Original Article

Biomechanical Properties and Biocompatibility of Implant-Supported Full Arch Fixed Prosthesis Substructural Materials

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Abstract

Objectives: The purpose of this study was to investigate the fracture resistance, biocompatibility, hardness, and transverse strength of non-precious metal alloy (chromium-cobalt; Cr-Co), titanium (Ti), zirconia (Zr), polymethylmethacrylate (PMMA), and polyetheretherketone (PEEK) when employed as substructure materials according to the implant supported full arch fixed prosthesis treatment concept. **Materials and Methods:** In total, 150 Cr-Co, Ti, Zr, PMMA, and PEEK samples (n = 30 per material) measuring 25 × 2 × 2 mm in size were produced. Of the samples, 50 (n = 10 for each material, all having dimensions of 6 × 3 mm) were subjected to biocompatibility tests. The Vickers hardness test and three-point bending test were performed; fracture resistance measurements were taken and the biocompatibility of the samples was evaluated by the XTT assay. **Results:** Vickers hardness was highest for Zr ($p < 0.05$). PEEK and PMMA had the lowest (and similar) fracture resistance values ($p < 0.05$). Cell proliferation on the surfaces of the materials was similar between PEEK and Zr ($p > 0.05$), which were the most biocompatible materials. **Conclusions:** Within the limitations of this study, the most favorable materials in terms of biocompatibility were found as PEEK and Zr. When biomechanical properties are evaluated, the most durable materials can be specified as Cr-Co and Zr. Also, further studies are needed to improve material stability.

Keywords: *Biocompatibility, biomechanical properties, implant supported full arch fixed prosthesis, PEEK, XTT assay*

Introduction

Implant-supported full-arch fixed prosthesis are well evaluated as a viable treatment option for the edentulous arch.[1] These restorations enable a screw-retained one-piece full-arch prosthesis that can only be removed by the dental professional and, thus, are meant to allow for higher patient satisfaction than removable prostheses.[2,3] These kinds of prostheses are usually made of an acrylic base, a special framework, and esthetic as well as functional prosthetic teeth.

The choice of prosthetic material and design are critical for the long-term clinical efficacy of implant-supported prostheses. The prosthetic material affects the stress mechanism during operation; this stress can be transmitted to prosthetic components, the implant-bone interface, or the implant itself.[4] Metal/PMMA, metal/ceramic and Zr/ceramic combinations are frequently preferred in implant-supported prostheses. However, there are limited studies on the most suitable material for implant-supported prostheses, particularly with respect to the substructure of the restoration. To the best of our knowledge, a few studies have evaluated the use of PEEK as an alternative to the materials currently used in implant-supported dental prostheses.[5,6] Therefore, the aim of this study is to compare the biomechanical properties and biocompatibility of various substructure

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materials (Zr, Cr-Co, Ti, PMMA) applied frequently using the implant supported full arch fixed prosthesis treatment concept to those of PEEK.

Materials and Methods

The Vickers hardness, transverse strength, fracture resistance, and biocompatibility of Cr-Co, Ti, PMMA, Zr, and PEEK were investigated as substructure materials. The Cr-Co (Methodical Dental, Germany), Ti (Whitepeaks Dental Solutions GmbH & Co. KG, Germany), PMMA (Alliance; Turquoise Dental, Izmir, Turkey), Zr (Katana Zirconia, Noritake Dental Supply; Miyoshi, Japan), and PEEK (Juvor Optima, Thornton-Cleveleys, UK) samples were 2 × 2 × 25 mm in size (American National Standards Institute/American Dental Association specification no. 27). For the biocompatibility test, 50 6 × 3 mm samples of each material were prepared according to the manufacturer's instructions. Cell culture media was prepared according to International Organization for Standardization (ISO) standard 10993-5.[7]

Zr, Ti, PEEK, and PMMA were produced from disc-shaped blocks by scraping using a CAD/CAM system (DC40-series; Yenadent Ltd., Istanbul, Turkey). All Zr samples were sintered using appropriate equipment (Programat 51 furnace; Ivoclar Vivadent AG, Germany). A TegraPol-11 (Struers, Ltd., Willich, Germany) grinding and polishing machine with a maximum speed of 120 rpm and a maximal grinding head force of 20 N was used for surface preparation.

