Journal of Prosthodontic Research

Official Journal of Japan Prosthodontic Society

Review article

The biocompatibility of glass-fibre reinforced composites (GFRCs) – a systematic review

Ting Wang[a,b], Jukka Pekka Matinlinna[b], Michael Francis Burrow[a], Khaled Elsayed Ahmed[c,*]

[a] *Prosthodontics, Division of Restorative Dental Sciences, Faculty of Dentistry, The University of Hong Kong, Hong Kong SAR, China*

[b] *Dental Materials Science, Division of Applied Oral Sciences and Community Dental Care, Faculty of Dentistry, The University of Hong Kong, Hong Kong SAR, China*

[c] *Prosthodontics Discipline, School of Dentistry and Oral Health, Griffith University, Gold Coast, Australia*

Abstract

Purpose: Fiber-reinforced composites (FRCs) have received considerable attention, owing to their potential use in dental prostheses or bone fracture fixation applications. The aim of this systematic review was to analyze and report the biological properties of FRCs reported in the existing literature.

Study selections: A systematic search of four databases (PubMed/MEDLINE, Scopus, Web of Science, and Cochrane library) was performed to identify all relevant studies published between 1962 and 2019. The search was limited to laboratory-based studies published in English. Citation mining was also performed through cross-referencing of included studies and hand searching of relevant journals.

Results: A total of 1283 potentially relevant articles were initially identified, and thirty-three articles were full-text screened. In the final ten studies included for review, four investigated bacterial adhesion and growth abilities on FRCs, four investigated the fibroblastic cytotoxicity of different surface-treated FRCs, and two investigated the osseointegration between bone and FRCs. Owing to the heterogeneity of fiber types, FRC-coating, and lack of standardized testing protocols, a meta-analysis was not feasible. The included studies indicated that glass fibers, and in particular E-glass fibers, are superior to ceramics and other FRCs in terms of bacterial adherence, fibroblast cytotoxicity, and cell viability.

Conclusions: Glass-fiber-reinforced composites are cytocompatible materials that possess satisfactory biological properties and can be used in dental prosthesis and craniofacial implants. Further research is necessary to regulate the matrix ion release/degradation of FRCs to prolong the initially demonstrated properties.

Keywords: *Fibre reinforced composites, Systematic review, Biocompatibility*

Received 5 April 2020, Accepted 1 October 2020, Available online 22 February 2021

1. Introduction

With the significant influence of minimally invasive dentistry, fiber-reinforced composites (FRCs) have proven to be a reliable, durable, and economical treatment alternative for conventional, metal-free management/treatment in various dental applications [1–6]. FRCs have been used to fabricate fixed partial dentures and as provisional fixed partial dentures during implant integration [7, 8]. Furthermore, chairside-fabricated FRCs can be designed in a customizable manner, which is particularly useful in endodontic post systems, fixed orthodontic retainers, or periodontal splint placement [9]. They have also been utilized in bone fracture fixation in facial and calvarial implants [3, 10].

Numerous studies have demonstrated that FRCs possess favorable mechanical properties in relation to their flexural strength and water sorption, bonding properties, and deformation behavior [11–14]. However, the mechanical properties of the fiber-reinforcement technology are dependent on certain features, such as the fiber type (continuous, chopped, and veil-type), the orientation and distribution of fibers, the resin matrix system employed, the volumetric ratio of fibers in a resin matrix, and the adhesion of fibers to the resin matrix [15]. It is also worth noting that methacrylate-based resins may cause hypersensitivity or contact dermatitis [16]. Furthermore, an increasing trend of FRC application in dentistry and orthopedics implies a pertinent and increasing need to investigate the biological properties of these biomaterials, including, but not limited to, the tissue response of bone and cellular and bacterial responses.

Although adverse reactions to resin-based dental materials are rare, the biological effects and limitations of resin-based materials have become a widely discussed topic [17–19]. Because FRCs typically have the same resin matrix as conventional resin composites, there are potential toxicity or biocompatibility issues of FRCs that must be considered. Given the hostile oral environment that FRCs are exposed to, there is a need to understand the biocompatibility of FRCs thoroughly. To date, there has been no systematic appraisal of the existing literature evaluating the biological properties of FRCs for dental applications. Therefore, the aim of the current review was to investigate the biological properties of FRCs by systematically reviewing and assessing the existing literature.

2. Materials and methods

2.1. Search strategy

A systematic search of four databases (PubMed/MEDLINE, Scopus, Web of Science, and Cochrane Library) was performed to identify relevant literature. The PICO statement was used to formulate the research question [20, 21]. The “population” was glass-fiber-reinforced resin-based composites (GFRCs). The “intervention” included different bacteria and cells. There was no control group selected, i.e., no “comparisons.” The

* Corresponding author at: School of Dentistry and Oral Health, Griffith University, Gold Coast Campus, Griffith Health Centre (G40) Room 7.59, QLD 4222, Australia.
E-mail address: khaled.ahmed@griffith.edu.au (K.E. Ahmed).

https://doi.org/10.2186/jpr.JPR_D_20_00031

"outcomes" were a positive result of cytotoxicity/antibacterial properties. Thus, the formulated research question was as follows:

Do GFRCs have a biological impact on the surrounding cells or bacteria?

The preferred reporting of 27 items for systematic reviews and meta-analyses guidelines (PRISMA) were consulted whenever applicable [22].

Articles published between January 1, 1962 and December 31, 2019 were searched using the search strategy (Table 1). The search was limited to studies that were laboratory-based tests published in English. Citation mining was also performed through cross-referencing of the included studies. Additional eligible articles were manually searched and added to the list of identified publications. Two reviewers (WT and KA) independently reviewed the titles and abstracts of the retrieved articles. The reviewers were not blinded to the identities of authors, institutions, or journal names.

2.2. Study selection

This review aimed to investigate the biological properties of GFRCs intended for use as a dental biomaterial. Studies investigating cytotoxicity and bacterial adherence properties were included. The inclusion and exclusion criteria were as follows:

Step 1: Criteria information source: abstract and title. Include: experimental studies of GFRCs as a subject (laboratory studies); studies investigating GFRC biological properties through antibacterial activity or cytotoxicity testing. When abstracts were not readily available, studies were included in further searches. Exclude: not of direct relevance to dentistry (e.g., application in craniofacial hip/knee surgery or orthopedics); not of direct relevance to biological properties (e.g., adhesion properties, glass ionomer cement (GIC), or resin composites without glass fiber reinforcement); descriptive studies (e.g., description of the technique, case report, clinical report, and reviews); clinical studies (e.g., involving prefabricated posts, dowels or fiber-reinforced fixed partial dentures); and studies using 3D finite element analysis (FEA) on anatomically designed GFRC.

Step 2: Criteria of information source: full text. Include laboratory-based studies investigating the biological properties of GFRCs. Exclude: in vivo only studies (including animals and/or humans); Studies that did not disclose the details of the chemical composition of the GFRC resin matrix system; studies that used GFRCs as a control group rather than the experimental group in the test protocol; and studies that evaluated the biological properties without any control groups.

2.3. Data extraction and management

Extracted data were collected, reviewed, and analyzed by two reviewers, and customized data collection forms were used to collect the required information, including study author, publication year, resin matrix system (monomer system), control groups (positive/negative control), tested cell/microbe types, tested properties, test methods, and results. Disagreements were resolved through further discussion. A meta-analysis was planned if satisfactory homogeneity was present across the included studies.

2.4. Quality assessment

The risk of bias of the included studies was assessed by two reviewers, according to the articles' methodological description, which was adapted from a similar systematic review [23]. Here, the descriptions are "sample size calculation," "duration of contact (more than two tested time points)," "positive control/ negative control," "test with biologically related properties," "more than one cell-line is used," "test with > 1 biological property," "following ISO (10993) protocol for cytotoxicity," "three independent tests at least." If the authors reported the above-listed items, the article receives "Y" (yes); if the required information was not available, the article received an "N" (no). Articles reporting one or two items were classified as having a high risk of bias, three or four items as a medium risk of bias, and more than five items as a low risk of bias.

