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Influence of Material and Surface Roughness of Resin Composite Cements on Fibroblast Behavior

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Clinical Relevance

A well-polished cement surface increases the viability and spreading of gingival fibroblasts. The tested resin composite cements did not reveal any cytotoxic effects.

SUMMARY

Objective: This *in vitro* study aimed to investigate the effect of cement type and roughness on the viability and cell morphology of human gingival fibroblasts (HGF-1).

Methods and Materials: Discs of three adhesive (Panavia V5 [PV5], Multilink Automix [MLA], RelyX Ultimate [RUL] and three self-adhesive (Panavia SA plus [PSA], SpeedCem plus [SCP], RelyX Unicem [RUN]) resin composite cements were prepared with three different roughnesses using silica paper grit P180, P400, or P2500. The cement specimens were characterized by surface roughness and energy-dispersive X-ray spectroscopic mapping. A viability assay was performed after 24 hours of incubation of HGF-1 cells on cement specimens. Cell morphology was examined with scanning electron microscopy.

Results: The roughness of the specimens did not differ significantly among the different resin composite cements. Mean Ra values for the three surface treatments were 1.62 ± 0.34 μm for P180, 0.79 ± 0.20 μm for P400, and 0.17 ± 0.08 μm for P2500. HGF-1 viability was significantly influenced by the cement material and the specimens' roughness, with the highest viability for PSA ≥ RUN = MLA ≥ SCP = PV5 > RUL ($p<0.05$) and for P2500 = P400 > P180 ($p<0.001$). Cell morphology did not vary among the materials but was affected by the surface roughness.

Conclusion: The composition of resin composite cements significantly affects the cell viability of HGF-1. Smooth resin composite cement surfaces with an Ra of 0.2-0.8 μm accelerate flat cell spreading and formation of filopodia.

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INTRODUCTION

Most dental restorations are fixed with cement material, and the selection of cement depends on the type of restoration, the restorative material, and the tooth or implant abutment surface contacting the restoration. Zinc phosphate cement has been used for decades for fixation of restorations, with exact fit providing thin cement layers of a minimum of 20 μm.[1] However, in restorations with larger cement margins, zinc oxide phosphate cement is more susceptible to hydrolytic degradation compared with resinous cements.[2] In laboratory and clinical situations, the cement margin width is reported to vary between 20 and 373 μm.[3-7] The introduction of resin composite cements provided materials that could adhere to etched enamel and to a lower degree also to dentin surfaces.[8] In addition, adhesion to the restoration was achieved, for instance, after etching and silanization of feldspar or lithium-disilicate ceramics. These adhesive characteristics of resin composite cements enabled the design of nonretentive restorations, even nonprep restorations, and facilitated a shift toward noninvasive preparation designs.[9] After inserting a reconstruction with resin composite cement, excess cement is initially removed with either foam pellets or by light curing for two seconds to achieve an initial polymerization followed by removal with a hand instrument of the consequently slightly hardened cement. Especially in the subgingival and interdental area, excess cement is difficult to eliminate, and residual cement is often found on parts of the reconstruction or surrounding tissue, acting as a potential cause of inflammation.[10,11] Afterward, the cement margin can be smoothed with fine burs and rubber polishers to remove the oxygen inhibition layer containing unreacted monomer and to prevent extensive biofilm formation.[12]

For implant reconstructions, screw retention is generally preferred because of the retrievability in cases with complications or need for restoration renewal.[13] Direct intraoral cementation can be indicated for compensation of divergent implant angulations or when zirconia implants are used, which are mainly one-piece designs.[14,15] Even with screw-retained implant restorations, resin composite cements are used for the fixation of computer-aided design/computer-aided manufacturing full-ceramic fixed-dental prostheses, which are extraorally cemented on titanium-bonding bases (Ti-Base) and then screwed onto the intraosseous implant part.[16]