Co-Cr dental alloy samples were fabricated with selective laser melting (SLM) (Concept Laser GmbH, Lichtenfels, Germany). All Cr-Co samples were sintered using a furnace (Protherm Furnaces, Ankara, Turkey) under a 8 h firing cycle with a maximum temperature of 1,150°C.

The surface hardness of the samples was measured by a microhardness tester (DuraScan; Emco-Test, Kuchl, Austria) with 1 kgf (9.8N) force applied in all Vickers hardness tests.

A hundred specimens of the five different substructure materials (n = 10 per material) were connected to a CS-4 chewing simulator device (SD Mechatronik GMBH, Feldkirchen-Westerham, Germany) and the samples were subjected to thermomechanical aging procedure. Each piston of the simulator was loaded with sufficient weight to exert a force of 140 N/sample. As an antagonist material, ceramic active tips with a hardness close to that of enamel were used. A total number of 1,200,000 cycles (equivalent to approximately 5 years of clinical service) and thermal cycles of +5°C to +55°C were applied with a frequency of 1.75 Hz.

Test specimens (n = 50) were subjected to a three-point bending test conducted using an Instron Universal Testing Machine (Model 3345; Instron, Canton, MA, USA) with a print speed of 1 mm/min. A vertical bending force was applied to the samples until complete bending or breaking. The force was automatically recorded in Newtons (N) as soon as any bending or breaking occurred. The transverse strength and elasticity modulus values were calculated according to the following respective formulas:

$S = 3PL/2bd^2$ and $E = PL^3/4bd^3$

where S = transverse strength (MPa), E = modulus of elasticity (MPa), P = maximum load before fracture (N), L = distance between supports (mm), b = sample width (mm), d = sample thickness (mm), and & = indicate the maximum bending (mm).

After the three-point bending test, a fracture test was carried out using The Instron Universal Testing Machine (n = 50). Force (N) was applied until the moment just prior to failure or complete failure.

Specimens were analysed with scanning electron microscopy (SEM) (Zeiss GeminiSEM 500, Germany) to investigate the surface morphology of the materials, to evaluate the deformations that may occur in the fracture surfaces after dynamic fatigue. One sample was selected each group randomly and a total of five samples were evaluated in SEM. The images were obtained at 1.00K X magnification in order to examine the surface properties of materials.

An XTT assay was used to evaluate the cytotoxicity of the implant supported full arch fixed prosthesis "substructure materials" (Cr-Co, Ti, Zr, PMMA and PEEK) in cultured cells. The aim was to determine the toxic effects of direct contact of products leaked from samples at different time points. Test media was prepared by adding foetal bovine serum (FBS), L-glutamine, and penicillin/streptomycin to phenol red-free Dulbecco's modified Eagle's medium (DMEM), as specified in ISO standard 10993-5.[7]

All samples were placed into tubes (three samples per tube), to which 5 ml of phenol red-free DMEM was added. The ratio of sample surface area to cell media volume was 0.65 cm^2/ml (the rate recommended by ISO = 0.5-6.0 cm^2/ml). DMEM containing leakage products was collected on days 1 and 2, and at weeks 1 and 2.

Cytotoxic potential was determined in the L929 mouse fibroblast cell line, grown in DMEM with addition of penicillin-streptomycin (1%), L-glutamine (1%), and FBS; 10%).

About 10 microliters of XTT Cell Proliferation Kit (Biological Industries, Beit-HaEmek, Israel) was added to wells and incubated for 2 hours at 37°C. After incubation, color change was analyzed using a microplate reader (Multiskan FC; Thermo Fisher Scientific, Waltham, MA, USA) at λ450 nm. To determine cytotoxic effects, all tests were repeated six times for each time period, on six samples.

The data were put into the formula below and mean cell proliferation percentages were calculated for test specimens.