Table 1. The search strategy for biological impact on GFRCs.

search (glass fibre OR glass fiber)
search (composite resin$ OR reinforce* composite$)
Search (Materials Testing OR biological)
Search ("Dental Materials" OR dentistry)
search (((((glass fibre OR glass fiber))) AND ((composite resin$ OR reinforce* composite$))) AND ((Materials Testing OR biological))) AND (("Dental
Materials" OR dentistry)) Filters: Publication date from 1962/01/01 to 2019/12/31

3. Results

3.1. Study selection

A total of 1950 potentially relevant articles were identified from the databases. After removal of duplicates, 1283 article titles/abstracts were screened, and 33 full-text articles were assessed, of which eight studies were included [24–31]. Hand searching and cross-referencing of relevant journals were also performed, resulting in the identification of additional two articles [32, 33]. After the assessment, a total of ten articles [24-33] fulfilled the inclusion criteria for this review (Fig. 1).

3.2. Summary

The components utilized in the experimental FRC groups and control groups of the ten included studies are presented in Table 2; the cells tested, test methods, and main findings are listed in Table 3.

3.3. Quality assessment and risk bias of included studies

Of the ten studies included in this review, four studies investigated the adhesion properties and growth ability of bacteria on FRCs [25–27, 30]; four studies investigated the cytotoxicity of various surface treatments on glass fiber composites on fibroblasts [24, 29, 31, 33], and the biological performance of bone or bone-like cells and FRCs was investigated in two studies. A number of the included studies also examined factors that influenced cell viability and proliferation from both biological and mechanical perspectives [25, 26, 28-30]. Table 4 shows three studies with a high risk of bias, one study has a low risk of bias, and the remaining studies that demonstrated a medium risk of bias.

3.4. Glass fiber materials

Most of the included studies [24, 26, 31, 32] evaluated the cytotoxicity, bacterial adhesion, and mechanical properties when using E-glass fibers, or compared different fiber types, such as experimental fibers including ultra-high molecular weight (UHMW) polyethylene fibers, aramid fibers, carbon/graphite fibers [26], and S2-glass and R-glass [31], with E-glass fibers. These glass fibers contain silicates, i.e., a family of anions consisting of silicon and oxygen, which is not pure silica (SiO2). One study used bioactive glass fibers [32]. Tanner et al. [26] indicated that carbon/graphite FRCs did not show a significant effect on bacterial adhesion, whereas polyethylene (PE) FRCs enhanced bacterial bonding more than other FRCs.

In addition, the biological effects of fiber surface treatments have also been assessed [24, 31]. Different surface treatments include epoxy silane-coated glass fibers, plasma-enhanced chemical vapor deposition (PECVD) [24], and methacrylate-coated glass fibers (so-called 'semi-IPN') [31]. Nonetheless, surface treatments of the fibers did not demonstrate any significant cytotoxic effects. The presence of a coating or coating material did not result in altered biocompatibility.

3.5. Resin materials

Most of the included studies [24-31, 33] used a resin matrix that contained methacrylates, such as PMMA [24, 25, 27, 29–31], bis-GMA/bis-EMA [26, 28, 30, 31, 33], UDMA [30, 31, 33], TEGDMA, BDMA [25,

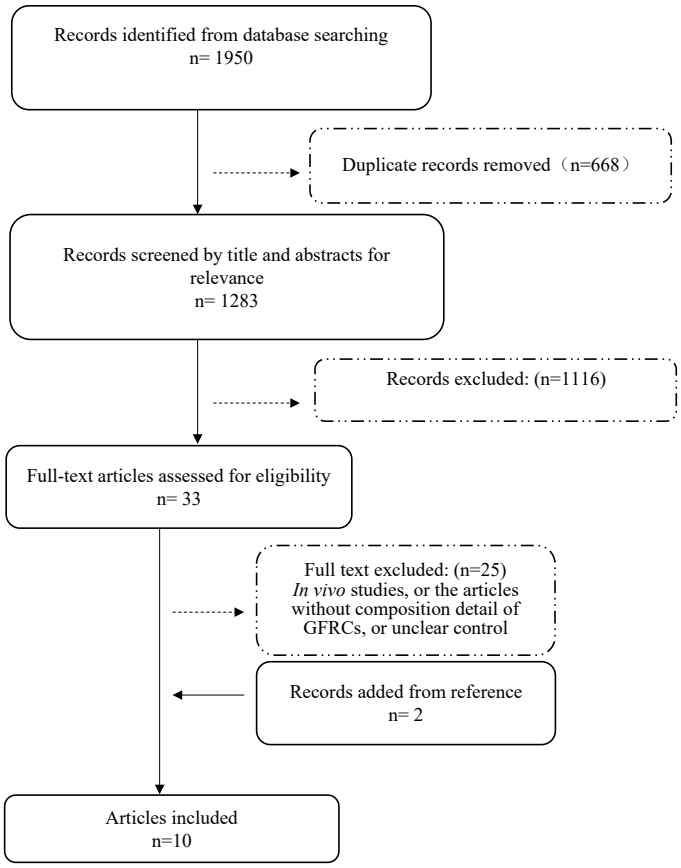

Fig. 1. A search strategy flows diagram for identification of studies to include in the review.

Table 2. Materials of the included studies.

Authors, Years	Fibre type	Resin system	Groups
Vallittu et al. 1999 [24]	Untreated E-glass fibre (EA, EH)	PMMA	Autopolymerized polymer*
	Epoxy–silane sized E-glass fibre (ESA and ESH)		Heat-cured polymer acrylic resin*
	Untreated silica fibre (SE)		
	Surface treated silica fibre (SSE)		Positive control: PVC-plastic portex Negative control: Polyethylene plastic Portex
	Methacrylate–chrome complex sized E-glass fibre (EVH)		
Tanner et al. 2001 [25]	Unidirectional silanized Eglass fibres	PMMA	Specimens were dry stored in a desiccator *
			After water-storage 14-30 days, specimens were coated with saliva
	No fibres	Palapress (PMMA /BDMA)	After water-storage 14-30 days, specimens coated with serum
			After water-storage 14-30 days, specimens left uncoated.
Tanner et al. 2003 [26]	Silanized E-glass fibres	bis-GMA/TEGDMA, BPO	High-leucite porcelain*
	UHMW polyethylene fibres		
	Aramid fibres		Restorative composite with bis-EMA*
	Sizing-treated carbon/graphite fibres		
Waltimo et al. 2004 [27]	Woven E-glass fibres	MMA, BDMA, a barbituric acid copper ion initiator system	FRC test specimens stored in water*
			FRC stored in 10% chlorhexidine digluconate solution for 24 h
			Pre-soaked in 20% chlorhexidine digluconate and dried before preparation followed by storage of the specimens in water
			Pre-soaked in 20% chlorhexidine digluconate and dried before preparation than 10% chlorhexidine digluconate for 24h.

Table 2. Materials of the included studies. (continued)

Ballo et al. 2008 [28]	Continuous unidirectional E- glass fibres pre-impregnated for 24h in bis-GMA/ TEGDMA	bis-GMA / TEGDMA	Commercially pure titanium*
	PMMA pre-impregnated bidirectional weave E-glass	bis-GMA / TEGDMA	Positive control: conventional tissue culture polystyrene wells
	Continuous unidirectional Eglass fibres	Bioactive glass particles and bis-GMA / TEGDMA	
Meric et al. 2008 [29]	Cross-linked PMMA sized silica glass fibre	EGDMA, 1,4-BDMA,	EGDMA, 1,4-BDMA, without fibre*
	Cross-linked PMMA sized silica glass fibre	DEGDMA	DEGDMA without fibre
	Linear PBMA sized silica glass fibre	DEGDMA	Negative control disks (PTFE,poly(tetrafluorethylene). Positive control disks (millipore glass fibre filter with 2% phenol solution)
Lassila et al. 2009 [30]	Silanized short E-glass fibres	bis -GMA/ PMMA	Enamel and dentin*
		BaAlSiO2-radio-opacity-fillers	
	E-glass fibres	PMMA, bis-GMA	
	No fibres	bis-GMA, UDMA,	
		bis -EMA	
		bis -GMA based hybrid composite resin	
		UHMW-polyethylene, bis-GMA	
		Aliphatic and aromatic dimethacrylates	
		Resin-modified Glassionomer cement	
		Silver, copper, tin, zinc and mercury	
		Leucite -reinforced glass ceramic	
Frese et al. 2014 [31]	Unidirectional epoxy silane coated E-glass	PMMA / bis-GMA	specimens were uncoated *
	Unidirectional silane coated R-glass	UDMA / TEGDMA	0.5 mm layer of flowable resin composite covered on fibres
	Unidirectional silane coated siliciumdioxide glass fibres	bis-GMA	1 mm thick resin composite layer covered on fibres
	Unidirectional silicate glass fibres and polyethylene fibres	bis-GMA	Positive control (HGF cell culture medium)
	Unidirectional Plasma enhanced chemical vapour deposition coated S2-glass	not specified	
Lazar. et al. 2016 [32]	E-glass Woven Roving	bis-GMA TEGDMA, HEMA UDMA, POB DHEPT	FRC1: bis-GMA (21%), TEGDMA (14%) FRC2: bis-GMA (21%), HEMA (14%) FRC3: bis-GMA (3.5%), UDMA (21%), TEGDMA (10.5%) FRC4: bis-GMA (3.5%), UDMA (21%), HEMA (10.5%) Cultures exposed to unconditioned medium*
Chan. et al. 2018 [33]	bioactive calcium phosphate silicate, double cladding: zinc barium silicate and borosilicate	Epoxy matrix	Ti6Al4V disk * Bio-A: bioactive calcium phosphate silicate, epoxy. Bio-B: bioactive calcium phosphate silicate, an epoxy matrix with 1% bioactive powder Bio-C: E-glass fibre, an epoxy matrix with 1% bioactive powder
	E-glass fibre		

