In Vitro Study on the Eligibility of Antibacterial Agents for Modification of Silicone in Implant-Abutment Interface Sealing

Inauguraldissertation

zur Erlangung des Grades eines Doktors der Zahnmedizin

des Fachbereichs Medizin

der Justus-Liebig-Universität Gießen

Eingereicht von Engelbrecht, Jeannine Dunila

aus Hamburg

Gießen (2020)

Aus dem Fachbereich Medizin der Justus-Liebig-Universität Gießen,

Zentrum für Zahn-, Mund- und Kieferheilkunde

Gutachter: Prof. Dr. B. Wöstmann

Gutachter: Prof. Dr. E. Domann

Tag der Disputation: 04.05.2021

Table of contents

1 Preamble

In case of missing teeth dental implants are used as anchorage of prosthetic devices in bone.[131] They depict an artificial dental root commonly made of titanium and imitate the functions of their natural counterpart.[43]

Most often implant systems consist of three parts: implant, abutment and superstructure. The implant is implemented in bone, the abutment connects the implant to the oral cavity and the superstructure is the prosthetic part, similar to those utilised on natural teeth, e.g. dental crowns.

These three parts are commonly separate from one another, which leads to some advantages and disadvantages. Exemplary advantages are the ability to change and adjust the prosthetic angle from the axis required for the implant implementation in bone to the desired axis for the superstructure by using angled abutments,[83] as well as enabling a subgingival healing period and osseointegration without the risk of implant loss through mechanical overload.[83, 142]
A disadvantage of this system is a microgap in between implant and abutment.[37, 96, 142] This microgap may function as a bacterial depot for the surrounding tissues,[37, 142] which would imply an increased amount of bacteria and their endotoxins in the peri-implant tissues.[112] Especially in cases of persistent peri-implantitis (a chronical inflammation of the surrounding tissues), an increase of the bacterial load would be counterproductive, as it is assumed to be a contributing factor.[142]
Peri-implantitis, partially caused by bacterial contamination surrounding the implant, may lead to bone loss, resulting in loosening of the implant with the possibility of implant loss.[59]

To avoid the possibility of a bacterial reservoir, there have been multiple attempts to seal the implant-abutment interface. One of them is sealing with a low viscosity dental silicone, which decreases the amount of bacteria.[113, 114]

This study examined whether three different antibacterial agents may be capable to grant the sealing-silicone antibacterial properties without infringing the fit in between implant and abutment, reducing the opening torque or leading to fracture of the screw.

2 Aim

The aim of this study was to test the suitability of a metal oxide and two metal salts as antimicrobial agents for modification of dental silicone – designed for sealing of the implant-abutment interface.
In a first step the antibacterial potential of the antibacterial agents mixed in low viscosity dental silicone was evaluated.

The examined antibacterial agents were:

1. Nano zinc oxide
2. Copper naphthenate
3. Silver 2-ethylhexanoate

In a second step, in case of successful antibacterial modification, the influence of the modified silicone on the applied torque whilst unbolting the abutment from the implant was examined, as well as whether the modified silicone inhibited the abutment's ability to reach the target position within the implant.

The following null-hypotheses were investigated:

1. Neither with nano zinc oxide nor copper naphthenate or silver 2-ethylhexanoate, it is possible to implement antibacterial properties in low viscosity dental silicones.
2. The application of modified silicone does not affect the positioning of the abutment in the implant, nor does it influence the opening torque or lead to breakage of the connecting screw.

3 Literature

3.1 Implants

3.1.1 Definition

The term “implant” derives from the old-Latin prefix “in-“ for “in/inside” and the Latin term “plantare” for “planting”.[41, 42] In total, the term describes something planted into the body. This definition is reflected most generally by the American Heritage® Dictionary of the English Language as “*Something implanted, especially a surgically implanted tissue or device*”,[41] by the definition of the Duden 2018 as (translation:) “*tissue, (part of an) organ or a different material - even microelectronic device, that assumes specific functions and is planted into the body*”.[42] In the Pschyrembel 2018 dental implants are defined as (translation:) “*replacement of a dental root usually shaped like a cylinder or a screw, predominantly made of titanium*”.[43]

3.1.2 History

The oldest find of a dental implant was found 1931 in Honduras during an archaeological research on the Mayan population. The find was a partial mandible with three of the incisors replaced by shells carved into the shape of incisors. The find was dated from around 600 AD. Implant placement must have taken place whilst the human was still alive, since the surrounding bone grew onto the implant.[104]

There has been a multitude of attempts on implantation with different materials and techniques over the course of time. For a long time human teeth were either transplanted or bridges made out of human teeth or alternatively ivory were fixed with golden ligatures as a replacement of missing teeth.[1, 104] Alloplastic materials for implants have mostly been used since the 18th century.[1, 104] A great variety of

materials were tested and used over the curse of time: gold, platinum, lead, silver, iridium, corrugated porcelain, vitallium, stainless steel, aluminium, cobalt-chromium, and the up to this date mainly used titanium and titanium alloys.[1, 104, 147] As a quite new development there is a lot of research on ceramic implants, especially to overcome the aesthetic limitations of titanium in combination with a slim fragile gingiva.[31]

Titanium implants have been studied by BRÅNEMARK et al. since the early 1960s and osseointegration in general since 1952, where the inability to separate titanium implants from surrounding bone tissue after they had healed in, unless cut away, was described.[21] In 1981 osseointegration was described by ALBREKTSSON et al. as "a direct – on the light microscopic level – contact between living bone and implant"[6] and by ADELL et al. as "Osseointegration implies a firm, direct and lasting connection between vital bone and screw-shaped titanium implants of defined finish and geometry – fixtures."[4] Multiple articles describing the potential for osseointegration of a mechanically loaded titanium implant in living bone were published, and lead to the regular use of titanium implants to this date.[4, 6, 21, 76]

Just like the materials, the shape and location of the fixtures changed a lot. The location of implants could be subperiosteal, transosteal or endosteal. There have also been some less popular tries with intramucosal implants.[45, 136] Subperiosteal implants were located in between the bone and the periost.[1, 7, 136] For this type of implant the bone did not need to be drilled since they held (similarly to a saddle) through being custom casted to fit perfectly on top of the bone.[7, 147] Transosteal implants were shaped like screws and reached all the way through the bone of the atrophic mandible.[147, 148] Endosteal implants are commonly used up to this day and showed the least amount of failures. But the shape changed a lot over the course of time: from implants shaped like plain tubes and cylinders to discs to hollow-basket designs (a hollow latticed cylinder) to spirals to blade implants, to the up to date commonly used more root shaped cylindrical and threaded implants, either with a more parallel, stepped or tapered form.[1, 7, 147]

3.1.3 **Structure**

A modern implant system commonly consists of three different parts: implant, abutment and superstructure (see fig. 1).

The implant is the endosseous part of an implant system and nowadays mainly made from titanium, in more rare cases from zircon dioxide.[31] The length varies in between 5 mm and 19 mm, the diameter in between 3,3 mm and 6,5 mm.[131] Shape wise, all modern implants are rotationally symmetric[122], with either more cylindrical (= parallel) or tapered forms, in more rare cases also stepped.[147] They can be threaded or in more rare cases straight and may have wings for further surface amplification.[147] There are multiple variations of outer surface treatment to enable the bone to grow onto the implant surface. As BRÅNEMARK already described in his studies in the 1960s, the outer surface is a crucial factor to the success of implants. It is the area providing the fixture of the implant in bone.[21] All of the different treatments have in common that they result in a rough surface. Depending on the desired depth of submersion into the bone, the implant shoulder may, or may not be polished. In case of total submersion, with the implant ending on bone level, the whole surface is treated. If the implant surpasses bone level, referring to the implant ending outside of the bone, the implant shoulder is polished to minimise the ability of bacteria to adhere to the surface.[143] The level of submersion depends on the gingival thickness. The desired distance from the prospective gingival margin to the implant shoulder should be 2 mm to 3 mm to allow enough space for a sufficient emergence profile (“emergence profile” describing the change from a round diameter to tooth shape).[131]

The abutment forms the transmucosal connection in between the implant fixed in bone and superstructure in the oral cavity.[97, 130, 142] There are various shapes and materials, depending on the desired superstructure. The shapes for abutments include, but are not limited to: imitations of tooth preparations – similar to primary crowns in double crown systems, customizable abutments, bar attachments, ballheads (locators) or magnets the superstructure can hold on to.[131, 132] In regards to material titanium is commonly used, but they can also be fabricated from zirconium (for better aesthetics in the front), gold (mainly bar attachments) and other metals, like non precious metal alloys.[131, 132] Most often the abutment is bolted onto the

implant. The alternative to bolting in fixing the abutment onto the implant is cementation, but the risk of inflammation due to cement residue and the disability to change the abutment if needed, may be some of the factors why this method was met with little approval and use.[111]

There are a lot of different variations on the connection in between implant and abutment. They can be either bolted or cemented,[94, 111] and show a multitude of different shapes of the implant-abutment interface, e.g. such as internal (hexagonal, conic)[37, 94, 96] and external (hexagonal) connections[37, 94]. On top of that there are one piece implant systems, which don't even have an interface, as they combine implant and abutment in one piece.[142] Two piece implant systems (or three piece implant systems if the superstructure is counted as well) are much more common than one piece implant systems, which are mainly used as temporary implants or in areas where it is possible to avoid mechanical load in the first months (e.g. front teeth restorations).[131, 132] Two piece implants enable a sound healing and osseointegration, since there is no risk of mechanical overload.[83, 142] They also provide the option for different angles in between the implant and abutment axis, an important feature in more complicated bone conditions and greater bridge spans.[83] On top of that it is possible to change the abutment if a different form of prosthesis is needed, e.g. change from a single tooth to a bridge with a different angle to the implant.
Nowadays there are predominantly three different shapes of the implant-abutment interface in the market: either polygonal (e.g. hexagonal), conical or parallel, with the options of an internal or external connection as well as with and without protection against rotation.[131]

The superstructure is the visible part of the implant system and forms the crown, bridge or prosthesis. Fixation of the superstructure on the abutment in case of crowns and bridges can be attained either by cementation, occlusal- or transversal bolting.[131] Cementation with temporary cements allows removal of the superstructure (e.g. single crown) and peri-implant care including changing or refastening of the screw connecting abutment and implant in case of loosening.[131] Cementation can also be conducted with permanent cements.[131, 132] In case of occlusal bolting, the superstructure and abutment form a single unit and the occlusal screw holding the abutment onto the implant fastens the superstructure on the implant as well. The screw is accessible through the superstructure by an occlusal opening, most often

sealed with resins.[132] For transversal fixation, the superstructure will have a similar opening, but in this case not in occlusal but transversal direction. This opening holds a screw which connects the superstructure to the abutment, so in the end two screws are used in this system.[131] Removable prostheses are fixed mechanically on the abutment in a similar manner to telescope prostheses.[132]

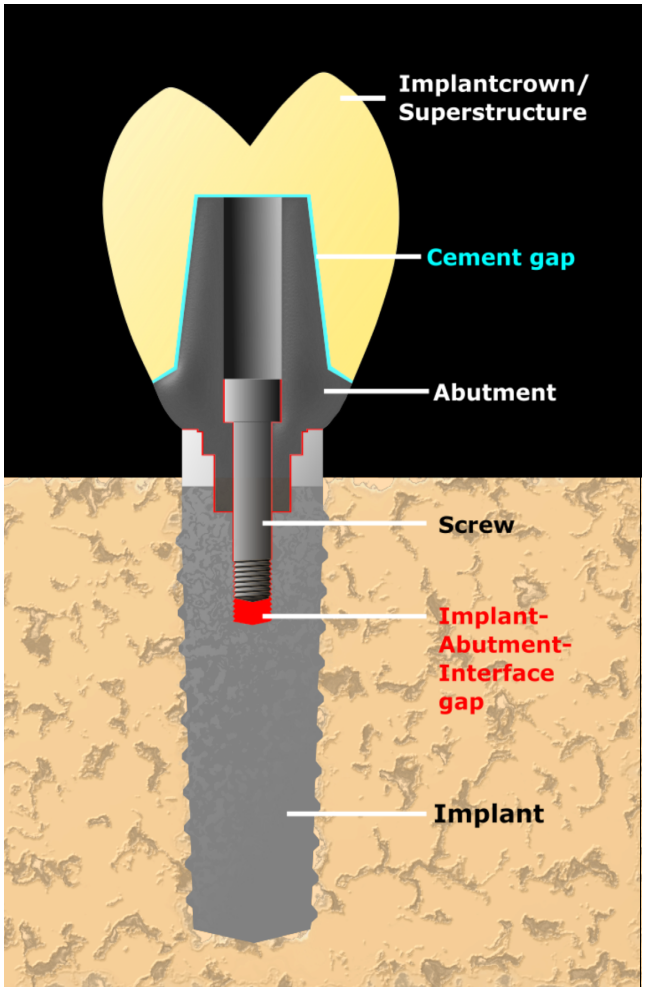


fig. 1) Structure of an implant with a screw retained abutment and cemented crown as superstructure

3.2 Peri-implantitis and peri-implant microbiome

3.2.1 Peri-implantitis

The biggest problem with implants is the risk of implant loss, either in early stages as a failure in osseointegration, or later after successful osseointegration due to bone loss through persisting inflammation[15, 16, 111, 123, 134], or overload[94, 111, 123]. An early failure in osseointegration may have a lot of different reasons, for example early (over-) loading, as a systemic disease that influences the tissues´ healing abilities, a host reaction to the implant material, or surgical contamination.[16] Late implant loss occurs most often due to inflammation in the surrounding tissues (see fig. 2).[16] If the inflammation affects the surrounding mucosa but not the bone, it is called peri-implant mucositis,[16, 124, 131] peri-implantitis if surrounding bone tissue is affected as well.[15, 16, 124]

Main cause for inflammation on bone level is bacteria on the implant surface. Oral cavities show a high number of bacterial species – at least 100 different ones per person.[16] In total, more than 700 different species were found in oral cavities.[16] These bacteria can become opportunistic pathogens if the circumstances change to a favourable environment – for example the formation of a new anaerobic niche with access to substrate.[15] Because of the absence of periodontal ligaments (Sharpey´s fibres) perpendicular to the implant surface and the alignment of collagen fibres in the mucosal connective tissues parallel to the implant, peri-implant tissues form deeper crevices than gingival ones and thus offer a weaker barrier to bacterial invasion than natural teeth.[15, 16, 36] Prolonged biofilm formation on the implant surface induces an inflammatory reaction in the adjoining tissues. A persisting and progressing inflammatory reaction close to the bone induces bone degeneration.[15, 16]

Bone degeneration leads at first to loosening and if untreated later to a loss of the implant.[15, 36, 59] The process of peri-implantitis is very similar to periodontitis in natural teeth. Both processes are slow, most often unnoticed, and show a shift towards a more gram-negative, anaerobic and motile bacterial flora.[15, 16, 97]

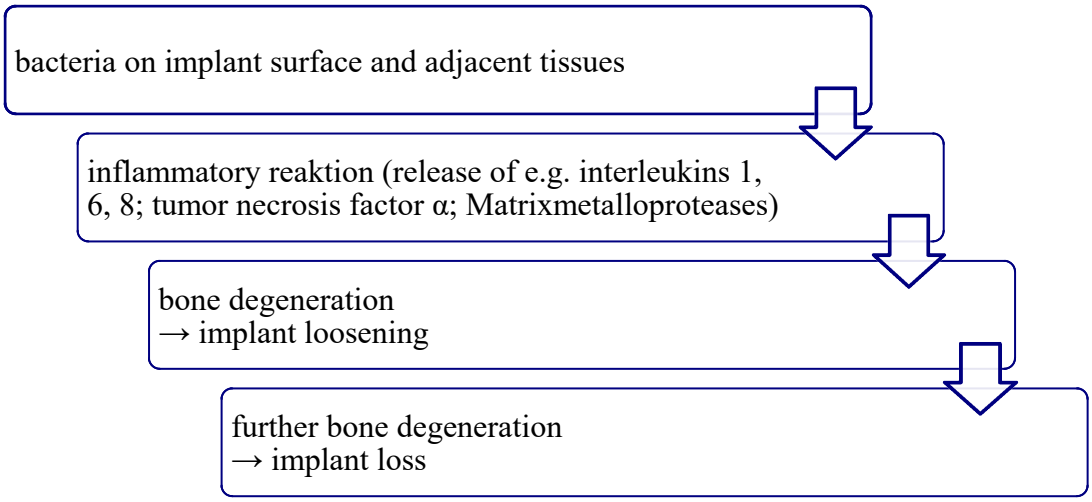


fig. 2) Pathogenesis of implant loss through peri-implantitis

3.2.2 **Peri-implant microbiome**

The peri-implant microbiome has been the focus of a lot of studies in the past years. It seems to show a shift to the anaerobe spectrum similar to the periodontal microbiome.

In 1999 LEONHARDT et al. detected *Porphyromonas gingivalis, Prevotella intermedia, Prevotella nigrescens* and *Actinobacillus actinomycetemcomitans* as typical periodontal germs and additionally *Enterics* (e.g. *Klebsiella pneumonie*), *Staphylococcus spp. (Staphylococcus epidermidis)* and *Candida spp. (Candida albicans).*[78]

2007, RENVERT et al. found a lot of species that teeth and implants had in common, such as *Neisseria mucosa, F. nucleatum sp. nucleatum, F. nucleatum sp. polymorphum, veillonella parvula, streptococcus intermedius, Staphylococcus aureus* and *M. micros.* Higher probing depths correlated with a presence of *Eikenella corrodens, F. nucleatum sp. vincentii, Porphyromonas gingivalis* and *Micromonas micros,* whilst *E. corrodens* was associated with peri-implant mucositis.[117]

In 2010 KOYANAGI et al. described a more diverse composition of bacteria in peri-implantitis in comparison to healthy implants and periodontitis, consisting mainly of gram-negative species. They detected *Chloroflexi, Ternericutes, Synergistes phyla*, *Parvimonas micra, Pseudoramibacter alactolyticus, Solobacterium moorei* and *Peptostreptococcus stomatis*. Also, they found *Fusobacterium nucleatum* and

Granulicatella adiacens in periodontitis and peri-implantitis sites but not healthy ones.[74]

PERSSON and RENVERT identified in 2013 nineteen different species that showed higher counts in the presence of peri-implantitis. These were *Actinomyces odontolyticus, Actinobacillus Actinomycetemcomitans, Campylobacter gracilis, Campilobacter rectus, Campylobacter showae, Helicobacter pylori, Haemophilus influenza, Leptothrichia buccalis, Porphyromonas intermedia, Propionybacterium acnes, Porphyromonas endodontalis, Porphyromonas gingivalis, Staphylococcus aureus, Staphylococcus anaerobius, Streptococcus intermedius, Streptococcus mitis, Tanerella forsythia, Tanerella denticola, Treponema socranskii*. Of these nineteen species they confirmed the association in between peri-implantitis and the presence of the following seven bacterial species: *Tanerella forsythia, Porphyromonas gingivalis, Treponema socranskii, Staphylococcus aureus, Staphylococcus anaerobius, Streptococcus intermedius* and *Streptococcus mitis*.[108]

In 2013 BELIBASAKIS et al. found the peri-implant microbiome to be very similar to the periodontal microbiome with a higher presence of enteric bacteria and staphylococci. In 2014 BELIBASAKIS described the healthy peri-implant microflora as consisting mainly of non-motile bacilli, gram-positive cocci and a few gram-negative anaerobic species. In peri-implantitis on the other hand he found more gram-negative, motile and anaerobic species, equivalent to periodontitis, with a higher count of *Porphyromonas gingivalis, Tanerella forsythia* and *Tanerella denticola*. Additionally some microorganisms uncommon in periodontitis could be identified, such as *Enterobacter aerogenes, Enterobacter cloace, Escherichia coli, Helicobacter pylori, Peptostreptococcus micra, Pseudomonas spp., Staphylococcus aureus*, *Staphylococcus epidermidis, and Candida spp. fungi.*[15]

In 2017, APATZIDOU et al. found the microbiome of healthy peri-implant sites to be much more diverse than diseased ones, similar to teeth. They associated a high quantity of *Actinomyces* and *Streptococci* with healthy sites and *Prevotella* and *Porphyromonas* with peri-implantitis.[12]

3.2.3 ***Staphylococcus aureus***

For these experiments there was a need for good biofilm forming properties. *Staphylococcus aureus* was chosen due to the germ being identified in peri-implant tissues,[15, 97, 108, 117] whilst being aerobe and therefore less complicated to work with in laboratory conditions than anaerobe organisms, as well as good experience in biofilm behaviour within our working group. The used strain has good biofilm forming properties, is aerobe and relatively harmless and safe to handle, whilst still being a human pathogen.

The name originates from “staphyle”, the Greek word for grape and describes the typical colony shape, due to growth and division into all directions in combination with being non-motile.[58] The second part of the name “coccus” is Latin for berry and describes the shape of the bacterium while the last part of the name “aureus” is Latin for golden and describes the often found yellow to orange tint of the otherwise gray/ white-gray colony colours.[14]

The Taxonomy of *S. aureus*:[14]

Superkingdom: *Bacteria*
↳Superphylum: *Terrabacteria group*
↳Phylum: *Firmicutes*
↳Class: *Bacilli*
↳Order: *Bacillales*
↳Family: *Staphylococcaceae*
↳Genus: *Staphylococcus*
↳Species: *Staphylococcus aureus*

S. aureus was discovered in 1878 by Robert Koch and is a non-motile, non spore-forming, facultative anaerobe, gram-positive spherical bacterium with a diameter of ca. 1 µm.[58, 67] The germ is on 20-35% of humans a permanent part of the microbial flora of skin and mucous membranes, as well as in another 30% transiently.[14] The preferred location is the nasal vestibule[14] but they are also found on the pharynx, armpits, perineum[14] and less commonly colon, rectum and vagina. Transmission occurs mainly through smear infection (e.g. hand contact), droplet infection and dust.[57]

Due to the expression of the intercellular adhesin Poly-N-Acetylglucosamine the germ is able to form biofilms, in which the influences of immune cells like macrophages are

reduced. In some strains this defence mechanism is further enhanced by the formation of a capsule.[58, 67]

Staphylococcus aureus expresses the extracellular protein coagulase, which binds to prothrombin in blood serum. The prothrombin-coagulase complex is proteolytic and induces the formation of fibrin from fibrinogen. This coagulase positivity is a great distinguishing factor, as most other staphylococci are coagulase negative, and *S. aureus* is almost the only coagulase positive staphylococcus that is important as a humane pathogen.[58, 67] If they are found on humans, they are most often transmitted through animals (pets), where they are more commonly found (e.g. *S. pseudointermedius*, *S. intermedius* and *S. schleiferi*).[57]

The coagulase effect is further enhanced by the expression of a clumping factor, a cell membrane bound receptor which promotes a conjunction between bacterium and fibrinogen.[58, 67]

When stored on blood agar, typical clear haemolytic zones will form around the colonies. This phenomenon happens due to the expression of haemolysin. In *S. aureus* four different types can be found: α-, β-, γ- and δ- haemolysin.[58, 67]

In dentistry *Staphylococcus aureus* is most relevant as a pathogen in causing oral abscesses and general abscesses in the head region. The abscesses develop most often in consequence of local tissue defects or general immune deficiency.[58] Also, as mentioned above, the germ was found around implants with peri-implantitis.[15, 117]

To the public eye *S. aureus* is most commonly known because of the emergence of MRSA: methicillin resistant *Staphylococcus aureus*.[14] Nowadays the abbreviation is also commonly used for multi-resistant *S. aureus*. These strains play a significant role in hospital-acquired infections.[14]

3.3 Microgap, micromotion and microleakage

Due to the limitations in precision in production, a microscopic gap in between abutment and implant, the so called "microgap", is unavoidable.[83, 94, 123, 124, 142, 157] The size of the microgap varies strongly depending on material of implant and abutment, type of connection and application of the recommended torque.[37, 83,124] There have been very different measurements for the microgap, ranging in the extremes from of 0.1 µm to 100 µm, most often in between 1 µm and 50 µm.[83, 127, 37] During mastication and therefore loading of the implant system, the abutment slightly moves within the implant, which may widen the microgap, enabling easier passage of bacteria and their toxins into and out of the implant-abutment interface into the surrounding tissue.[37, 55, 83, 127] This motion is called "micromotion" and the passage of bacteria into and out of the implants is called "microleakage" of the implant-abutment interface.[83] Microleakage might be enhanced by a so called "pumping effect", which describes the micromotion of the opening and closing microgap, causing more bacterial movement in between internal cavity of the implant and the implant-abutment interface surrounding tissues.[37, 124, 127, 142] The internal cavity of the implant may function as a bacterial reservoir, which might be a risk for prolonged and enhanced tissue inflammation.[37, 75, 94, 111, 118, 123, 124, 157]

Multiple studies have shown bacterial leakage through the implant-abutment interface.[36, 37, 53, 94, 96, 127, 130, 142, 157] For example BROGGINI et al. showed in a study in 2003 on foxhounds a higher bacterial count and bone degeneration surrounding two piece implant systems in comparison to one piece implant systems, which do not have an implant-abutment interface with possible microleakage from the microgap.[26] In a Follow-up study in 2006 BROGGINI et al. further elaborated these findings by demonstrating that the highest count of neutrophils, a marker of inflammation, could be found surrounding the implant-abutment interface, independent of position.[25]

Furthermore, studies have shown that the location of the implant-abutment interface in relation to bone is an important factor in regards to bone loss.[36, 75, 124, 130, 145] The further away the connection is located from crestal bone, the less bone loss was observed.[36, 124]

To accomplish high aesthetics, a more apical implant placement closer to the bone is required, especially in the front, to avoid visibility of the metallic implant through the gingiva. Thus the location of implant-abutment interface cannot be placed in the desired location for minimal inflammatory reaction. Microleakage may enhance bacterial contamination surrounding the implant abutment interface.[53, 75, 127] An inflammatory reaction surrounding an implant which affects crestal bone is the definition of the previously described peri-implantitis (see chapter 3.2.1).[124] The inflammatory reaction to bacteria and their toxins induces bone degeneration,[37] which in turn leads to loosening of the implant, in the worst case to implant failure.[36, 96, 118]

3.4 Antibacterial treatments of the implant-abutment interface

In multiple studies different materials were tested for implant-abutment interface sealing and antibacterial treatment, in an attempt to overcome bacterial leakage from the implant-abutment interface into the surrounding tissue, especially when placed in close proximity to the crestal bone (see chapter 3.3).

In general, investigated materials for antibacterial treatments, such as rinses, are most often on the basis of chlorhexidine[18, 28, 34, 39, 54, 113, 114, 132]. Examined materials for mechanical sealing - or in case of antibacterial modification mechanical and antibacterial sealing - against bacteria, were most often modified or pure silicones[39, 49, 50, 51, 61, 77, 94, 99, 106, 112-114].

The first published in vivo study regarding the topic of prevention of bacterial colonisation of access areas to the implant-abutment interface was realized by MCCARTHY and GUCKES 1993 with temporary cement as sealing material (Temp-Bond NE and Opotow) of the screw access canal of the abutment. Reason for the study was the commonly occurring bad odour and taste arising from the canal. The seal helped with both – odour and taste. The study did not investigate bacterial penetration any further.[91]

Studies regarding sealing of the implant-abutment interface itself started with an in vivo study by PATYK et al. 1997, where acetate linked silicone was investigated as a sealing material in regards to preventing plaque accumulation in implant system cavities, aging process and stability in an oral environment. They sealed in 464 implant systems the implant-abutment interface, as well as abutment-superstructure interface. The seal was removed and renewed after three months, a year and three years and examined with a reflected-light microscope with a magnification of 50:1, additionally in case of the margins with a scanning electron microscope. Over time the silicone displayed signs of aging at the margins, such as staining and after three years some plaque retention. They concluded a good sealing ability and resistance to an oral environment but recommended an annual renewal.[106]

In an in vitro study by JANSEN et al. 1997 thirteen different implant systems were examined and compared in their capability to stop bacteria from leaking out of the implant cavities. The internal cavities and screws were inoculated with *Escherichia coli* and the penetration into surrounding brain-heart solution noted. One of the examined systems (Frialit-2) was equipped with a silicone washer, and was the only system which stopped leakage on the first day. The conclusion of the experiment was an inability of the existing implant systems to prevent bacterial leakage, but the silicone washer being a step in the right direction, since there was a reduction and delay in leakage in comparison to the other systems.[61]

1999 BESIMO et al. researched chlorhexidine, the to this day most frequently tested material for disinfecting and/or sealing the interface, in an in vitro study. They used a chlorhexidine varnish (Cervitec) to seal the implant-abutment interface, as well as abutment-crown (prefabricated) interface. Leakage from outside of the specimens (immersed in *Staphylococcus aureus* solution, totally and in a second series partially – no immersion of the transversal screw holding the prefabricated crown onto the abutment) into the internal cavities was tested after three to eight weeks of immersion by collecting bacteria with paper points on the inside cavities after outer surface sterilisation with ethanol for three minutes and careful disassembly. The collected bacteria were streaked on blood agar plates and incubated in tryptic soy broth, followed by a monitoring for bacterial growth. Leakage from the insides of implant systems to the outside was measured by inoculating in the inside of the internal hexagon and the upper cavity of the abutment with *Staphylococcus aureus* and then immersing the samples after assembly in sterile tryptic soy broth. Bacterial growth or lack thereof was recorded. In case of leakage into the implant system, the results showed no bacterial leakage after three to eleven weeks of partial immersion, whilst in case of total immersion one of five samples displayed a leak after four weeks. In case of leakage from the samples there was no leakage detected after one week, but neither were viable bacteria in the implants inside.[18]

In 2001 RIMONDINI et al. tested a seal with a silicone washer device in vivo. They sealed eight implants with a silicone washer and compared bacterial contamination of the connecting screw after two months to those of nine unsealed implants. On two of eight sealed implant screws bacterial contamination was found, in comparison to the higher rate of seven contaminations out of nine unsealed implants. Therefore the

conclusion was: the silicone washer was able to reduce but not prevent bacterial contamination.[118]

A 0.2% chlorhexidine rinse (Corsodyl) was used as a disinfectant in 2003 by GROENENDIJK et al. in a double-blinded split-mouth in vivo study, in which saline served as a control. Each group consisted of 23 implants. At baseline-measurement (before rinse) 46% of the implants were contaminated with viable bacteria. After rinse with either saline or 0.2% chlorhexidine and six weeks passing the second measurement showed 87% of the implants being contaminated, with the saline group having significantly higher counts of bacteria than the chlorhexidine group.[54]

2005 PROFF et al. concluded gutta percha to be an unsuitable sealing material, after sealing six implants with gutta percha and comparing them to six unsealed implants in regard to bacterial penetration into the interior spaces of the implants in vitro. Measurements all resulted in bacterial contamination and occurred after 24 and 72 hours of storage in a solution of thioglycolate boullion with haemin-menadione and *Porphyromonas gingivalis*.[115]

2005 BUZELLO et al. rinsed 48 implants in vivo first with saline as a reference and then with 0.1% chlorhexidine and 3% H_2O_2. Bacterial contamination was measured: 0.1% led to reduction of bacterial contamination (after 38.9 ± 4.7 days); H_2O_2 did not influence the contamination (after 37.8 ± 6.9 days).[28]

DUARTE et al. published 2006 an in vivo study in which they sealed 60 implant-abutment interfaces with a silicone (Dow Silastic) mixed with 1% chlorhexidine-thymol-varnish (Cervitec). After sealing, the implants were immersed and incubated in an *Enterococcus faecalis* suspension for 7, 14, 21, 35, 49 and 63 days. After incubation the internal contamination was measured. The varnish was not able to inhibit bacterial penetration.[39]

In 2007 and 2008 FRITZEMEIER et al. published an in vivo split-mouth study conducted from 1996 to 2000, where they compared the number of cases of peri-implantitis in 82 implants sealed with a petrolatum to 85 implants sealed with a thymol-silicone (GapSeal®). The results showed a reduction in both groups at the first measurement after six months, with the cases of peri-implantitis slowly increasing over the years in the group sealed with petrolatum, whilst the group sealed with GapSeal® displayed a continuous reduction.[50, 51]

D'ERCOLE et al. displayed in 2009 in an in vivo study the effect of 1% chlorhexidine gel on bacterial contamination of the implant-abutment interface of 15 implants in comparison to a negative control (10 implants). The result was less contamination in the chlorhexidine gel group in comparison to the empty negative controls, where there was an increase in contamination over time.[34]

In an in vitro study in 2014 NAYAK et al. sealed 15 implants with a silicone O-ring, 15 with a thymol-silicone (GapSeal®) and left another 15 unsealed as reference. All three variations showed contamination after incubation in an *Enterococcus* solution for five days, most in the unsealed reference, a little less in the silicone O-ring group and the least in the thymol-silicone group.[99]

2014 PIMENTEL et al. published an in vivo study, in which they sealed 15 implant-abutment interfaces with a silicone membrane (Medgel silicone gel) for 30 and 90 days and respectively screened for the internal presence of bacteria. The bacterial penetration was reduced in comparison to no seal.[112]

In 2015 MATEESCU et al. investigated the use of two different hydrogels containing cateslytin as an antibacterial substance for implant-abutment interface sealing in vitro. They assessed bacterial viability (*Porphyromonas gingivalis*) after treatment with the examined agent by analysing the ability of found bacteria to reduce resazurin to resorufin under the assumption that only viable bacteria would be able to do so. The result was a reduced viability after treatment with the hydrogels. Furthermore, they tested the hydrogels' ability to influence colony formation, with the result of inhibition of colony formation.[90]

PODHORSKY et al. treated 2016 two different implant systems in vitro with three different materials: silicone (KieroSeal), chlorhexidine (Chlorhexamed) and grease (Berutemp) (per system: 10 implants per material). The bacterial penetration into the implants was determined and compared to 10 untreated references: first after incubation for one week in an *Escherichia coli* suspension, then after thermocycling (5000 cycles) followed by the same incubation. The result was a reduction in bacteria with all three treatment types, especially after thermocycling.[113]

A similar set up was used in a second study 2016 by PODHORSKY et al., with the only difference being, that instead of thermocycling, a chewing simulation (240000

cycles of 120N/12N) was conducted. The results were reductions in bacterial counts with all pre-treatments.[114]

In 2016 FERREIRA et al. used a silicone (KieroSeal) to seal the implant-abutment interface of 20 implants and compared leakage to 20 unsealed implants in vitro. *Escherichia coli* suspension in sterile saline was placed in the inner cavity of the implant systems, which were sealed or just assembled and then placed in medium. Contamination of the medium (turbidity), deriving from the inner cavity of the implants, was measured after 24 hours, 48 hours, 5 days, 7 days and 14 days. They found no contamination in the sealed group on the first day, but 25% in the unsealed group. On the second day, the sealed group showed 5% contamination, which was also the case in all the subsequent measurements. The unsealed group on the other hand displayed 40% contamination, which increased on the fifth day to 50%, a level the group remained at for the subsequent measurements. They concluded the seal reduced bacterial contamination.[49]

2017 LAURITIANO et al. demonstrated in an in vitro study the effect of a coating with an antimicrobial silicone (antimicrobial through functionalization with chlorhexidine digluconate) of 20 implants' internal chambers, in comparison to non-coated implant systems. Half of the implants were used to investigate bacterial penetration from the outside to the inside: The assembled – and in case of test group sealed – implant systems, filled with sterile medium, were placed in a medium inoculated with *Tannerella forsythia* and *Porphyromonas gingivalis* and incubated for 48 hours. Afterwards contamination of the internal medium was measured. The other half of implant systems was used to measure leakage from the inside to the outside: a solution containing *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Enterococcus hirae* and *candida albicans* was placed in the internal cavity before assembly – and in case of test group sealing – and then the system was incubated in sterile medium surrounding the implants. Contamination of external medium was measured after 15 minutes. In both cases bacterial contamination was prevented.[77]

3.5 Silicones

Silicone was chosen as a matrix material for this experiment, due to low viscosity silicones already being used as a sealing material in the implant-abutment interface. Studies have shown silicones may reduce the amount of bacteria penetrating the inner lumen, though not eliminating them completely.[113, 114]
Advantages of (A-)silicones as a sealing materials are retrievability, low shrinkage, dimension stability, little interaction with the water-based surroundings due to their hydrophobic nature, biological inertness and the absence of odour.[149]

Silicones in general are silicon-oxygen-chains (-Si-O-Si-O-Si-) with organic groups, for example methyl, on free bonding sites of the silicon atoms (see fig. 3).[27] Depending on the organic group, cross-linking level and chain length, silicone may be in a liquid or rubbery form.[27] Silicones are hydrophobic[48, 92, 101, 149], causing a good miscibility with other hydrophobic materials, but a bad one with hydrophilic materials, as long as there are no amphiphilic components involved.

