer wichtiger Schritt hin zu der Entwicklung eines implantierbaren weichen Kunstherzens.


Kapitel 5 stellt ein neuartiges DNA-Datenspeichersystem mit hoher Informationsdichte in anorganischen Matrizen vor. Magnesiumchlorid, Calciumchlorid oder Calciumphosphat bilden schnell eine stabile mineralische Matrix, in welcher die DNA eingebettet wird, was zu einer Verlangsamung ihres thermischen Abbaus führt. Dieses DNA-Speichersystem wurde getestet, indem eine 115 kB große digitale Datei abgespeichert und nach beschleunigter Alterung durch Illuminia-Sequenzierung wieder erfolgreich ausgelesen wurde. Das Ergebnis unterstreicht die Bedeutung von festen Mineralmatrizen für die stabile und dichte Lagerung digitaler Informationen in der DNA.

In Kapitel 6 werden die Erkenntnisse aus den vorhergehenden Kapitel zusammengefasst. Des weiteren werden zukünftige Anforderungen für die Entwicklung eines weichen Kunstherzen und DNA-basierter Funktionsmaterialien vorgeschlagen.
Summary

This thesis describes the investigation of new tools to solve specific problems in the realm of soft pumps and DNA-based functional materials. On the one hand, the lack of durable soft machines requires the investigation of industrial manufacturing methods, to allow the production of long-lasting and high-performance soft pumps. One the other hand, the growing demand for detailed product information and digital information storage, requires alternative storage media.

Herein we first introduce two advanced molding techniques to manufacture durable soft pumps for potential use as heart replacement therapy. Furthermore, we studied the embedding and extraction of product information, and, extremely dense digital information storage via protected DNA. These applications for DNA required the use and development of new and existing DNA protection mechanisms to prevent the biomolecule from degrading.

Chapter 1 separately introduces the usage of soft machines and DNA to tackle specific issues. Soft machines or soft robots are particularly good at mimicking soft natural movement. Therefore, soft elastomeric pumps show great potential in mimicking the physiological movement and output of the human heart. Also known as a soft total artificial heart, soft pumps are discussed as an interesting alternative to current heart replacement therapies. The second part of this chapter presents DNA as a dense but degradable information carrier. Widespread DNA analysis techniques, in combination with effective DNA protection mechanisms, enables the development of functional DNA materials. Two specific applications of silica or magnetic nanoparticles encapsulated with DNA are presented — first, the use of DNA nanoparticles as taggant material, for example, in supply chains. Second, the DNA nanoparticles are introduced as dense DNA information storage media for future archival data storage systems.

Chapter 2 presents the production of a pneumatically actuated soft pump by adapting rubber compression molding technology. Long-term actuation and essential parameters influencing the soft pump’s performance (e.g., inflation and deflation times, fluid outlet pressures) were studied. Based on material selection and the use of an established manufacturing technique, the soft pump completed one million actuation cycles and conveyed more than 140’000 liters of water in less than 12 days. Moreover, the soft pump was assessed as a potential heart replacement therapy.
Chapter 3 focuses on using industrial rubber injection molding to produce a soft pump for use as a soft total artificial heart (sTAH). Manufactured from high-temperature vulcanizing silicone, the sTAH produced a total cardiac output of 6.4 L/min against physiological pressures on a hybrid mock circulation. This sTAH prototype was able to pump continuously for 180'000 cycles and is yet another step forward towards developing a soft heart replacement therapy.

Chapter 4 describes a method of detecting material mixing ratios by using DNA barcodes. To enable the distribution and protection of the DNA within the various matrixes, the biochemical is encapsulated in silica nanoparticles and distributed within the matrix of raw materials. DNA barcodes have been previously proposed as low-cost markers for product authenticity, and here the quantification of such barcodes via multiplex PCR to determine mixing ratios further enhances their functionality. The functional DNA material was tested in two-component liquid and polymeric products over a wide range of initial barcode concentrations.

Chapter 5 presents a novel high-density DNA information storage system in inorganic matrixes. Magnesium chloride, calcium chloride, or calcium phosphate create a stable biomineral matrix preventing thermal degradation of DNA, for a highly applicable DNA-based information storage system. This DNA storage system was tested by storing a 115 kB digital file in DNA and the successful readout of the file by Illumina sequencing after accelerated aging. This finding highlights the importance of investigating solid mineral matrices for high-density preservation of digital information in DNA.

Chapter 6 gives a general conclusion of this work and offers an outlook on possible future challenges and possibilities in the development of the soft total artificial heart and DNA-based functional materials.
1. An introduction to soft machines and DNA-based functional materials

The following introduction will first present materials and manufacturing processes to create soft machines. Moreover, general information about DNA, as well as specific DNA applications, are presented. In the same manner, the introduction covers soft machines and DNA-based functional materials separately, this thesis investigates two subjects independently: First, how the use of advanced manufacturing techniques permits the production of new long-term stable soft machines (e.g., soft pumps). Second, how nature-inspired DNA protection allows for the measurement of mixing properties of materials or, in another application, long-term storage of digital data in the biopolymer.
1.1. Soft machines: A new tool to mimic natural movement

Soft machines or soft robots are also often inspired by soft animals or soft animal structures found in nature. Indeed, some soft machines mimic the natural movement of an elephant trunk\(^1\), a tail of a fish\(^2\), or an entire octopus\(^3\) to grab objects or to locomote. George Whitesides used the following definition to describe soft robots:

\[\text{“Machines made of soft – often elastomeric – materials”}^{4}\]

In contrast to classical hard machines, soft machines are frequently built from very few, mostly elastomeric parts. Soft machines can be extremely flexible and perform complex movements.\(^5\)

These machines are often powered by electrical charge\(^6,7\), pneumatic expansion\(^8,9\), gas combustion\(^10-12\), or magnetic fields\(^13,14\). These energy sources are used to deform the elastomeric parts of the machines and thereby transform energy into motion. In many cases, pressurized air actuates soft machines. The design and the choice of material are critical factors to determine the soft robotics system’s response to pressure. A good example illustrating some advantages of a soft machine is the soft robotic tentacle. This device can imitate the natural movement of an octopus arm.\(^15\) The soft robotic tentacle can move in all directions of three-dimensional space, while still being able to handle fragile objects. Soft machines are ideal for handling delicate objects since the applied pressure to an object is distributed over a large surface without the need for sophisticated sensory equipment.\(^16\) Moreover, soft machines can travel rough terrain and conserve their functionality after deformation.\(^11\) In contrast, bending one single blade of a plane’s turbine could lead to irreversible functionality loss.

More recently, the domain of application for soft robotics is growing from manufacturing and process automation to other areas like cooperative human assistance and healthcare.\(^17\) Depending on their intended biomedical application, soft robots have to master different levels of biomimicry and biocompatibility (see Fig. 1.2.).\(^18\) Differences in biocompatibility requirements exist between occasional external use and long-term implantation of a soft machine.\(^18\) Whereas allergies might be an issue for wearable soft robots (e.g., assistive hand gloves), implantable soft machines (e.g., soft total artificial heart) should trigger no negative response from the body, leading to potential rejection of the implant. The possibility to copy the natural function of human organs (e.g., movement of the heart) is a crucial consideration for the application of soft robots in biomedical engineering. Since soft machines are mostly constructed of compliant materials, they are better in adapting to changing conditions (e.g., inside a human body).\(^16\) A comparable measure between materials used for classical machines
and soft robots is the modulus of elasticity or Young’s modulus (see Fig. 1.3).\textsuperscript{5,19} Most materials used for hard machines (e.g., left ventricular assist device) have Young’s moduli in the order of $10^9$-$10^{12}$ Pa, however soft tissue and organs in the human body (e.g., skin, artery, muscle) exhibit an elasticity modulus between $10^4$-$10^9$ Pa.\textsuperscript{5,16} With the right elastomer choice soft machines are a new tool to mimic bioinspired capabilities, allowing the flexible adaptation and interaction within a changing environment.

\textbf{Figure 1.2.} Comparison of different levels of biocompatibility and biomimicry for biomedical soft robots. Copyright (2018), with permission from Springer Nature.

\textbf{Figure 1.3.} Approximate Young’s moduli of selected engineering and biological materials. Soft robotic materials are in a similar range than biological materials such as skin, artery and so on. Copyright (2015), with permission from Springer Nature.
1.1.1. Elastomers for soft machines

Elastomers are a key material for the realization of soft machines. These viscoelastic polymers are made of long chains of repeating subunits and generally have weak intermolecular forces. These elastic materials with a low glass transition temperature offer a wide range of physical properties interesting for soft machines.\textsuperscript{20} The Young’s modulus is typically between $10^5$ and $10^7$ Pa for elastomers used in soft robotics.\textsuperscript{16,21} When applying an external force some elastomers can be reversibly deformed up to 1000\%\textsuperscript{22,23}

Elastomers are usually thermosets, which irreversibly harden during a process called vulcanization or curing. This process introduces covalent cross-links between individual polymer chains and can be initiated by heat, radiation, condensation, or a catalyst.\textsuperscript{20,24} Before curing, the starting material is usually malleable and can be modeled into a specific shape.

Different industrial manufacturing methods exist to model a thermoset: injection molding, extrusion molding, and compression molding.

In the following paragraphs, the focus will be on natural rubber and silicone rubber, two elastomers frequently used in industrial molding processes. However, natural rubber is not commonly used in the soft robotics community since advanced manufacturing techniques are required to mold this elastomer. Natural rubber (NR), or latex, is a polyisoprene polymer (see Fig. 1.4.) and is mainly collected from rubber trees (e.g., Hevea brasiliensis). Before NR manifests its desired elastomeric properties, it needs to undergo vulcanization. This cross-linking process, discovered by Charles Goodyear in 1839, was commonly performed by heating a mixture of NR with 8-10 wt\% of sulfur and different inorganic catalysts.\textsuperscript{20} This procedure required long hours of heating before the polymer was sufficiently vulcanized by creating sulfur cross-links of varying lengths.\textsuperscript{25} In the advent of new organic accelerators, sulfur addition could be significantly reduced to 0.5-3 wt\% by adding 0.2-2 wt\% accelerators.\textsuperscript{20} Moreover, the vulcanization time was reduced to well below an hour, with temperatures ranging from 100-180\degree C. NR is used in many industries because of its high elastic and tensile properties, as well as its low cost to produce, for instance, tires or belts.\textsuperscript{26} The tire industry alone consumes more than 60\% of the produced polymer.\textsuperscript{20} Other industries (e.g., biomedical industry) also investigated NR rubber to manufacture products like gloves or catheters.\textsuperscript{27} However, poor blood compatibility, cytotoxicity, and allergic reactions significantly limit the usage of the material for medium to long-term biomedical applications.\textsuperscript{27,28}
In contrast to NR, silicones are commonly used in soft robotics.\textsuperscript{4} Silicones are a class of synthetic rubbers with a backbone made of Si-O repeating units (siloxane structure) with a variety of different organic side groups (see Fig. 1.4.). The most common side group of siloxane is a methyl group (CH\textsubscript{3}), constituting the silicone elastomer polydimethylsiloxane (PDMS). In general, silicones are divided into two subgroups, room-temperature vulcanizing (RTV) and high-temperature vulcanizing (HTV) silicones. Vulcanizing processes that transform silicone resin into the desired rubbery material include radical curing, hydrosilylation curing or radiation curing.\textsuperscript{29,30} In contrast to the binding energy and bond length of carbon-based polymers with a C-C based backbone (84.9 kcal/mol, 1.53 Å), the Si-O bonds are relatively long yet strong
(106.0 kcal/mol, 1.64 Å), and are one reason among others that explain why silicones are very flexible and heat resistant.\textsuperscript{31} Therefore, many different industries use silicones, e.g., as insulators, sealants, or in medical devices.\textsuperscript{23} In 1960 Bernhart, reported the first use of silicones to manufacture an external prosthesis.\textsuperscript{32} Since then, the chemically inert and biocompatible material was used in many biomedical applications such as medical device drivelines or pacemaker leads.\textsuperscript{33} Its popularity among the soft robotics community is not only due to the excellent mechanical properties but also due to the simple handling and crosslinking of RTV silicone elastomer precursors. Summarizing, both natural rubber as well as silicones can be used to manufacture resilient long-term operating soft machines. The next chapter presents different soft machine manufacturing techniques, with a focus on commercial NR and silicone molding processes.

1.1.2. Elastomer molding and manufacturing of soft machines

Elastomers need to be molded into specific shapes to manufacture soft machines.\textsuperscript{4} When soft robotics started to emerge at the beginning of the new millennium, the production process of soft machines was performed by using soft lithography.\textsuperscript{34,35} With the help of computer-aided design software and fused deposition modeling (FDM) printers, elastomers were cast into 3D printed plastic molds (i.e., acrylonitrile butadiene styrene molds) and assembled into more complex structures (e.g., PneuNets) to build a soft robot.\textsuperscript{4,35} The simplicity of this manufacturing process allows rapid iteration of soft machine designs.

Fast forward a couple of years, soft machines can nowadays be manufactured with wide variety of new manufacturing systems, allowing, for example, to directly create 3D silicone structures without the need of a mold.\textsuperscript{36} 3D manufacturing techniques include: Direct ink writing (DIW)\textsuperscript{36}, digital light processing (DLP)\textsuperscript{37} and rapid liquid printing (RLP)\textsuperscript{38}. Compared to FDM and lamination based manufacturing, DIW can produce a single soft tentacle four times faster.\textsuperscript{36} Also, RLP technology can manufacture a soft robotic system fast and at larger scales.\textsuperscript{38} However, some of the presented methods are not yet commercially available and might never reach the market since other established elastomer molding techniques are already widely adopted in the industry. In factories, precise and large-scale production of elastomeric parts is dominated by compression and injection molding (see Fig. 1.5.).
The global blow molded market produced more than 68 million tons of plastics in 2016 and the four largest tire manufacturers (Bridgestone, Michelin, Goodyear, and Continental) had combined revenue of over 110 billion euros in 2018. To produce plastic products or rubber tires, companies rely on well-established compression and injection molding techniques. While both mentioned rubber molding techniques slightly differ from each other, they both offer high reproducibility and high throughput production. Moreover, these molding techniques enable access to high-temperature vulcanizing material. In general, either unvulcanized rubber (e.g., natural rubber or silicone rubber) is compressed into a specific shape (compression molding), or the rubber is injected into the metal mold (injection molding).

However, these advanced production tools are rarely used in the academic field of soft machine manufacturing since rubber mold production is expensive and slow, therefore limiting the possibility of rapid prototyping. In the advent of metal 3D printing, companies advertise that the cost of metal tool production could drop more than 90%. The potential decrease in metal mold manufacturing cost and time could allow more widespread adoption of these techniques in the soft robotics community, thus allowing the use of durable HTV rubbers enabling to develop long-term operable soft machines (e.g., soft pumps for heart replacement therapy).

**Figure 1.5.** Illustration of two commercially adopted elastomer molding techniques. Left, cross-sectional view of a compression mold filled with rubber before and after the vulcanization process. Right, cross-sectional view of an injection mold with a rubber charge outside and inside the mold before and after the vulcanization process.
1.1.3. Soft total artificial heart

Between the left and the right lung, approximately in the middle of the chest, beats the human heart. Roughly the size of a human fist, the heart is a double soft pump responsible for transporting blood to all the organs.\(^4\) It is composed of a left and right half separated by a thin septum. Each side consists of an atrium and a ventricle. Beating simultaneously, the right ventricle pumps oxygen-poor blood to the lungs, and the left ventricle pumps oxygen-rich blood to the body. The heart together with the blood vessels, forms the cardiovascular system.\(^4\) A healthy human heart beats approximately 3 billion times in a lifetime.\(^4\) From tiny mammals like a mouse (< 50g) to a blue whale, the largest known animal on earth (> 50'000 kg) there is a linear inverse semilogarithmic relationship between heart rate and life expectancy, except for the human heart (see Fig. 1.6.).\(^4\) Due to advances in science and healthcare, most human hearts have to operate for extended periods.

![Figure 1.6. Semilogarithmic relationship between rest heart rate and life expectancy in small to large mammals. Human life expectancy far exceeds the linear trend. Adapted from Levine et al. Copyright (1997), with permission from Elsevier.](image)

Today, the leading cause of death in the world are cardiovascular diseases (CVD).\(^4\) The world health organization listed 17.9 million deaths due to CVD in 2016, which represents 31% of deaths globally.\(^4\) One type of cardiovascular disease is heart failure. For people suffering from end-stage heart failure, treatment options are limited.\(^4\) These weakened hearts are not able to
pump enough blood to sustain all the necessary body functions of the patients. In theory, three different hard or soft treatment options exist: ventricular assist devices (VAD), total artificial hearts (TAH) and soft total artificial hearts (sTAH). Only VAD and TAH systems are currently available on the market.

For most patients suffering from end-stage heart failure, only the left ventricle needs support, and therefore assisting a weakened heart is easier than replacing it. Left ventricular assist devices (LVAD) are, in most cases the treatment of choice. Less frequently right ventricle support or biventricular assistance by VAD is required. Most implanted LVAD are continuous flow pumps and use a rapidly spinning impeller to pump blood. These mechanical pumps are, in most cases, smaller, quieter, and more durable than a TAH. Implanting a TAH requires the removal of the heart. Most developed TAH are positive displacement pumps and are often considered too large and have limited durability. Only one TAH system (Syncardia™ TAH) is currently approved as a bridge to transplant by the FDA and received CE mark in Europe. Other TAH systems exist (e.g., CARMAT® TAH or RheinHeart TAH), but they are still in clinical or animal trials. Moreover, VAD are implanted far more frequently than TAH. After more than 50 years of research, the gold standard to treat end-stage heart failure remains a heart transplantation.

**Figure 1.7** Comparison of different levels of biomimicry and softness for artificial blood pumps. HeartWare™ HVAD™ is a ventricular assist device (VAD). Syncardia™ TAH is a total artificial heart (TAH). Silicone heart is a soft total artificial heart (sTAH). Copyright (2019) of VAD image by Medtronic plc and of TAH image by Syncardia Systems.
All the invented treatment options have at least one major difference to the natural heart: they are built with rigid parts. Stiff regions are one reason among others why these treatment solutions fail to reproduce the movement of the real heart. **Figure 1.7.** highlights the relation of soft materials used for the construction of VAD or TAH and the level of biomimicry of these systems achieve in comparison to the natural heart. The most common side effects of VAD are infections, bleeding, and neurologic dysfunction. An interesting alternative to the current treatment options would be a completely soft heart-like biocompatible pump, which allows pulsatile pumping. A soft machine manufactured out of materials with similar elastic modules than heart tissue could enable better biomimicry of the natural movement of the human heart.