Cell Proliferation Percentages = A-B ÷ C-B x 100

A. Mean of optical values in wells of test specimens

B. Mean of optical values in wells used as blind

C. Mean of optical values of positive control group values

The normality of the data distribution was evaluated with the Shapiro-Wilk test and QQ plots were generated. Homogeneity of variance was evaluated by Levene's test. One-way analysis of variance (ANOVA) was used for comparing materials, with Tukey's test applied for post-hoc comparisons. A P value < 0.05 was considered statistically significant. Data analysis was carried out using TURCOSA software (Turcosa AnalyTics Ltd. Co., Kayseri, Turkey).

Results

Among all materials, the highest Vickers hardness value was observed for Zr ($p < 0.05$; Table 1).

Among all materials, the transverse strength was lowest for PEEK and PMMA, but there was no statistically difference between these two materials ($p > 0.05$). Among all materials, the transverse strength was highest for Cr-Co ($p < 0.05$; Table 2).

Among all materials, of PEEK and PMMA had the lowest modulus values, but there was no statistically difference between these two materials ($p > 0.05$). Zr had the highest elasticity modulus among all materials ($p < 0.05$; Table 3).

Among all materials, fracture resistance was lowest for PEEK and PMMA ($p < 0.05$), but there was no statistically difference between these two

Table 1: Vickers hardness values of the substructure materials

Variable	Groups					*P*
	PEEK (*n*=10)	Zr (*n*=10)	Ti (*n*=10)	Cr-Co (*n*=10)	PMMA (*n*=10)	
Vickers hardness values	27.76±1.00[a]	1733.83±64.60[b]	318.92±10.00[c]	446.06±24.03[d]	18.79±0.93[a]	<0.001

Data are expressed as mean±standard deviation

Table 2: Transverse strength of the substructure materials

Variable	Groups					*P*
	PEEK (*n*=10)	Zr (*n*=10)	Ti (*n*=10)	Cr-Co (*n*=10)	PMMA (*n*=10)	
Transverse strength	124.44±13.72[a]	494.17±193.46[b]	1293.12±168.40[c]	1570.09±71.75[d]	72.42±9.17[a]	<0.001

Data are expressed as mean±standard deviation

Table 3: Elastic modulus values of the substructure materials

Variable	Groups					*P*
	PEEK (*n*=10)	Zr (*n*=10)	Ti (*n*=10)	Cr-Co (*n*=10)	PMMA (*n*=10)	
Elastic modulus	3.02±0.34[a]	77.54±27.32[b]	33.23±11.82[c]	30.15±2.48[c]	2.18±0.39[a]	<0.001

Data are expressed as mean±standard deviation

Table 4: Fracture resistance of the substructure materials

Variable	Groups					*P*
	PEEK (*n*=10)	Zr (*n*=10)	Ti (*n*=10)	Cr-Co (*n*=10)	PMMA (*n*=10)	
Fracture resistance	46.39±7.53[a]	191.88±34.49[b]	262.71±80.59[c]	470.83±38.63[d]	21.55±4.12[a]	<0.001

Data are expressed as mean±standard deviation

Table 5: Cell proliferation percentages for the substructure materials

Variable	Groups					*P*
	PEEK (*n*=6)	Zr (*n*=6)	Ti (*n*=6)	Cr-Co (*n*=6)	PMMA (*n*=6)	
Cell proliferation	107.23±14.49[a]	99.14±17.33[a,c]	87.05±18.68[b,c]	77.54±17.92[b]	89.94±20.35[b,c]	<0.001

