Cobalt-chromium alloys in fixed prosthodontics

Manufacturing techniques, biological and mechanical aspects

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Gothenburg 2022

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ISBN 978-91-8009-594-5 (PRINT)
ISBN 978-91-8009-595-2 (PDF)

Printed in Borås, Sweden 2022
Printed by Stema Specialtryck AB

To my family

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ABSTRACT

Gold and its alloys have been the dominant framework materials in metal-ceramic constructions when substantial loss of tooth substance or missing teeth needs to be replaced. However, base metal alloys, such as cobalt-chromium (Co-Cr), have been introduced due to increased gold price. Co-Cr alloys possess beneficial mechanical properties because the alloys can, even with small dimensions, resist high chewing forces and exhibit an acceptable bond to the surface porcelain layer. Yet, the biocompatibility of Co-Cr alloys has been questioned. The overall aim of the present thesis is to increase our knowledge about Co-Cr alloys that are used in fixed prosthodontics.

Results from Study I, which was a survey directed to all dental laboratories in Sweden, demonstrated that more than thirty different Co-Cr alloys were reported to be used in fixed prosthodontics. Besides the various chemical composition among the reported alloys, they were also manufactured by four different techniques; cast, milled, laser melted and pre-sintered milled. Based on the results from Study I, five Co-Cr alloys manufactured by four techniques, together with commercially pure titanium (c.p. Ti) grade 4 and Ti6Al4V ELI, were further included in Studies II-IV. *In vitro* studies were conducted in order to evaluate ion release, cell viability and inflammatory response. The effect of material combinations was also investigated. The mechanical properties and material structure with regards to yield strength, elongation after fracture, hardness, elastic modulus, surface roughness and microstructure were evaluated. The effect of heat treatment was also investigated on the above-mentioned parameters. The total ion release from all materials was extremely low, yet highest for the cast Co-Cr alloys in acidic conditions. The combination of Co-Cr, Ti6Al4V ELI and c.p. Ti showed lower total ion release compared

with the non-presence of c.p. Ti. All tested materials demonstrated non-cytotoxic effects, although the highest inflammatory response from cells exposed to the materials was observed for the cast and pre-sintered milled Co-Cr materials. Overall, the laser melted Co-Cr demonstrated the highest values in mechanical properties.

Conclusion: *In vitro* biological aspects and mechanical properties are influenced by the choice of manufacturing technique, heat treatment and microstructure of the materials. In order to evaluate these findings, more clinical studies are needed.

Keywords: prosthodontics, cobalt-chromium, manufacturing techniques
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SAMMANFATTNING PÅ SVENSKA

Guld och dess legeringar har sedan länge använts som material för underkonstruktion vid ersättning av förlorade tänder eller tandsubstans, men har på grund av ökade kostnader ersatts av oädla legeringar, som exempelvis kobolt-krom (Co-Cr). Co-Cr kännetecknas av hög styvhet och sträckgräns men uppvisar även god bindningsförmåga till porslin, vilket har inneburit att det har kunnat användas framgångsrikt vid metall-keramiska fasta tand- och implantatkonstruktioner. Dock har dess biokompatibilitet varit ifrågasatt. Det övergripande syftet med denna avhandling är att öka kunskapen kring de Co-Cr legeringar som används inom den svenska tandvården.

Resultaten från delarbete 1, som är en enkätstudie riktad till alla tandtekniska laboratorier i Sverige, visade att fler än 30 olika Co-Cr legeringar används inom fast protetik. Förutom att kemiska sammansättningen mellan dem var olika, var de även framställda på olika sätt; gjutna, frästa, laser smälta och presintrat frästa. Baserat på resultaten från studie I, inkluderades fem Co-Cr-legeringar tillverkade med de fyra olika produktionsteknikerna. Även kommersiellt rent titan grad 4 och Ti6Al4V ELI inkluderades som jämförelsematerial. För att studera jonfrisättning, cellviabilitet och inflammatoriskt svar mellan dem, utfördes olika *in vitro*-test. Även jonfrisättningen när olika material kombinerades undersöktes. Skillnader i mekaniska och mikrostrukturella egenskaper jämfördes, samt om värmebehandling påverkade materialegenskaperna. Den totala jonfrisättningen från alla material var extremt låg, men högst för de gjutna Co-Cr legeringarna vid lågt pH. Jonfrisättningen visade sig vara lägre när c.p. titan var närvarande. Trots att *in vitro*-testerna pekade på att inget av materialen var cytotoxiska, fann vi skillnader mellan inflammationssvar från cellerna som hade exponerats för de olika materialen. Resultaten visade att gjutet och presintrat fräst Co-Cr provocerade ett högre inflammationssvar från celler jämfört med fräst, lasersmält och de båda titanmaterialen. Sammanfattningsvis, uppvisade lasersmält Co-Cr de högsta värden avseende de mekaniska egenskaperna.

Slutsats: Tillverkningsteknik, värmebehandling och materialstruktur påverkar materialens egenskaper avseende *in vitro*-biologiskt svar samt mekaniska egenskaper. Fler kliniska studier behövs för att utvärdera betydelsen av dessa fynd.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. Kassapidou M, Franke Stenport V, Hjalmarsson L, Johansson CB. Cobalt-chromium alloys in fixed prosthodontics in Sweden. Acta Biomaterialia Odontologica Scandinavica. 2017;3(1):53-62.

II. Kassapidou M, Hjalmarsson L, Johansson CB, Hammarstrom Johansson P, Morisbak E, Wennerberg A, Franke Stenport V. Cobalt-chromium alloys fabricated with four different techniques: Ion release, toxicity of released elements and surface roughness. Dental materials : official publication of the Academy of Dental Materials. 2020;36(11):e352-e63.

III. Kassapidou M, Franke Stenport V, Johansson C, Syverud M, Hammarström Johansson P, Börjesson J, Hjalmarsson L. Cobalt-Chromium alloys in fixed prosthodontics; investigations of mechanical properties and microstructure. 2021 (submitted).

IV. Kassapidou M, Stenport VF, Johansson CB, Östberg AK, Hammarström Johansson P, Hjalmarsson L. Inflammatory Response to Cobalt-Chromium Alloys Fabricated With Different Techniques J Oral Maxillofac Res 2021;12(4):e3.

CONTENT

ABBREVIATIONS

ADT	agar diffusion test
AFM	atomic force microscopy
Co-Cr	cobalt-chromium
CAD-CAM	computer-aided design-computer-aided manufacturing
CNC	computer numeric controlled
c.p.	commercially pure
DLMS	direct laser metal sintering
DET	dye exclusion test
ECHA	European chemical agency
EDS	energy-dispersive X-ray spectroscopy
ELI	extra-low interstitials
ELISA	enzyme-linked immunosorbent assays
FIB-SEM	focused ion beam-scanning electron microscope
FIP	fixed implant prosthesis
FDP	fixed dental prosthesis
GERD	gastroesophageal reflux disease
ht	heat treated
ICP-AES	inductively coupled plasma atomic emission spectrometry
ICP-EOS	inductively coupled plasma optical emission spectrometry

ICP-MS	inductively coupled plasma mass spectrometry
IET	impulse excitation technique
IL	interleukin
LD	lethal dose
MELISA	Memory Lymphocyte Immunostimulation Assay
MPA	Medical products agency
MDR	medical devices directive
MTT	3-(4.5-dimethylthiazol-2-yl)-2.5-diphenyltetrazolium bromide
non-ht	non-heat treated
NRU	neutral red uptake
PCR	polymerase chain reaction
PBMCs	peripheral blood mononuclear cells
PEEK	polyetheretherketone
RFDA	resonant frequency & damping analysis
REACH	registration, evaluation, authorisation and restriction of chemicals
SLS	selective laser sintering
SLM	selective laser melting
Ti	titanium
WST-8	2-(2-methoxy4-nitrophenyl)-3-(4-nitrophenyl)-5-(2.4-disulfophenyl)-2H tetrazolium, monosodium salt

XTT	2.3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)carbonyl]-2H-tetrazolium hydroxide
ZrO_2	zirkoniumdioxide

1 INTRODUCTION

Tooth loss can affect a person's life, impairing oral function, reduced self-esteem, and social isolation. Thus, replacing missing teeth can positively influence the quality of life (1, 2).

Since the last decades of the 20th century, gold has been the standard material for frameworks of fixed prostheses to replace missing teeth (3). As a consequence of the increased global gold price, alternative metal alloys, i.e. palladium (Pd) and cobalt-based alloys, were introduced (4). Cobalt-chromium (Co-Cr) alloys possess several advantages regarding mechanical properties for intraoral use, i.e. high strength, hardness, resistance to corrosion and tarnish (5-13). Although Co-Cr alloys exhibit favourable mechanical properties to be used as a framework material in oral prosthodontics, its biocompatibility has been questioned regarding metal wear particles and ion release (14). In contrast, commercially pure (c.p.) titanium (Ti) is considered a high biocompatible material (15). However, the lower elastic modulus and higher rates of porcelain chip off fractures for c.p. Ti contribute to a lower usage in fixed prosthodontics compared to Co-Cr alloys (15-17). A high rate of chipping of the veneering porcelain has also been reported on frameworks made of zirconia (18). Constructions made by computer-aided design-computer-aided manufacturing (CAD/CAM) with full-anatomic monolithic zirconia frameworks have been suggested to overcome the chip off rates of both titanium and veneered zirconia prostheses (19). However, limitations in long-term follow-up studies, especially related to the decreased fracture strength after ageing of zirconia needs to be further investigated (20).

1.1 COBALT CHROMIUM ALLOYS

Cobalt (Co) was first isolated in 1735 by Georg Brandt and is a grey and brittle metal (21). One of the most important properties of Co, is its contribution as a strengthener in alloys at high temperatures (up to the melting point 1495°C) (21). For this reason, Co is used in the aerospace and defence area, in rechargeable batteries and manufacturing medical devices (21). Co is also known for creating an intense blue colour (21).

The Co metal does not exist in a pure state in nature and is mainly found in nickel- and copper ores in the Democratic Republic of Congo, Zambia, Canada, Russia and New Caledonia (21, 22). The refining of the Co metal from

the origin ores is, to a large extent (90 %), globally taken place in other locations, such as China (~67 %), followed by Finland (~11 %) and Canada (~5 %) (22). Yet, bad working conditions, child labour, corruption, and violent ethnic conflicts have been associated with cobalt mining in the Democratic Republic of Congo (23, 24). Furthermore, since the future demands of Co are predicted to increase, issues of the sustainable use of metals are discussed (22, 25). Chromium (Cr) is a shiny, brittle and hard metal that Nicholas-Louis Vauquelin isolated in 1797 (26). Cr is mostly extracted from the chromite mineral in South Africa (27, 28). The addition of Cr provides alloys with a higher strength and corrosion resistance (29).

The World Health Organization (WHO) defines Co and Cr as essential elements with a recommended daily uptake (RDI) of Co=6 µg and Cr=120 µg (30). Co is a part of vitamin B12 and is necessary for erythropoiesis and nerve repair or regeneration. Although it has been reported that chromium acts as a facilitator in metabolic mechanisms, i.e., by helping insulin to bind on receptors at the cell surface, the functions of chromium in the human body are not fully understood (31). Doses of more than 200 µg Cr per day are considered toxic, although no upper levels are known for Co (30).

Chromium and oxygen form Cr-based oxides (thickness~1-4 nm) on the surface, making the alloy corrosion-resistant (14, 32, 33). Co-Cr alloys usually contain other elements as well as Mo (molybdenum), Ce (cerium), Nb (niobium), Ru (ruthenium), Ga (gallium), Fe (iron), B (boron), W (tungsten), Si (silicon), V (vanadium), Ni (nickel), N (nitrogen), C (carbon) and Mn (manganese) (12, 14, 29, 33-35).

1.2 TITANIUM AND ITS ALLOYS

Titanium (Ti) is mainly found in mineral sources of rutile (TiO_2) and ilmenite ($FeTiO_3$) in South Africa, Australia, Mozambique, Sierra Leone and Ukraine (36). The element was discovered in 1791 in Cornwall (UK) by William Gregor. Later (in 1795) it was identified as an oxide by Martin Klaproth (37). It was named titanium from Greek mythology as the Titans were the powerful sons of the earth (15, 37, 38). Titanium is highly corrosion-resistant, light, and shows high strength values and low elastic modulus (*E)* (39). When titanium is exposed to oxygen, it reacts, and a thin surface oxide layer (approximately 4 nm) is formed, contributing to high corrosion resistance and biocompatibility (15).

The production of the highly pure and ductile Ti is difficult because Ti strongly reacts with oxygen and nitrogen. Early attempts to create a ductile and purified

Ti were less successful and led to a brittle material (37). It was not until 1937-1940 that a process called Kroll was invented. It involved that TiO_2 was converted to titanium tetrachloride ($TiCl_4$) and later to the highly porous elemental titanium, known as "titanium sponge" (15, 37, 40). Commercially pure Ti is produced when the titanium sponge is purified by pulverization followed by acid leaching or vacuum distillation (15). Most of the titanium ore is used to produce high purity TiO_2 used as a paint pigment. Only about 5 % of the titanium ore is finally used to produce the metallic titanium, making the production process from the mineral source to c.p. Ti both high-energy wasting and extremely costly (15). Titanium ores are found all over the world, but the main exporters of titanium sponge, and TiO_2 are the US (18 %), China (13 %), Japan, Germany (9 %), the Netherlands (8 %), Belgium (7 %), UK (6 %) and Russia (4 %) (36). Despite the refining and processing of titanium from the ores, a small number of impurities, such as iron and oxygen, remains in c.p. Ti (41).

Commercially pure Ti exists in four grades (1, 2, 3, 4) that differ in oxygen and iron content (Table 1) (39). Even small differences in oxygen and iron content among the c.p. titanium materials affect the physical and mechanical properties among them (39, 42). Strength and hardness increase as the oxygen and iron content increase (17, 41). However, the addition of impurities such as iron or oxygen is not recommended due to the negative impact on mechanical properties, i.e. the decrease of ductility, thermal stability, and creep resistance (41).

Titanium	N	C	H	Fe	O
c.p. grade 1	0.03	0.08	0.015	0.2	0.18
c.p. grade 2	0.03	0.08	0.015	0.3	0.25
c.p. grade 3	0.05	0.08	0.015	0.3	0.35
c.p. grade 4	0.05	0.08	0.015	0.5	0.40

Table 1. Impurity limits of c.p. titanium (wt %) (17).

In order to enhance the mechanical properties of c.p. titanium, alloying elements such as Al (aluminium) (3.0-6.5 wt %), V (vanadium) (5.5-6.5wt %) and Nb (niobium) (6.5–7.5wt %) are added (Table 1) (17). In prosthodontics, Ti6Al4V and Ti6Al7Nb are also being used in addition to c.p. titanium (17, 39, 43). Ti6Al4V alloys are available in two grades, grade 5 and 23, where grade 23, also known as Ti6Al4V ELI (extra-low interstitials) is mostly used in medical devices due to a lower content of oxygen, nitrogen and iron that

induces higher ductility and fracture toughness compared to Ti6Al4V (44-46). Titanium and its alloys are also widely used for aerospace, marine and chemical plant materials and as products such as golf clubs and glasses (47). In general, titanium metals (c.p. and alloys) are considered as relatively inert materials with the ability to have favourable biocompatibility and therefore successfully used as biomaterials in several medical applications, for example, in dental implants, maxillofacial devices, hip/knee implants, pacemakers, cochlear implants, defibrillators, intravascular stents, sutures and brackets/wires in orthodontics (15). However, concerns of possible corrosion of toxic elements as V, Al and Nb from titanium alloys have been reported (48, 49).