The soft total artificial heart (sTAH) is a silicone soft pump aiming at the requirements mentioned above. Manufactured with the aid of 3D printing technology, the sTAH is an innovative concept intended as a new treatment option for patients suffering from end-stage heart failure. This new sTAH concept promises two advantages over existing TAH: First, the simple production process resulted in a soft low complexity monoblock sTAH, which can adapt to the physiological surroundings inside the body. Second, the use of soft silicone elastomer to manufacture the silicone heart, allows the entire soft pump to move during actuation. Therefore, it can mimic the movement of the real heart (resulting in physiologically shaped signals for blood flow and pressure) and potentially eliminating dead spots of the flow. However, current sTAH are still lacking the required cardiovascular output and have a limited lifetime (approximately 3000 beats). By changing the size of the ventricles and the actuation chamber of the sTAH, new designs could enable higher pumping performance. Moreover, previously presented HTV elastomers (NR or silicone rubber) manufactured by industrial compression or injection molding techniques could allow overcoming the limited longevity of the first sTAH prototype.

The use of HTV rubber and silicone, as well as industrial molding techniques, are presented in chapters 2 and 3 to manufacture long-term stable soft pumps, which are assessed as potential soft total artificial hearts.
1.2. DNA: An information storage tool

For millions of years, nature has relied on nucleic acids as the primary information storage tool. In 1869, the same year as the Suez Canal was inaugurated, the Swiss chemist Friedrich Miescher identified “nuclein” (later called deoxyribonucleic acid, DNA) inside the nuclei of human cells. In the following century Phoebus Levene, who discovered the three major components of DNA (phosphate-sugar-base), and other scientists laid the groundwork for the discovery of the three-dimensional double-helical model of DNA published by James Watson and Francis Crick in 1953.

DNA is a biopolymer composed of a deoxyribose phosphate backbone and four different bases: adenine (A), guanine (G), cytosine (C), and thymine (T), whereas A and G are purines, and C and T are pyridines. Also known as Chargaff’s rule, the amount of A is almost equal to T, and the same is true for G and C, no matter what organism. The anti-parallel DNA strands that form the double-helical structure of DNA are held together by hydrogen bonds between

Figure 1.8. The double-helical structure of deoxyribonucleic acid (DNA). The sugar-phosphate backbones of the two DNA single strands run antiparallel and are held together by hydrogen bonds. Three hydrogen bonds connect guanine (G) to cytosine (C) and two hydrogen bonds connect thymine (T) to adenine (A). Copyright (2013), with permission of Springer Nature.
complementary bases (A with T and G with C), which form a base pair (see Fig. 1.8.). In the human body, most cells contain 23 pairs of chromosomes containing approximately 3 billion base pairs, which include all the information needed to grow a human being.53

Today, the understanding of DNA is impacting many different fields: In agriculture, DNA of plants is edited to create gene-modified crops with increased yields (e.g., BT cotton).54 In juridical investigations, DNA is used to identify criminals or in genealogy to establish biological relationships.55,56 Advances and cost reduction in DNA synthesis and sequencing facilitate the creation of new DNA tools, such as DNA tagging of goods and DNA data storage.57–59

1.2.1. DNA synthesis, amplification, and sequencing

The application of DNA in diagnostics, forensics, and other fields is due to the advances and more widespread adoption of three different techniques: DNA synthesis, DNA amplification, and DNA sequencing. The next few paragraphs will shortly explain the mentioned techniques.

Oligonucleotide synthesis is the chemical synthesis of small sequences of nucleic acids with a specific sequence. In contrast to the early days of DNA synthesis, when the technique was still confined to academic labs, today, a particular sequence of DNA can be ordered commercially online. Currently, the predominant synthesis technique is based on solid-phase phosphoramidite chemistry.60 In automated instruments, this four-step chemical process, adds one base at a time per cycle to grow oligo chains. Column-based oligo synthesis routinely synthesizes up to ~100 nucleotides (nt) with costs varying between ~$0.05-0.15 per nt, whereas modern spatially localized DNA synthesis methods allow the price per nt to drop by 2-4 orders of magnitude.60 These array-based oligo synthesis techniques produce DNA with varying lengths, scale, and error rates depending on the different vendors.60 Due to synthesis error rates and yield limitations typical lengths for oligonucleotides used in DNA data storage applications are 150-230 nt long.57

The second technique, DNA amplification is a method used to produce many copies of a specific DNA or DNA segment and is commonly referred to as polymer chain reaction (PCR). Since the first publication of PCR in 1985, the reaction is nowadays an established technique in many laboratories.61 In short, a DNA target in combination with heat-stable DNA polymerase (e.g. Taq polymerase), a forward and reverse primer (short oligo sequences), and a master mix
(buffered mixture of dNTPs) are repeatedly cycled through three different thermal cycling steps to amplify the DNA target exponentially. Each step has a specific function and temperature: In the denaturation phase (≈ 98°C), double-stranded DNA melts into a single-stranded DNA. During the annealing phase (≈ 60°C) primers anneal to the single-strand DNA target and in the elongation phase (≈ 72°C) DNA polymerase synthesizes a new complementary DNA strand to the template DNA. For example, starting with two DNA strands and performing 10 PCR cycles (assuming 100% efficiency) one creates $2^{10}=1024$ DNA strands, allowing rapid DNA multiplication.

The third technique, DNA sequencing, is the process of determining the order of nucleotides in DNA. Since Frederick Sanger introduced a rapid procedure to identify DNA sequences in the 1970s, significant advances in the field lead to the development of next-generation sequencing (NGS) methods. At the moment, the company Illumina®, using an NGS method called sequencing-by-synthesis (SBS), holds the largest market share for sequencing instruments. In brief, different short single-stranded DNA sequences flood a microarray chip. After the DNA strands attach to the flow cell surface, they form clusters of different DNA sequences via bridge amplification. Next, a step-by-step process attaches one complementary fluorescently tagged nucleotides to the DNA strand at the time and is imaged (one read) before the next nucleotide is attached. In small benchtop units like the Illumina® iSeq™ 100, this step-by-step sequencing-by-synthesis process generates approximately four million reads per run.

In 2012, the combined techniques (DNA synthesis, DNA amplification, and DNA sequencing) and new encoding strategies enabled Church et al. to encode 5.27 MB of digital data into 54,898 159-nt DNA strands. However, without adequate protection, the 54,898 DNA strands can rapidly decay during storage. Therefore, specialized storage techniques have to prevent that information stored in DNA becomes worthless. In Nature, DNA in ancient bone and tooth has shown that the biopolymer can be stable for thousands of years. The next subchapter will discuss in more detail DNA stability and DNA protection against degradation.

### 1.2.2. DNA stability and protection

In 1993, the Nobel laureate Tomas Lindahl stated: “All biological macromolecules spontaneously decompose”. DNA is no exception. DNA decay mechanisms include hydrolytic cytosine deamination, oxidative damage, alkylation, depyrimidination, and...
Since DNA generally exists in aqueous environments or is accessible by water to a certain extent, it frequently undergoes hydrolysis. This decay pathway leads to a loss of bases (depurination or depyrimidination). The loss of purines (depurination) is more likely than the loss of pyrimidines (depyrimidination). The loss rate of pyrimidine bases (C & T) was determined to be 5% of the rate of purines (A & G).[^70] The depurination of a base is not only a loss of information but also leads to a subsequent β-elimination, leading to single-strand breaks.[^70][^72]

If DNA is not stored under well-controlled environmental conditions (ideally dry and at low temperatures), any information saved therein can be lost in short periods (months to years). In vivo, DNA degradation is limited due to efficient DNA repair processes, and DNA is generally physically separated in the cell.[^73][^74] Also, the incorporation of DNA in inorganic matrices has proven to be effective against DNA decay. DNA can be preserved for thousands of years in bones, which are mostly composed of calcium phosphate.[^68][^69][^75]

![Figure 1.9. State of the art long-term high capacity DNA storage on Layer-by-Layer (LbL) DNA encapsulation in magnetic nanoparticles. Initially, carbon-coated iron nanoparticles are negatively functionalized before an alternating LbL loading of polyethylenimine (PEI) and DNA. Finally, a protective silica layer encapsulates the DNA. Copyright (2019), with permission of John Wiley and Sons.](image)

[^70]: The depurination of a base is not only a loss of information but also leads to a subsequent β-elimination, leading to single-strand breaks.
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[^75]: Also, the incorporation of DNA in inorganic matrices has proven to be effective against DNA decay. DNA can be preserved for thousands of years in bones, which are mostly composed of calcium phosphate.
Different storage methods exist to preserve the biomolecule (e.g., on filter paper, embedded in trehalose or stored in a freezer).\textsuperscript{76-78} Even though these methods prevent DNA decay, they don’t reproduce the chemical and physical sealing found in naturally occurring fossils. In 2013, Paunescu \textit{et al.} reported a DNA storage method mimicking fossils that encapsulated DNA in silica nanoparticles.\textsuperscript{79} On these nanoparticles, a thin (12-15 nm) silica layer separates the DNA from the environment, water, and reactive oxygen species. Thereby, a total of 0.2-0.7 wt\% DNA was effectively protected against degradation.\textsuperscript{80} In 2019, Chen \textit{et al.} reported a similar but improved DNA storage on magnetic nanoparticles.\textsuperscript{81} Using a layer-by-layer approach, a total of 3.4 wt\% DNA was loaded onto the particles (see \textbf{Fig. 1.9}). DNA protection for both the silica and magnetic nanoparticles is achieved by the polycondensation of tetraethoxysilane (TEOS), which forms a dense silica layer on top of the DNA. This protective shield allows DNA to be potentially stable for hundreds of years.\textsuperscript{82} Using a fluoride-containing release solution that dissolves the protective silica layer, DNA can be removed and analyzed. DNA stored in silica nanoparticles not only prevents DNA from decay but also allows DNA to be used as a novel tracing tool.\textsuperscript{83} The next chapter will introduce DNA nanoparticles as a new material tracing technique and advanced product information carrier.

\subsection*{1.2.3. Quality control via DNA barcodes}

DNA barcodes (or DNA taggant/tracer) are a chemical species that can be added to a product to identify it. The specific sequence of nucleotides in the DNA can be used to identify particular products, in a similar way that Universal Product Code (UPC) barcodes are used in the supermarket to identify specific products. DNA sequences used for DNA barcodes are generally less than 100 base pairs long, and recently, a news article proclaimed that an existing DNA barcode system called safeTracers\textsuperscript{TM} was affirmed G.R.A.S. (Generally Regarded as Safe) by the FDA.\textsuperscript{84} DNA barcodes offer some advantages over other tagging systems: an unlimited number of barcodes, naturally standardized biomaterial, and the tracer can be on or inside the product rather than on the exterior of the package.

The quality of a product is a common subjective understanding or a normed value (e.g., ISO standard) to differentiate or authenticate the superiority or inferiority of a product. In other words, the consumer generally wants to compare the quality of products. Tracing products enables companies to track a product’s supply chain, ensure product authenticity, and create
more transparency. Moreover, material transformation and mixing can be traced along the supply chain, as shown in Figure 1.10.

In general, almost any product can be tagged with DNA barcodes. Products that are often counterfeit and would benefit from a further product authentication system are pharmaceutical agents. Today, anti-counterfeit options exist; however, they are usually on the package and not on the pill or capsule itself. Recently, Jung et al. reported a rapid authentication of pharmaceutical via DNA fragments introduced into pharmaceutical ink.85 Thereby, the molecular taggant was attached directly onto the capsule containing the pharmacological agent.

The use of unprotected DNA as taggant might be insufficient since DNA can degrade. The previously introduced DNA protection technique, also called silica particles encapsulated with DNA (SPED), was initially invented to tag products and protect DNA.79 Thereby, SPED were made compatible, for example, to tag polymers and withstand harsh conditions like injection molding at 200°C.79 Recently the ETH spin-off Haelixa successfully tagged valuable gemstones throughout their value chain by using SPED. Several other studies also used this DNA barcode system to tag materials like olive oil, milk, and pesticides.58,86,87 The information carrier tool DNA can not only be used as a taggant but also as a sensor. Caged DNA nanosensor particles were used to measure light exposure in single cells or as groundwater tracers.88,89

Figure 1.10. Material and product tagging/tracing with DNA barcodes in the supply chain. During material mixing or transformation, DNA barcodes remain inside the material.
With stronger customer demand for supply chain transparency, tracing products from their origin of production, throughout the supply chain and until their purchase, might become standard practice. Concepts like from Farm to Fork will allow customers to review the entire journey of the food they are eating. Many food products are a combination of various ingredients. In the case of multicomponent products, SPED tagging could not only be used as a tracer but also as a composition detection tool. Chapter 4 introduces a SPED-based mixing and composition detection technique in more detail.

1.2.4. DNA data storage

The previous chapters highlighted that DNA is used as a tool to tackle various problems. The next paragraphs introduce DNA as a means to solve future digital data storage limitations.

Today, more than 20 billion devices are connected to the internet, accessing and creating new data. The Large Hadron Collider of CERN generates approximately 15 PB of data per year, and the IDC predicts that by 2025, we will create about 163 ZB of data (that is more than one trillion gigabytes). The installed base of mainstream storage is not keeping up with an exponentially growing data generation. Most generated data is discarded; nevertheless, some information is only valuable when stored. Today more than 60% of information is kept on hard drives. However, the lifetime of these devices is short, and most long-term/ archival storage of data is done on magnetic tape. This storage medium offers high storage density, low energy consumption, but the typical lifetime of this storage media is only a couple of decades. Tapes have to be repeatedly copied to keep data safe. To tackle the data storage problem, scientists investigated alternatives for dense, low power consuming, and long-term archival data storage.

DNA has one of the best track records as a long-term information storage tool. Moreover, as long as there will be humans, DNA will probably not lose its significance as an information carrier. Not only is DNA a standardized molecule among all self-reproducing cellular organisms, but its information storage density (10^{19} \text{bytes per cm}^3) is orders of magnitude higher than conventional storage media. Stored in the right conditions, DNA can be stable for hundreds to thousands of years. Moreover, PCR allows fast and cheap DNA replication. These characteristics of DNA make it an interesting alternative for future long-term archival data storage.
In the 1960s, the idea of storing data in DNA first appeared, but DNA synthesis and sequencing were still in their infancies and did not allow scientists to pursue the idea further. In the early 2010s, following major improvements in both DNA synthesis and sequencing, Church et al. and Goldman et al. independently stored hundreds of kilobytes of data in DNA. In short, the process of storing digital data in DNA involves the following steps: Encoding, synthesis, storage, sequencing, and decoding (see Fig. 1.11). First, digital information is encoded in DNA sequences (≈ 150 nt long). Next, the DNA sequences are physically synthesised before they can be stored. DNA storage generally includes physical DNA encapsulation and subsequent release. Finally, a sequencing machine can read the released DNA. Therefore, each DNA sequence is analyzed before the information stored in the molecules is decoded back into digital information. In other words, binary information in the form of 0 and 1 can be transformed into a sequence of nucleotides (A, G, T, and C), which can be converted back into binary data. Recently, DNA data storage systems became more advanced and larger in data storage size by introducing error-correction, different encoding schemes, and random access. In 2018, Organick et al. presented digital information storage in DNA of 200 MB in more than 13 million DNA oligonucleotides.

![Figure 1.11. Illustration of digital information storage in DNA. In general the storage process is composed of 5 steps: Encoding, DNA synthesis, DNA storage, DNA sequencing and decoding.](image)

The computational side of DNA data storage (encoding and decoding) has undergone many improvements; however, few researchers have focused on developing high density and stable
DNA storage media. With state of the art DNA storage in nanoparticles achieving DNA loadings up to 3.4 wt%, there is room for improvement in terms of DNA loading and encapsulation speed.\textsuperscript{81,82} Previously introduced inorganic matrices could potentially increase DNA loading beyond 10 wt%, while simultaneously simplifying the DNA encapsulation and release process.

In the near future, DNA could become an interesting alternative to magnetic tape storage since the biomolecule fulfills the requirements of dense, low power consuming, and long-term stable data storage. Cost comparisons between magnetic tape storage and DNA data storage, allowed Goldman et al. to predict that DNA data storage is more cost-efficient for storing several megabytes of data over a time horizon between 50-500 years.\textsuperscript{91}

1.3. Conclusion and outlook

Soft pumps have great potential to become an alternative treatment option for patients suffering from end-stage heart failure. However, the current sTAH prototype has a limited lifetime and pumping performance. To overcome these limitations, Chapter 2 reveals a rubber compression molding technique to produce soft pumps with longer lifetimes and improved volumetric flowrates. However, this soft pump was produced with a non-biocompatible natural rubber. Based on these findings, Chapter 3 introduces an injection molded silicone soft pump. Since silicone is used as a biocompatible material for medical devices, this soft machine is considered as a next-generation sTAH prototype.

DNA-based technologies are a powerful tool to accelerate progress in many different research areas. The widespread availability of DNA reading, writing, and manipulation techniques, allow the biomolecule to be used for new applications. In Chapter 4, we propose a DNA-based mixing detection technique that extends DNA functionality beyond the application of track and tracing of goods. Moreover, Chapter 5 reveals how inorganic matrixes limit DNA degradation. These high-density storage matrixes for DNA were tested as a novel DNA data storage system.
2. Long-Term Performance of a Pneumatically Actuated Soft Pump, Manufactured by Rubber Compression Molding

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2.1. Introduction

Soft machines have gained considerable attention during the last decade. In a field traditionally dominated by mechanical “hard” systems, soft machines add an entirely new dimension to tackle various engineering challenges. The intrinsic softness of these devices facilitates the imitation of human or animal motion and reduces the complexity of system control. Most soft machines are actuated by pressurized air. Also actuation by gas combustion, electrical charge or magnetic fields has been reported. Very complex machine or robot shapes can be realized by 3D printing the designated casting mold. Further, manufacturing soft robots is comparably inexpensive due to the large availability of curable elastomer systems and progress on 3D printing technology. Therefore, the applicability of soft robots and machines seems limitless and offers great potential for developing new alternatives for traditional machine tasks.