Around teeth and implants, the junctional epithelium and the gingival fibroblasts in the connective tissue compartment provide a seal between the oral cavity and the bone by their hemidesmosomes, thus preventing bacteria and bacterial toxins from migrating along the interface between soft tissue and reconstruction. The cement and restorative materials, which are in contact with soft tissues, should therefore promote fibroblast cell adhesion and growth while also impeding extensive biofilm formation.[17] *In vitro* tests with human gingival fibroblasts (HGF-1) cells are commonly performed to evaluate the biocompatibility of transgingival implant components.[18-21] On dental cements, the highest viability of fibroblasts was found on noneugenol zinc oxide, followed by zinc phosphate, zinc oxide, and resin composite cement.[22] Furthermore, fibroblast adhesion to resin composite cement was found to be lower than to dentin and to titanium but higher than to zirconia.[23] Previously, it was demonstrated that the bacterial adhesion on dental cement is significantly influenced by the material composition[12,22,24] and the surface roughness.[22] Biofilm formation was significantly lower on resin composite cement specimens having a surface roughness with an Ra value of 0.1 μm compared to those with an Ra value of 1.2 μm.[12] However, it is currently unknown if the composition and roughness of resin composite cements affect the behavior of the fibroblasts, as was found for bacterial biofilm formation. Hence, the purpose of this comparative experimental *in vitro* study was to evaluate whether the material composition and surface roughness of composite cements influence the viability and morphology of gingival fibroblasts. The null hypothesis was that the viability of HGFs does not differ among resin composite cements of different composition and roughness.

METHODS AND MATERIALS

The viability and morphology of HGF-1 cells were investigated on three adhesive (Panavia V5 [PV5], Multilink Automix [MLA], RelyX Ultimate [RUL]) and three self-adhesive (Panavia SA plus [PSA], SpeedCem plus [SCP], RelyX Unicem 2 Automix [RUN]) resin composite cements with three different surface roughnesses. Cell viability assays and scanning electron microscopy (SEM) were performed after 24 hours of cell cultivation on the cement specimens. The cement materials were chosen in accordance with a previous study of biofilm formation[7] and are listed in Table 1.

Table 1: *Composition of Resin Composite Cement Materials*

Code	Name	Manufacturer	Type	Monomers	Fillers	Initiators
PV5	Panavia V5	Kuraray	Adhesive resin composite cement	Paste A: Bis-GMA, TEGDMA, hydrophobic aromatic dimethacrylate, hydrophilic aliphatic dimethacrylate Paste B: Bis-GMA, hydrophobic aromatic dimethacrylate, hydrophilic aliphatic dimethacrylate	38 vol% • Silanated barium glass filler • Silanated fluoroalminosilicate glass filler • Colloidal silica • Silanated aluminum oxide filler Particle size: 0.01-12μm	dl-Camphorquinone
PSA	Panavia SA plus	Kuraray	Self-adhesive resin composite cement	Paste A: 10-MDP, Bis-GMA, TEGDMA, hydrophobic aromatic dimethacrylate, HEMA Paste B: hydrophobic aromatic dimethacrylate, hydrophobic aliphatic dimethacrylate	40 vol% • Silanated barium glass filler • Silanated colloidal silica Particle size: 0.02-20 μm	dl-Camphorquinone
MLA	Multilink Automix	Ivoclar Vivadent	Adhesive resin composite cement	Base paste: Bis-GMA, HEMA, 2-dimethylaminoethyl methacrylate Catalyst paste: ethyoxylated bisphenol A dimethacrylate, UDMA, HEMA	40 vol% • Barium glass • Ytterbium trifluoride • Spheroid mixed oxide Particle size: 0.25-3.0 μm	Dibenzoyl peroxide
SCP	SpeedCem Plus	Ivoclar Vivadent	Self-adhesive resin composite cement	Base paste: UDMA, TEGDMA, polyethylene glycol dimethacrylateCatalyst paste: polyethylene glycol dimethacrylate, TEGDMA, methacrylated phosphoric acid ester, UDMA 1,10-decandiol dimethacrylate	40 vol% • Barium glass • Ytterbium trifluoride • Silicium dioxide Particle size: 0.1-7 μm	Dibenzoyl peroxide
RUL	RelyX Ultimate	3M ESPE	Adhesive resin composite cement	Base paste: methacrylate monomers containing phosphoric acid groups, methacrylate monomers Catalyst paste: methacrylate monomers	43 vol% • Silanated fillers • Alkaline (basic) fillers Particle size: 13 μm	Sodium toluene-4-sulphinate, disodium peroxodisulphate, Tert-butyl 3,5,5-trimethylperoxyhexanoate
RUN	RelyX Unicem 2 Automix	3M ESPE	Self-adhesive resin composite cement	Base paste: phosphoric acid modified methacrylate monomers, bifunctional methacrylate Catalyst paste: methacrylate monomers	43 vol% • Alkaline (basic) fillers • Silanated fillers Particle size: 12.5 μm	Sodium toluene-4-sulphinate, sodium persulfate, tert-butyl 3,5,5-trimethylperoxyhexanoate

Abbreviations: 10-MDP, 10-methacryloyloxydecyl dihydrogen phosphate; Bis-GMA, bisphenol A-glycidyl methacrylate; HEMA, 2-hydroxyethyl methacrylate; TEGDMA, triethylene glycol dimethacrylate; UDMA, urethane dimethacrylate.

