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The effect of new anti-adhesive and antibacterial dental resin filling materials on gingival fibroblasts

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ABSTRACT

Objective. Aim of this study was to evaluate the biocompatibility of four experimental anti-adhesive and antibacterial dental filling composites on human gingival fibroblasts (HGFs).
Methods. For these experimental resin composites a delivery system based on novel polymeric hollow beads, loaded with Tego Protect (Aa1), Dimethicone (Aa2), Irgasan (Ab1) and methacrylated polymerizable Irgasan (Ab2) as active agents was used. The cultured HGFs' cell integrity, proliferation, viability, collagen synthesis and cytokine release were measured. For this purpose, human gingival fibroblasts were treated with eluates from all four composites and compared with an experimental standard composite (ST). Eluate extraction times 24 h and 168 h were chosen.
Results. Statistical analysis was conducted via a mixed model. Both antibacterial composites reduced proliferation, collagen and cytokine synthesis significantly ($p < 0.05$), increasing with time of elution. Ab1 did also have a damaging effect on the membrane and on cell viability.
Significance. Overall, it can be concluded that the antiadhesive composites showed clear advantages over the antibacterial composites in terms of biocompatibility. This study also continues to show the potential of the new poly-pore system, as it can be used for a variety of other applications in future composite mixtures.

1. Introduction

Due to increased aesthetic expectations of patients and enhanced long-term stability, the share of dental resin fillings has risen steadily, so that the former standard material amalgam is in steady decline since the 1980s and surpassed by resin materials [1]. Nevertheless, the scientific interest in further developments is high since the properties of dental resin composites have also given rise to new problems. Even after a short period of wear, they are subject to significant plaque accumulation, which follows initial pellicle formation [2,3]. It is still not possible to assume an absolutely tight margin, which is a risk of bacterial penetration and formation of secondary caries [4]. This problem leads to secondary caries being the main reason for failures of composite fillings in the molar and premolar region [5–7]. For this reason, several novel composites were developed to avoid or at least to

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diminish pellicle and bacterial adherence by modifying them with either antibacterial or anti-adhesive additives [8–10]. These composites use a delivery system of novel polymer hollow beads (Poly-Pore, AMCOL Health & Beauty Solutions, Arlington Heights, IL, USA) which can be used as a carrier material for either anti-adhesive or antibacterial additives [8–10]. Since there are studies suggesting that a lower surface free energy (SFE) significantly reduces bacterial adherence [11–15], the approach of anti-adhesive composites is to lower the SFE by incorporating low-surface tension agents (Tego Protect 5000, Evonik Tego Chemie GmbH, Essen, Germany and Dimethicone, Dow Corning Corp., Midland, MI, USA), which were added in small quantities of 5 wt.% to experimental dental resin materials. Furthermore, two antibacterial approaches were implemented, the first using the same delivery system loaded with Irgasan (5-chloro-2-(2,4-dichlorophenoxy)phenol) and the second using a methacrylated and polymerizable version of Irgasan (5-chloro-2-(2,4-dicholorophenoxy)phenyl methacrylate). The effectiveness of these materials in reducing the number of vital bacteria as well as their sufficient physical properties have already been proven in vitro [8,9].

The aim of this study was to test the biocompatibility of these new dental composites on human gingival fibroblasts. The focus of this study was on parameters of cell proliferation, membrane damage and cell viability. In addition, collagen synthesis using TGF-ß1 and possible inflammatory reactions by measuring IL-8 were investigated. This was accomplished through the production of eluates of the experimental composites as well as an experimental standard composite for comparison. Another point of investigation was the effect of different elution times, with 24 h or 168 h respectively on the aforesaid parameters, based on publications investing elution times and release rates [16,17].

The null hypothesis was (a) that none of the eluates differ in their effects on human gingival fibroblasts, (b) that a longer extraction time of 168 h does not show different effects in comparison to 24 h extraction time and (c) that there is no difference between antibacterial or anti-adhesive composites regarding their effects on human gingival fibroblasts.

2. Materials and methods

2.1. Cell cultivation

Primary human gingival fibroblasts (Gingiva #121 0412 Product HFIB-G Cryo, ProVitro, Berlin, Germany) were used and cultured in Dulbecco's Modified Eagle Medium (DMEM, Thermo Fisher Scientific, Waltham, MA, USA) with 10% new born calf serum (NCS, Thermo Fisher Scientific, Waltham, MA, USA), 1% penicillin/streptomycin solution (Thermo Fisher Scientific, Waltham, MA, USA) and 1% Glutamax. The cells were incubated at 37 °C and 5% CO_2.