1.3 MANUFACTURING TECHNIQUES

Cast Co-Cr alloys have been used in dentistry since the beginning of the 20th century (50). The *casting* technique involves the formation of a wax pattern that is placed in a suitable investment and, furthermore, is placed in a furnace that burns out the wax pattern. The hollowness is created after the diminished wax is filled with the melted alloy. Afterwards, the alloy is removed from the investment and left to cool (51). The cast Co-Cr alloys were dominantly used as framework materials in removable partial dentures but were later introduced in tooth- and implant-supported prosthodontics (7, 52, 53). However, early challenges related to the casting process due to the higher solidification shrinkage and the lower accuracy in fit for Co-Cr alloys compared to gold alloys have been reported (54). In order to overcome the casting problems, pre-machined gold alloy cylinders were cast and soldered together in a framework of gold alloy or silver palladium to achieve “passive fit” of the implant-retained framework (55, 56). Later on, the milling technique was applied on titanium frameworks and a lower degree of distortion in full-arch fixed implant constructions compared to the cast implant frameworks was reported (57).

Disadvantages of the *milling* technique, irrespective of material, also called *subtractive manufacturing*, are the high material waste that has a negative environmental impact, the limitation of the number of parts that can simultaneously be manufactured and the high maintenance costs due to the rapid tool wear when milling hard alloys as Co-Cr (58, 59). As a consequence of the high material waste when milling, it has been suggested that dental laboratories, to some extent, may reuse alloys when casting (60). Even so, some manufacturers only recommend the use of new alloys (61). It has been suggested that lower cell viability is observed to recast Co-Cr alloys compared to new Co-Cr alloys (62-64). However, there is no consensus on the possible effects of various recasting protocols on alloys concerning mechanical and

corrosion properties (65-68). The so-called soft milling or pre-sintered milling has been applied to fabricate Co-Cr frameworks, where a soft metal block is milled and afterwards sintered to its final shape to overcome the high costs of new tools (69-72). The pre-sintered Co-Cr block is manufactured in a similar way as zirconia; it contains binders and shrinks (~11 % for Co-Cr and ~25 % for zirconia) while it is sintered at a high temperature (73-75). The knowledge about Co-Cr alloys manufactured by this new technique is scarce (10, 34, 35, 76).

In contrast, the *AM (additive manufacturing)* technique, where the framework is three-dimensional (3D) built according to a digitally predesigned shape, is considered as a more sustainable and cost-effective manufacturing process compared to the subtractive technique (77-79). AM only uses the amount of metal powder needed to create geometrically complex 3D-dimensional parts and provides the possibility to manufacture parts in large quantities (77, 78). The most commonly reported AM of Co-Cr alloys in dentistry includes direct laser metal sintering (DLMS), selective laser sintering (SLS) and selective laser melting (SLM) (80-91). In direct laser metal sintering (DLMS) and selective laser sintering (SLS), the metal powder is only partially melted, causing a final metal part with larger porosities and a rougher surface compared to SLM, where the metal powder layers are fully melted together (92-95). It has been mentioned that the alloys processed with AM have an increased risk to exhibit residual stress and poor ductility (94, 96). Although these challenges may be overcome after the alloy is post-heat-treated (94, 96). However, a need to standardize or regulate the production of the starting powder due to observed heterogeneities among the AM materials has been requested (97, 98). Another future challenge for AM technology is the high energy consumption demand that is acquired compared to traditional manufacturing techniques (97, 99, 100).

For an optimal bonding between the porcelain and the alloy and after a suitable thickness of oxide layer is established, the dental technician applies a thin layer of a bonding agent before porcelain veneering (101). After that, different porcelain shades are applied to ensure optimal aesthetics (102). This procedure has many steps, and a sintering is performed between each layering. When the porcelain layers are sintered, the framework is placed in a vacuum furnace and undergoes a firing schedule recommended by the manufacturer. Some of the factors that may prohibit porcelain cracks or chip off fractures are 1) a lower CTE (coefficient of thermal expansion) of the veneering porcelain compared to the underlying alloy and 2) a lower sintering temperature compared to the alloy's melting point (102). *In vitro* studies investigating the porcelain bond strength to various Co-Cr alloys manufactured by different techniques did not reveal any statistically significant differences between them (103-105). Yet,

the differences in metal-porcelain bonding strength presented in previous studies are difficult to interpret since dissimilar test methods (shear bond test, loading under fracture and three-point bend test) has been used (106-109).
Despite a heterogeneity among the studies that evaluate fit among different Co-Cr alloys in fixed prosthodontics (both tooth- and implant-supported), it has been proposed that milled and laser melted frameworks demonstrate improved fit compared to cast ones (85, 110-117). However, one must keep in mind that fit evaluation among tooth-supported and implant-supported fixed constructions differs due to different levels and manufacturing processes when connected to teeth or implants, which makes the comparison of manufacturing techniques and fit difficult. In tooth- or cementable implant-supported fixed prosthodontics, the framework is cemented directly on the tooth or abutment. Yet, in screw-retained implant-supported prosthodontics, irrespective of the manufacturing technique used, the part of the framework that is connected to the implant or abutment is machined (118). Misfit in implant-supported frameworks may induce internal stress in the implant and the surrounding bone and lead to technical complications, such as screw loosing or fracture (85, 111, 112, 119). Furthermore, biological changes in terms of bone remodelling have been discussed, yet there is no evidence regarding the correlation between misfit in implant-supported frameworks and bone loss (119, 120). An unfavourable marginal accuracy in tooth-supported prostheses may lead to an increased risk of biological complications, such as secondary caries, periodontal problems, and pulpitis (121).

1.4 BIOLOGICAL ASPECTS

In fixed prosthodontics, the metal is usually covered with porcelain, except for non-visible areas as the lingual part of the constructions exposed to the oral *milieu* (Figure 1).

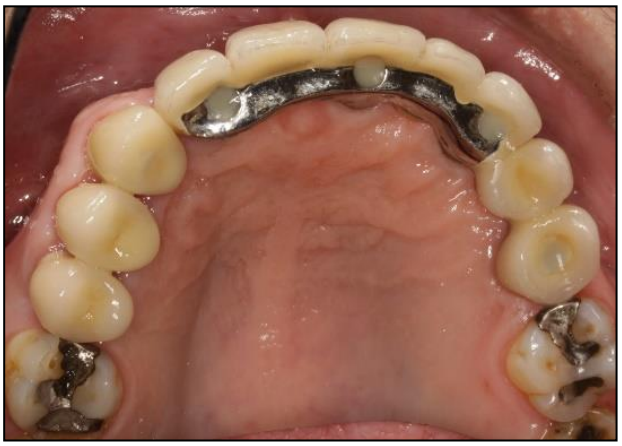

Figure 1. Implant-supported screw-retained prosthesis in Co-Cr alloy where Co-Cr is exposed to the oral cavity.

The hardness of Co-Cr alloys, causes a time-consuming process for the dental technician to finish and polish the construction (4). Moreover, the polishing of Co-Cr alloys without adequate ventilation has also been discussed to potentially increase the risk of allergies, sensitization and lung disorders (122-124). Yet, another disadvantage that has been reported is the risk of high ion release in acidic environments, which have caused concerns related to the biocompatibility of Co-Cr alloys (4, 14, 125-130).

Metal ions, such as Co and Cr, may be released in the oral cavity and evoke local and systemic adverse reactions (Figure 2) (14, 131). Dental materials are reported to release ions or particles intraorally (132, 133). Allergic reactions are divided into four different types: type I, II, III, IV. Type I, II, III occur quickly and are regulated by eosinophils, mast cells and B-lymphocytes that produce antibodies. Allergy reactions related to metal ions are referred to as a delayed hypersensitivity cell-mediated reactions, type IV, where the metal ions bind to host molecules that mediates cells, such as monocytes and T-cells that induces a type IV reaction (contact dermatitis) (132, 133). The systemic toxicity related to metal ions or particles is mainly evaluated by *in vivo* animal studies (133). Survival rates of the animals and pathological findings are registered after oral administration of a metal at a fixed dose over time, lower than LD_{50} (lethal dose) (133). Possible local reactions to metal ions can be

evaluated by *in vivo* and *in vitro* tests. *In vitro* tests of dental materials may include cell viability tests, the dentin-barrier test and other molecular toxicological methods such as fluorescence-activated cell sorting (FACS), Western blotting test and gene expression analysis (133-135). Although the extern validity of *in vitro* cell tests is debatable, *in vitro* tests can be standardized, which is of great importance for the reproducibility of the tests (136-138).

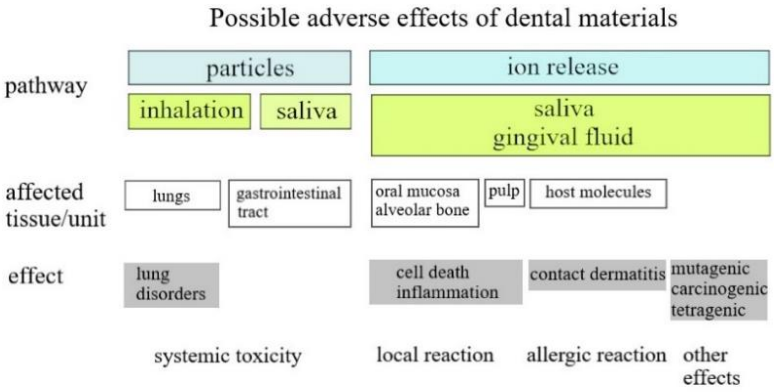

Figure 2. Overview of possible adverse effects from particles and ions in the human body (132, 133).

1.4.1 REGULATIONS AND REPORTS

Cobalt-chromium alloys mainly consist of Co (52.5-63.0 %) and Cr (27.4-29 %) (12, 14, 29, 34). According to ISO 22674:2016, manufacturers' are not obliged to declare elements contained <0.1 % (mass fraction), except for hazardous elements that should be reported at lower limits (139).

Due to the introduction of the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) regulation in 2007, new requirements of the Medical Devices Directive (MDR) (EU) 2017/745 regulations are now included:

a. establishment of a Unique Device Identification (UDI) for each device to obtain traceability within the supply chain. Also, the name and address of the carrier should be labelled on the device or packaging
b. substances with a content >0.1 % wt, where scientific evidence shows serious effects to human health, as carcinogenic, mutagenic or toxic effects or adverse effects on the reproduction and endocrine system, must be declared. However, a benefit-risk ratio analysis by the notified body and the manufacturer may justify the use of the specific substance in the device and state that the device is safe to use for its purpose (140, 141).

The European Chemicals Agency (ECHA) has listed Co as a CMR (carcinogenic, mutagenic and toxic for reproduction) substance (14, 142). A WHO report from 2006 concluded inadequate evidence that Co metal (without tungsten carbide) may cause cancer in humans (143). Moreover, it was concluded that the metallic form of Cr is not to be considered carcinogenic (144). Yet, Cr exists in different oxidation states where the trivalent Cr (III) play a key role in glucose metabolism. It has been reported that Cr (VI) and metallic nickel are carcinogenic to humans (144). These reports are based on animal studies and a few case reports on workers exposed to welding gases and fumes (144).

Due to the classification of Co as a CMR substance by ECHA, the European Union (EU) Medical Devices Regulation (MDR) (2017/745) in May 2021, suggest limited use of Co (145). Several manufacturers have claimed the necessity of Co use in Co-Cr alloys in dentistry due to their high corrosion resistance, strength, elongation after fracture, elastic modulus, and limited data on the possible carcinogenicity of Co in humans and the non-presence of alternative materials to Co-Cr alloys. Until May 2025, MDR invites both manufacturers and specialists to discuss the continued use of Co or to find a material that will replace Co in prosthodontics (14, 146).

1.4.2 BIOCOMPATIBILITY

Biocompatibility was initially mostly focused on material properties but was later extended to a wider definition including the host, and the interaction between the material and the host (147). Biocompatibility is usually evaluated by *in vitro* or *in vivo* investigations and clinical studies (133). The biocompatibility related to the metal-based tooth- or implant-supported constructions is mostly evaluated by their corrosion properties with (a) an electrochemical test that measures the electrode potential properties, or (b) a static immersion test where the amount of metal ions released is measured and their possible impact on cell morphology and cell viability and (c) a sulfide tarnish test that is usually used in alloys containing silver (148-156). Also, the mutagenic potential of different alloys has been investigated (152). Most studies investigating biocompatibility related to materials used in prosthodontics refer to toxic and allergic responses to ions caused by corrosion (157).

Although the wide use of Co-Cr alloys in dentistry, the biocompatibility of Co-Cr is questioned (14, 142, 144). Commercially pure titanium is known as the most biocompatible metal for prosthodontics (15). However, concerns related to biocompatibility for titanium alloys have also been reported (17, 133, 158, 159). Few studies investigate the biocompatibility of zirconia as a framework material in tooth-supported constructions (160). Most of the studies investigate biological aspects related to bone response to implants or different bone formation/bone healing applications (161-167). However, short-time clinical follow-up studies have shown promising results when monolithic translucent zirconia was used (168).

1.4.3 ADVERSE REACTIONS

Metals ions may cause an inflammatory response that will result in either a toxic or an allergic reaction (153). Although toxic reactions are usually considered dose-dependent and allergic reactions as dose-independent, their distinctions are unclear (153). For example, it has been suggested that metal ions in a very low concentration will not induce an allergic reaction (153). When metal ions contact body fluids, such as saliva or blood, they bind to proteins and activate T-cells (169-171). In some cases, this may cause allergic contact dermatitis in the skin adjacent to the medical device (Figure 2) (169, 172, 173).

Most studies investigating adverse effects in dentistry refer to case reports (174, 175). The reported prevalence of possible negative health effects from dental materials, i.e. systemic toxicity and local- and allergic reactions are very

low (<0.001 %) compared to the reports from the cosmetic industry (up to 23 %) (176). However, older reports declare that the prevalence of work-related occupational health effects was estimated at up to 27 %, mainly caused by the use of latex gloves and acrylates (174, 177, 178). Some of the symptoms that have been reported related to adverse effects to dental materials are burning mouth syndrome, taste disorders, dry mouth, gingivitis, lichenoid reactions, tongue pain, erythema on hands and feet, contact dermatitis and "abnormal sensation" (14, 153, 175, 179-181). Some case reports have also mentioned allergic symptoms, such as eczema related to titanium dental implants (178). The estimated prevalence of titanium allergy is low (<0.6 %) (174, 178, 182-184). Reports declare that up to 28 % are sensitive to nickel, and 1-8 % are sensitive to chromium and cobalt (153, 169, 185). Cobalt allergy often combines with nickel sensitivity, especially in women (133). Commercially pure titanium has been mentioned as a material for patients with already known metal allergies (158, 186, 187). It has been suggested that the low numbers of adverse reactions related to dental materials were due to underreporting (188). Some possible explanations for underreporting have been mentioned, such as (a) not knowing how to report, (b) lack of time or (c) not feeling obliged to report (189-191).