Cement Specimens

Discs of each cement were produced using a ring-shaped Teflon mold with an inner diameter of 13 mm and a thickness of 1 mm. The dimension of the specimens was chosen to allow them to be fitted into a 24-well plate, where cells were later cultivated on the specimens. The mold was placed on Mylar foil located on a glass object slide. The cement was filled into the mold, which was again covered with a Mylar foil and a glass slide and fixed with clamps from two sides. The Mylar foil was used to obtain an even specimen surface and to simulate the clinical situation, in which the initial oxygen inhibition layer would be removed by polishing the cement margin.

The cement was light cured from both sides for 60 seconds each (Elipar DeepCure-S, 3M ESPE, Seefeld, Germany) and then stored in an incubator (CTS T-4025, CTS Clima Temperatur Systeme GmbH, Hechingen, Germany) at 37°C for 15 minutes according to ISO 4049 to await further polymerization. Cement specimens were then removed from the molds, and the surfaces were wet polished (Minitech 265, Presi, Hagen, Germany) with silica carbide paper (Presi). Three different surface structures were created to imitate the following clinical situa-

tions: 1) polishing the cement margin with a rough red proxoshape diamond bur (silica carbide paper P180), 2) polishing with a coarse polisher (P400), and 3) polishing with a fine polisher (P2500).[12]

After grinding, the specimens were cleaned with distilled water in an ultrasonic bath (TPC-15, Telsonic, Bronschhofen, Switzerland) for four minutes, followed by four minutes in 70% ethanol and another four minutes in distilled water; air dried; and stored in sterile 24-well plates. This cleaning process was required to avoid contamination of cement specimens used for cell experiments.

To determine the composition of the cement materials, one specimen per cement was polished with P1200/P4000 and observed with SEM followed by an energy-dispersive X-ray (EDX) spectroscopy mapping (FEI Nova NanoSEM230, Thermo Fisher Scientific, Waltham, MA, USA) at 15 kV and 5000× magnification over 12 hours. The surface roughness of three specimens of each cement type and roughness was measured three times using a tactile profilometer (T1000/TKK50, Hommelwerke, Schwenningen, Germany). Measurements were taken over a distance of 4.8 mm (T1E, tip 5 μm 90°, 1.6 mN, Hommel-Etamic/Jenoptik, Jena, Germany). Ra values were determined as the arithmetic average of the absolute values of the profile heights (peaks/valleys) over the evaluation length.

Cell Cultivation

Human primary gingival fibroblast cells (HGF-1, ATCC American Type Culture Collection, Manassas, VA, USA) were cultivated in Dulbecco's Modified Eagle Medium (DMEM high glucose, Sigma-Aldrich, Darmstadt, Germany) with 1% L-glutamine (Gibco, Thermo Fisher Scientific), 1% sodium-pyruvate (Gibco, Thermo Fisher Scientific), 1% penicillin-streptomycin (Sigma-Aldrich), and 1% amphotericin B solution (Sigma-Aldrich) as well as 10% fetal calf serum (bioswisstec, Schaffhausen, Switzerland) at 37°C with 5% CO_2. The cultures were kept for an average of three weeks in the incubator (B5060 EK/CO_2, Heraeus, Hanau, Germany) until confluence, while the culture medium was changed every second day. Cells were then washed twice with phosphate-buffered saline (PBS) solution (Gibco, Thermo Fisher Scientific) without calcium and magnesium and detached using 0.5% trypsin/0.2% EDTA solution (Sigma-Aldrich). In 24-well plates, 10^4 cells in a drop of 140 μL cell culture medium were seeded per specimen surface. To prevent cells from growing on well bottoms, the drops were carefully placed on each specimen. After one hour in the incubator, an additional 200 μL of cell culture medium was added, and cells were cultivated for another 23 hours in the incubator. Polystyrene discs (Thermanox, Faust Laborbedarf, Schaffhausen, Switzerland) served as the control.

