



Various Surface Treatments to Implant Provisional Restorations and Their Effect on Epithelial Cell Adhesion: A Comparative *In Vitro* Study

Darya Luchinskaya, DDS, MS,* Rong Du, PhD,† David M. Owens, PhD,‡
Dennis Tarnow, DDS,§ and Nurit Bittner, DDS, MS¶

Historically, osseointegration has been the major topic of interest in dental implant research.^{1,2} Successful bone-to-implant contact can be predictably achieved through various surface treatments of the implant fixture potentially accelerating the osseointegration process. Recently, a parallel shift of attention occurred toward studying soft tissue integration in the peri-implant cuff.^{3–32} In addition, the focus evolved from a surgically driven approach to a prosthetically driven approach and now to a more biologically driven approach with the ultimate goal of optimizing and maintaining esthetics.^{16,24} However, a paucity of data exists on the clinical factors and procedures for establishing a stable soft tissue profile and achieving long-term esthetic success.

Modern techniques of implant dentistry include placing the implants at bone level to provide adequate space for

Purpose and Objective: The aim of this *in vitro* study was to investigate the ability of epithelial cells to attach to or proliferate on various mechanical or chemical surface treatments of an implant provisional material.

Materials and Methods: Polyethyl methacrylate discs 10 mm in diameter and ~0.2 to 0.75 mm in width were used in the study. Experimental discs were treated with either a mechanical (pumice, varnish for shine, or high polishing) or a chemical agent (alcohol, chlorhexidine, or steam) to provide cleaning and/or polishing. Using primary human epidermal keratinocytes, experiments were performed to test the adhesion or proliferation of cells on the discs with various surface treatments.

Results: Scanning electron microscope analysis, rhodamine staining, and cell counting using a hemocytometer corroborated all findings

and illustrated that the highest cell adhesion was found to be in the smooth surface treatment groups and the poorest adhesion was found to be in the rough surface groups and chemical treatment group.

Conclusion: Within the limitations of this study, the following clinical protocol is recommended for finishing, polishing, and disinfecting implant provisional restorations: coarse, medium, fine pumice → high polishing (if desired) → steam. It is recommended to avoid applying varnish in the perimucosal area near the epithelium. This study could establish the most appropriate way to handle provisional restorations in the peri-implant sulcus for improved soft tissue health, esthetics, and long-term stability. (*Implant Dent* 2017;26:12–23)

Key Words: immediate loading, interim prosthesis, biomaterials, disinfection

*Private Practitioner, New York, NY.

†Associate Research Scientist, Department of Dermatology, Columbia University, New York, NY.

‡Associate Professor of Epithelial Cell Biology, Department of Dermatology, Pathology and Cell Biology, Columbia University, New York, NY.

§Clinical Professor, Columbia University, New York, NY.

¶Assistant Professor, Columbia University, New York, NY.

Reprint requests and correspondence to: Darya Luchinskaya, DDS, MS, Columbia University College of Dental Medicine, 630 W 168th Street, PH-7E, Room 119, New York, NY 10032, Phone: (646) 732-4591, E-mail: dluchinskaya@gmail.com

ISSN 1056-6163/17/02601-012

Implant Dentistry

Volume 26 • Number 1

Copyright © 2017 Wolters Kluwer Health, Inc. All rights reserved.

DOI: 10.1097/ID.0000000000000538

the restoration's emergence profile. With this practice, the abutment usually traverses the soft tissues and is in direct contact with epithelium and connective tissue.³³ To obtain long-term success of the restoration, with stable soft tissue and therefore a stable esthetic result, the prosthetic materials have to be in homeostasis with the adjacent biological

tissues and should not be irritating or toxic to the tissues. Ideally, the prosthetic connection should establish a biological seal and integration to the surrounding tissues, similar to that provided by nature.^{4,9,10,17,34}

The interface between the gingival soft tissue and the tooth or implant is composed of the epithelium and

connective tissue, which form the biological width.^{4,10,33} The epithelial attachment around natural teeth has been well described in the literature.^{34–37} Light and electron microscopy studies illustrate similarities between the natural tooth sulcus and the peri-implant sulcus in both animal²⁵ and human models.²¹ The tissue at the most coronal aspect and adjacent to the implant abutment consists of the free gingival margin, which is covered by stratified squamous epithelium. As the epithelium progresses apically down the implant abutment surface, the epithelium becomes nonkeratinized.²⁵ Below the junctional epithelial attachment lies the connective tissue attachment. The most critical difference between periodontal and peri-implant tissue is the absence of Sharpey fibers extending into the implant. This results in a compromised perigingival defense mechanism, which must rely primarily on the adhesive quality of the junctional epithelium. There is no fiber “backup” system as that observed on the natural dentition because the directions of the fibers are usually described as parallel to the long axis of the implant and not perpendicular as seen in teeth.⁴ In effect, the periodontal and peri-implant soft tissues are histologically similar but not identical. The epithelial attachment is similar; however, the connective tissue fibers do not attach as they do in the natural tooth and therefore may alter the peri-implant tissue’s susceptibility to disease. For this reason, in this particular study, we decided to focus on the epithelial attachment as it serves to be the primary source of defense for implant restorations as well as shares most similarity with natural dentition.

An important method of establishing and stabilizing the soft tissue profile around dental implants is through the healing phase of provisionalization. Dental implant provisionalization can be performed at the time of implant placement or after implant osseointegration allowing for the recommended manufacturer healing time. Implant provisionals provide significant and invaluable benefits such as enhanced patient comfort and satisfaction, as well as the ability to contour peri-implant

tissues.³⁸ This procedure is critical in the esthetic zone, where the restorative dentist is able to contour the soft tissues and provide an ideal emergence profile to aid in both esthetics and phonetics.^{14,16,38} In nonesthetically critical areas of the mouth, the provisional is necessary for occlusion refinement, establishing critical gingival embrasure areas to prevent or reduce food impaction and to provide an ideal emergence profile to ensure proper oral hygiene around the implant platform. In effect, proper provisionalization allows for both diagnosis and treatment of implant prosthetics. If the provisional restoration is not handled with proper care, it may negatively impact the final restoration, as well as the integrity and health of the implant body. Complications such as gingival recession, inflammation, crestal bone loss, poor access to proper hygiene, and even implant failure are possible.¹⁰

Some of the most popular materials for dental implant provisional restorations are polymethyl methacrylate (PMMA) and polyethyl methacrylate (PEMA) because of their ease of manipulation, strength, ability to conform to tissues, and relatively nontoxic properties.^{39,43} Ethyl methacrylates were formulated as an alternative to methyl methacrylates to overcome some of their disadvantages. Some of the improved characteristics include good polishability, improved stain resistance, lower exothermic setting reaction, and lower polymerization shrinkage.^{40–42} Information related to implant-fixed provisionalization is limited and is based on natural tooth provisionalization techniques.^{44–46} Existing dental literature, regarding cell adhesion or cell proliferation, primarily focuses on permanent abutment materials such as titanium, gold alloy, and ceramic.^{5,9,15,19,21,22,25,27–29} Material composition, surface topography, surface tension, and surface energy have been studied in the past.^{3,5,7,9,22,23,27,29}

Regarding surface topography, the literature is controversial, as it has been shown that both rough and smooth surfaces allow for epithelial cell adhesion to a substrate.^{3,7,9,15,22,27,29,47,48} However, a slight majority of studies show increased attraction of epithelial cells toward a smooth surface as

opposed to a rough surface.^{9,22,27,29} Bacterial plaque was not examined in this study; however, as demonstrated in early studies by Waerhaug, a rough surface facilitates the retention of bacterial plaque.⁴⁸ This is another critical source of irritation to peri-implant tissues; therefore, surface topography may play an important role in cell adhesion, cell proliferation, and therefore soft tissue stability around dental implants.

In addition, it has been well documented that epithelial tissue attachment is significantly improved when a clean and smooth surface is introduced, such as scaling the root surface of the tooth or cleaning the restoration.²⁷ A strong epithelial attachment prevents both bacterial down growth as well as soft tissue stability both esthetically and structurally around the implant restoration.¹⁰ Therefore, the method with which the restorative dentist treats the provisional restoration may impact the short- and long-term outcome and success of overall implant therapy.

