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https://www.usuhs.edu/pdc

THESIS APPROVAL PAGE FOR MASTER OF SCIENCE IN ORAL BIOLOGY

Title of Thesis: "The Inflammatory Potential of Bis-Acrylic Composite and Polymethyl Methacrylate with Varying topography and in the Presence of LPS"

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Master of Science Degree
June 13, 2018

THESIS/MANUSCRIPT APPROVED: DATE:

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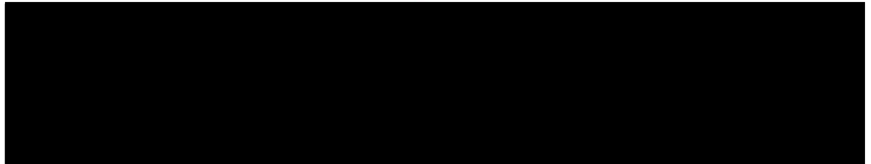

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The inflammatory potential of bis-acrylic composite and polymethyl methacrylate with varying topography and in the presence of lipopolysaccharide

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ACKNOWLEDGMENTS

This work was supported by funds from the US Army Medical Command and US Army Dental Activity. The authors thank Dr. Peter Liacouras at 3D Medical Applications Center, Bethesda, MD. for his support in creating the equipment for the disc fabrication. The authors thank Ms. Rosas Militza and Ms. Jessica Neder for help with cell culture and LTC Jeremy Goodin for the helpful discussions and critical reading of the manuscript. The authors thank COL Cynthia Aita-Holmes and COL (retired) Van Ramos for their ideas and support with this study.

ABSTRACT

Statement of problem. No studies to date have reported the potential of surface roughness of bis-acrylic composite (BAC) and polymethyl methacrylate (PMMA), with or without lipopolysaccharide (LPS) surface contamination, to elicit enhanced inflammatory activity.

Purpose. This study sought to determine if there is a difference in inflammatory potential between PMMA and BAC, and to examine any interactive effects between surface roughness of the provisional dental materials and bound LPS on induction of an inflammatory response.

Material and Methods. Fifty-two discs of BAC and PMMA were fabricated. Twelve discs of each material were used to assess intrinsic inflammatory activity. HEK-blue hTLR4 cells, Null2 cells (devoid of TLR4 receptor) or THP1-Xblue cells (human monocyte cell line), all carrying the NF-κB-induced secreted embryonic alkaline phosphatase (SEAP) reporter gene, were plated on these discs. Induced SEAP activity was assessed as substrate hydrolysis measured by optical density at 650 nm (OD_{650}). To investigate the effects of surface roughness, discs of each material were roughened with coarse, medium, fine or extra fine polishing discs (thirty-two material discs total). The 8 remaining discs of each material were used as control. Roughness was measured, as a Ra value (μm) using a prolifometer. Discs were coated with purified *E. coli* LPS, then HEK TLR4 cells were plated and incubated, followed by SEAP detection.

Results. PMMA induced a significantly greater NF-κB activity than BAC. For both LPS-treated and untreated surfaces, PMMA showed a significantly higher NFκB activity than the corresponding BAC surfaces. For BAC, there was a moderate correlation between NF-κB activation and Ra value, while PMMA showed little correlation of NF-κB activation with Ra value.

Conclusions. PMMA was estimated to be more pro-inflammatory than BAC, regardless of the BAC surface roughness or presence of LPS, and polishing BAC reduces inflammation when compared to unpolished BAC.

CLINICAL IMPLICATION

When the finish line of a crown preparation is at or below the gingival margin, bis-acrylic composite should be preferable to fabricate the provisional crown, as it has less inflammatory potential than polymethyl methacrylate. Polishing with extra-fine polishing discs would minimize the inflammatory activity of the BAC crown.

INTRODUCTION

Restoration of damaged teeth through placement of a crown is a common clinical practice. Once a tooth is prepared, a provisional crown is cemented temporarily while a laboratory fabricates the definitive crown. This provisional restoration serves to maintain the space for the crown, the gingival health, function and esthetics, as well as, provide thermal protection until the permanent restoration is placed. The provisional restoration can be fabricated using several different materials, including, but not limited to, bis-acrylic composite (BAC) and polymethyl methacrylate (PMMA).