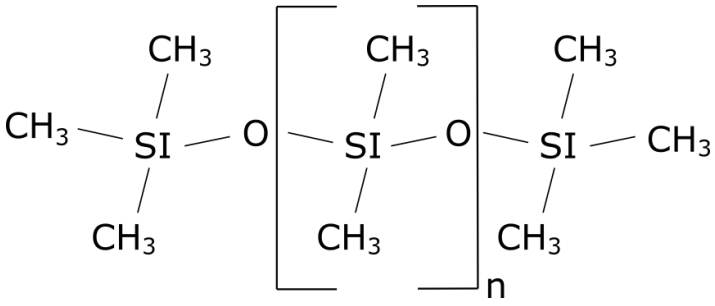


fig. 3) General chemical structure of silicones

Two types of silicone curing modes are commonly used in dentistry: addition curing and condensation curing, with addition curing being the more modern and more commonly used curing mode.

3.5.1 **Addition curing silicones**

Addition curing silicones (Synonyms: A-silicones; vinyl polysiloxanes) are quite common in dentistry, predominantly used as impression materials, and are the form of setting type used in this study.

A-silicones are sold as paste-paste compositions. They consist of a base paste and a catalyst paste, which are mixed together to initiate curing. Simplified, they are polydimethylsiloxane chains with some of the methyl groups being replaced by vinyl groups (in the base and catalyst paste) or hydrogen groups (in the base paste). When both pastes are mixed, a platinum component (commonly used catalyst in dental A-silicones) in the catalyst paste initiates the setting reaction: a polymerisation and cross-linking without formation of volatile components (e.g. ethanol; in contrast to condensation curing silicones; see fig. 4) resulting in low shrinkage (less than 0.05% linear).[149] This generates the desired characteristic of a lasting dimensional stability.[149]

Viscosity, elasticity, and hardness depend on the chain length and amount of vinyl groups. Shorter chains may “move” more easily within the silicone before setting and result in a lower viscosity and a denser network when set, thus more rigidity. Longer chains are not as mobile resulting in a higher viscosity and after setting a wider network with a higher flexibility. Another factor influencing consistency after setting is the cross-linking rate.[11]

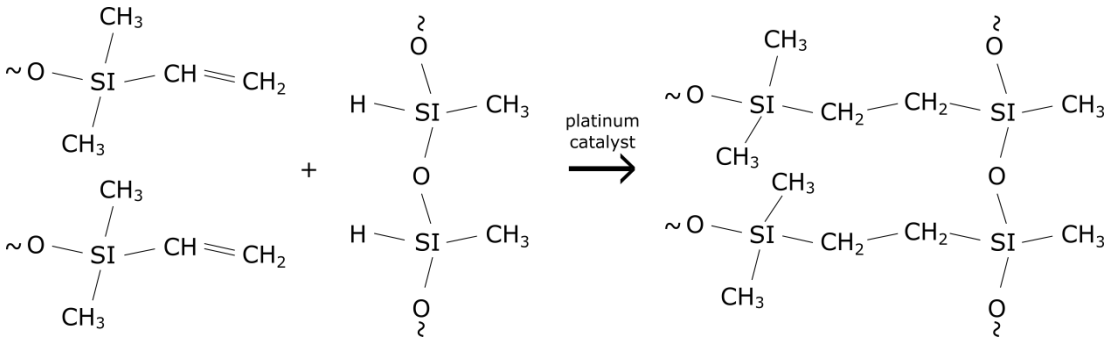


fig. 4) A-silicone, simplified setting reaction

3.5.2 **Condensation curing silicones**

Condensation curing dental silicones consist of two components which are mixed together to initiate curing: base paste and catalyst fluid or paste. Consistency is determined by the base paste. Essentially, the base consists of polydimethylsiloxane chains ending with hydroxy groups, and the catalyst paste or fluid of alkyl silicates for crosslinking and a tin component for catalytic activity. Curing creates volatile ethanol (see fig. 5), causing shrinkage and a lack of long-term dimensional stability.[149]

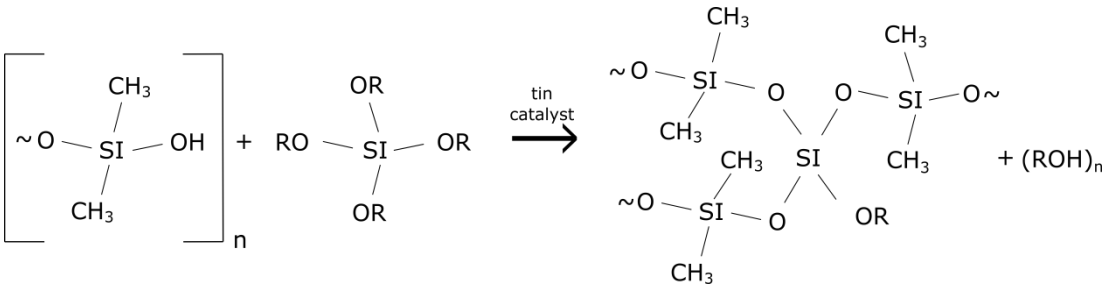


fig. 5) C-silicone, simplified setting reaction

3.6 Metal oxides and metal salts as antibacterial agents

With the rise of resistances in bacteria against antibiotics, metal oxides as antibacterial agents have come into focus once again as an alternative to antibacterial treatments. In particular silver, copper, and zinc are studied in different forms and complexes, along with other metals and metal salts, have been investigated in the past few years.[63] Nanoparticles generally seem to have better antibacterial properties than their macroscopic counterparts due to their higher surface area,[79] but also carry a higher risk to harm the surrounding tissues, especially if the size falls below a critical level. The threshold which separates great antibacterial properties from the increased risks for the host differs for the metals.

3.6.1 Zinc oxide

Zinc oxide is the oxidised form of the metal zinc and comes in the form of an odourless, white to yellowish tinted powder.

Especially nano zinc oxide is known to have antibacterial properties[8, 64, 82, 128, 151] and zinc oxide in general is used in pharmaceutical and medical products (e.g. wound dressings).[128] In dentistry zinc oxide powder is commonly used, for example in multiple forms of cements: zinc phosphate cement, zinc polycarboxylate cement, zinc oxide-eugenol cement[35, 47, 126] and additionally as a common additive, e.g. in alginates[125] and toothpastes[8, 110]. Additionally, there have been multiple experimentations with nano-zinc oxide as an antibacterial additive in composites[8, 13, 68, 129, 135], glass ionomer cements[20, 129, 138], temporary cements[9] and adhesives[156]. Outside of dentistry zinc oxide can be found in dermatological and cosmetic products,[103, 128] sunscreens[8, 98], and topical pastes with multiple indications (eczema, ulcers[80], warts, dermatitis etc.)[56] - due to the photoprotective (UV filter)[56, 80, 98], soothing and antipruritic effects[56], as well as the ability to supply zinc over a longer period of time to enhance wound healing[80, 128] and in case of necrotic wounds, increase collagen degradation[80].

The mechanisms of zinc oxide effecting bacteria are not fully known to this day. But damage to bacterial cell membrane[22, 64, 82, 128, 154] with increase in permeability[22] was shown with the effect of inhibited growth and reproduction after zinc oxide accumulation[22] in the bacterial cells.[128] In particular the integrity of the cell membrane is shown to be in disarray, the expression of oxidative-stress resistance genes down regulated – increasing the cells' susceptibility to oxidative stress – and the cell surface hydrophobicity reduced.[105] Also apoptosis may be induced[128] and biofilm formation disrupted[105]. The mechanisms of these antibacterial effects are either a permeation of zinc oxide into the cell with a direct binding of zinc ions to biomolecules (e.g. proteins) rendering them inactive,[128] or zinc oxide generating reactive oxygen species[120, 121, 151] within the cells which in turn damage lipids[69], DNA[69], proteins[69] and carbohydrates[69].[128] Also the antibacterial potential depends on particle size[3, 151] and might be activated/enhanced by the presence of light[3, 64, 151], as well as influenced by but not dependent on oxygen[139].

In 2012 zinc oxide was shown by VARGAS-REUS et al. to have antimicrobial properties against four peri-implant pathogens: *Prevotella intermedia*, *Porphyromonas gingivalis*, *Fusobacterium nucleatum* and *Aggregatibacter actinomycetemcomitans* under anaerobic circumstances. They found a minimal inhibitory concentration, as well as a minimum bactericidal concentration for zinc oxide of 250-1000 µg/ml, depending on the pathogen.[139]

3.6.2 **Copper naphthenate**

Copper naphthenate is a metallic salt of naphthenic acids containing copper. Other names for the substance are naphthenic acids and copper salt. Copper naphthenate is retrieved as a by-product from oil-refineries, more specific from petroleum distillates and consists of cycloaliphatic carboxylic acids (see fig. 6).[23, 107] It presents as a dark green viscous liquid with a strong turpentine odour. As for use in dentistry: copper salts used to be, and still are in some cases up to this day, part of starter systems, where they act as catalysts activators.[40] Also it is used as a catalyst in Polysulphides.[17] Outside of dentistry, copper naphthenate is used as an antimicrobial agent, for example in wood preservatives[93, 107, 146], outdoor textiles[107, 150] paper and

cardboard[107]. Copper naphthenate is insoluble in water, but easily soluble in organic solvents and petroleum products.[93, 107] This should enhance the solubility and movement of the copper salt within silicone and enable a more homogenous mixture with a more frequent exchange of the surface particles for a long term release of copper ions.

Copper is known to have antibacterial and antifungal properties.[46, 60, 103, 141, 150] There is proof of copper being used for its antimicrobial properties as early as 2600-2200 B.C..[52] In small portions it is harmless for humans, even necessary as it is an essential trace element,[46, 144, 153] necessary for more than thirty copper containing proteins.[52] A normal diet contains on average 2 to 5 mg copper per day.[27]

The antibacterial effect of copper seems to be similar to that of zinc, but the mechanisms are not completely clarified yet. In the same fashion as for zinc oxide more than one mechanism of antibacterial activity was found: Copper seems to either directly induce damages in the bacterial cell membrane leading to their death, comprise cellular respiration or cause DNA degeneration.[46, 141] Most studies conducted on this subject found the formation of reactive oxygen species to be the primary cause of cell deaths as they damage lipids, proteins and DNA.[10, 46, 52, 86] Alternative discussed mechanisms were a destruction of the cell membrane ("contact killing")[52, 86] with a to this day not fully known mechanism, followed by the damage of the oxidative stress, or in a first step the oxidative stress, followed by inhibition of cell respiration and DNA damage.[52, 144] Another primary mechanism in antibacterial properties was found by MACOMBER and IMLAY in 2009: copper competing for important protein binding sites against other essential metal ions, more specifically competing against iron on iron-sulphur clusters on enzymes of the catabolic and biosynthetic pathways.[85]

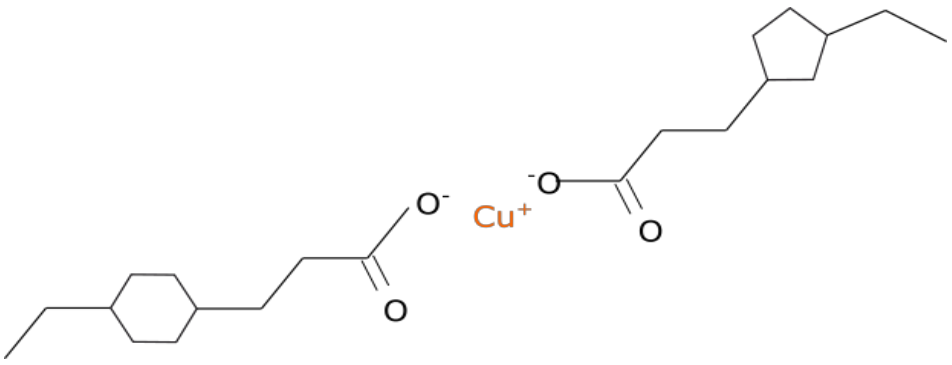


fig. 6) Copper naphthenate, exemplary chemical structure

3.6.3 **Silver 2-ethylhexanoate**

Silver 2-ethylhexanoate is an odourless, white to off-white metal salt. The molecular formula is $C_8H_{15}AgO_2$ (see fig. 7). In case of mechanical stress the powder's colour turns from white to brown. Silver is known for outstanding antibacterial properties, especially in direct contact[150] and the hexanoate form was chosen to enable the agent's movement within the silicone matrix.

Silver and silver salts have been known to have antibacterial properties for a long time.[71, 103] They have been used as antiseptic compounds for example in catheters[33, 84], ceramics[84], cosmetics[84], surgical devices[84] and wound dressings[33, 84, 103]. In dentistry silver has been and still is a compound of amalgam[133] and there are silver and silver salts containing materials, such as glass ionomer cements[133]. There have been multiple studies with materials modified with different forms of silver for antimicrobial purposes, such as endodontic irrigants[5, 137], endodontic sealers[140], composites[29, 68, 81], prosthetic resin[2, 87], adhesives[62, 73] and titanium coatings in implantology[30, 38, 155].

Just like with zinc oxide and copper, the antibacterial mechanisms of silver are not fully understood yet. One of the biggest effects silver exhibits on bacteria seems to be the disruption of the cell membrane, causing cell death.[40, 71, 84] The thicker murein layer in gram-positive bacteria is theorized to act as a protective layer and slightly reduce the antibacterial effect of silver in comparison to the effect on gram-negative bacteria.[40] Another mechanism was seen through transmission electron microscopy: silver interacted with the DNA, hindering replication.[40] Also silver was shown to bind to enzymes/peptides, either competing against the native metal (e.g. copper) on binding sites, or changing them entirely, rendering them useless; as well as generating reactive oxygen species, leading to damage of DNA, RNA, lipids and proteins.[40, 81] The antibacterial efficiency, as well as toxicity depends on particle size and shape.[70] But in general silver is known for a wide antibacterial effect.[33, 70, 81]

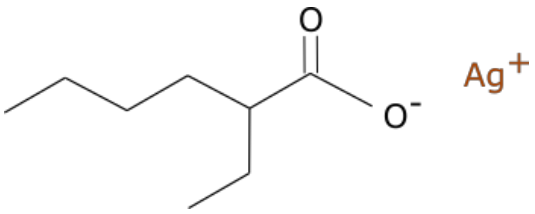


fig. 7) Silver 2-ethylhexanoate, chemical structure

3.7 Bacterial growth

3.7.1 Bacterial growth curve

In most bacterial species growth is achieved through binary fission. The time interval in between divisions varies, depending on multiple factors, such as species, medium and temperature. Bacterial proliferation in a culture is characterized by four distinct phases, describing the amount of living bacteria in the culture in a "bacterial growth curve".[66]

The first Phase is the "lag phase": a small amount of bacteria start growing in a new medium. There is no increase in numbers, bacteria are alive and synthesising the needed enzymes to survive in the new medium. The second phase is the "log phase", a phase of exponential proliferation (therefore "log" – deriving from logarithmic). The exponential growth is followed by the "stationary phase", characterized by a steady amount of bacteria, forming a plateau in diagrams. The last phase describes a decrease of the bacterial amount in the medium through the bacteria dying off, the so called "death phase".[66]

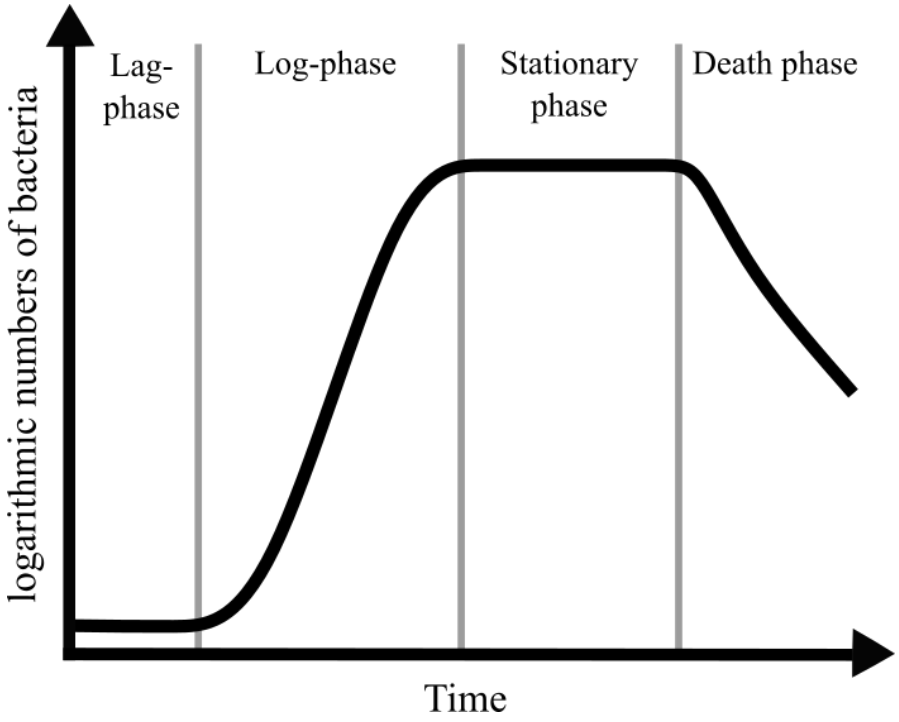


fig. 8) Phases of bacterial growth

3.7.2 **Measurement methods**

3.7.2.1 Agar diffusion test (filter paper disk)

Agar diffusion test with impregnated filter paper disks is an accepted method for testing bacteria's susceptibility to antibiotics.[65] Standardised filter paper discs are impregnated with antibiotic solutions and placed upon an agar plate, with a seeded bacterial lawn. The plate is incubated at 35°C ± 2°C for 18-24 hours. Then the plate is examined for formation of so called "inhibition zones" surrounding the disks, where the bacterial growth was inhibited and therefore no lawn formed. The diameter of inhibition zone is measured with a ruler. In theory, the bigger the inhibition is, the higher the germ's susceptibility to the tested antibiotic. Some factors (e.g. solubility in agar) may falsify this theory.

3.7.2.2 Spectrophotometry

Spectrophotometry is a common tool in microbiology. The optical density (= OD) of a liquid – and change herein – is measured for bacterial growth most commonly at a wavelength of 600 nm.

In general a spectrophotometer shines a light – most often either in the visible or ultraviolet spectrum (UV-vis spectrophotometry) – through a substance and measures the amount of light transmitted to the other side.[116] The transmitted light reaching the photometer is compared to the emitted light from the spectrometer.[116] The more particles – or bacteria – are within the substance, the more light will be absorbed or scattered, and therefore not reach the photodetector on the other side of the examined substance. The unit for the amount of light not reaching the measuring tool on the other side of the sample is "absorbance". In chemistry the light is usually absorbed by the molecules within the examined substance. In case of measurement of bacterial growth in microbiology, it is the bacteria within the liquid medium mainly scattering the light.

To eliminate the influence of the optical density of the holding device (medium), and just measure the desired matter (bacteria), the measurements are conducted against a reference, e.g. pure medium. The reference will be subtracted from the measured density, referred to as "blank".

In case of *S. aureus* an optical density of 1,000 A (A = absorbance), at 600 nm, indicates reaching the logarithmic phase in bacterial growth.

3.7.2.3 Microtiter plate biofilm assay

The microtiter plate biofilm assay in a 96-well plate was one of the first standardised methods for studying early stages of biofilm formation: the attachment of microbes to a surface.[95, 109] Depending on chosen incubation time either the initial attachment of bacteria to a surface (incubation of 1-2 hours) or formation of biofilms (incubation of 20-24 hours) may be measured.[109] The basic protocol consists in summary of the following steps:[95, 102, 109]

Step 1: A bacterial culture is grown either directly in the desired microtiter plates or first grown in a separate culture and then diluted (most commonly 1:100) before transfer into the desired microtiter plate (commonly 96-well).

Step 2: Incubation of the plate with the bacterial culture for the desired period of time.

Step 3: Removal of planktonic bacteria (together with medium) from the microtiter plate and wash of biofilm in wells with deionised water and optional further washing steps.

Step 4: Staining of the biofilm with 0.1% crystal violet solution.

Step 5: Removal of excess dye not bound to biofilm through washing with deionised water.

Step 6: Biofilm discolouration by addition of a suitable solvent such as 96% ethanol or 30% acetic acid.

Step 7: Measuring the optical density of solvent after discolouration process in a new flat-bottomed microtiter dish (96-well) at a wavelength in between 500-600 nm (depending on plate reader).

The theory behind this measurement is: the higher the absorbance, the higher the biofilm formation, since more colour is bound to the biofilm, which in turn dissolves in the measured solvent. Through the washing processes, the planktonic bacteria not bound in a biofilm are eliminated.

4 Materials and methods

Experimental outline

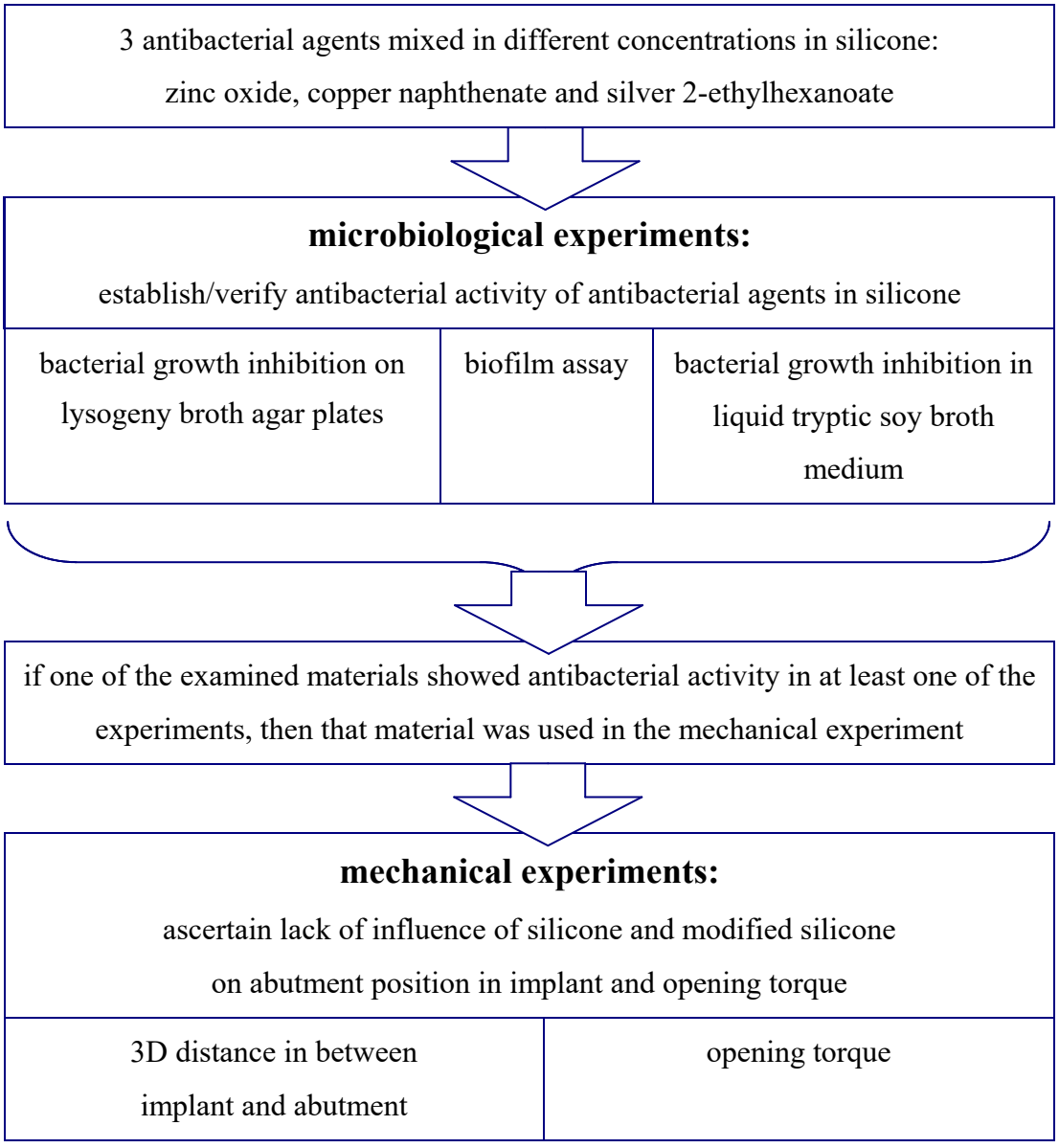


fig. 9) Experimental outline

4.1 Materials

4.1.1 Silicone matrix

4.1.1.1 IMPLANTO SEAL: P.L. Superior Dental Materials GmbH, Hamburg, Germany, Lot: 41701053. Low viscosity addition curing silicone, working time (at 23°C room temperature): 5 minutes ± 1 minute.

4.1.2 Antibacterial agents

4.1.2.1 Nano zinc oxide: PCM Products GmbH, Krefeld, Germany, Lot.: VA0809.

4.1.2.1 Copper naphthenate: Strem Chemicals, New Buryport, MA 01950, USA, Lot: 138537-S. Copper (II) naphthenate 77% in mineral spirits with 8% copper.

4.1.2.2 Silver 2-ethylhexanoate: Strem Chemicals, New Buryport, MA 01950, USA, Lot: A1918077.

4.1.3 Bacterium

4.1.3.1 *Staphylococcus aureus:* EDCC 5055,[88] culture collection from Prof. Dr. E. Domann, Institute of Medical Microbiology, Giessen, Germany. Stored at 5° C on columbia-agar plates with sheep's blood plus. (Available at Leibnitz Institute, DSMZ – German Collection of Microorganisms and Cell cultures GmbH, DSM number 28763)

4.1.4 Water (for dilutions and washing)

4.1.4.1 Purified water: Water was purified by using the Milli-Q® HR 7000 High-Throughput Central Water Purification System, Merck KGaA, 64293 Darmstadt, Germany.

4.1.5 **Culture media**

4.1.5.1 Tryptic Soy Broth: Merck KGaA, 64293 Darmstadt, Germany, Lot: VM754459644. Liquid medium, 30 grams of powder per litre purified water (Milli-Q®).

4.1.5.2 Columbia-agar, with sheep's blood plus: Thermo Fisher Scientific Inc., Oxoid Deutschland GmbH, 46483 Wesel, Germany, Ref: PB5039A. Agar plate, stored at 5°C.

Lysogeny broth agar plate:

1. **10g/L Bacto™ Tryptone:** Becton, Dickinson and Company, 38800 Le Pont du Claix, France, Lot: 7030910. Pancreatic digest of Casein.
2. **5g/L Bacto™ Yeast Extract:** BD Biosciences Advanced Bioprocessing, Miami, FL 33169, USA, Lot: 6295747. Extract of autolysed yeast cells.
3. **10g/L Sodium Chloride:** Carl Roth GmbH + Co. KG, 76185 Karlsruhe, Germany, Lot: 118267491.
4. **1-1.5% Bacto-Agar:** Becton, Dickinson and Company, 38800 Le Pont du Claix, France, Lot: 8057949.
5. **Purified water** (Milli-Q®).

4.1.6 **Biofilm stabiliser**

4.1.6.1 Buffer:

1. **2mM Calcium chloride dehydrate:** Carl Roth GmbH + Co. KG, 76185 Karlsruhe, Germany, Lot: 01521695
2. **2mM Magnesium chloride hexahydrate:** Merck KGaA, 64271 Darmstadt, Germany, Lot: TA179435 905.
3. **Purified Water** (Milli-Q®)

4.1.7 **Colouring agent**

4.1.7.1 Crystal violet (0.1%):

1. **0.1% Crystal Violet**: Sigma-Aldrich, Inc., St. Louis, MO, USA 63103; Sigma-Aldrich Chemie GmbH, 89555 Steinheim, Germany, CAS 548-62-9, Lot: C3886-25G.
2. **Purified Water** (Milli-Q®)

4.1.8 **Discolouring agent**

4.1.8.1 Ethanol (96%):

1. **96% Ethanol**: Sigma-Aldrich Chemie GmbH, 89555 Steinheim, Germany, Lot: SZBF0910V. Purity of 99.99%
2. **4% Distilled Water**

4.1.9 **Implant systems**

4.1.9.1 Xive® S Implant System: (see fig. 10, fig. 11) Dentsply Implants Manufacturing GmbH, 68229 Mannheim, Germany. The implant-abutment interface is an internal connection and supplies anti-rotation protection through an internal hexagon. Components:

1. **Xive® S Practice Implant:** Non sterile. Lot: 434329. The Implant has a threaded and tapered shape with a diameter of 3.8 mm and a length of 11 mm.
2. **Friadent® EstheticBase straight abutment:** Ref: 46-2142, Lot: B190006235. Diameter of 3.8 mm, gingival height of 2 mm, no angulations.
3. **Friadent® Screw for EstheticBase:** Ref: 46-4305, Lot: B190005138.

fig. 10) Xive® implant system

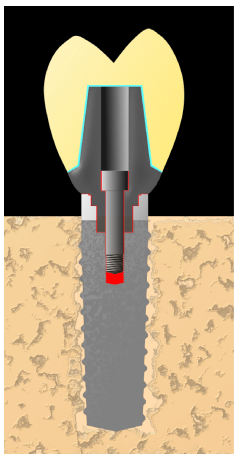


fig. 11) Simplified Xive® implant system

4.1.9.2 Straumann Bone Level Implant System: (see fig. 12 and fig. 13)

Institut Straumann AG, 4002 Basel, Switzerland. The implant-abutment interface consists of a conical connection ending in a hexagon for anti-rotation protection. Components:

1. **Straumann Bone Level Implant**: non sterile course material. Ref: 4200.0001V5, Lot: VJ246. The implant has a threaded, parallel shape with a diameter of 4.1 mm and a length of 10 mm.
2. **Regular Cross Fit® abutment for cemented restorations**: Ref: 022.4327, Lot: WW465. Emergence profile of 5 mm diameter, 3 mm gingival height, no angulations.
3. **Regular Cross Fit® Basal Screw for abutment for cemented restorations**: Ref: 025.4908, Lot: WG256.

fig. 12) Straumann implant system

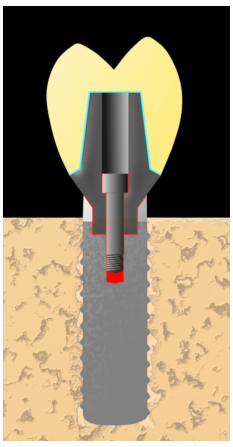


fig. 13) Simplified Straumann implant system

4.2 Microbiological methods

4.2.1 General conditions

The microbiological experiments were performed in the Institute of Medical Microbiology in the biomedical research centre Seltersberg (BFS) of the Justus-Liebig University, Giessen. The rooms were air conditioned at 23°C ± 2°C. Sterile working was enabled by usage of a sterile bench (MSC-Advantage, Thermo Scientific Inc., Waltham, MA 02454, USA) for every step where bacteria were handled or there were risks of contamination. Equipment was either dry sterilised for 3 h ± 1 h at 160°C ± 8°C or steam autoclaved at 134°C and 3 bar for five minutes.

4.2.2 Preparation

4.2.2.1 Specimen production:

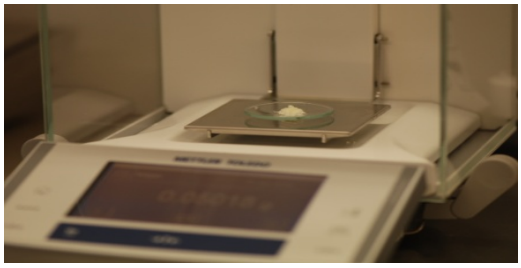


fig. 14) Weighing of antibacterial agent

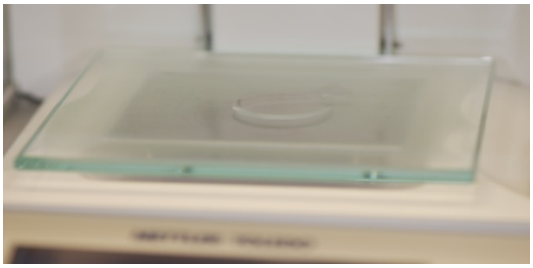


fig. 15) Weighing of silicone

The silicone (see chapter 4.1.1.1) and desired corresponding percentage by weight of antibacterial agent (see chapter 4.1.2) were weighed on a precision balance (Kern 770, KERN & SOHN GmbH, 72336 Balingen-Frommern, Germany) on an autoclaved glass mixing plate (see fig. 14, fig. 15).

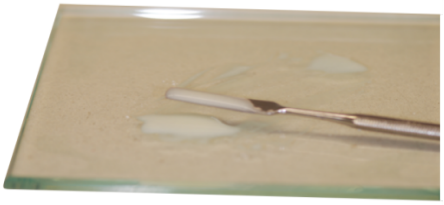


fig. 16) Mixing of the components

fig. 17) Stainless steel mould

After weighing, the glass plate was removed from the precision balance, and base and antibacterial agent were mixed. After achieving a homogenous mixture of the two components the catalyst was mixed in as well (see fig. 16). The mixture was filled into autoclaved, round, stainless steel moulds with a depth of 1 mm, and a diameter of 10 mm (see fig. 17). The moulds warranted a standardised volume and surface.