2.2. The composites

All experimental dental resin composites are based on a standard (ST) which consists of typical methacrylate variations commonly used in dental composites, namely Bis-GMA, UDMA and TTEGDMA. Antiadhesive or antibacterial components were added to the glass filler or the matrix [8–10].

Two different approaches were chosen: The first approach was to decrease the surface tension via introducing polyacrylic hollow beads (Poly-Pore, AMCOL Health & Beauty Solutions, Arlington Heights, IL, USA), loaded with hydroxy-functional polydimethylsiloxane (Tego Protect 5000 = Aa1) or polydimethylsiloxane (Dimethicone = Aa2) and therefore to inhibit bacterial adhesion [8,10]. The second approach included adding antibacterial properties via Irgasan, either using the same carrier system with hollow beads (Ab1) or methacrylated polymerizable Irgasan (Ab2) as part of the matrix [9].

All four experimental composites fulfil the minimum values for flexural strength, water sorption and solubility as requested by EN ISO 4049 [9,10]. Table 1 shows different compositions with the bioactive components replacing parts of the glass filler or matrix, resulting in adding 4 wt.% of active agents to each experimental composite.

2.3. Specimen preparation

All composites were heated to 50 °C to ensure better processing than at 4 °C storage temperature. The respective material was applied to prefabricated molds of 10 mm diameter and 1 mm thickness. Size of the specimen was according to ISO 10993-12 [18,19]. The molds were covered by a polyethylene film and metal plates (University Laboratory, Goethe-Universität Frankfurt, Frankfurt am Main, Germany) and compressed at 2000–3000 bar for 60 s with a hydromatic press (Reco Hydromatic Press HMP 1251-4, Reco-Dent International Co., LTD., Taipei, Taiwan). Both heating and compression were used to minimize irregularities, create a smooth surface and to achieve standardized conditions. After removing the metal plates, each side of the specimen was cured for 40 s with a halogen polymerization lamp (Spectrum 800, Dentsply Sirona, Pennsylvania, USA) at >850 mW/cm^2. The output of the curing device was routinely checked (Bluephase Meter, Ivoclar vivadent, Schaan, Liechtenstein) and no significant decrease of the output was observed. The test specimen were taken out of the molds and excess was removed using a scalpel (Carbon Steel Scalpel #15, Aesculap AG, Tuttlingen, Germany). In the next step they were mounted on a scale (PT300, Sartorius, Göttingen, Germany) and polishing was carried out using Al_2O_3-coated polishing discs (Super-Snap Buff Disks, L501 Green, L502 Red, Shofu Dental, Ratingen, Germany) at a speed of 10,000 rpm and while pressure of 40−50 g was applied. Each side was polished with a grain size of 20 μm (green) and 7 μm (pink) for 40 s. The test specimen were then disinfected by wipes (Mikrozid AF wipes premium 200 mm × 200 mm, Schülke, Norderstedt, Germany) and placed under the workbench under sterile conditions, where they were disinfected again using alcohol (Ethanol 70%, Carl Roth, Karlsruhe, Germany) before getting in contact with the nutrient solution.

2.4. Eluates and treatment

All specimen were incubated in accordance with DIN EN ISO 10993-12 [18,19] and an extraction ratio of 3 cm^2/ml for 24 ± 2 h or 168 ± 2 h in the nutrient solution (DMEM, Thermo

Table 1 – Composition of the experimental dental resin composites.