Patch tests are usually used when allergic contact dermatitis related to dental materials is suspected (Figure 3) (131, 192). The patch test identifies the delayed-type hypersensitivity (type IV reaction) caused by the etiologic agent that induces allergic contact dermatitis (133). In patch tests, salt solutions containing metal ions are applied to the skin (176). The investigated allergens are distributed below the patches with standard concentrations, in commercially available dental series (133, 193). Evaluation of the skin reactions occurs after two to three days and after five to seven days in case of late reactions of T-lymphocytes (133). However, factors such as (a) the type of salt (chloride, sulfate or nitrate), (b) the concentration of metal ions in the salt, (c) the oxidation state of the metal ion and (d) the type of patch may influence the results (153, 194). For instance, the concentration of 0.5 % cobalt chloride in Sweden was changed to 1.0 % according to worldwide standards due to a lower risk for false-positive results (195, 196). Another factor that may complicate the allergic investigation is that some metal salts contain other allergen elements than the elements tested, i.e. titanium (IV) oxalate hydrate used for detecting titanium allergy in patch tests for dental alloys has been shown to contain chromium (197). It has been observed that a small number of impurities, such as Cr, Cu, Fe, Mn, Ni and V were present in c.p. titanium grade 1 and 2, and titanium alloy (Ti6Al4V, Ti6Al7Nb) which makes it more difficult to determine which element caused the allergic reaction (198). Moreover, some patients that showed allergic reactions to nickel according to the patch test, did not show any clinical signs of adverse effects from their

nickel-containing alloys orally (133). In another study it was reported that the majority of patients (86 %) who consulted a specialist for suspected dental allergy and were subjected to the patch-test had symptoms that could not be related to a confirmed allergy (181, 199).

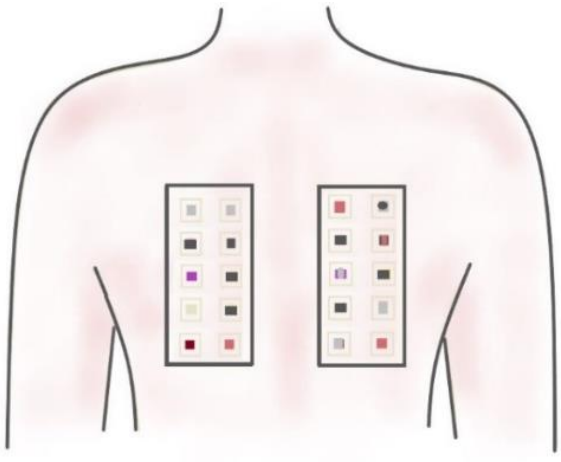

Figure 3. Suspected allergens are applied on the back in different patches. Registration of possible skin reactions after two to three days and five to seven days according to a graduation scale (193).

?+	*doubtful reaction*
+	*weak*
++	*strong*
+++	*extreme positive reaction*
IR	*irritant reaction*

One major difference between skin and mouth is the presence of saliva that plays an important role as a "cleaner" and surface protector in biofilm formation, which may cause changes in pH, electrolytic concentrations, and oxygen levels and affect the corrosion properties of the metallic surface (200). Also, the lower permeability in oral mucosa compared to the skin would need a 5-12 times higher concentration of allergens to provoke a tissue reaction in oral mucosa compared to the skin (184). Moreover, it is not recommended to use patch tests as screening tests since the test itself may induce a sensitization. In fact, the use of patch tests for investigating allergies related to dental materials is considered having a low sensitivity and is therefore controversial to use in dentistry (133, 153, 174, 201). Other tests, such as the Lymphocyte Transformation test and the MELISA (Memory Lymphocyte Immunostimulation Assay) test use monocytes from patient´s blood to detect increased levels of lymphocytes when the allergen is present, and has been proposed as the recommended test to detect titanium sensitization (133, 174,

184, 202-205). However, after the recommended removal of the restauration that contained the metal allergen that was previously confirmed with a patch test, only one patient out of 22 became symptom-free (175, 182).

Toxicity tests for dental materials are usually *in vitro* cytotoxicity tests that measure the proportion of viable cells after cells have been exposed (direct or indirect) to the investigated material (137, 206). A material is considered as having a cytotoxic effect when more than 30 % of the cells are reduced (137).

1.4.4 CORROSION

Corrosion of a material is defined as "an electrochemical process that depends on the ability to conduct electrical current, either using free electrons in metals or via ions in solutions" (157). Corrosion in the oral cavity is usually defined as aqueous corrosion (galvanic corrosion). It is described by two reactions: a) anodic (oxidation of metal) $M \rightarrow M^{(n+)} + n$ (electrons), and b) cathodic (where the electrons are absorbed) $2H^+ + 2e^- \rightarrow H_2$ (reduction of hydrogen) or $O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$ (reduction of dissolved oxygen) or $O_2 + 2H_2O + 4e^- \rightarrow 4OH^-$ (in acidic solutions) or in neutral and basic solutions, depending on the electrolyte (207). The greater the material's tendency to oxidize (ionize), the lower electrode potential value (V) will be achieved. A typical metallic property is their ability to release electrons when they come in contact with solutions (208). Ion release is more likely to occur for a metallic material with a more negative electrode potential (207). The influence of voltage between metallic constructions on biological structures has been discussed, yet no conclusions could be drawn (133).

Corrosion tests demonstrated higher corrosion resistance for the AM and pre-sintered Co-Cr alloys compared to the cast (155, 209, 210). It has been suggested that Co-Cr alloys with a high corrosion resistance could be used for patients diagnosed with gastroesophageal reflux disease (GERD) (211). The rapid cooling and strong temperature gradients for AM alloys causes a lower precipitate content, a lower presence of hcp phase and more uniformly distributed precipitates compared to cast which may explain the higher corrosion resistance for the AM Co-Cr alloy compared to the cast ones (94, 155, 212-214). However, the cast and pre-sintered milled Co-Cr alloy had a lower corrosion resistance after simulated porcelain firing (209). Alterations in the surface topography for cast specimens, such as the increased thickness and the heterogeneity of the oxide layers, explained this lower corrosion resistance (209).

1.4.5 ION RELEASE AND INFLAMMATORY RESPONSE

Elevated levels of metal ions both locally and systematically in patients with orthopaedic implants has been reported (215). An animal study reported an increased Ti amount in the bone after insertion of a dental implant (216). When Co is administrated orally to animals it is transferred to the blood and finally eliminated in the urine within 48 hours (217, 218). On the other hand, Cr binds to proteins (i.e. albumin) and accumulates in red blood cells and tissues (217). *In vitro*, it has been shown that there is a similar protein binding ability both for Co and Cr (219). The metal-protein complex has been suggested to be either reversible, where the metal is eliminated via the urine, or irreversible, where the ions are maintained and processed metabolically (217). Yet, metal ions that bind to proteins may be difficult to detect; therefore, the metal ion release may be under-reported (220). In the orthopaedic field, metal degradation from a metal implant is stated as one possible cause of periprosthetic bone loss (215, 221).

Although there are differences in environmental conditions between orthopaedic implants and oral prostheses, as to the absence of saliva, exposure to bacteria and the dissimilar combination of materials, connection type and loading circumstances, failures related to ion release from hip prosthesis made of Co-Cr alloys have been suggested (214, 222, 223). In implant-supported prosthodontics, Co-Cr supra constructions are sometimes connected to the titanium implants without any intermediate abutment, resulting in direct contact of the Co-Cr-alloy to the peri-implant mucosa. It has been reported that when ions from Co-Cr and c.p. titanium are present simultaneously, the ion release and inflammatory response decrease indicating a possible mechanism of passivation from them (224, 225). Moreover, it has been reported that ion release decreases over time (226). In contrast, a positive correlation between time and metal ion release has been described in another report (126). It has also been suggested that below pH 4, metal ion release increases quickly (126). Furthermore, the outcome of several studies that measured ion release demonstrated a total metal ion release below the limit level. However, higher levels were found for the cast compared to the laser melted Co-Cr (125, 130, 206, 227).

Metal ion release can be evaluated by inductively coupled plasma optical emission spectrometry (ICP-EOS) or inductively coupled plasma mass spectrometry (ICP-MS) (125, 126, 206, 227-229). ICP-OES measures the excited atoms and ions related to the characteristic wavelength of the investigated element while ICP-MS quantifies elements by mass spectrometry (228).

Lower cell viability for fibroblast cells was observed for AM Co-Cr alloys when exposed to metal ions compared to pre-sintered milled alloys (150). However, no statistically significant differences in cytotoxic effects were found between cast and AM Co-Cr alloys (230). When epithelial and fibroblast cells were exposed to cast Co-Cr and c.p. titanium grade 4 specimens, both cell types were more viable when exposed to c.p. titanium (129).

Moreover, metal ions may cause up-and down-regulation of inflammatory regulators, i.e. cytokines, with subsequent tissue destruction around dental- or orthopaedic implants (218, 231-233). Cells involved in the innate immune response, such as neutrophils, macrophages, mast cells, and eosinophils, can generate cytokines within seconds that further regulate the inflammatory response (234-237). In non-inflammatory conditions, monocytes circulate in the blood, yet in tissue injury, chemokine secretion, or other pathogen stimuli, the monocyte infiltrates the affected inflamed tissue (238). The importance of evaluating the cytokine expression from monocytes/macrophages in cardiac diseases, breast and lung cancer, and dental and orthopaedic implants have been suggested (238-241).

Pro- and anti-inflammatory cytokines, such as interleukins (IL)-1, IL-6, IL-4 and tumour necrosis factor-α (TNF-α), are reported to play an important role as regulators in the inflammatory process (242, 243). As pro-inflammatory cytokines activate and cause the migration of inflammatory cells such as neutrophils, monocytes and lymphocytes, the anti-inflammatory cytokines control the pro-inflammatory cytokines and depresses the immunological response (243, 244). Increased IL-6 levels were observed when cells were exposed to metals (149). It has also been proposed that implant debris activate macrophages that induce a pro-inflammatory response (169, 245). Furthermore, a correlation between the upregulation of IL-8 and the severity of oral lichenoid lesions OLL has been proposed (246). Still, knowledge about the possible negative effects of metal ion release in the oral environment is limited (215, 218, 247-250).

1.5 MECHANICAL PROPERTIES

In the oral environment, materials are exposed to different pH and temperatures, bacteria and mechanical loading. Mechanical loading occurs when teeth or constructions replacing teeth are in contact, i.e., during chewing, swallowing or bruxism. In general, the mechanical requirements for oral constructions in the molar area are 20 % higher compared to the frontal area (251, 252). In fact, the constructions in molar areas may be subjected to forces

between 600-1200 N, although forces up to 3500 N has been recorded (252-254). For metals used in oral prosthetic constructions, the minimum requirements for mechanical properties, such as yield strength, percentage of elongation after fracture, elastic modulus and hardness are presented in Table 2 (139).

	Yield strength (MPa)	Elongation after fracture (%)	Hardness (HV)	Elastic modulus (GPa)
The minimum value for type 4	360	2	-	-
The minimum value for type 5	500	2	-	150
Co-Cr (cast)	410-618	7.9-12	215-390	188-260
Co-Cr (milled)	438-672	2.3-12	230-353	223-253
Co-Cr (AM)	410-1058	2.4-32	335-1010	98-271
Co-Cr (pre-sintered milled)	426-549	14-29	230-242	169-270
c.p. Ti grade 1	170	24	126	110
c.p. Ti grade 2	280	20	178	110
c.p. Ti grade 3	380	18	221	110
c.p. Ti grade 4	480	15	263	110
Ti6Al4V	860	10-15	280-320	117

Table 2. Overview of mean values for mechanical properties of the cast, milled, AM and pre-sintered milled Co-Cr alloys and titanium presented in other studies, with limits for Type 4 and 5 applications in dentistry (32, 34, 35, 81, 82, 87, 88, 139, 255-270).

Yield strength represents the amount of stress that is achieved when 0.2 % of plastic strain occurs (Figure 4) (271). The percentage of elongation after fracture is measured after fracture of the specimen (Figure 3) (271). The high elastic modulus of Co-Cr alloys compared to gold alloys and c.p. titanium provides exceptional possibilities for the dental technician to reduce the framework thickness in cases where the vertical dimension is limited without jeopardizing their strength (12, 39). Elastic modulus or Young´s modulus describes the measure of rigidity or stiffness of a material. The stiffer the material, the steeper the slope (Figure 4) (254).

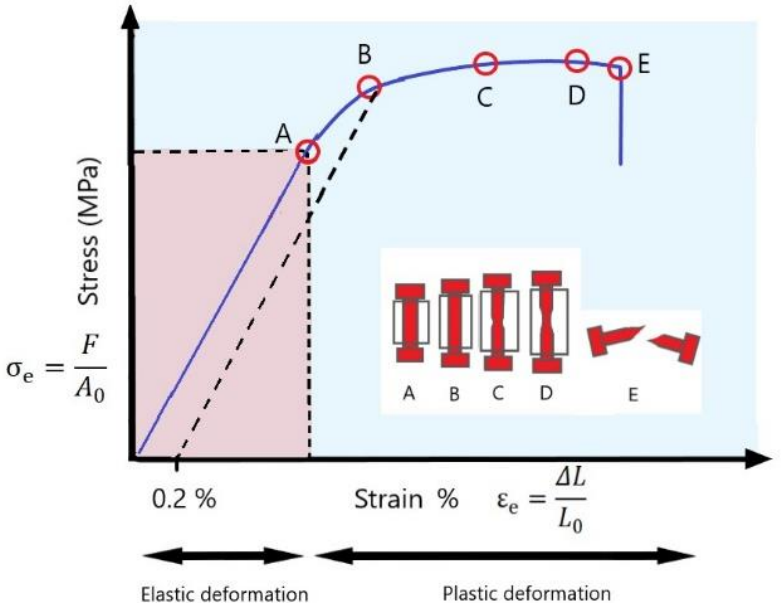

Figure 4. Illustration of a stress-strain diagram. When the stress exceeds the proportional limit (A) the specimen deforms plastically (blue=plastic deformation area) until it brakes (E). Elastic modulus is calculated by the slope equation in the elastic region (red=elastic deformation area) (254).

Moreover, Co-Cr alloys possess beneficial mechanical properties such as high hardness values. However, the high hardness may cause difficulties for the dental technician to finish or polish the frameworks. Hardness is defined as the "resistance to indentation" related to a specified load and time (272).

When comparing mechanical properties among Co-Cr alloys manufactured with different techniques, AM Co-Cr alloys demonstrate higher values of mechanical properties, such as yield strength, hardness, elongation after fracture and elastic modulus values compared to cast, milled and pre-sintered milled (Table 2) (34, 81, 88, 257, 260, 268, 273-275). However, it has been reported that the AM process should be more optimized due to the higher presence of microstructural defects compared to other techniques (262). For AM Co-Cr alloys, heat treatment, for example, annealing, is recommended in order to reduce the brittleness of the alloys (268).