A particularly interesting application of soft robotic principles and soft machines are medical devices. In a medical setting, these soft devices have to interact with organs (e.g., skin, muscle tissue, and blood). Within the human body, machine components of “hard” systems are also in contact with blood. The interaction between the typically rigid metal surfaces of ventricular assist devices (VAD) with blood components and an unphysiological actuation of blood (such as in rotary pumps) result in numerous negative side effects. These include high shear stress, blood trauma, and thrombus formation leading to embolic complications, infection and bleeding complications due to the necessary anticoagulation. The incompatibility between machine and patient can partially be explained by the difference of implant material stiffness and soft patient tissue. Therefore, a soft machine might have advantages compared to classical, rigid devices as they can be manufactured with the same stiffness range as the surrounding tissue. Soft devices also have to fulfill the durability requirements and provide accurate operation inside the human body over a large range of physiological conditions.

In literature, various pumps, which were manufactured almost entirely from elastomers, have been presented. Some of these pumps were designed to be applied as an artificial heart. Enabled by their flexibility, soft pumps can adapt to their environment and cope well with high degrees of mechanical deformation. So far, soft pumps lack durability and do not reach the performance goals of a biological soft pump, the human heart (Table 2.1.). The combustion driven diaphragm pump by Stergiopulos et al. has weak spots, at the glued bonding surfaces, which prevented long-term operation. The monoblock soft pump designed by Loepfe et al. removes bonding surfaces but failed after only 30’000 combustion cycles.
pneumatically actuated poroelastic foam pump by Mac Murray et al. generates flow rates of up to $430 \text{ mL/min}$, but no long-term operation has been shown. The soft artificial heart by Cohrs et al. is operated by pressurized air, however, it only has a limited lifetime of $3'000 \text{ cycles}$ under realistic physiological conditions.

**Table 2.1.** Performance and durability of different soft pumps found in the literature compared to the human heart. The maximum pump rate of the human heart is given for one ventricle of a healthy young individual during maximal exercise. The weight of the soft pumps is given without any connected parts.

<table>
<thead>
<tr>
<th>Pump</th>
<th>Material</th>
<th>Maximum pump rate (ml/min)</th>
<th>Actuation</th>
<th>Cycles</th>
<th>Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stergiopulos et al.</td>
<td>Diaphragm pump</td>
<td>Silicone rubber$^{[100]}$</td>
<td>Combustion (air/methane)$^{[100]}$</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Loepfe et al.</td>
<td>Soft silicone pump</td>
<td>Silicone rubber$^{[10]}$</td>
<td>Combustion (air/methane)$^{[10]}$</td>
<td>$30'000^{[10]}$</td>
<td>$600^{[10]}$</td>
</tr>
<tr>
<td>Mac Murray et al.</td>
<td>Poroelastic foam pump</td>
<td>Silicone rubber$^{[102]}$</td>
<td>Pneumatic$^{[102]}$</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cohrs et al.</td>
<td>Soft artificial heart</td>
<td>Silicone rubber$^{[49]}$</td>
<td>Pneumatic$^{[49]}$</td>
<td>$3'000^{[49]}$</td>
<td>$390^{[49]}$</td>
</tr>
<tr>
<td>-</td>
<td>Human Heart</td>
<td>Cardiac Muscle$^{[41]}$</td>
<td>Muscle contraction$^{[41]}$</td>
<td>$3 \text{ billion}^{[42]}$</td>
<td>$250-350^{[41]}$</td>
</tr>
</tbody>
</table>

Both the soft artificial heart presented by Cohrs et al. and a soft robotic sleeve presented by Roche et al. offer possible solutions for mechanical circulatory support of patients suffering from end-stage heart failure. These systems utilize the intrinsic advantages of soft robotics by performing complex motion without the need for difficult control mechanism. However, as illustrated in Figure 2.1, soft machines have the significant drawback of a limited lifetime. Whereas the human heart or centrifugal pumps operate for billions of cycles, soft pumps and soft heart assist devices do not present similar results yet.

Improvements in design and choice of material have to be considered in order for soft pumps to compete against traditional rigid systems. The testing of various alternative materials for soft pumps requires the investigation of different manufacturing techniques. These techniques would enable the transition from room temperature vulcanizing silicones to hot vulcanizing rubbers (e.g., styrene-butadiene rubber (SBR)). Hot vulcanizing materials have already proven long lifetimes, when for example being used as a material in car tires. Moreover, new manufacturing techniques could possibly enable high throughput manufacturing of soft machines.
In this study, we present a pneumatically actuated soft pump (SP), which was able to pump >8.5 L/min of water and to run for >1 million actuation cycles over a period of more than 11 days. To our knowledge for the first time, an entirely soft pump was manufactured by rubber compression molding in a hot table press. This manufacturing method enabled the use of extremely durable hot vulcanizing rubber as SP material. We investigated the pump performance and analyzed the pump behavior (in situ) by ultrasonic imaging. In order to validate the SP potential as a soft artificial heart prototype, it was tested against physiological in- and outlet pressures of the human cardiovascular system on a hybrid mock circulation.\textsuperscript{106}

### 2.2. Material and methods

#### 2.2.1. Pump design, actuation, and manufacturing

The SP was manufactured by rubber compression molding. This technique enables the manufacturing of entirely soft complex monoblock forms made of vulcanized materials. In detail, we designed two symmetrical fluid ejection chambers (volume 168 cm\(^3\)) in a computer-aided software (Autodesk Inventor, Autodesk Inc., USA) (Fig. A.1.1. and A.1.2.). An expansion chamber (volume 74 cm\(^3\)) was placed between the fluid ejection chambers, which enabled the pushing of the fluid from the ejection chambers by alternating in- and deflation of the expansion chamber with pressurized air (200 kPa). The pneumatic actuation cycle of the SP consisted of two phases (inflation and deflation phase). The inflation of the expansion chamber resulted in the compression of the two ejection chambers, thereby displacing the fluid and pushing it towards the outlets. During the deflation phase, the displaced ejection chambers returned to their initial form, enabling the refill with fluid. A detailed schematic overview of the design is shown in Figure 2.2.B. Unidirectional flow was maintained by mechanical heart

**Figure 2.1.** Images of different soft, mechanical or biological pumps and comparison of their expected lifetime. (A) Commercial centrifugal pump\textsuperscript{105} (B) Human heart\textsuperscript{42} (C) Soft total artificial heart\textsuperscript{60} (D) Soft pump.
valves (Björk-Shiley type). These valves were placed inside an aluminium tube at the inlets and outlets of the ejection chambers.

A schematic overview of the manufacturing process is shown in Figure 2.2. The mold was machined out of aluminium blocks with a 3-axis computer numerical control machine. Excess rubber openings were placed in the mold at various locations (Fig. A.1.5.B). The resulting aluminium mold was filled with unvulcanized rubber sheets (LK07-Kal, Kraiburg Holding GmbH & Co. KG, Germany) and placed in a hot table press (TBH 400, Fontune Holland, The Netherlands) at 160°C and 100 kN for 14 minutes. A temperature profile of the vulcanization process can be seen in Figure A.1.3. After the rubber had vulcanized, the aluminium mold was removed manually to release the SP. The air in- and outlet of the expansion chamber was manufactured in a second vulcanization step with another aluminium mold (Fig. A.1.4.). Additional details about the manufacturing process including a time-lapse video of the most important manufacturing steps are provided in the Appendix.

**Figure 2.2.** (A) Schematic overview of the manufacturing process. Design and manufacturing of the rubber compression mold and production of an entirely soft vulcanized rubber form. (B) gives a picture of the soft pump with the aluminium housings of the mechanical heart valves and cut-through pictures of the soft pump showing the ejection and expansion chambers and schematic representation of the unidirectional flow through the ejection chambers.
2.2.2. Performance characterization

The SP performance was tested with distilled water in two distinct settings. In the first test-setup, the water outlet pressure on the ejection chambers stayed constant at 10.4 kPa (78 mmHg). This value is in the range of the diastolic pressure regime of the human aorta. Once the SP was actuated, the outflowing water filled up two water reservoirs. These reservoirs were placed above the SP and connected to the inflow of the SP. Thus, they generated a water inlet pressure of 3.2 kPa (24 mmHg). The water pressure difference in this setup was 7.2 kPa (54 mmHg) (Fig. A.1.6.). In order to investigate the pumping characteristics of the SP, different ratios of inflation and deflation times were tested, and an inflation/deflation ratio for maximum flow and minimum stress on the pump was determined. These experiments were repeated at different rates, between 40 and 70 beats per minute (bpm). The flow was measured using ultrasonic flow probes (Sonoflow CO.55/190, Sonotec, Germany). Once an optimal operating condition for inflation and deflation times as well as the beat rate had been determined, a long-term test was performed to validate the durability of the SP design. The optimal operating condition (60 bpm, ratio of inflation/deflation time of 25% and air pressure of 200 kPa), was chosen at 60 bpm, since this beat rate represents a healthy physiological heart rate at rest. Also, previous experiments had shown, that high flowrates were achieved at this beat rate and inflation/deflation ratio.

To examine the performance of the SP as a potential prototype for a soft artificial heart, it was tested on a hybrid mock circulation.\textsuperscript{106} For a detailed description of the test setup we refer to the study of Petrou et al.\textsuperscript{106} Briefly, the second test setup consisted of four pressure-controlled water reservoirs connected with plastic tubes to each in- and outlet of the SP ejection chambers. The specific test conditions on the hybrid mock circulation were maintained through in-house developed software. The pressures at the outlets were varied between 3.3 and 20.6 kPa (25 and 155 mmHg), whereas the inlet pressure stayed constant at 0.7 kPa (5 mmHg). The flows were measured with ultrasonic flow probes, at each in- and outlet, as described above. The SP was actuated at 200-300 kPa, 60 bpm and an inflation/deflation ratio of 25%.

The diaphragm movement of the expansion chamber inside the SP was investigated by means of ultrasonography (Acuson Antares, Siemens Medical Solutions, USA). The SP was actuated at 200 kPa, 60 bpm, and an inflation/deflation ratio of 25%, while ultrasonic images were taken.
A Shimadzu Universal Testing Instrument AGS-X with a 50 N load cell was used to determine the elastic modulus and the continuous deformation of the used rubber upon repetitive strain at a constant stress of 1.62 MPa.

2.3. Results and discussion

2.3.1. Rubber compression molding

The rubber compression molding yielded a monoblock soft rubber pump with a mass of 162 g as shown in Figure 2.3.A. The monoblock design proved to be tear-resistant upon strong deformation by hand (Fig. 2.3.B and 2.3.C). As stated previously by Schumacher et al., the proposed monoblock manufacturing technique avoids weak spots such as bonding surfaces, which can depress the lifetime and durability of the structure. In order to characterize the rubber of the SP, the elastic modulus was determined. The measured elastic modulus \( E \) of the rubber was measured as \( E = 9.44 \pm 0.29 \text{ MPa} (n = 4) \) (Table A.1.1.). The rubber is approximately 3 times stiffer when comparing its elastic modulus to the silicone rubber of the soft total artificial heart presented by Cohrs et al. The design and manufacturing process of the molds resulted in several versions thereof with increased complexity. The first aluminium mold prototypes did not include excess rubber openings, and therefore, the resulting SP presented defects. These openings prevented the inclusion of air and thereby allowed the free flow of vulcanizing rubber into all cavities of the mold (Fig. A.1.5.B). A detailed protocol of the manufacturing process is available in the Appendix.

![Figure 2.3. Pictures of flexible soft pump: (A) Soft pump (SP) (B) Torsion of SP and (C) Bending of SP.](image-url)
2.3.2. Pump performance

Various rates and injection/deflation ratios of the pressurized air were investigated in order to determine optimum pump performance. Figure 2.4.A. shows the flow rate of one ejection chamber, whereas Figure 2.4.B shows the corresponding stroke volume. The experimental results demonstrated an increased flow rate with an increasing ratio of injection and deflation time and, thus, of pressurized air fed to the SP. The flow rates showed a steep increase in the region of 10-25% inflation/deflation ratio as depicted in Figure 2.4.A. This increase was observed at all tested rates. For one expansion chamber, the maximum flow rate of 4.5 L/min, was reached at 60 bpm and inflation/deflation ratios of 33 to 43%. Sufficient deflation of the expansion chamber could not be guaranteed when applying inflation/deflation ratios of more than 43%. Above this value, the soft pump continuously expanded with each beat, as the pressurized air inflow seems to be greater than the air outflow. Moreover, with high inflation/deflation ratios of more than 43%, the overall stress on the soft pump increased drastically, making long-term operation questionable. At comparably low rates of 40 bpm, the maximum stroke volume of 87 mL was measured. This maximum deformation of the expansion chamber emptied 52% of the ejection chamber volume. The smallest deformation was calculated at 70 bpm. Figure 2.4.C. schematically explains the expansion chambers deformation at different rates. The detected limiting factor for maximizing the flow rate at high rates was the deflation phase. During this phase, the passive relaxation of the expansion chamber limited the rapid refilling with water. Thus, only a sufficient deflation time allowed maximum refilling of the ejection chamber before the next actuation cycles began.
Figure 2.4. Pump performance of one ejection chamber against a water outlet pressure of 10.4 kPa (78 mmHg), actuated with pressurized air at 200 kPa at varying ratios of injection/deflation time and at varying rates. (A) The flow rate of one ejection chamber at different conditions. (B) The stroke volume of one ejection chamber at different conditions. (C) Schematic representation of the deformed expansion chamber at different rates.

2.3.3. Pump lifetime

In order to persist in most applications, reliable operation (a sufficient lifetime) is a key requirement for a pump. Therefore, we tested the SP over 1 million cycles. This increase in the lifetime of 2-3 orders of magnitude compared to previously developed and published soft pumps, represents a new long-term stability benchmark.\textsuperscript{12,49,100–102} So far, soft actuators like pneumatic artificial muscles reported actuation up to 229’000 cycles, while lifetimes of fluidic muscles up to 10 million cycles were reported.\textsuperscript{107,108} The long-term test was performed in the same test setup as the pump performance characterization. During testing, we observed no deterioration of the ejection chambers of the SP. Repetitive elongation of the material on a tensile tester (>200% strain, 1’500 cycles) resulted in significant wear out. In order to reach the engineering stress of 1.62 MPa, the strain continuously increased with the number of cycles. The strain of cycle 1’500 was 31% larger than the strain of cycle 1 (Fig. A.1.7.C). Since the
actuation of the soft pump does not cause a deformation of the expansion chambers diaphragm of >100%, we do not expect wear out of the diaphragm. **Figure 2.5.B** shows that constant flow rates of more than 4 L/min from one ejection chamber were measured up to 1 million cycles. However, the last measurement in **Figure 2.5.B** is below 4 L/min. The lower flow rate was not caused by a malfunction of the SP, but due to a leaking water tank. This reduced the pumping performance due to decreased water inlet pressure. Taking the average pumping performance of 4.27 L/min and assuming that both expansion chambers eject the same amount of water, a total volume of 142’283 L was pumped during 1 million cycles (11.57 days). A commercial pump with a performance of 10 L/min against 10 kPa, weighs 1.2 kg as compared to 162 g for the SP, without any connected parts.\(^{109}\)

**Figure 2.5.** (A) **Test bench for long-term testing and performance characterization of the soft pump.** A picture of the test bench can be found in the Appendix (Fig. A.1.6). The flow rates were measured with a flow sensor (FS). (B) **Flow rate of the soft pump, measured for one ejection chamber, operated for more than 1’000’000 cycles.** Actuation was performed at 60 bpm, 200 kPa and an inflation/deflation ratio of 25%.
2.3.4. Pump performance under physiological conditions

In order to assess to which degree the developed SP could be used as a possible soft total artificial heart prototype, the performance of the SP was evaluated on a hybrid mock circulation (Fig. 2.6.A). The SP had to pump against varying outlet pressures, at a beat rate of 60 bpm and at an inflation/deflation ratio of 25%. We observed a decrease of flow with increased outlet pressures and determined that the pumping performance is not yet sufficient for a soft total artificial heart prototype. Table 2.2. lists the mean flow rates for both ejection chambers exposed to different outlet pressures. For discussion purposes, we refer from now on to the right ventricle (RV) and left ventricle (LV) for the different ejection chambers of the soft pump. During the experiments, the RV performance stayed comparably constant against a constant outlet pressure head, while the LV pumping performance decreased with increasing water pressure head. At 60 bpm and physiological outlet pressures of 3.3 and 10 kPa (25 and 75 mmHg) for the RV and LV, respectively, flow rates of 2.5 and 1.8 L/min were measured. This flow is higher than the one of the soft total artificial heart, previously reported by Cohrs et al. However, the performance is still below 8 L/min maximum output value, required for artificial hearts by the National Heart, Lung, and Blood Institute. Moreover, applying different outlet pressure heads to each ejection chamber must not lead to different pumping performances if the SP should be used as a heart replacement. With the current design, equal pumping performance cannot be achieved, as the ejection chambers were designed symmetrically. The different flow patterns at different pressure conditions for the RV and LV are depicted in Figure 2.6.B-C. The RV was able to pump more than the LV, which seems reasonable due to the smaller outlet pressure (see Fig. 2.6.B-C). In a human body, this pumping behavior would be lethal, as the RV pumps excess volume into the lungs leading to pulmonary edema. Moreover, a small backflow occurs after every beat, which is inherent to the design of the used artificial heart valves. The backflow shall prevent dead spots of flow, thus preventing possible blood clots. We further observed that increasing the air pressure from 200 to 300 kPa increased the flowrate. However, we could visually observe that the stress on the pump was intense and no long-term operation would be feasible.
Figure. 2.6. Beat-by-beat pumping performance of the soft pump actuated at a beat rate of 60 bpm, 200 kPa pressurized air and an inflation/deflation ratio of 25%. (A) Simplified schematic of the software-controlled hybrid mock circulation (HMC) from Petrou et al. The HMC applied constant pressures to the pulmonary circulation, right atrial pressure (RAP) and pulmonary arterial pressure (PAP), and to the systemic circulation, left atrial pressure (LAP) and aortic pressure (AoP), thus mimicking the inlet and outlet pressures of a total artificial heart supported circulation. (B) The flow pattern of the right ventricle (right ejection chamber of the SP) operated against an outlet pressure of 3.3 kPa (25 mmHg). (C) The flow pattern of the left ventricle (left ejection chamber of the SP) operated against an outlet pressure of 13.3 kPa (100 mmHg).

Table 2.2. Mean flow rate of the right ventricle (RV) and the left ventricle (LV) of the soft pump measured at different outlet pressure for the RV, pulmonary arterial pressure (PAP), and for the LV, aortic pressure (AoP) on a hybrid mock circulation.