	ST	Aa1	Aa2	Ab1	Ab2
Glass	73.00	68.00	68.20	68.00	73.00
Poly-Tego 80%	–	5.00	–	–	–
Poly-Dimeth 80%	–	–	5.00	–	–
Poly-Irga	–	–	–	5.00	–
Methacryl-Irga	–	–	–	–	4.00
Matrix	27.00	27.00	26.80	27.00	23.00
Active agent	–	4.00	4.00	4.00	4.00
Matrix:	UDMA = 44.10, Bis-GMA = 30.00, TTEGDMA = 25.00, photoinitiator = 0.30, CQ = 0.20, amine = 0.10, stabilizer = 0.10				

Fisher Scientific, Waltham, MA, USA; Newborn Calf Serum, Gibco #16110-159, Thermo Fisher Scientific, Waltham, MA, USA; Gibco Penicillin/Streptomycin, Thermo Fisher Scientific, Waltham, MA, USA) on a rocking shaker at 37 °C and 5% CO_2 in the incubator. To adjust for LDH and TGF-β1 contained in the Newborn Calf Serum (NCS), according to the manufacturer's manual only 1% NCS was used for these tests while 10% NCS were used for BrdU, MTT and IL-8. Glass bottles (ND10 1,5 ml, Rotilabo, Carl Roth GmbH, Karlsruhe, Germany) with metal lids (ND18, Neolab, Heidelberg, Germany) were used and not closed completely during the first 24 h to allow gas exchange. After the extraction time, the test specimen were removed from the bottles under sterile conditions via forceps and the eluates were stored at 4 °C. HGF were seeded and incubated for 24 ± 2 h at 37 ± 1 °C until confluent and then exposed 24 ± 2 h to the eluates.

2.5. *Parameters*

2.5.1. *Brom-2′-desoxyuridin incorporation*

To determine the influence on the proliferative potential a Cell Proliferation ELISA BrdU (5-Brom-2′-desoxyuridin, Roche, Mannheim, Germany) was conducted. The test principal relies on the incorporation of the thymidine analogue BrdU during DNA replication. The amount of BrdU incorporation can be detected with the help of specific antibodies against BrdU (anti-BrdU) and used as analytical parameter for cell proliferation. The optical density was measured with an ELISA reader (Fluostar Omega, BMG Labtech, Ortenberg, Germany).

For this assay, the cells were seeded in a 96-well plate (Cellstar #655160 #656171, Greiner, Kremsmünster, Austria) with $7{,}5 \times 10^5$ cells per 100 µl. Wells treated only with medium were used as negative control (NC). One half of the NCs were used as such, the other half (designated as "blank") were treated with labeling reagent to adjust for noise.

2.5.2. *Cell integrity and cell viability*

Cell integrity was determined by testing the cytosolic lactate dehydrogenase enzyme activity. It was measured with the Cytotoxicity Detection Assay (Roche, Mannheim, Germany). Triton-X (1%) was used as a positive control (PC), cell medium as NC.

Cell viability was analyzed using a Cell Proliferation Assay (Cell Proliferation Kit I, Roche, Mannheim, Germany). Yellow tetrazolium salt is intracellularly reduced to purple formazan and can be measured after cell-lysis.

Cells were seeded in a 96-well plate with 10^4 cells per 100 µl. All assays were performed according to the manufacturers' instructions and absorptions were measured with an ELISA-Reader.

2.5.3. *Enzyme-linked immunosorbent assay of TGF-β1 and IL-8*

The secretion of TGF-β1 and IL-8 was assessed. HGF were seeded in a 96-well plate with 10^4 cells per 100 µl. Cell-free supernatants were assayed using ELISA test kits (R&D Systems Europe, Abingdon, UK) according to the manufacturer's protocol.

2.6. *Presentation of data and statistical analysis*

All data were displayed as mean values ± standard deviation. A Kolmogorov–Smirnov–Lilliefors test was performed to determine whether the data were normally distributed. Since all data were normally distributed, a mixed model analysis was used. The tests were conducted with "R" (R Foundation, Vienna, Austria). Each set of data was related to ST. Differences were considered significant at $p < 0.05$.

The statistical analysis was conducted in cooperation with the Institute of Biostatistics and Mathematical Modelling of the J.W. Goethe University, Frankfurt am Main (Tables 2 and 3).

3. Results

3.1. *LDH-assay*

After 24 h and 168 h extraction time, only Ab1 deviated significantly from the standard showing a significantly increased LDH. No significant differences within the groups were shown. In direct comparison of 24 h and 168 h extraction time, there was no difference between the standard composite and Aa1 and significant differences were found for the other composites. The mean values of LDH release of all composites decreased with increasing extraction time (Fig. 1).

3.2. *BrdU-ELISA*

The differences between the standard composite and the individual experimental composites were significant in all cases. The antiadhesive composites showed an increased incorporation rate of BrdU, while the antibacterial composites showed a reduced incorporation rate.