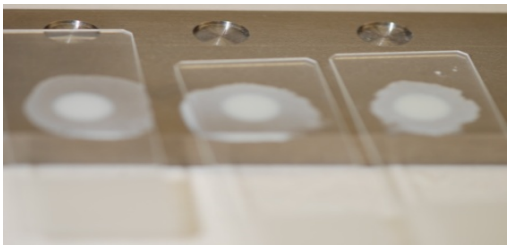


fig. 18) Openings covered with microscopic slides

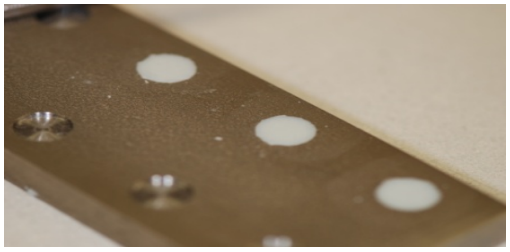


fig. 19) Specimens after excess removal

For standardisation and smoothness of the opening, it was covered with a sterilised microscopic slide (see fig.18). After setting, the excess was removed with a sterile scalpel (see fig. 19) and the specimens carefully transferred: either into a sterile petri dish for storage (see fig.20) until the experiment started (with a maximal storage time of one week) or transferred directly into the 12-well plates used for the experiments.

fig. 20) Finished specimens

The antibacterial agents affected the silicone very differently, so it was not possible to use the same weight percentages for all three antibacterial agents. While nano zinc oxide (see chapter 4.1.2.1) caused more rigidity with increasing amount, the other two materials lead to a softening of the mixture. On top of that, a higher percentage than 25% (per weight) of copper naphthenate (see chapter 4.1.2.2) and more than 10% (per weight) of silver 2-ethylhexanoate (see chapter 4.1.2.3) inhibited the setting reaction of the silicone. These circumstances lead to the following concentrations (per weight) of antibacterial agents:

Nano zinc oxide:	0.0%, 0.1%, 0.5%, 1.0%, 2.0%, 5.0%, 7.0%, 10.0%, 15.0%, 20.0%, 25.0%, 30.0%
Copper naphthenate:	0.0%, 0.1%, 0.5%, 1.0%, 2.0%, 5.0%, 7.0%, 10.0%, 15.0%, 20.0%, 25.0%
Silver 2-ethylhexanoate:	0.0%, 0.1%, 0.5%, 1.0%, 2.0%, 5.0%, 7.0%, 10.0%

In the inhibition zone experiment further concentrations from 2.5% to 10% with intervals of 0.5% were additionally prepared for silver 2-ethylhexanoate.

The following table shows a summary of test series per experiment and material:

tab. 1) Number of test series per material and experiment

Experiment / Material	**Agar diffusion Test**	**Biofilm assay:**	**Bacterial growth in liquid medium:**
Nano zinc oxide	3 test series	Regular: 3 test series Negative control: 3 test series	4 test series
Copper naphthenate	3 test series	Regular: 4 test series Negative control: 3 test series	4 test series
Silver 2-ethyl-hexanoate	Preliminary: 3 test series Follow-up: 3 test series	Regular: 3 test series Negative control: 4 test series	4 test series

4.2.2.2 Tryptic soy broth liquid medium

All three microbiological experiments required a liquid medium, at least for preparation of the bacterial culture. In this study, the used medium was tryptic soy broth (see chapter 4.1.5.1). For preparation of the medium, the recommended ratio of tryptic soy broth-powder and distilled water (= 15 g tryptic soy broth-powder to 500 ml aqua dest.) was mixed in a Schott-bottle and autoclaved for 15 min at 121°C and 3 bar (Tuttnauer Autoclave-Steam Sterilizer 2540EL N, Systec GmbH Labor-Systemtechnik, 35435 Wettenberg, Germany). After cooling down the medium was ready to use.

4.2.2.3 *S. aureus* overnight culture

In the afternoon before the first day of a microbiological experiment, overnight cultures of *S. aureus* (see chapter 4.1.3.1) were prepared: depending on the colony size, two to three colonies of *S. aureus* (see chapter 4.1.3.1) were taken of the blood agar plate (see chapter 4.1.5.2) via a sterile loop and dispensed in 20 ml tryptic soy broth medium (see chapter 4.1.5.1) in a 100 ml Erlenmeyer flask in sterile conditions (sterile working Bench, MSC-Advantage, Thermo Scientific Inc., Waltham, MA 02454, USA). The suspension was incubated (KS 4000 i control, IKA®-Werke GmbH & Co. KG, 79219 Staufen, Germany) for 18 h ± 45 min over night at 37°C while shaking with 180 rpm.

4.2.3 Agar diffusion test / bacterial growth from underneath specimens

Outline:

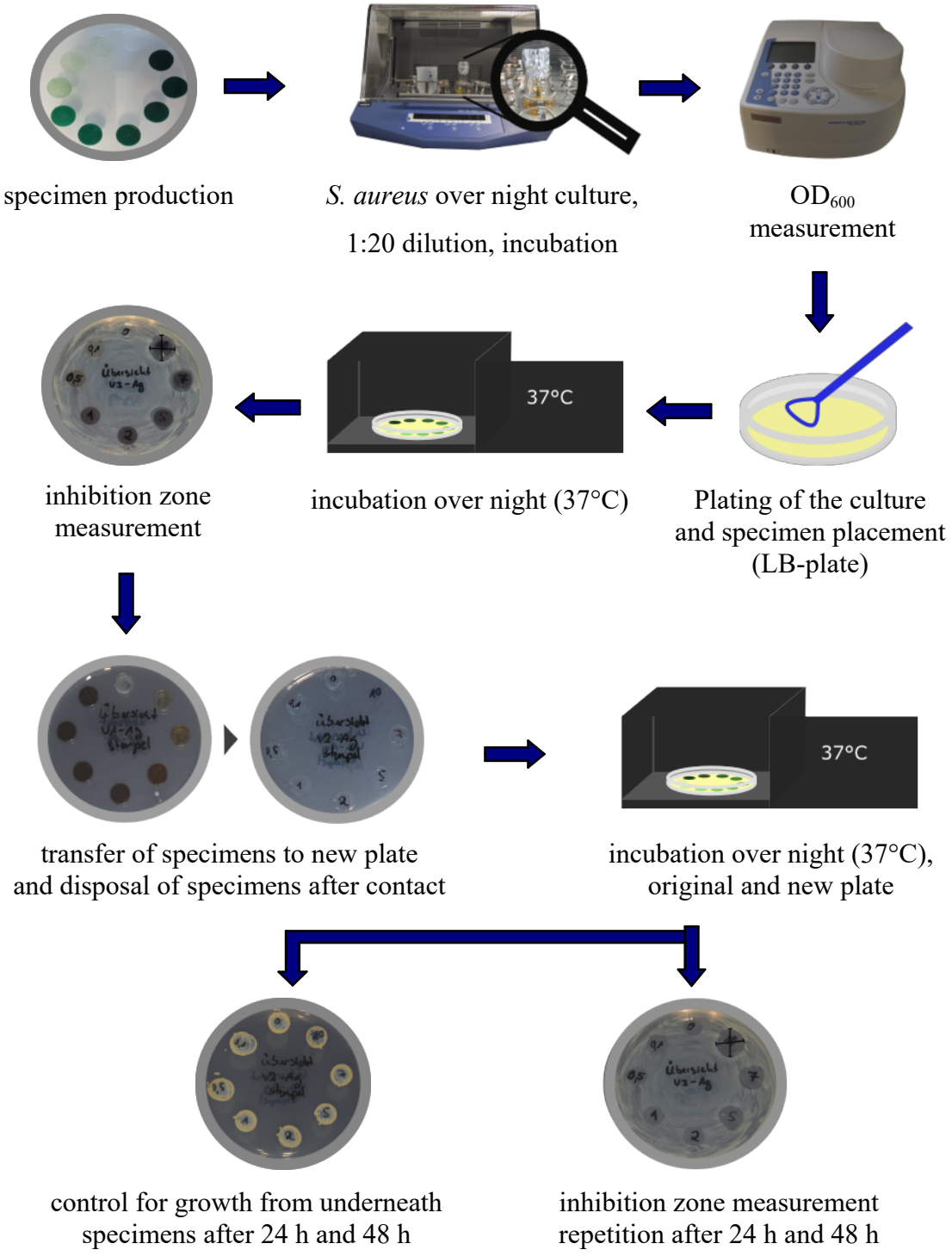


fig. 21) Outline experiment "agar diffusion test / bacterial growth inhibition on agar", (OD_{600} = optical density at 600 nm; LB = lysogeny broth)

Day one

As a first step, a 1:20 dilution of the *S. aureus* (see chapter 4.1.3.1) overnight culture was prepared in a 100 ml Erlenmeyer flask on a sterile working bench (MSC-Advantage, Thermo Scientific Inc., Waltham, MA 02454, USA) and further incubated at 37°C and 180 rpm until the logarithmic phase of bacterial growth was reached. This was verified by measuring the optical density at a wavelength of 600 nm in the program for cell growth in a spectrophotometer (Genesys 10S UV-Vis, Thermo Scientific, Thermo Fisher Scientific Inc., Waltham, MA 02454, USA). The logarithmic phase was reached once the optical density had an absorbance of 1 A, (in our case with an accepted deviation of ± 0.130 A) which happened 1 h ± 15 min after preparing the dilution. To determine the right moment, multiple measurements were performed around the expected time frame, until the desired optical density was reached. To perform the measurement, 900 µl of the dilution was transferred into a cuvette (SARSTEDT AG & Co., 51588 Nümbrecht, Germany, Ref.: 67.742). The same amount of pure tryptic soy broth (see chapter 4.1.5.1) was measured first and set as baseline reference (blank).

Once the logarithmic phase of bacterial growth was reached, the diluted culture was plated onto lysogeny broth agar plates (see chapter 4.1.5.3): the agar plates were set on a rotary disc (Sensor turn pro, WLD-TEC GmbH, 37318 Arenshausen, Germany) and 100 µl of the bacterial dilution per plate were spread evenly with a cell spreader until it was completely drawn into the agar.

Thereupon the specimens were carefully placed using breamed forceps, which were breamed again in between every contact with specimens to prevent contamination.

Once all the specimens were placed, the plates were incubated at 37°C for 22 h ± 2 h (Kelvitron® t, Heraeus Instruments, 63450 Hanau, Germany).

Day two

On the second day the plates were examined in regards to the formation of inhibition zones. If present, the diameter was measured two times using a ruler: narrowest and broadest stretch.

To examine whether there were still living bacteria underneath the specimens, they were carefully transferred onto a new lysogeny broth plate (see chapter 4.1.5.3), so any bacteria adhering to the specimens would transfer to the new lysogeny broth

plate (see chapter 4.1.5.3). Once there was contact with the new medium, the specimens were removed and disposed of.

Both sets of lysogeny broth plates, the original and those for growth control from underneath the specimens, were incubated at 37°C (Kelvitron® t, Heraeus Instruments, 63450 Hanau, Germany) over night for 22 h ± 2 h.

Day three

On the last day both sets of plates were examined once more. In the first set the inhibition zones were measured if present once again to inspect whether there was a change in diameter. The second set was examined in regards to the growth of bacteria from underneath the specimens.

In case of a formation of inhibition zones the experiment was repeated with more detailed intervals of 0.5% increase in the concentration of the antibacterial agent, starting from the last concentration without formation in inhibition zone, to determine which concentration is the starting point of formation of inhibition zones and ending with the highest concentration.

4.2.4 **Biofilm assay**

Outline:

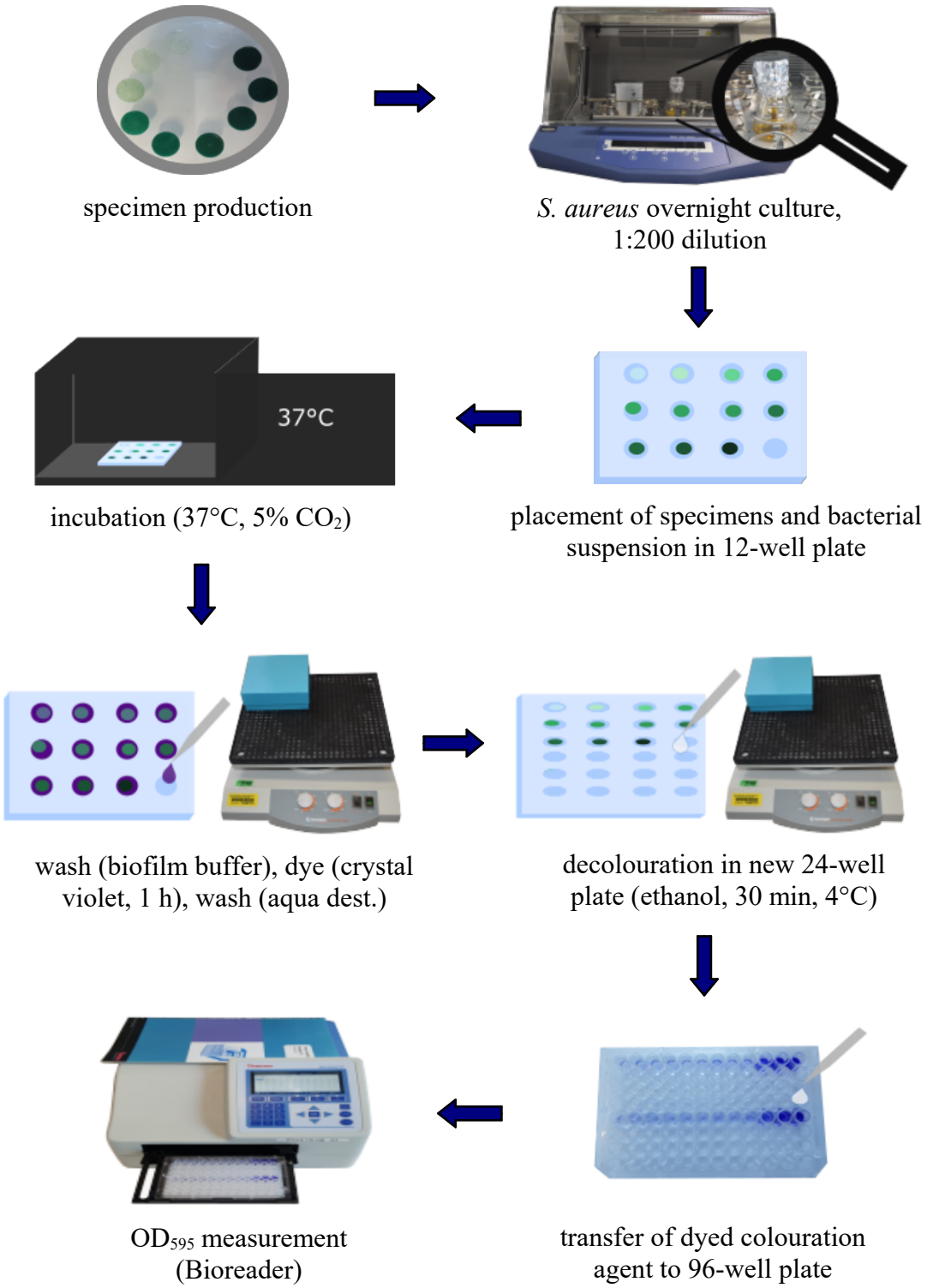


fig. 22) Outline experiment "biofilm assay" (OD_{595} = optical density at 595 nm)

Day one

The first day of the experiment started on the sterile working bench with the preparation of a 1:200 dilution of the *S. aureus* (see chapter 4.1.3.1) overnight culture with tryptic soy broth (see chapter 4.1.5.1) medium in a new 100 ml Erlenmeyer flask.

The specimens were placed one per well in a sterile 12-well plate (Falcon Multiwell 12 Well, Flat Bottom with Low Evaporation Lid, Corning Incorporated, New York 14831, USA, Ref: 353225) in ascending order. 1000 µl of the diluted *S. aureus* culture per well were added and ascertained that the specimens were floating in the liquid. The plates were removed from the sterile working bench after ensuring they were properly closed to avoid contamination and incubated over night for 22 h ± 2 h at 37°C and 5% CO_2 (Water Jacketed Incubator 3250, Forma Scientific).

Day two

On the second day the 12-well plates were removed from the incubator and slewed to dissolve the settled bacteria from the bottoms of the wells. In sterile conditions 500 µl medium per well were extracted and replaced by the same amount of fresh tryptic soy broth medium (see chapter 4.1.5.1) and the specimens were turned upside down. Afterwards the incubation was continued for further 22 h ± 2 h.

Day three

On day three the procedure was the same as on day two with the only difference being that 700 µl (instead of 500 µl) medium were removed per well but only 500 µl were refilled to concentrate bacteria in the liquid.

Day four

The last day started with retrieving the plates from incubation and bringing them to the sterile working bench for careful transfer of the specimens into a new sterile 24-well plate (Falcon Multiwell 24 Well, Flat Bottom with Low Evaporation Lid, Corning Incorporated, New York 14831, USA, Ref: 353226).

In the new 24-well plate the specimens were washed with 200 µl Biofilm buffer (see chapter 4.1.6.1) per well and afterwards dyed with 300 µl of 0.1% crystal violet (see chapter 4.1.7.1) per well. The dyeing took place in a dark box on a platform shaker

with a rotating wave movement (Polymax 1040, Heidolph Instruments GmbH & CO. KG, 91126 Schwabach, Germany) on level 10, after the specimens were turned two times in the dye.

After dyeing for an hour, the crystal violet (see chapter 4.1.7.1) was taken off and the specimens carefully washed with purified water (see chapter 4.1.4.1) until the specimens no longer exuded any colour and only the dye bound to the biofilm was left. The amount of water needed until clarity of the washing water was reached was very different in the materials. The specimens with nano zinc oxide (see chapter 4.1.2.1) needed around 2 ml, while those with copper naphthenate (see chapter 4.1.2.2) needed around 10 ml.

For the next step, the sterile working bench was left in favour of a 4°C refrigeration room to minimize ethanol evaporation. 200 µl of 96% ethanol (see chapter 4.1.8.1) were applied per well for biofilm discolouration. A self-adhesive plastic sheet (MicroAmp™, Optical Adhesive Film, Applied Biosystems, Life Technologies Corporation, Carlsbad, CA 92008, USA, Ref: 4311971) was adhered to the well-orifices as an additional seal against evaporation. The discolouration was conducted for 30 minutes in a dark box on a platform shaker with a (rotating) wave movement (Polymax 1040, Heidolph Instruments GmbH & CO. KG, 91126 Schwabach, Germany) on level 10.

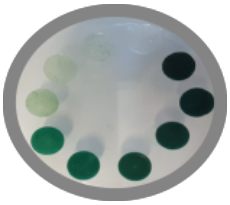
Subsequently 125 µl of the – by then coloured – ethanol (see chapter 4.1.8.1) per well were transferred into a 96-well plate (96-well microwell plate, flat bottom without lid, Thermo Fisher Scientific Nunc A/S, 4000 Roskilde, Denmark., Ref: 269787 and 96-well microwell lid, Thermo Fisher Scientific Nunc A/S, 4000 Roskilde, Denmark., Ref: 264122) and the optical density was measured at 595 nm (Multiskan FC, Thermo Fisher Scientific Oy, Microplate Instrumentation, 01621, Finland).

Reference

The experiment was repeated without bacteria as a reference for possible change in optical density in correlation to increased antibacterial agent without biofilm formation.

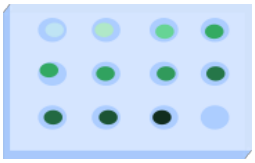
4.2.5 **Bacterial growth in liquid medium**

Outline:

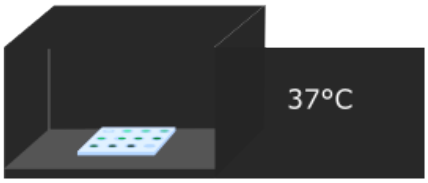


specimen production

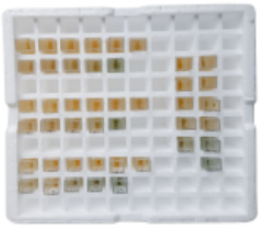
S. aureus overnight culture,
1:200 dilution



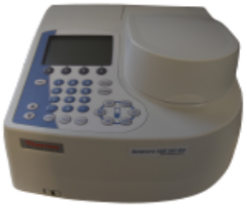
placement of:
specimens and TSB (= reference) &
specimens and bacterial suspension
in 12-well plate



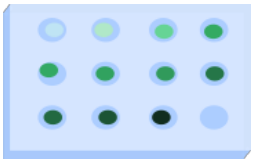
overnight incubation (37°C, 5% CO_2)



transferal of medium to micro cuvettes



OD_{600} measurement



replenishment of TSB
in 12-well plate

repetition of 24 h incubation,
transfer to micro cuvettes and
OD_{600} measurement

fig. 23) Outline experiment "growth inhibition in liquid medium" (TSB= tryptic soy broth; OD600= optical density at 600 nm)

On day two, three and four of the biofilm assay, the tryptic soy broth medium (see chapter 4.1.5.1) noticeably took the green tinge of copper naphthenate (see chapter 4.1.2.2), with the intensity being subject to the copper naphthenate concentration within the specimens. For further examination of the leftover tryptic soy broth medium (see chapter 4.1.5.1), the differences in between the greatest and smallest concentration were monitored after transferring them into caps. There was a noticeable difference in the size of the bacterial pallet at the bottom of the caps and the colour of the medium, both characteristics being stable over days on end (see fig.24). Those observations lead to the following experiment.

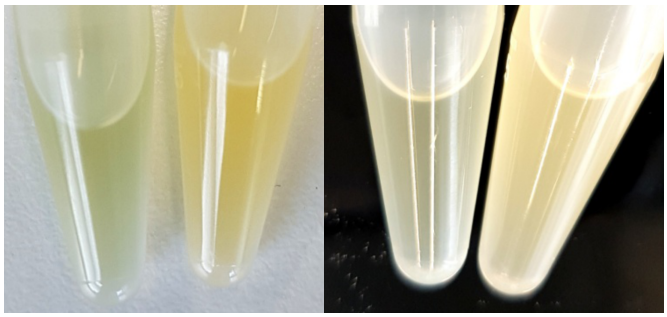


fig. 24) Surrounding tryptic soy broth of 25% copper naphthenate (see chapter 4.1.2.2) specimen (green tinge) and 0% copper naphthenate specimen (yellow tinge), at the end of biofilm incubation in front of black and white backgrounds.

The start of the experiment was the same as the biofilm assay with 24 h incubation of specimens in tryptic soy broth (see chapter 4.1.5.1), in a 12-well plate at 37°C, with an additional plate of specimens and tryptic soy broth (see chapter 4.1.5.1), but no bacteria as a reference.

On the **second day** 900 µl per well were transferred into cuvettes (SARSTEDT AG & CO., 51588 Nümbrecht, Germany, Ref.: 67.742) and their optical density (= OD) measured against their counterparts without bacteria. The medium in the wells was refilled with 900 µl per well. For the OD-measurement the spectrophotometer (Genesys™ 10S UV/Vis-Spectrophotometer, Thermo Fisher Scientific Inc., Waltham, MA 02454, USA) was set on the programme for cell growth at 600 nm. The references without bacteria were set as blanks and the bacteria containing liquid then measured against their blank counterparts.

The same process was repeated on the **next day**, to assure the antibacterial effect did not stop after removal of 90% of the liquid.

4.3 Mechanical methods

Outline:

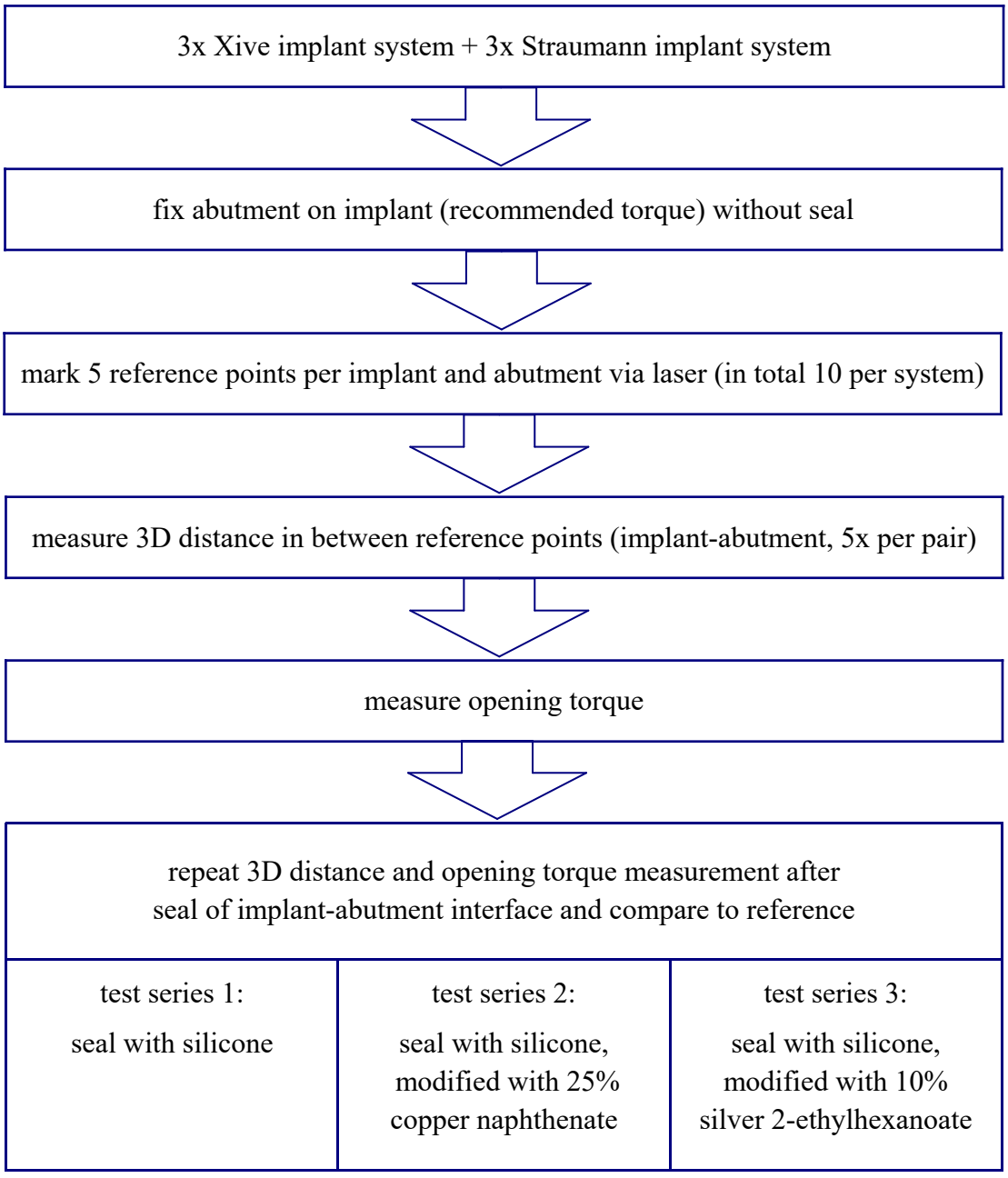


fig. 25) Outline mechanical method

4.3.1 **General conditions**

The mechanical experiments were performed in the Department of Prosthodontics of the Justus-Liebig University, Giessen, Germany. The experiment took place under controlled conditions, in an air-conditioned laboratory with a room temperature of 23°C ± 2°C.

4.3.2 **Bolting abutment onto implant**

The implants (see 4.1.9) were fixed in a three-point holding device (three-jaw chuck, dk FIXIERSYSTEME GmbH & Co. KG, 72770 Reutlingen, Germany, Ref: 329700) (see fig. 26), which was mounted on a metal board, which in turn was fastened on a table by two screw clamps (see fig. 27). Once the implant was stabilized, the abutment was carefully screwed onto the implant (see fig. 28) with the recommended torque, using a digital torque screwdriver, Model STC2-G by TOHNICHI MFG.CO., LTD, Tokyo Japan. The recommended torque for the Friadent® EstheticBase Abutments on Xive® S implants (see chapter 4.1.9.1) is 24 Ncm and for Regular Cross Fit® abutment on Straumann Bone Level Implant (see chapter 4.1.9.2) 35 Ncm. After setting of the silicone (and after laser process, before measuring the 3D distance) the screws were retightened. In both tightening processes, heed was paid to maintaining a perpendicular position of the digital torque screwdriver, so there was as little movement in horizontal directions as possible. For every test series a new set of screws was used to prevent falsification of the torque values.

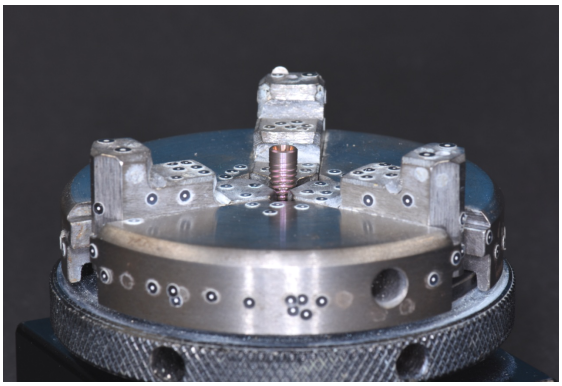


fig. 26) Three point holding device with implant

fig. 27) Torque measuring and bolting set-up

fig. 28) Bolting abutment onto implant

4.3.3 **Marking reference points by laser**

After bolting the abutments to the implants for the first time, the implant systems (see chapter 4.1.9) were removed from the holding device and marked by laser with ten reference points (Reinhardt Bretthauer GmbH, 35684 Dillenburg, Germany) per implant system surrounding the implant-abutment interface: five on the implant and five on the abutment. A special bracket (see fig. 29) was used for exact positioning of the implant in the laser device. The bracket had a slot for a fitted pentagon, which in turn held the implant and ensured marking in defined positions with equal spacing – by rotation of the pentagon. After marking the torque was once more applied to ensure there was no loosening in the connection.

fig. 29) Laser bracket for marking reference points and 3D distance measurements

4.3.4 **Measuring 3D distance**

The distance in between the reference points was measured with the digital 3D Zeiss Smart Zoom 5 microscope with the PlanApo D 1.6x/ FWD 36 mm objective lens in a 270x magnification. The positioning underneath the microscope was standardised by using the laser bracket.

In the Xive® implant system (see chapter 4.1.9.1), the starting point for the first measurement was the reference point on the highest part of the emergence profile. In the Straumann implant system (see chapter 4.1.9.2) the first measuring point was the first reference point to the right of the “RC” inscription on the abutment. The following measurements were conducted in a counter-clockwise rotation of the five defined pentagon positions.

A multilayer scan was realized and calculated into a 3D picture with an extended depth of focus. To achieve the extended depth of focus, the highest and lowest focal points were selected, and a scan was realized every 4 nm in between.

Once the 3D picture was constructed, the distance in between the lowest points on the laser reference points was measured (see fig. 30). To find the lowest points, the picture was inverted, so the lowest points became the highest ones and surveyed from multiple perspectives (see fig. 31). The 3D distance in between the selected points was measured.

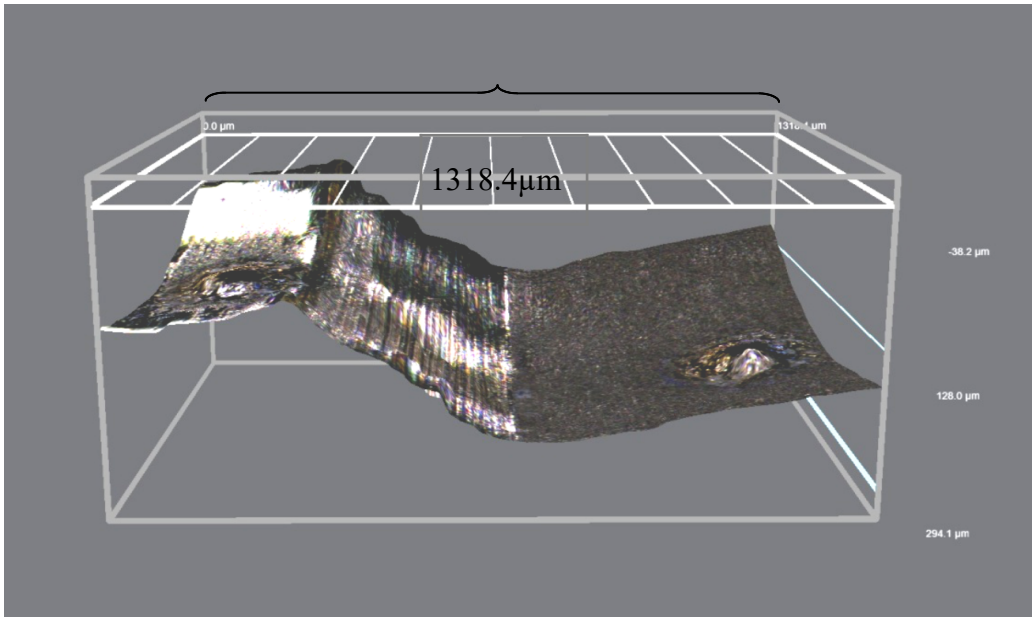


fig. 30) Inverted 3D reconstructed image of the implant-abutment interface

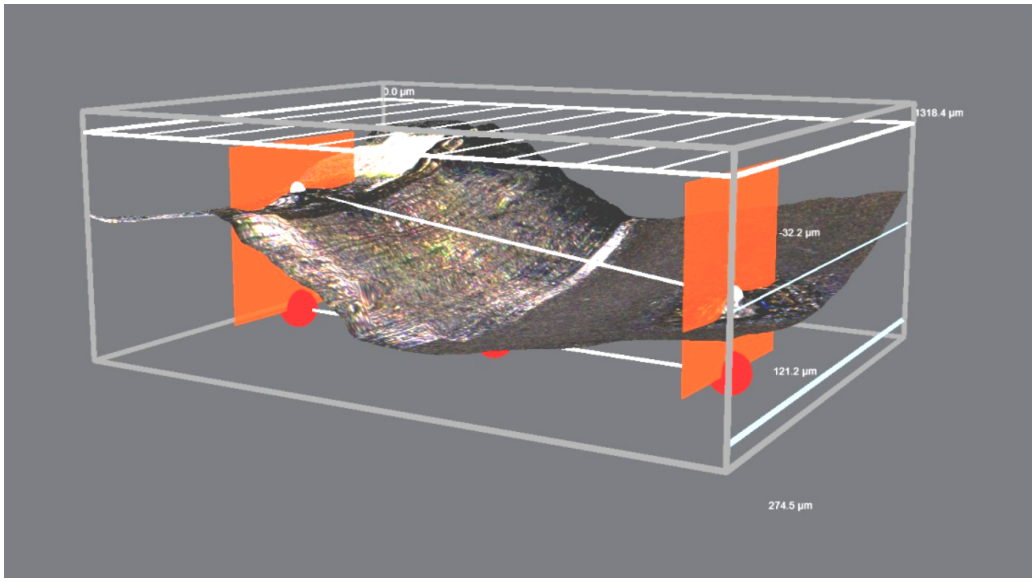


fig. 31) Applied 3D distance measuring tool

‘

4.3.5 **Opening torque**

Before unfastening, the positioning of the implant in relation to the abutment was indicated by a simple dot from a felt tip pen on the peripheral end of the implant perpendicular to the indicator on the abutment, which was the same as the indicator for the starting point of 3D distance measurements. This enabled assembly in the same position later on. For opening the implant-abutment connection, the implant system was mounted in the three-point holding device in the same manner as for tightening the connection (see fig. 26). The highest torque when disjoining abutments and implant was measured using the digital torque screwdriver, Model STC2-G (TOHNICHI MFG.CO., LTD, Tokyo Japan) (see fig. 32). The opening torque is indicated by a negative value.

fig. 32) Unfastening the implant-abutment connection

4.3.6 **Sealing the implant-abutment interface**

The void spaces of the implant-abutment interface were sealed and then the 3D distance measurements, as well as the opening torque measurements repeated and compared to the reference (= empty implant-abutment interface). In the first test series the material used for sealing was pure silicone (see chapter 4.1.1.1), in the second series 25% copper naphthenate (see chapter 4.1.2.2) modification and in the third 10% silver 2-ethylhexanoate (see chapter 4.1.2.3) modification. The preparations of the modifications of the silicone (see chapter 4.1.1.1) were conducted in the same manner as in the microbiological experiments. Per implant 0.5 g sealant was prepared. The material was applied into the implant lumen via a clean 1 ml plastic syringe (non-sterile) with a conventional luer lock tip (20 Gauge).