Some of the anecdotal protocols for treatment of implant provisional restorations include mechanical polish using fine laboratory burs with pumice, applying a varnish as well as high polishing.^{40,41} Some of the common chemical treatments include treatment with alcohol, chlorhexidine gluconate, or steam. However, there is no report in the literature of studies completed to determine which technique is most efficient in improving the ability of epithelial cells to attach to the provisional restorative material. Improving epithelial cell adhesion could potentially strengthen the peri-implant cuff and it can create a biological seal that prevents bacterial invasion, peri-implant disease, and furthermore implant loss. In addition, it could allow for the soft tissues surrounding the implant to keep a good spatial relationship and might play an important role in esthetics. Biocompatibility is therefore an important factor for treatment success.

The purpose of this *in vitro* study was to investigate the ability of epithelial cells to attach to and proliferate on various surface topographies of temporary implant material after different mechanical and chemical treatments.

To date, there is no evidence-based clinical protocol for proper handling of provisional restorations traversing the soft tissues of an implant restoration.

MATERIALS AND METHODS

PEMA samples (Super-T Temporary Crown and Bridge material; Amco International, Conshohocken, PA) were all prepared by the same operator using vinyl polyvinyl siloxane (PVS) duplicating material (PolyPour; GC America, Alsip, IL) to duplicate a model plate (Permanox Cell Culture Dish; Thermo Scientific, Waltham, MA). The model plate that was duplicated for all the samples had the following parameters: 10 mm in diameter and ~2 to 0.75 mm thick. Thickness varied among samples because of the rebound effect of the PVS material. This was not a concern because the thickness of the disc did not affect the cell behavior or the results, as the cells were added directly to the surface.

A total of 84 experimental PEMA discs were prepared and were mechanically finished to smooth out any irregularities using laboratory carbides (H251.11.060 HP TC cutter carbide; Brasseler, Savannah, GA) and diamond burs (368.11.023 HP medium football diamond; Brasseler, Savannah, GA). The control discs were not treated any further. The remaining discs were divided into 6 additional groups based on the surface treatment as follows: group 1—control (C), group 2—Pumice (Pum), group 3—Varnish (Palaseal; Heraeus Kulzer, South Bend, IN), (Var), group 4—High polishing (Acristre; Buffalo Dental, Syosset, NY), (HPol), group 5—Chlorhexidine gluconate 0.12% (CHX), group 6—Alcohol (Al), and group 7—Steam (St). (Table 1). All discs on groups 2 to 7 were initially polished mechanically starting with coarse, medium, and fine laboratory pumice (Henry Schein; Melville, NY) using a wet rag wheel (Muslin Wheel; Kerr, Orange, CA), before they were treated further mechanically or chemically. The discs were washed with water and mild soap (Moist Sure; Sultan York, PA) in between each polishing cycle. Hand polishing was used to best simulate clinical conditions as would occur in a dental office. Vinyl gloves (Kimberly-Clark, Irving, TX) were used throughout

Table 1. Surface Treatments Groups Description

Groups	Surface Treatments
1. No Treatment/Control (C)	Mechanical finishing with laboratory carbide and diamond burs.
2. Pumice (Pum)	Mechanical finishing with laboratory carbide and diamond burs. Polishing using coarse, medium, and fine pumice. Washed with water and mild soap between each polishing cycle.
3. Varnish (Var)	Mechanical finishing with laboratory carbide and diamond burs. Polishing using coarse, medium, and fine pumice. Washed with water and mild soap between each polishing cycle. Application of 1 layer of varnish followed by curing cycle.
4. High Polishing (HPol)	Mechanical finishing with laboratory carbide and diamond burs. Polishing using coarse, medium, and fine pumice. Washed with water and mild soap between each polishing cycle. High polishing application using a dry rag wheel.
5. Chlorhexidine gluconate 0.12% (CHX)	Mechanical finishing with laboratory carbide and diamond burs. Polishing using coarse, medium, and fine pumice. Washed with water and mild soap between each polishing cycle. Soaked in chlorhexidine for 10 min.
6. Alcohol (Al)	Mechanical finishing with laboratory carbide and diamond burs. Polishing using coarse, medium, and fine pumice. Washed with water and mild soap between each polishing cycle. Soaked in alcohol for 10 min (leaving some alcohol behind vs drying it).
7. Steam (St)	Mechanical finishing with laboratory carbide and diamond burs. Polishing using coarse, medium, and fine pumice. Washed with water and mild soap between each polishing cycle. Disinfected using steam.

all the treatment procedures, and discs were wrapped in sterile gauze when not in use.

The surface treatments were selected based on those that are most commonly used in the dental practice. To simulate clinical conditions, groups 5 (CHX) and 6 (Al) were soaked for 10 minutes and group 7 (St) was treated for 10 seconds. Immersion time was not a critical factor in this particular experiment as the aim was to evaluate cell adhesion or cell proliferation on different surfaces as opposed to the ability of the chemical treatment to disinfect the surface in the given period. According to the Centers for Disease Control and Prevention (CDC), immersion times vary depending on the level of disinfection that is required. In this particular study, clinical conditions were simulated for a 10-minute disinfection soaking.²⁰

The same operator performed all surface treatment procedures. Because

this was an *in vitro* study, the samples that were not chemically treated, which included groups 1 (C), 2 (Pum), 3 (Var), and 4 (HPol), had to be sterilized under ultraviolet (UV) light for 10 minutes before the cell attachment assay. This is due to the fact that the cells are isolated in media and do not have an immune system to ward off any potential contaminant that may be present on the surface of the discs. The limitation of this treatment will be discussed in further sections.

The sample size consisted of 3 discs per group for each experiment. A total of 4 experiments were performed: 2 attachment assays, 1 proliferation assay, and 1 chemical treatment comparison assay.

Primary human epidermal keratinocytes at low passage were used in all the experiments. One advantage of using human cells is easier correlation

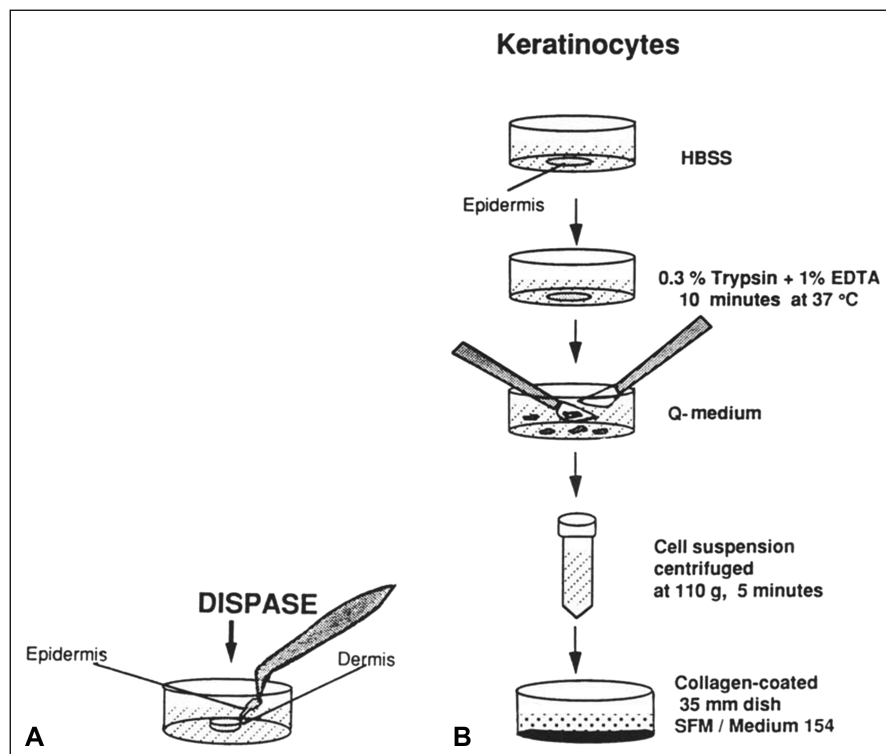


Fig. 1. Schematic diagram for the isolation of keratinocytes from human skin. **A**, Separation of the epidermal and dermal compartments. **B**, Isolation of keratinocytes. Adopted from Normand and Karasek,⁶⁷ 1995.