Collectively, studies have shown that crown margins with poor adaptation will tend to induce an inflammatory response over time due to plaque development.[1-5] Since a provisional crown is commonly fabricated chairside, it is less precise than the definitive crown when compared to its definitive counterparts, provisional materials will have greater susceptibility to plaque adherence and potentially elicit an inflammatory response where the restorative material is in close proximity to the gingival margin. When inflammation is present and bleeding occurs,

impression material is unable to capture the sulcular area properly. This may result in a die that does not have the correct emergence profile and/or an accurate and defined finish line.[6] Also, when an adhesive resin cement is used for cementation, the bond strength of the cement decreases in the presence of blood and other contaminants.[7] As a consequence, distortion of the final impression or contamination of a cement/resin-bonding agent in a site with inflammation can lead to an early failure of the definitive crown due to inadequate adaptation of the crown, secondary caries, or crown dislodgement.

Bacteria, as well as bacterial debris found in plaque, can cause periodontal disease by triggering an inflammatory reaction sub-gingivally. Bacteria and their debris interact with the junctional epithelium and penetrate into the underlying connective tissue, causing the blood vessels in the vicinity to become inflamed and enabling leukocytes to enter the area.[8] There are many types of bacteria found in the oral cavity including gram-negative bacteria, such as *Aggregatibacter actinomycetemcomitans and Porphyromonas gingivalis*.[9] Lipopolysaccharides (LPS) are endotoxins found in the outer membrane of gram-negative bacteria; they are potent inflammatory agents.[9] LPS is comprised of covalently linked lipid A, a core oligosaccharide, and an outer O-antigen. The lipid A binds to the toll-like receptor 4 (TLR-4) of immune cells, such as macrophages, in turn activating nuclear factor kappa B (NF-κB), resulting in the synthesis of pro-inflammatory cytokines, such as tumor-necrosis factor-α (TNF-α) and interleukin-1-β (IL1-β). These factors are involved in perpetuating and amplifying the inflammatory process, leading to periodontal destruction.[10] TLR-4 receptors are predominantly found in fibroblasts and inflammatory cells in the connective tissue, and at lower levels in the junctional epithelium .[16] As LPS molecules transverse the junctional epithelial barrier into the underlying connective tissue, a strong inflammatory response can ensue.

LPS adherence to dental materials in the sulcus can elicit an inflammatory response, and the magnitude of this adherence could be affected by the chemical nature of the material, as well as the surface topography. PMMA is prepared from polymethyl methacrylate (powder) and methyl methacrylate (liquid). BAC is a paste-paste mixture consisting of an organic resin, such as bisphenol-A-glycidyl methacrylate (bis-GMA), and an inorganic filler, such as zirconia silica and fumed silica. BAC and PMMA demonstrate lower *Streptococcus mutans* adhesion than methacrylates, regardless of surface roughness.[11] However, although bacterial adherence to BAC and PMMA has been reported, the ability of LPS to adhere to these provisional dental materials and to activate an inflammatory response has not been investigated. Additionally, to date no studies have examined the potential inflammatory effects of surface roughness of these materials and any interaction with LPS to elicit enhanced inflammatory activity. The purpose of this study was to determine if there is a difference in inflammatory potential between PMMA and BAC, and to examine any interactive effects between surface roughness of the provisional dental materials and binding LPS on induction of an inflammatory response.

MATERIALS AND METHODS

Preparation of PMMA and BAC discs with variable surface roughness: Fifty-two discs of PMMA (PMMA; Jet Tooth Shade™ Powder and Jet Liquid, Lang Dental Manufacturing Co., Inc. Wheeling, IL) and fifty-two discs of BAC (Protemp™ Plus, 3M ESPE, St. Paul, MN) were fabricated using a template with dimensions of 15.4 mm diameter and 2 mm depth. The discs were cleaned with 70% ethanol swabs for 15 seconds and stored in 50 mL endotoxin-free conical polypropylene tubes until use. Twelve discs of each material were used without further processing to assess intrinsic inflammatory activity. To investigate the effects of surface roughness, polishing discs (Sof-Lex™, 3M ESPE) were used to roughen the smooth material

surface. For each material, eight discs were roughened with either coarse, medium, fine, or extra fine polishing discs disc (thirty-two material discs total). A new polishing disc was used each time and stroked 20 times to provide total surface roughening. The 8 remaining discs of each material were used as the control. The discs were cleaned of debris with 70% ethanol and stored until surface roughness was measured. A profilometer (Perthometer M2, Mahr GmbH, Göttingen, Germany) was used to measure the roughness of each discs as an Ra value (μm). Five measurements of 5.6 mm length were taken for each disc and averaged. Once the roughness of each disc was recorded they were cleaned with 70% ethanol solution.