For sealing, the implant was mounted in the three-point holding device used for bolting. Immediately after application of the sealant (see fig. 33) the abutment was screwed onto the implant.
Excess spilling over the implant-abutment interface was removed with foam-pellets.

fig. 33) Seal application

4.4 Statistical Method

IBM SPSS Statistics for Windows version 26 was used for statistical analysis of all experiments. Diagrams were either drawn with the same program or Microsoft Office Exel 2007. The statistical analysis was conducted in consultation with Dr. Johannes Herrmann (Asterweg 60, 35390 Giessen, Germany).

Aim of the analysis was to determine whether the tested materials showed antibacterial effects and if they did, whether the concentration was a modulating factor and if the antibacterial characteristics remained for more than one day. Also, the influence of the application of a seal on positioning of abutment in implant and opening torque were investigated.

Significance level for all analyses was set at $p < 0.05$.

4.4.1 Bacterial growth inhibition on agar plates

In case of inhibition zone formation – which induced a repetition of the experiment with further concentrations in regular intervals – a mixed ANOVA of within (time factor; comparison over three days) and between (concentration factor) effects was conducted and the corresponding effect size (n_p^2) calculated. Furthermore, Pearson correlation was calculated for analysis of the relationship of concentration and inhibition zone size. Aim of the analysis was to investigate the interrelationship of agent-concentration and size of inhibition zone, along with the size-development over time. To obtain one diameter for inhibition zone size per specimen for statistical analysis, the mean of both measured diameters (per specimen) was determined and the specimen's size (10 mm) subtracted.

4.4.2 Biofilm assay

An independent samples test was used to compare the measured absorbance (A) for the different concentrations with the respective negative control, to determine whether the recorded change in absorbance was statistically significant. In case of heterogeneity of

variances, the Satterthwaite corrected t-test was reported. Furthermore, standardised effect size *r* was calculated. For the interpretation of *r*, COHEN formulated general suggestions: small effect: r = 0.1; medium effect: r = 0.3; strong effect: r = 0.5.[32, 119] For the results of this study the scale for interpretation was shifted due to very high values in case of strong effects: small effect: *r* = 0.1, medium effect: *r* = 0.5 and strong effect: *r* = 0.9. (Formula for effect size: $r = \sqrt{\frac{t \times t}{t \times t + df}}$, with "t" being the t-test result and "df" the degrees of freedom.[119])

4.4.3 **Bacterial growth inhibition in liquid medium**

In a similar manner to the biofilm assay analysis, an independent samples t-test was conducted for the analysis of influence on bacterial growth in liquid medium, too. But in this case the absorbance (A) for the different concentrations of the different materials was compared to the absorbance (A) for pure silicone. Apart of that, the test was followed through in the same manner as for the biofilm assay.

4.4.4 **Mechanical experiments**

The overall changes in 3D distance were analysed by a two-factorial ANOVA with the implant system as an additional disruptive factor. 3D distance was one of the factors and the sealing material (no seal, pure silicone, 25% copper naphthenate and 10% silver 2-ethylhexanoate) the other.

The changes in opening torque, or lack thereof, were analysed by paired-samples t-tests, comparing the different sealing materials (pure silicone, 25.0% copper naphthenate and 10.0% silver 2-ethylhexanoate) to the empty reference.

5 Results

5.1 Microbiological experiments

5.1.1 Agar diffusion test / bacterial growth from underneath specimens

5.1.1.1 Formation of inhibition zones

In the basic experiment an overview over the antibacterial behaviour in regards to inhibition zone formation was examined with the concentrations 0.0%, 0.1%, 0.5%, 1.0%, 2.0%, 5.0%, 7.0%, 10.0% for silver 2-ethylhexanoate, with addition of 15.0%, 20.0% and 25.0% for copper naphthenate and in case of zinc oxide a further addition of 30.0% (see fig. 37).

There was no formation of inhibition zones around specimens modified with zinc oxide and copper naphthenate (see fig 34, fig. 35).

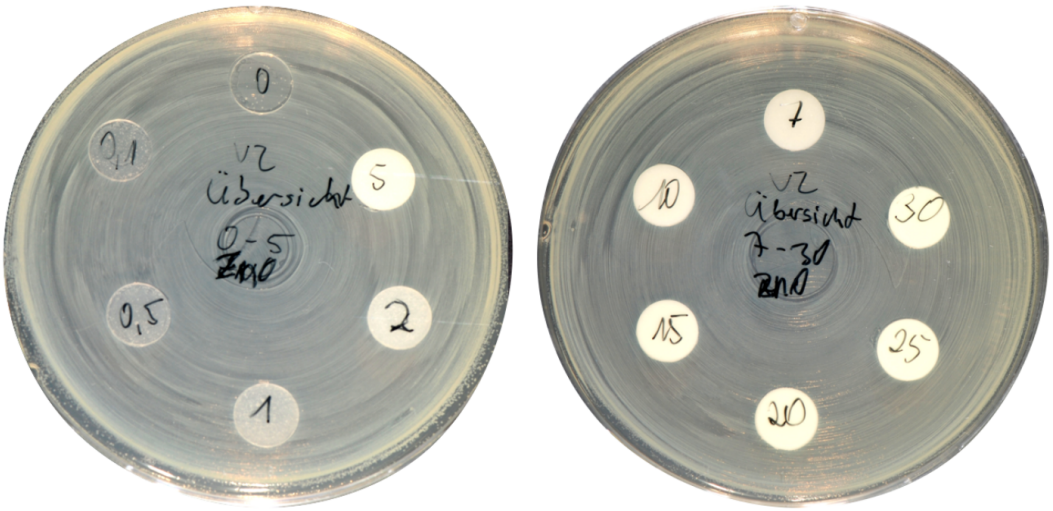


fig. 34) Zinc oxide, no inhibition zones. Test series two.

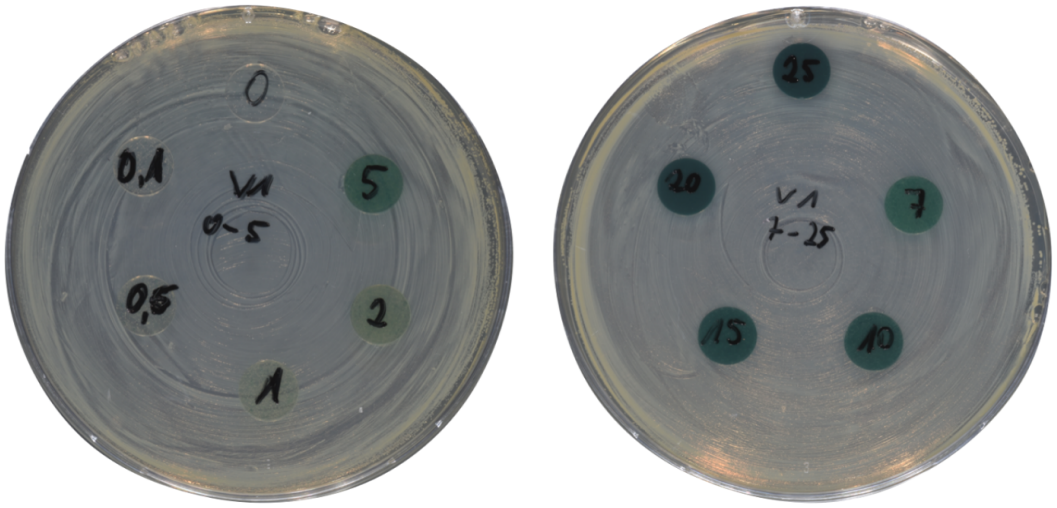


fig. 35) Copper naphthenate, no inhibition zone. Test series one.

With silver 2-ethylhexanoate on the other hand, there was formation of inhibition zones, starting from 5% and continuing in varying sizes up to the maximum amount of 10% with the biggest inhibition zones being found at 7.0% and 10.0% (see fig. 36). Therefore, the starting point of inhibition zone formation was somewhere in between 2.0% and 5.0% of silver 2-ethylhexanoate.

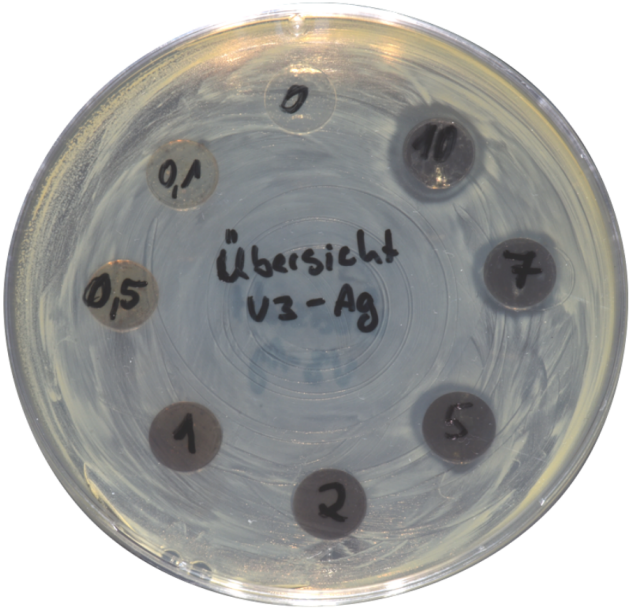


fig. 36) Inhibition zones on silver 2-ethylhexanoate preliminary concentrations. Test series one.

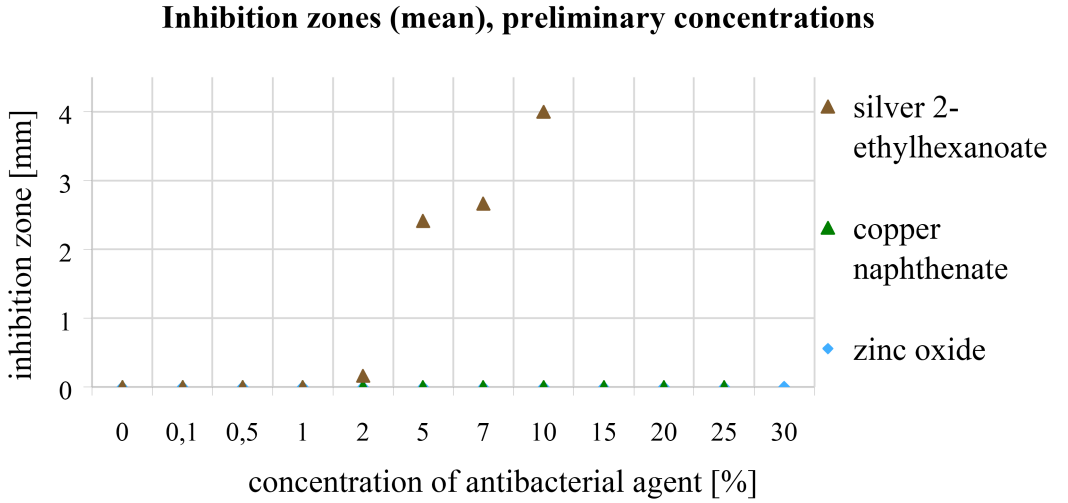


fig. 37) Mean of inhibition zone sizes per preliminary concentrations of antibacterial agent.

A follow-up experiment with silver 2-ethylhexanoate further examined the concentrations from 2.5% to 10.0% in intervals of 0.5% (see fig. 38). Influence of concentration on inhibition zone size, as well as changes in the size over two days (see fig. 39) were investigated.

2.5% and 3.5% silver 2-ethylhexanoate did not form inhibition zones. 3.0% along with 5.5% formed inhibition zones in just two of the three test series and 4.5% only in one of them. From 6.0% to 10.0% there were inhibition zones in all three test series, as well as for 4.0% and 5.0%. (For inhibition zone sizes see chapter 11.1.1)

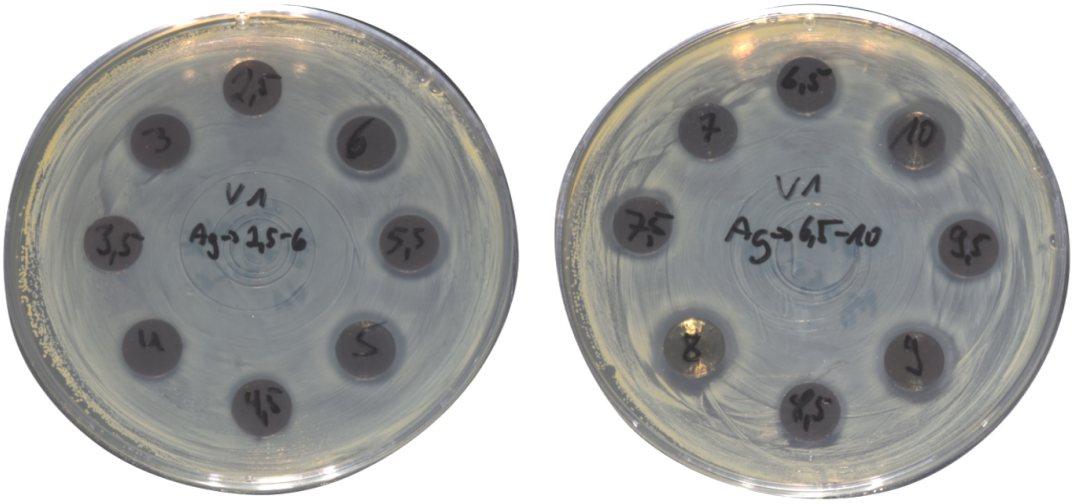


fig. 38) Silver 2-ethylhexanoate inhibition zones, concentrations of 2.5% - 10.0%. Test series one.

A mixed-design ANOVA was conducted with the concentration as the between subjects factor and time (measurements at starting point, 24 and 48 hours later) as within subjects factor; to analyse the differences in inhibition zone sizes due to changes in concentration and time.

The within factor "time" did not lead to any significant changes in inhibition zone sizes $F(2) = 1.161$, $p = .322$, $n_p^2 = .049$ (see fig. 39). Also interaction between time and concentration was insignificant $F(2) = 2.795$, $p = .066$, $n_p^2 = .087$.
In more detail, the test of within-subjects'-effects displayed no significant change in size of inhibition zones at the first measurement and after 24 and 48 hours, $F(2) = .948$, $p = .391$, $n_p^2 = .020$. No correction was needed as sphericity could be assumed (Mauchly's sphericity test $p = .188$).

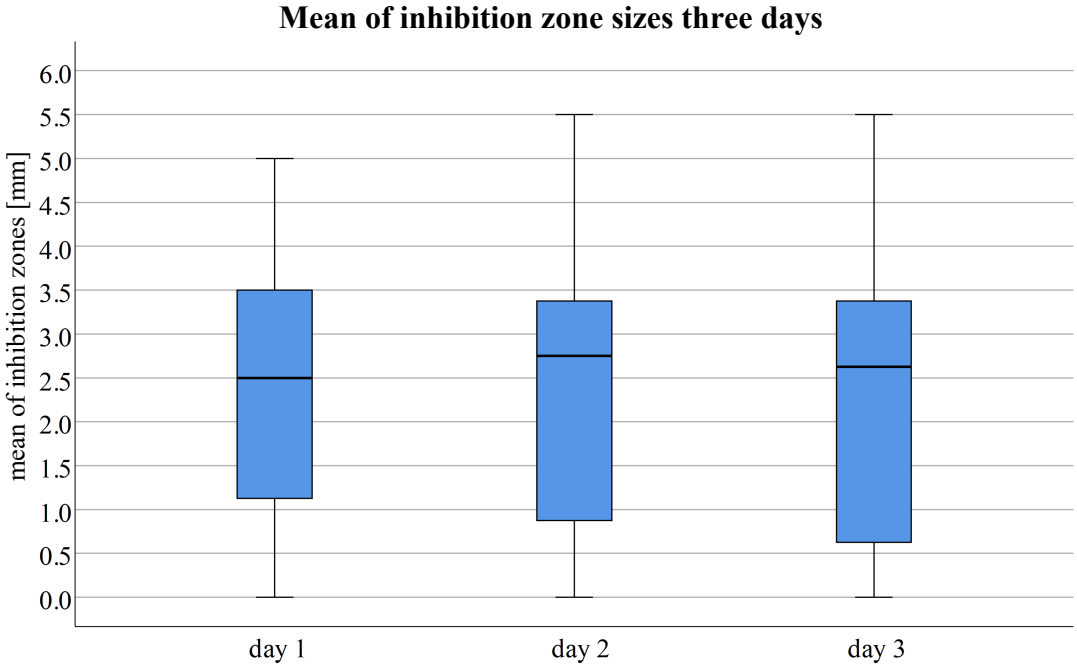


fig. 39) Mean of silver 2-ethylhexanoate inhibition zone sizes over three days

In contrast, the test of between subjects' effects (with the factor concentration) displayed highly significant differences in inhibition zone sizes, $F(1) = 82.115$, $p < .001$, $n_p^2 = .641$ (see fig. 40, fig. 41, fig. 42). To further investigate the influence of concentration on size of inhibition zone, Pearson correlation was analysed.
Correlational analysis revealed a strong positive relationship between the silver 2-ethylhexanoate concentration and antibacterial effect, $r = .786$, $p < .01$ (Pearson correlation).

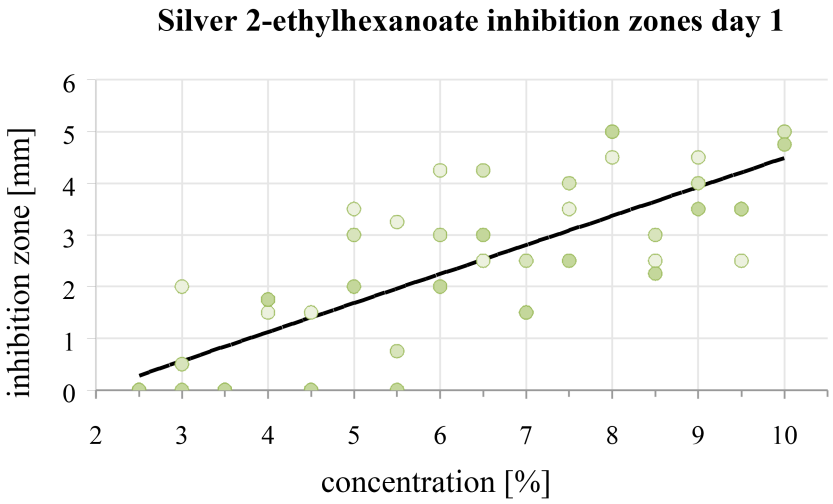


fig. 40) Silver 2-ethylhexanoate specimens' inhibition zones sizes, day one, with linear trend line.

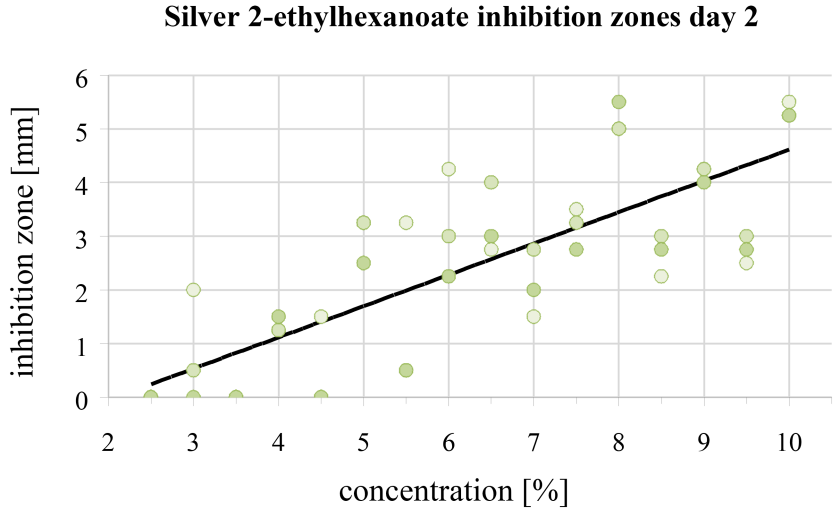


fig. 41) Silver 2-ethylhexanoate specimens' inhibition zones sizes, day two, with linear trend line.

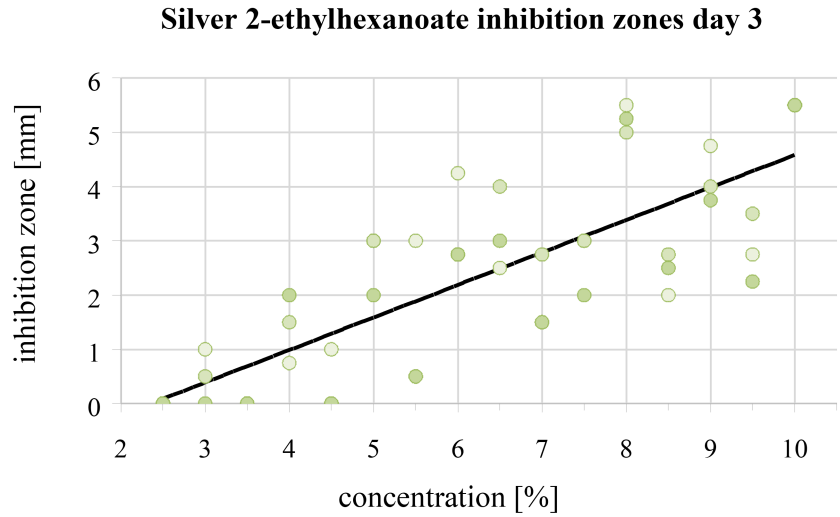


fig. 42) Silver 2-ethylhexanoate specimens' inhibition zones sizes, day three, with linear trend line.

5.1.1.2 Growth control from underneath the specimens

Zinc oxide:

An evenly spread bacterial lawn formed from underneath zinc oxide specimens, independent of the added concentration of zinc oxide (see fig. 43). Also, after specimen removal, an equable bacterial smear layer was left where the specimens were positioned beforehand, independent of the agent concentration (see fig. 44).

fig. 43) Growth control from underneath the zinc oxide specimens

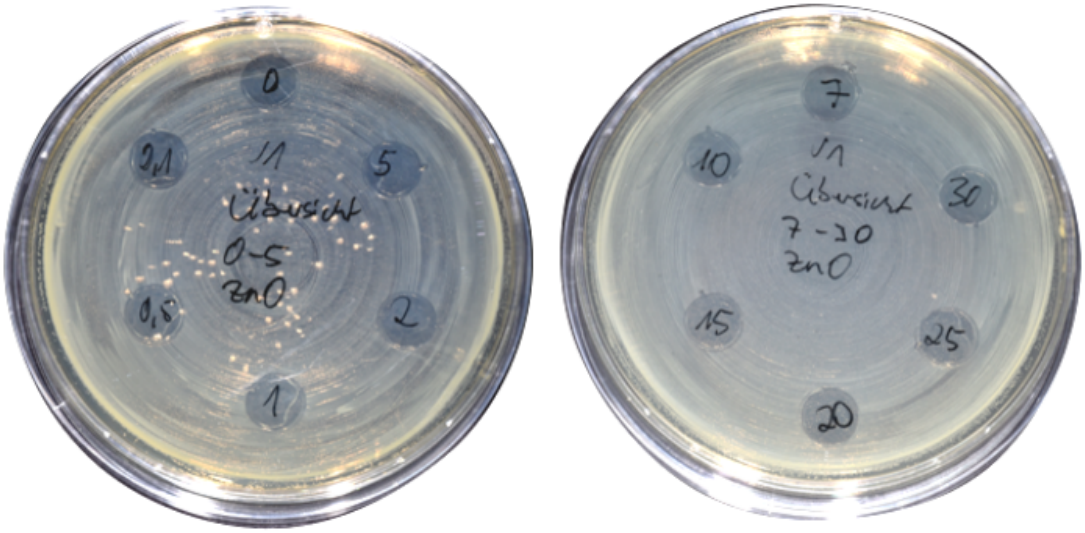


fig. 44) Original agar plates after zinc oxide specimen removal

Copper naphthenate:

There was a bacterial lawn formation from underneath all the copper naphthenate specimens, but at 25% and slightly at 20%, the bacterial lawn seemed to be a little less dense in the centre than from underneath the lesser copper naphthenate concentrations (see fig. 45, fig. 46). On the original agar plate, the remaining bacterial smear layer seemed reduced at 25% and slightly at 20% as well (see fig. 47, fig. 48).

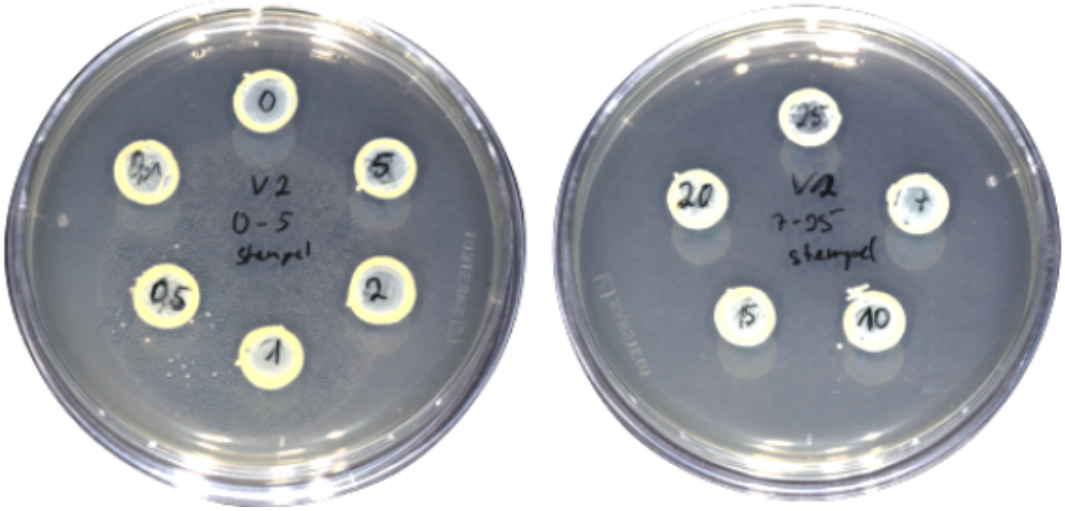


fig. 45) Growth control from underneath the copper naphthenate specimens

fig. 46) Close-ups from the growth control plate, 0%-15% had no change in density of bacterial lawn, slight decrease in the centre at 20%, and more of a decrease at 25% copper naphthenate.

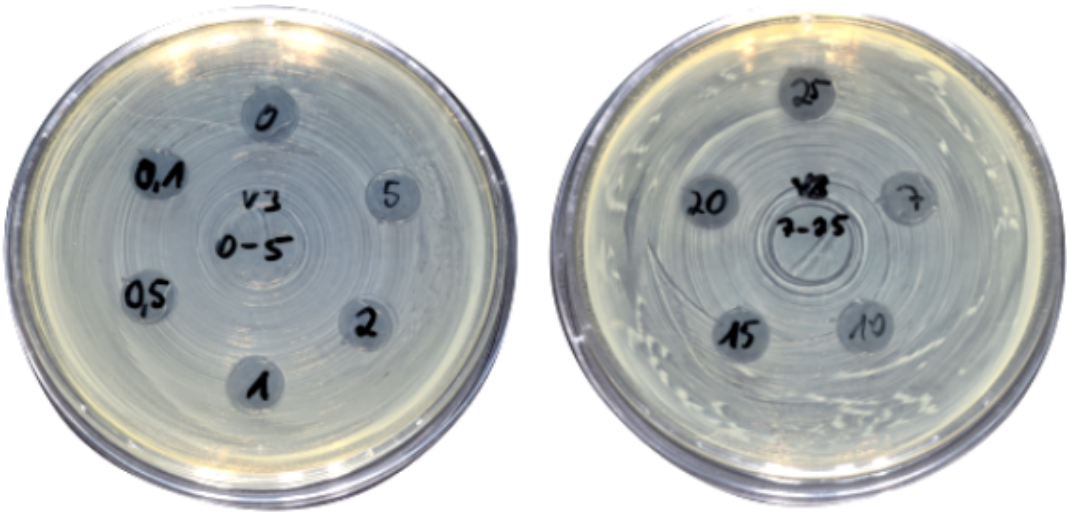


fig. 47) Original agar plates after copper naphthenate specimen removal.

fig. 48) Close-ups from the growth control plate. 0-15% copper naphthenate similar bacterial smear layers, at 20% and more noticeably at 25% reduced smear layer.

Silver 2-ethylhexanoate:

In the preliminary experiment with fewer concentrations (0.0%, 0.1%, 0.5%, 1.0%, 2.0%, 5.0%, 7.0%, 10.0%), there was – similar to copper naphthenate – a less dense bacterial lawn in the centre of 10.0% silver 2-ethylhexanoate (see fig. 49).

In the more detailed examination of the concentrations 2.5% - 10.0% silver 2-ethylhexanoate, the lower concentrations still showed a quite evenly spread bacterial layer, but at 8.0% and 10.0% silver 2-ethylhexanoate, the bacterial amount transferred to the new plate was highly reduced, with visibly separated colonies (see fig. 50).

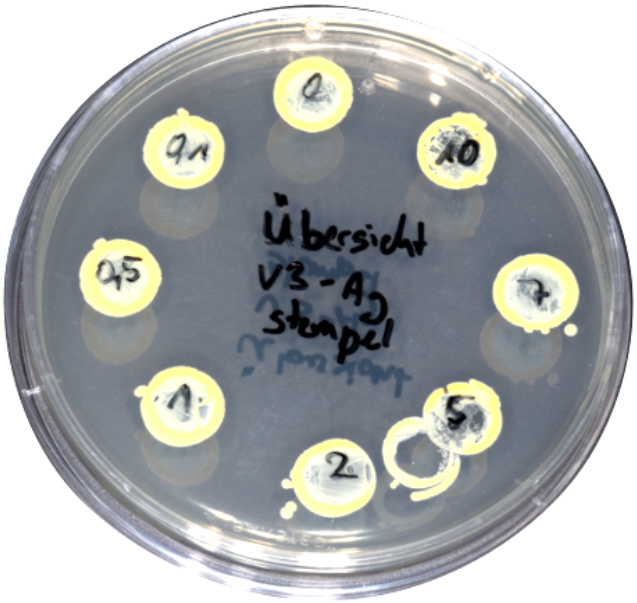


fig. 49) Growth control from underneath the specimens' preliminary experiment. Reduced density of bacteria in the centre at 10% silver 2-ethylhexanoate.

fig. 50) Growth control from underneath the specimens, follow up experiment – agar plate with high concentrations. High reduction of bacteria at 8.0% and 10.0% silver 2-ethylhexanoate. Test series one.

5.1.2 **Biofilm assay**

5.1.2.1 Zinc oxide

Zinc oxide led to a slightly increasing optical density of the solvent, depending on the amount of colour deriving from the dye on the specimens, in comparison to the negative control (see fig. 51). T-test in between sample and control group displayed a significant difference only in the concentrations where the statistical spread was little 0.0%, 0.5%, 5.0%, 15.0%, 20.0% and 25.0%.

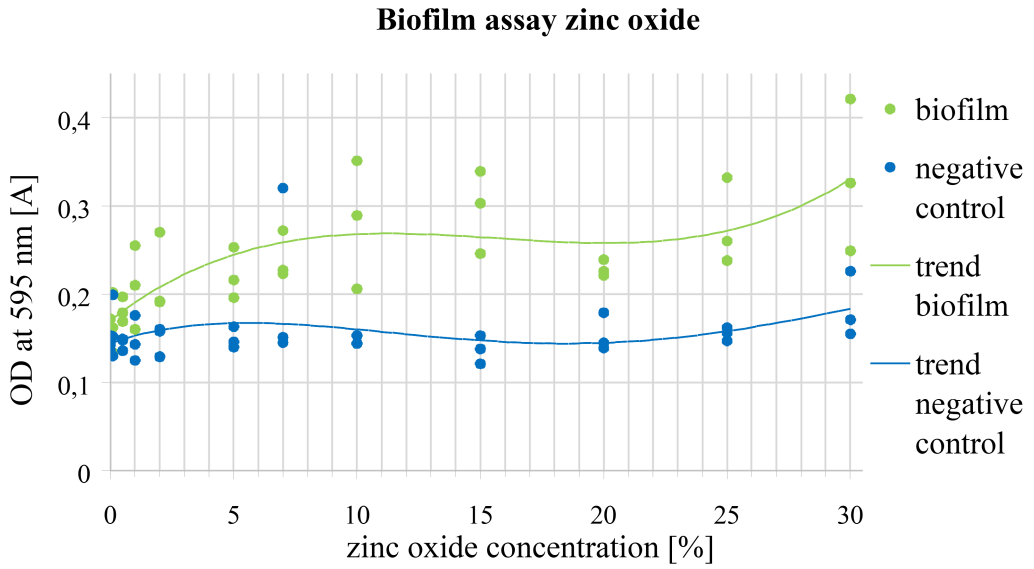


fig. 51) Scatter diagram "results biofilm assay, zinc oxide"

Independent Samples Test (for spreadsheet with values see chapter 11.2.1)

In the following section the results of the t-test will be elaborated. Absorbance, the unit for optical density, will be abbreviated to “A”, the mean values to “M” and standard deviation to “SD”. Optical density at 595 nm will be abbreviated to “OD_{595}”.

With **0.0% zinc oxide**, the biofilm series’ mean OD_{595} (M = 0.164 A, SD = 0.008 A) was higher than the negative controls’ mean OD_{595} (M = 0.144 A, SD = 0.009 A), resulting in a significant difference of optical density (t(4) = 2.960; p = .042; 95% confidence interval [0.001, 0.039]), with a big effect size (r = 0.829).

0.1% zinc oxide lead to the samples’ mean OD_{595} (M = 0.166 A, SD = 0.034 A) being very similar to the negative controls’ mean OD_{595} (M = 0.160 A,

SD = 0.035 A). The difference in means was insignificant (t(4) = 0.200; p = .851; 95% confidence interval [-0.073, 0.084]) and effect size very small (r = 0.099).

With **0.5% zinc oxide** the difference in between samples' mean OD_{595} (M = 0.182 A, SD = 0.014 A) and negative controls' mean OD_{595} (M = 0.144 A, SD = 0.007 A) was significant (t(4) = 4.060; p = .015; 95% confidence interval [0.012, 0.063]) and effect size high (r = 0.897).

At **1% zinc oxide** the biofilm series' mean OD_{595} (M = 0.208 A, SD = 0.048 A) was slightly higher than negative controls' mean OD_{595} (M = 0.148 A, SD = 0.026 A). The difference was not significant (t(4) = 1.931; p = .126; 95% confidence interval [-0.026, 0.147]) and the effect middle-sized (r = 0.695).

2% zinc oxide induced a similar result to 1%: samples' mean OD_{595} (M = 0.218 A, SD = 0.045 A) being slightly higher than negative controls' mean OD_{595} (M = 0.149 A, SD = 0.017 A) and the difference being insignificant (t(4) = 2.451; p = .070; 95% confidence interval [-0.009, 0.146]). The effect size was higher (r = 0.775).