After 168 h extraction time, no significant difference was found between the standard composite and the antiadhesive composites Aa1 and Aa2. The antibacterial composites Ab1

Table 2 – Extinction E and (standard deviations). Underlined numbers indicate significant differences between 24 h and 168 h incubation time. Same superscript numbers indicate no significant differences between different composites of the same incubation time.

	E_{LDH}		E_{BrdU}		E_{MTT}	
	24 h	168 h	24 h	168 h	24 h	168 h
ST	1,63 (0,34)[1]	1,46 (0,15)[1]	1,49 (0,46)[1]	1,63 (0,26)[1]	0,72 (0,06)[1]	0,76 (0,07)[1]
Aa1	1,52 (0,38)[1]	1,47 (0,17)[1]	2,03 (0,30)[2]	1,76 (0,29)[1]	0,84 (0,07)[2]	0,69 (0,04)[2]
Aa2	1,76 (0,20)[1]	1,40 (0,17)[1]	2,05 (0,26)[2]	1,81 (0,18)[1]	0,75 (0,09)[1,2]	0,82 (0,09)[3]
Ab1	2,40 (0,12)[2]	2,22 (0,15)[2]	0,48 (0,17)[3]	0,55 (0,14)[2]	0,48 (0,04)[3]	0,21 (0,01)[4]
Ab2	1,82 (0,30)[1]	1,46 (0,16)[1]	1,02 (0,28)[4]	0,96 (0,23)[3]	0,99 (0,05)[4]	0,94 (0,05)[5]

Table 3 – Concentration C in 10^2 pg/ml and (standard deviations). Underlined numbers indicate significant differences between 24 h and 168 h incubation time. Same superscript numbers indicate no significant differences between different composites of the same incubation time.

	$C_{TGF\text{-}\beta1}$* $(10^2$ pg/ml$)^{-1}$		$C_{IL\text{-}8}$* $(10^2$ pg/ml$)^{-1}$	
	24 h	168 h	24 h	168 h
ST	3.82 (3.95)[1]	3.81 (0.37)[1]	54.27 (12.68)[1]	37.73 (8.10)[1]
Aa1	4.38 (3.59)[2]	4.00 (0.17)[1]	50.70 (8.98)[1]	42.37 (3.33)[1]
Aa2	4.04 (3.12)[1]	3.46 (0.28)[2]	57.54 (10.50)[1]	42.05 (7.27)[1]
Ab1	0.12 (0.12)[3]	0.83 (0.10)[3]	23.75 (4.46)[2]	2.21 (1.11)[2]
Ab2	1.16 (0.60)[3]	1.06 (0.13)[4]	127.12 (12.90)[3]	22.10 (4.32)[3]

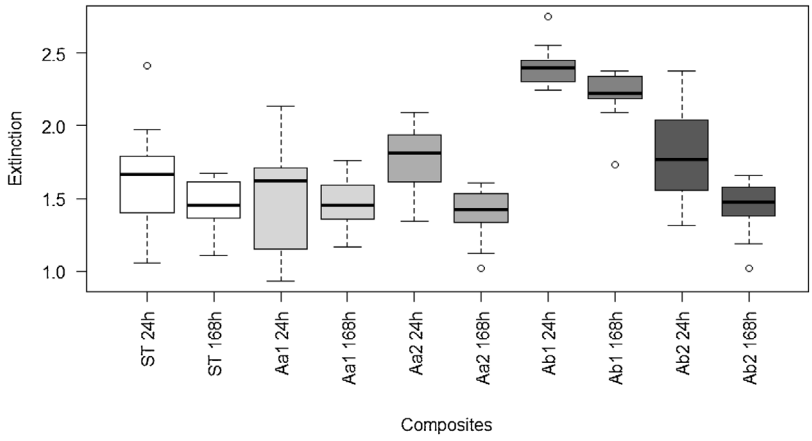

Fig. 1 – Extinction of LDH-assay.

and Ab2 differed significantly from the standard as well as from the antiadhesive composites.

Significant differences between the individual composites in the direct comparison after 24 and 168 h were only found within the antiadhesive composites, where the incorporation rate of BrdU decreased with increased extraction time (Fig. 2).