Assessment of inflammatory potential of PMMA and BAC using NF-κB reporter assays: Human embryonic kidney (HEK) cells expressing the TLR4-MD2-CD14 receptor complex and carrying the NF-κB-induced secreted embryonic alkaline phosphatase (SEAP) reporter gene (InvivoGen, San Diego, CA) were used to assess the NF-κB-stimulatory property of PMMA and BAC following the manufacturer's instructions, as described previously.[17] Unmodified discs (n=12 each) of PMMA and BAC were placed in a 24 well plate with the untouched surface facing up. Next, 1×10^6 HEK-blue hTLR4 cells, Null2 cells (devoid of TLR4 receptor) or THP1-Xblue cells (human monocyte cell line) were added to each well (total reaction volume, 750 μl/well) and the plates were incubated for 20 h at 37°C. For detection of induced SEAP, 20 μl of the supernatant was added to 180 μl of the QUANTI-blue substrate (InvivoGen) in 96-well microtiter plates. Supernatants from HEK-blue hTLR4 cells cultured without discs were used as negative control. The reaction mixture was incubated at 37°C for 30 min, and the SEAP activity was assessed by reading the optical density at 650 nm (OD_{650}).

LPS Binding Assay: After polishing, PMMA and BAC discs (40 total each, including control) were placed in 24 well endotoxin-free tissue culture plates. Fifty microliters of purified *E. coli*

LPS (10 ng/ml- resuspended in endotoxin free water) were placed on the surface of the discs and incubated for 1 hour, after which the unbound LPS was aspirated and the surface was washed with 100 μl of Dulbecco's phosphate-buffered saline to remove unbound, free LPS off the discs; a fresh pipette tip was used for each disc to avoid cross-contamination. HEK-blue hTLR4 cells were added to each well and incubated for 20 h at 37°C (total reaction volume, 750 μl/well), followed by SEAP detection.

RESULTS:

The inflammatory potential of PMMA was higher than BAC and dependent on TLR4 receptor: To determine whether the provisional dental materials per se could cause an inflammatory response through the activation of the TLR-4 receptor, and to measure the magnitude of any response, HEK cells that expressed the human TLR4-MD2-CD14 receptor complex and carried an NF-κB -induced SEAP reporter gene were cultured on the surface of the two materials. PMMA induced a significantly greater NF-κB activity than BAC (***$p<0.0001$, unpaired t-test) (Figure. 1A). To determine the proportion of this inflammatory response that was due to the presence of the TLR4 receptor, the two materials were incubated with HEK-blue Null 2 cells that carried an NF-κB -induced SEAP reporter gene but were devoid of TLR4. Even though both PMMA and BAC still induced some NF-κB activity, the differential induction of NF-κB by PMMA was lost in HEK-blue Null2 cells ($p = 0.4812$) (Figure 1B). Next, the inflammatory potential of PMMA and BAC were examined in THP1-Xblue cells (human monocytic cells). A differential induction of NF-κB by PMMA, in comparison to BAC, was also observed in these cells (****$p=0.0002$) (Figure 1C), although the magnitude of the NF-κB activation by PMMA in THP1 cells was lower than that in HEK-blue TLR4 cells.

PMMA showed a significantly higher NF-κB activity, for both LPS-treated and untreated surfaces, than the corresponding BAC surfaces. To determine the interaction of LPS with the provisional dental materials, unpolished disks of PMMA and BAC were treated with the absence or presence of LPS (10 ng/ml) and the NF-κB activity induced by the LPS loaded surfaces were compared to their corresponding unloaded surfaces. A two-way ANOVA was used to compare the effects of LPS exposure and material on TLR4-dependent surface activation of macrophages. There was a significant difference between materials (PMMA and BAC; $p<0.0001$) (Figure 2), and between LPS (with or without; $p=0.005$). PMMA +LPS activation was significantly higher than PMMA -LPS (Sidak's multiple comparisons; $p<0.0001$), but BAC +LPS was not significantly different from BAC -LPS ($p=0.14$) (Figure 2).