1.6 STRUCTURE

1.6.1 SURFACE ROUGHNESS

Surface roughness is often referred to "as the variation in the height of the surface relative to a reference plane," where the reference plane can be a single line or parallel lines, so-called surface maps (276). Studies investigating the influence of surface topography parameters, such as surface roughness, on biological response are mostly related to the possible correlation between dental implants and bone healing (277-284). It has been reported that increased surface roughness on dental implants may induce a higher bone-to-implant-contact and a faster bone healing as compared to implants with a turned surface (278-280, 282, 283, 285). However, dental implants with a rougher surface seemed to increase plaque accumulation in a short-term follow-up (286). Yet, no conclusions could be drawn when comparing the incidence of peri-implantitis among dissimilar roughness grades to implants (286). Furthermore, bacterial adhesion in the oral *milieu* is also suggested to be related to the type of bacteria and the formation of biofilm (287, 288). Although a rougher surface of an implant creates a larger surface area and is supposed to increase the ion release compared to a smoother surface, however no statistically significant differences in *in vitro* ion release among implants with different surface roughness parameters were observed (224, 277).

Moreover, no statistically significant differences in roughness nor cell viability to human cells exposed to cast, pre-sintered milled and AM Co-Cr specimens were revealed (134). However, it has been reported that fibroblasts compared to epithelial cells, prefer rougher surfaces of both zirconia and titanium (165). Also, manufacturing parameters within the AM technique may affect cell viability, i.e. the building angle in AM increased both surface roughness and fibroblast cell viability to AM Co-Cr specimens (289).

1.6.2 MICROSTRUCTURE

Metals generally consist of grains defined as randomly ordered crystals (208). Characteristics for metals or alloys are their typical metallic bonds created by a cloud of electrons around the positively charged nucleus (208). The crystals in metals are not perfectly arranged due to the presence of flaws called dislocations (208). When a force is applied to a metal, a sliding mechanism occurs, where the imperfect array of the crystals requires less force to move compared to if the crystals were in a perfect array. This movement related to dislocations in metallic materials leads to the material's deformation (208). Some of the factors that may suppress the movement of dislocation and therefore strengthen the metallic materials are: (a) the non-presence of

impurities, (b) grain and twin boundaries, (c) the presence of second phase particles or precipitates (carbides) and (e) alloying elements (4, 34, 39, 41, 79, 94, 208, 257, 271, 290-296). The faster solidification of Co-Cr alloys manufactured by AM, compared to the cast ones results in the typical microstructure for AM Co-Cr alloys with finer grains and the presence of precipitates (94, 297). On the other hand, the cast specimens demonstrate large dendritic structures related to lower elongation after fracture and ultimate tensile strength compared to AM Co-Cr alloys (94, 297). Furthermore, the rapid cooling rate and high-temperature gradient of AM generates a relatively smaller grain size, uniform distribution of precipitates and higher dislocation density compared to the cast ones (94, 212, 298, 299). However, it has been suggested that AM processing parameters, such as speed, power, powder quality and angulation of powder layer may affect the mechanical properties; i.e., yield strength, elongation, presence of pores and residual stress (79, 89, 94, 274, 300-304). Although the AM Co-Cr alloys exhibit higher mechanical strength than other manufacturing techniques, limitations such as anisotropy and porosities that affect their fatigue behaviour have arisen (88, 94, 275). Heat treatment has been suggested to decrease the residual stress in AM Co-Cr alloys (79, 155, 256, 259, 305-308). Although heat treatment is applied each time the porcelain layer is sintered, the heat treatment usually mentioned in studies occurs at a higher temperature (up to 1150°C) compared to the porcelain sintering (up to 1000°C) (87, 155, 256, 259, 305-309).

1.6.3 CO-CR ALLOYS

The microstructure of Co-Cr alloys is mainly composed of two different phases, (a) the unstable high-temperature face-centred cubic (fcc) γ and (b) the more stable low-temperature hexagonal close-packed (hcp) ε crystal structure (14, 310). When solely Co is cooled down slowly, it transforms from the unstable fcc γ to the hcp ε crystal structure at 417°C while this allotropic phase transformation for Co-Cr alloys occurs at a higher temperature of 970°C (12, 35, 290, 311). It has been reported that the transformation between fcc↔hcp in Co-Cr alloys is slow and therefore the cooled Co-Cr alloy may contain a metastable phase of fcc (Figure 5) (274).

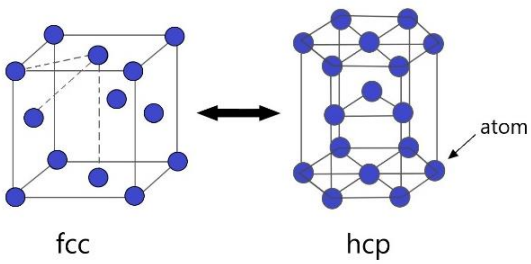

Figure 5. Examples of unit cells of space lattices for Co-Cr alloys. A unit cell is the smallest repetitive part of a crystal. Cubic lattices, i.e. fcc (face-centred cubic) are most common for metallic materials (312). The close-packed hexagonal is observed in Co- Cr alloys and titanium and zirconium (312). A missing atom (vacancy) in the unit cell is very common.

The retained fcc phase structure at room temperature is associated with favourable mechanical properties; (a) a high yield strength, (b) a high strain hardening rate, (c) high tolerance under cyclic stress and (d) absorption of stress (81). At low temperatures, the fcc phase in Co-Cr alloys may also be stabilized through fast cooling or by adding C and/or N (311, 313). The amount and ratio of different phases, as to the overall composition and microstructure of a metallic material, are influenced by (a) the temperature history, (b) the time of processing and (c) heat treatment of the metallic material (39). The Cr in Co-Cr alloys form oxides that create a corrosion-resistant surface (12). Moreover, both Cr and Mo increase the strength of an alloy by forming carbides and solid solution hardening (12, 94). Additionally, Mo and W form carbides as they act as stabilators for the hcp crystal phase (275, 314). The addition of Si improves the casting of Co-Cr alloys by lowering their melting point and increasing both corrosion resistance and hardness (266, 315, 316). Adding low amounts of elements, so-called grain refinement, of iridium (Ir) and Ru may increase the tensile strength and elongation after fracture due to the uniformity properties among the cast alloys. However, grain refinement

does not seem to affect the hardness and yield strength of a cast alloy (39). Still, precaution must be taken when adding Cr and W, in order to avoid the alloy becoming brittle (12, 317). Specifically, Cr (>30 %) forms a brittle and hard Cr-rich σ phase that decreases the corrosion resistance of the material (39, 255). Even small alterations in the composition of the added elements may significantly change the mechanical properties. For example, a reduction of 0.2 % of C reduces the yield and tensile strength to values to the extent that causes them unsuitable for usage in dental applications (39). The presence of C facilitates the formation of carbides that hardens the alloy, although C content above 1.0 % increases the second-phase particles always present in cast alloys (39). Chromium-rich second-phase particles in cast Co-Cr alloys may be related to a higher ion release from cast alloys compared to other manufacturing techniques (318). Although, it has been reported that Co-Cr alloys in metal-ceramic construction are strengthened to a higher extent by solution hardening rather than with carbide formation (39).

1.6.4 TITANIUM AND ITS ALLOYS

Commercially pure titanium exists at room temperature in a hcp (α-phase) crystal structure. When heated >883°C, it undergoes a phase transformation from the hcp crystal structure (α-phase) to a stronger but more brittle body-centred cubic (bcc) crystal structure (β-phase) (Figure 6) (39). Due to a lower number of slip systems (three) of the hcp α-phase compared to bcc β-phase (twelve), the hcp α-phase exhibits some limitations in formability and toughness compared to bcc β-phase that demonstrates better plastic deformation ability.

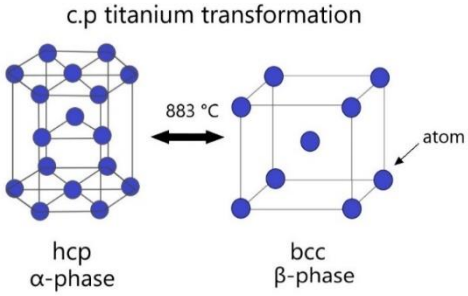

Figure 6. Examples of unit cells of space lattices for c.p. titanium. The hcp (left) presents a lower number of slip systems compared to the bcc β-phase (right) (40, 312).

Yet, the bcc β-phase also presents some limitations, such as lower creep resistance compared to the hcp α-phase. When alloying c.p. titanium with

atoms smaller than Ti, i.e. Al or when interstitially dissolved atoms are present, such as H, C, N, and O, the material becomes more densely packed and more slip systems can be induced (40, 319). In titanium alloys, Al, C, N, Ga stabilizes the α-phase meaning that the transformation from α to β occurs at a higher temperature than 883°C when heated (17). In contrast, V, Mo, Co, Ni, Nb, copper (Cu), Pd and tantalum (Ta), acts as β-phase stabilators (17). It causes a transformation from β to α to occur at a lower temperature than 883°C when cooling (17). At room temperature, the α-phase titanium is more weldable although difficult to form compared to the β-phase titanium that is more malleable and easy to form, i.e. orthodontic wires (17). Ti6Al4V is a two-phase alloy (α+β) and transforms to a bcc crystal structure (β-phase) at a higher temperature (>975°C) compared to c.p. titanium. Various thermal treatment procedures determine the amount of α and β phase in the titanium alloy that further contributes to their superior mechanical properties compared to c.p. titanium (except for elastic modulus) (Table 2) (17, 39).

2 AIM

The overall aim of the present thesis was to increase the knowledge about different Co-Cr alloys and manufacturing techniques used in fixed prosthodontics concerning biological and mechanical aspects.

2.1 SPECIFIC AIMS

The specific aims were:

Study I — To investigate and report the usage of Co-Cr alloys in fixed prosthodontics (FP) among dental laboratories in Sweden according to manufacturing technique.

Study II — To investigate the metal ion release from Co-Cr alloys manufactured with different techniques in media with different pH, the possible impact of simultaneous presence of c.p. Ti grade 4 together with Co-Cr alloys and Ti6Al4V ELI, surface roughness and the possible impact of heat treatment on metal ion release and surface roughness. Furthermore, to investigate cell viability among materials.

Study III — To compare the yield strength, elongation after fracture, hardness, elastic modulus and microstructure of Co-Cr alloys manufactured with different techniques and compare the mechanical properties with c.p. Ti and Ti6Al4V ELI. Futhermore, to investigate if heat treatment affects mechanical properties and microstructure of the materials.

Study IV — To study the cytokine release from human PBMCs that were exposed to Co-Cr alloys manufactured with different techniques and compare them to c.p. Ti and Ti6Al4V ELI, with a known surface roughness for all materials.

2.2 HYPOTHESIS

The null hypotheses were that

Study II
-total ion release does not differ among the materials
-total ion release does not differ at different time occasions
-the presence of c.p. titanium will not affect the total ion release
-surface roughness does not differ among the materials
-heat treatment does not affect the surface roughness
-the viability of cells that were exposed to released ions does not differ among the materials

Study III
-yield strength, elongation of percentage after fracture and hardness do not differ among the materials.
-heat treatment does not affect the yield strength, percentage of elongation after fracture and hardness

Study IV
-there are no differences in cytokine release regardless of material and the surface roughness will be similar among materials

3 MATERIAL AND METHODS

3.1 STUDY I

All registered dental laboratories in Sweden in March 2015 that were received from the Swedish MPA (Medical Products Agency, https://www.lakemedelsverket.se/en) and a large private dental health group, Praktikertjänst (https://www.praktikertjanst.se/) were included. Two questionnaires (for tooth- and implant-supported fixed prosthodontics) with ten questions each were sent by post to 542 registered dental laboratories in Sweden with questions regarding:

a) type of material used for fixed prosthodontics; alloy name
b) manufacturing technique used; location, type
c) if inward/outward preparation of the alloys was done before porcelain layering; which steps
d) total preparation time after manufacturing and before porcelain layering
e) total unit production of fixed frameworks, irrespective of material/year
f) total unit production of fixed Co-Cr frameworks/year
g) total unit production of fixed Co-Cr frameworks manufactured by cast/milled/ laser sintered/other technique/year

Reminders to those that did not respond were sent up to three times. Due to a high initial degree of non-responders, an additional question was added to the questionnaires concerning the type of work the dental laboratory provided; fixed or removable. The total drop-out reached 45 %, 38 % were non-responders, and 7 % were returned due to unknown addresses.

3.2 STUDIES II, III, AND IV

3.2.1 SPECIMENS (STUDIES II, III, IV)

The studies involved Co-Cr specimens manufactured by techniques, chosen from those reported in the questionnaires in Study I; (a) cast Wirobond 280® (W280), remanium® star (Rc), (b) milled remanium® star MDII (Rm), (c) laser melted remanium® star CL (Rlm) and (d) pre-sintered milled Zirkonzahn® Sintermetall (Zz). Commercially pure titanium and TI6Al4V ELI were included for comparison. Four shapes of specimens were used for Studies II, III, IV (Figure 7) (Table 3).

Study	Specimens	Test/analysis method	Evaluation parameters
II	cylinder-shaped (8x8 mm)	▪ static immersion test I/ICP-MS ▪ MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide	▪ ion release in pH 7.03 ▪ cell viability
II, III	rectangular-shaped (34x13x1.5 mm)	▪ static immersion test II/ICP-OES ▪ optical interferometry ▪ impulse Excitation Technique (IET) ▪ combined focused ion beam/-scanning electron microscope (FIB-SEM)/Energy-dispersive X-ray spectroscopy (EDS) analysis	▪ ion release in pH 2.3 ▪ roughness ▪ elastic modulus ▪ microstructure
III	rod-shaped (diameter=3±0.1 mm, length=42 mm)	▪ tensile test ▪ Vickers Hardness HV5	▪ yield strength, percentage of elongation ▪ hardness
IV	disc-shaped (8x2 mm)	▪ cytokine assay	▪ cytokine release

Table 3. An overview of the specimen shapes, methods for analyses and parameters used for evaluation in the various studies.

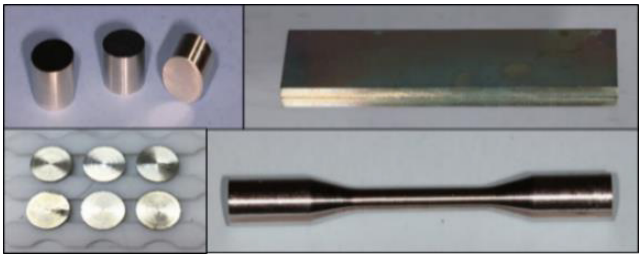

Figure 7. The different specimen shapes used in Studies II, III, IV; cylinder-, rectangular-, disc- and rod-shaped.

Half of the rectangular- and rod-shaped specimens underwent a firing process in a vacuum furnace (Jelenko Commodore 100 VPF, New York, USA), to simulate porcelain layering, according to the manufacturer's recommendation, (Table 4) (Figure 8).

	W280 (cast 1)	Rc, Rm, Rlm (cast 2, milled, laser melted)	Zz (pre-sintered milled)	c.p. Ti Ti6Al4V ELI
Firing X4	2 min 500ºC Vacuum 500 ºC 80 ºC/min to 960 ºC 1 min 960 ºC Vacuum 75 mm Hg	6 min 500°C Vacuum 500ºC 75 °C/min to 930 °C 1 min 930 °C Vacuum 75 mm Hg	6 min 500ºC 75 ºC/min to 870 ºC 1 min 870 ºC Vacuum 75 mm Hg	6 min 400ºC 67ºc/min to830 ºC 1min 830ºC Vacuum 75 mm Hg

Table 4. The firing process of the specimens according to the manufacturer's porcelain recommendation.