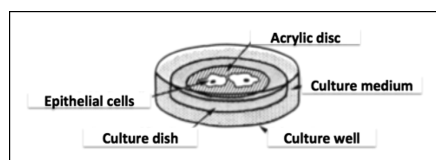


Fig. 2. Illustration of the attachment and proliferation experiments. Acrylic discs made of PEMA were placed in a culture dish and seeded with epithelial cells.

between the observations and clinical extrapolation. In particular, primary cell cultures more closely mimic the physiological state of cells *in vivo*. Epidermal keratinocytes (skin cells) are highly specialized epithelial cells designed to perform a very specific function, which is the separation and protection of the organism from its environment.²⁸ Although oral and skin keratinocytes have certain intrinsic differences, they share many histologic and functional characteristics.^{60,61} Ueda,⁵⁹ in 1995, was the first to describe the use of oral mucosal

keratinocytes for skin wound repair, therefore demonstrating the utility of epidermal keratinocytes as a model of cell adhesion with relevance to oral epithelial cells.

Techniques for isolation and propagation of keratinocytes from human skin have been established and are being routinely used in research.⁴⁹ The human primary epidermal keratinocyte isolation protocol was completed as follows: first, skin specimens were rinsed thoroughly in phosphate-buffered saline (PBS) (Gibco; Thermo Fisher Scientific, Waltham, MA) containing Pen/Strep (Gibco; Thermo Fisher Scientific, Waltham, MA), 0.1% betadine and 70% ethanol. After that, the tissue was transferred to a 100 mm petri dish with the epidermis side down. This allowed for access to remove adipose tissue from the sample. The skin was then transferred to another dish and sectioned into small fragments (1–3 mm in size). These fragments were then transferred to a tube containing 5 mL dispase (Gibco; Thermo Fisher

Scientific, Waltham, MA) and incubated overnight at 4°C. Dispase is a protease, which cleaves fibronectin and collagen, allowing for isolation of epithelia from the mesenchyme. The next day, the tissue was isolated from the dispase and the epidermis was separated from the remaining dermis with a sharp scalpel dissection. The isolated epidermis was placed into 5 mL of 0.25% trypsin (Gibco; Thermo Fisher Scientific, Waltham, MA) and incubated in a water bath for 30 minutes at 37°C. After this, 20 mL DMEM (Dulbecco's Modified Eagle Medium)/10% FBS (Fetal Bovine Serum) (Gibco; Thermo Fisher Scientific, Waltham, MA) was added to inactivate the trypsin. Dissociated cells were passed through sterile cell strainer and resuspended in CnT-07 keratinocyte growth media (CellnTech) and seeded onto P100 dish for mass propagation as described below (Fig. 1).

After the keratinocyte cells were isolated from the primary human foreskin, a process of subculturing (passaging) was performed to prolong their life and expand the number of cells in the culture. The human keratinocyte passage protocol was completed as follows: First, the cells were washed once using 3 mL versene solution (Gibco; Thermo Fisher Scientific, Waltham, MA). Versene was aspirated, and 3 mL 0.05% trypsin-EDTA (Ethylenediaminetetraacetic acid) (Gibco; Thermo Fisher Scientific, Waltham, MA) was then added and the mixture was incubated for 6 to 8 minutes at 37°C. Using a pipette, the cells were detached and 10 mL 10% FBS (Fetal Bovine Serum)/DMEM (Dulbecco's Modified Eagle Medium) was added and centrifuged at 1000 rpm for 7 minutes. The supernatant was aspirated and keratinocyte growth media were added. The cells were then resuspended and seeded.⁴⁹

Cellular adhesion is a necessary step for many cellular activities, such as differentiation, proliferation, and cell expression. Adhesion of the cell on a substrate will determine its fate. It will allow the cell to spread. The attachment assay in this study was primarily focused only on the number of cells that were attached to the substrate (Fig. 2).

The attachment assay (group 1A–group 7A) was the same as the

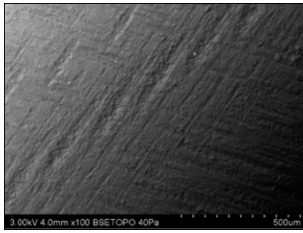


Fig. 3. Group 1 (No treatment/control group, rough surface)—PEMA disc ground with coarse carbide laboratory burs followed by fine diamond laboratory burs at $\times 100$ magnification. Evident rough surface before any polishing treatment.

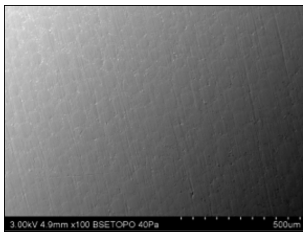


Fig. 4. Group 2 (Pumice and Water)—PEMA disc at $\times 100$ magnification after polishing with coarse, medium, and fine pumice. Polished effect evident as compared with group 1.

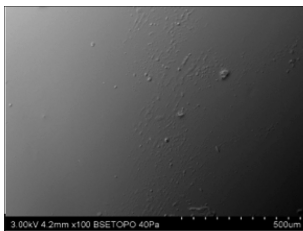


Fig. 5. Group 3 (Varnish)—PEMA disc at $\times 100$ magnification after applying 1 layer of light cure varnish. Spotty globular appearance with surface irregularities.

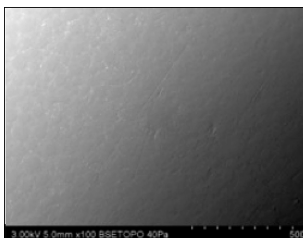


Fig. 6. Group 4 (High polishing)—PEMA disc at $\times 100$ magnification after high polishing with a compound applied on dry cloth buffs. This group appears to display the highest degree of homogeneity in surface texture, shallow grooves, and no apparent irregularities.

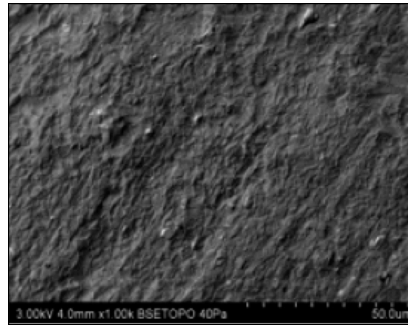


Fig. 7. Group 1 (No treatment/control group, rough surface)—PEMA disc at $\times 1000$ magnification, same disc as Figure 3. At increased magnification, the irregular rough surface is apparent when discs are finished with laboratory burs, without polishing.

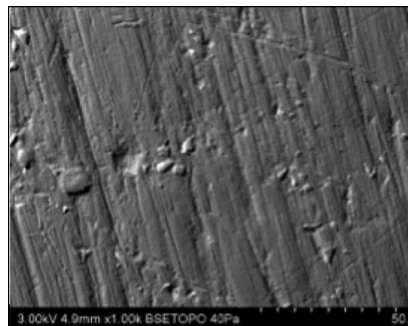


Fig. 8. Group 2 (Pumice and Water)—PEMA disc at $\times 1000$ magnification, same disc as Figure 4. Some homogeneity is accomplished due to polishing with coarse, medium, and fine pumice on wet rag wheel. However, the surface still exhibits evident grooves and irregularities of mechanically abraded material.

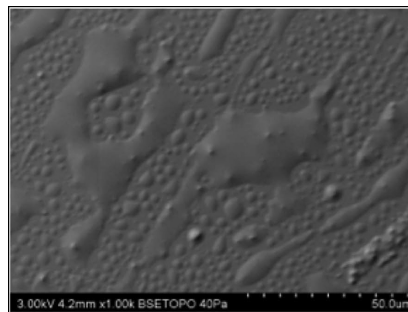


Fig. 9. Group 3 (Varnish)—PEMA disc at $\times 1000$ magnification, same disc as Figure 5. After application of 1 layer of light cure varnish, the surface displays a globular appearance with extensive irregularity. High magnification illustrates areas that may have previously appeared homogeneous, actually consisting of small bubbles on a microscopic level.