*Control group

Abbreviations: bis-GMA: (2.2-bis(4-(2-hydroxy-3-methacrylyloxypropoxy)phenyl)-propane; bis-EMA: 2,2-bis[(4methacrylyloxy)phenyl]-propane; BPO : bentzoyl peroxide 1,4-BDMA: 1,4-Butanediol dimethacrylate ; BMA: Butyl methacrylate; DEGDMA: Diethylene glycol dimethacrylate; EGDMA: Ethylene gylycol dimethacrylate; PMMA: Poly(methyl methacrylate); PBMA : Poly(butyl methacrylate); PVCAC: Poly(vinyl chloride-co-vinyl acetate) ; TEGDMA: triethyleneglycol dimethacrylate; UHMW: Ultra-high molecular weight; UDMA, urethane dimethacrylate; MMA: Methyl methacrylate.

Table 3. Main findings of the included studies.

Authors, Years	Tested cells /Microbe	Tested properties	Methods	Main results
Vallittu et al. 1999 [24]	*Mouse ibroblasts*	Cytotoxicity effect	Agar diffusion test	Positive controls showed lysis and an inhibition zone of the fibroblast cells.
				Positive controls showed unstained zone at the cell–material contact area, classified as moderate cytotoxicity.
Tanner et al. 2001 [25]	*Streptococcus mutans*	Initial adhesion experiment	Scanning electron microscopy	The highest number of adhered S. mutans cells was observed on saliva-coated specimens, particularly on the glass fibre.
		Growth experiment	Scanning electron microscopy	The growth number of adhered cells stayed the same on both materials.
		Contact angle	Optical contact angle meter	The contact angles for polymer specimens were more than twice as big than the contact angles for glass specimens
Tanner et al. 2003 [26]	*Streptococcus mutans*	Surface roughness	Two-dimensional height parameter	Aramid FRCs showed higher surface roughness (Ra=0.18 μm) in comparison with all materials (p <0.001)except polyethylene FRC.
		Protein adsorption	Immunoblot analysis	Aramid FRC bonded more proteins than the other materials. Bulk E-glass, restorative composite, and GFRC showed a stronger band of an agglutinin. No agglutinin was detected in samples from the polymer matrix.
		Adhesions test	Liquid scintillation assay	Ceramic and polyethylene FRC bond more bacteria than others.
		Scanning electron microscopy	Scanning electron microscopy	For GFRC, more bacteria adhere to saliva-coated fibres than to non-coated fibres.
Waltimo et al. 2004 [27]	*Candida albicans*	Adhesion assay	Light microscopy	There were significantly more adherent C. albicans cells found in the control group without chlorhexidine than in the remaining groups.
				The lowest number of C. albicans adherent with specimens pre-soaked in 20% chlorhexidine digluconate and then stored in water.
Ballo et al. 2008 [28]	*Rat bone marrow stromal cells*	Surfaces characteristic	Surface roughness tester	The blasting produced an overall Ra of 1.97 ± 0.26 μm for the FRC group, 4.90 ± 0.30 μm for the FRC-Net group, 3.28 ± 0.22 μm for the FRC-BAG group and 0.97 ± 0.10 μm for the titanium group.
		Silica and calcium ion concentration analysis	The ortho cresol phthalein complexone method	Ion concentrations with FRC-BAG substrates declined by day 9. Calcium depletion on FRC and FRC-Net started at day 21 in culture.
		cell proliferation	Alamar BlueTM assay / ELISA plate reader	The cell activity on FRC-BAG was significantly lower than on titanium and FRC.
		Alkaline phosphatase (ALP) activity	Micro BCATM protein assay reagent kit and ELISA plate reader	The maximal ALP activities on FRC, FRC-Net, and titanium were observed on day 21. FRC-BAG reached the maximal level on day 14. Bone sialoprotein(BSP) was observed with all materials after 7 days.
		Gene expression gene repression RT-PCR	FAM-labeled TaqMan Gene Expression Assays	FRCs showed fastest osteogenic differentiation while a prolonged differentiation process was observed on titanium, with a higher OC expression level than on any other tested material
Meric et al. 2008 [29]	*Human gingival fibroblasts*	Cytotoxic effects	Filter diffusion test	There was no change in staining intensity at the cell– material contact area with negative controls or the test samples.
		Cell viability	MTT assay	The viability of all groups including before and after thermal cycling was more than 90 %. No statistically significant difference among groups.
Lassila et al. 2009 [30]	*Streptococcus mutans*	Surface roughness	Surface profile meter	FC resin had a significantly higher Ra value than control groups. No association found between surface roughness and S. mutans adhesion
		Adhesion test	Colony forming units	Experimental composite materials showed similar adhesion of S. mutans, while adhesion to dentin and enamel was significantly higher. Saliva coating significantly decreased the adhesion for all materials.
		Scanning electron microscope	Scanning electron microscopy	Bacteria binding to non-coated and saliva-coated specimens revealed fewer bacteria on the specimens after saliva coating.

Table 3. Main findings of the included studies. (continued)

Frese et al. 2014 [31]	*Mouse fibroblasts*	Cell viability	MTT assay.	The E-glass FRC material displayed the lowest cytotoxicity followed by Silicate glass FRC and S-2 glass FRC.
		Cell viability	LIVE /DEAD assay.	The lowest mean percentage of dead cells (< 2%) was shown for Silicate glass FRC followed by E-glass FRC.
		Cell proliferation	MTT assay	After 3 days the cell number of all materials reached the level of the positive control. At day 7 a decrease of 7–23% in cells was detected compared to the control.
		Cytotoxic effects	Immunocytochemistry technique	Leached components of FRCs did not reveal an impact on the integrity of the cytoskeleton of HGFs after 48 h of exposure.
Lazar et al. 2016 [32]	*human dental pulp stem cells*	Viability	Tetrazolium dye colorimetric assay	The best results were obtained by FRC3 followed by FRC2, FRC4 and FRC1.
	dermal fibroblasts	cytotoxicity		
Chan et al. 2018 [33]	*MG-63 human osteoblast-like cell*	Surface roughness	2D profilometer	The Ra value of the Bio-C group was higher than that of the Bio-A and Bio-B; no statistical difference in Ra value was present among bioactive GFRC groups.
		Wettability	Optical measurement of the static contact angle of water	The surface hydrophilicity of Bio-A group was greater than those of the Bio-B and Bio-C groups ($p < 0.05$), while no significant difference for Bio-A group was noted compared to the Ti6Al4V group
		Cell proliferation	MTT assay	All three Bio-GFRC groups after 1 day, 3 day and 5 days of of culture was higher than that of MG-63 cells cultured on the Ti6Al4V samples ($p < 0.05$)
		Cell differentiation	Alkaline phosphatase (ALP) activity	No significant difference was found after 1 day culture between the groups, but after 5 days Bio groups all demonstrated a statistically significant higher ALP specific activity when comparing to the Ti6Al4V specimens ($p < 0.05$).
		Cell morphology	Scanning electron microscopy (SEM),	The cells cultured on the bioactive GFRC samples had more highly differentiated morphologically

Table 4. The quality assessment of the screened studies. (Y: authors reported the above-listed items; N: No information available in the article).