3.3. *MTT-assay*

The MTT assay showed significant differences between the standard and all composites except Aa2 after 24 h extraction time. Aa1 and Ab2 were above the standard, while Ab1 was significantly below. The composites also showed significant differences between each other; only between the antiadhesive variants, no significant differences were found.

After 168 h extraction time, significant differences were found between the standard composite and the antiadhesive and antibacterial variants as well as among the novel composites in direct comparison. Here, Aa1 and Ab1 were below average, with Ab1 clearly showing the lowest extinction, while Aa2 and Ab2 were above the standard in the mean value.

When comparing between 24 h and 168 h extraction time, the differences were significant for all composites, although the differences for the standard, Aa2 and Ab2 were smaller than for the remaining composites. The standard and Aa2 showed an increase in absorbance, while Aa1, Ab1 and Ab2 showed a decrease, which was most prominent in Ab1 (Fig. 3).

3.4. *TGF-ß1-ELISA*

In the TGF-β1-ELISA, the differences to the standard after 24 h extraction time were significant in all cases except Aa2. Aa1 showed an increased mean value compared to the standard, while Ab1 and Ab2 showed significantly reduced values. The different composites also showed significant differences between each other in most cases; only between the antibac-

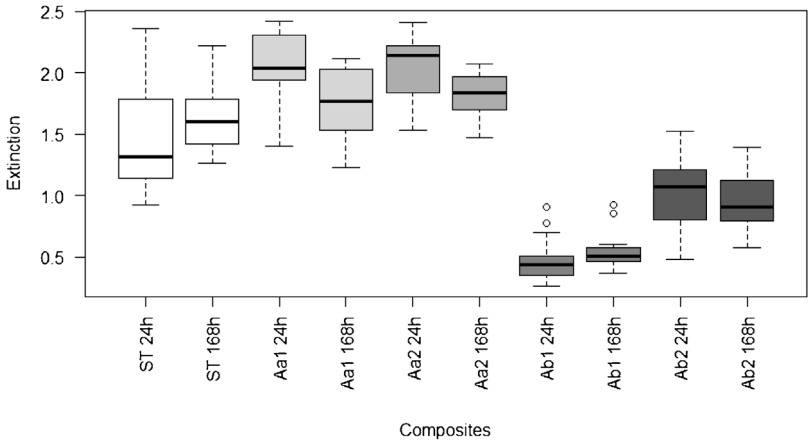

Fig. 2 – Extinction of BrdU-ELISA.

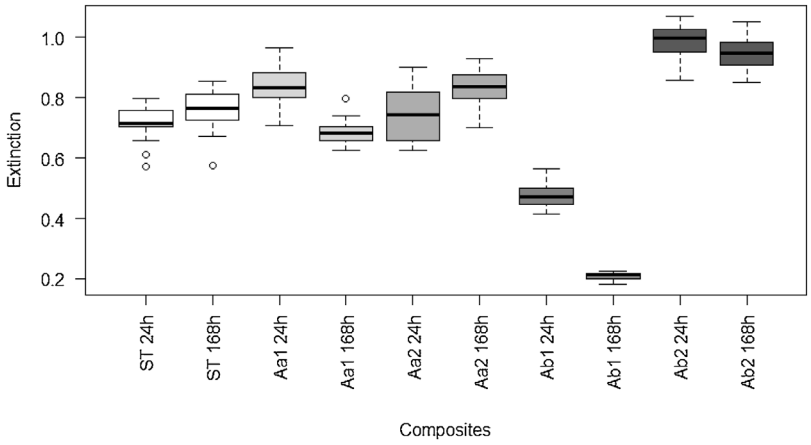

Fig. 3 – Extinction of MTT-assay.

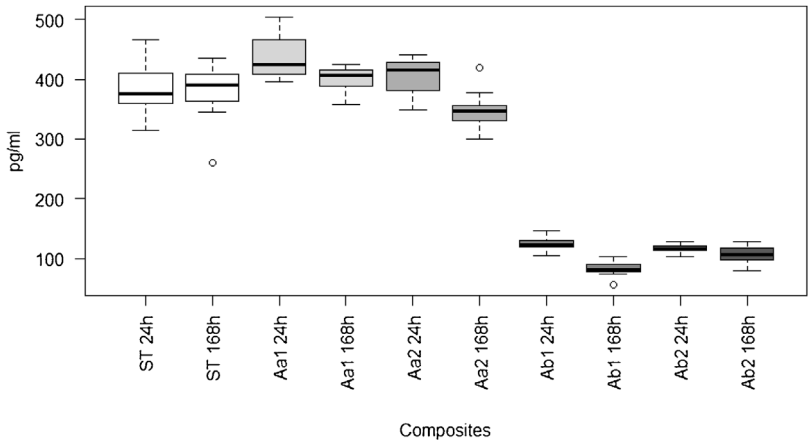

Fig. 4 – TGF-1 secretion in pg/ml.