Surface roughness of BAC correlated positively with LPS binding and induction of inflammatory response: The different polishing discs altered the surface roughness of PMMA and BAC (Figure 3). The Ra values for PMMA ranged from a low of 0.174 μm to a high of 0.602 μm, and for BAC from a low of 0.097 μm to a high of 1.357 μm. Unpolished PMMA discs showed moderate roughness (mean 0.330 $\pm$ 0.085 μm SD), but other polished categories were not markedly different in Ra value. In contrast, unpolished BAC discs were relatively smooth (mean 0.134 $\pm$ 0.036 μm SD), and polished discs were rougher, with the mean roughness paralleling the roughness category of the polishing discs. The individual measurements were relatively clustered within each polishing category, with no marked gaps or evident unusual values, indicating a relatively consistent polishing technique (Figure 3). Each polishing category passed the D'Agostino & Pearson and the Shapiro-Wilk tests for normality of distribution ($p\geq0.23$).

The NF-κB activation values were also relatively clustered within each polishing category, with no marked gaps or evident unusual values (Figure 4). Values in the polishing categories passed at least one, and generally both tests for normality ($p \geq 0.06$). The overall activation seen with BAC was relatively modest and there was a clear relationship to polishing disc grade and activation. The coarse category displayed a relatively strong activation (mean 0.990 ± 0.287 OD_{650}), and the other four surface categories displayed a progressive decline from a mean of 0.364 ± 0.287 OD_{650} (Medium) down to a mean of 0.130 ± 0.089 OD_{650} (Control). NF-κB activation of HEK TLR-4 cells on PMMA did not show a simple relationship to the polishing disc category. The coarse grade showed the highest value, and medium grade the lowest, with means then increasing towards extra fine grade, becoming comparable to the control.

Given the overlap in Ra values between groups, non-linear regression using a linear model was used to analyze the relationship between Ra and inflammatory activation following application of LPS. For BAC, there was a moderate correlation between NF-κB activation and Ra value ($r^2=0.51$), although 5/7 coarse values were statistical outliers to the model (ROUT method, Q=1%). The coarse outliers had about a two-fold increased inflammatory reaction in comparison to discs from other groups with similar Ra values. By omitting these values, a good correlation was obtained ($r^2=0.62$). The positive slope of the line excluding the coarse outliers, 0.329 (95% CI 0.232-0.426), was significantly different from zero ($p<0.0001$; F-test (F(DFn, DFd)=48.45 (1, 30)). The y intercept value (i.e., at Ra=0) was 0.099, also significantly different from zero ($p=0.003$; F-test (F(DFn, DFd)=10.20 (1, 30)) (Figure 5A). Separate linear regression with the five coarse group outliers gabe a very good fit ($r^2=0.86$) to a line with a y intercept significantly different to that of the main data (0.728, 95% CI 0.4067 to 1.048; $p=0.002$),

consistent with higher inflammatory activity in the coarse group. However, the fit was largely dictated by the inclusion of one or two of the remaining coarse group samples, and given the small group size, differences were not tested further.

In contrast to BAC, PMMA showed a different pattern of polishing response to the polishing disc grades with little correlation of activation with Ra value (r^2=0.19). The overall mean across all groups for NF-κB activation was 1.985 $\pm$ 0.978 OD_{650}, and excluding the coarse samples, the activation mean was 1.642$\pm$0.719 OD_{650} (Figure 5B).

DISCUSSION

Previous studies have shown that BAC has less plaque accumulation than PMMA provisional crowns.[5] Bacteria/bacterial debris (containing LPS) found in plaque adhering to dental materials can cause periodontal disease by triggering an inflammatory reaction sub-gingivally.[9, 14, 15] LPS are endotoxins found in the outer membrane of gram-negative bacteria and are potent inflammatory agents.[9] The roughness of a contact surface can also influence macrophage activation directly. For example, macrophages preferentially aggregate on rough surfaces over smooth ones.[12] Furthermore, the provisional crown surface roughness has the potential to interact with free LPS shed by bacteria.[13] However, the inflammatory potential of the dental materials per se and any interactive effect with LPS have not been investigated previously.