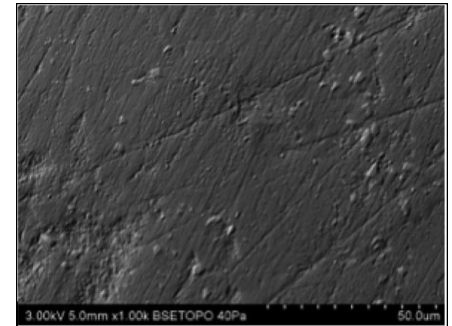


Fig. 10. Group 4 (High polishing)—PEMA disc at $\times 1000$ magnification, same disc as Figure 6, where high polishing was accomplished using a compound applied on dry cloth buffs. Improved homogeneity of surface texture is evident.

passaging assay and was completed as follows: First, the cells were washed with 3 mL versene solution (Gibco; Thermo Fisher Scientific, Waltham, MA). Then 3 mL of 0.05% trypsin-EDTA (Gibco; Thermo Fisher Scientific, Waltham, MA) was added and the mixture was incubated for 6–8 minutes at 37°C. The cells were then detached and 10 mL of 10% FBS (Fetal Bovine Serum)/DMEM (Dulbecco's Modified Eagle Medium) was added and centrifuged at 1000 rpm for 7 minutes. The supernatant was aspirated and keratinocyte growth media were added. The cells were plated at 500,000 per disc and allowed to attach for 30 minutes. After 30 minutes, trypsin was added and after 5 minutes, 10%

Table 2. First Attachment Experiment Results—Rhodamine Stain Illustrating the Degree of Epithelial Cell Adhesion to Each Experimental PEMA disc

No Treatment (rough)	Cell adhesion present
Pumice	Cell adhesion present
Varnish	No cell adhesion
High Polish	Cell adhesion present
Chlorhexidine	No cell adhesion
Alcohol	Cell adhesion present
Steam	Cell adhesion present

Highly pigmented "islands" of cells signify adhesion, as evidenced in the following groups: Pumice, High Polish, and Steam.

Table 3. Second Attachment Experiment Cell Count Results

	Group 1A (No Tx)	Group 2A (Pumice)	Group 3A (Varnish)	Group 4A (High Pol)	Group 5A (CHX)	Group 6A (Alcohol)	Group 7A (Steam)
Disc							
1	42,500	43,000	20,500	46,250	11,000	41,750	25,500
2	35,750	37,250	23,750	42,500	7000	44,500	25,500
3	48,250	45,500	22,500	45,250	18,000	45,500	24,750
Average	42,166.67	41,916.67	22,250	44,666.67	12,000	43,916.67	25,250
SD	6256.66	4230.35	1639.36	1941.86	5567.76	1941.86	433.01
P		0.9903	0.0014†	0.6918	$8.8 \times 10^{-07}‡$	0.7699	0.0068

Five hundred thousand cells were seeded and counted after 30 minutes. When compared with the control group, statistically significant differences with a *P* value of <0.01 were found only in groups 3A (Var), 5A (CHX). These groups had a significantly lower number of attached cells.

P value—all groups compared with group 1A.

*Significant at *P* < 0.05.

†Significant at *P* < 0.01.

‡Significant at *P* < 0.001.

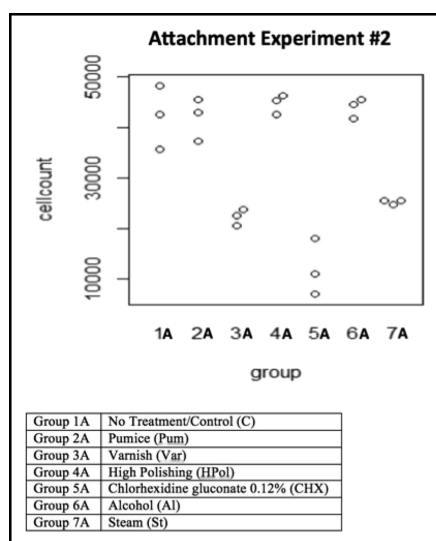


Fig. 11. Attachment experiment #2 results. Five hundred thousand keratinocyte cells were seeded on experimental discs and counted after 30 minutes. All groups showed initial attachment to the substrate. The number of attached cells varied among groups. Groups 3A (Var) and 5A (CHX) showed the least amount of attached cells. Groups 1A (C), 2A (Pum), 4A (Hpol), and 6A (Al) showed the most initial cell attachment.

donor bovine serum DMEM (Dulbecco's Modified Eagle Medium) (Gibco, Thermo Fisher Scientific, Waltham, MA) was added to neutralize the trypsin, then everything was collected to spin at 1200 rpm for 5 minutes. Supernatant was then aspirated and then 100 μ L media were added to resuspend the cell pellet. Then 10 μ L cell suspension was transferred to an Eppendorf tube, and 10 μ L trypan blue was added to the mix well. Then 10 μ L was pipetted to

the Hausser phase contrast hemocytometer (Hausser Scientific Partnership 3200, Thermo Fisher Scientific, Waltham, MA) to count the cells. The cells were counted in suspension under a microscope (Axiovert 40 CFL; Zeiss, Peabody, MA) and then the cell density was calculated.

The proliferation assay (group 1P–group 7P) was the same as the attachment assay except 20,000 cells were seeded per disc and allowed to proliferate for 1 week before trypsinization and counting of the cells with the use of the hemocytometer.

The chemical treatment comparison assay (group 1C–group 7C) was conducted to determine whether seeding cells on top of a chemically treated surface with and without washing with phosphate buffered saline (PBS) would affect final outcomes. Also, this assay tested whether UV sterilization would have any significant effect on cell proliferation. Groups 5 (CHX), 6 (Al), and 7 (St) were tested with the following parameters: group 1C (Al), group 2C (Al + PBS), group 3C (CHX), group 4C (CHX + PBS), group 5C (Al + PBS + UV), group 6C (CHX + PBS + UV), and group 7C (St). Groups 1C, 2C, 3C, and 4C were treated with the appropriate chemical agent and then cells were either immediately seeded on top of treated discs or washed with PBS and subsequently seeded. In groups 5C and 6C, the discs were also treated with UV light before seeding of cells.

An overall 0.05 level of type I error was set for the statistical analysis. A linear regression model was

used where the outcome is the log transferred cell counts, and the predictor is the grouping variable indicating which group it is. Statistical analysis consisted of comparing average log cell counts between groups. All measurements were made by an independent experimenter blinded to the groups and surface treatments.

RESULTS

Twenty-one discs were tested for each experiment. A total of 4 experiments were performed: 2 attachment assays, 1 proliferation assay, and 1 chemical treatment comparison assay.

First, scanning electron microscope (SEM) analysis at $\times 100$ and at $\times 1000$ magnification was performed on all samples. SEM analysis of group 1—(C) at $\times 100$ magnification revealed a rough surface, where the laboratory carbides and diamond burs were used. Bur marks are evident on the disc sample (Fig. 3). Group 2 (Pum) reveals a smoother surface than the control group sample; however, scratch marks are still visible from the carbide and diamond burs as well as from the pumice polish (Fig. 4). Group 3 (Var) reveals a smooth but patchy surface at $\times 100$ magnification. Large globules of cured material are evident and interspersed throughout the sample, providing a nonhomogeneous composition (Fig. 5). Group 4 (HPol) reveals a smoother surface than the pumice group, indicating that the rouge material provides for a smoother finish of the restorative material. This

Table 4. Proliferation Experiment Cell Count Results

	Group 1P (No Tx)	Group 2P (Pumice)	Group 3P (Varnish)	Group 4P (High Pol)	Group 5P (CHX)	Group 6P (Alcohol)	Group 7P (Steam)
Disc							
1	6500	66,000	500	62,100	3000	55,000	28,800
2	4400	51,300	370	57,500	3600	60,000	23,700
3	3600	48,900	420	45,000	3300	58,000	21,300
Average	4833.33	55,400	430	54,866.67	3300	57,666.67	24,600
SD	1497.78	9257.97	65.57	8848.92	300	2516.61	3830.14
P		$5.2 \times 10^{-11}\ddagger$	$7.5 \times 10^{-11}\ddagger$	$5.5 \times 10^{-11}\ddagger$	0.023*	$4.0 \times 10^{-11}\ddagger$	$1.0 \times 10^{-08}\ddagger$

Twenty thousand cells were seeded and counted after 1 week. When compared with the control group, statistically significant differences were found with group 3P (Varnish) and group 5P (CHX) because these groups had a significant reduction of cells over a 1-week period. In addition, statistically significant differences were found with group 2P (Pumice), group 4P (High Polish), group 6P (Alcohol), and group 7P (Steam) because these groups had a significant increase in proliferation of cells.

