

Submission ID #: 71357

Scriptwriter Name: Pallavi Sharma

Project Page Link: <https://review.jove.com/account/file-uploader?src=21763933>

Title: A Standardized *In Vitro* Procedure for Evaluating Surface Microhardness Changes in Nanohybrid Resin Composites After Whey Protein Beverage Exposure

Authors and Affiliations:

Ayşe Tugçe Tunac, Tugba Ayaz

University of Health Sciences, Hamidiye Dental Faculty, Department of Restorative Dentistry

Corresponding Authors:

Ayşe Tugçe Tunac (aysetugce.tunac@sbu.edu.tr)

Email Addresses for All Authors:

Ayşe Tugçe Tunac (aysetugce.tunac@sbu.edu.tr)

Tugba Ayaz (241002090@sbu.edu.tr)

Author Questionnaire

1. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

2. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes**

3. Filming location: Will the filming need to take place in multiple locations? **No**

4. Testimonials (optional): Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **No**

Current Protocol Length

Number of Steps: 32

Number of Shots: 44

Interviews

Videographer: Obtain headshots for all authors available at the filming location.

INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Tugba Ayaz:** We examine the impact of whey protein beverages on the surface microhardness of nanohybrid resin composites.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera *Suggested B roll: 4.1.1.*

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

- 1.2. **Tugba Ayaz:** Existing methods lack standardized beverage exposure conditions, limiting reproducibility. This protocol establishes controlled exposure conditions, enabling reliable, comparable microhardness assessments of resin composites.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Tugba Ayaz:** This method allows researchers to quantify microhardness changes caused by dietary protein beverages in restorative materials.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera *Suggested B roll: 6.5.1*

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.4. **Tugba Ayaz:** The most important consideration while performing this protocol is maintaining consistent specimen preparation and controlled immersion conditions to ensure reliable results.

- 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera *Suggested B roll: 2.2.2*

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

- 1.5. **Tugba Ayaz:** Future studies can investigate additional beverages, longer exposures, and other material properties.
- 1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

Videographer: Obtain headshots for all authors available at the filming location.

Protocol

Videographer: Please always record an audio slate while filming each shot mentioned in the shotlist.

2. Preparation of Nanohybrid Resin Composite Disc Specimens

Demonstrator: Tugba Ayaz

- 2.1. To begin, prepare a total of 90 disc-shaped specimens from three nanohybrid resin composites [1].
 - 2.1.1. WIDE: Talent placing the nanohybrid resin composites on the bench.
Videographer: Please obtain CU shot as well for this shot.
- 2.2. Fabricate the specimens using cylindrical clear silicone molds [1]. Place the composite material into the molds in a single 2-millimeter-thick increment [2]. Cover the surface with a clear microscope slide to obtain a flat surface [3].
 - 2.2.1. Talent placing cylindrical clear silicone molds on the work surface.
 - 2.2.2. Talent placing composite material into the silicone molds.
 - 2.2.3. Talent covering the mold surface with a clear microscope slide.
- 2.3. Polymerize the specimen for 20 seconds using a blue LED light-curing unit at 1000 milliwatts per square centimeter, positioned approximately 1 millimeter from the surface [1].
 - 2.3.1. Talent positioning the blue light-emitting diode light-curing unit above the specimen surface and polymerizing the specimen.
- 2.4. Verify the curing light intensity using a radiometer before each polymerization cycle to ensure consistent light exposure [1].
 - 2.4.1. Talent checking the curing light intensity with a radiometer.
- 2.5. Store the prepared specimens in distilled water [1] at 37 degrees Celsius for 24 hours to allow post-polymerization and simulate initial oral conditions [2].
 - 2.5.1. Talent placing the prepared specimens in distilled water for storage.
 - 2.5.2. LAB MEDIA: JoVE_LM_71537_Step2.5.2.StoredSamples.png

3. Specimen Polishing and Baseline Microhardness Measurement NOTE: The Title has been changed

- 3.1. Using medium, fine, and superfine aluminum oxide-impregnated discs, polish the upper surfaces of all specimens sequentially [1] for 10 seconds each under water cooling at 10,000 rpm using light pressure and a unidirectional rotational motion [2].