Figure 8. The firing of the specimens. After each firing process (n=four), the specimens were left to cool down at room temperature.

All specimens (included the heat treated (ht)) were ground (Knuth Rotor, Struers, Ballerup, Denmark) with SiC grinding paper (Struers A/S, Ballerup, Denmark) from 320 to 1200 grit size according to ISO 22674:2016.

3.2.2 STATIC IMMERSION TESTS (STUDY II)

In Immersion Test I, six ht and non-ht specimens of each material were immersed in the corresponded volume of 0.1 mol/L lactic acid (pH 2.3) for seven days at 37 ºC, according to ISO 22674:2016. After removing the specimens, the immersion solution was extracted and sent for ICP-OES analysis (ARL 3580, Thermo-Optek, Ecublens, Switzerland).

In Immersion Test II, none of the specimens were ht. Six cylinder-shaped specimens from each material were immersed in separate tubes of artificial saliva (pH 7.03). Furthermore, six cylinder-shaped specimens from each material were immersed simultaneously with c.p. Ti grade 4 in separate tubes of artificial saliva (pH 7.03). After 1, 4, 7, 14 and 21 days, the solution extracted from each tube was sent for ICP-MS analysis (Thermo Fisher Scientific Inc., Bremen, Germany).

3.2.3 ICP-OES AND ICP-MS (STUDY II)

In both atomic spectroscopic techniques of ICP-OES and ICP-MS, the sample (immerse solution) was heated into a gas of free atoms and ions (320).

Argon gas was supplied to an ICP torch, and together with a high-frequency current and the electric magnetic field created, argon gas was ionized (321). A spark and the argon gas started a chain reaction that caused free electrons to accelerate by the magnetic field and collide with more argon atoms, causing more free electrons (321). This ionization of argon gas forms a plasma contained by argon atoms, ions and electrons (320). The samples were pumped into a nebulizer that converted the liquid into an aerosol or mist (322, 323). The aerosol produced from the sample was then introduced to the ICP torch where intense heat broke down the sample into a hot gas containing free atoms and ions of the element of interest (321).

In ICP-OES, the hot plasma induced the atoms or ions from the sample to emit their characteristic light (Figure 9) (320). ICP-OES uses the characteristic energy level of every element, its emission spectra in the visible region for us, the ultraviolet (160-800 nm) (320). When the excited atoms or ions return to a lower energy level, emission rays are released, and the concentration of each specific element is analyzed (320).

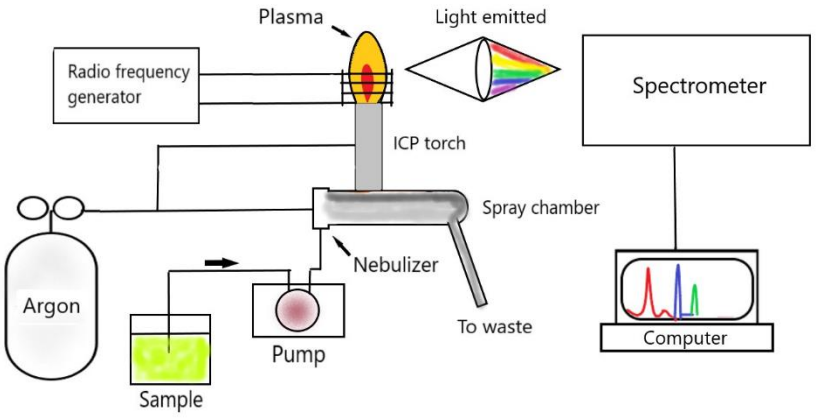

Figure 9. An overview of the ICP-OES technique (321, 322).

In ICP-MS, the mass of ions is analysed, usually with a mass filter called quadrupole (324). The quadrupole consists of metallic rods charged with different electric currents, creating electric fields that separate ions based on their m/z ratio (mass of an ion/ion charge) (324). For example, only an ion with a certain m/z ratio will pass through the quadrupole when applied to a certain current potential combination (324).

3.2.4 SURFACE ROUGHNESS (STUDY II)

The surface roughness was determined with white-light interferometry by an optical interferometer (smartWLI-extended, GBS, Ilmenau, Germany). A light source is separated into two light beams with a beam splitter. One light beam is reflected from the specimen surface, and the other is reflected from a reference plane (325). The 3D differences on each point of the specimen surface cause wave changes in the reflected light evaluated by Surfascan software (Somicronic Instrument, Lyon, France) (Figure 10). The surface of six rectangular-shaped specimens (half of them were ht) from each material was examined. The measurements included three randomly selected regions/specimens, resulting in nine measurements per material. To separate surface roughness from errors of form and waviness, a high-pass Gaussian filter of 50 μm x 50 μm was used (325).

The following parameters were measured:

- Sa (µm)– average roughness,
- Sds (1/µm^2)– summit density
- Sdr (%)– developed inter facial area ratio

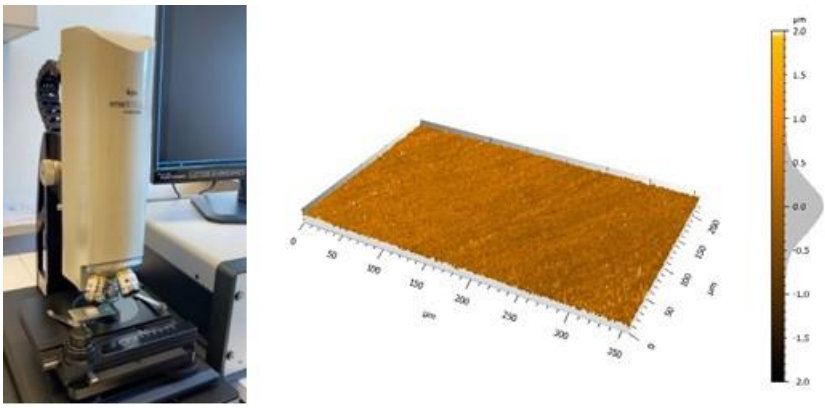

Figure 10. Surface roughness measurement. The optical interferometer (left) and surface roughness (Sa) for cast Co-Cr alloy (non-ht).

3.2.5 CELL CULTURES (STUDIES II, IV)

In Study II, cell lines of mouse fibroblasts (L929) and human bronchial epithelial cells (BEAS-2B) from the European Collection of Authenticated Cell Cultures (ECACC, Public Health, England) were used. In Study IV, buffy coats from peripheral blood mononuclear cells (PBMCs) were obtained from ten anonymous, healthy, volunteer blood donors at the Sahlgrenska University Hospital in Gothenburg, Sweden.

3.2.6 CELL VIABILITY TEST (STUDY II)

Cylindrical specimens of all materials were immersed in separate glass bottles with cell culture medium (pH 7.2-7.4) at 37 ºC in an agitated water bath (Julabo, Göteborg, Sweden) according to ISO 10993-12:2012. After 24 hours, the extract from the glass bottles was removed, and well-plates of cell lines L929 and BEAS-2B were exposed to the extract for another 24 h at 37 ºC. The extract was then removed, and cells were exposed to MTT (0.5 mg/ml diluted in phosphate-buffered saline, Sigma, St Louis, USA) for one hour at 37 ºC, 5 % CO2 and 95 % humidity (326). MTT converts the mitochondrial enzyme

dehydrogenases in viable cells to purple-coloured Formosan. After removing the culture medium, dimethyl sulfoxide (DMSO, VWR, Life science, Radnor, USA) that dissolves Formosan was added. After 20 minutes of agitation, the proportion of cell viability was evaluated in a spectrophotometer (Synergi H1, Biotek, Vermont, USA).

3.2.7 CYTOKINE ANALYSIS (STUDY IV)

The cytokine analysis tests were performed on buffy coats from PBMC cells obtained from ten anonymous, healthy donors at the Sahlgrenska University Hospital in Gothenburg, Sweden. PBMC cells were exposed to disc-shaped specimens from all materials. Supernatants were collected on three occasions; after four h, 24 h and 72 h. Analysis of the cytokine concentration was made by the detection of antibodies specific to each cytokine through a colour-coded magnetic bead conjugated with capture antibodies and a fluorescent reporter. The cytokine concentrations in the culture supernatants were measured by Luminex xMAP technology using the 21-plex, and 27-plex screening panels of the Bio-Plex Pro™ Human Cytokine Assay (Bio-Rad Laboratories, Hemel Hempstead, UK) and the analysis and calculations were made using the BioManager analysis software (Bio-Rad Laboratories, Hemel Hempstead, UK) (327).

3.2.8 MICROSTRUCTURE (STUDY III)

The microstructural investigation of one rectangular Co-Cr specimen from each group (cast, milled, laser melted, pre-sintered milled) was performed by combined focused ion beam/-scanning electron microscope (FIB-SEM) operating at 15kV with Tescan Lyra3 (Tescan Orsay Holding, Kohoutovice, Czech Republic. In FIB, a beam of ions is used while SEM uses electrons to form an image (328, 329). A four-quadrant backscatter electron detector obtained micrographs with grain channelling and atomic number contrasts. The EDAX Octane Plus detector (EDAX, Mahwah, New Jersey, USA) was used for energy-dispersive X-ray spectroscopy (EDS) analysis.

3.2.9 YIELD STRENGTH, ELONGATION AFTER FRACTURE (STUDY III)

The 0.2 % yield strength and elongation after fracture of twelve rod-shaped specimens for all materials (half of them were ht) from each material were performed in Zwick/Roell BZ1-MM11210.IN01, the capacity of 10 kN, Zwick GmbH &Co. KG, (Ulm, Germany) using an extensometer with a gauge length of 10 mm, a preload of 2 MPa and a speed of 1.5 mm/min until the specimen fractured (Figure 11).

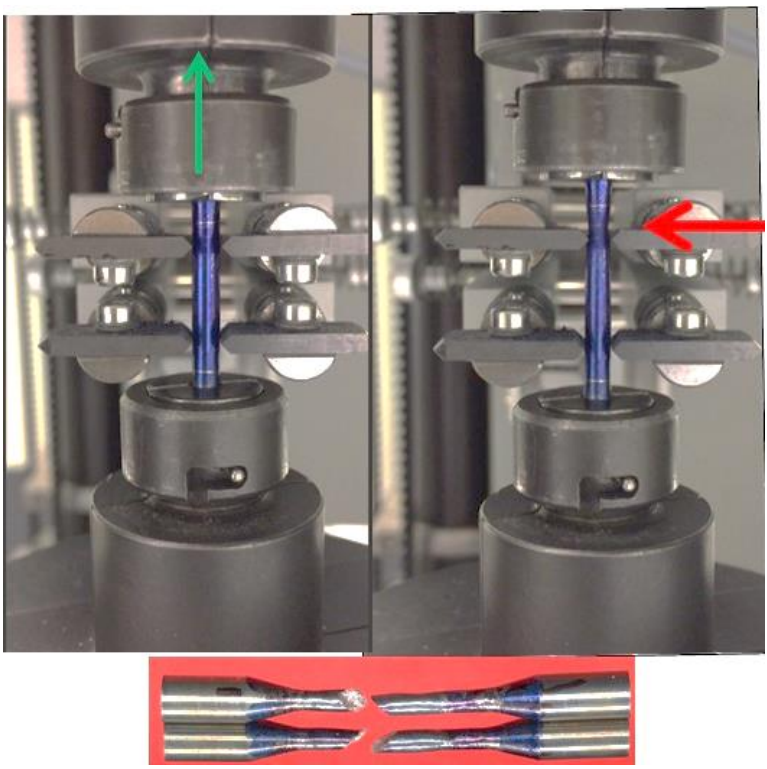

Figure 11. A uniaxial tensile force (green arrow, left side) is applied to the test specimen until it brakes. Notice the narrowing of the rod-shaped specimens as the applied force increases (red arrow, right side).

3.2.10 ELASTIC MODULUS (YOUNG´S MODULUS) PILOT STUDY (STUDY III)

The elastic modulus for three non-ht and three ht rectangular-shaped specimens from all materials were investigated by impulse excitation technique (IET) (330-332). This non-destructive method excites impulses and detects the vibrations from the tested material. Since metallic specimens are monolithic and isotropic there is a mathematical correlation formula between (a) geometry, (b) mass, (c) mechanical resonant frequencies and (d) elastic properties of tested specimens which calculates elastic modulus after the resonance frequency is measured (Figure 12).

$$E = 0{,}9465\left(\frac{mf_f^2}{b}\right)\left(\frac{L^3}{t^3}\right)T_1$$

Figure 12. E=Dynamic Young´s modulus, m=mass, b=width, L=length, t=thickness, ff=fundamental resonant frequency of bar in flexure, T1=correction factor for the fundamental flexural mode to account for finite thickness of the bar, Poisson´s ratio.

The vibration is induced by a light impact with a small hammer on the tested specimens. The created signal is recorded by a microphone and transmitted to Resonant Frequency& Damping Analysis (RFDA), mechanical spectroscopy based on IET (Figure 13) (333).

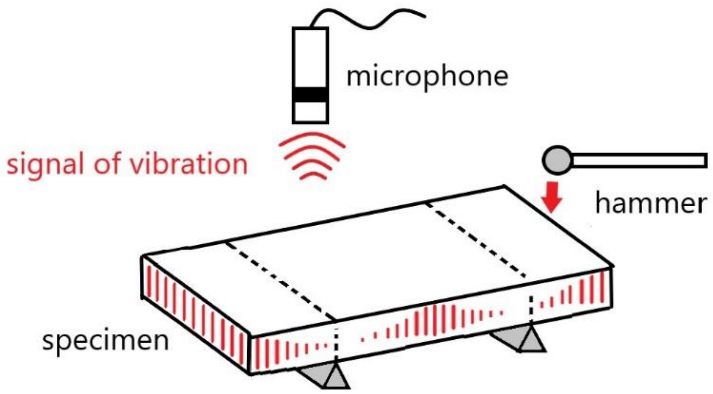

Figure 13. Schematic overview of the IET that through mechanical excitation creates signals that measures resonance frequency and by RFDA Software calculates elastic modulus for the tested specimen (334).

3.2.11 HARDNESS (STUDY III)

Vickers hardness test HV5 was performed on three non-ht and three ht rod-shaped specimens (five measurements per specimen), from each material with a 136-degree diamond edge and a weight of 5 kg during dwell time of 20 seconds, Innovatest (Nova330, Maastricht, Netherlands) (Figure 14) (335).