Results from NF-κB activation assays from HEK-hTLR4 cells and THP-1 cells reported here revealed that in the absence of LPS, PMMA per se caused a greater inflammatory response than BAC. When BAC and PMMA discs +/- LPS were plated with HEK-hTLR4 cells, PMMA discs with LPS elicited a higher NF-κB activity than PMMA-LPS. However, the BAC +/-LPS discs showed little difference in NF-κB activation, suggesting unaltered BAC has poor binding

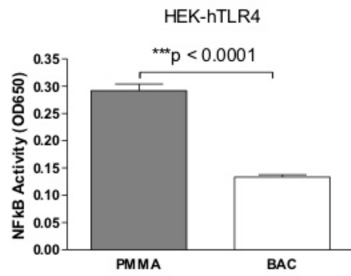
affinity to LPS. When the surface profile of the material was altered by polishing and then exposed to a fixed concentration of LPS, the coarser profiled discs exhibited a greater inflammatory reaction. This was potentially due to more LPS adhering to the discs due to an increased surface area. However, without further testing, a potential synergy between surface roughness and LPS activation cannot be excluded.

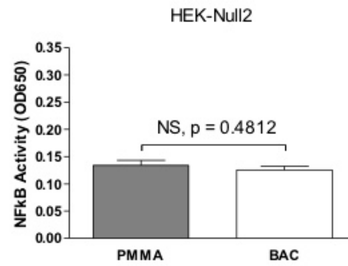
The findings of this study demonstrate that the polishing discs caused an increase in roughness of the samples the coarser the polishing discs. However, the polishing discs did not cause great variation with PMMA except for the coarsest polishing disc. The variation of roughness between PMMA and BAC with each polishing disc group indicates that the material's physical properties play a role in creating roughness. The Ra values for the different roughness categories were limited to a linear measurement of the topography of five random areas on each disc. Using a 3-D profilometer could provide a Ra value that encompasses the entirety of the disc.

Overall, PMMA was estimated to be more pro-inflammatory than BAC, regardless of the BAC surface roughness or presence of LPS. A future study should include clinical observation of the periodontal health and surface roughness measurement pre and post provisional crown insertion to determine clinical translation of this in vitro study.

CONCLUSION

BAC appeared to be less pro-inflammatory than PMMA, regardless of BAC surface roughness. If there is a need to polish BAC, it should be to at least an extra fine grade. Polishing either PMMA or BAC with only a coarse disc appeared to be unadvisable regarding pro-inflammatory NF-κB activation. In the presence or absence of LPS, BAC exhibits less inflammatory potential than PMMA.





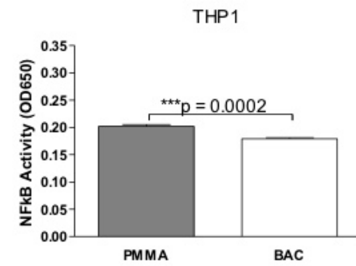


Figure 1A Figure 1B Figure 1C

Figure 1: comparison of induced SEAP activity in HEK-blue hTLR4 (1A), HEK-blue Null 2 cells (1B), and THP1-Xblue cells (1C) using the two different materials.

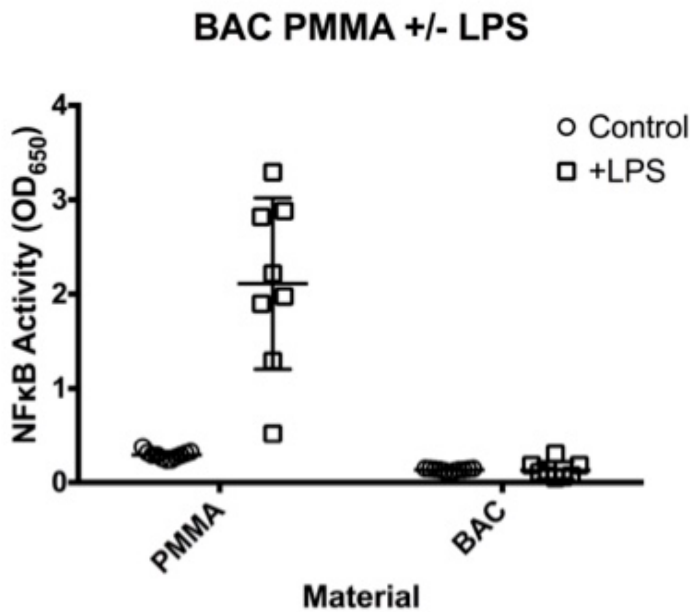


Figure 2: comparison of NF-κB activity when HEK-blue hTLR4 cells are exposed to PMMA +/- LPS and BAC +/- LPS