- 3.1.1. Talent showing medium, fine, and superfine aluminum oxide-impregnated discs
- 3.1.2. Talent polishing the specimen surface with a medium polishing disc under water cooling.
- 3.2. Replace the polishing discs after every five specimens to maintain consistent abrasive efficiency and ensure surface standardization [1].
 - 3.2.1. Talent replacing the polishing disc after polishing five specimens.
- 3.3. After each polishing step, rinse the specimens with distilled water for 10 seconds to remove debris [1]. Gently air-dry the specimens for 5 seconds before proceeding to the next disc [2].
 - 3.3.1. Talent rinsing the polished specimens with distilled water.
 - 3.3.2. Talent places the specimens aside for air drying.
- 3.4. Mark the unpolished surface of each specimen with a unique identification code to ensure consistent orientation during subsequent testing [1]. Then, store the specimens in distilled water [2] at 37 degrees Celsius for 24 hours prior to baseline microhardness testing [3].
 - 3.4.1. Talent marking the unpolished surface of the specimen with an identification code.
 - 3.4.2. Talent placing the specimens in distilled water for storage.
 - 3.4.3. LAB MEDIA: JoVE_LM_71537_Step3.4.3.StoredSamples.png

~~4. Baseline Microhardness Measurement~~ NOTE: Please merge this section with section 3

- 4.1. Perform baseline Vickers microhardness or VHN (*V-H-N*) testing using a VHN tester [1]. Apply a 200 g load for 15 seconds during the microhardness measurement [2].
 - 4.1.1. LABMEDIA/SCREEN:
JoVE_LM_71537_Step4.1.1.PositioningSpecimenVickers.jpg
 - 4.1.2. LAB MEDIA/SCREEN: JoVE_LM_71537_Step4.1.2.PerformingVickers.jpg
- 4.2. Make three indentations per specimen with at least 1 millimeter spacing between each indentation [1]. Perform the indentations on the central surface area of each specimen to avoid edge effects [2].
 - 4.2.1. LAB MEDIA/SCREEN: JoVE_LM_71537_Step4.2.1(1).MultipleIndentations.jpg,
JoVE_LM_71537_Step4.2.1(2).MultipleIndentations.jpg,
JoVE_LM_71537_Step4.2.1(3).MultipleIndentations.jpg
 - 4.2.2. LAB MEDIA/SCREEN:
JoVE_LM_71537_Step4.2.2.IndentationsCentralSurfaceArea.jpg
- 4.3. Calculate the mean baseline VHN for each specimen, with all measurements performed by a single calibrated operator to minimize variability [1].

4.3.1. SCREEN: JoVE_SCREEN_71357_STEP4.3.1.BaselineMeanVHN.MOV: 00:02-end

5. Immersion Solution Preparation and Immersion Procedure

- 5.1. Dissolve 25 grams of whey protein powder in 200 milliliters of distilled water to prepare immersion solutions [1].
 - 5.1.1. Talent adding whey protein powder to distilled water in a container.
- 5.2. Mix the solutions using a laboratory shaker for 60 seconds until complete dissolution is achieved [1]. Allow the solutions to equilibrate at room temperature for 5 minutes to minimize foam formation and ensure homogeneity [2].
 - 5.2.1. Talent placing the solution containers on a laboratory shaker.
 - 5.2.2. Shot of the prepared whey protein solutions resting at room temperature.
- 5.3. Measure and record the pH of each solution using a calibrated digital pH meter before immersion [1].
 - 5.3.1. Talent measuring the pH of the whey protein solution with a digital pH meter.
- 5.4. Prepare fresh whey solutions prior to each immersion cycle to ensure consistent chemical composition and avoid protein degradation [1]. Use 10 milliliters of immersion solution per specimen to ensure complete submersion during each cycle [1].
 - 5.4.1. Shot of the prepared solutions.
 - 5.4.2. Talent dispensing immersion solution into a container.
- 5.5. Divide each composite group into three subgroups containing 10 specimens each [1].
 - 5.5.1. Talent organizing the composite specimens into three separate subgroups.
- 5.6. Immerse the specimens in the assigned solutions for 10 minutes twice daily [1]. Select the immersion duration and frequency based on established in vitro beverage-exposure models that simulate daily consumption patterns [2].
 - 5.6.1. Talent placing the specimens into the assigned immersion solutions.
 - 5.6.2. Shot of the immersed specimens remaining in the solutions during the exposure period.
- 5.7. For whey-exposed groups, after each immersion cycle, rinse the specimens with distilled water [1]. Place the specimens in artificial saliva for 10 minutes to simulate short-term salivary exposure [2].
 - 5.7.1. Talent rinsing the specimens with distilled water.
 - 5.7.2. Talent placing the specimens into artificial saliva.
- 5.8. Store the specimens in distilled water at 37 degrees Celsius between immersion cycles until the next exposure cycle. Continue the immersion protocol for 30 days [1].