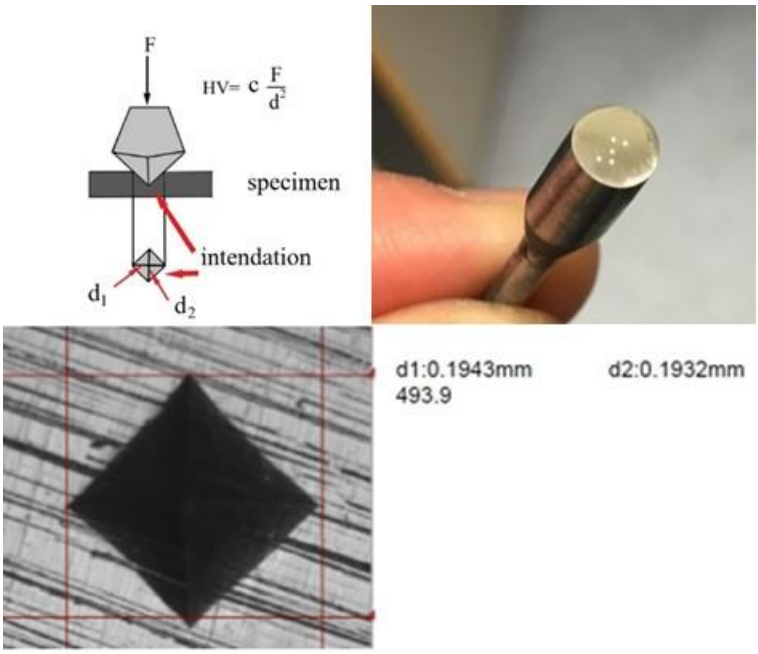

Figure 14. Overview of the Vickers hardness test. The HV score is calculated by the indentation of the diamond edge at the surface of the specimen through applying the formula (see left upper drawing). F= test force, in newtons, d=mean millimetres, of the two diagonal lengths d1 and d2, c=0.1892. Five indentations per specimen (right upper). One indentation from a ht laser melted Co-Cr specimen is shown below.

3.2.12 STATISTICAL ANALYSES

Study I — No statistical analysis was performed.

Study II — The comparison of cell viability and metal ion release among materials was tested using Kruskal Wallis. Metal ion release between different test occasions was calculated with Friedman's test. The Mann-Whitney U test was performed to analyze the total ion release difference between the presence of titanium or not and non-ht and ht specimens. The difference in surface roughness among materials was tested by one-way ANOVA, and roughness difference between non-ht and ht specimens was compared using the independent samples t-test. The Bonferroni correction method was used for post-hoc testing.

Study III — One-way ANOVA analysis was performed to compare the difference in yield strength and percentage of elongation after fracture among the materials. The independent samples t-test was used to compare the yield strength and elongation after fracture between non-ht and ht specimens. Tukey's significance test was used for post-hoc testing. The difference in hardness among the materials was evaluated using a mixed-effects model followed by the Bonferroni correction method for post-hoc testing.

Study IV — The cytokine levels were compared using Friedman's non-parametric test, and Dunn´s multiple correction test was performed for post-hoc testing. The difference in surface roughness data was tested by one way ANOVA, and pairwise post-hoc testing was performed by the Tukey test.

Statistical significance was evaluated by SPSS version 25 (Study II), 26 (Study III), 27 (Study IV) (IBM Corp, Armonk, New York, USA) and GraphPad Prism version 9.1.0 software (GrapdPad Software Inc., La Jolla, CA, USA) (Study IV).

4 RESULTS

4.1 STUDY I

From the surveys that were sent to 542 dental laboratories in Sweden, 299 (55 %) answers were received. Three reminders were sent. Of the 542 sent envelopes, 206 (38 %) did not respond, and 37 (7 %) were returned because of an unknown address. In total, 22 dental laboratories were excluded due to that (a) they were not dental laboratories (4 %), (b) they were inactive (4 %), (c) they were taken over by another company (0.3 %), and (d) they had their production abroad (0.3 %). Due to a high number of missing answers to questions related to the preparation and the production volume of the Co-Cr alloys, these answers were excluded.

Higher frequency of non-answered surveys for the questions related to fixed implant prosthesis (FIP) (14 %), compared to the questions related to the fixed dental prosthesis (FDP) (5 %). The 299 received surveys showed that 180 (60 %) and 135 (45 %) worked with FDP and FIP, respectively. Eighty-six (29 %) of the dental laboratories worked with both FDP and FIP, 47 (16 %) worked only with FDP and 3 (1 %) only with FIP. Most of the dental laboratories reported the use of Co-Cr and ZrO_2 in both FDP and FIP. A larger part of dental laboratories reported the use of titanium (c.p. Ti and alloys) in FIP compared to FDP (Table 5). In total, from the 542 dental laboratories, 134 (45 %) and 89 (30 %) that were reported to use Co-Cr in FDP and FIP, respectively, were included.

	181 dental laboratories					
	Co-Cr	ZrO_2	gold*	c.p. Ti* *	Ti alloy	other***
FDP	135 (75 %)	138 (76 %)	84 (46 %)	38 (21 %)	13 (20 %)	105 (58 %)
FIP	91 (66 %)	90 (64 %)	40 (29 %)	50 (36 %)	25 (18 %)	29 (21 %)

Table 5. A summary of the reported construction type used (FDP/FIP) and material use from the included 181 dental laboratories.
** noble, **grade 1-4, *** noble alloys, palladium alloys and other ceramics.*

A high number of unidentified Co-Cr alloys were registered in the FIP survey compared to the FDP survey; 36 % of the laboratories reported up to nine unidentified Co-Cr alloys in the FIP survey compared to 18 % of laboratories that reported up to five unidentified Co-Cr alloys in the FDP survey. The included dental laboratories reported using up to 35 different Co-Cr alloys in FDP and up to 30 different in FIP. The five most commonly reported Co-Cr alloys in FDP were: Wirobond ®280 (28 %), Cara SLM (19%), Wirobond® C (13 %), Remanium® Star CL (11 %), Solibond C plus (10 %) and Heranium PW (10 %) and in FIP: Cara SLM (27 %), Cara Milled 26 %, Wirobond® 280 (19 %), Wirobond® C (14 %), Coron® (12 %), Remanium® Star CL (11 %). Besides differences in the chemical composition among the Co-Cr alloys reported, they were also manufactured by various techniques. In both FDP and FIP, three manufacturing techniques were reported: casting, milling and AM. A fourth technique was reported for the construction of FDP, the pre-sintered milled technique. Furthermore, some dental laboratories reported the usage of up to six different Co-Cr alloys in FDP and up to eight different Co-Cr alloys in FIP.

4.2 STUDY II

4.2.1 IMMERSION TESTS

A higher amount of ion release, ≤11.6 µg/cm^2, was observed in acidic conditions compared to physiological conditions, ≤0.08 µg/cm^2. Both immersion tests demonstrated a total ion release below the limit (200 µg/cm^2 per seven days) (139). In acidic conditions, the ht cast and milled Co-Cr demonstrated the highest ion release, and in physiological conditions, the pre-sintered milled Co-Cr showed the highest ion release. The results generally showed a total decrease of released ions from Day 1 to Day 21, and an ion release decrease as c.p. Ti grade 4 was present. In all tests, Co ion was detected in the highest concentration. Except for Co ions, Cr and Si ions were detected in the cast and milled Co–Cr alloys in the acidic immersion test. Also, the ICP-MS test detected small amounts of Mo, Mn, Al and Ti ions from all materials (Figure 15).

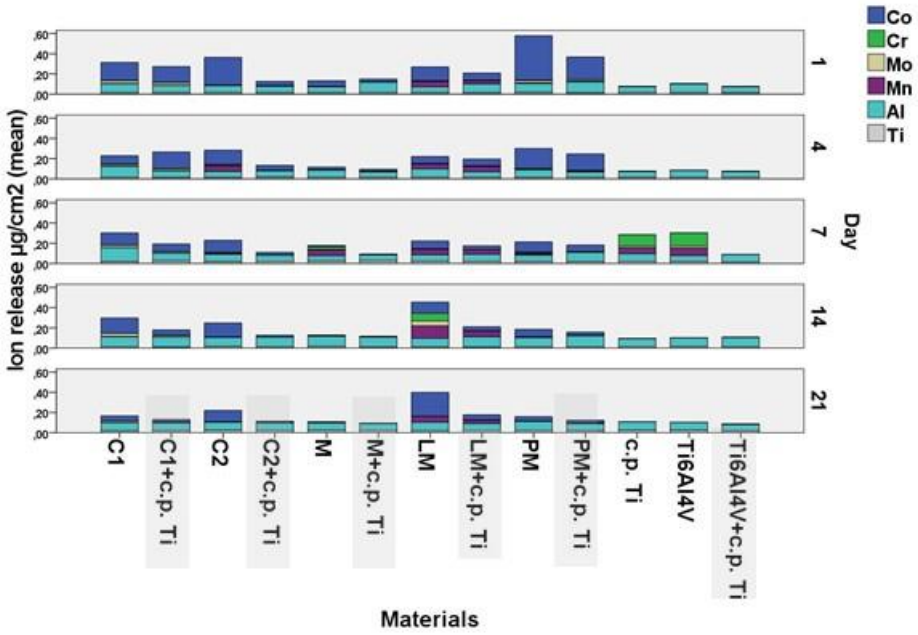

Figure 15. Ion release from the ICP-MS (pH 7.03, with and without c.p. Ti at different time points. C1=cast Co-Cr alloy (Wirobond ®280), C2=cast Co-Cr alloy (remanium® Star), M= milled Co-Cr alloy (remanium® Star MDII), PM=pre-sintered milled Co-Cr alloy (Zirkonzahn® Sintermetall). The grey areas in the y-axe represent the materials with the simultaneous presence of c.p. Ti in the artificial saliva.

4.2.2 SURFACE ROUGHNESS

The surface roughness analyses of all tested materials, irrespective of heat treatment or not, presented a smooth surface, Sa < 0.5 μm (325). Minor differences were demonstrated between the materials in all surface parameters; mean range difference for S_a (0.02-0.1 μm), Sd_s (0.03-0.21/ μm^2), Sd_r (1.2-15.7 %), $p < 0.01$. Commercially pure titanium (non-ht) demonstrated a statistically significant higher surface roughness (Sa, Sdr) compared to all other non-ht groups. Furthermore, the Ti6Al4V ELI (ht) group also showed a statistically significant higher mean surface roughness (Sa, Sdr) compared to cast, milled, laser melted and pre-sintered milled Co-Cr as well as c.p. Ti. Among the Co-Cr alloys, the milled Co-Cr (non-ht) presented statistically significant higher Sa and Sds values than non-ht cast, laser melted, and pre-sintered milled Co-Cr specimens.

The ht cast, laser melted Co-Cr alloys and Ti6Al4V ELI specimens presented a statistically significant rougher surface compared to their corresponding non-ht specimens.

4.2.3 MTT (CYTOTOXICITY) ASSAY

The results demonstrated that none of the epithelial cells and fibroblasts cells, that were indirectly exposed to Co-Cr, c.p. Ti and Ti6Al4V ELI, showed a cytotoxic reaction according to ISO 10993-5:2009.

4.3 STUDY III

4.3.1 MECHANICAL ASPECTS

The results demonstrated a statistically significant higher yield strength value for ht laser melted Co-Cr compared to all other ht materials, except for the Ti6Al4V ELI. The latter material demonstrated a higher yield strength mean value compared to the laser melted Co-Cr, $p<0.05$. The ht laser melted Co-Cr specimens showed the highest mean values in hardness compared to all other materials, $p<0.05$. A higher elongation after fracture was observed for the ht c.p. Ti compared to all other ht materials, $p<0.05$.

A minor decrease in yield strength was recorded after heat treatment for the cast (W280) and milled Co-Cr specimens, $p<0.05$. No statistically significant differences in hardness were recorded between non-ht and ht specimens. A minor increase in percentage of elongation after fracture was found for c.p. Ti after heat treatment, $p<0.05$.

4.3.2 MICROSTRUCTURE

The qualitative analysis of the Co-Cr specimens demonstrated a larger grain size for the cast and milled Co-Cr specimens compared to pre-sintered milled and laser melted Co-Cr specimens. The cast and milled Co-Cr materials showed dendritic segregation and second phase interdendritic particles in the last solidified areas. No secondary phase particles or segregation could be observed in the pre-sintered milled Co-Cr specimens. However, a high presence of pores was registered in the pre-sintered milled Co-Cr specimen. The cast and milled Co-Cr presented similar degree of segregation of Cr and W. Additionally, the cast Co-Cr specimen demonstrated the segregation of Nb in the secondary phase particles.

Both inter- and intragranular precipitations were shown for the laser melted Co-Cr specimen. The white precipitates were enriched in W and Nb, with no signs of Co and Cr. Heat treatment did not alter the grain morphology or the number of secondary phase particles after heat treatment.

4.4 STUDY IV

All cells secreted low cytokine levels after four hours, except for RANTES, which was upregulated for all materials (including the controls). Interleukin-4 and IL-8 were upregulated after four hours of exposure to the pre-sintered milled Co-Cr alloy. The expression of cytokines was highest for cast and pre-sintered Co-Cr specimens compared to the milled and laser melted Co-Cr materials and both titanium materials. The expression of pro-inflammatory cytokines IL-1β, IL-6, IL-8, IL-17, IFN-γ, and TNF-α were highest when cells were exposed to cast Co-Cr specimens compared to milled, laser melted, and titanium specimens (c.p. Ti and Ti6Al4V) after day 1 and 3. Moreover, the anti-inflammatory cytokine IL-4 was upregulated when cells were exposed to cast Co-Cr specimens compared to milled and laser melted Co-Cr specimens. Lower anti-inflammatory cytokine levels of IL-4, IL-10, IL-12 and IL-ra were observed at Day 3 when cells were exposed to c.p. Ti compared to cast Co-Cr specimens.

Furthermore, an upregulation of the pro-inflammatory cytokine levels of IL-1β, IL-17, TNF-α and IFN-γ and the anti-inflammatory cytokines of IL-4 and IL-12 were observed for the pre-sintered Co-Cr specimens compared to the milled and laser melted Co-Cr specimens. When comparing c.p. Ti and Ti6Al4V, no statistically significant difference in pro- and anti-inflammatory cytokine release were found.

5 DISCUSSION

5.1 DISCUSSION OF METHODS

5.1.1 STUDY I

The Swedish MPA was contacted to include all dental laboratories in Sweden in **Study I**, and a list of the registered dental laboratories in 2015 in Sweden was received. Moreover, dental laboratories incorporated to a large private dental concern, Praktikertjänst, were included. The information obtained was the name of the dental laboratory and the postal address. An attempt to find email addresses through the websites was made. However, the questionnaires were sent by post due to a high number of dental laboratories without an existent website or email address. In total, 542 dental laboratories were included.

In order to finalize the questionnaires, initial questions were asked to local dental technicians from two dental laboratories in Jönköping, Sweden. No validated questionnaires related to prosthetic material use were available at that time. Two surveys, one for FDP and one for FIP, with ten questions each, were sent by post to the dental laboratories. Because of a high initial number of non-responders, an additional question was added which was related to if the dental laboratories worked with fixed prosthodontics or not. Three reminders were sent. The response rate of 55 %, was considered acceptable since the response rate compared to similar national surveys in other countries ranged between 32-60 % (336-341).

Study I aimed to obtain the most frequently used Co-Cr alloys, although due to a high drop-out, the most commonly reported Co-Cr alloys were registered. Theoretically, an increased response rate within and among questionnaires may have been seen if the questionnaires had been validated, thus achieving higher reliability and validity (342). Higher response rates from dental technicians for paper surveys (40-80 %) compared to web-based surveys (14-57 %) were observed between the years 2003-2021, with an increase of web-based surveys over time (6, 337-339, 341, 343-347). A lower response rate and lower missing values were observed for web-based surveys in 2005 compared to paper surveys (348, 349).