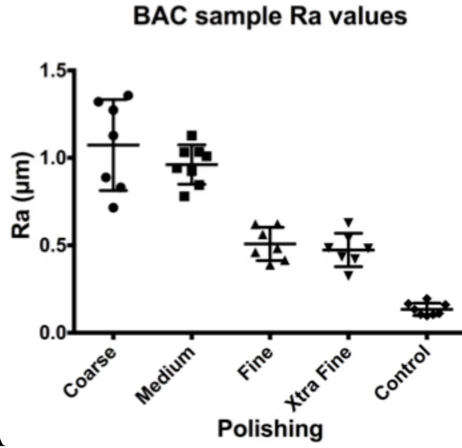


Figure 3A

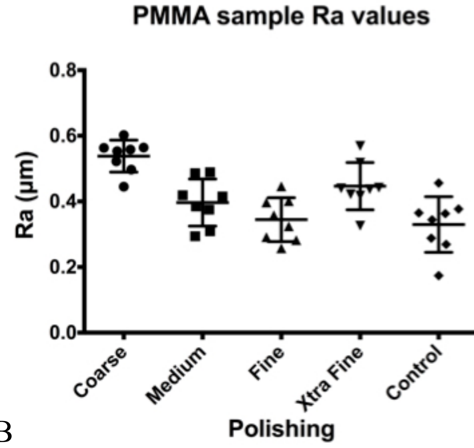


Figure 3B

Figure 3: Roughness values (Ra) measured in µm for BAC and PMMA discs polished with different polishing discs

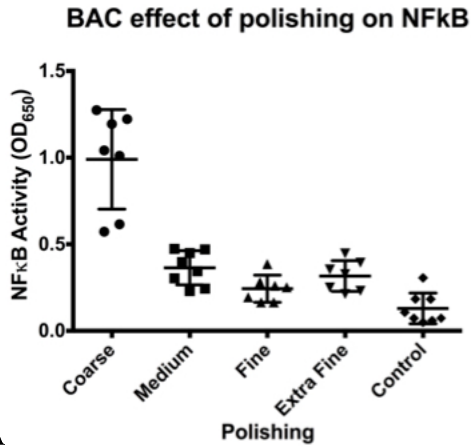


Figure 4A

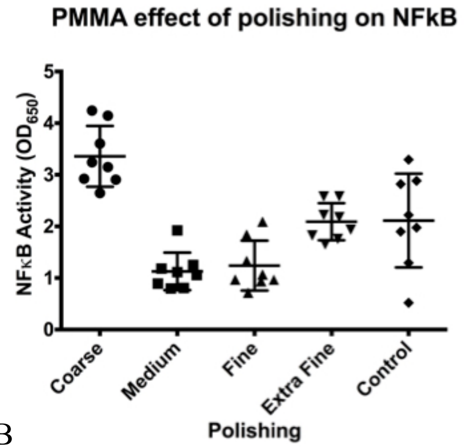


Figure 4B

Figure 4: NF-κB activity of HEK-blue hTLR4 cells when plated on different roughness categories of BAC or PMMA discs. All discs were exposed to 10 ng/ml LPS.

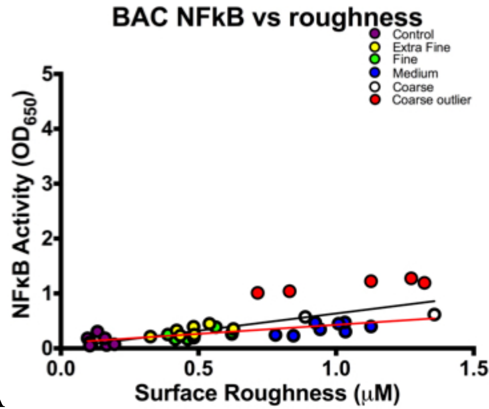
Figure 5A 

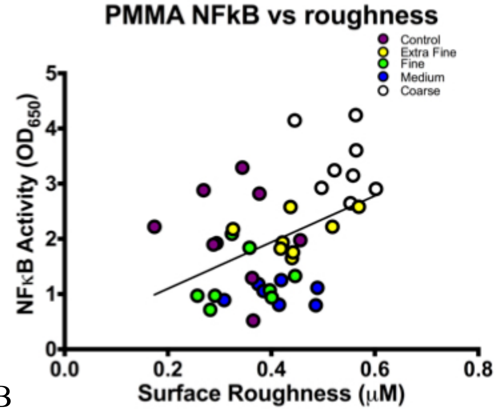
Figure 5B 

Figure 5: NF-κB activity of HEK TLR4 cells when plated on BAC or PMMA disc of different roughness with 10 ng/ml LPS.

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