Included articles	Sample size calculation	Duration of contact (More than two tested time points)	Positive control/ Negative control	Test with biologically related properties	More than one cell-line is used	Test with > 1 biological property	Following ISO protocol	Three independent tests repeat at least	***Risk of bias***
Vallittu et al. 1996 [24]	N	N	Y	N	N	N	Y	N	**H**
Tanner et al. 2001 [25]	N	Y	Y	Y	N	Y	N	N	**M**
Tanner et al. 2003 [26]	N	N	Y	Y	N	Y	N	N	**M**
Waltimo et al. 2004 [27]	N	N	N	N	N	N	N	N	**H**
Ballo et al. 2008 [28]	N	Y	Y	Y	N	Y	N	N	**M**
Meric et al. 2008 [29]	N	Y	Y	N	N	Y	Y	N	**M**
Lassila et al. 2009 [30]	N	N	N	Y	N	Y	N	N	**H**
Frese et al. 2014 [31]	N	Y	N	N	N	Y	Y	N	**M**
Lazar et al. 2016 [32]	N	Y	N	Y	Y	Y	Y	N	**L**
Chan et al. 2018 [33]	N	Y	N	Y	N	Y	N	N	**M**

27, 29], or epoxy resin [32]. In respect to the polymerization process, six articles used light activation [25, 26, 28, 30, 31, 33], while the remaining studies used high temperature with high pressure for polymerization [24, 27, 29]. One study that used epoxy resin [32] with pre-formed FRCs did not require curing.

3.6. Bacterial/fungal adhesion

Four studies investigated the initial adhesion and growth properties of oral bacteria Streptococcus mutans (S. mutans) and yeast Candida albicans (C. albicans) on the surface of GFRCs [25–27, 30]. Three studies compared the binding ability of S. mutans to different FRCs and evaluated the effects of water storage and salivary pellicle presence on adherence [25, 26, 30]. The findings indicated that the glass fiber composites bound more S. mutans than commercial restorative resin composite materials when specimens were coated with a salivary pellicle. It was also shown that exposed glass fibers promoted greater adhesion of S. mutans in comparison with the polymer matrix alone. Specimens that were not coated with saliva exhibited fewer colonies of S. mutans [26]. Moreover, polyethylene and aramid FRCs demonstrated significantly greater binding of S. mutans compared to serum-coated glass FRC specimens, which showed the lowest adhesion.

Bacterial adhesion to dentin and enamel has been demonstrated to be more significant than that identified for the FRCs examined [25, 30]; remarkably, this finding is contrary to other studies when resin composite was used [34]. The surface characteristics of the materials were shown to influence bacterial adhesion, either by direct physical influence or adsorption of the pellicle, when surface roughness and contact angle were assessed as variables. One study [25] demonstrated that the contact angles of water on PMMA specimens were more than twice as large as those for glass fiber specimens, i.e., glass FRC is more hydrophilic than PMMA. In contrast, the presence of glass fibers seemed to bind more than twice as many S. mutans than the polymer matrix in all saliva-coated specimens, whereas the proteins for bacterial adhesion favored a hydrophobic environment [35]. However, although all these studies used S. mutans as the tested bacteria, each utilized a different method to grow S. mutans on the material surfaces. For instance, some studies [26, 36] have used saliva from humans, whereas other studies [37, 38] used standard chemicals such as phosphate-buffered saline (PBS). The saliva-coated specimens might provide a more clinically relevant environment than PBS, but there is the potential for saliva from different human subjects to vary slightly. Consequently, given the inconsistency in the method, it cannot be conclusively argued that glass FRCs increase or reduce bacterial adhesion. Further studies are necessary to understand bacterial adhesion to FRCs better.

A further study evaluated and compared the condition of C. albicans in contact with PMMA-based GFRCs that were stored in 10% and 20% chlorhexidine digluconate solutions or water. As these concentrations of chlorhexidine are not used clinically, there are significant limitations to the relevance of this work [27]. The study demonstrated that pretreating the porous polymer pre-impregnated glass fibers for reinforcement of the composite with 20% chlorhexidine digluconate resulted in a significant reduction in the number of adherent yeast cells on the GFRC surface.

3.7. Fibroblasts

Four studies investigated the cytotoxic effects of FRCs using mice, human gingival, and human dermal fibroblasts [24, 29, 31, 33] In particular, FRCs with E-glass fibers demonstrated the lowest cytotoxicity compared with the other glass fibers containing FRCs [24]. For the silicate glass FRCs, cell viability was not influenced by thermal cycling (12,000 cycles between 5 °C and 55 °C), with cell viability of all groups remaining more than 90% before and after thermal cycling [29]. Nevertheless, the resin matrix was shown to affect the viability of fibroblasts. A high concentration of bis-GMA or HEMA significantly suppressed the growth of fibroblasts, while matrix resins containing UDMA and TEGDMA could maintain good cell growth [33].

Coating of the FRCs with flowable resin composite and uncoated viscous resin matrix (PMMA, bis-GMA, UDMA) was also investigated in one study [31]. The findings indicated that coating did not increase cell viability, while cell proliferation increased similarly in the first seven days, and then decreased by 7%–23% compared with the control, i.e., culture medium. Furthermore, this study suggested that the cytotoxic effects of resin monomers might cause disintegration of cytosolic fibers, i.e., actin filaments, microtubules, and intermediate filaments, of the cytoskeleton in living cells, which are made of protein fiber coils and form an intermediate component of the cytoskeleton. The leached components of FRCs did not show any relation to the integrity of the cytoskeleton of human gingival fibroblasts (HGFs) after 48 h of exposure in one study [31].

3.8. Bone-like cells

Two studies investigated the responses of bone-like cells on the surface of GFRCs [28, 32]. Different bone-related cells were assessed, including rat bone marrow-derived stromal cells and human osteosarcoma MG63 cells. One study [28] compared osteoblast proliferation and maturation on a bioactive glass-modified FRC (BAG-FRCs) surface, GFRCs, and titanium. This study demonstrated that on days 14 and 21, the cell activity on BAG-FRCs was significantly lower ($p < 0.05$) than that on both titanium and GFRCs. However, cells on the BAG-FRCs were able to reach the maximum level of alkaline phosphatase activity (ALP) on day 14, which was faster than the other materials tested. Additionally, the fastest osteogenic differentiation seemed to take place on the GFRCs, while titanium had a higher osteocalcin expression level than that of any other tested material [28]. Another study [32] compared BAG-FRC to MG63 cells. The data showed that BAG-FRC had a more differentiated and significantly higher viability of cells than the tested titanium alloy (Ti6Al4V) on days 1, 3, and 5. In addition, ALP was significantly higher by day 5; however, a decreasing trend of ALP activity was observed as the number of days increased. In general, bioactive glass-type FRC could stimulate osteoblast-like cells better than titanium and its alloys in the early stages.

4. Discussion

Dental materials are used to replace physiologically/pathologically damaged dental hard tissues, and as such, materials need to be chemically inert, nontoxic, and physically stable in the oral environment [39]. Accordingly, laboratory tests provide an initial assessment for identifying whether a material fulfills the biological requirements outlined above and is suitable for further clinical testing and application [40, 41]. In the case of dental resin composites, studies have shown that these materials can potentially dissolve, leach, and degrade monomers over time [42]. Therefore, the current review focused on laboratory testing, identifying studies investigating the biological properties of GFRCs centered on the effects of surface characteristics related to cells/bacteria/yeast adhesion and growth, chemical toxicity of monomers, and the possible interactions between them.

4.1. Surface Properties

The findings of this systematic review did not identify a significant association between the surface roughness of GFRCs and bacterial adhesion [25, 26]. In the case of conventional dental resin composites (Filtek Z350 (3M ESPE, St Paul, MN, USA)), Park et al. compared the cured composites polished with 400- and 800-grit silicon carbide papers to glass slides. This study found that biofilm formation by S. mutans significantly increased on the rougher 400-grit polished surface [43]. In this case, the rougher surface might be a result of the ease of trapping the bacteria and protein on the polished surface, i.e., topographical reason and increased surface area. A more recent study demonstrated that certain topographical microstructures on dental resin composite surfaces could significantly influence the adhesion of oral bacteria [44], with higher bacterial adhesion observed on composite samples that had linear surface trapezoid structures, followed by flat pyramids and cubic shape. This indicates that the roughness per se can be the most significant factor

influencing bacterial adhesion, but it is not always the case.

In addition to the physical parameters (e.g., surface roughness) that might trap serum proteins such as albumin, fibronectin, fibrinogen, and others, binding or inhibiting the adhesion of bacteria, or suspended in the fluid flow condition [45], the initial cell adhesion is highly dependent on surface chemical interactions and charge (e.g., hydrophobic/hydrophilic, electrostatic, and van der Waals forces) between the substrate and bacterial/yeast cells [45]. This effect might be the same when stored and exposed to saliva. The reviewed studies [25–27, 30] have shown that matrix resins and fiber types are factors able to induce different degrees of bacterial adhesion.