The difference in means at **5% zinc oxide** was significant (t(4) = 3.986; p = .016; 95% confidence interval [0.022, 0.122]) with samples' mean OD_{595} (M = 0.222 A, SD = 0.029 A) being higher than negative controls' mean OD_{595} (M = 0.150 A, SD = 0.012 A). Effect size was also quite high (r = 0.894).

At **7% zinc oxide** the biofilm series' mean OD_{595} (M = 0.241 A, SD = 0.027 A) displayed an insignificant (t(2.298) = 0.594; p = .606; 95% confidence interval [-0.191, 0.262]) difference to negative controls' mean OD_{595} (M = 0.205 A, SD = 0.099 A). Effect size was also low (r = 0.365). Levene's Test indicated unequal variances (F = 7.778; p = .049). Therefore, degrees of freedom were adjusted from 4 to 2.298.

A concentration of **10% zinc oxide** lead to a higher samples' mean OD_{595} (M = 0.282 A, SD = 0.073 A) than negative controls' mean OD_{595} (M = 0.147 A, SD = 0.005A). The difference was significant (t(4) = 3.206; p = .033; 95% confidence interval [-0.018, 0.252]) and effect size high (r = 0.848).

At **15% zinc oxide** biofilm series' mean OD_{595} (M = 0.296 A, SD = 0.047 A) was higher than negative controls' mean OD_{595} (M = 0.137 A, SD = 0.016 A) again. The

difference in means was significant (t(4) = 5.546; p = .005; 95% confidence interval [0.079, 0.238]). Size of effect was high (r = 0.941).

20% zinc oxide induced the results for biofilm series' mean OD_{595} (M = 0.229 A, SD = 0.009 A) to be higher than negative controls' mean OD_{595} (M = 0.154 A, SD = 0.022 A). The difference was significant (t(4) = 5.482; p = .005; 95% confidence interval [0.037, 0.112]). Effect size was high (r = 0.939).

Akin to 10-20% zinc oxide, with **25% zinc oxide** the samples' mean OD_{595} (M = 0.277 A, SD = 0.049 A) was higher than negative controls' mean OD_{595} (M = 0.155 A, SD = 0.008 A) and the difference was significant (t(4) = 4.236; p = .013; 95% confidence interval [0.042, 0.201]), as well as high effect size (r = 0.904).

30% zinc oxide caused biofilm series' mean OD_{595} (M = 0.332 A, SD = 0.086 A) to be higher than the negative controls' mean OD_{595} (M = 0.184 A, SD = 0.037 A), too. The difference on the other hand was insignificant (t(4) = 2.731; p = .052; 95% confidence interval [-0.002, 0.298]), but with a high effect size (r = 0.807).

5.1.2.2 Copper naphthenate

Copper naphthenate induced an increase in optical density of the solvent, depending on the amount of colour deriving from the dye on the specimens, from 0.0% copper naphthenate to 15.0%, with a peak at 7%, but fell below the negative control at 20.0% and 25.0% copper naphthenate (see fig. 52) . Independent sample test revealed all values for optical densities at 595 nm from bacteria-containing samples, apart from 0.0% and 0.1% copper naphthenate, to be significantly different from the negative control.

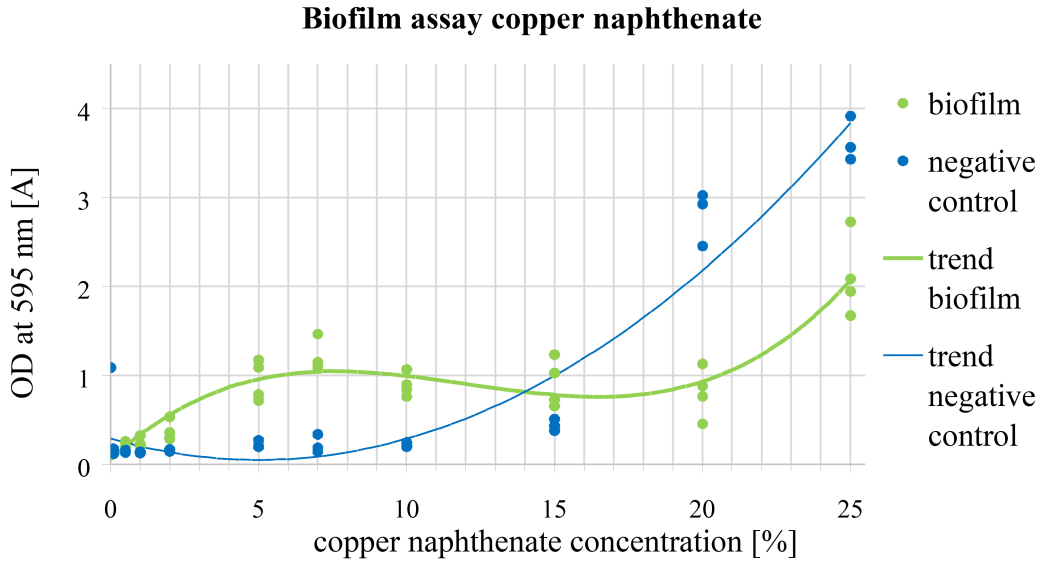


fig. 52) Scatter diagram "results biofilm assay, copper naphthenate"

Independent Samples Test (for spreadsheet with values see chapter 11.2.2)

In the following section the results of the t-test will be elaborated. Absorbance, the unit for optical density, will be abbreviated to “A”, the mean values to “M” and standard deviation to “SD”. Optical density at 595 nm will be abbreviated to “OD_{595}”.

At **0.0% copper naphthenate** the biofilm series’ mean OD_{595} (M = 0.150 A, SD = 0.011 A) was lower than the mean of the negative controls’ mean OD_{595} (M = 0.461 A, SD = 0.544 A). This difference was not significant (t(2.001) = -0.989; p = .427; 95% confidence interval [-1.662, 1.040]), with a medium effect size (r = 0.573). Levene’s test indicated that equality of variances could not assumed (F = 21.898; p = .005) and therefore, degree of freedom was corrected from 5 to 2.001.

A complementary case presented for **0.1% copper naphthenate**, with a rather similar samples’ mean OD_{595} (M = 0.145 A, SD = 0.016 A) and negative controls’ mean OD_{595} (M = 0.4607 A¸ SD = 0.028 A). The difference in between means was also insignificant (t(5) = -0.305; p = 0.773; 95% confidence interval [-0.047, 0.037]) with a lower effect size (r = 0.135).

The first significant difference (t(4.668) = 5.130; p = .004; 95% confidence interval [0.043, 0.132]) emerged from **0.5% copper naphthenate**, with the biofilm series’ mean OD_{595} (M = 0.232 A, SD = 0.029 A) displaying a higher optical density than

the negative controls' mean OD_{595} (M = 0.144 A; SD = 0.015 A). Levene's test showed that equality of variances could not be assumed (F = 6.790; p = .048), and degree of freedom was corrected accordingly from 5 to 4.668. Effect size was high (r = 0.922).

Optical density at **1.0% copper naphthenate** started the increasing height of samples' mean OD_{595} (M = 0.246 A, SD = 0.053 A) in comparison to the rather steadily low mean of negative controls' mean OD_{595} (M = 0.134 A, SD = 0.006 A), with the difference being significant (t(5) = 3.572; p = .016; 95% confidence interval [0.031, 0.192]) and the effect size high (r = 0.848).

The continuation of the rising height of optical of biofilm series' mean OD_{595} (M = 0.369 A; SD = 0.115 A) was also displayed at **2% copper naphthenate**. The negative controls' mean OD_{595} (M = 0.159 A, SD = 0.011 A) was still low. The difference was significant (t(5) = 3.072; p = .028; 95% confidence interval [0.034, 0.385]) and effect size high (r = 0.808).

5% copper naphthenate induced an even higher samples' mean OD_{595} (M = 0.942 A, SD = 0.029 A), whilst the negative controls' mean OD_{595} (M = 0.223 A, SD = 0.042 A) was still quite low. Levene's Test indicated that equality of variances could not be assumed (F = 35.464; p = .002) and the difference in between means was highly significant (t(3.275) = 6.272; p = .006; 95% confidence interval [0.371, 1.068]), as well as the effect size very high (r = 0.961).

With **7% copper naphthenate** the difference in means was highly significant (t(5) = 8.743; p < .001; 95% confidence interval [0.697, 1.277]), with the samples' mean OD_{595} (M = 1.209 A, SD = 0.172 A) being much higher than the one of negative controls' mean OD_{595} (M = 0.222 A, SD = 0.102 A). Effect size was very high (r = 0.969).

At **10% copper naphthenate** there was a slight decrease compared to 7% in optical density of biofilm series' mean OD_{595} (M = 0.892 A, SD = 0.128 A) and still no real change in density of negative controls' mean OD_{595} (M = 0.2173 A, SD = 0.026 A). The difference in means was highly significant (t(5) = 8.773; p < .001; 95% confidence interval [0.477, 0.873]) and effect size just as high (r = 0.969).

15% copper naphthenate marked the starting increase in OD_{595} of the negative controls' mean OD_{595} (M = 0.441 A, SD = 0.066 A), whilst OD_{595} of samples' mean

OD_{595} (M = 0.911 A, SD = 0.270 A) stayed at a similar level as at 10% copper naphthenate. The difference in means was less than it was at 10% copper naphthenate, but still significant (t(3.459) = 3.342; p = .036); 95% confidence interval [0.054, 0.884]) and effect size still quite high (r = 0.874).Equality of variances could not be assumed, as indicated by Levene's test (F = 10.060; p = .025) and degree of freedom was adjusted accordingly from 5 to 3.459.

From **20% copper naphthenate** on, the negative controls' mean OD_{595} (M = 2.802 A, SD = 0.305 A) started to supersede the samples' mean OD_{595} (M = 0.808 A, SD = 0.279 A) as the higher density. The difference fell into the negative realm and was highly significant (t(5) = -9.009; p < 0.001; 95% confidence interval [-2.563, -1.425]). Effect size was very high (r = 0.971).

Just like 20% copper naphthenate, **25% copper naphthenate** displayed the negative controls' mean OD_{595} (M = 3.6363 A, SD = 0.249) being higher than the biofilm series' mean OD_{595} (M = 2.107 A, SD = 0.446 A). The difference in means was very significant (t(5) = -5.271; p = .003; 95% confidence interval [-2.275, -0.783]) and effect size (r = 0.921) high.

5.1.2.3 Silver 2-ethylhexanoate

In higher concentrations, silver 2-ethylhexanoate led to elevated levels of absorbance in the solvent, depending on the amount of colour deriving from the dye on the specimens, in both, test series and negative control, with high dispersion and the differences in mean being insignificant (see fig. 53). Significant differences in between test series and control group were only reached at concentrations with little spread: of 0.5% and 2.0% silver 2-ethylhexanoate.

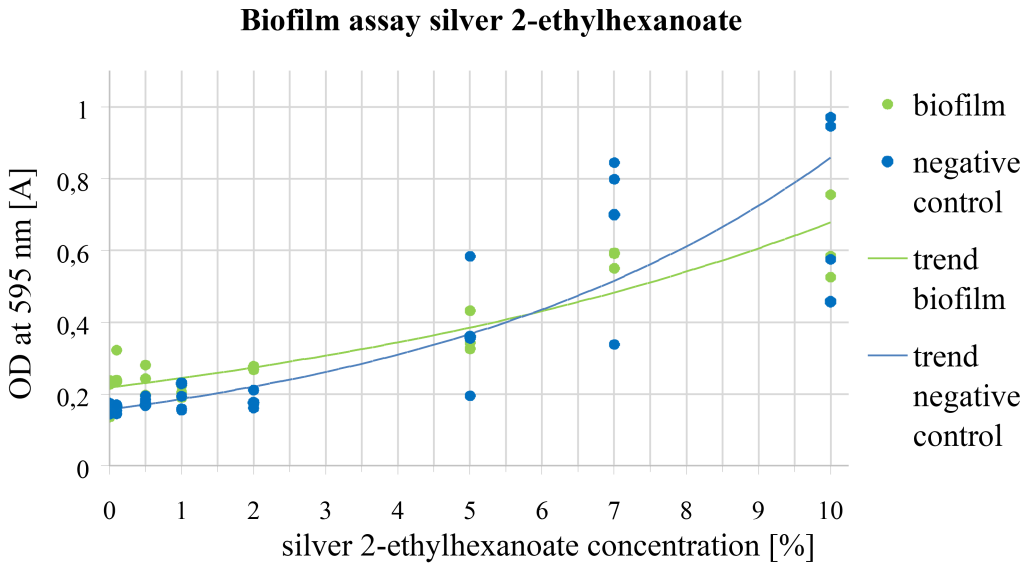


fig. 53) Scatter diagram "results biofilm assay, silver 2-ethylhexanoate"

Independent Samples Test (for spreadsheet with values see chapter 11.2.3)

In the following section the results of the t-test will be elaborated. Absorbance, the unit for optical density, will be abbreviated to “A”, the mean values to “M” and standard deviation to “SD”. Optical density at 595 nm will be abbreviated to “OD_{595}”.

At **0.0% silver 2-ethylhexanoate**, samples’ mean of OD_{595} (M = 0.200 A, SD = 0.056 A) started higher than negative control’ mean of OD_{595} (M = 0.163 A, SD = 0.013 A). The difference in between means was insignificant (t(2.174) = 1.146; p = .362; 95% confidence interval [-0.094, 0.170]) with a medium effect size (r = 0.614). Levene’s test indicated that equality of variances could not be assumed (F = 11.759; p = .024). Degree of freedom was adjusted accordingly from 5 to 2.174.

In case of **0.1% silver 2-ethylhexanoate**, the samples’ mean of OD_{595} (M = 0.265 A, SD = 0.049 A) was also higher than the negative controls’ mean of OD_{595} (M = 0.159 A, SD = 0.011 A). The difference was insignificant (t(2.161) = 3.651; p = .060; 95% confidence interval [-0.010, 0.223]) but effect size high (r = 0.928). Equality of variances could not be assumed as shown by Levene’s test (F = 11.759; p = .019), and degree of freedom corrected from 5 to 2.161.

0.5% silver 2-ethylhexanoate induced a similar result: a higher samples’ mean of OD_{595} (M = 0.240 A, SD = 0.042 A) and lower negative controls’ mean of OD_{595} (M = 0.182 A, SD = 0.011 A), with a significant difference (t(5) = 2.755; p = .040;

95% confidence interval [0.004, 0.114]). Effect size was lower than at 0.1% silver 2-ethylhexanoate, but still on the higher spectrum (r = 0.776).

With **1% silver 2-ethylhexanoate**, biofilm series' mean of OD_{595} (M = 0.206 A, SD = 0.017 A) was rather similar to negative controls' mean OD_{595} (M = 0.185 A, SD = 0.035 A). Significance in difference was not given (t(5) = 0.964; p = .379; 95% confidence interval [-0.036, 0.079]). Effect size was small (r = 0.396).

The last significant difference (t(5) = 7.246; p = .001; 95% confidence interval [0.060, 0.126]) was shown by **2% silver 2-ethylhexanoate**, with a higher samples' mean of OD_{595} (M = 0.274 A, SD = 0.005 A) than negative controls' mean of OD_{595} (M = 0.181 A, SD = 0.021 A). Effect size was high (r = 0.956).

At **5% silver 2-ethylhexanoate** the biofilm series' mean of OD_{595} (M = 0.367 A, SD = 0.057 A) was very similar to that from negative controls' mean of OD_{595} (M = 0.373 A, SD = 0.159 A), with the difference being insignificant (t(5) = -0.064; p = .952; 95% confidence interval [-0.259, 0.246]) and the effect size very small (r = 0.028).

A concentration of **7% silver 2-ethylhexanoate** lead to similar results: the samples' mean of OD_{595} (M = 0.578 A, SD = 0.024 A) and negative controls' mean of OD_{595} (M = 0.670 A, SD = 0.229 A) were very similar with the difference being insignificant, too (t(5) = -0.674; p = .530; 95% confidence interval [-0.442, 0.258]), with a small effect size (r = 0.289).

Akin to 5% and 7%, **10% silver 2-ethylhexanoate** displayed biofilm series' mean of OD_{595} (M = 0.621 A, SD = 0.119 A) and negative controls' mean of OD_{595} (M = 0.737 A, SD = 0.260 A) to be very similar to one another. The difference was insignificant (t(4.406) = -0.785; p = .472; 95% confidence interval [-0.509, 0.278]). Effect size was small (r = 0.350). Levene's test indicated equality of variances could not be assumed (F = 12.385; p = .017) and degree of freedom was corrected accordingly from 5 to 4.406.

5.1.3 **Bacterial growth in surrounding liquid medium**

There was no significant change in the optical density of medium surrounding specimens modified with different concentrations of 0.0% to 30.0% zinc oxide (see fig. 54). With silver 2-ethylhexanoate there was an increase in the optical density of the medium surrounding specimens modified with different concentrations of 0.1% to 7.0% silver 2-ethylhexanoate, with a slight decrease starting at 10.0%, but never falling below the absorbance of 0.0% silver 2-ethylhexanoate (see fig. 54). Copper naphthenate increased the optical density of the medium surrounding specimens modified with 0.1% to 15.0% copper naphthenate in comparison to 0.0%, but returned to similar values to 0.0% copper naphthenate at 20.0% and then decreased further at 25.0% copper naphthenate, falling below the optical density of 0.0% copper naphthenate (see fig. 54). Effect size correlates with the significance levels.

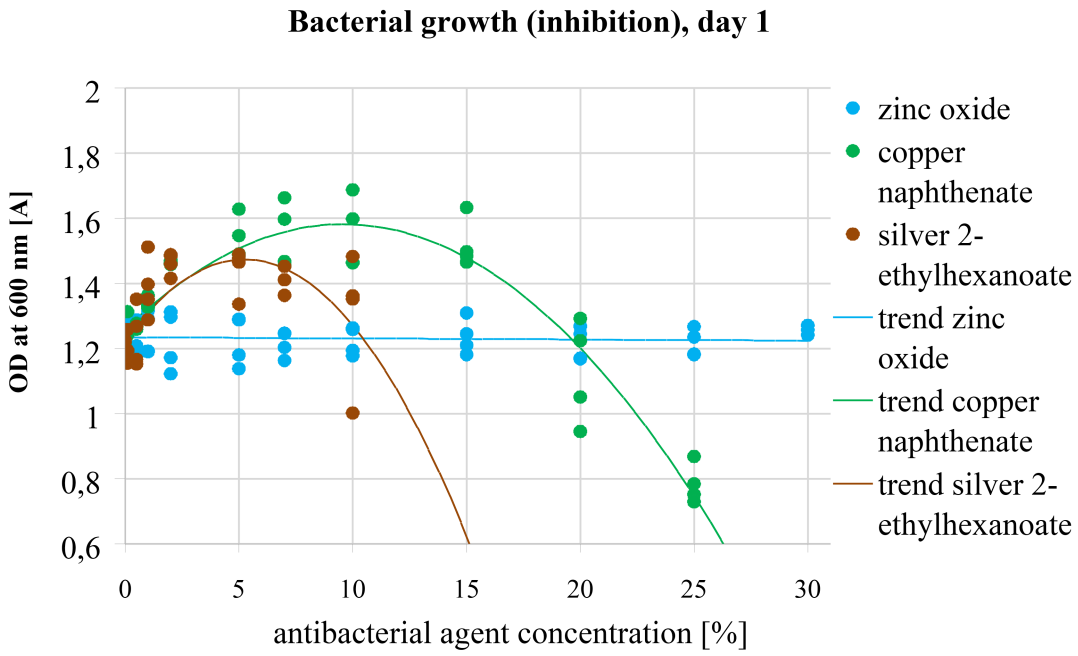


fig. 54) Bacterial growth (inhibition) in liquid medium, day one

On the second day the curves looked similar to the first day, just with a generally elevated bacterial concentration (see fig. 55):

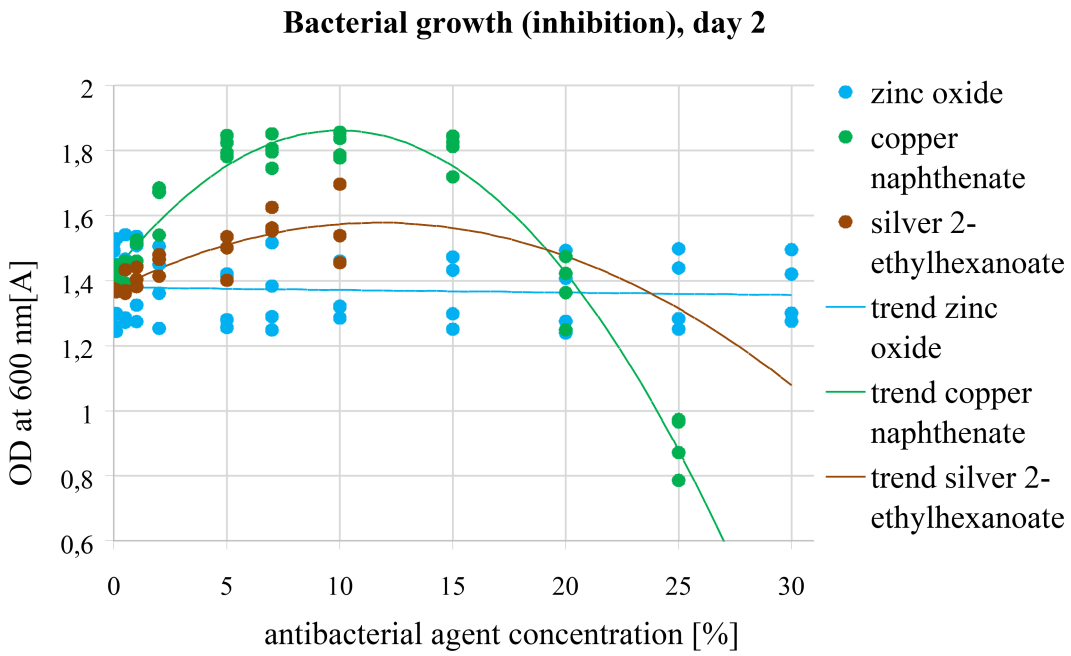


fig. 55) Bacterial growth (inhibition) in liquid medium, day two

Bacterial growth on day one with unmodified specimens as reference (see tab. 2):

tab. 2) Results bacterial growth inhibition in liquid medium

Material	**Concentration [%]**	**Mean OD_{600} [A]**	**Difference to 0.0% agent**	**Effect size [r]**
Zinc oxide	0.0	M = 1.248 SD = 0.064	--- reference	---
Zinc oxide	0.1	M = 1.234 SD = 0.063	t(6) = 0.313; p = .765; 95% confidence interval [-0.095, 0.123] → insignificant difference	0.127
Zinc oxide	0.5	M = 1.237 SD = 0.052	t(6) = 0.225; p = .808; 95% confidence interval [-0.091, 0.112] → insignificant difference	0.103
Zinc oxide	1.0	M = 1.247 SD = 0.066	t(6) = 0.016; p = .987; 95% confidence interval [-0.111, 0.113] → insignificant difference	0.007

Zinc oxide	2.0	M = 1.226 SD = 0.093	t(6) = 0.394; p = .707; 95% confidence interval [-0.116, 0.160] → insignificant difference	0.159
Zinc oxide	5.0	M = 1,224 SD = 0.077	t(6) = 0.475; p = .652; 95% confidence interval [-0.097, 0.146] → insignificant difference	0.190
Zinc oxide	7.0	M = 1.215 SD = 0.040	t(5.050) = 0.875; p = .421; 95% confidence interval [-0.059, 0.125] (Levene's test significant: F = 8.013, p = .030, degree of freedom correction from 6 to 5.050.) → insignificant difference	0.363
Zinc oxide	10.0	M = 1.223 SD = 0.044	t(5.318) = 0.639; p = .550; 95% confidence interval [-0.073, 0.123], (Levene's test significant: F = 20.604, p = .004, degree of freedom correction from 6 to 5.318.) → insignificant difference	0.267
Zinc oxide	15.0	M = 1.236 SD = 0.055	t(6) = 0.273; p = .794; 95% confidence interval [-0.092, 0.115] → insignificant difference	0.111
Zinc oxide	20.0	M = 1.213 SD = 0.051	t(6) = 0.854; p = .426; 95% confidence interval [-0.065, 0.135] → insignificant difference	0.329
Zinc oxide	25.0	M = 1.217 SD = 0.042	t(5.181) = 0.818; p = .449; 95% confidence interval [-0.062, 0.128], (Levene's test significant: F = 9.559, p = .021, degree of freedom correction from 6 to 5.118.) → insignificant difference	0.338
Zinc oxide	30.0	M = 1.244 SD = 0.028	t(4.079) = 0.108; p = .919; 95% confidence interval [-0.092, 0.100] (Levene's test significant: F = 21.225, p = .004, degree of freedom correction from 6 to 4.079.) → insignificant difference	0.053

Copper naphthenate	0.0	M = 1.202 SD = 0.027	--- reference	---
Copper naphthenate	0.1	M = 1.249 SD = 0.050	t(6) = 1.647; p = .151; 95% confidence interval [-0.116, 0.023] → insignificant difference	0.558
Copper naphthenate	0.5	M = 1.268 SD = 0.008	t(6) = -4.586; p = .004; 95% confidence interval [-0.100, -0.030] **→ significantly higher**	0.882
Copper naphthenate	1.0	M = 1.338 SD = 0.017	t(6) = -8.459; p < .001 ; 95% confidence interval [-0.175, -0.097] **→ significantly higher**	0.961
Copper naphthenate	2.0	M = 1.264 SD = 0.005	t(6) = -18.665; p < 0.001 ; 95% confidence interval [-0.295, -0.226] **→ significantly higher**	0.991
Copper naphthenate	5.0	M = 1.538 SD = 0.066	t(6) = -9.389; p < 0.001 ; 95% confidence interval [-0.423, -0.248] **→ significantly higher**	0.968
Copper naphthenate	7.0	M = 1.546 SD = 0.101	t(3.438) = -6.539; p = .005; 95% confidence interval [-0.499, -.188] (Levene's test significant: F = 16.881, p = .006, degree of freedom correction from 6 to 3.438.) **→ significantly higher**	0.962
Copper naphthenate	10.0	M = 1.553 SD = 0.110	t(3.375) = -6.212; p = .006; 95% confidence interval [-0.520, -0.182] (Levene's test significant: F = 12.500, p = .012, degree of freedom correction from 6 to 3.375.) **→ significantly higher**	0.959
Copper naphthenate	15.0	M = 1.520 SD = 0.077	t(6) = -7.801; p < 0.001; 95% confidence interval [-0.417, -0.218] **→ significantly higher**	0.954
Copper naphthenate	20.0	M = 1.128 SD = 0.159	t(3.179) = 0.992; p = .421; 95% confidence interval [-0.174, 0.323] (Levene's test signifi-	0.459

			cant: F = 16.905, p = .006, degree of freedom correction from 6 to 3.179.) → insignificant difference	
Copper naphthenate	25.0	M = 0.784 SD = 0.061	t(6) = 12.557; p < 0.001 ; 95% confidence interval [0.337, 0.501] → **significantly lower**	0.981
Silver 2-ethyl-hexanoate	0.0	M = 1.193 SD = 0.022	--- reference	---
Silver 2-ethyl-hexanoate	0.1	M = 1.200 SD = 0.043	t(6) = -0.301; p = .773; 95% confidence interval [-0.066, 0.052] → insignificant difference	0.122
Silver 2-ethyl-hexanoate	0.5	M = 1.235 SD = 0.093	t(3.339) = -0.872; p = .441; 95% confidence interval [-0.186, 0.102] (Levene's test significant: F = 10.501, p = .018, degree of freedom correction from 6 to 3.339.) → insignificant difference	0.431
Silver 2-ethyl-hexanoate	1.0	M = 1.387 SD = 0.094	t(6) = -4.013; p = .007; 95% confidence interval [-0.312, -0.076] → insignificant difference	0.854
Silver 2-ethyl-hexanoate	2.0	M = 1.462 SD = 0.034	t(6) = -13.366; p < 0.001; 95% confidence interval [-0.318, -0.220] → **significantly higher**	0.984
Silver 2-ethyl-hexanoate	5.0	M = 1.442 SD = 0.071	t(6) = -6.672; p = .001; 95% confidence interval [-0.341, -0.158] → **significantly higher**	0.939
Silver 2-ethyl-hexanoate	7.0	M = 1.397 SD = 0.043	t(6) = -8.463; p < 0.001; 95% confidence interval [-0.264, -0.145] → **significantly higher**	0.961
Silver 2-ethyl-hexanoate	10.0	M = 1.300 SD = 0.207	t(6) = -1.027; p = .344; 95% confidence interval [-0.362, 0.148] → insignificant difference	0.506

5.2 Mechanical experiments

5.2.1 3D distance

To analyse whether there was a change in the 3D distance measurements after sealing with pure silicone, 25% copper naphthenate and 10% silver 2-ethylhexanoate in comparison to an unsealed empty reference and to one-another, a two factorial ANOVA was conducted. The 3D distance was one factor, the sealing material a second factor and the implant system an additional disruptive factor. The result of the ANOVA showed a significant difference by comparison of the implant systems, $F(1) = 441.141$; $p < 0.001$; $\eta_p^2 = .809$, but no significant change in the 3D distance (within the implant system groups) with the different seals, $F(3) = .019$; $p = .996$; $\eta_p^2 = .001$; and the difference in implant systems did not change significantly with the different seals, $F(3) = .007$; $p = .999$; $\eta_p^2 = .000$ (see fig. 56). (For more detailed informations on measurements see chapter 11.3)

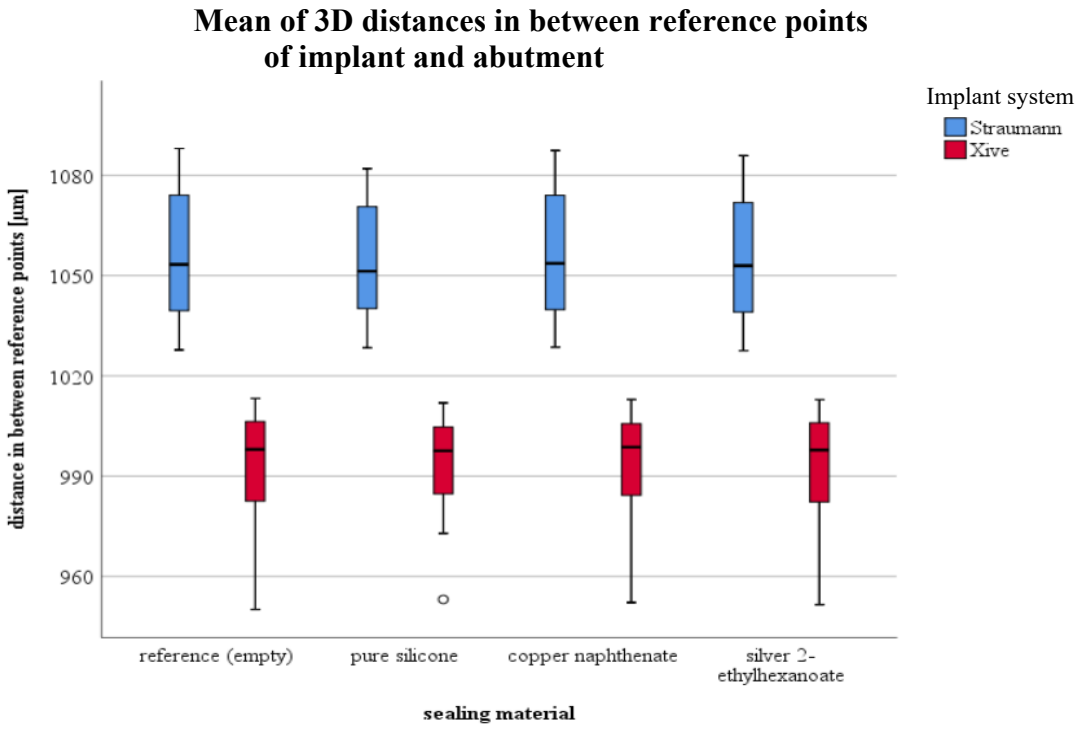


fig. 56) Comparison of means of distance in 3D measurement

5.2.2 **Opening Torque**

The opening torque showed high deviations, especially with the Straumann implant system, but paired-samples t-test showed no significant change in comparison to the empty reference in both implant systems (see fig. 57). No breakage of screws occurred. (For more detailed information on measurements see chapter 11.4)

The opening torque mean of empty Straumann implant systems (= reference) (M = -32.63 Ncm, SD = 1.49 Ncm) was insignificantly different to the opening torque mean of the sealed Straumann implant systems:

- pure silicone (M = -32.48 Ncm, SD = 1.45 Ncm, t(2) = -0.140; p = .902),
- copper naphthenate (M = -31.33 Ncm, SD = 1.80 Ncm), t(2) = 0.797; p = .509),
- silver 2-ethylhexanoate (M = -31.50 Ncm, SD = 3.33 Ncm), t(2) = 0.651; p = .582).

The opening torque mean of empty Xive® implant systems (= reference) (M = -19.85 Ncm, SD ± 0.38 Ncm) was not significantly different to the opening torque mean of the sealed Xive® implant systems:

- pure silicone (M = -32.48 Ncm, SD = 1.45 Ncm), t(2) = -0.096; p = .932),
- copper naphthenate (M = -20.03 Ncm, SD = 1.97 Ncm), t(2) = -0.198; p = .861),
- silver 2-ethylhexanoate (M = -19.92 Ncm, SD = 0.87 Ncm), t(2) = -0.210; p = .853).

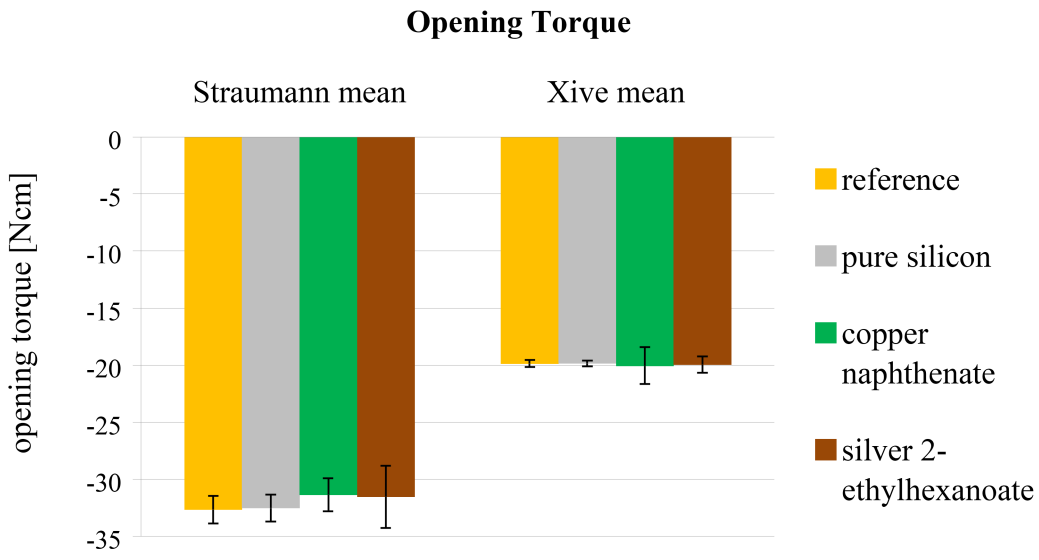


fig. 57) Comparison of means (with standard deviation) of opening torque.