terial composites, no significant differences were found. Between the antiadhesives, the significance was reduced, but still significant (Fig. 4).

After 168 h of extraction time, no significant difference was found between the standard and Aa1 and a reduced significance between the standard and Aa2, with Aa2 being slightly below the standard. The other composites deviated from the standard with significantly reduced mean values and the differences between the composites were significant.

Only the standard composite showed no significant deviation between the values of 24 and 168 h extraction time in direct comparison. For the novel composites, the difference was significant, although it was less pronounced for Ab2 than for the other composites. In all experimental compos-

ites, the extended extraction time resulted in a lower TGF-β1 release.

3.5. *IL-8-ELISA*

After 24 h extraction time, the IL-8 ELISA showed no significant differences between the standard and both antiadhesive composites. Only the antibacterials deviated significantly from the standard, but here Ab1 with significantly less pg/ml IL-8 than the standard, while Ab2 showed a significantly increased output. The difference between the antiadhesives was also not significant, in contrast to all other compositions (Fig. 5).

After 168 h extraction time, this experiment showed a clear change. Although the antiadhesive composites still did not deviate significantly from the standard, after the longer incubation period the deviation of Ab2 from the excess changed to a reduced ejection of interleukin compared to the standard. Both antibacterial composites thus showed a significantly reduced IL-8 release compared to the standard composite. Among themselves, the antiadhesives did not differ significantly from each other as in 24 h extraction time, while all other combinations came to significantly different mean values.

The direct comparison between the extraction times showed significant differences for all composites, only for Aa1 this difference was not as pronounced. It is noticeable that the values decreased with the extended extraction time, especially in Ab2, where an increased IL-8 release turned into a reduced release.

4. Discussion

Aim of this study was to evaluate the biocompatibility of these new antibacterial and antiadhesive composites. Regarding the parameters, the experimental composites showed different behavior compared to ST and to each other. In most cases both antibacterial composites showed significant lower biocompatibility levels, while the antiadhesive composites seem to have less impact.

After 24 h extraction time only Ab1 showed a significant release of LDH and therefore damage to the membrane. Neither the antiadhesive composites, which are equipped with the same carrier system, nor Ab2, which contains the same antibacterial agent in a different form, damaged the cells in a comparable way. It should be noted that the other components of the composites have also been proven to have a membrane-damaging effect [20,21]. Bis-GMA is often present in the plastic matrix in greater proportions than TEGDMA, as is also the case in the new types of composites tested here [8,9]. Biocompatibility in this sense can therefore only be achieved to the extent that the anti-adhesive or antibacterial additives do not have an additional damaging effect. For this reason, the composites Aa1, Aa2 and Ab2 can be classified as having no increased membrane damage in comparison with modern commercially available filling materials.

The antibacterial composites express a significant reduction of proliferation, while the antiadhesive composites show increased integration of BrdU. When looking at the range of values, it is noticeable that the standard composite can also achieve similar proliferation rates as Aa1 and Aa2, but the average is lower. The lowest proliferation rate with Ab1, followed by Ab2, suggests that the carrier system can achieve a higher release of the active ingredient and thus bigger effect than a methacrylated variant. Secondly, the antiadhesive additives Tego Protect 5000 and Dimethicone are significantly less damaging than Irgasan. This is in line with expectations, as Triclosan has already been classified as potentially DNA damaging in recent studies [22,23]. Currently there are no studies available that investigate the direct relationship between Tego Protect or Dimethicone and DNA synthesis. However, polysiloxanes as a parent group have been studied for some time [24–27]. Prasad et al. used polysiloxanes to influence surface roughness [24]. As in this study, the use of polysiloxanes there also demonstrated decreased surface roughness and concomitant increased fibroblast proliferation compared with the standard [24]. In other publications, polysiloxanes have been shown to have good biocompatibility and low toxicity both in vitro and in vivo [25–27]. Thus, it can be concluded that an increased proliferation of fibroblasts under the influence of polysiloxanes is reproducible. With regard to the novel composites, this can be interpreted as at least equal biocompatibility.