Cell Viability Assay

To evaluate the effect of cement material and roughness on gingival fibroblasts, a WST-1 cell viability assay (cell proliferation reagent WST-1, Sigma Aldrich) was performed after 24 hours. In this assay, the stable water-soluble tetrazolium salt WST-1 is converted to a soluble formazan by a cellular mechanism, and the amount of formazan directly correlates to the number of metabolically active cells. Cells were seeded on all cements with three different roughnesses in triplicates, meaning three specimens per cement and roughness were seeded, and the experiments were repeated three times (n=9 per group; 162 specimens in total).

Polystyrene discs with a diameter of 13 mm served as control (n=9). In addition, one specimen per group and experiment was not seeded with cells and served as a blank (n=3 per group). After 24 hours, specimens were rinsed once with PBS, and 300 μL of WST-1 solution mixed with DMEM (1:3) was added to each well and incubated for two hours. Afterward, 200 μL of the supernatant was transferred to a 96-well plate, and the optical density (OD) was recorded at 490 nm with a microplate reader (RT-2100C Microplate Reader, Versamax, Molecular Devices LLC, San Jose, CA, USA). A cell viability of 100% was attributed to the cells grown on polystyrene control discs. The relative cell viability was calculated using the following equation:

$$\text{Relative cell viability} = (\text{OD}_{\text{specimen}} - \text{OD}_{\text{blank specimen}})/(\text{OD}_{\text{control}} - \text{OD}_{\text{blank control}}).$$

Cell Morphology

Cell morphology after 24 hours was visualized on one additional specimen per group for three repetitions (n=3 per group) using SEM (FEI Nova NanoSEM230, Thermo Fisher Scientific) at 15 kV. Cells on the specimens were rinsed with PBS, fixed with 2.5% glutaraldehyde (Sigma-Aldrich) for 20 minutes at room temperature, rinsed with PBS and distilled water, dehydrated with ethanol (30%, 50%, 70%, 80%, 90%, 96%, abs), critical point dried (Autosamdri-815, Tousimis Research Corporation, Rockville, MD, USA), and gold sputtered.

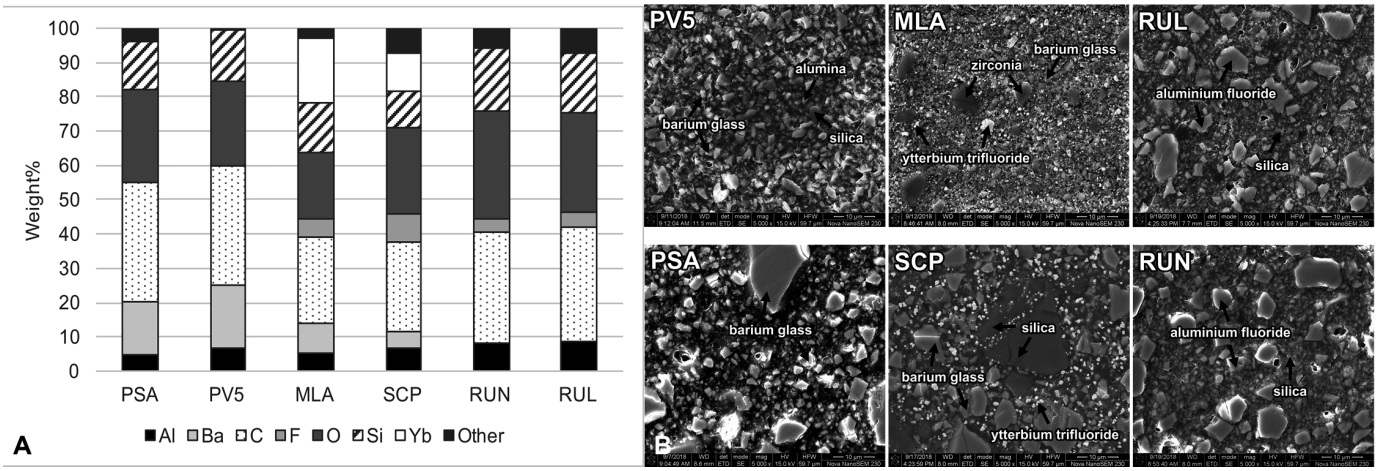

Figure 1. *(A): EDX analysis of elements present in weight% at resin composite cement surfaces. (B): SEM images of resin composite cement surfaces. Ceramic fillers determined with EDX mapping are indicated with black arrows. PV5, Panavia V5; PSA, Panavia SA plus; MLA, Multilink Automix; SCP, SpeedCem plus; RUL, RelyX Ultimate; RUN, RelyX Unicem 2 Automix.*

Statistical Analysis

Values of surface roughness and cell viability were tested for normal distribution using Shapiro-Wilk test. Two-way analysis of variance was applied afterward to determine the effect of roughness and material. Differences within subgroups were evaluated with post hoc Fisher least significant difference test. The level of significance was set to $\alpha = 0.05$.