5.3 Summary of the results

Even though none of the tested antibacterial modification agents showed antibacterial effects in all three microbiological experiments, there were still measurable antibacterial effects deriving from high concentrations of copper naphthenate (starting at 20%, better at 25%) and silver 2-ethylhexanoate (best at 10%). The only tested agent that displayed no antibacterial efficiency was zinc oxide. Thus, the first null hypothesis was partially rejected. Neither silicone by itself, nor the modified versions with 10% silver 2-ethylhexanoate and 25% copper naphthenate affected positioning of abutment in implant, influenced the opening torque or led to breakage of the connecting screw. Therefore, the second null hypothesis could not be rejected.

6 Discussion

6.1 In vitro study set-up

For this study, an in vitro set-up was chosen for proof of principal if it was possible to attain measurable antibacterial activity from modified silicone, and to investigate whether there would be measurable change in the positioning of the abutment in relation to the implant or change in opening torque. It would be ethically questionable to investigate such initial questions in vivo with the risk of damaging integrated implants and implant loss. Now after conclusion of this study, in vivo experiments might be a reasonable next step, but it would also be advisable to further investigate in vitro first, e.g. by choosing a similar setup to PODHORSKY et al.[113, 114] to test the seal and effect against bacteria when applied to abutment-implant interfaces and under stress.

6.2 Materials

6.2.1 Silicone as a matrix material

Silicone was chosen as a matrix material by reason of low viscosity silicone being used as a sealing material on its own already, and being quite inert to the surrounding tissues thanks to the hydrophobic nature in water-based surroundings. There are multiple studies regarding silicone on its own as a sealing material[39, 49, 61, 106, 112-114] or with an antibacterial modification[50, 77, 99]. Overall previous studies concluded that silicone could reduce the bacterial contamination, but the sealing ability could still be improved. A different matrix material might ease the integration of an antibacterial

agent, or enable a higher concentration of integrated agent. The silicone's polarity (hydrophobicity) impedes an easy integration, as well as movement of hydrophilic or neutral components within. Nonetheless, silicone does not interact with the water-based surroundings, is easily applied and retrievable, whilst not being volatile, enabling a more long-term seal. In PODHORSKY et al.'s studies they also tested grease as a sealing agent with acceptable bacterial reduction,[113, 114] but complications in retrieval from the implants' cavity and breakage of connecting screws in a follow-up study by BISCOPING et al., led to dismissal of grease as a sealing material. The study by BISCOPING et al. investigated the effect sealing materials had on the abutment-implant attachment (change in size of microgap between implant and abutment) and found cases of fractures of the connecting screw when grease was used as a sealing material.[19] Established silicone was chosen as a matrix material for this study instead. Complications in handling silicone specimens were such as a difficult control in positioning and transfer, as they tended to bounce unexpectedly of the forceps. This led to high rate of loss of test series.

The different modification agents influenced the silicone very differently. Whilst nano zinc oxide hardened the specimens, copper naphthenate and silver 2-ethylhexanoate softened them. The limit of antibacterial agent that could be integrated also derived from these differences. For zinc oxide, the viscosity became too rigid to shape standardised specimens at more than 30% zinc oxide integration. For copper naphthenate and silver 2-ethylhexanoate the setting reaction was malfunctioning at respectively more than 25% copper naphthenate and more than 10% silver 2-ethylhexanoate.

6.2.2 **Nano zinc oxide as an antibacterial agent**

In this study, the antibacterial effect of nano zinc oxide when integrated in silicone was low. Reasons for this might be an insufficient ability of the crystalline nano particles to move through silicone to reach the surface area, or the antibacterial activity of nano zinc oxide on the surface being insufficient.

The size of the particles is an important factor in antibacterial efficiency of zinc oxide[3, 151], suggesting that the antibacterial effects might be achievable with a smaller particle size. Some studies display a dependence on the presence of light for

activation of antibacterial mechanisms.[3, 64, 151] Since light does not reach a sealing material of the implant-abutment interface, the antibacterial activity needs to be strong even in the absence of light. The used nano zinc oxide might lack this ability.
Furthermore, nano zinc oxide displays a better antibacterial effect on gram positive than gram negative bacteria.[3] *Staphylococcus aureus* is gram-positive, the effect on gram negative bacteria in the peri-implant tissues being worse than the results found in this study cannot be ruled out.
In general, zinc oxide is a popular antibacterial agent in dentistry. It is widely spread in dental materials, and multiple studies have investigated different forms. There are little toxicological qualms due to zinc oxide being used for such a long time in the zinc oxide form and in so many medical products (see chapter 3.6.1) with no major negative effects.
The use in a silicone matrix in this study led to more rigidity of the specimens. This might have impacted the fit of abutment in implant negatively if the materials would have been tested in the mechanical tests.

6.2.3 **Copper naphthenate as an antibacterial agent**

Copper in general is known for great antibacterial properties (see chapter 3.6.2). And the naphthenate form seemed to greatly enhance the agents' ability to move through silicone in comparison the nano zinc oxide crystals. Since copper naphthenate has a very strong turpentine odour, which may lead to headaches if the air is not circulated proficiently during placement, and can be toxic to organisms living in water in higher concentrations, it would be advisable to test the toxicity levels before application in vivo. More specifically it would be advisable to measure the toxicology levels of the antibacterial effective concentrations (> 20%) in surrounding tissues, when used as a sealing material for the implant-abutment interface, since the space is quite closed off and might pose no risks for significant impact on surroundings. Also, the odour isolation should be tested fist to prevent patients' discomfort. Since the active component might be dissolved from the silicone, questions in regards to longevity of the antibacterial effect arise. The effect might be more temporary than from a more bound component with a more controlled agent release – since it might be washed away quickly. Also, as soon as the agent was mixed into silicone, gas formation

within the silicone started. And if the agent was mixed into just one component ahead of time and the mixture was then used for an attempt at preparing specimens, the setting reaction was impaired. Hence, the antibacterial agent had to be incorporated into the base immediately before mixing with the catalyst paste. This would complicate an in vivo application.
The gas formation led to a very rough surface, the higher the copper naphthenate concentration, the rougher the surface (larger gas blebs set on the surface area). Further in vitro testing would be advisable before in vivo testing to investigate the agent's ability to shield the implant-abutment interface from bacterial leakage under stress.

6.2.4 **Silver 2-ethylhexanoate as an antibacterial agent**

Silver by itself is well known for antibacterial effects (see chapter 3.6.3). In the microbiological experiments of this study, silver 2-ethylhexanoate was the only modification agent which induced formation of inhibition zones in direct contact on agar plates. In liquid medium, there seemed to be no notion of dissolution of antibacterial components in the surrounding medium, since there was no positive measurable antibacterial effect. The positive aspect of the agent not getting dissolved into surrounding medium might be a prolonged time the agent can have an antibacterial effect on bacteria in immediate contact – in comparison to better soluble agents which could migrate away from the desired location. One of the greatest problems with disinfection of the implant-abutment interface with chlorhexidine is the great solubility, resulting in an effect that does not seem to supersede more than three months. Less solubility might result in a longer lasting effect.
Even though there was no noticeable dissolution into surrounding liquid, the agent's movement within silicone seemed to be sufficient, as the mixture seemed to increase in homogeneity of the mixtures' colour over the subsequent days of the experiment, and there was a good antibacterial effect in formation of inhibition zones on agar plates.
Other positive sides are the agent's odourlessness, and silver complexes already being successfully used in medicine, thus raising fewer concerns in regards to biocompatibility/toxicology with negative effects on health. But even if there are less

concerns in regards to toxicology than with copper naphthenate, the most effective 10% silver 2-ethylhexanoate is still not a low dosage, and testing toxicology levels deriving from the closed of implants would be advisable before in vivo application.
Just as in case of copper naphthenate, in vitro testing of bacterial penetration into, or out of the internal cavity of implants would be preferable before in vivo application to ensure the desired antibacterial effect works when applied to implant-abutment interface sealing, and to investigate the risk of increased bacterial proliferation due to increased surface roughness.

6.2.5 **Staphylococcus aureus**

The search for a germ for this studies' experiments was defined by looking for a bacterium native to the oral microbiome, in particular that of the peri-implant microbiome, whilst being aerobe and able to form reliable biofilms. *Staphylococcus aureus* EDCC 5055 /DSM 28763 meets these requirements. *S. aureus* has been found in multiple studies to be part the oral microbiome, and sometimes even surrounding implants.[8, 62, 117] It is a common germ for in vitro studies and has been used in other studies regarding implant-abutment sealing materials.[18, 77] Alternative bacteria which are more frequently found in the peri-implant microbiome, such as *Porphyromonas gingivalis*, are usually anaerobe, therefore much more complex to work with in vitro, and not applicable for the experiments of this study. *Escherichia coli* is another germ frequently used in in vitro studies concerning sealing materials,[48, 60, 77, 113, 114] since it is a simple standard bacterium, but the biofilm formation of *Escherichia coli* was not strong enough for the biofilm assay experiment.

6.3 Methods

6.3.1 Agar diffusion test

The agar diffusion test is a simple established microbiological routine test with filter disks impregnated with antibacterial agents such as antibiotics. (see chapter 3.7.2) In this study the filter disks were replaced by the (modified) silicone disks. A similar method was also used by HOJATI et al. in 2012 for composite discs modified with zinc oxide,[135] as well as FEROZ et al. for dental luting cements[47] and ANDRADE et al. for modified temporary luting cement[9]. The difficult handling of the silicone specimens led not only to a high rate of loss of test series, but also to problems evaluating the growth from underneath the specimens, since it was unavoidable to slightly move the specimens on the agar plate whilst taking hold of them for transfer and consequently re-contaminating them with living bacteria at the margins. This was most likely the reason for the high concentrations of bacteria at the margins on the control plate for growth from underneath the specimens. The contact time with the antibacterial components of the silicone disks was not long enough to kill reintroduced bacteria before contact with the fresh agar plate, where the specimens were removed from shortly after, and with them the component which might have prevented bacterial growth. Therefore, the bacterial growth from underneath the specimens' margins on fresh agar might not display whether growth was inhibited in prolonged contact or not. If growth on the margins was inhibited in comparison to specimens without modification, it was strong evidence for effective antibacterial activity.

Instead of lysogeny broth agar plates, it is more common for *Staphylococcus aureus* to be grown on brain-heart-infusion, which enables ideal growth. But lysogeny broth was chosen instead, due the bacterial growth still being sufficient and even though nutrient composition in oral environment depends on diet, the composition of lysogeny broth might be slightly more similar to the nutrients the bacteria would encounter in an oral environment, than the high amount of protein from the brain-heart-infusion.

The capability of the bacterial agent to lead to formation of inhibition zones greatly depends on the agents' ability to move within the silicone and agar. Movement within silicone determines the utilizable amount of agent on the specimens' surface. Another factor is the solubility and diffusion in agar. Hydrophobic agents such as pure nano zinc oxide might be hindered in doing so.

The phase in bacterial growth in which the bacterial solution is spread on the agar plates determines amount and density of bacteria on the plate and is verified by measuring the optical density. Due to the transfer of specimens taking quite a while and the high rate of loss, it was impossible to plate all of the test series with the same solution, whilst still abiding to the set parameters and necessary comparable timing. Thus, multiple *S. aureus* cultures had to be set up for the different test series and materials. With each test series there was a minor variation in optical density at time of plating. This might have led to slight variations in density and amount of bacteria in the bacterial lawn, and thence slightly different levels of required antibacterial activity for a measurable effect.

6.3.2 **Biofilm assay**

For accuracy of the biofilm assay, a standardised lining of 96-well plate's wells would have been preferable, since all the steps would have been performed in the same plate and errors due to contamination from changing the plate would have been eliminated. So, preliminary to the main experiment, the practicability of lining the wells of a 96-well plate with a standardised layer of modified silicone was investigated, to try to resemble the original biofilm test as much as possible. But the modifications' setting times and viscosity with corresponding flow behaviour varied considerably, hindering the formation of a standardised layer of modified silicone along the wells' walls with a smooth surface and standardised volume. The volume left within the coated wells, even in case of achievement of a very thin layer, was too small to warrant sufficient nutrition of bacteria for the 24 hours of incubation for ample biofilm formation.

Thus, the standardised specimens as they were used in the other experiments were chosen instead. In a similar manner, CHENG et al.[29] used discs (in their case of

modified composite) in wells filled with medium and inoculated them with bacteria for measurement of biofilm formation.

Since the specimens were alike those in the agar diffusion experiment, the same difficulties in handling the specimens also applied to this experiment: The specimens tended to bounce off the forceps, either into other wells or completely off the plate. Both led to cut of test series.

One of the disadvantages of the applied method was the lack of distinction in between viable and dead bacteria in the biofilm. Both were measured. But viable bacteria may lead to much higher risks in a host than dead ones, which may be disposed of over time by the immune system, through such complications as proliferation, toxin formation and induction of inflammatory reactions.

Another problem that might have led to inaccuracy was the inability to perform the experiments with exactly the same bacterial suspension and at the same time. Small differences in bacterial density and in timing may have had an influence on the measured biofilm. Furthermore, it took more time to conduct the first few test series than the later ones, due to gain of experience in handling the specimens and working in sterile conditions. This might have had an influence on the results. Also, the need for a negative control in this experiment arose only after the test series with the modified materials were carried out, and the very different behaviour of the colour dissolving at the washing process from the modifications was seen, leading to questions in regards to the materials ability to retain colour even without formation of a biofilm. Due to the delay in display of the necessity for a negative control, it was not possible to use the same bacterial suspension for test series and negative control. But even within the test series and negative controls, not all test series could be performed at the same time whilst still abiding to the same timing within the experiment. Another factor prohibiting the use of the same bacterial suspension was the high loss rate of specimens, resulting in the need for multiple repetitions of the experiment, and in case of the negative control, some repetitions were necessary due to contaminations as well. This might explain the differences in values measured for unmodified silicone in the negative controls and test series.

In case of copper naphthenate, the reduction of the amount of colour retrieved from specimens with bacteria – and therefore a possible existence of a biofilm – was less than the amount of colour deriving from negative control specimens without bacteria – and thus no possibility of biofilm formation – leading to the fundamental question

if this experiment can be used for sensible information on biofilm formation on specimens modified with copper naphthenate, and if it gives us reliable information in general – independent of the tested modification agent. The surface texture may play to big a role in colour retention and may obstruct this experiment from obtaining meaningful data on biofilm prevalence.

6.3.3 **Bacterial growth in surrounding liquid medium**

This experiment was conducted after an observation in the biofilm assay, that the media surrounding 20.0% and 25.0% copper naphthenate specimens had taken on a green tinge and were clearer than their unmodified counterparts. Thus, this experiment investigating the optical density – due to bacterial density in the medium – followed. The method used in this experiment is a very simple and common method to evaluate bacterial density through spectroscopy (see chapter 3.7.2). The higher the absorbance of light, the higher the bacterial count within the medium. To eliminate the influence of the media's colour, each concentration in the test series was measured against a blank. The blank went through the same procedure as the test series, only without bacteria – ensuring the colouring in the control series (blank) being the same as in the test series. The only difference left to influence absorbance was the bacterial density.

A study by FEROZ et al. used a similar approach, where wells of a 96-well plate were evenly coated with a measured amount of zinc oxide eugenol and zinc polycarboxilate cement, and a bacterial suspension was incubated in the coated wells.[46] After set time spans (1 hour, 24 hour, 1 week, 1, 3 and 6 months) the changes of optical density were recorded by transferring the suspension into adjacent empty wells and measuring optical density with an enzyme-linked immunosorbent assay (ELISA) in an ELISA plate reader.[47] This set-up could not be achieved with silicone as a matrix material, as an even and comparable coating of 96-well plate wells with modified silicone was not possible, as described above (see chapter 6.3.2). Therefore, the chosen method was employed to measure the change in optical density and hence bacterial density in the suspension.

A contact killing may not be detectible through bacterial density in medium, since the bacteria not in direct contact to the specimens can still proliferate freely.

6.3.4 **Mechanical experiment**

The materials used for sealing in this last experiment were those which displayed antibacterial properties in the previous experiments (silver 2-ethylhexanoate and copper naphthenate), and pure silicone as well as empty implants as references. Only the highest concentrations of the investigated modifications were subjected to this test, as it was assumed that if neither the highest concentrations, nor pure silicone led to problems in positioning or torque, the concentrations in between would not either.

The method used in this study was based on a previous study by BISCOPING et al., where the gap in between abutment and implant – after sealing with different materials – was measured and compared to an empty reference.[19] In this study, an exact replication of the aforementioned study's method was not possible with the selected implant systems, by reason of the Straumann implant system not providing a clean reference line on the abutment, which the systems in the aforementioned study by BISCOPING et al. did. The implant systems of this study were selected in consideration of them commonly being used and having their implant-abutment interface on bone level, where the risk of bone degeneration is high.[36] To enable measuring the change in distance in comparison to the reference (= empty implant), abutment and implant were marked with reference points via laser. These reference points were one of the factors leading to less accuracy, since the positioning of the reference points was not be as precise as desired. Thus, there were some small deviations in between the different implants, even within the same system. On top of the positioning, especially in the Straumann implant systems, the angulations of the abutment surface to the laser when held in the laser device were unfavourable, resulting in more blurred edges of the reference points. To negate the disparity of the reference points, calibration of the operator measuring the distance (the author) was necessary to enable comparable measurements with little standard deviations. Also, due to the shapes of the abutments being quite different in the selected implant systems, the reference points were located in quite different 3D distances in the two systems. The 3D distances between reference points were generally much farther on the Straumann® implant system, than on the Xive® implant system. If further studies should be conducted, a more precise technique for creating reference points would be preferable.

Using a digital screwdriver for measurement of the counter torque is a method used in multiple studies.[19, 72, 89, 100, 152] The precision in torque application and measurement depended greatly on the conductor. To meet the required torque values (as recommended by the manufacturer) with the used digital screwdriver, a lot of practice was needed to attain the required precision, as the screwdriver only measured the applied torque, but did not stop automatically when the desired torque was reached. The human error might have been decreased by use of a fully automated system. But with practice and caution, the desired precise torque values could be reached by manual assembly as well. An alternative to the used digital screwdriver would have been the wrench provided by the manufacturer, but they have high standard deviations, depending on the system:[24] ERDEM et al. for example, found a deviation of 14.34% in case of Straumann systems.[44]

Drawbacks of the three point clasp are the risk of damaging the implant, and a loosening of the hold during bolting – resulting in reduced control during bolting. An alternative to the three point holding device used in this experiment would have been embedding the implants in resin, similar to an osseointegrated implant being embedded in bone, as was done in a study by MARTINS et al.[86] That method was discarded, as it would have complicated the handling of the implants during the 3D distance measurements. This far, holding the implant in a wench, or a two or three point clasp is the most common method for fixation of implants during bolting.[19, 24, 72, 113, 152]

6.4 Results

6.4.1 Zinc oxide

Nano zinc oxide did not display any antibacterial effects in the microbiological experiments. There was no formation of inhibition zones surrounding specimens modified with nano zinc oxide, nor reduction in bacterial growth beneath the specimens. This finding was in accordance with HOJATI et al.'s results in composite modified with zinc oxide.[135] They used a matrix of composite, which also sets and is hydrophobic, too. The lack of movement of zinc oxide nano particles within the matrix might be similar. Another study that showed similar results, as in no formation of inhibition zones, was a study by ANDRADE et al., in which inhibition zone formation on agar was investigated around temporary cement specimens, modified with nano zinc oxide (against *Streptococcus mutans*).[9] A study by BOYD et al. on the other hand found inhibition zones surrounding glass polyalkenoate cements modified with zinc oxide.[20] These contradicting results suggest a high influence of the chosen matrix material, or great differences in forms of zinc oxides on antibacterial effectiveness.

The biofilm assay displayed a modest biofilm formation on modified silicone, riding on the edge of significance, with a minor increase in significance in higher agent concentrations. A possible explanation for this effect might be the slightly rougher surface. A study by AYDIN SEVINC and HANLEY showed different results with zinc oxide modifications of composite disks: a lessened biofilm formation in the first day, but no noticeable difference in comparison to the unmodified control group after three days.[13] The differences could be explained on one hand by the different matrix material – polished composite may have a much smoother surface and therefore inhibit bacterial attachment more effectively – and a different zinc oxide. Also, they used a different method in measuring biofilm formation, in which only viable bacteria were counted, which were mechanically removed from the specimen surface.[13] The method applied in this experiment did not differentiate in between viable and dead bacteria.

Furthermore, there was no measurable inhibition of bacterial growth in liquid medium surrounding specimens. The slightly elevated values on the second day, in comparison to the first day, were most likely due to the concentration of bacteria (when 90% of the medium was removed for measurements of optical density on day one, and the remaining bacteria were supplied with new nutrients).
FEROZ et al. found antibacterial growth inhibition (change in optical density) in medium in direct contact with zinc oxide based products (different forms of zinc containing cements),[47] but this study did not. This suggests the influence of the matrix material (silicone) on antibacterial efficiency of (nano) zinc oxide particles in bacterial growth inhibition in surrounding liquids.
Since no measurable antibacterial effects could be found, zinc oxide was disregarded as an antibacterial modification agent in a silicone matrix and not included in the mechanical tests. Zinc might be usable for silicone modification in different forms than zinc oxide, a form that permits more movement within the silicone, or as a modification agent in different matrixes.

6.4.2 **Copper naphthenate**

High concentrations of copper naphthenate, starting at 20%, but with better results at 25%, displayed antibacterial effects in liquid environments (found in the biofilm assay and bacterial growth inhibition in liquid medium). Copper naphthenate was the only component with a visible inhibition of bacterial growth in surrounding liquid medium. Either components of copper naphthenate, or copper naphthenate as a whole, went into solution in surrounding liquid medium, resulting in a visible green tinge (see fig. 24). Only very high concentrations of copper naphthenate, starting at 20%, showed antibacterial effects. The antibacterial activity on agar did not show such promising results. But even though there was no formation of inhibition zones, the density of the bacterial lawn at the centre underneath the specimens of 25%, and slightly at 20%, was less than underneath unmodified specimens, or those with low concentrations, implying an antibacterial effect on bacteria in direct contact. But the effect is not distinct enough to accredit antibacterial efficiency on agar, as the sample size of n = 3 per concentration was too small for proficient evaluation of such minor distinction. But if the antibacterial effect functions mainly through dissolution of

components out of the material into the surrounding tissues, the risk of the agent diffusing away from the desired area should be investigated before undertaking of in vivo experimentations.

The increased surface roughness of specimens modified with copper naphthenate (through gas formation from chemical reactions during blending of the components) may explain the negative impact of copper naphthenate modifications on biofilm formation (measured in optical density of decolouring agent) in lower concentrations than 20%, with a peak at 7%. The greater surface roughness may enable better bacterial adhesion. The roughness intensifies with increasing copper concentrations, but the effect may be negated by the antibacterial effect of copper naphthenate, leading to the decrease and, starting at 20% copper naphthenate, to less optical density in the decolouring agent than on the negative control (as discussed in chapter 6.3.2).

Another hypothesis for the digression of colour deriving from the specimens, to less than from the negative control, could be a filling of the deep dents in the surface in high concentrations – resulting in a more smoothed out surface, with a biofilm colouration and discolouration similar to the lower concentrations (the optical density does not change much in between the concentrations of 5% and 20%). The surface on the negative control may retain more colour in the deep dents, or the retained colour might be more easily disassociated from silicone than from bacterial surfaces into the discolouring agent. This might explain the measurement of lower optical density in the high concentrations, than in the negative control with impossible biofilm formation due to a lack of bacteria.

It is possible that the test for biofilm formation is generally not applicable to copper naphthenate specimens, for reasons described above (see chapter 6.3.2), mainly due to too much influence of the surface texture on colour retention.

In case of the measurement of bacterial growth in surrounding medium, the surface roughness is a plausible explanation for the increase of optical density at less than 20% copper naphthenate. In those concentrations the bacterial growth is not yet inhibited (with a peak at 10%), but instead either the bacterial proliferation is promoted, or the dissolution of colour is enhanced. The effect is negated with the sufficient antibacterial activity starting at 20%. The elevated level on the second day with a similar progression to the first day can be explained with the concentration of bacteria, when 90% of the medium is taken off for measurement of optical density on

the first day and then replenished with fresh medium, guaranteeing sufficient nutrition. The lesser amount of bacteria in medium surrounding 25% copper naphthenate specimens, in comparison to medium surrounding unmodified specimens, was visible to the naked eye, giving the measured decrease in bacteria credit. The liquid was much clearer at 25% than at 0% copper naphthenate and the bacterial pallet, that formed at the bottom of the caps in which remaining medium of the biofilm experiment was stored in, was also remarkably smaller.

It might be interesting to search for a different copper containing compound with a more controlled copper release, and which does not lead to gas formation through chemical reactions with silicone resulting in the rough surface.

There was no measurable mechanical influence on the implant system. The only significant difference in the 3D distance in between reference points was detected between the different implant systems, but that was due to the different implant shapes, in particular the much greater angulation of emergence profile of the Straumann abutment, resulting in different 3D distances of the reference points on the implant systems. The seal did not result in any significant change in the opening torque, neither in comparison to a seal with pure silicone, nor without application of a sealing material. Also, no breakage of screws occurred, which would have been one on of the more feared complications in implant treatments, which would have led to instant dismissal of the material. Retrieving broken screws from within the implant sets the implant up for risk of damage. Therefore, the assumption that there is no change in implant position after a seal with silicone modified with up to 25% copper naphthenate can be made, and there were no mechanical counterarguments found for usage of copper naphthenate modified silicone as a sealing material.

6.4.3 **Silver 2-ethylhexanoate**

Silver 2-ethylhexanoate was the only tested agent that induced formation of inhibition zones. The preliminary experiment revealed a start of inhibition zone formation in between 2% and 5%. The follow-up detailed experiment demonstrated a correlation in between concentration and inhibition zone size. The greatest and most reliable inhibition zones were achieved with 10% and 8% silver 2-ethylhexanoate. The specimens' transfer to a new plate and immediate disposal after contact with the

fresh medium revealed a reduced amount of viable bacteria from underneath the specimens. The least amounts of viable bacteria were found underneath 10% and 8% silver 2-ethylhexanoate. The remaining viable bacteria were all found at the specimens' edges. They might have been freshly transferred onto the specimens from the bacterial lawn in the original plate, as a little movement of the specimens across the plate at transfer was unavoidable. The movement also explains the general observation of much more bacteria growing from underneath the specimens' edges. Another additional reason might be a rougher surface at the specimens' edges in comparison to the centre, enabling more bacterial attachment.

In regards to biofilm formation, the impact of silver 2-ethylhexanoate modifications was minor. The only statistically significant differences were found in very low concentrations of silver 2-ethylhexanoate. In those concentrations, there were no measurable antibacterial activities in the agar diffusion test either. At higher concentrations – starting at 5% (the same starting point where there was formation of inhibition zones in the preliminary agar diffusion test, too) – the optical density, deriving from colour retained by the specimens, did not differ significantly from test series to negative control without bacteria. In the few concentrations that showed a statistically significant difference to the negative control, the differences might be explained by minor differences in the bacterial solutions (as the same one could not be used whilst abiding to the timing) or slight timing differences, as the conduction of the experiments had a learning curve in handling specimens and equipment, which led to a slightly more rapid procedure at the point in time when the negative controls were measured. Also silver 2-ethylhexanoate modification led to gas formation in the mixing process, similar to, but at a lower level as copper naphthenate. This resulted in a rougher specimen surface, too, even though not as severely as in copper naphthenate modifications. As discussed in chapter 6.3.2, the validity of the obtained information on biofilm formation is questionable, due to the unknown impact of the surface structure on the colour retention. But the results of the experiment do not imply a major impact of silver 2-ethylhexanoate on biofilm formation, therefore no increased formation would be expected.

In contrast to the great antibacterial effects high silver 2-ethylhexanoate concentrations showed in the agar diffusion test, and in contrast to the great effect copper naphthenate had on the bacterial density in surrounding liquid, the effect of silver 2-ethylhexanoate on surrounding liquid medium was not positive. The optical

density was elevated in comparison to the negative control and increased with higher agent concentrations. It is possible that silver 2-ethylhexanoate only kills bacteria in immediate contact, because the agent cannot be (sufficiently) dissolved out of the specimens to kill bacteria in surrounding medium, therefore showing no influence on proliferation of bacteria in not immediate contact with the specimens. But this does not explain the increase in optical density. Rather, no to little change would be expected in such a case, as it is seen in zinc oxide modifications. The curve of the first day suggests a development similar to copper naphthenate, maybe due to the rougher surface, or a stimulation of colour release from the specimens into the medium in the presence of bacteria, leading to an increased optical density of the medium. But since the silver 2-ethylhexanoate concentration could not exceed 10% without inhibiting the setting reaction, the antibacterial effect could not get strong enough to result in measurable bacterial reduction in the surrounding medium. The bacterial concentration is quite high, so no reduction is no proof of lacking antibacterial effect, but rather of an insufficient reach of the target in liquid medium, or of the amount of bacteria superseding antibacterial efficiency. But the exact reason for increase in optical density warrants further investigation before in vivo application, as a bacterial growth stimulation in surrounding tissues would be opposing to the desired effect, and the possibility should be precluded before in vivo experimentation. But the effect should be investigated after application in the implant abutment interface, as the impact might be quite low in comparison to the material lying openly, in direct contact to tissues, due to the closed-off nature of the interface.

The lack of the indicated decrease of optical density at 10% silver 2-ethylhexanoate in the measurements of the second day, in contrast to the first day, may be explained by a negation of the antibacterial effect through the concentration of bacteria and the new supply of nutrients. A repetition with a higher sample size would help to evaluate, if the decrease at 10% agent on the first day was by chance, or a reliable effect.

No mechanical influence on the implant systems could be detected in these experiments. Just like copper naphthenate modifications, silver 2-ethylhexanoate modifications did not lead to changes in abutment positioning in implant, or significant changes in the opening torque. Also, no damage to the screws or any other part of the implant systems occurred.

6.5 Conclusion

Based on this experimental set-up, silver 2-ethylhexanoate and copper naphthenate seem to be suitable for antibacterial modification of implant-abutment interface sealing silicone. Antibacterial activity could be measured from both modification agents, and there was neither an impairment of position of abutment in implant and opening torque, nor did the modifications lead to breakage in the connecting screw. Silver 2-ethylhexanoate would be preferable for patient's comfort (odourless, in contrast to the strong turpentine odour of copper naphthenate). But further research should be conducted in vitro, before in vivo experimentations. The toxicity of copper naphthenate, when sealed in the implant-abutment interface, should be investigated due to the necessity of high concentrations (25%) for reliable antibacterial efficiency. Also, the prospect of silver 2-ethylhexanoate having a negative influence on bacterial proliferation in adjoining liquids or tissues should be refuted before in vivo experimentation. Additionally, the bacterial seal of the implant-abutment interface after stress could be investigated in vitro before in vivo application.

7 Summary

In search of a solution to the insufficient seal of implant-abutment interfaces, with persisting bacterial penetration into the inner cavity of implants and therefore the possibility of bacterial leakage into surrounding tissues, an antibacterial modification of sealing silicone for the implant-abutment interface was investigated. One of the main reasons bacterial leakage should be avoided is an increased inflammation (peri-implantitis) with the risk of implant loss. Three agents for modification were microbiologically tested in regards to their ability to maintain their antibacterial properties and bestow them to a silicone matrix after integration. In case of microbiological success, further mechanical tests followed with the material being applied to the implant-abutment interface. The chosen agents were zinc oxide, copper naphthenate and silver 2-ethylhexanoate. Pure zinc oxide, copper and silver are known for their antibacterial properties.

The microbiological experiments consisted of an agar diffusion test, biofilm assay and a measurement of bacterial growth (inhibition) in liquid medium.

Zinc oxide, integrated into the silicone matrix, did not display antibacterial activity in any of the three experiments. Thus, zinc oxide was dismissed for subsequent mechanical experiments.

High concentrations (> 20%) of copper naphthenate displayed great antibacterial efficiency in inhibiting bacterial growth in surrounding liquid medium. Also, there was a measured decrease in biofilm formation, but the results may rather be due to an unexpected change in colour retention, than due to less biofilm formation. Agar diffusion test did neither show inhibition zone formation around specimens, nor a reliable inhibition of bacterial growth in direct contact (underneath the specimens).

Silver 2-ethylhexanoate led to formation of inhibition zones in the agar diffusion test with a positive correlation between agent concentration and inhibition zone size. The best results were achieved at 10% and 8% silver 2-ethylhexanoate. Biofilm formation was not too greatly influenced by the modification and there was no measurable bacterial growth inhibition in surrounding liquid medium, but rather more of an optical density (the method of measuring bacterial density).

In the subsequent mechanical experiments the change in opening torque, as well as the change in the 3D distance between two measuring points – one on the implant, one on the abutment – were measured to evaluate whether the desired positioning was still reached after modification. Seals with 25% copper naphthenate modifications were compared to seals with 10% silver 2-ethylhexanoate and references: a pure silicone seal and empty implants.

There was no significant influence of any of the tested materials on opening torque and positioning. Also, no breakage of connecting screws occurred.

Within the limits of this study, silver 2-ethylhexanoate and copper naphthenate seem to be suitable for antibacterial modifications of silicone in implant abutment sealing, but further in vitro research should be conducted before in vivo application: in case of silver 2-ethylhexanoate to rule out a negative effect on bacterial counts in surrounding tissues, and in case of copper naphthenate mainly in regards to toxicology and odour.

8 Zusammenfassung

Auf der Suche nach einer langfristigen Lösung des Problems der Undichtigkeit des Implantat-Abutment-Interfaces, mit bakterieller Besiedelung des Implantat-Innenraums und damit auch der Gefahr des Austritts von Bakterien aus dem Implantat-Inneren in das umgebende Gewebe, wurde eine antibakterielle Modifikation eines Abdichtungs-Silikons untersucht. Ein Austritt von Bakterien aus dem Implantat-Innenraum sollte vermieden werden, da sie zu einer verstärkten Entzündung des umgebenden Gewebes (Periimplantitis) mit dem Risiko des Implantatverlusts führen könnten. Drei Modifikations-Agenzien wurden mikrobiologisch untersucht, um festzustellen, ob sie in der Lage sind ihre antibakterielle Wirkung auch nach Integration in Silikon beizubehalten und auszuwirken. Im Falle von messbarem Erfolg wurden die Materialien im Implantat-Abutment-Interface mechanisch untersucht. Bei den untersuchten Agenzien handelte es sich um Zinkoxid, Kupfernaphthenat und Silber 2,Ethyl-Hexanoat. Pures Zinkoxid, Silber und Kupfer sind bekannt für ihre guten antibakteriellen Eigenschaften.

Die mikrobiologischen Experimente waren ein Agar-Diffusions-Test, Biofilm Assay und die Messung des bakteriellen Wachstums (Inhibition) im flüssigen Medium.

In Silikon integriertes Zinkoxid zeigte keine messbare antibakterielle Wirkung in den mikrobiologischen Experimenten und wurde daher ausgeschlossen von den weiterführenden mechanischen Experimenten.