Data are expressed as mean±standard deviation

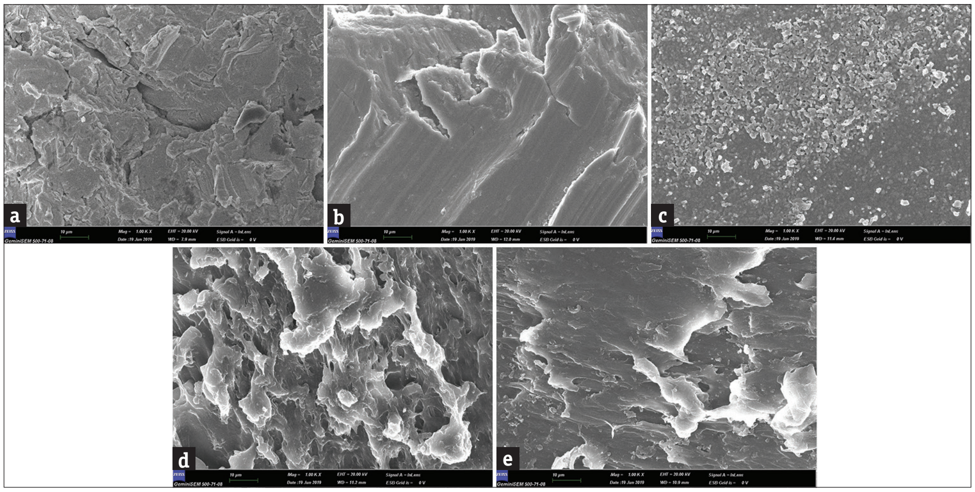

Figure 1: SEM images of test specimens after thermomechanical aging at magnification of 1.00K X. (a) Cr-Co. (b) Ti. (c) Zr. (d) PMMA. (e) PEEK

Table 6: Cell proliferation by time point for the substructure materials

Variables	Time				*P*
Cell proliferation	1.day (*n*=6)	2.day (*n*=6)	1.week (*n*=6)	2.week (*n*=6)	
PEEK proliferation	113.18±16.11	111.33±19.08	109.05±6.56	95.39±8.00	0.127
Zirkonya proliferation	111.92±14.88^{a}	105.15±16.31^{a}	79.56±10.99^{b}	99.93±8.63^{ab}	0.002
Titanyum proliferation	108.92±10.15^{a}	67.20±13.30^{b}	82.58±9.47^{bc}	89.51±12.57^{c}	<0.001
Cr-Co proliferation	94.65±12.50^{a}	61.72±11.83^{b}	63.96±7.57^{b}	89.83±8.38^{a}	<0.001
PMMA proliferation	105.09±11.51^{a}	60.51±11.48^{b}	92.19±8.33^{a}	101.95±8.80^{a}	<0.001

Data are expressed as mean±standard deviation

materials ($p > 0.05$). Cr-Co had the highest fracture resistance among all tested materials ($p < 0.05$; Table 4).

SEM images that obtained to evaluate the morphology of fractured samples (Cr-Co, Ti, Zr, PMMA and PEEK respectively) are presented in Figure 1(a-e).

The cell proliferation percentage of mouse fibroblasts differed among all five materials ($p < 0.05$). The cell proliferation percentage was highest for PEEK ($p < 0.05$; Table 5).

Proliferation of mouse fibroblasts grown with restorative materials was measured on days 1 and 2, and at weeks 1 and 2. Proliferation percentages on PEEK did not change over time ($p > 0.05$). For Zr, proliferation at week 1 was significantly lower compared to days 1 and 2 ($p < 0.05$). Mouse fibroblasts cultured with Ti showed lower cell growth, but increased again at day 2 ($p < 0.05$). For Cr-Co, proliferation was similar between day 2 and week 1, but day 1 percentages were significantly lower versus week 2 ($p < 0.05$). For PMMA, proliferation was lower on day 2 compared to day 1 and week 2 ($p < 0.05$; Table 6).

Discussion

Different types of substructure materials are now available for use in implant-supported full-arch fixed prostheses. However, choosing the most appropriate prosthetic material for each patient is critical. Moreover, the way in which a material is shaped during prosthesis construction will affect material stress resistance during operation, where the stress can be transmitted to prosthetic components, implant-bone interfaces, or the implant body itself.[4] Our study compared the biocompatibility and biomechanical properties among five commonly used prosthetic materials: Cr-Co, Zr, Ti, PMMA, and PEEK.