P value—all groups compared with group 1P.

*Significant at $P < 0.05$.

†Significant at $P < 0.01$.

‡Significant at $P < 0.001$.

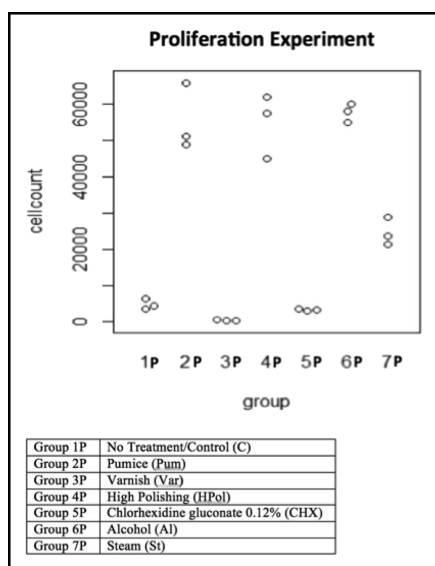


Fig. 12. Proliferation experiment results. Twenty thousand keratinocyte cells were seeded on experimental discs and counted after 1 week. Groups 1P (C), 3P (Var), and 5P (CHX) experienced a reduction from the original 20,000 number of seeded cells with the lowest cell count in group 3P (Var) of 370 cells; whereas groups 2P (Pum), 4P (HPol), 6P (Al), and 7P (St) experienced an increase and proliferation of cells from the original 20,000 number of seeded cells with the highest count of up to 66,000 in group 2P (Pum). The trends were similar to the results obtained from the attachment experiment.

surface appears to be the smoothest of all the treatment groups (Fig. 6). At $\times 1000$ magnification, new information is obtained. Group 1 (C) appears to be extremely rough (Fig. 7). Group 2 (Pum) appears to be rough with vertical lines spanning the sample

indicating bur marks as well as marks from the pumice wheel (Fig. 8). Group 3 (Var) revealed interesting findings as the globular patterns are very evident and the surface is not smooth (Fig. 9). Group 4 (HPol) even at $\times 1000$ magnification appears to have the smoothest appearance from all the other samples. Lines are evident and the surface is not perfectly smooth; however, it displays most homogeneity from all the other samples (Fig. 10).

Two attachment assays were conducted. Rhodamine staining was used during the first attachment assay to visualize the general trends in cell adhesion for each group tested.⁵⁰ The results of this assay are shown in Table 2, where the various surface treatments studied had a significant effect on epithelial cell adhesion to acrylic discs. The high concentration of rhodamine dye as an “island” signifies colonies of cells forming and attaching to the substrate. Discs without staining or with poor concentration of dye signify that the cells did not attach to the substrate. Highest degree of adhesion was found in groups 2 (Pum), 4 (HPol), and 7 (St). Poor cell adhesion was found in groups 1 (C), 3 (Var), 5 (CHX), and 6 (Al). The second attachment assay was conducted to quantify the exact number of cells that adhered to each disc. Five hundred thousand keratinocyte cells were plated for the second attachment assay and after 30 minutes the cells were trypsinized and counted. All groups tested showed initial attachment to the substrate. The number of attached cells varied among groups. Groups 3A

(Var) and 5A (CHX) showed the least amount of attached cells. Groups 1A (C), 2A (Pum), 4A (HPol), and 6A (Al) show the most initial cell attachment (Table 3 and Fig. 11).

The proliferation assay was conducted to observe cell attachment and growth over a longer period. In the proliferation assay, 20,000 cells were seeded and counted after 1 week. Groups 1P (C), 3P (Var), and 5P (CHX) experienced a reduction from the original 20,000 number of seeded cells with the lowest cell count in group 3P (Var) of 370 cells; whereas groups 2P (Pum), 4P (HPol), 6P (Al), and 7P (St) experienced an increase and proliferation of cells from the original 20,000 number of seeded cells with the highest count of up to 66,000 in group 2P (Pum). The trends were similar to the results obtained from the attachment experiment.

As stated above, in the attachment assay, epithelial cells attached to all surface treatments with various affinities. However, in the proliferation assay, where 20,000 cells were seeded, various groups showed significant continued cell growth. The Pumice group had an average of 55,400 cells; the High Polish group had an average of 54,867 cells; the Alcohol group had an average of 57,667 cells; and the Steam group had an average of 24,600 cells. This illustrates that these conditions were favorable to allow cells to continue to grow and replicate in a non-toxic environment; whereas the No treatment (rough) group, the Varnish group, and the Chlorhexidine group all showed a decline in cell activity and growth.

Table 5. Chemical Treatment Comparison Cell Count Results

	Group 1C (Alcohol)	Group 2C (Alc + PBS)	Group 3C (CHX)	Group 4C (CHX + PBS)	Group 5C (Alc + PBS + UV)	Group 6C (CHX + PBS + UV)	Group 7C (Steam)
Disc							
1	50,000	48,750	500	1250	44,750	250	83,750
2	52,500	46,250	250	1500	37,250	250	80,000
3	48,750	41,000	250	3500	41,500	250	57,750
Average	50,416.67	45,333.33	333.33	2083.33	41,166.67	250	73,833.33
SD	1909.41	3955.48	144.34	1233.22	3761.09	0	14,054.21
P	0.12	0.05	$7.0 \times 10^{-13}\ddagger$	$1.5 \times 10^{-10}\ddagger$	0.022*	$4.0 \times 10^{-13}\ddagger$	

Twenty thousand cells were plated and counted after 1 week. Groups treated with chlorhexidine had statistically significantly lower cell proliferation as compared with the group treated with steam. P value—all groups compared with group 7C.

*Significant at $P < 0.05$.

†Significant at $P < 0.01$.

‡Significant at $P < 0.001$.

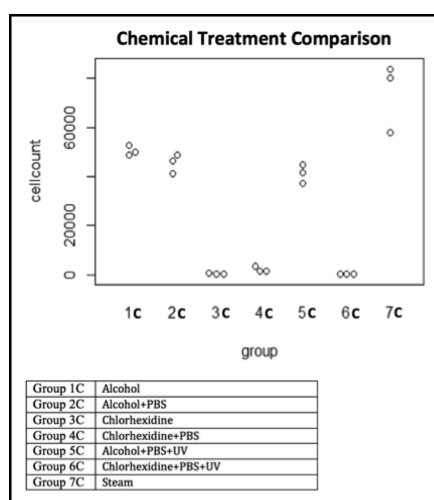


Fig. 13. Chemical treatment experiment results. Twenty thousand cells were seeded on each disc and counted after 1 week. Keratinocytes in the Chlorhexidine groups, whether seeded after washing off the CHX with PBS or using UV light before seeding, exhibit cell decline from the original 20,000 cells plated. Keratinocytes in the Alcohol groups did not show a significant difference between each other. Group 7C (St) illustrates the highest cell count with an average of 73,833 cells.

Descriptive statistics were completed using linear regression models. To compare the groups, the outcome variable cell count was first log transformed. In the attachment and proliferation experiments, group 1A (C) and group 1P (C) are the reference groups and the P value is set to <0.05 , <0.01 , and <0.001 . In the chemical comparison experiment, group 7C (St) is the reference group. All other groups are compared with the reference group.

For the second attachment experiment, when compared with the control

group, statistically significant differences with a P value of <0.01 were found only in groups 3A (Var), 5A (CHX). It is important to note that group 1A (C) exhibited high initial cell attachment. Therefore, groups 2A (Pum), 4A (HPol), 6A (Al), and 7A (St) did not demonstrate a significant difference as compared with group 1 because these groups also showed a high initial cell attachment. Group 3A (Var) and group 5A (CHX) had a significantly lower number of attached cells (Table 3 and Fig. 11).