Hohe Konzentrationen von Kupfernaphthenat (> 20%) zeigten sehr gute antibakterielle Wirkung in der Inhibition des bakteriellen Wachstums in umgebendem flüssigen Medium. Es wurde auch eine Abnahme in der Biofilm-Bildung gemessen, aber die Ergebnisse kommen wahrscheinlicher durch eine unerwartete Änderung in der Retention von Farbe an Probekörpern, als durch tatsächliche Minderung der Biofilm-Bildung. Der Agar-Diffusions-Test zeigte weder eine Bildung von Inhibitions-Zonen um Probekörper, noch eine verlässliche Inhibition von bakteriellem Wachstum in direktem Kontakt unter den Probekörpern.

Silber 2,Ethyl-Hexanoat führte mit einer positive Korrelation zwischen Agens-Konzentration und Inhibitionszonen-Größe zu Bildung von Inhibitionszonen um die

Probekörper. Die besten Ergebnisse wurden mit 10% und 8% Silber 2,Ethyl-Hexanoat erreicht. Die Biofilm-Bildung wurde nicht messbar beeinflusst durch Modifikation und die bakterielle Dichte in umgebendem flüssigen Medium wurde nicht verringert, eher scheint die gemessene optische Dichte des Mediums (Methode zum Bestimmen der Menge der Bakterien im Medium) sogar erhöht zu sein.

In den nachfolgenden mechanischen Experimenten wurde die Veränderung im Drehmoment beim Öffnen der Implantat-Abutment-Verbindung, sowie in der 3D-Distanz zwischen zwei Messpunkten auf Implantat und Abutment – zur Beurteilung von Veränderungen in Positionierung des Abutments zum Implantat – nach Anwendung von modifiziertem Silikon untersucht. 25% Kupfernaphthenat und 10% Silber 2,Ethyl-Hexanoat wurden mit purem Silikon und leeren Implantaten verglichen.

Es gab keine signifikante Veränderung von Drehmoment beim Öffnen der Verbindung und in der Positionierung. Es trat auch kein Schraubenbruch auf.

Unter den Limitationen dieser Studie scheinen Silber 2,Ethyl-Hexanoat und Kupfernaphthenat geeignete antibakterielle Modifikationsagenzien zu sein für die Abdichtung des Implantat-Abutment-Interfaces mittels Silikon. Sie sollten jedoch vor Anwendung in-vivo weitergehend in-vitro untersucht werden: bei Silber 2,Ethyl-Hexanoat um auszuschließen, dass es zu einer vermehrten bakteriellen Proliferation in umgebendem Gewebe kommt und bei Kupfernaphthenat sollte hauptsächlich die Toxikologie und Geruchsabriegelung vor in vivo Anwendung abgeklärt werden.

9 Figure, picture and table index

9.1 Figures and pictures

9.2 Tables

10 Register of literature

1. **Abraham, C. M.** A Brief Historical Perspective on Dental Implants, Their Surface Coatings and Treatments. *Open Dent J*; 8: 50-55; 2014. doi:10.2174/1874210601408010050

2. **Acosta-Torres, L. S.; Mendieta, I.; Nunez-Anita, R. E.; Cajero-Juarez, M.; Castano, V. M.** Cytocompatible antifungal acrylic resin containing silver nanoparticles for dentures. *Int J Nanomed*; 7: 4777-4786; 2012. doi:10.2147/IJN.S32391

3. **Adams, L.; Lyon, D.; Alvarez, P.** Comparative Eco-Toxicity of Nanoscale TiO2, SiO2, and ZnO Water Suspensions. *Water Res*; 40(19): 3527-3532; 2006. doi: 10.1016/j.watres.2006.08.004

4. **Adell, R.; Lekholm, U.; Rockler, B.; Branemark, P. I.** A 15-year study of osseointegrated implants in the treatment of the edentulous jaw. *Int J Oral Surg*; 10(6): 387-416; 1981. doi: 10.1016/s0300-9785(81)80077-4

5. **Al-Madi, E. M.; Al-Jamie, M. A.; Al-Owaid, N. M.; Almohaimede, A. A.; Al-Owid, A. M.** Antibacterial efficacy of silver diamine fluoride as a root canal irrigant. *Clin Exp Dent Res*; 5(5): 551-556; 2019. doi: 10.1002/cre2.222

6. **Albrektsson, T.; Branemark, P. I.; Hansson, H. A.; Lindstrom, J.** Osseo integrated titanium implants. Requirements for ensuring a long-lasting, direct bone-to-implant anchorage in man. *Acta Orthop Scand*; 52(2): 155-170; 1981. doi: 10.3109/17453678108991776

7. **Aldhayan, I. ; Qassadi, W. ; AlShehri, T. ; Alshehri, A.; ALonazi, K**. Review on Dental Implantology. *Egypt J Hosp Med*; 71(1): 2217-2225; 2018. doi:10.12816/0045293

8. **Allaker, R. P.** The use of nanoparticles to control oral biofilm formation. *J Dent Res*; 89(11): 1175-1186; 2010. doi: 10.1177/0022034510377794

9. **Andrade, V.; Martinez, A.; Rojas, N.; Bello-Toledo, H.; Flores, P.; Sanchez-Sanhueza, G.; Catalan, A.** Antibacterial activity against Streptococcus mutans and diametrical tensile strength of an interim cement modified with zinc oxide nanoparticles and terpenes: An in vitro study. *J Prosthet Dent*; 119(5): 862.e861-862.e867; 2018. doi: 10.1016/j.prosdent.2017.09.015

10. **Angele-Martinez, C.; Nguyen, K. V.; Ameer, F. S.; Anker, J. N.; Brumaghim, J. L.** Reactive oxygen species generation by copper(II) oxide nanoparticles determined by DNA damage assays and EPR spectroscopy. *Nanotoxicology*; 11(2): 278-288; 2017. doi: 10.1080/17435390.2017.1293750

11. **Anusavice, K. J.; Brantley, W. A.** Physical Properties of Dental Materials. In: I Anusavice, K.J. (ed.). Phillips' Science of Dental Materials. St. Louis (US): *Saunders, an imprint of Elsevier*; Edition 11. Chapter 3: 41-72; 2003.

12. **Apatzidou, D.; Lappin, D. F.; Hamilton, G.; Papadopoulos, C. A.; Konstantinidis, A.; Riggio, M. P.** Microbiome of peri-implantitis affected and healthy dental sites in patients with a history of chronic periodontitis. *Arch Oral Biol*; 83: 145-152; 2017. doi: 10.1016/j.archoralbio.2017.07.007

13. **Aydin Sevinç, B.; Hanley, L.** Antibacterial activity of dental composites containing zinc oxide nanoparticles. *J Biomed Mater Res B Appl Biomater*; 94(1): 22-31; 2010. doi: 10.1002/jbm.b.31620

14. **Becker, K.; Skov, R.L.; Eiff, C. von**. Bacteriology - Staphylococcus, Micrococcus and Other Catalase-Positive Cocci. In: Jorgensen, J. H.; Pfaller, M. A.; Caroll, K. C.; Funke, G.; Landry, M. L.; Richter, S. S., et al. (ed.). Manual of clinical microbiology. Washington D.C. (US): *ASM Press*; Vol. 1. Edition 11. Chapter 21: 354-364; 2015.

15. **Belibasakis, G. N.** Microbiological and immuno-pathological aspects of peri-implant diseases. *Arch Oral Biol*; 59(1): 66-72; 2014. doi: 10.1016/j.archoralbio.2013.09.013

16. **Belibasakis, G. N.; Charalampakis, G.; Bostanci, N.; Stadlinger, B.** Peri-implant infections of oral biofilm etiology. *Adv Exp Med Biol*; 830: 69-84; 2015. doi: 10.1007/978-3-319-11038-7_4

17. **Bell, J. W.; Fraunhofer, J. A. von**. The handling of elastomeric impression materials: a review. *J Dent*; 3(5): 229-237; 1975. doi: 10.1016/0300-5712(75)90129-3

18. **Besimo, C. E.; Guindy, J. S.; Lewetag, D.; Meyer, J.** Prevention of bacterial leakage into and from prefabricated screw-retained crowns on implants in vitro. *Int J Oral Maxillofac Implants*; 14(5): 654-660; 1999.

19. **Biscoping, S.; Ruttmann, E.; Rehmann, P.; Wöstmann, B.** Do Sealing Materials Influence Superstructure Attachement in Implants? *Int J Prosthodont*; 31(2): 163-165; 2018. doi: 10.11607/ijp.5530

20. **Boyd, D.; Li, H.; Tanner, D. A.; Towler, M. R.; Wall, J. G.** The anti-bacterial effects of zinc ion migration from zinc-based glass polyalkenoate cements. *J Mater Sci Mater Med*; 17(6): 489-494; 2006. doi: 10.1007/s10856-006-8930-6

21. **Branemark, P. I.** Osseointegration and its experimental background. *J Prosthet Dent*; 50(3): 399-410; 1983. doi: 10.1016/s0022-3913(83)80101-2

22. **Brayner, R.; Ferrari-Iliou, R.; Brivois, N.; Djediat, S.; Benedetti, M. F.; Fievet, F.** Toxicological impact studies based on Escherichia coli bacteria in ultrafine ZnO nanoparticles colloidal medium. *Nano Lett*; 6(4): 866-870; 2006. doi: 10.1021/nl052326h

23. **Brient, J. A.; Wessner, P. J.; Doyle, M. N.** Naphthenic Acids. In: Kirk-Othmer Encyclopedia of Chemical Technology. *John Wiley & Sons, Inc.* 2000. doi: 10.1002/0471238961.1401160802180905.a01.

24. **Britton-Vidal, E.; Baker, P.; Mettenburg, D.; Pannu, D. S.; Looney, S. W.; Londono, J.; Rueggeberg, F. A.** Accuracy and precision of as-received implant torque wrenches. *J Prosthet Dent*; 112(1): 811-816; 2014. doi: 10.1016/j.prosdent.2016.07.005

25. **Broggini, N.; McManus, L. M.; Hermann, J. S.; Medina, R.; Schenk, R. K.; Buser, D.; Cochran, D. L.** Peri-implant inflammation defined by the implant-abutment interface. *J Dent Res*; 85(5): 473-478; 2006. doi:10.1177/154405910608500515

26. **Broggini, N.; McManus, L. M.; Hermann, J. S.; Medina, R. U.; Oates, T. W.; Schenk, R. K.; Buser, D.; Mellonig, J. T.; Cochran, D. L.** Persistent acute inflammation at the implant-abutment interface. *J Dent Res*; 82(3): 232-237; 2003. doi:10.1177/154405910308200316

27. **Brown, T. L.; LeMay, H. E.; Bursten, B. E.** Chemie der Nichtmetalle. In: Chemie - Die zentrale Wissenschaft. München (DE): *Pearson Studium*; Edition 10. Chapter 22: 1081-1146; 2007.

28. **Buzello, AM; Schütt-Gerowitt, H; Niedermeier, W**. Desinfizierende Spüllösungen zur Keimzahlreduktion im Interface zwischen Implantat und Aufbau. *Z Zahnarztl Implantol*; 21(4): 216-223; 2005.

29. **Cheng, L.; Weir, M. D.; Xu, H. H.; Antonucci, J. M.; Lin, N. J.; Lin-Gibson, S.; Xu, S. M.; Zhou, X.** Effect of amorphous calcium phosphate and silver nanocomposites on dental plaque microcosm biofilms. *J Biomed Mater Res B Appl Biomater*; 100(5): 1378-1386; 2012. doi:10.1002/jbm.b.32709

30. **Choi, S.-H.; Jang, Y.-S.; Jang, J.-H.; Bae, T.-S.; Lee, S.-J.; Lee, M. H.** Enhanced antibacterial activity of titanium by surface modification with polydopamine and silver for dental implant application. *J Appl Biomater Funct Mater*; 17(3): 228080001984706; 2019. doi:10.1177/2280800019847067

31. **Cionca, N.; Hashim, D.; Mombelli, A.** Zirconia dental implants: where are we now, and where are we heading? *Periodontol 2000*; 73(1): 241-258; 2017. doi:10.1111/prd.12180

32. **Cohen, J.** A Power Primer. *Psychol Bull*; 112(1): 155-159; 1992. doi:10.1037//0033-2909.112.1.155

33. **Corrêa, J. M.; Mori, M.; Sanches, H. L.; da Cruz, A. D.; Poiate, E., Jr.; Poiate, I. A. V. P.** . Silver nanoparticles in dental biomaterials. *Int J Biomater*; 2015: 485275; 2015. doi:10.1155/2015/485275

34. **D'Ercole, S.; Tete, S.; Catamo, G.; Sammartino, G.; Femminella, B.; Tripodi, D.; Spoto, G.; Paolantonio, M.** Microbiological and biochemical effectiveness of an antiseptic gel on the bacterial contamination of the inner space of dental implants: a 3-month human longitudinal study. *Int J Immunopathol Pharmacol*; 22(4): 1019-1026; 2009. doi: 10.1177/039463200902200417

35. **Daugela, P.; Oziunas, R.; Zekonis, G.** Antibacterial potential of contemporary dental luting cements. *Stomatologija*; 10(1): 16-21; 2008.

36. **Dibart, S.; Warbington, M.; Su, M. F.; Skobe, Z.** In vitro evaluation of the implant-abutment bacterial seal: the locking taper system. *Int J Oral Maxillofac Implants*; 20(5): 732-737; 2005.

37. **do Nascimento, C.; Miani, P. K.; Pedrazzi, V.; Goncalves, R. B.; Ribeiro, R. F.; Faria, A. C.; Macedo, A. P.; de Albuquerque, R. F., Jr.** Leakage of saliva through the implant-abutment interface: in vitro evaluation of three different implant connections under unloaded and loaded conditions. *Int J Oral Maxillofac Implants*; 27(3): 551-560; 2012.

38. **do Nascimento, C.; Nogueira Fernandes, F. H. C.; Teixeira, W.; Pedrazzi, V.** Iodoform and silver-coated abutments preventing bacterial leakage through the implant-abutment interfaces: In vitro analysis using molecular-based method. *Arch Oral Biol*; 105: 65-71; 2019. doi: 10.1016/j.archoralbio.2019.06.009

39. **Duarte, A. R.; Rossetti, P. H.; Rossetti, L. M.; Torres, S. A.; Bonachela, W. C.** In vitro sealing ability of two materials at five different implant-abutment surfaces. *J Periodontol*; 77(11): 1828-1832; 2006. doi:10.1902/jop.2006.060101

40. **Eckhardt, S.; Brunetto, P. S.; Gagnon, J.; Priebe, M.; Giese, B.; Fromm, K. M.** Nanobio silver: its interactions with peptides and bacteria, and its uses in medicine. *Chem Rev*; 113(7): 4708-4754; 2013. doi:10.1021/cr300288v

41. **Editors of the American Heritage® Dictionaries**. "Implant". In: The American Heritage® Dictionary of the English Language. Edition 5. *Houghton Mifflin Harcourt Publishing Company*; 2020

42. **Editors of the Duden (Dictionary), [o.J.]** "Implantat, das". In: Duden online. *Dudenverlag*; Access Date: 09.08.2018; Link: https://www.duden.de/node/705885/revisions/1201904/view.

43. **Editors of the Pschyrembel: Clinical Dictionary**. "Implantat, dentales". In: Pschyrembel: Klinisches Wörterbuch. Edition 267. *Walter de Gruyter*; 2017

44. **Erdem, M. A.; Karatasli, B.; Dinçer Kose, O.; Kose, T. E.; Çene, E.; Aydın Aya, S.; Cankaya, A. B.** The Accuracy of New and Aged Mechanical Torque Devices Employed in Five Dental Implant Systems. *Biomed Res Int*; 2017: 8652720; 2017. doi:10.1155/2017/8652720

45. **Evasic, R. W.** Intramucosal implants: A review of concepts and techniques - Single inserts and tandem denserts. *J Prosthet Dent*; 49(5): 695-701; 1983. doi:10.1016/0022-3913(83)90399-2

46. **Febré, N.; Silva, V.; Báez, A.; Palza, H.; Delgado, K.; Aburto, I.; Silva, V.** Comportamiento antibacteriano de partículas de cobre frente a microorganismos obtenidos de úlceras crónicas infectadas y su relación con la resistencia a antimicrobianos de uso común. *Rev Med Chil*; 144(12): 1523-1530; 2016. doi:10.4067/S0034-98872016001200003.

47. **Feroz, S.; Bhoyar, A.; Khan, S.** Comparative Evaluation of Antibacterial Effect of Dental Luting Cements on Streptococcus mutans and Lactobacillus acidophilus: An In vitro Study. *J Contemp Dent Pract*; 17(12): 973-977; 2016. doi:10.5005/jp-journals-10024-1966

48. **Ferracane, J. L.** Composition of Impression Materials. In: Materials in Dentistry: Principles and Applications. Maryland (US), Pennsylvania (US): *Lippincott Williams & Wilkins*; Edition 2. Chapter 8: 177-183; 2001.

49. **Ferreira, L. A.; Carlos Eduardo Francischone, C. A.; Bruno Salles Sotto-Maior, B. S.; Cecília Pedroso Turssi, C. P.; Milton Edson Miranda, M. E.; de Vasconcellos, A. A.; de Carvalho, R. S.** Effectiveness of kiero seal material against the bacterial infiltration on the implant/abutment interface. *Int J Clin Dent*; 10(1): 61-66; 2017.

50. **Fritzemeier, C. U.** Periimplantitisprophylaxe bei zusammengesetzten Implantaten - Versiegelung von Implantatinnenräumen. *DZW*;46: 1-6; 2008.

51. **Fritzemeier, C. U.; Schmüdderich, Wolfgang**. Periimplantitis-prophylaxe durch Versiegelung der Implantatinnenräume mit GapSeal®. *Implantologie*; 15(1): 71-79; 2007.

52. **Grass, G.; Rensing, C.; Solioz, M.** Metallic copper as an antimicrobial surface. *Appl Environ Microbiol*; 77(5): 1541-1547; 2011. doi:10.1128/AEM.02766-10

53.. **Grecchi, F.; Di Girolamo, M.; Cura, F.; Candotto, V.; Carinci, F.** A new system of implant abutment connection: how to improve a two piece implant system sealing. *Oral Implantol (Rome)*; 10(3): 234-240; 2017. doi:10.11138/orl/2017.10.3.234

54. **Groenendijk, E.; Dominicus, J. J.; Moorer, W. R.; Aartman, I. H.; van Waas, M. A.** Microbiological and clinical effects of chlorhexidine enclosed in fixtures of 3I-Titamed implants. *Clin Oral Implants Res*; 15(2): 174-179; 2004. doi:10.1111/j.1600-0501.2004.00977.x

55. **Gross, M.; Abramovich, I.; Weiss, E. I.** Microleakage at the abutment-implant interface of osseointegrated implants: a comparative study. *Int J Oral Maxillofac Implants*; 14(1): 94-100; 1999.

56. **Gupta, M.; Mahajan, V. K.; Mehta, K.r S.; Chauhan, P.r S.** Zinc therapy in dermatology: a review. *Dermatol Res Pract*; 2014: 709152; 2014. doi:10.1155/2014/709152

57. **Hanselman, B. A.; Kruth, S. A.; Rousseau, J.; Weese, J. S.** Coagulase positive staphylococcal colonization of humans and their household pets. *Can Vet J*; 50(9): 954-958; 2009.

58. **Hof, H.; Dörries, R.** Medizinische Mikrobiologie. In: Duale Reihe. *Georg Thieme Verlag*; Edition 6. Chapter D2: 319 - 329; 2017.

59. **Ibrahim, Z.; Gassmann, G.; Zöllner, A.; Tschernitschek, H.; Jackowski, J.** Mikroleakage der Implantatkomponenten – Klinische Bedeutung und Behandlung durch Versiegelung. *ZWR*; 117(12): 648-652; 2008. doi:10.1055/s-0028-1121876

60. **Inkinen, J.; Makinen, R.; Keinanen-Toivola, M. M.; Nordstrom, K.; Ahonen, M.** Copper as an antibacterial material in different facilities. *Lett Appl Microbiol*; 64(1): 19-26; 2017. doi:10.1111/lam.12680

61. **Jansen, V. K.; Conrads, G.; Richter, E. J.** Microbial leakage and marginal fit of the implant-abutment interface. *Int J Oral Maxillofac Implants*; 12(4): 527-540; 1997.

62. **Jiang, M.; Mei, M. L.; Wong, M. C. M.; Chu, C. H.; Lo, E. C. M.** Effect of silver diamine fluoride solution application on the bond strength of dentine to adhesives and to glass ionomer cements: a systematic review. *BMC Oral Health*; 20(1): 40; 2020. doi:10.1186/s12903-020-1030-z

63. **Jo, J. H.; Kim, H. C.; Huh, S.; Kim, Y.; Lee, D. N.** Antibacterial activities of Cu-MOFs containing glutarates and bipyridyl ligands. *Dalton Trans*; 48(23): 8084-8093; 2019. doi: 10.1039/c9dt00791a

64. **Jones, N.; Ray, B.; Ranjit, K. T.; Manna, A. C.** Antibacterial activity of ZnO nanoparticle suspensions on a broad spectrum of microorganisms. *FEMS Microbiol Lett*; 279(1): 71-76; 2008. doi: 10.1111/j.1574-6968.2007.01012.x

65. **Jorgensen, J. H.; Turnidge, J. D.** Antibacterial Agents and Susceptability test methods - Susceptability test methods: dilution and disk diffusion methods. In: Jorgensen, J.H.; Pfaller, M.A.; Caroll, K.C.; Funke, G.; Landry, M.L.; Richter, S.S., et al. (ed.). Manual of Clinical Microbiology. Washington, DC (US): *ASM Press*; Vol. 1. Edition 11. Chapter 71: 1253-1273; 2015.

66. **Josenhans, C.; Hahn, H.; Gatermann, S.** Bakteriologie - Bakterien: Vermehrung und Stoffwechsel. In: Suerbaum, S.; Burchard, G.-D.; Kaufmann, S. H. E.; Schulz, T.F. (ed.). Medizinische Mikrobiologie und Infektiologie. Berlin (DE), Heidelberg (DE): *Springer-Verlag*; Edition 8. Chapter 23: 183-186; 2016.

67. **Josenhans, C.; Hahn, H.; Gatermann, S.** Bakteriologie - Staphylokokken. In: Suerbaum, S.;Burchard, G.-D.; Kaufmann, S. H. E.; Schulz, T.F. (ed.). Medizinische Mikrobiologie und Infektiologie. Berlin (DE), Heidelberg (DE) *Springer-Verlag*; Edition 8.Chapter 24: 187-196; 2016.

68. **Kasraei, S.; Sami, L.; Hendi, S.; Alikhani, M.-Y.; Rezaei-Soufi, L.; Khamverdi, Z.** Antibacterial properties of composite resins incorporating silver and zinc oxide nanoparticles on Streptococcus mutans and Lactobacillus. *Restor Dent Endod*; 39(2): 109-114; 2014. doi:10.5395/rde.2014.39.2.109

69. **Kelly, K. A.; Havrilla, C. M.; Brady, T. C.; Abramo, K. H.; Levin, E. D.** Oxidative stress in toxicology: established mammalian and emerging piscine model systems. *Environ Health Perspect*; 106(7): 375-384; 1998. doi:10.1289/ehp.98106375

70. **Kim, D. H.; Park, J. C.; Jeon, G. E.; Kim, C. S.; Seo, J. H.** Effect of the size and shape of silver nanoparticles on bacterial growth and metabolism by monitoring optical density and fluorescence intensity. *Biotechnol Bioproc E*; 22: 210-217; 2017. doi:10.1007/s12257-016-0641-3

71. **Kim, J. S.; Kuk, E.; Yu, K. N.; Kim, J. H.; Park, S. J.; Lee, H. J.; Kim, S. H.; Park, Y. K.; Park, Y. H.; Hwang, C. Y.; Kim, Y. K.; Lee, Y. S.; Jeong, D. H.; Cho, M. H.** Antimicrobial effects of silver nanoparticles. *Nanomedicine*; 3(1): 95-101; 2007. doi:10.1016/j.nano.2006.12.001

72. **Kim, K. S.; Lim, Y. J.** Axial Displacements and Removal Torque Changes of Five Different Implant-Abutment Connections under Static Vertical Loading. *Materials (Basel)*; 13(3):699; 2020. doi:10.3390/ma13030699

73. **Ko, A. K.; Matsui, N.; Nakamoto, A.; Ikeda, M.; Nikaido, T.; Burrow, M. F.; Tagami, J.** Effect of silver diammine fluoride application on dentin bonding performance. *Dent Mater J*; 39(3): 407-414; 2020. doi:10.4012/dmj.2019-057

74. **Koyanagi T, Sakamoto M, Takeuchi Y, Ohkuma M, Izumi Y.** Analysis of microbiota associated with peri-implantitis using 16S rRNA gene clone library. *J Oral Microbiol*; 2: 5104; 2010; doi: 10.3402/jom.v2i0.5104

75. **Koutouzis, T.** Implant-abutment connection as contributing factor to peri-implant diseases. *Periodontol 2000*; 81(1): 152-166; 2019. doi:10.1111/prd.12289

76. **Laney, W. R.** Glossary of Oral and Maxillofacial Implants. *Int J Oral Maxillofac Implants*; 32(4): Gi-G200; 2017. doi: 10.11607/jomi.2017.4.gomi.

77. **Lauritano, D.; Bignozzi, C. A.; Pazzi, D.; Cura, F.; Carinci, F.** Efficacy of a new coating of implant-abutment connections in reducing bacterial loading: an in vitro study. *Oral Implantol (Rome)*; 10(1): 1-10; 2017. doi:10.11138/orl/2017.10.1.001

78. **Leonhardt, A.; Renvert, S.; Dahlen, G.** Microbial findings at failing implants. *Clin Oral Implants Res*; 10(5): 339-345; 1999. doi:10.1034/j.1600-0501.1999.100501.x

79. **Li, Y.; Zhang, W.; Niu, J.; Chen, Y.** Mechanism of photogenerated reactive oxygen species and correlation with the antibacterial properties of engineered metal-oxide nanoparticles. *ACS Nano*; 6(6): 5164-5173; 2012. doi:10.1021/nn300934k

80. **Lin, P.-H.; Sermersheim, M.; Li, H.; Lee, P. H. U.; Steinberg, S. M.; Ma, J.** Zinc in Wound Healing Modulation. *Nutrients*; 10(1): 16; 2017. doi:10.3390/nu10010016

81 **Liu, T.; Song, X.; Guo, Z.; Dong, Y.; Guo, N.; Chang, X.** Prolonged antibacterial effect of silver nanocomposites with different structures. *Colloids Surf B Biointerfaces*; 116: 793-796; 2014. doi:10.1016/j.colsurfb.2014.01.010

82. **Liu, Y.; He, L.; Mustapha, A.; Li, H.; Hu, Z. Q.; Lin, M.** Antibacterial activities of zinc oxide nanoparticles against Escherichia coli O157:H7. *J Appl Microbiol*; 107(4): 1193-1201; 2009. doi:10.1111/j.1365-2672.2009.04303.x

83. **Liu, Y.; Wang, J.** Influences of microgap and micromotion of implant-abutment interface on marginal bone loss around implant neck. *Arch Oral Biol*; 83: 153-160; 2017. doi:10.1016/j.archoralbio.2017.07.022

84. **Lu, X.; Ye, J.; Sun, Y.; Bogale, R. F.; Zhao, L.; Tian, P.; Ning, G.** Ligand effects on the structural dimensionality and antibacterial activities of silver-based coordination polymers. *Dalton Trans*; 43(26): 10104-10113; 2014. doi:10.1039/c4dt00270a

85. **Macomber, L.; Imlay, J. A.** The iron-sulfur clusters of dehydratases are primary intracellular targets of copper toxicity. *Proc Natl Acad Sci U S A*; 106(20): 8344-8349; 2009. doi:10.1073/pnas.0812808106

86. **Mahmoodi, S.; Elmi, A.; Nezhadi, S.** Copper Nanoparticles as Antibacterial Agents. *J Mol Pharm Org Process Res*; 6(1): 140; 2018. doi: 10.4172/2329-9053.1000140

87. **Malic, S.; Rai, S.; Redfern, J.; Pritchett, J.; Liauw, C. M.; Verran, J.; Tosheva, L.** Zeolite-embedded silver extends antimicrobial activity of dental acrylics. *Colloids Surf B Biointerfaces*; 173: 52-57; 2019. doi:10.1016/j.colsurfb.2018.09.043

88. **Mannala, G. K.; Hain, T.; Sproer, C.; Bunk, B.; Overmann, J.; Alt, V.; Domann, E.** Complete Genome and Plasmid Sequences of Staphylococcus aureus EDCC 5055 (DSM 28763), Used To Study Implant-Associated Infections. *Genome Announc*; 5(8): e01698-16; 2017. doi:10.1128/genomeA.01698-16

89. **Martins, C.; Ramos, E.; Kreve, S.; de Carvalho, G.; Franco, A.; de Macedo, L.; Silva, A.; Dias, S.** Reverse torque evaluation in indexed and nonindexed abutments of Morse Taper implants in a mechanical fatigue test. *Dent Res J (Isfahan)*; 16(2): 110-116; 2019.

90. **Mateescu, M.; Baixe, S.; Garnier, T.; Jierry, L.; Ball, V.; Haikel, Y.; Metz-Boutigue, M. H.; Nardin, M.; Schaaf, P.; Etienne, O.; Lavalle, P.** Antibacterial Peptide-Based Gel for Prevention of Medical Implanted-Device Infection. *PLoS One*; 10(12): e0145143; 2015. doi:10.1371/journal.pone.0145143

91. **McCarthy, G. R.; Guckes, A. D.** Preventing bacterial colonization associated with two types of implant abutments. *J Prosthet Dent*; 70(5): 479; 1993. doi:10.1016/0022-3913(93)90089-7

92. **McGabe, J. F.; Walls, A. W. G.** Elastic Impression Materials: Synthetic Elastomeres. In: Applied Dental Materials. Oxford (UK): *Blackwell Publishing*; Edition 9. Chapter 19: 163-177; 2008.

93. **Melnikov, N. N.** Alicyclic carboxylic acids and their derivatives. In: Melnikov, N.N.; Gunther, F.A.; Gunther, J.D. (ed.). Chemistry of Pesticides. New York (US): *Springer US*; Edition 1. Chapter 8: 130-140; 1971.

94. **Mencio, F.; Papi, P.; Di Carlo, S.; Pompa, G.** Salivary bacterial leakage into implant-abutment connections: preliminary results of an in vitro study. *Eur Rev Med Pharmacol Sci*; 20(12): 2476-2483; 2016.

95. **Merritt, J. H.; Kadouri, D. E.; O'Toole, G. A.** Growing and analyzing static biofilms. *Curr Protoc Microbiol*; Chapter 1: Unit-1B.1; 2005. doi:10.1002/9780471729259.mc01b01s00.

96. **Mishra, S. K.; Chowdhary, R.; Kumari, S.** Microleakage at the Different Implant Abutment Interface: A Systematic Review. *J Clin Diagn Res*; 11(6): Ze10-Ze15; 2017. doi:10.7860/JCDR/2017/28951.10054

97. **Mombelli, A.; Decaillet, F.** The characteristics of biofilms in peri-implant disease. *J Clin Periodontol*; 38 Suppl 11: 203-213; 2011. doi:10.1111/j.1600-051X.2010.01666.x

98. **Morganti, P.** Use and potential of nanotechnology in cosmetic dermatology. *Clin Cosmet Investig Dermatol*; 3: 5-13; 2010. doi:10.2147/ccid.s4506

99 **Nayak, A. G.; Fernandes, A.; Kulkarni, R.; Ajantha, G. S.; Lekha, K.; Nadiger, R.** Efficacy of antibacterial sealing gel and O-ring to prevent microleakage at the implant abutment interface: an in vitro study. *J Oral Implantol*; 40(1): 11-14; 2014. doi:10.1563/AAID-JOI-D-10-00167

100. **Neto, D.; Silva, G.; Cerutti-Kopplin, D.; Pereira, J.; Pereira, K.; Valle, A.** Measurement of loosening torque of prosthetic components in morse taper connection implants. *J Res Dent*; 2(4): 335-342; 2014.

101. **Noort, R. van**. Clinical Denatal Materials - Impression Materials. In: Introduction to Dental Materials. *London (UK): Mosby, imprint of Elsevier Limited*; Edition 3.Chapter 2.7, 187-208; 2007.

102. **O'Toole, G. A.** Microtiter dish biofilm formation assay. *J Vis Exp*; (47): 2437; 2011. doi:10.3791/2437

103. **OpenStax; Parker, N.; Schneegurt, M.; Thi Tu, A.-H.; Forster, B. M.; Lister, P.** Control of Microbial Growth - heavy metals. In: Microbiology. Edition 3. 2016. *Open Educational Resources Collection*; access date: 11.02.2020; link: https://irl.umsl.edu/oer/3

104. **Pasqualini, U.; Pasqualini, M. E.** . History of Implantology. In: Treatise of Implant Dentistry: The Italian Tribute to Modern Implantology. Carimate (IT) *Ariesdue*; Chapter 1; 2009. access date: 09.10.2018; link: https://www.ncbi.nlm.nih.gov/books/NBK409631/

105. **Pati, R.; Mehta, R. K.; Mohanty, S.; Padhi, A.; Sengupta, M.; Vaseeharan, B.; Goswami, C.; Sonawane, A.** Topical application of zinc oxide nanoparticles reduces bacterial skin infection in mice and exhibits anti bacterial activity by inducing oxidative stress response and cell membrane disintegration in macrophages. *Nanomedicine*; 10(6): 1195-1208; 2014. doi:10.1016/j.nano.2014.02.012

106. **Patyk, A.; Laugisch, J.; Steinberg, J.M.** Hohlraumversiegelung bei implantatgetragenen Suprakonstruktionen mit Hilfe acetatvernetzter Silikone. *Z Zahnarztl Implantol*; 13(4): 195-198; 1997.

107 **Paulus, W.** Acids. In: Paulus, W. (ed.). Directory of Microbicides for the Protection of Materials: A Handbook. Dordrecht (NL): *Kluwer Academic Publishers*; Edition 1. Chapter: 8: 574-597; 2005.

108 **Persson, G. R.; Renvert, S.** Cluster of bacteria associated with peri-implantitis. *Clin Implant Dent Relat Res*; 16(6): 783-793; 2014. doi:10.1111/cid.12052

109 **Peterson, S.; Irie, Y.; Borlee, B.; Murakami, K.; Harrison, J. A.; Colvin, K.; Parsek, M.** Different Methods for Culturing Biofilms In Vitro. In: Bjarnsholt, T.; Moser, C.; Østrup Jensen, P.; Høiby, N. (ed.). Biofilm Infections. New York (US): *Springer Science+Business Media*; Edition 1. Chapter 15: 251-266; 2011.