<table>
<thead>
<tr>
<th>Air Inlet Pressure (kPa)</th>
<th>RV Outlet Pressure PAP (kPa)</th>
<th>LV Outlet Pressure AoP (kPa)</th>
<th>RV Mean Flow (L/min)</th>
<th>LV Mean Flow (L/min)</th>
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<td>200</td>
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<td>3.3</td>
<td>2.50</td>
<td>2.83</td>
</tr>
<tr>
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<td>10</td>
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</tr>
<tr>
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<tr>
<td>200</td>
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<td>0.08</td>
<td>0.20</td>
</tr>
<tr>
<td>300</td>
<td>20.7</td>
<td>20.7</td>
<td>0.61</td>
<td>0.74</td>
</tr>
</tbody>
</table>
2.3.5. Ejection chamber diaphragm visualization

With the help of an ultrasound device, we observed the non-uniform movement of the inner ejection chamber diaphragm. Upon actuation, sonography is able to record the ejection chamber’s movement in situ. The array transducer was placed such that the imaging plane resulted in a cut-through image of the expansion chamber wall. In Figure 2.7, the three different positions, which were chosen to analyze the movement, are indicated with yellow lines on the SP. The blue area in the ultrasound images indicates the water inside the ejection chamber. As explained above, the actuation cycle can be separated into two different phases: first the inflation phase and, second the deflation phase. During the inflation phase, a rather symmetrical expansion of the expansion chamber diaphragm was observed. After the maximum expansion is reached, and, thus displacement of fluid, the expansion chamber diaphragm collapsed unsymmetrically during the deflation phase (Fig. 2.7). This uncontrolled collapse led to an s-shape folding of the expansion chamber’s diaphragm. We observed this s-shape folding in the series of images taken in position 2 of the array inducer on the SP. Not only is the passive relaxation of the expansion chamber the limiting factor of the pump performance, but this uncontrolled motion could lead to turbulences inside the SP fluid chambers. Turbulences inside a soft pump would be a clear downside, when it should be considered as a potential soft total heart, due to possible hemolysis at high shear stresses in turbulent flows.¹¹¹
Figure 2.7. Ultrasound images of one diaphragm of the expansion chamber of the soft pump (SP). Three array transducer positions are indicated on the SP with yellow lines. One actuation cycle (inflation phase and deflation phase) is shown with five sonograms of the moving ejection chamber diaphragm.
2.4. Conclusion

In this study, we demonstrated a soft pulsatile pump manufactured by rubber compression molding, which could be operated over 1 million actuation cycles. The rubber compression molding process enabled the production of soft pumps of reproducible quality. The introduction of this new manufacturing process for soft robots and soft machines makes the production of long-lasting, highly durable systems possible. There are two major advantages of the presented rubber compression molding manufacturing process: first, the use of highly deformable and more importantly long lasting high-temperature vulcanizing materials was possible, and second, the soft pumps could be manufactured to rubber monoblocks without any seams. This manufacturing technique avoids weak spots, such as bonding surfaces.

In contrast to 3D printed injection molds, the manufacturing of complex metal molds is not cheap and not readily available. In the advent of metal 3D printing and widely available design software, rubber compression molding could develop into a commonly used soft robotics manufacturing technique, if the costs of metal 3D printing are appropriate for the application. Moreover, a variety of new materials for soft machines and soft robotics could be used with the rubber compression technique.

Besides the long lifetime of more than >1M cycles, >140’000 liters pumped and maximum stroke volumes of >87 mL, the performance of the soft pump is insufficient when evaluated under physiological in situ conditions. Further design improvements have to be undertaken, not only to increase the pumping performance but also to control an equal pumping output when different pressure heads are applied to the ejection chambers. Future work has to investigate two individually controlled expansion chambers, thus enabling equal pumping performance of the individual ejection chambers under physiological pressure conditions. For these reasons among others, the current design is not yet suitable to be further investigated as a soft total artificial heart prototype.

Our findings point out that the next milestone of soft pumps to cycle 10-100 million times is in reach. With the help of the new manufacturing technique, enabling longevity of soft pumps, one game breaker for further developments is resolved. We believe future soft pump designs could reach the physiological pumping performance of a human heart and potentially offer a new heart replacement therapy.
3. Increased Longevity and Pumping Performance of Injection-Molded Soft Total Artificial Heart

Submitted manuscript:


*The manuscript was written by L.G. and X.K. with input from all authors. L.G. performed all experiments during his master thesis.
3.1. Introduction

Heart failure is a serious health condition where the heart is unable to pump a sufficient amount of blood to oxygenate all organs. Worldwide, over 26 million people are affected; a number which will continue to increase due to an aging population. Potential symptoms of such a condition are constant tiredness, coughing, an excessive amount of fluid in and around the lungs and swelling of the abdomen, ankles, and legs. The symptoms lead to patient death in 20-35% of the cases within only one year and there is a 27% 10-year survival rate.

Two categories of devices are available to treat patients suffering from severe heart failure: The Ventricular Assist Device (VAD) and the Total Artificial Heart (TAH). A VAD is a mechanical pump attached to one or both ventricles to support the heart’s pumping performance. A TAH is a pump that completely replaces the heart: its implantation requires the heart’s removal. Metals and hard plastics are used in the fabrication of both devices and therefore these devices fail to reproduce the physiological movements of a real human heart. Patients implanted with a VAD or a TAH suffer from adverse effects such as infection, bleeding and neurologic events.

To address the side-effects of current heart failure treatments, research has focused on developing alternatives using nature-inspired soft machines. By building biocompatible soft machines, which mimic the natural movement of the heart, stress on the blood will be reduced, potentially leading to fewer side effects. Cohrs et al. presented a proof of concept of a soft machine: the soft total artificial heart (sTAH). This silicone-based soft pump was developed with the aid of 3D printers and the lost-wax cast technique and achieved flows of 1 L/min and was actuated for 3000 cycles against physiological pressure heads. Compared to the lifespan of a real heart, around 3 billion beats, the performance of this prototype is insufficient. The longevity of soft pumps can be increased by producing an elastomer monoblock with well-established manufacturing techniques. Recently a soft pump manufactured with industrial rubber via compression molding was continually pumping for 1 million actuation cycles. However, current sTAH lacks the longevity and pumping performance of a real human heart, which can pump more than 20 L/min at peak performance.

To increase both longevity and flow rates of the sTAH, robust engineering methods need to be applied to the soft heart development. Finite element analysis (FEA) can be used in the design process of the sTAH to predict weak points and estimate the pumping performance of the device. Moreover, a robust manufacturing method such as injection molding could produce a seamless elastomer monoblock, which reduces the probability of introducing weak points during manufacturing.
Using injection molding and FEA, we present the design, manufacturing, and performance of an injection-molded sTAH. The pump was actuated for 180,000 cycles in series and conveyed a total of 6.4 L/min of water against physiological pressures.

### 3.2. Material and methods

**Figure 3.1.** CAD images and pictures of the soft total artificial heart (sTAH). In A-D, different perspectives on the sTAH design process are shown. (A) CAD model of a real human heart. (B) Outer shape design of the lower half of the sTAH. (C) Complete sTAH design. (D) Cross-sectional view of the sTAH with three separate chambers (left/ right ventricle and actuation chamber. (E) Squeezing of the flexible injection-molded sTAH. (F) Side-view of the sTAH. (G) The inflow canals (approximated as cylinders) of the sTAH are shown, revealing the Björk-Shirley mechanical heart valves. (H) Schematics of the injection mold showing its seven different parts. (I) Picture of the injection mold. (J) Pile of sTAHs produced by injection molding.

A computer-aided design (CAD) file of a real human heart was used as the blueprint for the physiological design of the sTAH (Fig. 3.1.A-D). The shape of the sTAH was modified using CAD software (Solidworks 2018, Dassault Systems, France). The sTAH consists of three chambers (two ventricles of 152 cm³, each and one actuation chamber of 25 cm³), separated by membranes of 1.9 and 2 mm thickness (See Appendix for calculations). Emptying the large capacity of the ventricle chambers by 55% (83 cm³) should provide a blood flow of 5 L/min at 60 beats per minute (bpm). The membrane thicknesses were chosen to provide equal pumping against different pressure heads. Each ventricle has an in- and outflow opening housing the mechanical heart valves. The heart valves (Björk-Shirley type, 23 mm diameter) surrounded by
rubber rings are integrated into predesigned structures of the sTAH (Fig. 3.1.G), to enable a directional flow of the liquid.

To simplify the manufacturing by injection molding, the ventricular openings were approximated as cylinders, which could potentially be reattached to the real blood vessels. The oblong-shaped opening of the actuation chamber serves as both the inflow and outflow of pressurized air (200 kPa), which actuates the pump. Once the air is injected into the actuation chamber, it expands the membranes propelling the liquid outside the ventricular chambers.

An external injection molding company (ACI Rubber, France) built a metallic injection mold made of 7 different parts (Fig. 3.1.H-I) and used a custom-made HTV silicone with an ultimate tensile strength of 9 Mpa and an elongation at break of 600% to manufacture the sTAH. The material properties also stayed constant after repeated testing (see Fig. A.2.1.). Using this mold, sTAH could be reliably produced in large quantities, with repeatable properties (Fig. 3.1.J). This manufacturing method allows for easy change of the material properties, such as hardness, elasticity, or color. All the produced soft pumps (Fig. 3.1.E-G) had a total volume of 447 cm³ and weighed 136 g (See Table A.2.1. for details on volume). Considering the size range between 400 – 800 cm³ for the adult human heart, the silicone-based soft pump would fit most patients.

3.3. Results and discussion

Three aspects are of major importance in the development process of a new sTAH made out of one silicone monoblock. First, the design feedback from heart surgeons must provide guidelines to create an implantable sTAH prototype. Second, the operational lifetime of the soft pump must significantly increase in comparison to previous prototypes. Third, the pumping performance of the sTAH needs to be in the physiological range.

To evaluate the new sTAH pumping performance against different in- and outlet pressures, a hybrid mock circulation was used. Against physiological pressures, the soft pump conveyed 3.95 L/min (RV) and 2.45 L/min (LV) at 60 bpm (Table 3.1.).
Compared to the silicone sTAH presented by Cohrs et al., the left ventricle far exceeds the 1 L/min performance of the previous prototype. However, the flow output is still below the human heart’s mean total cardiac output of 10.5 L/min, and it is unequal. Equal pumping is a major concern since an increased pumping of the RV would cause an excess volume in the lungs, leading to pulmonary edema.

Different parameters were investigated to understand the limited and unequal pumping performance. First, a pressure sensor was attached to the actuation chamber to measure the pressure during an actuation cycle consisting of 0.2 s of inflation followed by 0.8 s of deflation time. The air actuation pressure was set to 200 kPa but due to a slow pressure build-up inside the actuation chamber, the maximum measured pressure was 155 kPa (See Fig. A.2.). Since the geometry of the sTAH was designed to operate at a higher air pressure, the observed pressure loss is one reason for limited and unequal pumping.

FEA is a powerful tool to potentially predict the pumping performance of a given sTAH design. The measured pressure of 155 kPA was used to model the deformation of the inner membranes using FEA software (Ansys Mechanical; Ogden model with steady-state conditions; see Appendix and Fig. A.2.3.) to estimate the pumping performance. With the modeled membrane deformation and volumetric displacement calculations (see Fig. A.2.4-6), we predicted that the right ventricle (RV) and left ventricle (LV) should convey 4.9 L/min and 3.9 L/min, respectively, against outlet pressures of 3.3 kPa and 13.3 kPa (25 and 100 mmHg). The flow estimations were 18% and 38% higher than the measured ones.

Small differences in the inner membrane thicknesses caused by the injection molding process is another reason which explains the unequal flow. Since membranes thicknesses need to be precise to account for different outlet pressures of the ventricles, slight variations (mm range, see Fig. A.2.7.) lead to unequal pumping performance. Furthermore, ultrasound imaging was

<table>
<thead>
<tr>
<th>Right outlet pressure [kPa]</th>
<th>Left outlet pressure [kPa]</th>
<th>RV mean flow [L/min]</th>
<th>LV mean flow [L/min]</th>
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<td>2.45</td>
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</table>

Table 3.1. Mean flow rate of the right and left ventricles of the soft total artificial heart measured at different outlet pressures on a hybrid mock circulation.
performed during an actuation cycle to understand how membrane expansion differs from the simulations. Inflated by the air from the pneumatic actuation system, the membranes expand until they contact the walls of the ventricles. As the air is removed from the actuation chamber, the expanded membranes fail to return to their initial resting state, constantly remaining in a semi-inflated state (Fig. 3.2.A-D). This behavior reduces the amount of fluid which can reenter the ventricle and therefore reduces the pumping performance. The inability of the membranes to return to the resting state can arise from three different factors: the time allowed for deflation, the material properties, and the diameter of the air inlet and outlet.

Figure 3.2. (A) Cut-through of the sTAH, showing the resting state at the point of imaging. (B-C) Series of ultrasound images where the liquid has been colored in blue for better visibility. The ultrasound image was taken over one inflation-deflation cycle at 60 beats per minute and a 25% inflation-deflation ratio. The deflated membranes (B) quickly expands to touch the outer wall of the sTAH (C) and return to their original state (D). (E) Colored cut through image of the finite element model shows the maximal principal stress in MPa. (F) Cut through image of the real sTAH, after long-term testing, showing where the crack initiated and a zoom-in on the crack.
The ratio between inflation and deflation time has little room for improvement because increasing the deflation time means also changing the beat rate, which decreases overall performance. Concerning the material properties, the best performing silicone provided by ACI Rubber, with the highest possible Young’s Modulus, was used. The high restoring force of this HTV silicone allows a fast relaxation time of the membrane. sTAH made of silicone with higher Young’s Modulus could not be removed from the injection mold. Moreover, the size of the air inlet and outlet limits the airflow rate and thus the relaxation of the membranes. A possible solution to reduce the deflation time could be to replace the passive deflation system by an active deflation system (e.g., usage of a vacuum sucking device to deflate the actuation chamber actively).

The new sTAH has a better overall pumping performance and increased longevity. Contrary to the sTAH of Cohrs et al., which suffered from inner membrane fragility, the new sTAH did not show wear or crack initiation on inner membranes on any of the three thoroughly tested sTAH. All sTAH showed similar pumping performance (Fig. 3.3) and ruptured due to fatigue at the same location: next to the air inlet and outlet, in the external shell. After up to 180'000 cycles or two orders of magnitude higher than the previous silicone sTAH, the sTAH stopped working. FEA was used to compute the maximal principal stress of the sTAH during inflation and deflation. The analysis identified a region of high stress, approaching the material’s ultimate tensile strength (7.1 MPa compared to 9 MPa). The identified weak point exactly corresponds to the region where the hole formation occurred during testing of the sTAH (Fig. 3.2.E-F). FEA can, therefore, be used to predict the failure location of such a complex design. These findings show great hope in using predictive engineering techniques such as FEA, to better design the future sTAH iteration to further increase its longevity.

In more detail, the weak point is located at the intersection between the air oblong and the outer shape of the sTAH. Two elements are indicating that this region is prone to stress concentration. First, there is a difference in the rigidity of the two components because the oblong is very thick compared to the outer wall of the sTAH. Second, there is an angle close to 90° between the two components, so the transition is sharp. With the aid of FEA, the oblong design can be smoothed enough to lower the stress and enhance longevity. The next sTAH porotype with increased pumping performance, lifetime, and better actuation control could also be tested dynamically on the hybrid mock circulation.
3.4. Conclusion

In summary, we present the first injection molded soft total artificial heart, showing improved pumping performance over any previously presented heart-inspired soft pumps. Injection molding allowed the manufacturing of several complex silicone monoblocks, which were actuated for up to 180,000 actuation cycles. As shown in other studies, the monoblock design may be key in achieving extended lifetimes and allow movement of the entire soft pump in a natural heart-like manner. However, the sTAH performance is still below the required flow rate of 10.5 L/min and longevity of several weeks to months. Upon demonstrating further sTAH development progress, animal trials can be considered as a next step. Future sTAH design should focus on increasing pumping performance by changing the thickness of the actuation membranes. Moreover, more complex pneumatic active inflation and deflation systems could

**Figure 3.3.** Flow rate of the right (in blue) and left (in red) ventricles of three sTAH (sTAH 1, 2, and 3) after different actuation cycles. Error bars represent standard deviation. Actuation of the sTAH was performed up to 180,000 cycles at 60 bpm, against a pressure of 2 kPa, with an inflation/deflation ratio of 25%. The empty squares are a symbolic representation of the point where the sTAH broke, not actual measurements.
also be designed to increase performance. With the help of FEA, pumping performance of future sTAH designs could be estimated, and weak spots eliminated before beginning the production process. Also, production with medical-grade silicone could ensure sTAH biocompatibility. The progress on the sTAH presented in this work provides the groundwork to build a biocompatible soft pump, which could reach the next longevity milestone of 10-100 million (≈ 10-100 days) actuation cycles. We further show how industrial mold manufacturing, mechanical modeling, and failure analysis assist future large-scale production of soft machine components.
4. DNA Barcodes Quantification as a Robust Tool for Measuring Mixing Ratios in Two-Component Systems

Manuscript published in part as:

A. Xavier Kohll, Julian Koch, Weida Chen, Conor O'Dwyer, Gediminas Mikutis, Wendelin J. Stark, Robert N. Grass

4.1. Introduction

Measuring material properties and detection of the authenticity of physical products is of great interest to confirm their quality. In general, many different factors describe the quality of goods. Some of those are origin, purity, mechanical properties, or composition of the different materials. One way to guarantee specific product quality and authenticity is by tagging and tracing it. An ideal tagging and tracing technology protecting against adulteration should be imperceptible within the material matrix and should not alter the properties of the product. Some existing tagging technologies include isotopic tracers, fluorescent labels, polypeptides, and nucleic acids.