For the proliferation experiment, when compared with the control group (rough surface), statistically significant differences were found with group 3P (Var) and group 5P (CHX) because these groups had a significant reduction of cells over a 1-week period. In addition, statistically significant differences were found with group 2P (Pumice), group 4P (High Polish), group 6P (Alcohol), and group 7P (Steam) because these groups had a significant increase in proliferation of cells. (Table 4 and Fig. 12). These preliminary *in vitro* results demonstrate that surface treatment modification to the PEMA material, whether mechanical or chemical, has a statistically significant effect on epithelial cell proliferation on the substrate.

A third proliferation assay was performed comparing the chemical treatments, the effect of washing off the chemical before seeding the cells, and the effect of UV light. Twenty thousand cells were seeded on each disc and counted after 1 week. Groups 3C (CHX), 4C (CHX + PBS), and 6C

(CHX + PBS + UV) with average cell counts of 333 cells, 2083 cells, and 250 cells, respectively, illustrate the lowest cell count of all chemically treated groups and this was found to be statistically significant with a P value of <0.001 when compared with group 7C (St). Epithelial cells in the Chlorhexidine group, whether seeded after washing off the CHX with PBS or using UV light before seeding, exhibit cell decline from the original 20,000 cells plated. Group 7C (St) illustrates the highest cell count with an average of 73,833 cells. This is a significant growth of cells from the original 20,000 cells that were plated. Groups 1C (Al), 2C (Al + PBS), and 5C (Al + PBS + UV) with average cell counts of 50,417 cells, 45,333 cells, and 41,167 cells, respectively, did not show a significant difference between each other. In addition, chemical treatment with alcohol allowed for cells to continue to proliferate on the disc whether the disc had residual alcohol or whether it was thoroughly washed off. This comparison illustrates that UV light had no effect on epithelial cell affinity for the substrate. (Table 5 and Fig. 13).

DISCUSSION

For a long time, osseointegration was identified as the main factor that determines the success of dental implants.² However, it is now well known that the soft tissues, and especially the epithelial biological seal adjacent to the implant, are just as crucial for the long-term success of dental implants.^{3,5-7,10,16,44-46} Proper dimension and quality of the soft tissue

seal around dental implants is considered to be a prerequisite for achieving long-term stable peri-implant conditions.⁵ The biocompatibility as well as proper handling of the material used in the transmucosal part of the implant is therefore a factor of importance for treatment success. In this *in vitro* study, epithelial cell adhesion to PEMA discs was investigated. The discs were treated with different mechanical and chemical surface treatments. Electron microscopy, rhodamine staining, attachment assays, and proliferation assays were used. A statistically significant difference in cell adhesion and in cell proliferation was found between the groups tested.

To better understand the surface topography of a PEMA material after it has been treated with various mechanical surface treatments, electron microscopy was conducted on the samples. A formal investigation on microtopography of these surfaces was not part of the experiment, but the SEM observations demonstrated subjectively a clear difference between each surface in terms of topography. The $\times 1000$ magnification in particular revealed significant differences between the groups. Although the group treated with varnish looks smooth without any magnification, it presented a globular and rough surface under magnification. This is an important finding because as discussed above and as the results of this study show, the epithelial cells prefer a smooth surface for adhesion.¹⁵ However, the high polishing sample under $\times 1000$ magnification revealed that the surface was the smoothest of all the samples tested. Cell affinity for attachment to these plates was the highest and this was illustrated with the hemocytometer cell count that was obtained where this group had one of the highest cell adhesion counts of all the samples tested. Hormia et al studied gingival epithelial cells growth on cell culture glass and on titanium plates with various surface topographies. The results showed that epithelial cells attached and spread more readily on smooth than on rough, sandblasted titanium surfaces.²² This indicates that a smooth surface would be optimal for the

perimucosal penetrating parts of dental implants.

Rhodamine staining provided visual evidence for cell adhesion to PEMA discs. The findings from this assay corroborated with the findings from the SEM analysis and adhesion studies. The highest adhesion was found to be in the smooth surface treatment groups such as Pumice, High polishing, and Steam, and the poorest adhesion was found to be in the rough surface groups such as No treatment and Varnish. Furthermore, the groups that were chemically treated in addition to mechanical treatment showed statistically significant differences. The Chlorhexidine group showed significantly lower epithelial cell adhesion than other groups.

A literature search did not show any discussion of an evidence-based treatment protocol for cleaning or disinfecting implant provisional restorations or final abutments. Only one study actually followed a "sterilization" technique for the provisional restoration, using a chlorhexidine solution for chemical sterilization.³⁸ Another study, which introduced a periimplantitis treatment protocol, found that cleaning with hydrogen peroxide slightly enhances human epithelial cell growth on titanium surfaces, in contrast to chlorhexidine gel.⁵¹

It has been well documented that chlorhexidine is toxic to various human cell types including skin epithelial cells.^{52,53} The antibacterial activity of chlorhexidine results from its positive charge at physiological pH, which accounts for its nonspecific bindings to the negatively charged sites on the surfaces of bacterial cells.⁵⁴ Similar electrostatic interactions may account for the noxious effects of chlorhexidine on mammalian cells, probably by disruption of the eukaryotic plasma membrane. This also prevents the cells from recovering after the insult if they are subsequently placed in a chlorhexidine-free medium.⁵² According to Mariotti and Rumpf,⁵⁵ chlorhexidine gluconate induces a dose-dependent reduction in cellular proliferation and reduces both collagen and noncollagen protein production of human gingival fibroblasts

in vitro. Thus, the introduction of commonly available 0.12% chlorhexidine gluconate to surgical sites for short periods before wound closure can have serious toxic effects on gingival fibroblasts and may negatively affect wound healing.⁵⁵ The results obtained in this study are in agreement with these previous studies as the cells that were seeded onto discs disinfected with chlorhexidine showed very poor cell growth.

Isopropyl alcohol is a chemical disinfectant and is an irritant to skin. Disinfectants are not used for skin antisepsis because they can injure skin and other tissues.⁵⁶ However, in this study, alcohol showed adequate cell adhesion to the discs. The most likely explanation for this result is the rapid evaporation of the alcohol, allowing for the cells to attach. Therefore, if alcohol is used for disinfection, it is recommended to allow the provisional restoration to dry before reinserting it into the mouth.

The attachment assay aimed at providing quantitative data on the affinity of epithelial cells to various tested surfaces. Surface topography as well as the chemical treatments have been shown to influence cell adhesion and behavior. It should be noted that different cell types attach with different affinities when exposed to various surface treatments. For example, it has been shown that osteoblasts have a great affinity for rough surfaces.² Fibroblasts behave similarly to osteoblasts as they have a higher affinity toward rough surfaces. As for epithelial cells, published literature has proven to be contradictory; however, more studies show a higher affinity of epithelial cells to smooth surfaces rather than rough surfaces.^{9,22,27,29} In addition, a rough surface, as demonstrated by Waerhaug⁴⁸ in 1956, facilitates the retention of bacterial plaque and tissue inflammation. Therefore, a surface that is highly polished is preferred especially when traversing the peri-implant cuff.

Na An et al investigated the influence of surface hydrophilicity of titanium implant surfaces on the behavior and differentiation of epithelial cells. Their results suggest that surface hydrophilicity might positively influence the

epithelial seal around dental implants. Proliferation, spreading, and moving of epithelial cells were enhanced on hydrophilic Ti surfaces compared with hydrophobic Ti surfaces. In addition, the relatively smoother surfaces were preferred.²⁷ In this study, epithelial cells showed initial attachment to all the surface treatments tested. Variations in affinity to the different surfaces are evident as some surface treatments had higher attachment than others. In particular, the Varnish group proved to have the poorest cell adhesion from all the groups studied. Some studies show that visible light-polymerized denture base resin systems affect various cell metabolic processes with varying degrees of cytotoxicity.^{57,58} The dual polymerization resins seem to be less toxic than those polymerized only by light. Future studies have to be performed on the cytotoxicity of the varnish used around dental implant provisionals and their effect on epithelial cells. In this particular study, we were not able to determine whether the cells did not adhere to the varnish surface due to extreme roughness or whether additional cytotoxic properties of the material prevented cell affinity and adhesion. According to the results obtained in this study, it is recommended that when using a provisional varnish, especially after staining the PEMA, that the varnish is not added to the subgingival aspect of the abutment to obtain a more stable epithelial cell behavior.