110. **Phan, T. N.; Buckner, T.; Sheng, J.; Baldeck, J. D.; Marquis, R. E.** Physiologic actions of zinc related to inhibition of acid and alkali production by oral streptococci in suspensions and biofilms. *Oral Microbiol Immunol*; 19(1): 31-38; 2004. doi:10.1046/j.0902-0055.2003.00109.x

111 **Piattelli, A.; Scarano, A.; Paolantonio, M.; Assenza, B.; Leghissa, G. C.; Di Bonaventura, G.; Catamo, G.; Piccolomini, R.** Fluids and microbial penetration in the internal part of cement-retained versus screw-retained implant-abutment connections. *J Periodontol*; 72(9): 1146-1150; 2001. doi:10.1902/jop.2000.72.9.1146

112 **Pimentel, A. C.; Manzi, M. R.; Sartori, S. G.; da Graca Naclerio-Homem, M.; Sendyk, W. R.** In vivo effectiveness of silicone gel sheets as barriers at the inner microgap between a prosthetic abutment and an external-hexagon implant platform. *Int J Oral Maxillofac Implants*; 29(1): 121-126; 2014. doi:10.11607/jomi.3177

113 **Podhorsky, A.; Biscoping, S.; Rehmann, P.; Streckbein, P.; Domann, E.; Wostmann, B.** Transfer of Bacteria into the Internal Cavity of Dental Implants After Application of Disinfectant or Sealant Agents In Vitro. *Int J Oral Maxillofac Implants*; 31(3): 563-570; 2016. doi:10.11607/jomi.4408

114 **Podhorsky, A.; Putzier, S.; Rehmann, P.; Streckbein, P.; Domann, E.; Wostmann, B.** Bacterial Contamination of the Internal Cavity of Dental Implants After Application of Disinfectant or Sealant Agents Under Cyclic Loading In Vitro. *Int J Prosthodont*; 29(5): 493-495; 2016. doi:10.11607/ijp.4546

115. **Proff, P.; Steinmetz, I.; Bayerlein, T.; Dietze, S.; Fanghanel, J.; Gedrange, T.** Bacterial colonisation of interior implant threads with and without sealing. *Folia Morphol (Warsz)*; 65(1): 75-77; 2006.

116. **Rendina, G.** Absorption and Analytical Spectrophotometry - Principles of Spectrophotometry In: Experimental methods in modern biochemistry. Philadelphia (US): *Saunders*; Edition 1.Chapter 3: 46-47; 1971.

117. **Renvert, S.; Roos-Jansaker, A. M.; Lindahl, C.; Renvert, H.; Rutger Persson, G.** Infection at titanium implants with or without a clinical diagnosis of inflammation. *Clin Oral Implants Res*; 18(4): 509-516; 2007. doi:10.1111/j.1600-0501.2007.01378.x

118. **Rimondini, L.; Marin, C.; Brunella, F.; Fini, M.** Internal contamination of a 2-component implant system after occlusal loading and provisionally luted reconstruction with or without a washer device. *J Periodontol*; 72(12): 1652-1657; 2001. doi:10.1902/jop.2001.72.12.1652

119. **Rosenthal, R.; Rubin, D.** r equivalent: A Simple Effect Size Indicator. *Psychol methods*; 8(4): 492-496; 2004. doi:10.1037/1082-989X.8.4.492

120. **Sawai, J.; Kawada, E.; Kanou, F.; Igarashi, H.; Hashimoto, A.; Kokugan, T.; Shimizu, M.** Detection of active oxygen generated from ceramic powders having antibacterial activity. *J Chem Eng Jpn*; 29(4): 627-633; 1996. doi:10.1252/jcej.29.627

121. **Sawai, J.; Shoji, S.; Igarashi, H.; Hashimoto, A.; Kokugan, T.; Shimizu, M.; Kojima, H.** Hydrogen peroxide as an antibacterial factor in zinc oxide powder slurry. *J Ferment Bioeng*; 86(5): 521-522; 1998. doi:10.1016/S0922-338X(98)80165-7

122. **Scacchi, M.** The development of the ITI Dental Implant System. Part 1: A review of the literature. *Clin Oral Implants Res*; 11 Suppl 1: 8-21; 2000. doi:10.1034/j.1600-0501.2000.011s1008.x

123. **Scarano, A.; Mortellaro, C.; Mavriqi, L.; Pecci, R.; Valbonetti, L.** Evaluation of Microgap With Three-Dimensional X-Ray Microtomography: Internal Hexagon Versus Cone Morse. *J Craniofac Surg*; 27(3): 682-685; 2016. doi:10.1097/SCS.0000000000002563

124. **Sebastien, B.; Tenenbaum, H.; Etienne, O.** Pénétration microbienne dans la connectique pilier-implant : revue de littérature. *Rev Stomatol Chir Maxillofac Chir Orale* 117(1): 20-25; 2015. doi:10.1016/j.revsto.2015.11.004

125. **Shen, C.** Impression Materials. In: I Anusavice, K.J. (ed.). Phillips' Science of Dental Materials. St. Louis (US): *Saunders, Elsevier*; Edition 11. Chapter 9: 205-254; 2003.

126. **Shen, C.** Dental Cements. In: I Anusavice, K.J. (ed.). Phillips' Science of Dental Materials. St. Louis (US): *Saunders, Elsevier*; Edition 11. Chapter 16: 443-494; 2003.

127. **Siadat, H.; Arshad, M.; Mahgoli, H. A.; Fallahi, B.** Microleakage Evaluation at Implant-Abutment Interface Using Radiotracer Technique. *J Dent (Tehran)*; 13(3): 176-183; 2016.

128. **Siddiqi, K. S.; Ur Rahman, A.; Tajuddin; Husen, A.** Properties of Zinc Oxide Nanoparticles and Their Activity Against Microbes. *Nanoscale Res Lett*; 13(1): 141; 2018. doi:10.1186/s11671-018-2532-3

129. **Song, W.; Ge, S.** Application of Antimicrobial Nanoparticles in Dentistry. *Molecules (Basel)*; 24(6): 1033; 2019. doi:10.3390/molecules24061033

130. **Steinebrunner, L.; Wolfart, S.; Bossmann, K.; Kern, M.** In vitro evaluation of bacterial leakage along the implant-abutment interface of different implant systems. *Int J Oral Maxillofac Implants*; 20(6): 875-881; 2005.

131. **Strub, J. R.; Kern, M.; Türp, J.C.; Witkowski, S.; Heydecke, G.; Wolfart, S.** Einführung in die dentale Implantologie. In: Strub, J. R.; Kern, M.; Türp, J.C.; Witkowski, S.; Heydecke, G.; Wolfart, S. (ed.). Curriculum Prothetik. Berlin (DE): *Quintessenz Verlag*; Vol. 3. Edition 4. Chapter 41: 923-972; 2011.

132. **Strub, J. R.; Kern, M.; Türp, J.C.; Witkowski, S.; Heydecke, G.; Wolfart, S.** Implantologie: Klinisches und labortechnisches Vorgehen. In: Strub, J. R.; Kern, M.; Türp, J.C.; Witkowski, S.; Heydecke, G.; Wolfart, S. (ed.). Curriculum Prothetik. Berlin (DE): *Quintessenz Verlag*; Vol. 3. Edition 4. Chapter 43: 989-1038; 2011.

133. **Svanberg, M.; Krasse, B.; Ornerfeldt, H. O.** Mutans streptococci in interproximal plaque from amalgam and glass ionomer restorations. *Caries Res*; 24(2): 133-136; 1990. doi:10.1159/000261255

134. **Tabanella, G.; Nowzari, H.; Slots, J.** Clinical and microbiological determinants of ailing dental implants. *Clin Implant Dent Relat Res*; 11(1): 24-36; 2009. doi:10.1111/j.1708-8208.2008.00088.x

135. **Tavassoli Hojati, S.; Alaghemand, H.; Hamze, F.; Ahmadian Babaki, F.; Rajab-Nia, R.; Rezvani, M. B.; Kaviani, M.; Atai, M.** Antibacterial, physical and mechanical properties of flowable resin composites containing zinc oxide nanoparticles. *Dent Mater*; 29(5): 495-505; 2013. doi:10.1016/j.dental.2013.03.011

136. **Tetsch, P.** Implantatsysteme. In: Tetsch, P. (ed.). Enossale Implantationen in der Zahnheilkunde: ein Atlas und Lehrbuch. München (DE), Wien (AT): *Carl Hanser Verlag*; Edition 2. Chapter 5: 73-79; 1991.

137. **Tonini, R.; Giovarruscio, M.; Gorni, F.; Ionescu, A.; Brambilla, E.; Mikhailovna, I. M.; Luzi, A.; Maciel Pires, P.; Sauro, S.** In Vitro Evaluation of Antibacterial Properties and Smear Layer Removal/Sealer Penetration of a Novel Silver-Citrate Root Canal Irrigant. *Materials (Basel)*; 13(1): 194; 2020. doi:10.3390/ma13010194

138. **Tüzüner, T.; Dimkov, A.; Nicholson, J. W.** The effect of antimicrobial additives on the properties of dental glass-ionomer cements: a review. *Acta Biomater Odontol Scand*; 5(1): 9-21; 2019. doi:10.1080/23337931.2018.1539623

139. **Vargas-Reus, M. A.; Memarzadeh, K.; Huang, J.; Ren, G. G.; Allaker, R. P.** Antimicrobial activity of nanoparticulate metal oxides against peri-implantitis pathogens. *Int J Antimicrob Agents*; 40(2): 135-139; 2012. doi:10.1016/j.ijantimicag.2012.04.012

140. **Vilela Teixeira, A. B.; de Carvalho Honorato Silva, C.; Alves, O. L.; Cândido Dos Reis, A.** Endodontic Sealers Modified with Silver Vanadate: Antibacterial, Compositional, and Setting Time Evaluation. *Biomed Res Int*; 2019: 4676354; 2019. doi:10.1155/2019/4676354

141. **Vincent, M.; Duval, R. E.; Hartemann, P.; Engels-Deutsch, M.** Contact killing and antimicrobial properties of copper. *J Appl Microbiol*; 124(5): 1032-1046; 2018. doi:10.1111/jam.13681

142. **Wachtel, A.; Zimmermann, T.; Spintig, T.; Beuer, F.; Müller, W.-D.; Schwitalla, A.** A novel approach to prove bacterial leakage of implant-abutment connections in vitro. *J Oral Implantol* 42(6): 452-457; 2016. doi:10.1563/aaid-joi-D-16-00065

143. **Wagner, W.** Implantatmaterialien. In: Tesch, P. (ed.). Enossale Implantationen in der Zahnheilkunde: Ein Atlas und Lehrbuch. München (DE), Wien (AT) *Carl Hanser Verlag*; Edition 2. Chapter 4: 29-72; 1991.

144. **Weaver, L.; Noyce, J. O.; Michels, H. T.; Keevil, C. W.** Potential action of copper surfaces on meticillin-resistant Staphylococcus aureus. *J Appl Microbiol*; 109(6): 2200-2205; 2010. doi:10.1111/j.1365-2672.2010.04852.x

145. **Weng, D.; Nagata, M. J.; Bell, M.; Bosco, A. F.; de Melo, L. G.; Richter, E. J.** Influence of microgap location and configuration on the periimplant bone morphology in submerged implants. An experimental study in dogs. *Clin Oral Implants Res*; 19(11): 1141-1147; 2008. doi:10.1111/j.1600-0501.2008.01564.x

146. **Williams, G. R.** Industrial wood protection. In: Paulus, W. (ed.). Directory of Microbicides for the Protection of Materials: A Handbook. Dordrecht (NL) *Kluwer Academic Publishers*; Edition 1,Chapter 5.17: 419-439; 2005.

147. **Wingrove, S. S.** Implants 101: History, Implant Design, Parts, and Pieces. In: Peri-Implant Therapy for the Dental Hygienist. Ames (US), Chichester (UK), Oxford (UK): *Wiley-Blackwell, imprint of John Wiley & Sons*; Edition 1. Chapter 1: 1-13; 2013.

148. **Wolfinger, G. J.; Rogoff, G. S.; Harrison, J. A.; Callum, J. O.** Prosthodontic Management of a Combination Transosteal/Endosteal Implant Reconstruction: A Clinical Report. *J Prosthodont*; 5(2): 76-83; 1996. doi:10.1111/j.1532-849x.1996.tb00278.x

149. **Wöstmann, B.** Abformmaterialien. In: Rosentritt, M.; Ilie, N.; Lohbauer, U. (ed.). Werstoffkunde in der Zahnmedizin - Moderne Materialien und Technologien. Stuttgart (DE): *Georg Thieme Verlag*; Edition 1. Chapter 2: 23-50; 2018.

150. **Wypkema, A. W.** Microbicides for the protection of textiles. In: Paulus, W. (ed.). Directory of Microbicides for the Protection of Materials: A Handbook. Dordrecht (NL): *Kluwer Academic Publishers*; Edition 1. Chapter 5.16: 411-417; 2005.

151. **Yamamoto, O.** Influence of particle size on the antibacterial activity of zinc oxide. *Int J Inorg Mater*; 3(7): 643-646; 2001. doi:10.1016/S1466-6049(01)00197-0

152. **Yi, Y.; Heo, S.-J.; Koak, J.-Y.; Kim, S.-K.** Comparison of CAD/CAM abutment and prefabricated abutment in Morse taper internal type implant after cyclic loading: Axial displacement, removal torque, and tensile removal force. *J Adv Prosthodont*; 11(6): 305-312; 2019. doi:10.4047/jap.2019.11.6.305

153. **Zhang, E.; Li, F.; Wang, H.; Liu, J.; Wang, C.; Li, M.; Yang, K.** A new antibacterial titanium-copper sintered alloy: preparation and antibacterial property. *Mater Sci Eng C Mater Biol Appl*; 33(7): 4280-4287; 2013. doi:10.1016/j.msec.2013.06.016

154. **Zhang, L.; Jiang, Y.; Ding, Y.; Povey, M.; York, D.** Investigation into the antibacterial behaviour of suspensions of ZnO nanoparticles (ZnO nanofluids). *J Nanopart Res*; 9: 479-489; 2007. doi: 10.1007/s11051-006-9150-1

155. **Zhao, L.; Chu, P. K.; Zhang, Y.; Wu, Z.** Antibacterial coatings on titanium implants. *J Biomed Mater Res B Appl Biomater*; 91(1): 470-480; 2009. doi:10.1002/jbm.b.31463

156. **Zhou, W.; Liu, S.; Zhou, X.; Hannig, M.; Rupf, S.; Feng, J.; Peng, X.; Cheng, L.** Modifying Adhesive Materials to Improve the Longevity of Resinous Restorations. *Int J Mol Sci*; 20(3): 723; 2019. doi:10.3390/ijms20030723

157. **Zipprich, H.; Miatke, S.; Hmaidouch, R.; Lauer, H. C.** A New Experimental Design for Bacterial Microleakage Investigation at the Implant-Abutment Interface: An In Vitro Study. *Int J Oral Maxillofac Implants*; 31(1): 37-44; 2016. doi:10.11607/jomi.3713

11 Annexe

11.1 Silver 2-ethylhexanoate inhibition zone sizes

tab. 3) Spreadsheet day one inhibition zone sizes per concentration

Silver 2-ethylhexanoate inhibition zones day one

concentration [%]	inhibition zone [mm]		
	test series 1	test series 2	test series 3
2.5	0.00	0.00	0.00
3.0	2.00	0.50	0.00
3.5	0.00	0.00	0.00
4.0	1.50	1.75	1.75
4.5	1.50	0.00	0.00
5.0	3.50	3.00	2.00
5.5	3.25	0.75	0.00
6.0	4.25	3.00	2.00
6.5	2.50	4.25	3.00
7.0	1.50	2.50	1.50
7.5	3.50	4.00	2.50
8.0	4.50	5.00	5.00
8.5	2.50	3.00	2.25
9.0	4.50	4.00	3.50
9.5	2.50	3.50	3.50
10.0	5.00	5.00	4.75

tab. 4) Spreadsheet day two inhibition zone sizes per concentration

Silver 2-ethylhexanoate inhibition zones day two

concentration [%]	inhibition zone [mm]		
	test series 1	test series 2	test series 3
2.5	0.00	0.00	0.00
3.0	2.00	0.50	0.00
3.5	0.00	0.00	0.00
4.0	1.25	1.25	1.50
4.5	1.50	0.00	0.00
5.0	3.25	3.25	2.50
5.5	3.25	0.50	0.50
6.0	4.25	3.00	2.25
6.5	2.75	4.00	3.00
7.0	1.50	2.75	2.00
7.5	3.50	3.25	2.75
8.0	5.00	5.00	5.50
8.5	2.25	3.00	2.75
9.0	4.25	4.25	4.00
9.5	2.50	3.00	2.75
10.0	5.50	5.25	5.25

tab. 5) Spreadsheet day three inhibition zone sizes per concentration

Silver 2-ethylhexanoate inhibition zones day three

concentration [%]	inhibition zone [mm]		
	test series 1	test series 2	test series 3
2.5	0.00	0.00	0.00
3.0	1.00	0.50	0.00
3.5	0.00	0.00	0.00
4.0	0.75	1.50	2.00
4.5	1.00	0.00	0.00
5.0	3.00	3.00	2.00
5.5	3.00	0.50	0.50
6.0	4.25	2.75	2.75
6.5	2.50	4.00	3.00
7.0	1.50	2.75	1.50
7.5	3.00	3.00	2.00
8.0	5.50	5.00	5.25
8.5	2.00	2.75	2.50
9.0	4.75	4.00	3.75
9.5	2.75	3.50	2.25
10.0	5.50	5.50	5.50

11.2 Biofilm: detailed information

11.2.1 Zinc oxide

tab. 6) Zinc oxide group statistics: OD_{595} mean/standard deviation

Concentration [%]	Series	N	Mean	Std. Deviation
0.0	1 biofilm	3	0.1640	0.0080
	2 negative control	3	0.1440	0.0085
0.1	1 biofilm	3	0.1660	0.0342
	2 negative control	3	0.1603	0.0353
0.5	1 biofilm	3	0.1817	0.0142
	2 negative control	3	0.1443	0.0072
1.0	1 biofilm	3	0.2083	0.0475
	2 negative control	3	0.1480	0.0259
2.0	1 biofilm	3	0.2177	0.0453
	2 negative control	3	0.1490	0.0174
5.0	1 biofilm	3	0.2217	0.0289
	2 negative control	3	0.1497	0.0119
7.0	1 biofilm	3	0.2407	0.0272
	2 negative control	3	0.2053	0.0994
10.0	1 biofilm	3	0.2820	0.0728
	2 negative control	3	0.1470	0.0052
15.0	1 biofilm	3	0.2960	0.0469
	2 negative control	3	0.1373	0.0160
20.0	1 biofilm	3	0.2287	0.0093
	2 negative control	3	0.1543	0.0216
25.0	1 biofilm	3	0.2767	0.0492
	2 negative control	3	0.1550	0.0076
30.0	1 biofilm	3	0.3320	0.0862
	2 negative control	3	0.1840	0.0372

tab.7) Zinc oxide: independent sample test & effect size

Concentration	Levene's Test for equality of variances			T-test						Effect size
								95% confidence interval of difference		
[%]	F	Sig.	Equality	t	df	Sig.	Mean difference	lower	upper	r
0.0	0.033	.865	yes	2.960	4.000	.042	0.02000	0.00124	0.03876	0.829
0.1	0.017	.902	yes	0.200	4.000	.851	0.00567	-0.7303	0.08437	0.099
0.5	1.290	.319	yes	4.060	4.000	.015	0.03733	0.01180	0.06286	0.897
1.0	0.651	.465	yes	1.931	4.000	.126	0.06033	-0.02640	0.14706	0.695
2.0	5.304	.083	yes	2.451	4.000	.070	0.06867	-0.00913	0.14646	0.775
5.0	2.101	.221	yes	3.986	4.000	.016	0.07200	0.02185	0.12215	0.894
7.0	7.778	.049	no	0.594	2.298	.606	0.03533	-0.19122	0.26188	0.365
10.0	4.520	.101	yes	3.206	4.000	.033	0.13500	0.01808	0.25192	0.848
15.0	2.474	.191	yes	5.546	4.000	.005	0.15867	0.07924	0.23810	0.941
20.0	3.660	.128	yes	5.482	4.000	.005	0.07433	0.03668	0.11198	0.939
25.0	7.650	.051	yes	4.236	4.000	.013	0.12167	0.04193	0.20140	0.904
30.0	1.252	.326	yes	2.731	4.000	.052	0.14800	-0.00256	0.29846	0.807

11.2.2 **Copper naphthenate**

tab.8) Copper naphthenate group statistics: OD_{595} mean/standard deviation

Concentration	Series	N	Mean	Std. Deviation
0.0%	1 biofilm	4	0.1498	0.0110
	2 negative control	3	0.4607	0.5442
0.1%	1 biofilm	4	0.1450	0.0158
	2 negative control	3	0.1500	0.0279
0.5%	1 biofilm	4	0.2315	0.0291
	2 negative control	3	0.1443	0.0153
1.0%	1 biofilm	4	0.2455	0.0527
	2 negative control	3	0.1337	0.0060
2.0%	1 biofilm	4	0.3690	0.1148
	2 negative control	3	0.1597	0.0114
5.0%	1 biofilm	4	0.9420	0.2242
	2 negative control	3	0.2227	0.0419
7.0%	1 biofilm	4	1.2090	0.1717
	2 negative control	3	0.2220	0.1021
10.0%	1 biofilm	4	0.8923	0.1284
	2 negative control	3	0.2173	0.0255
15.0%	1 biofilm	4	0.9105	0.2703
	2 negative control	3	0.4413	0.0657
20.0%	1 biofilm	4	0.8075	0.2793
	2 negative control	3	2.8017	0.3050
25.0%	1 biofilm	4	2.1073	0.4461
	2 negative control	3	3.6363	0.2495

tab. 9) Copper naphthenate: independent sample test & effect size

Con-cen-tra-tion	Levene's Test for equality of variances			T-test						Ef-fect size
								95% confidence in-terval of difference		
[%]	F	Sig.	E-qua-lity	t	df	Sig.	Mean differ-ence	lower	upper	r
0.0	21.898	.005	no	-0.989	2.001	.427	-0.31092	-1.66225	1.04041	0.573
0.1	1.459	.281	yes	-0.305	5.000	.773	-0.00500	-0.04715	0.03715	0.135
0.5	6.790	.048	no	5.130	4.668	.004	0.08717	0.04254	0.13179	0.922
1.0	4.811	.080	yes	3.572	5.000	.016	0.11183	0.03135	0.19231	0.848
2.0	3.801	.109	yes	3.072	5.000	.028	0.20933	0.03416	0.38451	0.808
5.0	35.464	.002	no	6.272	3.275	.006	0.71933	0.37109	1.06758	0.961
7.0	0.833	.403	yes	8.743	5.000	.000	0.98700	0.69679	1.27721	0.969
10.0	2.294	.190	yes	8.773	5.000	.000	0.67492	0.47717	0.87266	0.969
15.0	10.606	.025	no	3.342	3.459	.036	0.46917	0.05420	0.88413	0.874
20.0	0.100	.765	yes	-9.009	5.000	.000	-1.99417	-2.56318	-1.42515	0.971
25.0	0.554	.490	yes	-5.271	5.000	.003	-1.5908	-2.27486	-0.78330	0.921

11.2.3 **Silver 2-ethylhexanoate**

tab.9) Silver 2-ethylhexanoate group statistics: OD_{595} mean/standard deviation

Concentration	Series	N	Mean	Std. Deviation
0.0%	1 biofilm	3	0.2003	0.0560
	2 negative control	4	0.1625	0.0134
0.1%	1 biofilm	3	0.2650	0.0494
	2 negative control	4	0.1588	0.0114
0.5%	1 biofilm	3	0.2403	0.0421
	2 negative control	4	0.1815	0.0111
1.0%	1 biofilm	3	0.2060	0.0171
	2 negative control	4	0.1845	0.0350
2.0%	1 biofilm	3	0.2737	0.0049
	2 negative control	4	0.1808	0.0213
5.0%	1 biofilm	3	0.3670	0.0569
	2 negative control	4	0.3733	0.1595
7.0%	1 biofilm	3	0.5780	0.0243
	2 negative control	4	0.6698	0.2293
10.0%	1 biofilm	3	0.6213	0.1195
	2 negative control	4	0.7368	0.2596

tab. 10) Silver 2-ethylhexanoate: independent sample test & effect size

Concentration	Levene's Test for equality of variances			T-test						Effect size
								95% confidence interval of difference		
[%]	F	Sig.	Equality	t	df	Sig.	Mean difference	lower	upper	r
0.0	10.272	.024	no	1.146	2.174	.362	0.03783	-0.09387	0.16954	0.614
0.1	11.759	.019	no	3.651	2.161	.060	0.10625	-0.01044	0.22294	0.928
0.5	3.190	.134	yes	2.755	5.000	.040	0.05883	0.00393	0.11373	0.776
1.0	2.549	.171	yes	0.964	5.000	.379	0.02150	-0.03584	0.07884	0.396
2.0	2.444	.179	yes	7.246	5.000	.001	0.09292	0.05995	0.12588	0.956
5.0	0.980	.368	yes	-0.064	5.000	.952	-0.00625	-0.25884	0.24634	0.028
7.0	3.886	.106	yes	-0.674	5.000	.530	-0.09175	-0.44175	0.25825	0.289
10.0	12.385	.017	no	-0.785	4.406	.472	-0.11542	-0.50911	0.27828	0.350

11.3 3D distance: detailed Information

tab. 12) group statistics: 3D distance [µm] mean/standard deviation

Implant system		Sealing material	Posi-tion	N	Mean [µm]	Std. Deviation
Straumann	implant 1	empty	1	5	1074.9	3.5
			2	5	1088.0	3.4
			3	5	1050.4	3.3
			4	5	1044.4	2.1
			5	5	1072.1	1.8
		pure silicone	1	5	1074.5	1.5
			2	5	1081.8	1.7
			3	5	1051.3	0.8
			4	5	1043.9	1.1
			5	5	1070.3	1.0
		copper naphthenate	1	5	1074.4	0.3
			2	5	1087.4	2.0
			3	5	1050.5	1.5
			4	5	1043.7	1.5
			5	5	1071.4	1.0
		silver 2-ethylhexanoate	1	5	1071.5	1.3
			2	5	1085.9	3.6
			3	5	1047.4	3.7
			4	5	1043.9	2.2
			5	5	1072.2	1.6
	implant 2	empty	1	5	1053.3	1.2
			2	5	1035.7	0.2
			3	5	1034.4	0.3
			4	5	1066.3	2.3
			5	5	1074.0	5.0
		pure silicone	1	5	1050.8	1.1
			2	5	1035.7	1.2

			3	5	1034.3	0.8
			4	5	1062.5	1.7
			5	5	1071.0	3.8
		copper naphthenate	1	5	1053.6	0.7
			2	5	1035.5	0.9
			3	5	1034.3	0.7
			4	5	1066.4	0.6
			5	5	1073.6	1.7
		silver 2-ethylhexanoate	1	5	1053.0	2.4
			2	5	1034.8	0.3
			3	5	1034.2	1.5
			4	5	1065.1	1.3
			5	5	1071.5	3.8
	implant 3	empty	1	5	1080.9	2.0
			2	5	1038.8	3.1
			3	5	1027.7	1.5
			4	5	1040.2	3.8
			5	5	1074.1	3.1
		pure silicone	1	5	1082.0	1.2
			2	5	1044.6	2.2
			3	5	1028.4	2.0
			4	5	1036.4	4.0
			5	5	1069.0	3.7
		copper naphthenate	1	5	1081.3	1.5
			2	5	1039.5	2.0
			3	5	1028.6	1.9
			4	5	1040.2	2.7
			5	5	1074.9	1.8
		silver 2-ethylhexanoate	1	5	1080.6	1.4
			2	5	1038.3	1.5
			3	5	1027.5	1.1
			4	5	1039.9	2.0
			5	5	1073.6	1.8

Xive	implant 1	empty	1	5	1006.3	0.8
			2	5	1007.5	1.9
			3	5	1013.2	0.8
			4	5	973.3	3.1
			5	5	1001.2	1.0
		pure silicone	1	5	1006.5	0.4
			2	5	1007.2	0.5
			3	5	1011.9	0.9
			4	5	973.0	1.8
			5	5	1000.6	1.3
		copper naphthenate	1	5	1006.5	0.8
			2	5	1006.8	0.6
			3	5	1012.9	0.4
			4	5	973.5	1.2
			5	5	1001.5	1.2
		silver 2-ethylhexanoate	1	5	1006.5	0.4
			2	5	1006.0	3.7
			3	5	1012.9	1.0
			4	5	974.0	2.0
			5	5	1001.0	0.4
	implant 2	empty	1	5	997.6	1.1
			2	5	998.4	1.0
			3	5	1006.9	1.6
			4	5	982.5	1.5
			5	5	1002.1	2.5
		pure silicone	1	5	997.3	0.6
			2	5	997.8	1.5
			3	5	1004.7	1.5
			4	5	984.7	1.3
			5	5	1001.8	1.4
		copper naphthenate	1	5	998.7	0.3
			2	5	998.6	0.6
			3	5	1005.7	1.3

			4	5	984.2	0.7
			5	5	1002.1	2,4
		silver 2-ethylhexanoate	1	5	997.3	0.9
			2	5	998.3	0.8
			3	5	1006.5	0.8
			4	5	982.3	1.3
			5	5	1002.0	0.5
	implant 3	empty	1	5	292.4*	2.2
			2	5	972.5	3.6
			3	5	991.6	0.8
			4	5	996.6	1.5
			5	5	950.1	2.8
		pure silicone	1	5	290.7*	0.7
			2	5	972.9	1.4
			3	5	990.4	1.7
			4	5	996.1	1.2
			5	5	953.1	2.2
		copper naphthenate	1	5	292.8*	1.3
			2	5	973.4	2.2
			3	5	991.7	0.9
			4	5	996.6	1.6
			5	5	952.2	0.8
		silver 2-ethylhexanoate	1	5	292.3*	0.7
			2	5	972.1	0.7
			3	5	991.1	0.4
			4	5	996.6	0.3
			5	5	951.5	1.1

*Laser distance deviation, excluded in further statistical analysis. Not comparable with the other measurement points, but consistent within the same measurement point with different sealing materials.

11.4 Opening torque: detailed information

tab 13) Opening torque: paired samples statistics

Implant system		Sealing Material	N	Mean (cNm)	Std. Deviation
1 Straumann	Pair 1	reference/empty	3	-32.6333	1.48689
		pure silicone	3	-32.4833	1.44684
	Pair 2	reference/empty	3	-32.6333	1.48689
		copper naphthenate	3	-31.3333	1.79606
	Pair 3	reference /empty	3	-32.6333	1.48689
		silver 2-ethylhexanoate	3	-31.5000	3.32754
2 Xive	Pair 1	reference/empty	3	-19.8500	0.37749
		pure silicone	3	-16.8333	5.27549
	Pair 2	reference/empty	3	-19.8500	0.37749
		copper naphthenate	3	-20.0333	1.97758
	Pair 3	reference/empty	3	-19.8500	0.37749
		silver 2-ethylhexanoate	3	-19.9167	0.86939

12 Publication

Poster abstract, IADR 2020 (international association of dental research):

Engelbrecht, D.; Wöstmann, B.; Domann, E.; Antibacterial Modification of Silicone; *J Dent Res*; 99: Spec Iss A: abstract no 0835.

13 Declaration

"I hereby declare that I have produced the present work independently and without undue assistance or use of any aids other than those specified. All text passages taken verbatim or analogously from published or unpublished writings and all information based on oral information are marked as such. In the research I conducted and mentioned in my dissertation, I adhered to the principles of good scientific practice as laid down in the "Satzung der Justus-Liebig-Universität Gießen zur Sicherung guter wissenschaftlicher Praxis", as well as to ethical, data protection and animal welfare principles. I affirm that third parties have not received any direct or indirect monetary payments from me for work related to the content of the submitted dissertation, or have specified these below. The submitted work has not been submitted in the same or a similar form to any other examination authority for the purpose of a doctorate or other examination procedure, neither in Germany nor abroad. All material from other sources and material taken from other persons which was used in the thesis or to which direct reference is made was marked as such. In particular, all persons who were directly or indirectly involved in the creation of the present thesis were named. I agree to the verification of my work by plagiarism detection software or an internet-based software program.“

_____

Date

_____

Jeannine Dunila Engelbrecht

Erklärung zur Dissertation

„Hiermit erkläre ich, dass ich die vorliegende Arbeit selbständig und ohne unzulässige Hilfe oder Benutzung anderer als der angegebenen Hilfsmittel angefertigt habe. Alle Textstellen, die wörtlich oder sinngemäß aus veröffentlichten oder nichtveröffentlichten Schriften entnommen sind, und alle Angaben, die auf mündlichen Auskünften beruhen, sind als solche kenntlich gemacht. Bei den von mir durchgeführten und in der Dissertation erwähnten Untersuchungen habe ich die Grundsätze guter wissenschaftlicher Praxis, wie sie in der „Satzung der Justus-Liebig-Universität Gießen zur Sicherung guter wissenschaftlicher Praxis“ niedergelegt sind, eingehalten sowie ethische, datenschutzrechtliche und tierschutzrechtliche Grundsätze befolgt. Ich versichere, dass Dritte von mir weder unmittelbar noch mittelbar geldwerte Leistungen für Arbeiten erhalten haben, die im Zusammenhang mit dem Inhalt der vorgelegten Dissertation stehen, oder habe diese nachstehend spezifiziert. Die vorgelegte Arbeit wurde weder im Inland noch im Ausland in gleicher oder ähnlicher Form einer anderen Prüfungsbehörde zum Zweck einer Promotion oder eines anderen Prüfungsverfahrens vorgelegt. Alles aus anderen Quellen und von anderen Personen übernommene Material, das in der Arbeit verwendet wurde oder auf das direkt Bezug genommen wird, wurde als solches kenntlich gemacht. Insbesondere wurden alle Personen genannt, die direkt und indirekt an der Entstehung der vorliegenden Arbeit beteiligt waren. Mit der Überprüfung meiner Arbeit durch eine Plagiatserkennungssoftware bzw. ein internetbasiertes Softwareprogramm erkläre ich mich einverstanden.“

_____

Datum

_____

Jeannine Dunila Engelbrecht

14 Acknowledgements

First of all, many thanks to Prof. Dr. Bernd Wöstmann for giving me this opportunity and assigning me this interesting topic. Also, for giving me the chance to work in the department of Prosthodontics, as well as for the time and patience for and with the discussions about this dissertation and supervision of this study.

Next, I would like to thank Prof. Dr. Eugen Domann very much for giving me the opportunity to work on the experiments in his working group and laboratory in the department of medical microbiology, as well as for the patience and time for the supervision of and discussions on the microbiological parts of this study.

A lot of thanks are also due to Silke Zechel-Gran, for being a great mentor and supervisor in regards to handling microbiological equipment and conducting the experiments, as well as for being a friend and having lengthy discussions about methodical improvements.

Many thanks to Dr. Stephanie Biscoping for answering all the organisational questions and the general assistance, as well as Prof. Rehmann for the advice.

Many thanks to Dr. Johannes Herrmann for the statistical consultation, and Dr. Daniel Dürr for proofreading my statistical reporting.

Also, thanks to the department of Prosthodontics for a great working environment and a lot of new input, and to Mr. Müller for creating the moulds for specimen production.

I would like to thank Ann-Christin Krüger very much, for checking the spelling of the dissertation.

For fun breaks and great uplifiting of the mood in general, my thanks go to my break-time-microbiology group, so much laughter in such a short time of the day is rare.

Last but not least, I owe many to thanks to my parents and friends for all the patience, great emotional and mental support, and joy they have gifted me with over the years.