Since many products are a combination of different materials, detailed quantification of the material composition is an important factor to ensure a specific quality. Problems occurring during mixing processes can lead to faulty material texture, undesired mechanical properties, or incorrect composition ratios. For example, by changing the ratio of water to ethanol in printing inks or in perfumes, the quality of these products is altered. Moreover, the contamination or the incorrect mixing ratios of polymers and adhesives will lead to undesired physical properties, compromising the quality and performance of the final product. While in many cases mixing ratios can be determined by chemical analysis of a product, there are cases where this is no longer possible, or challenging. This is especially true for products that have gone through a transformation during their making and/or use. For example, a mixture of ethanol and water might have been mixed correctly during manufacture, but due to the difference in evaporation rate, an analysis sample may no longer have the same composition. Another relevant example is cross-linked polymers: only having access to the finally polymerized item, it is close to impossible to determine if the reagents were originally mixed in the correct proportions. A way to circumvent this problem is to add taggants to one or more components in the mixture. Having ensured that the taggants are homogeneous in the original components, the mixing detection problem is transferred to being able to measure the concentration ratio of the taggant(s) in the final product. While some of the existing systems rely on one taggant only (e.g., magnetic particles) the possibility of having access to two or more taggants simultaneously, enables a robust measurement system: the absolute concentration/amount of a taggant no longer needs to be measured, but the ratio of the taggant concentrations is sufficient.

Nucleic acids, especially deoxyribonucleic acid (DNA) has been previously proposed as a material taggant in anticounterfeiting applications as it is detectable at extremely low concentrations using polymerase chain reaction (PCR), and a near to infinite array of possible
tags can be generated by altering the nucleotide sequence in short synthetic DNA strands (e.g., 100 base pairs long). However, DNA lacks inherent long-term stability in various environments. The biopolymer is prone to chemically induced damage in the presence of an elevated temperature, reactive oxygen species, or non-neutral pH. Several methods have been reported to stabilize DNA (e.g., on clay, in polymers, or encapsulated in silica). From these, silica particles with encapsulated DNA (SPED) have been established as a reliable tagging system. This material essentially consists of silica particles, into which short DNA oligomers (50-200 base pairs) have been loaded. Due to the nonporous nature of the outer silica shell, the DNA within the particles is fully protected from the outside environment, such as reactive oxygen species and thermal degradation by hydrolysis. By exposing the DNA loaded particles to a fluoride-containing buffer (e.g., buffered oxide etch, BOE), DNA is easily released without suffering any harm and can be quantified by PCR. In previous studies, such DNA loaded particles were shown to be useful in tracing the ecological networks, underground reservoirs, tagging of food, oil, pesticides, and polymers. Therefore, nontoxic SPED tracing with unlimited possibilities of DNA barcodes and extremely low detection limits offers an attractive tracing method.

PCR analytics, however, not only offers the measurement of the presence or absence of a specific DNA sequence (yes/no); real-time PCR also enables the (relative) quantification of a specific DNA sequence. Utilizing internal standards and dilution curves, this relative reading can be further translated into an absolute DNA concentration measure (quantitative PCR = qPCR).

This makes DNA interesting as a taggant to measure compositions. Especially the possibility of measuring several DNA barcodes simultaneously in the same PCR well (multiplex PCR) promises robustness to experimental error and minimization of handling steps. In the present study, we introduced the novel concept of composition detection with nanometer-sized DNA taggants via relative concentration measurements with multiplex PCR. Therefore, we evaluated to which extent encapsulated DNA can be used to detect mixture compositions in liquid systems, and also in a cross-linked polysilicon matrix, an envisioned large-scale application of the novel technology.
4.2. Materials and methods

4.2.1. SPED synthesis and characterization

Two different DNA sequences (S1 and S2, see Appendix for full details) were separately encapsulated in silica particles. Desalted single-stranded DNA sequences were ordered from Microsynth AG (Balgach, CH). After annealing the DNA in TE buffer, an up-scaled SPED synthesis procedure adapted from Paunescu et al. was performed. The procedure included three main synthesis steps: First, the loading of 300 µg of DNA onto positively charged 0.4 g of SiO2-TMAPS functionalized nanoparticles. Second, addition of 40 µL N-trimethoxysilylpropyl-N,N,N-triethylammonium chloride (TMAPS, 50% in MeOH, ABCR) to the mixture. Third, 62.5 µL of tetraethoxysilane (TEOS, ≥99%, Aldrich) was added to grow a protective silica layer around the DNA attached to the nanoparticles. Scanning transmission electron microscopy images of silica particles encapsulated with either S1 DNA (SPED1) or with S2 DNA (SPED2) are shown in Figure 4.1.A, B. The SPED particles had a uniform size distribution with an average diameter of 73 ± 4 nm. The zeta potential was 15.5 mV for SPED1 and 19.2 mV for SPED2. DNA and primer sequences, as well as a more detailed SPED synthesis protocol, are found in the Appendix.

4.2.2. SPED mixing procedure in different mixtures

Liquid-Liquid Mixtures. SPED1 and SPED2 were dispersed in different water or ethanol solutions before being mixed in the following composition ratios: 80:20, 60:40, 50:50, 40:60, and 20:80. A 100 µL pipet was used to pipet together all the different mixtures (e.g., 80 µL of SPED1 in water with 20 µL of SPED2 in water). Upon the addition of both liquids in a 2 mL Eppendorf tube, the total volume of 100 µL was thoroughly mixed with a vortex. The concentration of SPED1 varied from 13 ppm to 13 ppb in water and SPED2 from 11 ppm to 11 ppb in water and ethanol.

Reactive polymer Mixtures. SPED particles (100 µL of 10 mg/mL (µg/µL)) were transferred from the original stock solution (water) to a THF dispersion. Transfer of SPED was achieved through sequential centrifugation, removal of the supernatant, and addition of the new solvent followed by thorough dispersion in an ultrasonic bath (Fig. A.3.4.). The used silicones (S5 and S50, Siliconesandmore, NL) are two-component silicones (components A and B, e.g., S5 A and S5 B) that start to cure upon addition of both components. SPED1 and SPED2 in THF were added to component S5 A and S50 A, respectively. The SPED and silicone were mixed for 30 s
at 3300 rpm in a 12 mL container in a SpeedMixer (Synergy Devices Ltd, U.K.). Before adding component B and mixing both tagged silicones together, THF was removed from the SPED containing silicone by putting the samples in a desiccator and applying a vacuum. After approximately 40 h, more than 90% of THF was removed. Next, the SPED1/SPED2 in silicone S5 A/S50 A solution was mixed with component S5 B/S50 B in a 1:1 ratio. Specific composition ratios of both silicones (e.g., 60:40) were prepared by mixing together SPED1 in S5 and SPED2 in S50. A total of approximately 1000 mg of silicone mixture (e.g., 600 mg SPED1 in S5 with 400 mg SPED2 in S50) was mixed in the SpeedMixer and then cured for several hours.

4.2.3. DNA release and PCR amplification protocol

**Liquid-Liquid mixtures:** DNA S1 and S2 was released from SPED containing water-water and water-ethanol mixtures by adding 400 µL of 1:50 buffered oxide etch (BOE), which is a fluoride-containing buffer, to 100 µL of the sample. Without the need for purification, the released DNA was amplified with a Roche Lightcycler 96. Each sample well was filled with a total volume of 12.5 µL containing: 2.5 µL sample volume, 6.25 µL of GoTaq Pro Master Mix, 0.625 µL (0.1 µM) Probe (Texas Red), 0.625 µL (0.1 µM) of Probe (HEX), 0.625 µL (0.2 µM) of forward primer and 0.625 µL (0.2 µM) of reverse primer. The multiplex PCR consisted of a two-step amplification protocol (95°C for 15 s and 56°C for 60 s), and each sample was analyzed in duplicates. The particle concentration of ≈10 ppm to 10 ppb was released and amplified following this protocol.

**Reactive polymer mixtures:** First 10-25 mg of SPED containing silicone samples were exposed to a 1 M tetrabutylammonium fluoride (TBAF, Sigma-Aldrich) solution in THF for 60 min at room temperature in a shaker at 800 rpm. After the destruction of the silicone network by TBAF, the samples were washed with 1 mL of THF before being treated with 300 µL of BOE for 30 min in a shaker at 800 rpm. Next, the samples were purified with QIAquick PCR purification columns, before 200 µL of the released DNA in elution buffer was analyzed via multiplex PCR (same protocol as mentioned above). **Figure A.3.5.** shows the release process of SPED from silicone and DNA from SPED.
4.2.4. Composition detection in two-component mixtures with SPED

**Scheme 4.1.** Schematic diagram explaining the mixing of two components, containing two different SPEDs, and subsequent composition analysis via multiplex PCR.

**Scheme 4.1.** shows a simplified schematic diagram explaining the general tagging of two solutions X and Y with SPED, mixing of both solutions and subsequent mixture composition detection via SPED. DNA released from SPED is measured with multiplex PCR, allowing the detection of the threshold cycle (CQ), which is the number of cycles required for the fluorescent signal of a specific DNA to cross an arbitrary threshold value. Measuring two different DNAs allows the calculation of the ΔCQ value, which is the difference of CQ of DNA S1 (CQ_{S1}) minus the CQ of DNA S2 (CQ_{S2}). This ΔCQ value can then be correlated to a specific mixture composition.
Results and discussion

Two nonidentical DNA taggants were prepared and characterized before they were used for mixture compositions detection. Therefore, we generated two different batches of silica particles with encapsulated DNA following adapted literature procedures (see experimental). STEM images revealed that the particles were near identical with an average size of ≈70 nm, but each contained a different short DNA sequence, named S1 and S2. (See Fig. 4.1.A,B). Both particles could be dissolved rapidly by a buffered oxide etch solution, releasing the encapsulated DNA. This DNA could then be quantified by real-time PCR reactions. With this measurement technology, the sample is temperature cycled in the presence of an enzyme (polymerase), primers (short DNA sequences), and a DNA specific fluorescent dye. Under idealized conditions, the enzyme enables the doubling (amplification) of the DNA concentration in every cycle, and the fluorescence signal is constantly recorded (see Scheme 1). The cycle at which the fluorescence reaches a predefined threshold (CQ value) can be used to calculate the relative concentration (C) of the DNA sequence in the original sample.

\[ C = 10^{\frac{CQ-b}{s}} \]  

with \( s = \text{slope} \), \( C = \text{concentration} \), and \( b = \text{y-intercept} \).

A comparison with the dilution curves of the particles in water (see Appendix) can then be used to evaluate the amplification efficiency and allow the absolute quantification of the particles in the aqueous solution. In a more advanced setup (multiplex PCR), the amplification curves of several DNA sequences can be measured simultaneously in one cycling reaction using various detection dyes and allows the simultaneous recording of multiple (2-4) amplification curves and the detection of the threshold cycle (CQ) of different DNAs. Due to the logarithmic dependence between DNA concentrations and CQ values, the difference between the CQ values of two DNAs (\( \Delta \text{CQ} \)) is proportional to the ratio of the DNA concentrations, and subsequently proportional to the ratio of DNA loaded particles. As a result, this can be used to measure the mixing ratios of multicomponent systems.
4.3.1. Composition detection of different liquid-liquid mixtures.

Since the dispersion of SPED in water and subsequent DNA release is well controllable, it was crucial to test and validate the composition detection via SPED in a two-component water-water mixture. Therefore, SPED1 and SPED2 were dispersed in two different water solutions, which were mixed together in different composition ratios, before the DNA was released and detected via BOE treatment and multiplex PCR. Scheme 1 illustrates the generalized workflow for the composition detection method. Indeed, different mixture compositions resulted in different CQ values for S1/S2 DNA (Fig. 4.2.A). A clear trend of CQ values for S1/S2 DNA in dependence of the mixture composition was observed proving mixture composition detection was possible. This result demonstrated that: First, SPED were well dispersed in the two separate water solutions. Second, DNA was released from the silica particles and CQ values changed proportionally to mixture composition.

In order to show the clear distinguishability between the different water-water mixtures and corresponding CQ values, ΔCQ (=CQ\textsubscript{S1}-CQ\textsubscript{S2}) was also plotted against the mixture composition ratio in Figure 4.2.B. All ΔCQ values could be correlated to specific composition ratios.
Furthermore, we carried out a two-sample t-test (see SI) between the ∆CQ values of the different mixtures. All ∆CQ values for different mixture compositions were significantly different (p < 0.05).

**Figure 4.2.** (A) CQ values for S1 and S2 DNA depending on different sample composition ratios in a water-water mixture. Broken lines are semilog fits of the collected data. (B) ∆CQ values in water-water mixtures (80:20, 60:40, 50:50, 40:60, and 20:80 of S1:S2 DNA) showing significant differences (two-sample t-test) between composition ratios: (*) p < 0.05 (n=3). (C) CQ values for S1 and S2 DNA depending on different sample composition ratios in a water-ethanol mixture. Error bars (in panel A and C) for each data point represent the standard deviation of measured CQ values of three samples. For each composition ratio in panel B, error bars represent standard deviations of ∆CQ values of three samples.

To show the potential applicability of this composition detection via SPED, another liquid-liquid system was tested. Printing inks and perfumes, are both composed of varying amounts of ethanol and water and therefore offer an ideal case to show the applicability of the composition detection method. Therefore, SPED1 was added to water and SPED2 to an ethanol solution. Both solvents were then mixed together in varying amounts and analyzed by multiplex PCR. In this case, also a clear trend of CQ values in dependence of mixture composition was observed (**Fig. 4.2.C**).

A statistical test can be used to detect if the average difference between two samples is significantly different (e.g., differences in material product compositions). Inverting the test allows for determining the minimal detectable average difference between two samples. In our case, we were interested in estimating the minimum detectable composition difference between two samples (i.e. at what difference of composition we could determine the change to a given statistical limit). We were especially interested in determining how many technical replicates
are required to detect a required composition difference. Therefore, we estimated the minimal distinguishable ΔCQ in dependence of sample size by using the $t$ test. In short, a dilution series of CQ values for S1 and S2 DNA in dependence of the sample composition ratios was used to calculate the ΔCQ values for any composition ratio (Fig. A.3.1.). Figure 4.3. shows how the minimum detectable composition difference changes with the number of samples. For example, a total number of 6 samples is required to distinguish an 80:20 from a 73:27 composition ratio (with a 95% confidence interval).

The limiting factors to detect small composition changes are the number of sample replicates and the sample standard deviations. If the standard deviations are too large, a higher number of sample replicates in qPCR could be used. These calculations highlight that precise detection of composition differences is theoretically possible with SPED and PCR quantification, although the CQ values have a logarithmic concentration dependence.

**Figure 4.3.** Minimum detectable composition difference between two different SPED tagged liquid-liquid mixtures in dependence on the number of samples. With $N=6$, the mixture B composition ratio of 73:27 could be significantly differentiated from mixture A composition ratio of 80:20. The solid line is a guide to the eye.

Tagging and tracing products with SPED are especially interesting since they are detectable over a large concentration range.

To investigate whether composition detection is also possible at lower SPED concentrations in liquid-liquid mixtures, a dilution series from 10 ppm to 10 ppb was prepared in the individual mixing components. Two different 80:20 and 40:60 composition ratios of SPED tagged water-
water mixtures were analyzed. All the measured CQ values of the different samples showed a clear dependence on SPED concentration in water (Fig. 4.4.A,B). In order to compare the specific composition ratios, ΔCQ values were plotted in Figure 4.4.C. Indeed, all ΔCQ values were similar over the measured concentration range for the specific samples with a 40:60 or 80:20 composition ratio. Such low tracer concentrations (10 ppb) allows price estimations for labeling products with SPED of around 9¢ per ton.$^{86}$ Moreover, for SPED concentrations below 1 ppm ($10^3 \mu g/L$), it may be expected that the presence of the SPED does not influence the physical properties of the matrix.$^{58}$

In order to show the robustness of the proposed method to the physical size of the sample taken for analysis, we measured the ΔCQ values of 3 different sample sizes (6, 30 and 60 µL) of water-ethanol mixtures. Data (Fig. 4.4.C) shows that for a given mixing sample, the ΔCQ value is fully independent of the physical size of the sample, even if 10 times more sample volume are taken for analysis. The reason for this robustness lying within the logarithmic relation between the individual CQ values and the concentrations of the two SPEDs.

Applying equation (1) for a mixing ratio results in equation (2), whereas for proportionality the intercepts $b_1$ and $b_2$ can be disregarded (see Eq. 3).

$$\frac{C_1}{C_2} = \frac{10^{\frac{CQ_1-b_1}{s_1}}}{10^{\frac{CQ_2-b_2}{s_2}}}$$  \hspace{1cm} (2)$$

$$\frac{C_1}{C_2} \propto \frac{10^{\frac{CQ_1}{s_1}}}{10^{\frac{CQ_2}{s_2}}}$$  \hspace{1cm} (3)$$

Applying the decadic logarithm, the relationship between the mixing ratio and the difference in CQ values (=ΔCQ) becomes evident.

$$\log \frac{C_1}{C_2} \propto \log 10^{\frac{CQ_1}{s_1}} - \log 10^{\frac{CQ_2}{s_2}}$$  \hspace{1cm} (4)$$

$$\log \frac{C_1}{C_2} \propto \frac{CQ_1}{s_1} - \frac{CQ_2}{s_2} = \Delta CQ_{corr}$$  \hspace{1cm} (5)$$

If further assuming equivalent amplification yields for the two DNA strands$^{132,133}$ ($s_1 = s_2$) the equation further simplifies to equation (6):

$$\log \frac{C_1}{C_2} \propto CQ_1 - CQ_2 = \Delta CQ.$$  \hspace{1cm} (6)
Figure 4.4. (A) Dilution series of SPED tagged water-water mixtures with composition ratios 80:20 and (B) with composition ratio 40:60 over 4 orders of magnitude. Broken lines are semilog fits. (C) ΔCQ values of water-water and water-ethanol mixtures with different SPED composition ratios, concentrations and physical sample size. CQ values for S2 DNA were corrected since the PCR efficiency for S2 (E_{S2}) was higher than for S1 DNA. (E_{S1} = 98% and E_{S2} = 104%; see Figure A.3.3.). For each bar, the error bar represents the standard deviation of the ΔCQ values of the three samples.

4.3.2. Composition detection of a solid-solid mixture.

Silicone is a commonly used polymer in industries ranging from automotive to aerospace and its physical properties can easily be modified. For example, two different room temperature vulcanizing (RTV) silicones with different hardness (shore hardness of 5 and 50) can be mixed together to obtain the desired stiffness.

In general, two-component composition detection via SPED in solid matrices can be challenging since the dispersion and recovery of the nanoparticles can be complicated. More specifically, SPED needs to be homogeneously dispersed and recovered from the matrix without being destroyed during the process. Here, we chose different RTV silicone mixtures to demonstrate that two-component composition detection in solids via SPED is possible, which is otherwise impossible (or very challenging) for cross-linked polymer systems after polymerization has occurred.