ISO standard 10993-5[7] recommends the use of specific cell cultures, such as L-929 or Balb/3T3, when investigating the cytotoxic effects of dental materials. Therefore, we used L-929 mouse fibroblasts in our study. To determine the cytotoxic potential of products leaked from the material samples, the proliferation of mouse fibroblasts was analyzed using of an XTT assay; among the materials, only cells grown with PEEK showed had no cytotoxicity. Cells grown in the presence of Zr showed increasing proliferation throughout week 1. For Ti, proliferation was lower on day 2 than day 1, but began to increase again at week 2. Cells proliferation in the presence of Ti was significantly lower compared to in the presence of PEEK. The contact surfaces of Ti dental implants open to the oral cavity are exposed to abrasion due to toothbrushing, acidic pH, and hard food particles.

Titanium-based structures can show degradation, wear, and corrosion in the physiological environment, with the debris thus produced being released into nearby tissues.[8]

Cell proliferation percentages for the Cr-Co material were similar between day 2 and week 1 but were also significantly lower than those on day 1 and week 2. Finally, for cells grown in the presence of PMMA, proliferation was lowest on day 2 ($p < 0.05$). Taken together, the results show no difference in biocompatibility between PEEK and Cr-Co, Zr, Ti, and PMMA. Our results also show that the substructure materials applied according to the implant supported full arch fixed prosthesis concept may carry an in-vivo cytotoxicity risk, and the cellular response to various frequently use dental materials is inconsistent and not always desirable.

Transverse durability, which is based on both tensile and compressive forces, reflects the chewing forces that dental materials are exposed to in the clinical setting.[9] We performed a three-point bending test using an Instron Universal Testing Machine to determine transverse strength, similar to previous studies. The transverse strength of PEEK and PMMA was significantly lower than that of the other three materials. Prior to the three-point bending test, the material samples were subjected to 1,200,000 chewing and thermal cycles in a chewing simulator, corresponding to 5 years of use. The CS-4 chewing simulator is a dual-axis chewing simulator that has been used to simulate wear on dental materials and allows a high level of standardization to be achieved, such as the type and shape of antagonist materials, contact load, number of cycles, and chewing frequency.[10] In this research, ceramic pieces were used as an antagonist and in order to simulate the situation closest to the oral cavity, aging was performed in the presence of fluid with a temperature change of +5°C to +55°C. Flat surfaces are preferred in different test samples in order to provide the force distribution on the contact surfaces equally. It was found in a previous in-vitro study that there was no difference in antagonist wear between flat and anatomical-shaped specimens after 120,000 cycles in a chewing simulator.[11]

We found that the highest elastic modulus was for Zr, and the lowest was for PMMA, followed by PEEK. The low elastic modulus of PEEK renders it a suitable substructure material for implant prostheses, particularly in overloaded areas, given its shock absorbing effect.[12,13] Previous studies showed that PEEK has an elastic modulus similar to that of bone[14]; therefore, PEEK can be expected to absorb some of the forces generated during chewing, limiting their spread to the cervical region of the periimplant bone. This prevents peri-implant marginal bone loss due to occlusal overload.[15] Materials with a high elastic modulus have high stress values and are far more resistant to bending and deformation.[16] Akca *et al*.[17] noted that the distribution of stress between bases depends on the type of alloy used in the prosthetic substructure. Materials with a low elastic modulus have low bending strength, while substrates made from rigid base alloys undergo less deformation. Assunção *et al*.[18] demonstrated that a rigid material, such as metal-supported porcelain, can increase the transmission of forces to the implant and surrounding bone tissue. However, Jacques *et al*.[19] reported that a material having a low modulus of elasticity also had low bending strength, and that structures made of rigid alloys suffered less deformation, thus not exerting excessive stress on retention screws and other prosthetic components.

In this study, the Vickers hardness was similar between PEEK and PMMA, but their hardness values were lower than those of the other materials. Rubo and Capello Souza[20] showed that a harder substructure permits better stress distribution, while Hussein and Rabie[21] used 3D finite element analysis to demonstrate that Zr prosthetic material on implants, applied according to the all-on-four concept, had higher compression and tensile stress values than the abutment, implant, and surrounding bone. Moreover, compression and tensile stresses were transferred to thicker cortical bone instead of to the softer trabecular bone. Favot *et al*.[22] also investigated four different prosthetic substructure materials (Zr, Ti, gold, and nickel-titanium [NiTi]) designed for implants placed in the edentulous mandible using the all-on-four concept. Zirconia substructures experienced the highest stress, while NiTi experienced the lowest stress; moreover, the stress in the substructure decreased as material hardness decreased, and stress in the Zr substructure was almost twice as high as that in the NiTi substructure.