Viability was influenced mostly by Ab1, showing a significant negative effect. All other compositions showed acceptable biocompatibility in this regard. Surprisingly Aa1 and even Ab2 showed higher means than ST did. While the effects of Ab1 were expected, the behavior of Ab2 seems contradictory. The negative impact of Ab1 is in line with previous studies [23,28,29], which showed the cytotoxic potential of Triclosan. On the other hand de Paula et al. [28] assessed their polymerized Triclosan as biocompatible, regardless of the lower viability. There are also other studies with MTT-assays that get higher results than the control group, which shows that this seems to be a common result [21,30]. Regarding these results, it can be concluded that while Triclosan acts as a cytotoxic ingredient when released through the Poly-Pore-System, its negative effects can be significantly reduced by using a polymerized form.

Considering collagen, the two antibacterial composites showed a significant decrease in TGF-β1 production. Compared to the standard, there is a significant excretion of TGF-β1 in Aa1, which could be explained by an increased irritation of the cells with subsequent initiation of a healing process. Thus, the cells are obviously able to show an adequate reaction after the exposed stress by the composite. It should be noted that although the deviation of the mean value is significant, it is still within the range of the standard. Aa2 showed no significant deviation from the standard after 24 h extraction time, which shows that its biocompatibility regarding TGF-β1 is equal to standard commercial products. This suggests that Irgasan negatively influences the protein biosynthesis and the synthesis of TGF-β1, while the antiadhesive variants have no additional damaging effect. This is in line with a previous study with different composite variations without Triclosan, where no significant influence on TGF-β1 synthesis was found [31].

Relating to IL-8 release, no significant differences between the standard and the antiadhesive composites after 24 h

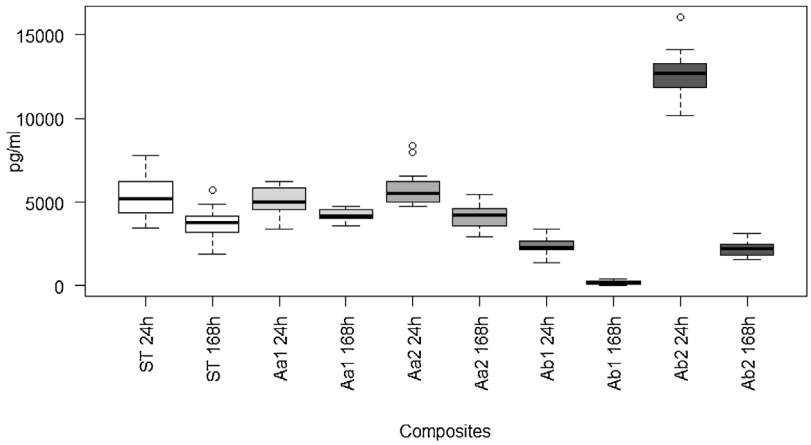

Fig. 5 – IL-8 secretion in pg/ml.

extraction time were shown. Thus, an increased inflammatory reaction seems not to be caused by the antiadhesive additives.

The behavior of the antibacterial composites differs: Ab1 decreases IL-8 secretion, while Ab2 increases it. A uniform reaction was expected, since Triclosan generally has an anti-inflammatory effect. Multiple publications describe a down-regulation of prostaglandin synthesis under Triclosan [32,33]. Composites can also inhibit IL-8 synthesis: a chemically activated polyethyl methacrylate composite also led to a significant reduction in IL-8 output, while the comparative composites did not show any significant differences [31]. While highly elevated levels of proinflammatory cytokines such as IL-8 impair wound healing and a reduction leads to improved outcomes [34], elevated IL-8 levels are also part of regular wound healing [35]. Accordingly, both the reaction of Ab1 and Ab2 can be classified as less biocompatible.