5.8.1. Talent placing the specimens at 37 degrees Celsius.

5.9. Maintain the control group continuously in distilled water at 37 degrees Celsius throughout the experimental period [1]

5.9.1. Shot the control group specimens placed at 37 degrees Celsius.

6. Post-Immersion Microhardness Measurement and Analysis

6.1. Rinse the specimens with distilled water and gently dry them [1].

6.1.1. Talent rinses the specimens with distilled water and gently dries them.

6.2. Perform VHN as demonstrated earlier and calculate the mean post-immersion VHN [1].

6.2.1. SCREEN: JoVE_SCREEN_71357_STEP6.2.1.PostImmerionMeanVHN.MOV

6.3. Determine the change in VHN or Δ VHN (*Delta-V-H-N*) for each specimen [1].

6.3.1. SCREEN: JoVE_SCREEN_71357_STEP6.3.1.DeltaVHN.MOV

6.4. Analyze VHN values using a repeated measures ANOVA model, with time point as the within-subject factor and composite type and immersion solution as between-subject factors [1].

6.4.1. SCREEN: JoVE_SCREEN_71357_STEP6.4.1.RepeatedANOVA.MOV: 00:02-end
Video editor: Please speed it up!

6.5. When appropriate, calculate the Δ VHN values as descriptive outcome measures to express the magnitude of microhardness change for each specimen [1].

6.5.1. SCREEN: JoVE_SCREEN_71357_STEP6.5.1.DeltaVHN.MOV

6.6. Perform post-hoc pairwise comparisons using Tukey's honestly significant difference test [1].

6.6.1. SCREEN: JoVE_SCREEN_71357_STEP6.6.1.Post_hoc_tukey.MOV

6.7. Calculate 95 percent confidence intervals for all group means [1] and report effect sizes using partial eta-squared for ANOVA results [2].

6.7.1. SCREEN: JoVE_SCREEN_71357_STEP6.7.1.Confidence_intervals.MOV

6.7.2. SCREEN: JoVE_SCREEN_71357_STEP6.7.2.Partial_eta_squared.MOV

6.8. Compare hydrogen potential values between whey protein solutions using Welch's t-test [1] and set the level of statistical significance for the analysis [2].

6.8.1. SCREEN: JoVE_SCREEN_71357_STEP6.8.1.Welchs_ttest_comparison.MOV :
00:05-00:10, 00:24-00:33

6.8.2. SCREEN: JoVE_SCREEN_71357_STEP6.8.2.Welchs_ttest_significance.MOV:
00:01-00:05

6.9. In addition to absolute change in VHN values, calculate percentage microhardness change using the presented formula [1].

6.9.1. TEXT ON A PLAIN BACKGROUND

$$[(\text{baseline VHN} - \text{post-immersion VHN}) / \text{baseline VHN}] \times 100.$$

Results

7. Results

- 7.1. The lowest microhardness change was observed in the highly filled composite stored in distilled water [1], whereas the greatest change was recorded in the injectable composite exposed to whey concentrate [2].
 - 7.1.1. LAB MEDIA: Table 1. *Video editor: Highlight the row “EA” with the column “Distilled Water” and the value “ 0.9 ± 0.4 ”*
 - 7.1.2. LAB MEDIA: Table 1. *Video editor: Highlight the row “GUI” with the column “Whey Concentrate” and the value “ 10.4 ± 2.1 ”*
- 7.2. Exposure to whey protein solutions resulted in greater hardness loss compared to distilled water [1]. However, no statistically significant difference was observed for whey isolate in the highly filled composite [2].
 - 7.2.1. LAB MEDIA: Figure 1. *Video editor: Highlight the lines for “WI” and “WC” positioned above “DW” across the graph*
 - 7.2.2. LAB MEDIA: Figure 1. *Video editor: Highlight the blue “EA” points at “DW” and “WI” showing only a small separation*
- 7.3. The injectable composite exposed to whey concentrate demonstrated the highest hardness loss [1], whereas the highly filled composite stored in distilled water exhibited the lowest change [2].
 - 7.3.1. LAB MEDIA: Figure 2. *Video editor: Highlight the tallest bar labelled “GUI-WC”*
 - 7.3.2. LAB MEDIA: Figure 2. *Video editor: Highlight the shortest bar labelled “EA-DW”*
- 7.4. Immersion in whey concentrate caused significantly greater microhardness loss compared to distilled water for all tested composites [1].
 - 7.4.1. LAB MEDIA: Figure 2. *Video editor: Highlight the “WC” bars as visibly taller than the corresponding “DW” bars for each composite group*
- 7.5. Specimens exposed to whey isolate also showed higher ΔVHN values than those stored in distilled water [1].
 - 7.5.1. LAB MEDIA: *Figure 2. Video editor: Highlight the “WI” bars as visibly taller than the corresponding “DW” bars for each composite group*