Possible reasons for the high frequency of missing answers to several questions in **Study I**, may have been; (a) lack of time (a survey may take >20 minutes), (b) an avoidance in sharing sensitive information related to

production/economical profit and (c) that the surveys were not sent to dental technicians in person but to the laboratories which may have had an impact (when large laboratories) on the willingness and responsibility felt in answering the survey (350, 351). In comparison, a web-based survey that was conducted during the same time period (2017) in Sweden, with questions regarding radiography choice among orthodontic specialists (n=255) and not orthodontic clinics demonstrated a similar response rate (57 %) as in **Study I** (55 %) (350, 351).

5.1.2 STUDY II

Co-Cr materials were selected based on the manufacturing technique used and according to the most frequently reported (352). One Co-Cr alloy from each manufacturing technique was included. Furthermore, one additional cast Co-Cr alloy (from the same manufacturer) was selected (Rc) to match the chemical composition of the milled and laser melted Co-Cr alloys declared from the manufacturer. Commercially pure Ti grade 4 and Ti6Al4V ELI were included.

Immersion Tests I and II
Ion release was measured by two immersion tests in different pH (acidic and physiological) and was analyzed by ICP-OES and ICP-MS. In immersion test I, the ion release from specimens were analyzed by ICP-OES, in pH 2.3 ± 0.1 according to ISO 22674:2016 and ISO 10271:2011 (139, 353). In order to mimic the oral *milieu*, with regard to the variation of pH, a modified standard protocol to ISO 10993-15:2009 and 10271:2011 was applied in immersion test II where the ion release was analyzed by ICP-MS (137, 324, 353). Most studies have evaluated ion release from Co-Cr alloys with ICP-MS compared to ICP-OES, also named inductively coupled plasma atomic emission spectrometry (ICP-AES), due to higher sensitivity, detection capability and accuracy (125, 126, 128, 206, 220, 227-229, 354-356). However, due to the possibility of reactions between the plasma argon gas and the atoms investigated, i.e., Fe ions, in ICP-MS, there is a high risk for interferences which is considered a disadvantage in ICP-MS (324, 357). Half of the specimens in Immersion Test 1 in **Study II** were ht to simulate the porcelain firing. After the firing protocols, all specimens were ground on the surfaces (139).

Surface roughness analysis
In order to evaluate surface roughness analysis in **Study II**, three surface parameters (Sa (μm), Sds ($1/\mu m^2$) and Sdr (%)) were measured with an optical interferometer. Half of the specimens were ht.

Methods to measure surface roughness can be roughly divided in optic or mechanic techniques (287). The SEM technique has also been suggested to evaluate surface roughness. Yet, as it has limitations in creating 3D data, the SEM is preferable for morphological investigations rather than in surface roughness evaluations (134, 287, 325, 358).

In order to measure the surface roughness of a solid surface, (a) a mechanical contact profilometer, (b) an optical profiling instrument, or (c) a scanning probe microscope can be used (325). The mechanical contact profilometer uses a diamond tip that is always in contact with a constant speed along the surface and records and scans the vertical motions of the tip, creating 3D data (276, 288, 325). One of the disadvantages of the mechanic profilometers is that the contact between the tip and surface may damage the tested surface, leading to underestimating the surface dimensions (276, 325). Consequently, mechanical profilometers are recommended to be used on flat surfaces and not on "soft" materials, i.e., c.p. Ti (276, 278, 325). However, optical profiling is considered a fast method capable of producing high-resolution images of the surface without any mechanical contact (224, 277, 280, 325, 358). There are several types of methods within the optical profiling technique, such as (a) the focus detection system, (b) the confocal laser scanning microscopy and (c) the white light interferometry method where the latter being the most commonly reported (224, 289, 325, 358, 359). Another method that measures surface roughness and is mainly applied to non-conducting materials is the Atomic Force Microscopy (AFM) (360). The AFM technique measures force between a sharp probe and the surface to provide a nanoscale 3D image and is preferred for rough surfaces (359-361). However, only an extremely small area can be measured, resulting in data that are not representative of the total specimen surface (325).

Cell viability assay

According to standards, the evaluation of *in vitro* cytotoxicity of medical devices is recommended to be determined by quantitative methods, such as (a) neutral red uptake (NRU) cytotoxicity test, (b) colony formation cytotoxicity test, (c) MTT cytotoxicity test, and (d) XTT cytotoxicity test (2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)carbonyl]-2H-tetrazolium hydroxide) (137). The NRU test is a membrane integrity assay and has been reported to be used in evaluating toxicity to endodontic materials (135). MTT and XTT assays utilise the mitochondrial activity in viable cells to evaluate cell viability (362, 363). Yet, the MTT formazan is insoluble compared to the formazan product from XTT that is soluble (363). In order to measure the cell viability by a spectrophotometer, the MTT formazan needs to be dissolved with an additional step compared to XTT (363). A qualitative evaluation of the *in vitro* cytotoxicity of medical devices is only preferred for screening (137).

Other techniques evaluating *in vitro* toxicity by cell viability, i.e. WST-8 (2-(2-methoxy4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H tetrazolium, monosodium salt) and Resazurin (that is also classified as a colourimetric assay as MTT), dye exclusion test (DET), agar diffusion test (ADT) and Resazurin have been reported (Table 6) (129, 134, 148, 152, 230, 289, 363-366). It has been reported that DET has low sensitivity compared to other methods that evaluate cell viability (367). Also, a possibility of an overestimation of cell toxicity due to toxic effects from the fluorescent test compounds on cells in the Resazurin has been mentioned (363).

In **Study II,** the evaluation of cell viability was performed by the MTT assay, which is in accordance with other studies that compared the cell viability among Co-Cr alloys (149, 150, 154, 206, 230, 326, 366, 368). Standards recommend the use of established cell lines from recognized repositories, i.e. mouse fibroblasts (L929, Balb/3T3 clone A31) and human fibroblasts (MRC-5 and WI-38) (137, 369-371). Except for L929 mouse fibroblasts commonly used in other studies, human epithelial cells (BEAS-2B) were used in **Study II** to include the major cells surrounding the oral epithelium (129, 148, 150, 152, 206, 289, 372, 373).

Cell viability assays	Cell lines	Evaluation method
MTT (149, 150, 154, 206, 230, 365, 366, 368)	▪ L929 mouse fibroblasts (150, 206, 372) ▪ Balb/C 3T3 mouse fibroblasts (366, 374) ▪ Human epithelial cells from mucosa (368) mouse 3T3 fibroblasts (154) ▪ Human fibroblasts (149) ▪ MRC-5 human lung fibroblasts (230)	Quantitative colourimetric evaluation
WST-8 (134, 148, 373)	▪ Human Adipose Derived Stem Cells (hADSC) (134) ▪ L929 mouse fibroblasts (148, 372)	Quantitative colourimetric evaluation
Dye exclusion test (DET) (230)	▪ MRC-5 human lung fibroblasts (230)	Quantitative staining evaluation (375)
Agar Diffusion Test (ADT) (152, 230)	▪ MRC-5 human lung fibroblasts (230) ▪ L929 mouse fibroblasts (152, 372)	Qualitative evaluation grade 0-4 (none-severe) (137)
Resazurin (129, 289, 365, 368)	▪ Human epithelial cells from oral mucosa (368) ▪ L929 mouse fibroblasts (129, 289, 372) ▪ Human epithelial cells (129)	Quantitative colourimetric evaluation

Table 6. A summary of methods used in assays for evaluating cell viability.

5.1.3 STUDY III

Microstructure

In **Study III,** cast, milled, laser melted and pre-sintered milled Co-Cr specimens were evaluated using a combined focused ion beam/-scanning electron microscope (FIB-SEM) (259, 328, 329, 376). The advantage of using a combined FIB-SEM compared to solely SEM is that in a combined FIB-SEM, FIB, with the help of Ga ions, mills the specimens cross-sectional before analysing the microstructure by SEM (35, 81, 88, 212, 256, 259, 265, 266, 269, 274, 298, 299, 306, 329, 377-379). Thus, no other tool is needed to prepare the specimens before the analysis (380). Some studies advocate the specimens' cutting to investigate microstructure (306, 381). However, the definition of microstructure is not always well-defined since some studies refer to microstructural investigations, even though the surfaces were examined (34, 35, 256, 258, 382, 383).

Yield strength, percentage of elongation after fracture

A tensile testing machine with extensometer was used to perform the 0.2 % yield strength and elongation after fracture in **Study III** (32, 34, 35, 89, 139, 257, 258, 263, 266, 269, 296, 300, 304, 379, 381, 383-386). Elongation after fracture is one of the variables, except for the per cent reduction of the area that describes the ductility of a material (387). Elongation after fracture is defined as the difference in gauge length before and after the tensile test (388). The gauge length can be measured both manually and automatically. The majority of similar studies as the present one, that presented data of elongation after fracture to Co-Cr specimens, does not always declare the method used, yet, one may assume that the automatically received data from the tensile test were used (34, 35, 257, 263, 266, 269, 296, 300, 384, 385). It has also been reported that a non-contact optical measurement of strain values should be preferred in order to minimize the uncertainty of methods evaluating the strain value of the material (32, 258, 304, 383, 389). However, standards do not specify how to register the gauge length difference for obtaining the percentage of elongation after fracture (139). Except for the automatic measurement obtained from the tensile test, the manual gauge length measurement has been reported, which was also done in **Study III** (381). After the specimens had been fractured, they were re-measured. The new gauge length was recorded, and the apparatus software calculated the percentage of elongation after a fracture. However, due to a high degree of uncertainty in the manual reading of the gauge length after fracture, because of difficulties putting back the fractured dumb-bell specimens together in a straight line, only

the obtained automatically data were incorporated in the calculations (Figure 16).

Figure 16. The manual measurement of gauge length before and after specimens´ fracture. The gauge area was coloured with a marker, and two positions were curved with a pointy tip and measured before the test (upper). After the test specimens fractured, the two pieces were put together, and a re-measurement of the gauge length was done (down).

Elastic modulus (Young´s modulus) pilot study

Due to a high degree of uncertainty as well as heterogeneity among results and methods from the various tests used for evaluating the elastic modulus (tensile and flexure test method), other methods such as the acoustic resonance method, have been suggested (32, 34, 82, 139, 257, 258, 263, 384, 390-392). In study III, elastic modulus was evaluated by impulse excitation technique (IET) (330-333).

Hardness

In **Study III**, hardness was evaluated using the Vickers hardness test, according to other studies (35, 87, 88, 257, 258, 262, 265, 266, 269, 335, 382, 385, 393). Except for the Vickers hardness test, other test methods evaluating hardness has been reported, such as the (a) Brinell hardness test, (b) Rockwell hardness test, and (c) the Knoop hardness test (257, 272, 298, 299). The difference among them is the variation in indent materials used and their shape design (272). For example, both the Vickers and Knoops hardness tests utilize a pyramidal shaped diamond point, while the Brinell test involves a cylindrical shaped steel point (272).

5.1.4 STUDY IV

In **Study IV**, human PBMCs cells (monocytes) were used according to recommendations (394). The life-span of monocytes in blood is short, approximately 24-48 hours (234). After that, monocytes die or become macrophages that have a longer life-span than the monocyte, months to years (234). However, it has been reported that the short-life of monocytes may cause false-negative results in cytokine release tests which should be considered when interpreting the results (234).

Some examples of assays that measure the *in vitro* cytokine release are; (a) ELISA, PCR and (b) multiplexed based immunoassays (236). The ELISA test detects cytokines directly from the cells and is a fast method with high sensitivity (236). In contrast, PCR detects cytokines indirectly by determining the gene expression for cytokine production (232, 233, 236, 246, 395-398). The multiplexed-based immunoassays were also used in **Study IV**, which detects cytokines rapidly but at a lower detectable limit than the ELISA test (236, 399, 400).

5.2 DISCUSSION OF RESULTS

5.2.1 STUDY I

Besides new materials, the traditional cast technique has been replaced by newer methods using the CAD-CAM workflow, such as subtractive and AM techniques (94, 110, 401, 402). Web-based questionnaires that were sent to dental technicians in the United Kingdom and Ireland in 2015 demonstrated that the majority of dental technicians that worked in "large" dental laboratories utilized CAD-CAM workflow in the production of the implant- and dental retained constructions to a higher extent than those who worked in "smaller" laboratories (344). However, no information was provided regarding the number of employees (344). Dental technicians reported that high initial cost and the absence of CAD-CAM technology were the main reasons that smaller dental laboratories were not involved in the CAD-CAM workflow (344). Furthermore, "large" dental laboratories used zirconia more frequently, but also titanium and base metal alloys, contrary to the "smaller" dental laboratories that, to a higher extent, used noble alloys (344). Results from **Study I** demonstrated that both Co-Cr and zirconia were most commonly reported to be used as framework materials in FDP and FIP. Yet a higher frequency of dental laboratories reported using zirconia in FDP while Co-Cr alloys were most reported in FIP. Locally-distributed surveys with a response rate of 42 % and 84 % respectively in Greece and Saudi Arabia demonstrated that the majority of the dentists preferred base metal alloys, yet areas of weakness in communication between dental technicians and dentists were reported (403, 404). In general, base metal alloys were most commonly reported to be used as framework material for metal-ceramic fixed prostheses (337, 339). No information was available neither for the type of construction, dental- or implant-supported nor for the alloy type (339). Results from **Study I** reported that the use of c.p. Ti and Ti alloys were more frequently reported in FIP compared to FDP, probably due to the well-documented biocompatibility of titanium (15, 405, 406).

Furthermore, results from **Study I** demonstrated that the dental laboratories reported a higher number of used Co-Cr alloys; up to 35 different Co-Cr alloys in FP and up to 30 different in FIP. Dental laboratories reported using up to eight different Co-Cr alloys compared to another study that reported the use of up to seven different base-metal alloys per dental laboratory (407). Three different manufacturing techniques for Co-Cr alloys were reported in both FDP and FIP, namely cast, milling and AM, yet a fourth technique, the pre-sintered milling technique, was reported in FDP. No surveys could be found that investigate the applied manufacturing technique for Co-Cr in FP among dental

laboratories. Moreover, results from **Study I** demonstrated that Co-Cr FDPs were more commonly reported to be cast than Co-Cr FIPs, which were more commonly reported to be manufactured by the additive and the subtractive technique.

Due to a higher ability in AM technique to produce shapes with complex forms and undercuts, makes AM a more suitable production technique for both FDP and FIP compared to cast and milling (77, 78, 90).

5.2.2 STUDIES II, III, IV

The main results from Study II, III, IV are presented in Table 7.

		Results
Biological aspects	Study II	▪ most ions released from cast and milled in acidic conditions ▪ fewer ions when c.p titanium also was present ▪ Co ions were released the most ▪ all tested materials were non-toxic
	Study IV	▪ stronger inflammatory response from cast and pre-sintered milled Co-Cr compared to laser melted and milled Co-Cr
Mechanical aspects	Study III	▪ higher yield strength and hardness for laser melted compared to cast, milled and pre-sintered milled ▪ higher elongation after fracture for laser melted compared to cast and pre-sintered milled
Material structure	Study II	▪ milled Co-Cr were rougher compared to cast, pre-sintered and laser melted Co-Cr
	Study III	▪ similar microstructure for milled and cast Co-Cr regarding grain size, dendritic segregations, second phase interdendritic particles ▪ segregations of Cr and W in cast and milled ▪ segregations of Nb in cast ▪ pores in pre-sintered milled Co-Cr
Heat treatment	Study III	▪ rougher surface for cast and laser melted Co-Cr specimens after ht ▪ lower yield strength for cast Co-Cr after ht

Table 7. Main results obtained for Co-Cr in Studies II, III, IV. Note that only the statistically significant differences are presented, except for the microstructural investigation in Study IV.