Wetting is a surface phenomenon that occurs when a solid and liquid interact and creates an interface between the two, i.e., balancing the surface energies at the interfaces between air, liquid, and solid, for a system. Thus, different surface physiochemical conditions would contribute to different surface wettabilities. The contact angle (CA) was used to evaluate the surface wettability. The wettability of dental materials seems to be partly associated with bacterial adhesion, with hydrophilic materials being more resistant to bacterial adhesion than hydrophobic ones [30, 46]. In contrast, Kang et al. demonstrated that C. albicans adhered better and in larger numbers on hydrophilic materials [47], while S. mutans seemed to adhere better to saliva-coated FRCs than to non-coated FRCs [25]. Thus, water sorption and the type of bacterium seem to influence bacterial adhesion ability. At present, there are few reports investigating the relationship between the wettability of FRCs and cell proliferation. In general, hydrophilic surfaces promote extracellular matrix protein adsorption and interchange, which ultimately directly influences cell activity on surfaces [48].

Regarding surface roughness, different surfaces could cause various outcomes for different cells. One study suggested that nanoscale roughness could promote cell adhesion and lead to proliferation and differentiation of the cells better than a micrometer-scale modification since the surface could alter the cell's extracellular matrix protein binding to the surface [49]. Hallab et al. compared metallic materials, glass, and polymers based on their surface roughness and surface energy. It was reported that polymers with a lower surface free energy showed increased fibroblast adhesion strength, which was also associated with an increased surface roughness [50]. Osteoblast cells, such as rat calvarial osteoblasts, were found to favor rough surfaces, with the proliferation rate significantly increasing with increased surface roughness. In contrast, human gingival fibroblasts (HGF) demonstrated a decrease in proliferation with increased roughness [51]. Tsui et al. [52] and Li et al. [53] reported that nano-features on resinous surfaces would shred the bacteria, i.e., serving as an antibacterial function. To conclude, these findings demonstrate that cell adhesion varies depending on the surface (e.g., morphology and energy) and cell (e.g., types and species) interactions.

4.2. Biocompatibility

In general, biocompatibility is a property that implies that materials or medical devices are compatible with living tissues when in direct contact. If the materials or devices have no toxic or immunological response acting on the tissues, they can be claimed to be biocompatible. Appropriate tests are necessary to protect humans from potential biological risks. The current standards include ISO 10993, a set of tests for evaluating the biocompatibility of medical devices, and ISO 7405, a dental-specific standard for biocompatibility evaluation of medical devices used in dentistry. According to ISO 10993-2009, cytotoxicity needs to be evaluated for all medical devices. Various cell culture techniques can be applied to devices and materials for cytotoxicity to determine cell lysis (cell death), inhibition of cell growth, colony formation, and other effects on cells.

From the literature [24, 29, 31, 33], E-glass fibers appear to be the most suitable for GFRCs in terms of biocompatibility. Moreover, the resin should ideally be UDMA-TEGDMA based. This concurs with various previous studies. For example, Schweikl et al. demonstrated that cytotoxicity on hamster lung fibroblasts (V79 cells) with unpolymerized resin monomers was ranked from high to low toxicity for bis-GMA, UDMA, TEGDMA, HEMA, and MAA [54], which are the most commonly used monomers in FRCs. Another study also confirmed that the monomer bis-GMA was more toxic than TEGDMA, with UDMA being the least toxic when tested against three different human fibroblasts and immortalized HaCaT keratinocytes [55, 56]. Silica-glass-based FRCs possessed a limited quantity of residual MMA (0.37 ± 0.007% (wt/wt)), demonstrating good cytotoxic properties with mouse fibroblasts [25, 57, 58].

The reviewed studies [28, 32] included bioactive glass (BAG)-containing GFRCs, and the results varied. The different designs of the glass fibers and FRCs might have caused the different results observed. Other studies [59, 60] used fiber and FRC designs, similar to the study [28], and the results indicated that the use of BAG-FRCs (Fiber: E-glass fiber) has a satisfactory potential to promote interactions and proliferation of osteoblast-like cells. It was concluded that the results were comparable to those when titanium was used [61]. In contrast, Chen et al. [32] attempted to use the BAG fiber or the addition of bioactive glass particles in an epoxy resin to augment the osteoblastic performance of pre-fabricated implant material (as indicated in MG63 and ALP), which was deemed to be a different approach. These two studies were not comparable.

In terms of osteoblast-like cells, rat bone marrow-derived stromal cells [28] or human osteosarcoma MG63 cells [32] were used. Bone marrow-derived stromal cells are multipotent and need to be regulated by chemicals such as dexamethasone [62-65], glycerophosphate [62, 65], and ascorbic acid [65] to form osteoblasts. However, even the standardized method using dexamethasone [63] can split the stromal cells into other cells such as osteoclasts. Thus, other osteoblast-like cells, e.g., MG63, MC3T3-E1, and SaOs-2, are preferred to reduce the error of differentiation [66].

MC3T3-E1 is a pre-osteoblast that becomes a mature osteoblast [67], while MG63 and SaOs-2 are osteosarcoma cells. It was shown that MC3T3-E1 is a preferable choice for proliferation, ALP activity, and mineral deposition compared to the other two cells because MC3T3-E1 has similar functionality to primary human osteoblasts (HOB) [66]. Using HOB might be suitable for research institutes, but they are not easily obtained, and there are ethical concerns related to the use of human subjects in a test house. In addition, MC3T3-E1 was used to evaluate dental resin composites for years [68–70] to assess cytotoxicity, but has never been used to evaluate FRCs to the authors' knowledge. Therefore, MC3T3-E1 seems to be a good screening cell for cytotoxicity.

Insufficient light polymerization of resin composites can potentially show higher toxicity owing to the leaching of residual monomers and their interaction with surrounding tissues, possibly leading to adverse effects such as postoperative sensitivity or pulp inflammation [71]. This might be caused by two mechanisms: the resin monomers are released because of the low degree of conversion when a low light output occurs, or unreacted peroxy radicals as the oxygen inhibitor layer are exposed on the resin's surface, which inhibits the polymerization process [72, 73]. Imazato et al. [70] evaluated eluted or unreacted monomers such as HEMA, which have been shown to decrease osteoblastic proliferation, differentiation, and mineralization of MC3T3-E1 cells.

In contrast, even though monomers, such as BPA and MMA, are eluted from composites, little or no bacteriostatic/bactericidal effects were found against oral bacteria [74, 75]. Thus, conventional FRCs that use common dimethacrylates in the resin matrix do not possess antibacterial properties, unless antibacterial fillers/functional groups are added, such as silver compounds [76], fluoride-containing fillers [77], or 12-methacryloyloxy dodecylpyridinium bromide (MDPB) [78]. These components have been tested in resin composites but are yet to be utilized in FRCs, given that their effect on the FRC material properties, such as mechanical properties, release profile, and efficiency, has not been investigated.

4.3. Biomechanics

The biological response of FRCs correlated to certain mechanical properties has also been investigated [29, 79-81]. These biomechanical properties, identified as the structural biocompatibility by Vallittu et al. [71], include tensile, flexural, compressive, and shear strengths. The mismatch of the strength and modulus of elasticity may transfer an unstable mechanical strain onto the surrounding bone or tissue. In contrast, from a clinical perspective, biomechanical behavior such as stress-shielding is associated with wound healing and implant stability [82–84]. Therefore, considering

this aspect, FRCs have demonstrated the potential to replace metallic materials used in implants, owing to their better matching of the modulus of elasticity with bony tissues. The unidirectional glass fibers possess a similar modulus (20 GPa) to that of longitudinal cortical bone (17.7 GPa) and transverse cortical bone (12.8 GPa), particularly when compared with titanium (110–120 GPa) or zirconia (210 GPa) [71, 83, 84].

4.4. Study bias and quality

There are no standard parameters to assess the quality of laboratory studies. In the present study, self-developed parameters were used to assess the risk of bias in the included studies. These studies had a high, medium, and low risk of bias, demonstrating that variables that could influence the results of the studies were not controlled by researchers favoring the high heterogeneity of the findings of this study.