Despite the high stress exerted on prosthetic substructures, rigid substrate materials transmit less stress to other components. Sertgöz[23] recommended the use of harder materials in prosthetic substructure to avoid failure of the implant support system. This method leads to less stress in the cortical bone, implants, abutments, and screws. Substructures made of materials such as Zr and Cr-Co will cause less displacement of the implant support system. Previous studies have shown that harder materials exert less stress on bone,[4,24] implants,[25] and retention screws.[4,23]

Bhering *et al*.[16] compared the stress distribution on an atrophic maxilla caused by placement of an all-on-four implant-retained prosthesis, according to the use of

Cr-Co, Ti and Zr as the substructure material. Cr-Co and Zr showed low stress levels and good biomechanical properties; Ti had the worst biomechanical properties. Nazari *et al*.[26] examined the fracture strength of implant-supported three-member fixed prostheses produced from Zr, PEEK, and metal ceramics. Despite heavy occlusal forces, the metal ceramic restorations showed the highest fracture strength. Other studies indicated that prosthetic complications are more frequent in metal-PMMA prostheses compared to metal-ceramic fixed prostheses.[27,28] According to the results of this study, it may be appropriate to reinforce the PMMA material with a metal substructure in permanent restorations or to use it as a temporary prosthesis material in immediate loading protocols due to the low fracture resistance of PMMA.

We also examined the fracture resistance of the tested materials and found that Zr, Ti, and Cr-Co had significantly higher resistance than PEEK. PMMA showed the lowest fracture resistance. Taken together, these results show that neither of our null hypotheses are supported, i.e., there are significant differences in biomechanics and biocompatibility between PEEK and other common dental prosthetic materials. Neumann *et al*.[15] evaluated the fracture strength of abutment screws produced from Ti and PEEK; PEEK abutment screws showed lower fracture strength than Ti. Malo *et al*.[5] also used PEEK as a substructure material, applied via the all-on-four treatment concept, but reported that greater thickness is needed to compensate for the softer nature of PEEK. In the aforementioned study, when PEEK material is used as substructure material, the minimum anterior bucocolingual width is indicated as 4 mm. The PEEK material was less rigid and more flexible, and the width of the titanium sleeve areas (to compensate for flexion as it represents a weak spot) required a minimum of 6 mm according to the pilot study result. Thickness is related to interoclusal space. Malo *et al*. stated that in cases where PEEK material is used as substructure, if the interoclusal distance is minimum 5 mm and there is a limitation about interoclusal distance, materials with higher rigidity such as zirconia and metal should be preferred instead of peek.[5]

These results also suggest that, if PEEK and PMMA are used as final restorative materials in implant-retained prostheses, their thicknesses must be increased to strengthen the fracture durability. There are also many articles in the literature advocating this view.[29,30]

The present study had some limitations. First, the materials used were not designed according to the implant supported full arch fixed prosthesis concept (although they had the same dimensions), due to the high cost of both manufacturing and standardizing the test steps. Our study was also limited to an *in vitro* analysis, and the extent of the effects of thermomechanical fatigue on these materials remains debatable.

With consideration of the limitations detailed above, the following conclusions can be drawn from our study:

- The highest cell proliferation percentages were observed when fibroblasts were grown in the presence of PEEK. The cell cytotoxicity rate was significantly lower with this material compared to the others, i.e., it was the most biocompatible material.
- The transverse strength and fracture resistance of PEEK were significantly lower than those of Ti, Zr, and Cr-Co; further studies are needed to improve material stability.
- Although the Cr-Co samples showed the highest transverse strength and fracture resistance, they had the lowest cell growth and viability, suggesting higher cytotoxicity compared to the other materials.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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