RESULTS

Cement Characterization

EDX mapping of the cements provided the weight% of the elements present on the surfaces of the specimens (Figure 1A). Based on the distribution of the elements displayed in the corresponding SEM images, the filler composition was determined (Figure 1B).

There was no statistical difference in Ra values between the different cement types when polished to the same roughness (p=0.204). The three different polishing approaches resulted in significantly different Ra values, with Ra = 1.62 ± 0.34 μm for P180, which was significantly higher than Ra = 0.79 ± 0.20 μm for P400 (p<0.001), and significantly higher than Ra = 0.17 ± 0.08 μm for P2500 (p<0.001).

Cell viability

Cement material (p=0.002) and surface roughness (p<0.001) significantly affected the viability of HGF-1 cells. Cell viability was significantly highest for PSA ≥ RUN = MLA ≥ SCP = PV5 > RUL (p<0.05). Surface treatment with silica carbide paper grit 180 resulted in significantly lower cell viability (p<0.001) than treatment with grit 400 and 2500, which did not differ significantly (p=0.606). Mean cell viability varied between 73% ± 6% for RUL (P180) and 92% ± 13% for PSA (P400) and was smallest for P180 for all cements (Table 2). The correlation between cell viability and the surface roughness of the specimens on which the cells were cultured is displayed in Figure 2.

Cell Morphology

SEM images of cells on the specimens revealed no visible differences in cell morphology or growth pattern of the fibroblasts between the different cements with the same surface roughness. On rough specimens (P180 Ra=1.62 μm), fibroblast cells aligned themselves parallel to the direction of the grinding grooves and displayed an elongated morphology, while on smooth surfaces (P2500 Ra=0.17 μm), cells spread planar and without a specific orientation (Figure 3). On surfaces treated with silica paper grit P400 (Ra=0.79 μm), both cell morphologies were observed. Filopodia were formed by cells on all surfaces but most prominently by cells on surfaces treated with silica paper grit P2500.

DISCUSSION

This *in vitro* study investigated the influence of different resin composite cements with varying surface roughnesses on the viability of gingival fibroblasts. The results demonstrated that cell viability was significantly higher on surfaces with a roughness Ra value of between 0.2 and 0.8 μm;

Table 2: *Relative Cell Viability (%) of HGF-1 on Resin Composite Cements PV5, PSA, MLA, SCP, RUL, and RUN With Surface Pretreatment Grits P180, P400, and P2500*[a]

%	PV5	PSA	MLA	SCP	RUL	RUN
P180	$78 \pm 11^{A,B,a}$	$78 \pm 11^{A,B,a}$	$80 \pm 9^{A,a}$	$76 \pm 9^{A,B,a}$	$73 \pm 6^{B,a}$	$78 \pm 6^{A,B,a}$
P400	$83 \pm 14^{A,a,b}$	$92 \pm 13^{B,b}$	$88 \pm 12^{B,b}$	$88 \pm 8^{B,b}$	$78 \pm 12^{A,a}$	$90 \pm 8^{B,b}$
P2500	$87 \pm 15^{A,b}$	$91 \pm 21^{A,b}$	$87 \pm 11^{A,b}$	$85 \pm 6^{A,b}$	$80 \pm 8^{B,a}$	$88 \pm 10^{A,b}$

[a] *Data are mean ± standard deviation. Statistically significant differences ($p<0.05$) between groups determined with post hoc Fisher least significant difference test are indicated with superscript letters (uppercase horizontal comparison, lowercase vertical comparison).* PV5: Panavia V5; PSA: Panavia SA plus; MLA: Multilink Automix; SCP: SpeedCem plus; RUL: RelyX Ultimate; RUN: RelyX Unicem 2 Automix[a]

hence, the null hypothesis was rejected. On these smoother surfaces, cells spread flat, and the formation of filopodia was observed.