The findings of the current review systematically illustrate that FRCs possess satisfactory biological properties that support their use in dental applications. Their biological properties are related to both biocompatibility and surface compatibility. However, some issues remain to be resolved. First, a standardized approach for processing and testing FRCs' experimental specimens is lacking. This was obvious given the differences in polymerization techniques employed (e.g., light curing, water-heat curing, or oven curing), time period (e.g., water heated at 70 °C for 90 min, at 70 °C for 60 min, or at 80 °C for 60 min), and specimen configuration (e.g., bar, square, rhomboid, or cylindrical). All of these parameters can contribute to the differences between material and material interactions and, thus, the variability of the test results. Second, the biocompatibility of FRCs remains a concern that is worthy of being thoroughly investigated. Moreover, the surface properties of FRC and their influence on bacterial adhesion with different bacterial strains over long-term observation periods require further research.

5. Conclusion

The data evaluated in this review remain limited, albeit promising. FRCs have been demonstrated to be biocompatible materials, promoting fibroblast cell interactions and bone-like cell adhesion. Nonetheless, further research is necessary to understand the resin matrix monomer release/degradation process of FRCs better and to identify mechanisms to prolong their early (short-term) acceptable biological properties. The introduction of antibacterial components into the FRC matrix system can also be considered in future studies to improve the FRC-response to various microbial/biofilm challenges.

Conflict of interest

NIL

References

[1] Ayna E, Celenk S. Polyethylene fiber-reinforced composite inlay fixed partial dentures: two-year preliminary results. Journal of Adhesive Dentistry. 2005;7(4).

[2] Garoushi S, Lassila LV, Tezvergil A, Vallittu PK. Load bearing capacity of fibre-reinforced and particulate filler composite resin combination. Journal of Dentistry. 2006;34(3):179-84. doi:http://doi.org/10.1016/j.jdent.2005.05.010.

[3] Aitasalo KM, Piitulainen JM, Rekola J, Vallittu PK. Craniofacial bone reconstruction with bioactive fiber-reinforced composite implant. Head and Neck. 2014;36(5):722-8. doi:http://doi.org/10.1002/hed.23370.

[4] Ahmed KE, Li KY, Murray CA. Longevity of fiber-reinforced composite fixed partial dentures (FRC FPD)—Systematic review. Journal of Dentistry. 2017;61:1-11. doi:http://doi.org/10.1016/j.jdent.2016.08.007.

[5] Kumbuloglu O, Saracoglu A, Özcan M. Pilot study of unidirectional E-glass fibre-reinforced composite resin splints: up to 4.5-year clinical follow-up. Journal of Dentistry. 2011;39(12):871-7. doi:http://doi.org/10.1016/j.jdent.2011.09.012.

[6] Scribante A, Sfondrini MF. Orthodontic retainers. A Clinical Guide to Fibre Reinforced Composites (FRCs) in Dentistry: Elsevier; 2017. p. 187-202.

[7] Meiers JC, Freilich MA. Use of a prefabricated fiber-reinforced composite resin framework to provide a provisional fixed partial denture over an integrating implant: a clinical report. The Journal of Prosthetic Dentistry. 2006;95(1):14-8. doi:http://doi.org/10.1016/j.prosdent.2005.10.020.

[8] Shinya A, Yokoyama D, Lassila LV, Shinya A, Vallittu PK. Three-dimensional finite element analysis of metal and FRC adhesive fixed dental prostheses. Journal of Adhesive Dentistry. 2008;10(5).

[9] Kargul B, Çaglar E, Kabalay U. Glass fiber-reinforced composite resin as fixed space maintainers in children: 12-month clinical follow-up. Journal of Dentistry for Children. 2005;72(3):109-12.

[10] Kurunmäki H, Kantola R, Hatamleh MM, Watts DC, Vallittu PK. A fiber-reinforced composite prosthesis restoring a lateral midfacial defect: a clinical report. The Journal of Prosthetic Dentistry. 2008;100(5):348-52. doi:http://doi.org/10.1016/S0022-3913(08)60235-8.

[11] Fujii K, Arikawa H, Kanie T, Hamano T, Nishi Y, Nagaoka E. Dynamic viscoelastic properties of commercial glass-fibre reinforced resin used for crowns and bridges. J Oral Rehabil. 2002;29(9):827-34. doi:http://doi.org/10.1046/j.1365-2842.2002.00887.x.

[12] Lassila LV, Tezvergil A, Lahdenpera M, Alander P, Shinya A, Shinya A, et al. Evaluation of some properties of two fiber-reinforced composite materials. Acta Odontol Scand. 2005;63(4):196-204. doi:http://doi.org/10.1080/00016350510019946.

[13] Garoushi S, Kaleem M, Shinya A, Vallittu PK, Satterthwaite JD, Watts DC, et al. Creep of experimental short fiber-reinforced composite resin. Dental Materials Journal. 2012;31(5):737-41. doi:http://doi.org/10.4012/dmj.2011-247.

[14] Yla-Soininmaki A, Moritz N, Lassila LV, Peltola M, Aro HT, Vallittu PK. Characterization of porous glass fiber-reinforced composite (FRC) implant structures: porosity and mechanical properties. J Mater Sci Mater Med. 2013;24(12):2683-93. doi:http://doi.org/10.1007/s10856-013-5023-1.

[15] Zhang M, Matinlinna JP. E-glass fiber reinforced composites in dental applications. Silicon. 2012;4(1):73-8. doi:http://doi.org/10.1007/s12633-011-9075-x.

[16] Mallineni SK, Nuvvula S, Matinlinna JP, Yiu CK, King NM. Biocompatibility of various dental materials in contemporary dentistry: a narrative insight. Journal of Investigative and Clinical Dentistry. 2013;4(1):9-19. doi:http://doi.org/10.1111/j.2041-1626.2012.00140.x.

[17] Fan C, Chu L, Rawls HR, Norling BK, Cardenas HL, Whang K. Development of an antimicrobial resin—A pilot study. Dent Mater. 2011;27(4):322-8. doi:http://doi.org/10.1016/j.dental.2010.11.008.

[18] Luo W, Huang Q, Liu F, Lin Z, He J. Synthesis of antibacterial methacrylate monomer derived from thiazole and its application in dental resin. J Mech Behav Biomed. 2015;49:61-8. doi:http://doi.org/10.1016/j.jmbbm.2015.04.026.

[19] Miki S, Kitagawa H, Kitagawa R, Kiba W, Hayashi M, Imazato S. Antibacterial activity of resin composites containing surface pre-reacted glass-ionomer (S-PRG) filler. Dent Mater. 2016;32(9):1095-102. doi:http://doi.org/10.1016/j.dental.2016.06.018.

[20] Schardt C, Adams MB, Owens T, Keitz S, Fontelo P. Utilization of the PICO framework to improve searching PubMed for clinical questions. BMC Med Inform Decis Mak. 2007;7:16. doi:http://doi.org/10.1186/1472-6947-7-16.

[21] Sayers A. Tips and tricks in performing a systematic review. Br J Gen Pract. 2008;58(547):136. doi:http://doi.org/10.3399/bjgp08X277168.

[22] Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. Annals of Internal Medicine. 2009;151(4):264-9. doi:http://doi.org/10.1371/journal.pmed.1000097.

[23] Sarkis-Onofre R, Skupien J, Cenci M, Moraes R, Pereira-Cenci T. The role of resin cement on bond strength of glass-fiber posts luted into root canals: a systematic review and meta-analysis of in vitro studies. Operative Dentistry. 2014;39(1):E31-E44. doi:http://doi.org/10.2341/13-070-LIT.

[24] Vallittu PK, Ekstrand K. In vitro cytotoxicity of fibre-polymethyl methacrylate composite used in dentures. J Oral Rehabil. 1999;26(8):666-71. doi:http://doi.org/10.1046/j.1365-2842.1999.00431.x.

[25] Tanner J, Vallittu PK, Soderling E. Effect of water storage of E-glass fiber-reinforced composite on adhesion of Streptococcus mutans. Biomaterials. 2001;22(12):1613-8. doi:http://doi.org/10.1016/s0142-9612(00)00314-8.

[26] Tanner J, Carlén A, Söderling E, Vallittu PK. Adsorption of parotid saliva proteins and adhesion of Streptococcus mutans ATCC 21752 to dental fiber-reinforced composites. Journal of Biomedical Materials Research Part B: Applied Biomaterials. 2003;66(1):391-8. doi:http://doi.org/10.1002/jbm.b.10012.

[27] Waltimo T, Luo G, Samaranayake LP, Vallittu PK. Glass fibre-reinforced composite laced with chlorhexidine digluconate and yeast adhesion. Journal of Materials Science-Materials in Medicine. 2004;15(2):117-21. doi:http://doi.org/10.1023/B:JMSM.0000011811.15602.3a.