In all the experiments that were conducted for this study, group 7 (St) consistently had high epithelial cell attachment and proliferation. Dry steam vapor deodorizes and disinfects using heat and moisture. This treatment does not contain any chemical agent that may interfere with cell affinity toward the substrate. In addition, all discs in this group were polished using coarse, medium, and fine pumice, which allowed for a moderately smooth surface for cell adhesion. Because this method serves to disinfect the provisional without adding unnecessary and potentially harmful chemicals, it is recommended to steam the implant provisional before insertion in the patient's mouth to improve cell adhesion and therefore

the biological connection around the peri-implant cuff. No adverse effects were observed when steam was applied to the acrylic discs.

Cell attachment can be further studied on a microscopic level examining the presence of a hemidesmosomal attachment. Studies of the peri-implant epithelium-implant abutment interface are scarce and controversial; however the presence of a hemidesmosomal attachment has been previously shown.¹⁹ No studies have been conducted on PEMA and epithelial adhesion until now. In this particular study, we were able to illustrate cell adhesion. Further studies would be needed to evaluate a true hemidesmosomal attachment.

This experiment presents a number of limitations. First, it is an *in vitro* study design and results cannot be readily extrapolated to the oral environment where the immune system, saliva, other cell types, and many other biological processes can have an effect on cell adhesion. However, an *in vitro* experiment may give a first impression of probable reactions that might also occur in the clinical situation and they allow studies under defined and more controlled conditions.

Second, the mechanically treated groups such as No treatment, Pumice, Varnish, and High polishing had to be treated with UV light before seeding the cells. This ensured that the cells would survive because they do not have the protection of the immune system to ward off a possible antigen that is present on the surface of a possibly contaminated disc. This means that not all samples were treated equally; however, all discs were disinfected either by UV light or by chemical disinfection.

In addition, primary human keratinocyte cells were used for the study. Although this cell line is similar to oral epithelial cells, we are not able to draw direct extrapolation to oral cells. Another limitation is that we did not use any titanium surfaces, which the literature shows to be the best surface material for cell adhesion. Future studies should be performed using the most effective surface treatments for PEMA and comparing them with a smooth hydrophilic titanium surface or other final abutment materials.

CONCLUSION

Although the provisional restoration usually serves a temporary function and will be removed for the fabrication of the final prosthesis, it is important to establish a strong biological seal at this stage of the restorative phase around the implant-abutment interface. If provisional restorations are handled with proper care, they may facilitate and even enhance the quality of soft tissue. However, due to improper handling, the soft tissues may be affected, often times irreversibly. This study aimed to establish a protocol that is most appropriate for a predictable long-term soft tissue outcome.

Within the limitations of this study, we recommend the following clinical protocol for finishing, polishing, and disinfecting implant provisionals: coarse, medium, fine pumice → high polishing (if desired) → steam. If steam is not available, alcohol may be used to disinfect the provisional restoration before insertion. It is recommended to avoid applying varnish in the perimucosal areas near the epithelium. Also it may be contraindicated based on the finding from this study to soak restorations in chlorhexidine before placing them intraorally.

DISCLOSURE

The authors claim to have no financial interest, either directly or indirectly, in the products or information listed in the article.

This study was completed with the help of an ICOI grant from IDREF.

REFERENCES

1. Albrektsson T, Brånemark PI, Hansson HA, et al. Osseointegrated titanium implants. Requirements for ensuring a long-lasting, direct bone-to-implant anchorage in man. *Acta Orthop Scand*. 1981;52:155-170.
2. Abrahamsson I, Zitzmann NU, Berglundh T, et al. The mucosal attachment to titanium implants with different surface characteristics: An experimental study in dogs. *J Clin Periodontol*. 2002;29:448-455.
3. Berglundh T, Lindhe J, Ericsson I, et al. The soft tissue barrier at implants and teeth. *Clin Oral Implants Res*. 1991; 2:81-90.

4. Abrahamsson I, Berglundh T, Lindhe J. The mucosal barrier following abutment dis/reconnection. An experimental study in dogs. *J Clin Periodontol.* 1997;24:568–572.
5. Abrahamsson I, Berglundh T, Glantz PO, et al. The mucosal attachment at different abutments. An experimental study in dogs. *J Clin Periodontol.* 1998;25:721–727.
6. Abrahamsson I, Zitzmann NU, Berglundh T, et al. Bone and soft tissue integration to titanium implants with different surface topography: An experimental study in the dog. *Int J Oral Maxillofac Implants.* 2001;16:323–332.
7. Arvidson K, Fartash B, Hilliges M, et al. Histological characteristics of peri-implant mucosa around Brånemark and single-crystal sapphire implants. *Clin Oral Implants Res.* 1996;7:1–10.
8. Baharloo B, Textor M, Brunette DM. Substratum roughness alters the growth, area, and focal adhesions of epithelial cells, and their proximity to titanium surfaces. *J Biomed Mater Res A.* 2005;74:12–22.
9. Bauman GR, Rapley JW, Hallmon WW, et al. The peri-implant sulcus. *Int J Oral Maxillofac Implants.* 1993;8:273–280.
10. Berglundh T, Lindhe J, Ericsson I, et al. The soft tissue barrier at implants and teeth. *Clin Oral Implants Res.* 1991;2:81–90.
11. Cairo F, Pagliaro U, Nieri M. Soft tissue management at implant sites. *J Clin Periodontol.* 2008;35:163–167.
12. Canullo L, Pellegrini G, Allievi C, et al. Soft tissues around long-term platform switching implant restorations: A histological human evaluation. Preliminary results. *J Clin Periodontol.* 2011;38:86–94.
13. Chai WL, Moharamzadeh K, Brook IM, et al. A review of histomorphometric analysis techniques for assessing implant-soft tissue interface. *Biotech Histochem.* 2011;86:242–254.
14. Chee WW. Provisional restorations in soft tissue management around dental implants. *Periodontol 2000.* 2001;27:139–147.
15. Chehroudi B, Gould TR, Brunette DM. Effects of a grooved titanium-coated implant surface on epithelial cell behavior in vitro and in vivo. *J Biomed Mater Res.* 1989;23:1067–1085.
16. Chu SJ, Salama MA, Salama H, et al. The dual-zone therapeutic concept of managing immediate implant placement and provisional restoration in anterior extraction sockets. *Compend Contin Educ Dent.* 2012;33:524–532, 534.
17. Donley TG, Gillette WB. Titanium endosseous implant-soft tissue interface: A literature review. *J Periodontol.* 1991;62:153–160.
18. Fujii N, Kusakari H, Maeda T. A histological study on tissue responses to titanium implantation in rat maxilla: The process of epithelial regeneration and bone reaction. *J Periodontol.* 1998;69:485–495.
19. Gould TR, Westbury L, Brunette DM. Ultrastructural study of the attachment of human gingiva to titanium in vivo. *J Prosthet Dent.* 1984;52:418–420.
20. Grusovin MG, Coulthard P, Worthington HV, et al. Maintaining and recovering soft tissue health around dental implants: A Cochrane systematic review of randomised controlled clinical trials. *Eur J Oral Implantol.* 2008;1:11–22.
21. Hansson HA, Albrektsson T, Brånemark PI. Structural aspects of the interface between tissue and titanium implants. *J Prosthet Dent.* 1983;50:108–113.
22. Hormia M, Könönen M, Kivelahti J, et al. Immunolocalization of proteins specific for adherens junctions in human gingival epithelial cells grown on differently processed titanium surfaces. *J Periodontol Res.* 1991;26:491–497.
23. Iglhaut G, Becker K, Golubovic V, et al. The impact of dis-/reconnection of laser microgrooved and machined implant abutments on soft- and hard-tissue healing. *Clin Oral Implants Res.* 2013;24:391–397.
24. Mankoo T. Single-tooth implant restorations in the esthetic zone—contemporary concepts for optimization and maintenance of soft tissue esthetics in the replacement of failing teeth in compromised sites. *Eur J Esthet Dent.* 2007;2:274–295.
25. McKinney RV Jr, Stefflik DE, Koth DL. Evidence for a junctional epithelial attachment to ceramic dental implants. A transmission electron microscopic study. *J Periodontol.* 1985;56:579–591.
26. Moon IS, Berglundh T, Abrahamsson I, et al. The barrier between the keratinized mucosa and the dental implant. An experimental study in the dog. *J Clin Periodontol.* 1999;26:658–663.
27. An N, Rausch-fan X, Wieland M, et al. Initial attachment, subsequent cell proliferation/viability and gene expression of epithelial cells related to attachment and wound healing in response to different titanium surfaces. *Dent Mater.* 2012;28:1207–1214.
28. Piattelli A, Scarano A, Piattelli M, et al. Histologic aspects of the bone and soft tissues surrounding three titanium non-submerged plasma-sprayed implants retrieved at autopsy: A case report. *J Periodontol.* 1997;68:694–700.
29. Räisänen L, Könönen M, Juhanoja J, et al. Expression of cell adhesion complexes in epithelial cells seeded on biomaterial surfaces. *J Biomed Mater Res.* 2000;49:79–87.
30. Ruggeri A, Franchi M, Marini N, et al. Supracrestal circular collagen fiber network around osseointegrated nonsubmerged titanium implants. *Clin Oral Implants Res.* 1992;3:169–175.
31. Sitbon YAD. *Epithelial Cells Attachment on Five Different Dental Implant Abutment Surface Candidates [MS thesis].* University of Iowa; 2009.
32. Weber HP, Buser D, Donath K, et al. Comparison of healed tissues adjacent to submerged and non-submerged unloaded titanium dental implants. A histometric study in beagle dogs. *Clin Oral Implants Res.* 1996;7:11–19.
33. Hermann JS, Buser D, Schenk R, et al. Biologic width around titanium implants. A physiologically formed and stable dimension over time. *Clin Oral Implants Res.* 2000;11:1–11.
34. Schroeder HE, Listgarten MA. The gingival tissues: The architecture of periodontal protection. *Periodontol 2000.* 1997;13:91–120.
35. Kobayashi K, Rose GG, Mahan C. Ultrastructure of the dento-epithelial junction. *J Periodontol Res.* 1976;11:313–330.
36. Listgarten MA. Electron microscopic study of the dentogingival junction of man. *Am J Anat.* 1966;19:147–178.
37. Schroeder HE. *The Periodontium. Handbook of Microscopic Anatomy.* Berlin, Germany: Springer-Verlag; 1986:233–323.
38. Hui E, Chow J, Li D, et al. Immediate provisional for single-tooth implant replacement with Brånemark system: Preliminary report. *Clin Implant Dent Relat Res.* 2001;3:79–86.
39. Frazer RQ, Byron RT, Osborne PB, et al. PMMA: An essential material in medicine and dentistry. *J Long Term Eff Med Implants.* 2005;15:629–639.
40. Sen D, Göller G, İşsever H. The effect of two polishing pastes on the surface roughness of bis-acryl composite and methacrylate-based resins. *J Prosthet Dent.* 2002;88:527–532.
41. Kuhar M, Funduk N. Effects of polishing techniques on the surface roughness of acrylic denture base resins. *J Prosthet Dent.* 2005;93:76–85.
42. Hernandez EP, Oshida Y, Platt JA, et al. Mechanical properties of four methylmethacrylate-based resins for provisional fixed restorations. *Biomed Mater Eng.* 2004;14:107–122.
43. Burns DR, Beck DA, Nelson SK. A review of selected dental literature on contemporary provisional fixed prosthodontic treatment: Report of the Committee on Research in Fixed Prosthodontics of the Academy of Fixed Prosthodontics. *J Prosthet Dent.* 2003;90:474–497.