Mechanical aspects and material structure

In **Study II**, a rougher surface was demonstrated for c.p titanium grade 4 and Ti6Al4V ELI compared to cast, laser melted and pre-sintered milled Co-Cr ($p<0.01$). Another finding from **Studies II** and **III** was that the laser melted Co-Cr alloy demonstrated higher hardness values and a smoother surface than the milled Co-Cr alloy ($p<0.05$). The topographical analyses were performed to assure that a similar surface roughness was obtained on tested specimens after a standardized protocol, so the ion and cytokine release results were not influenced by dissimilar surface roughness.

Hardness tests are often used to obtain initial general measurements on the strength of materials to decide if further strengthening processes are needed, i.e., heat treatment. A correlation between the hardness and yield strength of materials has been suggested (408). This relation trend could also be seen for the Co-Cr alloys in **Study III**, although no statistical correlation analysis was made (408). Hardness is usually classified as indentation hardness, although other classifications exists, such as wear or abrasive hardness (408). If the antagonizing restorative material to enamel exhibits higher hardness values than enamel, there is a risk of extensive loss of tooth substance (409, 410). The hardness value of enamel has been reported to reach 400 (HV), which is higher compared to the results from **Study III** for the cast, milled and pre-sintered milled Co-Cr but lower compared to the laser melted Co-Cr, 564 (HV) (409, 411). Approximately similar values for hardness are reported for the veneered zirconia from an *in vitro* study, as the hardness values for laser melted Co-Cr in **Study III** (410). On the other hand, the hardness value of full anatomic zirconia has been reported to be more than four times harder than enamel (up to 1700 HV) (410). Suppose these *in vitro* results are applied in a clinical situation. In that case, it could mean that both laser melted Co-Cr, and veneered and full anatomic zirconia may cause higher tooth wear than cast, milled, pre-sintered Co-Cr, c.p titanium and Ti6Al4V (411). However, enamel wear is not only caused by the hardness of the opposite material, but other factors, such as bruxism, quantity/quality of saliva and pH may influence the wear process in dentition (412).

If a metallic material is isotropic ("uniform properties in all directions") the measured hardness on the surface will be equivalent to the hardness in the bulk of the material (408). Hardness measurements in **Study III** were only performed on one surface of each specimen. This situation might be a general limitation in the present **Study III**, due to the reported anisotropy in mechanical properties of AM Co-Cr (87, 413). It has been reported dissimilar results of hardness values in AM when surfaces were evaluated on different surfaces (upper or side) of the AM Co-Cr specimen (87).

The higher yield strength and hardness values for AM Co-Cr compared to cast, milled and pre-sintered milled reported in the literature were also confirmed in **Study III** (Table 2) (32, 34, 255, 257, 260, 264, 265, 267, 269, 270). The high hardness values of laser melted Co-Cr alloys is explained by the fine-distributed phases when rapid cooling occurs in the melt pools (414). When comparing results from mechanical tests for AM Co-Cr alloys in other studies, one can notice that the data for yield strength and hardness values varies, i.e., for yield strength=410-1058 MPa and hardness=335-1010 HV (Table 2). The wide range in yield strength and hardness values are probably caused by the applied post-processing heat treatment that reduces the yield strength and hardness and increases the ductility of AM Co-Cr and is applied in some studies (81, 257-259, 262, 267, 296, 306-308, 382). The heat treatment temperature applied in **Study II** and **Study III,** but also in other studies, is referred to as a porcelain firing process that occurs at a lower temperature (<960 ºC) than the corresponding post-processing heat treatment (up to 1050 ºC) mentioned in the literature (81, 139, 257, 258, 382, 413). It has also been reported that a high cooling rate during the post-processing heat treatment for AM Co-Cr causes smaller grain size, lower number of precipitation microstructure and higher ductility (306). A slight increase of elongation after fracture was observed in **Study II** after heat treatment, but only for c.p. Ti ($p<0.05$).

Results from the elastic modulus test in **Study III** demonstrated higher mean elastic modulus values for the laser melted and lowest for the c.p. Ti and Ti6Al4V ELI. Due to a high degree of uncertainty of the results, no statistical analysis was performed (333, 415). However, we found these results of interest and great value for designing up-coming and further studies related to the emerging techniques applied for materials for various devices (331).

In general, the results from the mechanical tests in **Study III** demonstrated values within the range of mechanical properties that has been reported in earlier studies using Co-Cr-alloys (Table 2). One exception is the pre-sintered milled Co-Cr that had a slightly higher yield strength and hardness value in **Study III** compared to the results presented in other publications. However, this result might be an effect of the limited number of investigations on the pre-sintered milling technique (also called “soft milling”) applied on Co-Cr (34, 35, 81, 270).

All the tested materials, irrespective of manufacturing technique, except for the milled Co-Cr met the requirements for being classified as Type 4. The laser melted, pre-sintered milled, one of the cast Co-Cr alloys and both titanium materials was classified as type 5, which is the highest according to standards.

Type 5 alloy can be used for very thin sections, i.e., clasps and the fabrication of removable partial dentures.

According to results from **Study III** and other studies, Co-Cr alloys that are manufactured by different techniques demonstrates microstructural differences as to larger grain size for cast and milled Co-Cr compared to AM and pre-sintered milled Co-Cr (34, 35, 81, 82, 269, 275, 401, 416). Furthermore, a more homogeneous and dense structure was observed for the laser melted compared to cast Co-Cr in **Study III**, which is in accordance with the literature (34, 35, 81, 82, 269, 275, 401, 416).

Biological aspects

In general, this thesis demonstrated that irrespective of the manufacturing technique and composition of the tested materials in different conditions, pH or when c.p. Ti is present or not, the total ion release was far below the limit value of 200 μg/cm^2 (139). Despite the extremely low total ion release among all materials, results from **Study II** demonstrated a generally higher ion release in acidic conditions than physiologic conditions, following other *in vitro* studies (125-128, 417-419).

In general, a higher total ion release and low corrosion resistance were demonstrated in **Study I** for cast Co-Cr alloys compared to milled and AM Co-Cr alloys (420). Also, a higher total ion release in acidic conditions was shown in **Study II** compared to physiologic conditions following other studies (128, 420). The pH=2.3 is extreme low to mimic oral conditions, as the physiologic pH in saliva may range from 5.3 up to 7.8 (421, 422). In patients suffering from GERD, the pH may vary between 4.9-6.5, yet when gastric fluid (pH=1-1.5) leaks back in the oesophagus and oral cavity, it may cause an acidic environment lower than pH 2.3 (423-425). Based on these findings and the results from **Study II**, it can be speculated that the cast and milled Co-Cr alloys should be avoided in patients with a history of GERD or signs of erosion to avoid possible ion release. In physiologic conditions, results from **Study II** demonstrated the highest ion release (<0.6 μg/cm^2) for pre-sintered milled Co-Cr. Comparison with other studies is difficult due to limited investigations on ion release from pre-sintered milled Co-Cr (81, 148, 150). Higher corrosion resistance was presented for milled and AM Co-Cr compared to cast (227, 426, 427). Due to variations in the outcome from other studies and the nearly undetectable ion levels**,** the decrease in ion release over time from **Study II** is difficult to interpret (87, 125, 126, 427-430). The ion release levels were very low, and thus their clinical relevance is as yet unknown. Furthermore, the results from studies investigating ion release are difficult to compare because of variations in the test methods and related to the number of elements that

have been investigated (209, 227). Consequently, if the investigated number of ions are lesser or more, a variation of the total ion release will be achieved and probably also various statistically significant results will be obtained (125, 128, 206, 220, 227, 355, 427).

Additionally, results from **Study II** demonstrated that the total ion release was lower for all materials when c.p. Ti was present compared to when materials were immersed solely. Another study demonstrated a higher ion release of Co, Cr, Al, and Ti when a c.p. Ti grade 4 implant was coupled directly to a Co-Cr crown compared to an implant coupled to a Co-Cr crown with an abutment underneath (417). Cobalt was the ion detected in the highest concentrations in both immersion tests for Co-Cr, which is also demonstrated by other studies (128, 220, 227, 300). A high level of Fe ions was detected in Co-Cr alloys in studies that used ICP-MS as an ion detection test (125, 127). The analysis of Fe was discarded in **Study II** because of the interference of Fe ions with argon ions in the high-sensitive IPS-MS technique that may cause an overestimation of the Fe values (125, 126, 128, 206, 220, 227-229, 354-356, 430).

An interesting finding from **Study II** was that except for the elements expected to be found (according to the manufacturer's data sheet) in all materials, a small amount of Mo, Mn, Al and Ti were found (Figure 15). The presence of "impurities" is earlier described in implant materials of Ti alloys and c.p. Ti grade 1 and was mentioned as inevitable due to production processes (198). In order to obtain fewer impurities, the manufacturers are proposed to improve their refinement process of materials (198). It has been suggested that these impurities may trigger allergic reactions in allergy-sensitive patients (431).

In accordance to other studies, the cell viability assay in **Study III** demonstrated that all tested materials had a non-toxic effect on epithelial cells and fibroblasts (206, 230, 366, 368).

A higher inflammatory response was observed from PBMC cells exposed to the cast and pre-sintered Co-Cr specimens compared to the milled and laser melted Co-Cr materials and c.p. Ti and Ti6Al4V ELI. Despite the non-toxic effect on epithelial cells and fibroblasts observed in **Study II**, a difference in inflammatory response among materials was shown in **Study IV**. Yet, no limit values have been reported for the cytokine level *in vitro*. However, it has been proposed that the quantification of cytokines can be used as a diagnostic tool for diseases and their treatment in the near future (234). To the best of our knowledge, no previous studies have investigated differences in inflammatory response among the cast, milled, pre-sintered and laser melted Co-Cr alloys (246).

Limitations in **Study IV** are the unknown data of the donors regarding age and sex, which can have influenced the results. It has been suggested that women and ages among both women and men (>51-60 years) induced a higher pro-inflammatory response for IL-6 compared to men and younger men and women (<51 years) (432).

A higher ion release and a higher inflammatory response were observed in **Studies II** and **III** for the cast compared to the AM Co-Cr (125, 128, 213, 433). It has been reported in the literature that ions may initiate an inflammatory response, that could speculatively induce peri-implant inflammation and tissue destruction around dental or orthopaedic implants (218, 231-233, 397, 434).

Microstructural differences, such as the type of secondary phases and the presence of segregations in cast Co-Cr alloys may explain the higher ion release from cast Co-Cr alloys compared to laser melted Co-Cr alloys (155, 212, 269, 433, 435).

6 CONCLUSION

I. Four different manufacturing techniques (cast, milled, pre-sintered milled and laser melted) and more than thirty different Co-Cr alloys were reported to be used to produce Co-Cr frameworks for fixed prostheses in Sweden 2017.

II. Total ion release was depended on the manufacturing technique used, pH and combination of materials. Minor differences in surface roughness among the materials and after heat treatment were observed. All tested materials demonstrated a non-cytotoxic effect.

III. The production technique, the alloy content, and heat treatment had an impact on the mechanical properties and microstructure of the materials.

IV. The production technique and material type had an impact on the inflammatory response from PBMC cells.

7 FUTURE PERSPECTIVES

New manufacturing techniques have been applied in the manufacturing of Co-Cr alloys. Despite the similar chemical composition that the manufacturers declare, microstructural differences are observed among them that influences both the mechanical properties as *in vitro* properties (ion release and inflammatory response).

More basic research is needed before the materials are used in patients. Standards need to be updated to simulate the clinical conditions and make research data among various studies more comparable. More clinical studies are needed to compare metallic materials with various manufacturing techniques.

Future studies should include a wide range of patient groups to investigate factors within the population that may affect the prognosis of Co-Cr frameworks manufactured with different techniques. Furthermore, more clinical research is needed to compare what happens when different materials are combined or the difference in porcelain bond among them.

Allergies with metallic materials have been reported, although an underreporting of adverse effects has been mentioned. Also, there is a knowledge gap in the clinical significance of the non-declared substances that are found to be released from Co-Cr alloys.

Based on *in vivo* animal studies, Co has been classified as a carcinogenic substance. Therefore, the future use of Co in prosthodontics is under suspension, and the replacement of Co in dentistry is a future challenge.

Furthermore, all involved “consumers” of medical devices, from the mine to the patient, i.e., manufacturers, dental laboratories and dentists, should be aware of the origin of any material from the perspective of sustainability and human rights.

ACKNOWLEDGEMENT

I wish to express my sincere gratitude to everyone who has been involved in the work of this thesis and with whom I had the pleasure of collaborating with. In particular, I would like to thank:

Victoria Franke Stenport, my main supervisor, for inspiration and for always supporting me and sharing your knowledge. I am grateful for all the interesting discussions we have had, both on site and on Zoom. Thank you for always finding time for me.

Carina Johansson, my supervisor, for believing in this project from the beginning and for all your support during this journey.

Lars Hjalmarsson, my supervisor, for your guidance and support and for always being positive.

My co-author Petra Hammarström-Johansson, for your support in helping me with preparation of specimens, surface analysis and all kinds of matters. Thank you!

To all the staff in NIOM, Oslo and especially to Jon Dahl and my co-authors Else Morisbak and Morten Syverud, for helping me with the *in vitro* studies and mechanical tests. Thank you for your warm welcome.

To my co-author Ann Wennerberg for your valuable help and guidance in surface analysis.

To my co-author Anna-Karin Östberg, for your guidance in the world of cytokines.

To Johan Börjesson, my co-author for your valuable help in the microstructure analysis.

To Ola Norderyd, Peter Nilsson and Agneta Bartoll, my former and current manager, who supported me in this project.

Katarina Sondell, for giving me the opportunity, and guiding me in the world of research and prosthodontics.

To my colleague Catharina Göthberg, for all your support and pep-talk.

To all staff at the Institute for Postgraduate Dental Education in Jönköping, and especially the Department of Periodontology, Endodontics and Oral Prosthodontics, Jönköping, for your support and encouraging.

To all staff at the Department of Prosthodontics/Dental Materials Science, Göteborg, for your support and valuable feed-back in this thesis.

To Pia Karlsson, dental technician, for your help with the specimen preparation.

To all dental laboratories, that participated in the survey.

To the librarians at Medicinska Fackbiblioteket, Ryhov, Jönköping, for your valuable help when I needed you most.

To my beloved brother Labriano, for always supporting me.

To my late father Pavlos. I wish you were here dad, you would be proud of me! I will always love you. Thank you for everything you taught me.

To my mother Anastasia, for learning me to always aim high and that nothing is impossible. For your endless love and support.

To my beloved daughters, Christina and Anna, for your love and support in this journey. I am done now girls!

To my beloved husband Christos, my best friend, my mentor, for always believing in me and supporting me in all ways. This project would never have been accomplished without you! I love you! Σε αγαπώ!

This project received financial support from Futurum-the Academy for health and care, Region Jönköping County, Sweden, the Wilhelm and Martina Lundgren Research Foundation, Sweden, the Hjalmar Svensson Research Foundation, Sweden, the Sylvan Foundation, Sweden, Folktandvården Sörmland AB, Nyköping, Sweden and Swedish Dental Society, Stockholm, Sweden.

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