[28] Ballo AM, Kokkari AK, Meretoja VV, Lassila LL, Vallittu PK, Narhi TO. Osteoblast proliferation and maturation on bioactive fiber-reinforced composite surface. J Mater Sci Mater Med. 2008;19(10):3169-77. doi:http://doi.org/10.1007/s10856-008-3453-y.

[29] Meriç G, Dahl JE, Ruyter IE. Cytotoxicity of silica–glass fiber reinforced composites. Dent Mater. 2008;24(9):1201-6. doi:http://doi.org/10.1016/j.dental.2008.01.010.

[30] Lassila LV, Garoushi S, Tanner J, Vallittu PK, Söderling E. Adherence of Streptococcus mutans to fiber-reinforced filling composite and conventional restorative materials. The Open Dentistry Journal. 2009;3(1):227-32. doi:http://doi.org/10.2174/1874210600903010227.

[31] Frese C, Wolff D, Zingler S, Krueger T, Stucke K, Lux CJ, et al. Cytotoxicity of coated and uncoated fibre-reinforced composites. Acta Odontologica Scandinavica. 2014;72(5):321-30. doi:http://doi.org/10.3109/00016357.2013.826381.

[32] Chan YH, Lew WZ, Lu E, Loretz T, Lu L, Lin CT, et al. An evaluation of the biocompatibility and osseointegration of novel glass fiber reinforced composite implants: In vitro and in vivo studies. Dent Mater. 2018;34(3):470-85. doi:http://doi.org/10.1016/j.dental.2017.12.001.

[33] Lazar MA, Rotaru H, Baldea I, Bosca AB, Berce CP, Prejmerean C, et al. Evaluation of the Biocompatibility of New Fiber-Reinforced Composite Materials for Craniofacial Bone Reconstruction. J Craniofac Surg. 2016;27(7):1694-9. doi:http://doi.org/10.1097/SCS.0000000000002925.

[34] Van Dijken J, Persson S, Sjostrom S. Presence of Streptococcus mutans and lactobacilli in saliva and on enamel, glass ionomer cement, and composite resin surfaces. European Journal of Oral Sciences. 1991;99(1):13-9. doi:http://doi.org/10.1111/j.1600-0722.1991.tb01017.x.

[35] Wei J, Igarashi T, Okumori N, Maetani T, Liu B, Yoshinari M. Influence of surface wettability on competitive protein adsorption and initial attachment of osteoblasts. Biomed Mater. 2009;4(4):045002. doi:http://doi.org/10.1088/1748-6041/4/4/045002.

[36] Atkinson JC, Diamond F, Eichmiller F, Selwitz R, Jones GJDM. Stability of bisphenol A, triethylene-glycol dimethacrylate, and bisphenol A dimethacrylate in whole saliva. 2002;18(2):128-35. doi:http://doi.org/10.1016/s0109-5641(01)00031-8.

[37] Kurata S, Hamada N, Kanazawa A, Endo T. Study on antibacterial dental resin using tri-n-butyl (4-vinylbenzyl) phosphonium chloride. Dental materials journal. 2011:1111220217-. doi:http://doi.org/10.4012/dmj.2011-157.

[38] Tsui K-H, Li X, Tsoi JK, Leung S-F, Lei T, Chak WY, et al. Low-cost, flexible, disinfectant-free and regular-array three-dimensional nanopyramid antibacterial films for clinical applications. 2018;10(22):10436-42. doi:http://doi.org/10.1039/c8nr01968a.

[39] Beyth N, Yudovin-Fearber I, Domb AJ, Weiss EI. Long-term antibacterial surface properties of composite resin incorporating polyethyleneimine nanoparticles. Quintessence International. 2010;41(10).

[40] Savoldi F, Tsoi JKH, Paganelli C, Matinlinna JP. Evaluation of rapid maxillary expansion through acoustic emission technique and relative soft tissue attenuation. J Mech Behav Biomed. 2017;65:513-21. doi:http://doi.org/10.1016/j.jmbbm.2016.09.016.

[41] Savoldi F, Tsoi JKH, Paganelli C, Matinlinna JP. Biomechanical behaviour of craniofacial sutures during distraction: An evaluation all over the entire craniofacial skeleton. Dent Mater. 2017;33(7):E290-E300. doi:http://doi.org/10.1016/j.dental.2017.04.025.

[42] Teuten EL, Saquing JM, Knappe DR, Barlaz MA, Jonsson S, Björn A, et al. Transport and release of chemicals from plastics to the environment and to wildlife. Philosophical Transactions of the Royal Society B: Biological Sciences. 2009;364(1526):2027-45. doi:http://doi.org/10.1098/rstb.2008.0284.

[43] Park J, Song C, Jung J, Ahn S, Ferracane J. The effects of surface roughness of composite resin on biofilm formation of Streptococcus mutans in the presence of saliva. Operative Dentistry. 2012;37(5):532-9. doi:http://doi.org/10.2341/11-371-L.

[44] Frenzel N, Maenz S, Beltrán VS, Völpel A, Heyder M, Sigusch BW, et al. Template assisted surface microstructuring of flowable dental composites and its effect on microbial adhesion properties. Dent Mater. 2016;32(3):476-87. doi:http://doi.org/10.1016/j.dental.2015.12.016.

[45] Katsikogianni M, Missirlis YF. Concise review of mechanisms of bacterial adhesion to biomaterials and of techniques used in estimating bacteria-material interactions. Eur Cell Mater. 2004;8:37-57. doi:http://doi.org/10.22203/ecm.v008a05.

[46] Azam MT, Khan AS, Muzzafar D, Faryal R, Siddiqi SA, Ahmad R, et al. Structural, Surface, in vitro Bacterial Adhesion and Biofilm Formation Analysis of Three Dental Restorative Composites. Materials. 2015;8(6):3221-37. doi:http://doi.org/10.3390/ma8063221.

[47] Kang S-H, Lee H-J, Hong S-H, Kim K-H, Kwon T-Y. Influence of surface characteristics on the adhesion of Candida albicans to various denture lining materials. Acta Odontologica Scandinavica. 2013;71(1):241-8. doi:http://doi.org/10.3109/00016357.2012.671360.

[48] Scotchford CA, Gilmore CP, Cooper E, Leggett GJ, Downes S. Protein adsorption and human osteoblast-like cell attachment and growth on alkylthiol on gold self-assembled monolayers. Journal of Biomedical Materials Research Part A. 2002;59(1):84-99. doi:http://doi.org/10.1002/jbm.1220.

[49] Mendonça G, Mendonça DB, Aragao FJ, Cooper LF. Advancing dental implant surface technology–from micron-to nanotopography. Biomaterials. 2008;29(28):3822-35. doi:http://doi.org/10.1016/j.biomaterials.2008.05.012.

[50] Hallab NJ, Bundy KJ, O'Connor K, Moses RL, Jacobs JJ. Evaluation of metallic and polymeric biomaterial surface energy and surface roughness characteristics for directed cell adhesion. Tissue Engineering. 2001;7(1):55-71. doi:http://doi.org/10.1089/107632700300003297.

[51] Kunzler TP, Drobek T, Schuler M, Spencer ND. Systematic study of osteoblast and fibroblast response to roughness by means of surface-morphology gradients. Biomaterials. 2007;28(13):2175-82. doi:http://doi.org/10.1016/j.biomaterials.2007.01.019.

[52] Tsui KH, Li X, Tsoi JKH, Leung SF, Lei T, Chak WY, et al. Low-cost, flexible, disinfectant-free and regular-array three-dimensional nanopyramid antibacterial films for clinical applications. Nanoscale. 2018;10(22):10436-42. doi:http://doi.org/10.1039/c8nr01968a.

[53] Li X, Tsui K-H, Tsoi JKH, Green D, Jin X-z, Deng YQ, et al. Nano-structured anti-biofilm surface widens efficacy against spindle-shaped and chain forming rod-like bacteria. Nanoscale. 2020. doi:http://doi.org/10.1039/d0nr03809a.

[54] Schweikl H, Schmalz G, Spruss T. The induction of micronuclei in vitro by unpolymerized resin monomers.Journal of Dental Research, Jul 2001;80(7):1615-20. doi:http://doi.org/10.1177/00220345010800070401.

[55] Yoshii E. Cytotoxic effects of acrylates and methacrylates: relationships of monomer structures and cytotoxicity. Journal of Biomedical Materials Research. 1997;37(4):517-24. doi:http://doi.org/10.1002/(sici)1097-4636(19971215)37:4<517::aid-jbm10>3.0.co;2-5.