Figure 4.5. A illustrates the simplified mixing, curing, and releasing procedure of SPED particles from the silicone. Detailed step-by-step procedures are shown in Figure A.3.4. and A.3.5. To release the particles from the solid polysilicone elastomer, we developed a chemical procedure that etches the polymer with a nonaqueous fluoride source but leaves the silica particles intact. In a second etching step with an aqueous fluoride solution, the DNA is released from the particles and can be quantified via multiplex PCR.
Figure 4.5.B visualizes the CQ values for specific composition ratios of S1 and S2 DNA released from the respective SPED silicone mixture. A trend of CQ values of S1 and S2 in dependence of the silicone composition ratio is observable. The CQ values of 50:50 mixture of silicone S5 and S50 are slightly below the linear trend line, which can be explained by delayed sample preparation, which led to premature silicone curing for this sample. Due to the advanced silicone curing of the 50:50 sample, SPED could have been incorporated differently in the cured silicone matrix. Nevertheless, when plotting the ∆CQ values (Fig. 4.5.C) a clear trend is observable. This again shows the robustness of utilizing the ∆CQ, because as long as the change in processing affects the two particles, in the same manner, the difference in CQ will remain the same, even if the absolute values differ. A two-sample t-test (Table A3.1) between all the ∆CQ values for different silicone composition ratios showed that the composition difference between all samples could be statistically determined (p < 0.05).

Figure 4.5. (A) Mixing and curing of SPED containing silicone S5 and S50 mixtures and a simplified two-step release procedure of S1 and S2 DNA from the silicone matrix. (B) CQ values for S1 and S2 DNA depending on different sample composition ratios of silicone mixtures. Broken lines are semilog fits. Error bars for each data point represent the standard deviation of measured CQ values of nine samples. (C) ∆CQ values of different ratios of silicone mixtures (80:20, 60:40, 50:50, 40:60, and 20:80 of S1:S2 DNA) showing significant differences (two-sample t-test) between composition ratios: (*) p < 0.05 (total number of samples 12, or 18). For each bar, the error bar represents standard deviations of ∆CQ values of nine samples.
Many different analytical methods exist to measure composition or detect adulterations of products. Chemical and composition analysis can be performed by gas chromatography-mass spectrometry (GC-MS) or inductively coupled plasma – mass spectrometry (ICP-MS) and other techniques.\textsuperscript{134} For example, proton nuclear magnetic resonance (\textsuperscript{1}H-NMR) is commonly used to detect illegal dilution of honey with cheaper sugar sources.\textsuperscript{135} Nevertheless, expensive equipment and compound depending sampling methods limit the broad applicability of these methods. Since PCR devices are available for 10’000 USD or less and SPED tagging price of 9\textcent per ton,\textsuperscript{86,136} DNA barcode composition detection method is an interesting alternative. The method is of added value, if the mixture goes through a chemical transformation following the mixing process, as it allows the determination of the mixing ratio prior to chemical transformation. If DNA taggant removal from solid mixtures is possible, commercially available DNA strands as well as low-cost PCR devices, could allow rapid adoption of this composition detection method. As a possible application example, we see two-component building materials (e.g., polysilicones, polyurethanes, epoxy glues) containing the DNA particles in both components to enable postfailure analysis of the mixing process.

\subsection*{4.4. Conclusion}

In this study, we report a method for two-component composition detection of liquid-liquid and solid-solid mixtures via DNA taggants and a quantitative PCR detection method. This composition detection method enhances the functionality of DNA tracers beyond the tagging and tracing of materials, to an enhanced authenticity and composition control method. The usage of an individual taggant for every component in the mixture further allows for a robust determination of the mixing process, which is independent of the physical size of the sample, and the absolute concentration of the taggants in the mixture. Very low required tracer concentrations and the compatibility of silica particles with a variety of material matrixes enable a cost-effective technology with the potential of industrial application, e.g., in polymeric construction materials. For this, the required next steps will involve scaled-up and application-specific testing as well as market research.
5. The Influence of Earth Alkaline Salts and Calcium Phosphate on DNA Stability for a Simple and Stable DNA-Based Information Storage System
The volume of digital data is growing at an unprecedented pace, triggering journals to claim that the world’s most valuable resource is no longer oil but data. However, some digital data is only relevant when it can be stored. In archival storage, one is more concerned about information density, durability, and energy cost than access speed to the data. In this storage domain, DNA data storage has become an attractive idea to substitute traditional media such as magnetic tape. The natural biopolymer offers some unique advantages: high data storage densities, ease of replication, and extended storage lifetimes. DNA can potentially store up to 455 exabytes of information per gram, is easily replicated via polymer chain reaction (PCR), and can be preserved for centuries to millennia. The advantages of DNA data storage should ideally be transformed into a simple, accessible, stable, and dense DNA-based information storage system.

Besides the encoding of digital information into DNA sequences and the subsequent decoding, a DNA-based information storage system is composed of three major parts: DNA synthesis, DNA storage, and DNA sequencing (Fig. 5.1.). Fast progress and cost reduction for DNA synthesis and sequencing, makes archival DNA data storage economically more and more attractive. Also, the total amount of digital data stored in DNA recently increased to over 200 MB. However, fewer efforts have been made to investigate a simple, high density, and stable DNA storage systems.

**Figure 5.1.** DNA-based information storage system composed of three major building blocks: DNA synthesis, DNA storage and DNA sequencing. Four different storage media are shown: DNA in bone, DNA in solution, DNA in nanoparticles and DNA in salt. Stability, DNA loading and handling simplicity are different in all systems. Colour code: Green = High, Orange = Medium, Red = Low, white = n.a.
Without any protection, DNA is a fragile biomolecule, prone to degradation, for example, by hydrolysis or oxidation. The prevention of DNA degradation is possible, for instance, by storing DNA in a dehydrated and anaerobic environment or at very low temperatures. Existing DNA storage solutions include DNA storage at low temperatures (e.g., freezer at -80°C) or room temperature under anoxic and anhydrous conditions (e.g., DNAshells®). However, all of these storage systems have either high maintenance costs or exhibit low DNA loading (DNA mass/total mass of the storage system). Hence, with low DNA loading, the information density per volume or mass is significantly reduced making these storage solutions less attractive for a potential DNA-based information storage system.

Nature exhibits several DNA preservation methods, for example, biomineralized DNA in a collagen/calcium phosphate matrix of bones. In this matrix, DNA is stable enough to be sequenced and used to reconstruct the mitochondrial sequence of a cave bear after an estimated 300'000 years of conservation. Currently, DNA data storage solutions in silica or magnetic nanoparticles show long DNA lifetime and DNA loadings of up to 3.4 wt% without the need of anhydrous storage conditions. However, these state of the art storage technologies require multiple handling steps, which are potentially difficult to automate, and DNA loadings are well below 10 wt%.

An effective DNA data storage system needs to fulfill the following requirements: High DNA loading, increased DNA stability, and simple sample handling (physical storage and accessibility). No current DNA storage media is optimal in all three aspects (Fig. 5.1.).

Herein, we have investigated the influence of different amounts of calcium phosphate (CaP), calcium chloride (CaCl₂), magnesium chloride (MgCl₂), and various other salts on DNA stability for reliable DNA data storage purposes. We primarily focused on high DNA loadings and simple DNA storage and retrieval. In this chapter, we suggest that several inorganic matrixes that significantly improve the thermal stability of DNA at high DNA loadings. Furthermore, we tested our storage method with a DNA pool encoding 115 kB on a cartridge system.

In the experiments, we routinely exposed model DNA to accelerated aging conditions, by drying 30 ng of dialysis cleaned DNA in salt solution in a 2 mL Eppendorf tubes. The model DNA is a synthetic 148mer double-stranded DNA sequence with specific priming regions. The DNA and primer sequences can be found in the Appendix. Accelerated aging-induced thermal degradation of DNA was performed by exposing the biomolecule to 60 or 70°C at 50% RH
humidity in a desiccator. Different DNA and salt combinations were tested over a range of DNA loadings (≈ 0.01 – 80 wt%). qPCR was used to determine the amount of intact and amplifiable DNA after exposure to accelerated aging for up to 33 days. For detailed protocols, see the Appendix.

As mentioned above, an ideal DNA storage system should be simple (in terms of handling), dense and stable. A rather simple system is possible when only water-soluble chemicals are used, which upon drying, stabilize the DNA. Moreover, standard lab equipment allows the sample deposition and recovery of such a system in a matter of seconds. **Figure 5.2.A** shows a simplified schematic of the used sample drying and accelerated aging process, to access different salt stability on model DNA.

![Schematic of sample drying and accelerated aging process](image)

**Figure 5.2.** (A) Schematic illustration of the storage process of DNA and DNA with a salt. Salts can protect DNA in accelerated aging conditions (AAC) from thermal degradation. (B) Relative DNA concentration after AAC for different DNA loadings with EDTA, Tris-HCl, NaCl, HEPES and calcium phosphate (CaP). (C) SEM picture of a sample with 18 wt% DNA loading with CaP.

Previous research showed that DNA stored in bones had exceptional longevity.\(^{68,142}\) Therefore, we were especially interested in reproducing the storage environment in bones. Since the mineral phase of bones is primarily composed of CaP, we tried to protect DNA by coprecipitating the biomolecule with CaP. This concept of trapping DNA in CaP has been used for decades by biologists for DNA transfection.\(^{143}\) Additionally, Lindahl *et al.* mentioned
stabilizing effects of adsorption of DNA onto hydroxyapatite (a particular form of calcium phosphate), and CaP also shields DNA from degradation by DNaseI (endonuclease that attacks DNA).\textsuperscript{144,145} DNA with CaP and a handful of organic and inorganic chemicals were therefore tested for their influence on DNA stability (\textbf{Fig. 5.2.B}). During these initial experiments, we searched for an optimum between DNA loading in CaP and DNA stability.

In general, it is comprehensible that the more protecting material is surrounding the DNA, the higher the DNA protection from its surroundings (e.g., water in humid air) should be. This concept was true for low DNA loadings ($\leq 1$ wt\%) in EDTA or NaCl (\textbf{Fig. 5.2.B}). However, the overall trend for DNA storage with CaP was quite different from all the other DNA salt systems. A steep stability increase from very low DNA loadings in CaP ($<1$ wt\%) to loadings beyond 10 wt\% was observed (see \textbf{Fig. 5.2.B}). Up to two orders of magnitude more DNA was protected in the 18 wt\% DNA in CaP samples in comparison to 1 wt\% samples. \textbf{Figure 5.2.C} and \textbf{A.4.4.} show SEM pictures of the DNA with CaP precipitate at 18 wt\% DNA loading. Water molecules, which are structurally essential for DNA to maintain its double helix conformation, are neglected in these DNA wt\% calculations.\textsuperscript{70} We further investigated high DNA loadings in CaP over extended periods. Therefore, 18 wt\% DNA in CaP was aged over 33 days at 60 and 70°C at 50%RH and compared to unprotected DNA (\textbf{Fig. 5.3.A-B}). These results also highlighted that in the presence of CaP, indeed, DNA exhibits much greater stability against thermal degradation for extended periods.

A DNA storage system comprised of an optimized DNA CaP coprecipitation system, with subsequent DNA release via the application of the calcium ion complexing agent EDTA, would be an elegant and straightforward way to store DNA. However, DNA stability with CaP was not always a robust storage system, since DNA stability results could vary between different experiments. One reason for the varying stability results was that the precipitation process of DNA with CaP was difficult to control. In extreme cases, DNA stability results varied a lot when changing the sample drying process to precipitate the DNA (e.g., changing from vacuum centrifuge drying to freeze-drying, see \textbf{Figure A.4.5.}). Such effects have already been discussed in DNA transfection literature, describing the difficulty to maintain precise conditions for CaP precipitation.\textsuperscript{146–149}
A control experiment was performed to gain further insights into the individual stabilizing effects of the chemical components used to produce the CaP system. Consequently, model DNA was stored separately with CaCl\(_2\) solution or KH\(_2\)PO\(_4\)/K\(_2\)HPO\(_4\) buffer under accelerated aging conditions (Fig. 5.3.C). The results showed that CaCl\(_2\) stabilizes DNA to a higher degree than CaP and the potassium phosphate buffer. CaCl\(_2\) significantly increased DNA stability especially at loadings beyond 20 wt% (Fig. 5.3.C). Since CaCl\(_2\) showed high stabilization potential, we were interested in testing all other earth alkaline salts as well, except for beryllium chloride (high toxicity) and radium chloride (radioactive). Whereas barium chloride and strontium chloride did not exhibit exceptional DNA stabiliziation effects, magnesium chloride effectively protected DNA from thermal degradation at high loadings (Fig. 5.3.D). Even though the samples were dried, the stabilization effect of MgCl\(_2\) is not unexpected since Lindahl et al. and Marguet et al. both highlighted the stabilizing impact of MgCl\(_2\) on DNA in solution.\(^{72,150}\) Our results indicate, that both the calcium and magnesium cation should, therefore, have some chemical interaction with the DNA backbone, or other DNA structures, to limit its thermal

**Figure 5.3.** Relative concentration of: (A) 18 wt% DNA in CaP and pure DNA stored at 60°C and 50%RH for 33 days. (B) 18 wt% DNA in CaP and pure DNA stored at 70°C and 50%RH for 33 days. (C) DNA with different amounts of CaCl\(_2\), CaP and potassium phosphate buffer stored for 5 days under accelerated aging conditions (AAC). (D) DNA with different amounts of earth alkaline salts stored for 3.8 days at AAC.
degradation at very high loadings. However, at the moment, sample analysis via STEM or XRD did not provide sufficient information to allow a better chemical understanding of the system.

The application of a salt-based DNA storage system can be assessed in more detail, when storing a DNA pool, encoding digital information, and transitioning from an Eppendorf tube test set-up to a potentially scalable and automatable glass slide set-up. Therefore, we changed from the previously described model DNA to DNA pool, consisting of 7′323 distinct DNA sequences, encoding a 115 kB picture, to create a more realistic application test. To assess the reproducibility of the salt-based storage system with different DNA, the DNA pool was first stored in Eppendorf tubes, with our selection of stabilizing salts (MgCl$_2$, CaCl$_2$, and CaP). Similar stability trends of the DNA pool in combination with the salts were measured, and the results are presented in the Appendix (Fig. A.4.6.). Second, a potentially scalable and automatable storage system (e.g., glass slides) was selected and tested with the DNA pool stored in the inorganic matrix. Therefore, we chose a recently published cartridge-based system presented by Newman et al. This system allows the automated manipulation, deposition, and retrieval of physically separated DNA spots with the help of a digital microfluidic (DMF) device. These spots on a glass slide can be dried and rehydrated separately and, therefore, allow to selectively address a specific DNA pool without rehydration of multiple DNA pools encoding many different DNA data files stored in, for example, a laboratory tube. As a simple

\[ \text{Figure 5.4. Schematic representation of DNA with salt deposition and drying on a glass slide. Relative concentration of DNA stored with or without MgCl}_2 \ (38 \text{ wt\%}), \text{ CaCl}_2 \ (35 \text{ wt\%}) \text{ and CaP} \ (18 \text{ wt\%}) \text{ for 5.8 days at 70°C and 50% RH.} \]
proof of concept, we limited ourselves to manually deposit and retrieve DNA salt spots from a Teflon-coated glass slide used in the previously mentioned automatable system. The measurement of DNA stability in the presence of MgCl$_2$, CaCl$_2$, and CaP after accelerated aging on the cartridge revealed again that DNA stored with MgCl$_2$ or CaCl$_2$ was degraded the least (Fig. 5.4.).

Finally, to demonstrate the salt-based DNA storage system on a glass slide, a successful readout (sequencing) of the stored DNA pool, after accelerated aging, is essential. Therefore, each of the five spots of the DNA pool with or without protection by MgCl$_2$ underwent accelerated aging. All the samples and a non-aged reference sample were prepared for sequencing on an iSeq 100 (Illumina). See Appendix for the detailed sequencing preparation protocol. The digital file in all the salt-protected DNA spots was successfully decodable. However, none of the unprotected DNA samples was successfully decodable after accelerated aging. Fig. 5.5.A schematically summarizes the results of the storage and sequencing experiment. Fig. A.4.7-9 shows detailed sequencing statistics.

Figure 5.5. (A) Schematic representation of the DNA storage and sequencing experiment. (B) Total sequences lost after sequencing of protected (with MgCl$_2$) and unprotected DNA samples. (C) Percentage of substitutions of different nucleobases in case of substitution (Probability ≈ 1%, see Appendix).
Storing a DNA pool by silica encapsulation takes about four days to perform.\textsuperscript{80} In comparison, the presented salt-based DNA storage is both faster and simpler to execute. One-step addition of a salt solution to DNA is done in a matter of seconds, and subsequent drying of the samples can be performed in a matter of hours. Furthermore, the DNA loading in the inorganic matrix was increased to up to 38 wt\% in comparison to the state of the art nanoparticle storage density of 3.4 wt\%. Also, DNA stability in salts at 70°C and 50% RH is comparable to the previously mentioned storage technique.\textsuperscript{81,82} Our research shows that minimal amounts of calcium chloride or magnesium chloride found in bones could be a potential cause for long-term stabilizing effects of DNA.\textsuperscript{68,142}

We have herein demonstrated that several salts (MgCl\textsubscript{2}, CaCl\textsubscript{2}, and CaP) increase DNA stability at high loadings in solid-state storage at accelerated aging conditions of 70°C and 50% RH. We introduced a novel and fast salt-based DNA storage system by depositing and retrieving DNA-salt spots from a potentially automatable cartridge-based system. A DNA pool was protected against thermal degradation by drying DNA with MgCl\textsubscript{2} at 38 wt\% DNA loading. The system was also tested by successful digital file read-out via Illumina sequencing of protected heat-treated samples and compared to unprotected heat-treated DNA samples. However, it remains challenging to predict DNA longevity in the salt-based system at ambient temperature, since the uncertainty related to the extrapolation of DNA stability data from high temperature to room temperature persists.\textsuperscript{152} These findings highlight that a solid-state, salt-based and high-density DNA storage system is an exciting concept for an alternative archival storage system. Future work could assess the system stability at room temperature by performing long-term storage at experiments at different temperatures. Moreover, the automation of this storage system with a DMF device could be tested, and different methods (e.g., NMR or IR) could provide a more detailed chemical characterization of the salts stabilizing effects on DNA.
6. Conclusion and Outlook

This thesis presents approaches to solve challenges in the realm of soft pumps and DNA-based functional materials. In chapters 2 and 3, two industrial manufacturing techniques were presented to produce a more durable and better performing soft pump for potential application as soft total artificial heart. In chapters 4 and 5, two different DNA protection techniques were presented, to develop a new mixing control method and a simple but stable DNA data storage system.