Based on the composition given by the manufacturers in combination with the mapping images determined with SEM/EDX, the respective inorganic fillers of the resin composite cements could be identified. The characterization of the specimens' filler contents and morphologies revealed that similar inorganic fillers were selected for the adhesive and self-adhesive cement by the same manufacturer, especially for RUL and RUN, which also presented identical surface structures. For PV5 barium glass, alumina and silica could be identified, while PSA was mainly composed of irregular-sized barium glass fillers. Barium glass and additionally ytterbium trifluoride were present in MLA and SCP. To MLA, zirconia fillers were added, while SCP contained chunks of silica fillers. Both RUN and RUL contained silica particles and aluminum fluoride fillers. The weight% of the analyzed elements corresponded to the fillers visible in the SEM images. Neither the type of cement, adhesive or self-adhesive, nor the cement composition correlated with cell viability among the investigated cements.

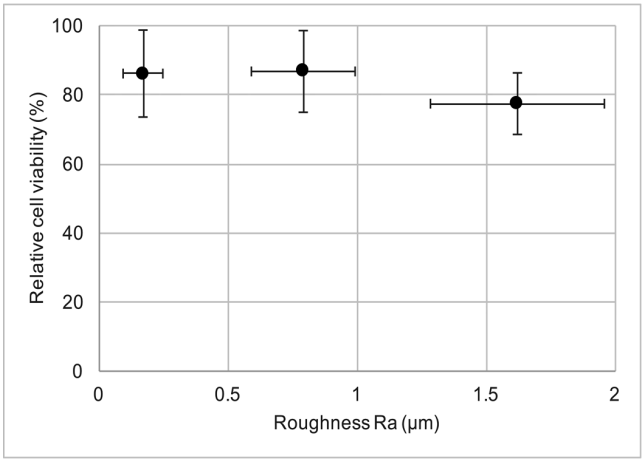

Figure 2. *Correlation between surface roughness and relative cell viability for HGF-1 on all cements.*

Using EDX analysis, only inorganic compositions could be determined, and organic compositions are displayed as contents of carbon and partially oxygen.

In addition, elements present in very small amounts, such as copper, which may have affected cell behavior, could not be captured because of the limited sensitivity of the detector and the presence of heavier elements that potentially covered the signal from lighter elements. For the organic components, it was reported that monomer release induces toxic effects, especially for cements that are not sufficiently polymerized.[25] The *in vitro* cytotoxicity of monomers to HGFs has been reported to be significantly highest for bisphenol A-glycidyl methacrylate (PV5, PSA, MLA) > urethane dimethacrylate (MLA, SCP) > triethylene glycol dimethacrylate (PV5, PSA, SCP) > 2-hydroxyethyl methacrylate (PSA, MLA).[26,27] However, in the present study, the contents of those monomers in the cements as indicated by the manufacturers were not correlated with cell viability. The manufacturer of the RUL and RUN cements did not specify their monomer contents. It was demonstrated that effective polymerization of methacrylate-based materials improves their biocompatibility, physical properties, and clinical performance.[28] Measurements of the polymerization degree of the cement specimens and consequently an estimation of potential monomers leaking from the surfaces would have allowed further interpretation of the results.

On all cements—except RUL on the roughest surface—fibroblast viability values of HGF-1 were greater than 75% compared with the control cells grown on polystyrene, which is categorized as noncytotoxic according to ISO standard 10993-5.

The effect of surface roughness on the behavior of HGF-1 is controversial.[29-32] A surface roughness with an Ra value between 0.15 and 0.25 µm has been proposed to provide optimal conditions for growth of HGF-1 cells.[31] The surface roughness of the specimens in the present study was chosen in accordance with the roughness relevant in a clinical situation by