After 168 h of extraction time, a significant decrease in the incorporation rate of BrdU and therefore an inhibited DNA synthesis by the antiadhesive substances could be shown for both antibacterial composites in comparison to the standard, while the LDH release remained unchanged. TGF-β1 release Ab1 and Ab2 also continued to show significantly lower cytokine release, which was also significantly lower than at 24 h extraction time. Aa2 did also show a reduced TGF-β1 release, which was also significant, but still resembled the standard in its range. A negative influence on collagen synthesis and regeneration of the gingival fibroblasts due to extended extraction time can therefore be attested for Aa2, Ab1 and Ab2. In a direct comparison of the total values, however, small amounts, measured in pg/ml, are shown, so that a clinically significant change due to the extended extraction time seems unlikely. The antibacterial composites also show significantly reduced IL-8 release compared to 24 h. Ab2 is particularly noticeable here: While an increased IL-8 release still took place after 24 h, a significantly lower release is shown after 168 h compared to the standard composite. However, the poly-pore system seems to elute more Triclosan into the nutrient solution, which is why the effect is significantly more distinctive with Ab1. Ab2 puts the cells under stress after 24 h extraction time rather than having an anti-inflammatory effect. On the other hand, it could also be an indication that the composites generally inhibit the IL-8 synthesis and that this increases with a prolonged period of use, as was also observed in another study [31]. Currently there exists no quantitative data regarding the releasing rates of the different systems and reactive agents. However, a significant influence and clinical significance was measured in multiple studies, including release after chewing simulations:

Single delivery particles are always present in the outer material's surface [9]. Occurring abrasion processes, simulated by polishing [9] or chewing simulations [36], destroy the delivery particles and release the active agents. While there was an average material loss of 2.4 μm after each chewing simulation process [36], especially the antiadhesive agents created a renewed "floating" surface that over-compensated the increase of surface roughness [36]. While there is no quantitative data available for the release mechanism, this study suggests a higher release through the poly-pore system, because it shows greater effects with the same active agent (Triclosan). This is an expected result, since the poly-pore system is designed to actively elute agents, while the methacrylated variant stabilizes the agent in a composite matrix.

Longer incubation periods might lead to enhanced effects because of higher concentration, but a clinical significance can be doubted since a longer incubation time is impossible in-vivo due to continuous saliva production.

Regarding the methods of this study, all ELISA-Kits used as well as the HGF were well-established commercial products. To ensure correct results with clinical relevance, the focus was on internal and external validity as defined by Campbell and Stanley [37]: Instrumentation bias was minimized by using the same instruments wherever possible and recalibration if necessary. For example to ensure maximum polymerization, the output of the LED was constantly controlled. To avoid maturation or mortality bias, the HGF passages were limited to 15 generations, as recommended by commercial distributers. For every sowing of HGF, the cell numbers and viability were controlled via microscope. Testing and pretesting effects could be excluded since every cell was only used once in one test. However, this results in the probability of different reactions of different cells. To acknowledge this effect, the mixed model

analysis was chosen. A selection bias can be disregarded since the cells were all cultivated to the fifth generation before the experiments started and randomly harvested and sowed, which allowed no identifying of different groups.

Limitations in external validity can be considered, since in-vitro studies as this do not have the capability to fully imitate the clinical environment. Neither were the cells in the environment of a fully-grown organism nor were any bacteria present. Although this was necessary to ensure that any differences in biocompatibility could be traced back to the different ingredients of the experimental composites rather than any bacteria, in-vivo studies seem necessary to back up the results of this study.

5. Conclusion

With the limitations of this study, it may be concluded that the addition of antiadhesive substances show sufficient biocompatibility in comparison to modern commercial products. While Ab1 shows negative effects in all parameters, no active destruction of the cell is visible by Ab2, but the metabolism, DNA and protein synthesis are significantly disturbed. Therefore, the antiadhesive composites should be preferred in further development of "active" materials. This study also continues to show the potential of the new poly-pore system, as it can be used for a variety of other applications in future composite mixtures. Based on these results, the null hypotheses were rejected.

Ethical approval

Not applicable. No animals or human beings were involved in this study.

CRediT authorship contribution statement

Philipp Landenberger: Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing - original draft, Visualization. **Linn Baumann:** Methodology, Writing - original draft, Conceptualization, Supervision. **Susanne Gerhardt-Szép:** Writing - review & editing, Conceptualization. **Stefan Rüttermann:** Methodology, Writing - review & editing, Conceptualization, Resources, Project administration, Funding acquisition.

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