We showed that rubber molding is a viable technique to produce longlasting soft pumps. To manufacture the first pump out of vulcanized natural rubber, we developed a compression mold. This soft device withstood actuation for more than 1 million actuation cycles and conveyed more than 140’000 liters of water. However, limited biocompatibility of the material and unsuited design for the potential application as heart replacement therapy, required the development of an alternative prototype. The second soft pump was manufactured by silicone injection molding. An improved design and the use of high-temperature vulcanizing silicone allowed the pump to run continuously for over 180’000 cycles. This new sTAH prototype maintained a flow of 6.4 L/min against physiological outlet pressures. Both soft pumps have increased durability and pumping performance in comparison to the sTAH presented by Cohrs et al.

The elasticity of the presented monoblock sTAH, allowed the entire sTAH structure to be in motion during actuation. This intrinsic softness of the pump shall enable two things: A more physiological blood flow and minimization of dead volume within the sTAH. However, the following limitations remain in the latest sTAH prototype: Limited durability, unequal pumping between the left and right ventricle, limited pump performance, as well as unclear biocompatibility. The following approaches could provide solutions to address these issues. An optimized design (e.g., smooth transition between stiffer and softer regions), with limited weak spots determined by finite element analysis, could allow the soft pump to be actuated beyond 10 million actuation cycles. Moreover, an adapted actuation system (e.g., active inflation and deflation) would allow a faster relaxation of the actuation membranes, thereby increasing the pump output. Furthermore, a new injection mold design (e.g., introduction of a second mount for the inner metal mold) could allow better control of inner membrane thicknesses to permit equal pumping. Finally, material biocompatibility and impact on hemolysis need to be explored.
By using industrial rubber manufacturing techniques, the goal of improved pumping performance along with increased longevity was achieved. The presented sTAH development progress, together with the identified challenges, build a solid foundation for exciting future research of a soft heart replacement therapy.

The protection of DNA against degradation enables its use in applications, where the stability of the molecule is required. In chapter 4, we presented silica particles encapsulated with DNA to detect material mixing ratios. Identifying and controlling of correct material mixtures allows confirming one dimension of a product’s quality. These so-called SPED enabled the composition analysis of two-component liquid-liquid and solid-solid mixtures via multiplex PCR. This detection method, via DNA barcodes, is cheaper and more facile in its application when compared to existing composition detection techniques. However, SPED release and dispersion stability need to be investigated if considering other materials. The future investigation could try to quantify multiple material compositions as well as limits of SPED detection. The new possibility of SPED to quantify mixing ratios broadens the functionality of the DNA nanoparticles, allowing potentially broader use of this tracing and detection technique in new material streams of supply chains.

Considering DNA data storage, information stability and density are two key factors. While SPED allow the protection of DNA from degradation, the encapsulation technique is time-consuming and is limited in its DNA loading. Therefore, in chapter 5, other inorganic matrices were investigated to create a fast and high-density DNA data storage system. We identified three different salts (MgCl₂, CaCl₂, and CaP) that decrease DNA thermal degradation at high loadings (> 10 wt%). These inorganic matrices outperform existing techniques such as DNAshell or SPED, in either sample handling and/or DNA loading. The new storage technique was also tested on a Teflon-coated glass slide (cartridge-based storage system), in combination with a DNA pool, encoding a digital file. In this case, DNA stored with MgCl₂ at 38 wt% DNA loading provided sufficient protection against DNA degradation, to successfully sequence and decode the digital file after accelerated aging. On a glass cartridge, this system is potentially automatable by connecting it to a digital microfluidic device, allowing a scalable DNA data storage system. However, automation of the storage process, as well as detailed long-term DNA degradation tests, are still necessary before a more detailed assessment of the presented technology is possible. This research should open the door to further development and investigation of salt-based DNA storage techniques that allow, automatable, scalable, and high-density DNA data storage.
7. Appendix
A.1 Supplementary Information Chapter 2

Soft pump mold design

The soft pump (SP) mold was made out of 16 aluminium pieces. The mold consists of an outer mold and the inner mold parts. The outer mold consisted of 6 pieces. The inner mold parts consisted of the ejection chambers, the expansion chambers and the metal holder for the expansion chamber. The inner and outer mold parts can be seen in the explosion view in Figure A.1.1. One expansion chamber was made out of 4 different pieces. It was necessary to build the ejection chamber out of multiple parts to ensure the removal of the metal pieces without damaging the rubber. For the same reason, the outer mold was built out of multiple pieces. In Figure A.1.2., the dimensions of the finished soft pump are shown.

Figure A.1.1. Different pictures from the Autodesk design software of the soft rubber pump mold. (A) Translucent picture of the closed metal mold. (B) Explosion view of metal mold parts. (C) Explosion view of the metal mold, where the outer mold parts are apart.
Figure A.1.2. Different Autodesk pictures of the soft rubber pump and the corresponding dimensions in mm.

Manufacturing Process

In general, the manufacturing process can be divided into two vulcanization steps in the hot press. First, the vulcanization of the main soft pump body and second the vulcanization of the air in- and outlet opening.

Vulcanization of the main soft pump body

The aluminium mold was preheated to 140°C before filling the mold in 8 min with precut rubber sheets. It is important to preheat the mold, to ensure uniform vulcanization of the rubber. During the filling step of the mold, the measured temperature inside the mold quickly dropped to 101°C before the mold was placed inside the hot table press. The temperature was continuously measured during the rubber filling, vulcanization and cooling phase, inside the mold with a long-stem digital thermostat. The temperature profile can be seen in Figure A.1.3. After the mold was placed in the hot table press, a pressure of up to 100 kN was applied. During the initial pressure build-up, most of the excess rubber flowed out of the mold. The rubber was vulcanized for 14 min before the hot table press was cooled down to 40°C with cold water. In
the last step, the SP was removed manually from the aluminium mold. Unnecessary rubber was removed with the help of scissors.

Figure A.1.3. The temperature profile of the soft pump mold. Initially, the mold was preheated in the table press to 140°C. Afterward, the mold cooled down when filled with unvulcanized rubber. During the vulcanization phase, the mold temperature increased to 160°C before being cooled down to 40°C.

Vulcanization of the soft pump air in- and outlet

In the second vulcanization step, the air in- and outlet opening was added to the SP. Therefore, the mold in Figure A.1.4 was attached to the SP, then filled with unvulcanized rubber before being placed inside the table press for 4 minutes at 160°C. After the mold was removed, the SP was ready to be tested.
Figure A.1.4. Autodesk pictures of the aluminium mold introducing the air in- and outlet to the soft pump during the second vulcanization step.

Rubber excess openings

Rubber excess openings were placed in the mold at various locations (Figure A.1.5.B). These openings allowed the free flow of vulcanizing rubber into all cavities of the mold and thereby prevented the inclusion of air. All of the excess rubber flowed out the mold trough these openings.

Figure A.1.5. Pictures of the metal mold used for the manufacturing of the soft pump. (A) Explosion view of the metal mold. (B) Pictures of two outer mold parts. In the upper picture the red circles indicate round 2 mm rubber excess openings. The red circles in the lower picture, indicate 2 by 2 mm rubber excess openings.
Soft pump test bench

A picture of the test bench used for the long-term test, as well as pump performance characterization, can be seen in Figure A.1.6.

**Figure A.1.6.** *Soft pump test bench used for long-term testing as well as pump performance characterization. The red and blue arrows indicated the flow of pumped water from the two ejection chambers of the soft pump.*
Measurement of the tensile properties

A Shimadzu Universal Testing Instrument AGS-X with a 50 N load cell was used to determine the elastic modulus of the used rubber. The sample size for the stress-strain curve in Figure A.1.7. was $n = 4$. The samples were taken from excess rubber in the aluminium mold after the vulcanization process.

Figure A.1.7. Mechanical stability tests of the used rubber. (A) Stress-strain curve of the used rubber. (B) Change of engineering strain when the is cycled between 0 - 1.62 MPa. Stress train curves are shown for cycles 1-5, 50, 100, 500, 1’000, and 1’500 (C) Maximum engineering strain for 0 - 1’500 cycles when maximum stress of 1.62 MPa is applied.
Table A.1.1. *Summary of the mechanical properties of rubber, which the soft pump is made of.*

<table>
<thead>
<tr>
<th>Property</th>
<th>SBR rubber</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tensile strength at max stress (MPa)</td>
<td>13.05 ± 0.73 (n = 4)</td>
</tr>
<tr>
<td>Percent elongation at max stress (%)</td>
<td>1141 ± 80 (n = 4)</td>
</tr>
<tr>
<td>Elastic modulus (MPa)</td>
<td>9.44 ± 0.29 (n = 4)</td>
</tr>
</tbody>
</table>
A.2 Supplementary Information Chapter 3

Calculations for membrane thickness estimation

On each membrane, the air pressure PA is applied on one side of the membrane and the blood pressure (right or left) PB(R/L) is applied on the other side. The resulting force PR/L on the membrane will be:

\[ p_{R/L} = p_A - p_{B(R/L)} \]

To achieve equal pumping performances on both sides, the displacements of each membrane, DR and DL must be equal. Assuming the displacement to be:

\[ d_{R/L} \approx \frac{p_{R/L}}{T_{R/L} \times E} \]

Where TR/L is the thickness of the right/left membrane, and E is the Young’s modulus of the material. For DR to be equal to DL then:

\[ T_R = T_L \frac{p_R}{p_L} \]

With a chosen inlet pressure (PA) of 2 bars (200 kPa), a right blood pressure (PBR) of 20 mmHg (2.66 kPa), a left blood pressure (PBL) of 120 mmHg (16 kPa), and a right membrane of 2 mm, the thickness of the left membrane had to be equal to 1.86 mm. With an inlet pressure (PA) of 155 kPa, the thickness of the membranes have to be 2 mm and 1.49 mm.

Volumetric measurements of the sTAH

Table A.2.1. The volumetric size of the total soft total artificial heart (sTAH), Left ventricle (LV), Right Ventricle (RV), Actuation Chamber (EC), and material only.

<table>
<thead>
<tr>
<th>Description</th>
<th>Volumetric size [cm³]</th>
</tr>
</thead>
<tbody>
<tr>
<td>sTAH</td>
<td>447</td>
</tr>
<tr>
<td>LV</td>
<td>152</td>
</tr>
<tr>
<td>RV</td>
<td>152</td>
</tr>
<tr>
<td>AC</td>
<td>25</td>
</tr>
<tr>
<td>sTAH minus chambers (silicone only)</td>
<td>118</td>
</tr>
</tbody>
</table>
Stress-strain curves for repeated cycling of HTV silicone

![Stress-strain curve of the silicone elastomer. The material was repeatedly (1, 10, 100, and 1000 times) elongated to 100% of its original size.](image1)

**Figure A.2.1.** Stress-strain curve of the silicone elastomer. The material was repeatedly (1, 10, 100, and 1000 times) elongated to 100% of its original size.

Measured pressure in the actuation chamber

![Relative pressure inside the actuation chamber during actuation at 60 beats per minute, 25% inflation-deflation ratio.](image2)

**Figure A.2.2.** Relative pressure inside the actuation chamber during actuation at 60 beats per minute, 25% inflation-deflation ratio.

Finite elements analysis parameters

For the silicone material, the 1st order Ogden model was used as a hyperelastic model. This model assumes incompressibility of the material and defines the strain energy function $W$, and principal stresses $\sigma_i$ by the following equation (general model)\textsuperscript{154}:

$$W = \sum_{p=1}^{n} \frac{\mu_p}{\alpha_p} \cdot \left( \lambda_1^{\sigma_p} + \lambda_2^{\sigma_p} + \lambda_3^{\sigma_p} - 3 \right)$$
\[ \sigma_i = -P + \lambda_i \frac{\partial W}{\partial \lambda_i}, i = 1, 2, 3 \]

And the shear modulus resulted from:

\[ 2\mu = \sum_{p=1}^{n} \mu_p \sigma_p \]

For the different modes, the principal stresses were calculated with the following equations:

**Uniaxial tension:**

\[ \sigma_1 = \sum_{p=1}^{n} \mu_p \left( \lambda^{\alpha_p} - \lambda^{-\alpha_p} \right) \]

\[ \sigma_2 = \sigma_3 = 0 \]

**Biaxial tension:**

\[ \sigma_1 = \sigma_2 = \sum_{p=1}^{n} \mu_p \left( \lambda^{\alpha_p} - \lambda^{-\alpha_p} \right) \]

\[ \sigma_3 = 0 \]

**Shear:**

\[ \sigma_1 = \sum_{p=1}^{n} \mu_p \left( \lambda^{\alpha_p} - \lambda^{-\alpha_p} \right) \]

\[ \sigma_2 = \sum_{p=1}^{n} \mu_p \left( 1 - \lambda^{-\alpha_p} \right) \]

\[ \sigma_3 = 0 \]

Where \( n \) was the order, \( \lambda_1, \lambda_2, \lambda_3 \), the principal stretches \([\text{m/m}]\), and \( \alpha_p \) [-] and \( \mu_p \) [N/m2]. The material constants were calculated using the following experimental data.
The material constants obtained from the uniaxial data were:

\[ \mu_1 = 5.73 \times 10^5 \text{[N/m}^2\text{]} \]
\[ \alpha_1 = 1.31 \]

**Calculations to estimate the theoretical heart flow**

The expansion membranes are approximated as disks with the same surface area. (52 cm² membranes approximated as a disk of diameter 8.136 cm)

When inflated, the membrane of the actuation chamber will expand, and its diameter D will convert to an arc of length E and central angle \( t \), as shown in Figure A.2.5. The ratio between E and D is called the expansion ratio. The volume of fluid displaced by the expansion of the membrane would be the one of a spherical cap of height h and radius R. Therefore, the displaced volume can be estimated by the following equation (displaced volume equation):
Finite element analysis of the sTAH actuated with a pressure of 1.55 kPa was used to calculate the displacement h of the membrane.

\[ V = \frac{\pi R^2}{3} (3R - h) \]

Figure A.2.5. Finite element analysis showing the displacement h of the membrane of the actuation chamber for an inlet air pressure of 155 kPa.

The deformation of the right membrane reached 27.26 mm and 22.63 mm for the right.

Figure A.2.6. The volume displaced (V) as a function of membrane displacement (h), using the displaced volume equation.
From Figure A.2.5., and Figure A.2.6., stroke volumes can be calculated for both ventricles, with 82 ml for the right and 65 ml for the left, resulting in a flow rate of 4.9 L/min for the right ventricle and 3.9 L/min for the left ventricle.

**Measurements of membrane thickness**

![Thickness distribution in cm of the left/right ventricle’s membrane measured with a caliper.](image)

**Figure A.2.7.** (Left/Right) Thickness distribution in cm of the left/right ventricle’s membrane measured with a caliper.
A.3 Supplementary Information Chapter 4

DNA Codes:

**SPED1:**

GM-06-S1
TTATGGGCTCTAAGGATCTCTCTGCCTGTTGAGGTTCCTGCGTTTTTCGATTCGA
GGGTGAGTT

GM-06-S1_rev
AACTCACCCTCGAATCGAAAAACGCAGGAACCTAACGACAACGAAGAGATCCTT
AGAGCCCATAA

Forward and reverse primer of GM-06-S1
GM-06-S1_pF ATGGGCTCTAAGGATCTC
GM-06-S1_pR CTCACCCTCGAATCGAA
GM-06-S1_probe ACGCAGGAACCTAACGACAACG

**SPED2:**

GM-06-S2
TATGCGCCTTTTATCTCTTATAGGTATCCTGTTGCTGGCACTTTTTTCTAGCAAAAG
TCTTCTCCT

GM-06-S2_rev
AGGAGAAGACTTTGCTAGAAAAAAGTGCCAGCAACAGGATACCTATAAGAGTAT
AAAGCGCGCATA

Forward and reverse primer of GM-06-S2
GM-06-S2_pF ATGCAGCTTTTATCTCCTTA
GM-06-S2_pR GGAGAAGACTTTGCTAGAA
GM-06-S2_probe AAGTGCCAGCAACAGGATACCT
Minimum detectable composition difference

The minimal detectable composition difference \( \delta \) was calculated by using an independent two-sample \( t \)-test with equal or unequal sample size and equal variance. \( \delta \) is dependent on a chosen signified level \( \alpha \), and where \( p(\delta) \leq \alpha \) and \( \alpha=0.05 \). Composition ratios were derived from \( \Delta CQ \) values plotted in Figure A.3.1. \( \Delta CQ \) values were obtained from a linear regression of 5 different water-water composition ratios shown in Figure 4.2.A. For example, with \( N=6 \) (\( N= \) total number of samples), a minimal detectable composition difference of \( \delta=7 \) is obtained. Hence, a 73:27 composition ratio can be significantly differentiated from an 80:20 composition ratio. In our calculations, we used \( N_b=2 \) (with \( N=N_a+N_b \)).

![Figure A.3.1. \( \Delta CQ \) value for different S1:S2 composition ratio for water-water mixtures.](image)
**SPED synthesis**

An upscaled synthesis procedure adapted from Paunescu et al. was used for SPED synthesis. In short, 2 mL of annealed DNA (S1 or S2) (NanoDrop concentration: 150 µg/mL) was added to 200 mL of mQ water before 0.4 g of SiO2-TMAPS functionalized particles were added. After shaking and 15 s sonication of the particles, 40 µL N-trimethoxysilypropyl-N,N,N-triethylammonium chloride (TMAPS, 50% in MeOH, ABCR) was added. After each addition of a new chemical to the particles, they were shaken and sonicated. Next, 62.5 µL tetraethoxysilane (TEOS, ≥ 99%, Aldrich) was added. Leave the particles shaking at 300 rpm for 5h. Finally, 500 mL of mQ water, 10 mL iProH, 20 mL of annealing buffer and 5.9 mL TEOS were added before shaking the particles for 4 days. The DNA loading on the SPED was measured with the Qubit dsDNA high-sensitivity assay. SPED1 had a DNA loading of 0.8 wt% and SPED2 of 0.9 wt%. Scanning electron microscopy images of the two different SPED particles can be seen in Figure A.3.2.