[56] Moharamzadeh K, Van Noort R, Brook IM, Scutt AM. Cytotoxicity of resin monomers on human gingival fibroblasts and HaCaT keratinocytes. Dent Mater. 2007;23(1):40-4. doi:http://doi.org/10.1016/j.dental.2005.11.039.

[57] Meriç G, Dahl JE, Ruyter I. Physicochemical evaluation of silica-glass fiber reinforced polymers for prosthodontic applications. European Journal of Oral Sciences. 2005;113(3):258-64. doi:http://doi.org/10.1111/j.1600-0722.2005.00209.x.

[58] Tanner J, Robinson C, Söderling E, Vallittu P. Early plaque formation on fibre-reinforced composites in vivo. Clinical Oral Investigations. 2005;9(3):154-60. doi:http://doi.org/10.1007/s00784-005-0317-4.

[59] Hoppe A, Güldal NS, Boccaccini AR. A review of the biological response to ionic dissolution products from bioactive glasses and glass-ceramics. Biomaterials. 2011;32(11):2757-74. doi:http://doi.org/10.1016/j.biomaterials.2011.01.004.

[60] Ballo AM, Akca E, Ozen T, Moritz N, Lassila L, Vallittu P, et al. Effect of implant design and bioactive glass coating on biomechanical properties of fiber-reinforced composite implants. European Journal of Oral Sciences. 2014;122(4):303-9. doi:http://doi.org/10.1111/eos.12133.

[61] Ballo A, Cekic-Nagas I, Ergun G, Lassila L, Palmquist A, Borchardt P, et al. Osseointegration of fiber-reinforced composite implants: Histological and ultrastructural observations. Dent Mater. 2014;30(12):e384-e95. doi:http://doi.org/10.1016/j.dental.2014.08.361.

[62] Maniatopoulos C, Sodek J, Melcher AH. Bone formation in vitro by stromal cells obtained from bone marrow of young adult rats. Cell and Tissue Research. 1988;254(2):317-30. doi:http://doi.org/10.1007/bf00225804.

[63] Udagawa N, Takahashi N, Akatsu T, Sasaki T, Yamaguchi A, Kodama H, et al. The bone marrow-derived stromal cell lines MC3T3-G2/PA6 and ST2 support osteoclast-like cell differentiation in cocultures with mouse spleen cells. Endocrinology. 1989;125(4):1805-13. doi:http://doi.org/10.1210/endo-125-4-1805.

[64] Porter RM, Huckle WR, Goldstein AS. Effect of dexamethasone withdrawal on osteoblastic differentiation of bone marrow stromal cells. J Cell Biochem. 2003;90(1):13-22. doi:http://doi.org/10.1002/jcb.10592.

[65] Deliloglu-Gurhan SI, Vatansever HS, Ozdal-Kurt F, Tuglu I. Characterization of osteoblasts derived from bone marrow stromal cells in a modified cell culture system. Acta Histochem. 2006;108(1):49-57. doi:http://doi.org/10.1016/j.acthis.2005.11.001.

[66] Czekanska EM, Stoddart MJ, Ralphs JR, Richards RG, Hayes JS. A phenotypic comparison of osteoblast cell lines versus human primary osteoblasts for biomaterials testing. J Biomed Mater Res A. 2014;102(8):2636-43. doi:http://doi.org/10.1002/jbm.a.34937.

[67] Rutkovskiy A, Stenslokken KO, Vaage IJ. Osteoblast Differentiation at a Glance. Medical Science Monitor Basic Research. 2016;22:95-106. doi:http://doi.org/10.12659/Msmbr.901142.

[68] Geurtsen W, Lehmann F, Spahl W, Leyhausen G. Cytotoxicity of 35 dental resin composite monomers/additives in permanent 3T3 and three human primary fibroblast cultures. J Biomed Mater Res. 1998;41(3):474-80. doi:http://doi.org/10.1002/(sici)1097-4636(19980905)41:3<474::aid-jbm18>3.0.co;2-i.

[69] Imazato S, Horikawa D, Ogata K, Kinomoto Y, Ebisu S. Responses of MC3T3-E1 cells to three dental resin-based restorative materials. J Biomed Mater Res A. 2006;76(4):765-72. doi:http://doi.org/10.1002/jbm.a.30422.

[70] Imazato S, Horikawa D, Nishida M, Ebisu S. Effects of monomers eluted from dental resin restoratives on osteoblast-like cells. J Biomed Mater Res B Appl Biomater. 2009;88(2):378-86. doi:http://doi.org/10.1002/jbm.b.31067.

[71] Vallittu PK, Närhi TO, Hupa L. Fiber glass–bioactive glass composite for bone replacing and bone anchoring implants. Dent Mater. 2015;31(4):371-81. doi:http://doi.org/10.1016/j.dental.2015.01.003.

[72] Quinlan C, Zisterer D, Tipton K, O'sullivan M. In vitro cytotoxicity of a composite resin and compomer. International Endodontic Journal. 2002;35(1):47-55. doi:http://doi.org/10.1046/j.1365-2591.2002.00456.x.

[73] Jagdish N, Padmanabhan S, Chitharanjan AB, Revathi J, Palani G, Sambasivam M, et al. Cytotoxicity and degree of conversion of orthodontic adhesives. The Angle Orthodontist. 2009;79(6):1133-8. doi:http://doi.org/10.2319/080808-418R.1.

[74] Kawai K. Effects of eluate from composite resins on Gtase and growth of Streptococcus mutans. Jpn J Conserv Dent. 1988;31:332-51.

[75] Imazato S. Antibacterial properties of resin composites and dentin bonding systems. Dent Mater. 2003;19(6):449-57. doi:http://doi.org/10.1016/s0109-5641(02)00102-1.

[76] Yoshida K, Tanagawa M, Atsuta M. Characterization and inhibitory effect of antibacterial dental resin composites incorporating silver-supported materials. Journal of Biomedical Materials Research Part A. 1999;47(4):516-22. doi:http://doi.org/10.1002/(sici)1097-4636(19991215)47:4<516::aid-jbm7>3.0.co;2-e.

[77] Yap A, Khor E, Foo S. Fluoride release and antibacterial properties of new-generation tooth colored restoratives. Operative Dentistry. 1999;24:297-305.

[78] Imazato S, McCabe J. Influence of incorporation of antibacterial monomer on curing behavior of a dental composite. Journal of Dental Research. 1994;73(10):1641-5. doi:http://doi.org/10.1177/00220345940730100901.

[79] Brauer DS, Russel C, Vogt S, Weisser J, Schnabelrauch M. Degradable phosphate glass fiber reinforced polymer matrices: mechanical properties and cell response. J Mater Sci Mater Med. 2008;19(1):121-7. doi:http://doi.org/10.1007/s10856-007-3147-x.

[80] Felfel RM, Ahmed I, Parsons AJ, Palmer G, Sottile V, Rudd CD. Cytocompatibility, degradation, mechanical property retention and ion release profiles for phosphate glass fibre reinforced composite rods. Mater Sci Eng C Mater Biol Appl. 2013;33(4):1914-24. doi:http://doi.org/10.1016/j.msec.2012.12.089.
[81] Liu X, Hasan MS, Grant DM, Harper LT, Parsons AJ, Palmer G, et al. Mechanical, degradation and cytocompatibility properties of magnesium coated phosphate glass fibre reinforced polycaprolactone composites. J Biomater Appl. 2014;29(5):675-87. doi:http://doi.org/10.1177/0885328214541302.
[82] Huiskes R. Failed innovation in total hip replacement: diagnosis and proposals for a cure. Acta Orthopaedica Scandinavica. 1993;64(6):699-716. doi:http://doi.org/10.3109/17453679308994602.
[83] Choi H-H, Chung C-H, Kim S-G, Son M-K. Reliability of 2 implant stability measuring methods in assessment of various periimplant bone loss: an in vitro study with the Periotest and Osstell Mentor. Implant Dentistry. 2014;23(1):51-6. doi:http://doi.org/10.1097/ID.0000000000000000.
[84] Du J, Lee J-H, Jang AT, Gu A, Hossaini-Zadeh M, Prevost R, et al. Biomechanics and strain mapping in bone as related to immediately-loaded dental implants. Journal of Biomechanics. 2015;48(12):3486-94. doi:http://doi.org/10.1016/j.jbiomech.2015.05.014.