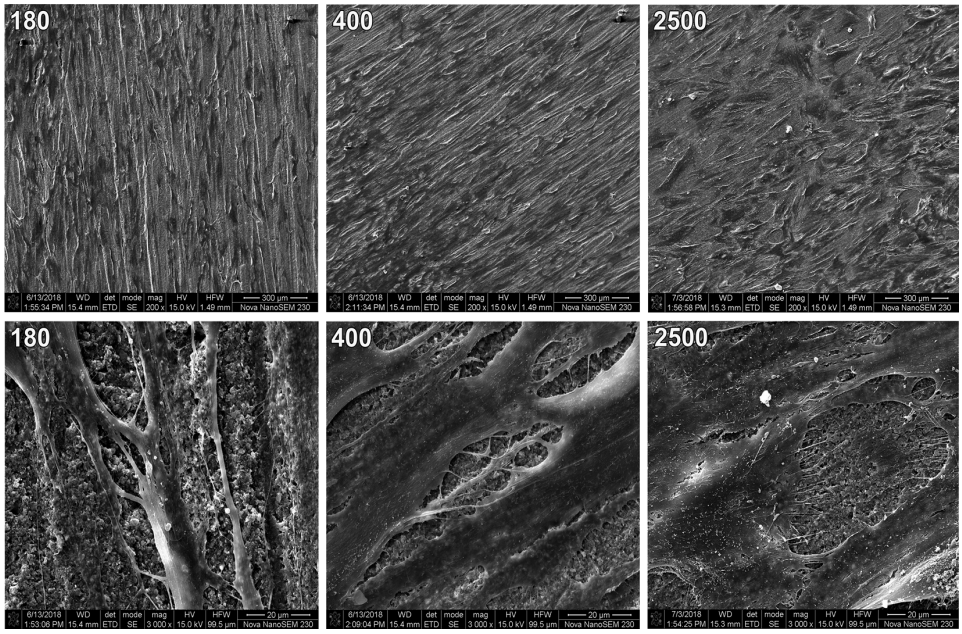

Figure 3. *SEM images of HGF-1 cells on a randomly selected cement (MLA: Multilink Automix) surface with pretreatment of grit P180, P400, or P2500 (first row 200×, second row 3000×).*

polishing the cement margin with a diamond bur (Ra=1.6 μm) or a coarse (Ra=0.8 μm) or fine intraoral polisher (Ra=0.2 μm).[12] Since the viability of HGF-1 cells on cement surfaces with an Ra value of 1.6 μm was significantly lower than on smoother surfaces, the cement margin should be finished with rubber polishers, which agrees with previous findings for biofilm formation on resin composite cements.[12]

Interestingly, it has been proposed that cell attachment is influenced by surface roughness between Ra 0.5 μm and 0.2 μm and that wettability plays a major role in the surface roughness at an Ra value of 0.2 μm or less.[33] It can be speculated that the cement's microstructure, defined by the filler size and distribution, which are not considered within the Ra measurements, may have also affected the cell viability. Since the morphology of RUL and RUN was very similar while different cell viability was observed, it is more likely that the difference between those cements is due to the chemical composition of the surface.

HGFs spread more widely, and the formation of filopodia was increased, on smooth surfaces with an Ra value <0.8 μm than on the rough specimens with an Ra value of 1.6 μm. The behavior of HGFs on rough compared with smooth surfaces has been described in several studies on titanium.[17,34,35] The current study confirms this behavior on all tested cements. HGFs were oriented on rougher surfaces along grooves, which is referred to as contact guidance.[17,34,35] Cells on smooth surfaces were randomly distributed and not oriented. The cell size of HGF-1 (15 to 150 μm)[36,37] was within the range of the width of the cement margin (20 to 373 μm).[3-7] During cementation in a clinical situation, cement is often smeared over larger areas of the reconstruction and is difficult to be fully retrieved. Therefore, the surface structure and cytotoxic potential of cements are important factors influencing the adaption of gingival tissue around a tooth or an implant abutment.

The present results on how the inorganic composition and surface roughness potentially affect cell behavior highlights the complexity of material influencing cell behavior. Numerous other factors such as surface wettability, aging, and the formation of an oxygen inhibition layer, as well as the interaction with saliva and blood at the cement surfaces, may also affect the behavior of fibroblasts in a clinical situation and should be evaluated in further studies.

CONCLUSIONS

This study is the first to provide *in vitro* data on how the surface roughness and composition of resin composite cements affect HGF cell viability. A smooth surface increases viability after 24 hours. Hence, clinicians should finish the surface of the cement margin with rubber polishers to obtain an optimal substrate for fibroblast growth. With respect to viability, all tested resin composite cements displayed no cytotoxic reactions and can be recommended for clinical use.

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Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of the University Center for Dental Medicine, University of Basel, Switzerland.

Conflict of Interest

In addition to his function as the head of the Division of Dental Materials and Engineering at the University of Basel, Switzerland, Jens Fischer is the manager of research and development at VITA Zahnfabrik, Bad Säckingen, Germany. All other authors declare no conflict of interest.

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