![Figure A.3.2](image_url)  
*Figure A.3.2. Scanning transmission electron microscope (STEM) images of (A-B) silica nanoparticles encapsulated with S1 DNA (SPED1) and (C-D) silica nanoparticles encapsulated with S2 DNA (SPED2).*
Dilution Series with SPED

**Figure A.3.3.** (A) Dilution series of S1 and S2 DNA in 40:60 water-water mixture and (B) and Dilution series of S1 and S2 DNA in 80:20 water-water mixture. Solid lines are linear fits.

**Independent Two-Sample T Test**

All the calculations were done using the following equations:

\[
t = \frac{\bar{X}_1 - \bar{X}_2}{s_p \cdot \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}
\]

Where

\[
s_p = \sqrt{\frac{(n_1 - 1)s_{x_1}^2 + (n_2 - 1)s_{x_2}^2}{n_1 + n_2 - 2}}
\]

\(\bar{X}_1, \bar{X}_2, n_1, n_2 \) and \(s_{x_1}, s_{x_2}\) are, respectively, the means, the sample sizes and the standard deviations of sample groups 1 and 2. The degree of freedom for this test is either \(2n-2\) (\(n = n_1 = n_2\) equal sample sizes) or \(n_1 + n_2 - 2\) (unequal samples sizes).
Table A3.1. $X_1$-$X_2$, $S_{X1X2}$, $n_1+n_2$ and $t$ values for water-water and silicone mixtures.

<table>
<thead>
<tr>
<th></th>
<th>water-water mixtures</th>
<th></th>
<th>silicone-silicone mixtures</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 vs Group 2</td>
<td>$X_1$-$X_2$</td>
<td>$S_{X1X2}$</td>
<td>$n_1+n_2$</td>
<td>$t$</td>
</tr>
<tr>
<td>80:20 vs 60:40</td>
<td>-1.41</td>
<td>0.20</td>
<td>6</td>
<td>-8.58</td>
</tr>
<tr>
<td>60:40 vs 50:50</td>
<td>-0.71</td>
<td>0.15</td>
<td>6</td>
<td>-5.94</td>
</tr>
<tr>
<td>50:50 vs 40:60</td>
<td>-0.79</td>
<td>0.16</td>
<td>6</td>
<td>-6.06</td>
</tr>
<tr>
<td>40:60 vs 20:80</td>
<td>-1.23</td>
<td>0.22</td>
<td>6</td>
<td>-6.89</td>
</tr>
</tbody>
</table>

Detailed SPED release procedure from silicone mixtures

Figure A.3.4. Transfer of SPED from water to THF and subsequent dispersion procedure into Silicone.

Figure A.3.5. Release of SPED from silicone with subsequent DNA release from SPED.
A.4 Supplementary Information Chapter 5

Comparison of DNA storage media

**Figure A.4.1.** Comparison of different DNA storage media. Estimates for stability (half-life), DNA loading and synthesis speed of the storage media are shown.\(^{72,75,78,79,81,82,155}\)

**DNA in bone**

Stability:

Half-life \((t_{1/2})\) of 242 bp mtDNA at 13.1 °C is 521 years.\(^{75}\) \(t_{1/2}\) scaled to 150 bp equals 841 years.

DNA loading:

\[
DNA \text{ loading in bones} = 0.05 \times 0.01 \text{ wt\%} = 0.005 \text{ wt\%}
\]

Calcified bones contain approximately up to 5 wt% cells.\(^{155}\) DNA mass in a human cell is about 10 pg/cell.\(^{156}\) The cell volume is around 1000 \(\mu\text{m}^3\) (= 1000 pg). Therefore, a cell is approximately composed of 1 wt% DNA.

**DNA in solution**

Stability:

DNA in solution has a reported activation energy of 129 kJ/mol.\(^{72}\) Extrapolation of the rate constant of depurination per nucleotide to 10°C allows the calculation of the half-life, which is equal to 33 years (scaled to DNA strands of 150 bp). All data was taken from Lindahl et al.\(^{72}\)
K₀ = 5.45 E12; Scaling to 150 bp strands: \( t_{1/2}^{150nt} = t_{1/2}^{1nt} / 75 \) (on average, every second nucleotide is a purine)

Oxford Gene Technology suggests storage of DNA samples at 4°C for only a limited number of weeks to ensure high-quality microarray results.⁷⁸

DNA loading:

\[
DNA \text{ loading in solution} = 500 \text{ ng/μL} = 0.05 \text{ wt%}
\]

The typical concentration of dsDNA from Microsynth AG is approximately 500 ng/μL.

Handling and synthesis time: DNA annealing time plus sample preparation time.

**DNA in nanoparticle**

Stability: Half-life of 500 years at 10°C was calculated with data from Grass et al. for 158 bp long DNA.⁸² \( t_{1/2} \) scaled to 150 bp equals 527 years.

DNA loading is reported by Chen et al.⁸¹ Handling time is reported by Paunescu et al.⁸⁰

**DNA in salt**

Stability:

DNA pool in the presence of MgCl₂ (38 wt%) was aged for three weeks and compared to data presented by Chen et al. (see Fig. A.4.2.). The experimentally determined DNA decay rate constant at 70°C was used to calculate the half-life of this storage method (109 - 753 years) for two different activation energies found in the literature (see Fig. A.4.3.).⁷²,⁸²,¹³⁹

Handling and synthesis time: Sample preparation time and drying time.
**Figure A.4.2.** The decay of the DNA pool with MgCl2 (38 wt%) by exposure to accelerated aging conditions. Pure DNA and encapsulated DNA decay data presented by Chen et al. were compared to this storage method.\(^81\)

**Figure A.4.3.** Half-lives for DNA stored with MgCl2 (38 wt%) extrapolated from experimental rate constant and activation energies from literature. Half-lives at 10°C vary between 109 and 753 years with different activation energies taken from Lindahl et al. (129 kJ/mol), Grass et al. (155 kJ/mol) and Bonnet et al. (155 kJ/mol).\(^72,82,139\)
DNA and primer sequences

148 BP MS DNA: Seq4235_tr_wpr_re

AGACGTGTGCTCTTCCGATCTCTTCTGACTTCTATGTAGCGCTATGGTAGTCGCG
AGCTAACTATATCGCGTGAGCACCGTTGAAGACGAGACTGCGAGGTGGAGATCT
ATCTAGTAGATGAGAGTCAGATCGG AAGAGCGTCGTGT

148 BP MS DNA: Seq4235_tr_wpr_fr

ACACGACGCTCTTCCGATCTCTTCTCATTACTAAGATAGATCTCCCATCGCAGT
CTCGTCTCTCAACGGGTGCTCAGCCGATATAGTTAGCTCGCGACTACCATAGCGCTA
CATAGAAGTCAGCAAGAGATCGGAAGAGCACACGTCT

Primer 0R: AGA CGT GTG CTC TTC CGA TCT
Primer 0F: ACA CGA CGC TCT TCC GAT CT

DNA storage with salt protocol

This protocol describes a method for encapsulating and de-encapsulating DNA into calcium phosphate. After the de-encapsulation of DNA from calcium phosphate, the nucleic acids were analyzed by qPCR. All other DNA salt samples were prepared following the same protocol by changing the used salt solutions.

Preparation of solutions:

Prepare 1 M solutions of chemicals (Calcium chloride dihydrate (Ca solution), Potassium Phosphate Monobasic, and Di-Potassium Hydrogen Orthophosphate Trihydrate) in mQ water. Mix both phosphate solutions 1:1. This mixture will be called KP solution. Dilution of Ca solution and KP solution to 0.1 mM.

DNA preparation

Add 20 µl of DNA on 0.025 µm Millipore filter.

Millipore filter is placed inside a closed 6-well plate filled with 3 mL of mQ water.

Wait for 20 minutes before removing the maximum amount of liquid from the filter.

Measure the DNA concentration with Nanodrop.

Dilute the DNA concentration to 15 ng/µl with mQ.
Sample preparation

Add 2 µl of DNA in a 2 ml Eppendorf tube (total of 30 ng of DNA).

Add 5 µl of Ca solution to the DNA.

Add 5 µl of KP solution.

Place the sample in a vacuum centrifuge to remove the water from the sample. Wait for a minimum of 2 hours or overnight (depending on the number of samples in the centrifuge).

De-encapsulation

Prepare 1 mM EDTA solution.

Add 100 µl of 1 mM EDTA to one sample and vortex the sample.

Dilute the sample 1:100 with mQ water, therefore remove 5 µl of the sample and add to 495 µl of mQ water.

Samples are ready for qPCR.

Accelerated aging experiments

All accelerated aging experiments were performed at 60 or 70°C at 50% relative humidity in a closed desiccator, filled with a saturated NaBr solution. The desiccators were placed inside an oven.

PCR amplification protocol

MS DNA and DNA pool encoding 115kB of data were amplified with a Roche Lightcycler 96. Each sample well was filled with a total volume of 20 µl containing: 5 µl sample volume, 10 µl of Kapa Sybr Fast qPCR Master Mix, 3 µL mQ water, 1 µl (10 µM) forward primer and 1 µl (10 µM) reverse primer. The qPCR for MS DNA consisted of a 3-step amplification protocol (95°C for 15 s, 56°C for 15 s and 72°C for 10 s), and each sample was analyzed in duplicates. The qPCR for the DNA pool also consisted of a 3-step amplification protocol (98°C for 20 s, 60°C for 15 s and 72°C for 20 s), and each sample was analyzed in duplicates.
Figure A.4.4. SEM pictures (A) and (B) at a different magnification of a dried sample of DNA with CaP (18 wt%) in a 2 ml Eppendorf tube.

Figure A.4.5. The relative concentration of DNA stored with different amounts of calcium phosphate after accelerated aging at 70°C and 50% RH for 5 days. Samples were dried in a freeze drier or a vacuum centrifuge.
Figure A.4.6. The relative concentration of DNA pool consisting of 7323 distinct oligonucleotide sequences (150 bp long) stored with different amounts of salts (CaP, MgCl$_2$, and CaCl$_2$) after accelerated aging at 70°C and 50% RH for 5 days.

Figure A.4.7. The average probability of DNA damage by insertion, deletion, or substitution per 110 base pairs (payload region) of DNA protected by MgCl$_2$ (red, n = 5), unprotected DNA (black, n = 5) and a reference sample (blue).
Figure A.4.8. The average distribution of insertions in DNA samples protected by MgCl₂ (red, n = 5), unprotected DNA (black, n = 5), and a reference sample (blue).

Figure A.4.9. The average distribution of deletions in DNA samples protected by MgCl₂ (red, n = 5), unprotected DNA (black, n = 5), and a reference sample (blue).
**Protocol for sequencing preparation**

In short, a random 25N region was added via PCR. Following an adapted TruSeq® Nano Library Prep protocol (Illumina, June 2015, Rev. D) and a TruSeq® ChiP sample preparation protocol (Illumina, October 2013, Rev. B), sequencing adapters were ligated to the samples. After quality control of the samples via Gel electrophoresis, samples were cut out from the gel and released with QIAx Gel Extraction Kit. Next, a Qubit concentration measurement was done, and the samples were diluted and spiked with 2% of Illumina’s PhiX control before being sequenced on the DNA sequencer iSeq 100 (Illumina).

**Figure A.4.10. Schematic overview of the main steps involved in DNA sequencing preparation.**

**Addition of 25N region by PCR amplification**

Amplify protected, and unprotected samples with two separate PCR runs.

Dilute the samples 1:500 with mQ water.

Prepare PCR mix:

- 5 μL DNA sample; 1 μl of 10 mM forward primer and 1 μl of 10 mM reverse primer, 10 μL of 2x Kapa cybr Fast mix, 3 μL of mQ water.

Thermocycling protocol: (1) 95 °C for 3 min, (2) 98°C for 20 s, (3) 62 °C for 20 s, (4) 72 °C for 15 s, repeat steps 2-4 as needed.

Stop the PCR when the signal reaches the plateau phase.

**Conversion to blunt ends (adapted from page 14 of TruSeq® Nano Library Prep protocol)**

Add 20 μL ERP2 to each well with 10 μL of PCR product and 20 μL of mQ water.

Pipette up and down to mix.
Place on the thermal cycler and run the ERP program (30 min at 30 °C) then place it on ice. Each well contains 50 μl.

Sample purification with AMPure XP beads (adapted from page 12 of TruSeq® ChiP sample preparation protocol)

Add 80 μl well-mixed AMPure XP beads to each well of the PCR plate containing 50 μl End Repair Mix.

Gently pipette the entire volume up and down 10 times to mix thoroughly.

Incubate the PCR plate at room temperature for 15 minutes.

Place the PCR plate on the magnetic stand at room temperature for 15 minutes or until the liquid is clear.

Using a 200 μl pipette set to 125 μl, remove and discard 125 μl of the supernatant from each well of the PCR plate.

5 μl are left in each well.

**NOTE** Leave the PCR plate on the magnetic stand while performing the following 80% EtOH wash steps (g–i).

With the PCR plate on the magnetic stand, add 100 μl freshly prepared 80% EtOH to each well without disturbing the beads. (*Pipette to the side of the wall*)

Incubate the PCR plate at room temperature for 30 seconds, and then remove and discard all of the supernatants from each well. Take care not to disturb the beads.

Repeat steps g and h one time for a total of two 80% EtOH washes.

Let the PCR plate stand at room temperature for 15 minutes to dry, and then remove the plate from the magnetic stand.

Resuspend the dried pellet in each well with 17 μl Resuspension Buffer (RSB). Gently pipette the entire volume up and down 10 times to mix thoroughly.

Incubate the PCR plate at room temperature for 2 minutes.

Place the PCR plate on the magnetic stand at room temperature for 5 minutes or until the liquid is clear.

Transfer 15 μl of the clear supernatant from each well of the PCR plate to the corresponding well of a new 96-well 0.2 ml PCR plate.
Ligation of adapters

Add 15 µl of ATL to the 15 µl of supernatant from the previous step.
Briefly centrifuge (3 min at 600 rpm).
Place on the thermal cycler and run the ATAIL70 program. Each well contains 30 µl
Choose the preheat lid option and set it to 100°C.
- 37°C for 30 minutes
- 70°C for 5 minutes
- 4°C for 5 minutes

Addition of the adapters: add the in the following order:
- RSB (2.5 µl)
- LIG2 (2.5 µl)
- DNA adapter (2.5 µl)

Pipette up and down, centrifuge briefly (3 min at 600 rpm).

Run lig program
Choose the preheat lid option and set to 100°C
30°C for 10 minutes
Hold at 4°C
Add 5 µl STL to each well, and then mix well.

NOTE: Can hold this mixture at -20°C overnight with no trouble.
Vortex SPB until well dispersed.
Add 42.5 µl of SPB (AMPure XP beads) to each well, and then mix thoroughly as follows.
Pipette up and down.
Incubate at room temperature for 5 minutes.
Place on a magnetic stand and wait until the liquid is clear (2–5 minutes).
Remove and discard all supernatant from each well.
Wash 2 times as follows.
Add 200 µl freshly prepared 80% EtOH to each well.
Incubate on the magnetic stand for 30 seconds.
Remove and discard all supernatant from each well.
Use a 20 µl pipette to remove residual EtOH from each well.
Air-dry on the magnetic stand for 5 minutes.
Add 52.5 µl RSB to each well.
Remove from the magnetic stand, and then mix thoroughly as follows. Pipette up and down.
Incubate at room temperature for 2 minutes.
Place on a magnetic stand and wait until the liquid is clear (2–5 minutes).
Transfer 25 µl supernatant to the corresponding well of the PCR plate.

**SAFE STOPPING POINT:** Ligation is done. If you are stopping, seal the plate and store at -25°C to -15°C for up to 7 days.

**Enrichment for sequencing:**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration</th>
<th>Volume (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA mix</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>PPC (PCR Primer Cocktail)</td>
<td>10X</td>
<td>3</td>
</tr>
<tr>
<td>EPM (Enhanced PCR Mix)</td>
<td>2.5X</td>
<td>12</td>
</tr>
<tr>
<td>H₂O</td>
<td></td>
<td>12</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>30</strong></td>
</tr>
</tbody>
</table>

Choose the preheat lid option and set to 100°C
95°C for 3 minutes
10 total cycles of:
- 98°C for 20 seconds
- 60°C for 15 seconds
- 72°C for 30 seconds

72°C for 3 min
Hold at 4°C
Quality control and dilution for sequencing:

Run a gel electrophoresis experiment (E-gel® EX, SYBR Gold II, 2% Agarose) with ligated samples and control samples with 25N region.

Cut out DNA band of ligated samples and release the DNA with QIAx Gel Extraction Kit (Qiagen).

Measure DNA concentration with dsDNA assay for Qubit Fluorometer.

Dilute each sample to 1 nmol/L.

Add 10 µl of each the samples to one tube.

Dilute the DNA solution to a total volume of 100 µl and 50 pmol/L and add 2 µl of 50 pmol/L PhiX. For details, see Illumina iSeq 100 protocol.
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Curriculum Vitae

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Education

04/2016 – current **PhD studies** at the Department of Chemistry and Applied Biosciences, Institute of Chemical- and Bioengineering, Functional Materials Laboratory, ETH Zurich, Zurich, Switzerland

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Title: *Advances in DNA-based functional materials and performance improvements of soft pumps*

03/2014 – 11/2015 **MSc studies** in Chemical- and Bioengineering, Department of Chemistry and Applied Biosciences, ETH Zurich, Zurich, Switzerland.


09/2010 - 08/2014 **BSc studies** in Chemical Engineering, Department of Chemistry and Applied Biosciences, ETH Zurich, Zurich, Switzerland.

09/2003 – 07/2010 **High-school**, Examen de fin d’études secondaires
Lycée Classique Robert-Schuman, Luxemburg, Luxemburg.

Core subjects: Mathematics, Biology, Chemistry, and Physics.
Refereed Journal Articles


Conference Presentations and Proceedings


Student supervision


2. Sebastian Sewing (Research Project, 02/2018 – 05/2019): The effect of contact time with acetone and mixing ratio of 2-component silicone elastomer on its mechanical properties.


Teaching

09/2016 – 08/2017 Lecture Assistant, ‘Chemieingenieurswissenschaften’, Bachelor course for Chemists and Chemical Engineers, ETH Zurich.

09/2016 – 11/2019 Course assistant, laboratory course ‘Chemical Engineering Laboratory I’, Institute for Chemical- and Bioengineering, ETH Zürich.

Professional experience

01/2017 – 08/2019 Development of DNA data storage technologies with industrial partner Microsoft, Functional Materials Laboratory, ETH Zürich.

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