44. Lewis S, Parel S, Faulkner R. Provisional implant-supported fixed restorations. *Int J Oral Maxillofac Implants*. 1995;10:319–325.
45. Moscovitch MS, Saba S. The use of a provisional restoration in implant dentistry: A clinical report. *Int J Oral Maxillofac Implants*. 1996;11:395–399.
46. Gratton DG, Aquilino SA. Interim restorations. *Dent Clin North Am*. 2004;48:487–497.
47. Waerhaug J, Zander HA. Reaction of gingival tissues to self-curing acrylic restorations. *J Am Dent Assoc*. 1957;54:760–768.
48. Waerhaug J. Effect of rough surfaces upon gingival tissue. *J Dent Res*. 1956;35:323–325.
49. Sen D, Göller G, İşsever H. The effect of two polishing pastes on the surface roughness of bis-acryl composite and methacrylate-based resins. *J Prosthet Dent*. 2002;88:527–532.
50. Kuhar M, Funduk N. Effects of polishing techniques on the surface roughness of acrylic denture base resins. *J Prosthet Dent*. 2005;93:76–85.
51. Hernandez EP, Oshida Y, Platt JA, et al. Mechanical properties of four methylmethacrylate-based resins for provisional fixed restorations. *Biomed Mater Eng*. 2004;14:107–122.
52. Hodosh M, Povar M, Shklar G. The dental polymer implant concept. *J Prosthet Dent*. 1969;22:371–380.
53. Shklar G, Hodosh M, Povar M. Tissue reactions to polymer-coated Vitalium pin implants. *J Prosthet Dent*. 1970;24:636–645.
54. Brånemark PI, Hansson BO, Adell R, et al. Osseointegrated implants in the treatment of the edentulous jaw. Experience from a 10-year period. *Scand J Plast Reconstr Surg Suppl*. 1977;16:1–132.
55. Jensen UB, Ghazizadeh S, Owens DM. Isolation and characterization of cutaneous epithelial stem cells. *Methods Mol Biol*. 2013;989:61–69.
56. Ungvári K, Pelsöczy IK, Kormos B, et al. Effects on titanium implant surfaces of chemical agents used for the treatment of peri-implantitis. *J Biomed Mater Res B Appl Biomater*. 2010;94:222–229.
57. Babich H, Wurzbürger BJ, Rubin YL, et al. An in vitro study on the cytotoxicity of chlorhexidine digluconate to human gingival cells. *Cell Biol Toxicol*. 1995;11:79–88.
58. Helgeland K, Heyden G, Rolla G. Effect of chlorhexidine on animal cells in vitro. *Scand J Dent Res*. 1971;79:209–215.
59. Hugo WB, Longworth AR. The effect of chlorhexidine on the electrophoretic mobility, cytoplasmic constituents, dehydrogenase activity and cell walls of *Escherichia coli* and *Staphylococcus aureus*. *J Pharm Pharmacol*. 1966;18:569–578.
60. Mariotti AJ, Rumpf DA. Chlorhexidine-Induced changes to human gingival fibroblast collagen and non-collagen protein production. *J Periodontol*. 1999;70:1443–1448.
61. Rutala W, Weber D, et al. *CDC Guideline for Disinfection and Sterilization in Healthcare Facilities*. 2008.
62. Lefebvre CA, Schuster GS. Biocompatibility of visible light-cured resin systems in prosthodontics. *J Prosthet Dent*. 1994;71:178–185.
63. Lefebvre CA, Knoernschild KL, Schuster GS. Cytotoxicity of eluates from light-polymerized denture base resins. *J Prosthet Dent*. 1994;72:644–650.
64. Ueda M. Formation of epithelial sheets by serially cultivated human mucosal cells and their applications as a graft material. *Nagoya J Med Sci*. 1995;58:13–28.
65. Drukala J, Zarzecka J, Gojniczek K, et al. Comparison of proliferation and motile activity between human keratinocytes isolated from skin and oral mucosa. *Folia Biol (krakow)*. 2005;53:21–28.
66. Turabelidze A, Guo S, Chung AY, et al. Intrinsic differences between oral and skin keratinocytes. *PLoS One*. 2014;9:e101480.
67. Normand J, Karasek MA. A method for the isolation and serial propagation of keratinocytes, endothelial cells, and fibroblasts from a single punch biopsy of human skin. *In Vitro Cell Dev Biol Anim*. 1995;31:447–455.