1. Electrophysiology
Pronunciation link: <https://www.merriam-webster.com/dictionary/electrophysiology>
IPA: /ɪˌlek.troʊˌfɪz.iˈɑː.lə.dʒi/
Phonetic Spelling: ihˌlekˌtrohˌfizˌeeˌolˌuhˌjee
2. Silver chloride
Pronunciation link: <https://www.merriam-webster.com/dictionary/silver%20chloride>
IPA: /ˈsɪl.vər ˈklɔːr.aɪd/
Phonetic Spelling: silˌver klorˌide
3. Unchlorinated
Pronunciation link: No confirmed link found
IPA: No verified IPA available
Phonetic Spelling: unˌklorˌuhˌnayˌtid
4. Polymethyl methacrylate
Pronunciation link: <https://www.merriam-webster.com/dictionary/polymethyl%20methacrylate>
IPA: /ˌpɑː.liˈmeθ.əl məˈθæk.rə.leɪt/
Phonetic Spelling: polˌeeˌmethˌuhl muhˌthakˌruhˌlayt
5. Hexadecane
Pronunciation link: <https://www.merriam-webster.com/dictionary/hexadecane>
IPA: /ˈhek.səˌdeɪn/
Phonetic Spelling: hekˌsuhˌdayn
6. Diphytanoyl
Pronunciation link: No confirmed link found
IPA: No verified IPA available
Phonetic Spelling: dyeˌfyˌtanˌoyl
7. Phosphocholine
Pronunciation link: <https://www.merriam-webster.com/medical/phosphocholine>
IPA: /ˌfɑːs.foʊˈkoʊ.liːn/
Phonetic Spelling: fosˌfohˌkohˌleen
8. HEPES
Pronunciation link: <https://www.howtopronounce.com/hepes>
IPA: /ˈhep.ez/
Phonetic Spelling: hepˌez
9. Electrolyte
Pronunciation link: <https://www.merriam-webster.com/dictionary/electrolyte>
IPA: /ɪˌlek.troˌlaɪt/
Phonetic Spelling: ihˌlekˌtruhˌlyte
10. Bilayer
Pronunciation link: <https://www.merriam-webster.com/dictionary/bilayer>
IPA: /ˈbaɪˌleɪ.ər/
Phonetic Spelling: byeˌlayˌer
11. Capacitance
Pronunciation link: <https://www.merriam-webster.com/dictionary/capacitance>
IPA: /kəˈpæs.ə.təns/
Phonetic Spelling: kuhˌpassˌuhˌtuhns

12. Faraday
Pronunciation link: <https://www.merriam-webster.com/dictionary/Faraday>
IPA: /'fer.ə.deɪ/
Phonetic Spelling: fair·uh·day
13. Solubilize
Pronunciation link: <https://www.merriam-webster.com/dictionary/solubilize>
IPA: /sə'lu:.bəˌlaɪz/
Phonetic Spelling: suh·loo·buh·lyze
14. Nitrotriacetic
Pronunciation link: <https://www.howtopronounce.com/nitrotriacetic>
IPA: /naɪˌtraɪˌlooˌtraɪ.ə'siː.tɪk/
Phonetic Spelling: ny·try·loh·try·uh·see·tik
15. Affinity
Pronunciation link: <https://www.merriam-webster.com/dictionary/affinity>
IPA: /ə'fɪn.ə.ti/
Phonetic Spelling: uh·fin·uh·tee
16. ancTREK1
Pronunciation link: No confirmed link found
IPA: No verified IPA available
Phonetic Spelling: ank·trek·one
17. Transfection
Pronunciation link: <https://www.merriam-webster.com/dictionary/transfection>
IPA: /træn'sfekʃən/
Phonetic Spelling: trans·fek·shuhn
18. Flickering
Pronunciation link: <https://www.merriam-webster.com/dictionary/flicker>
IPA: /'flɪk.ə.ɪŋ/
Phonetic Spelling: flik·er·ing
19. Symmetric
Pronunciation link: <https://www.merriam-webster.com/dictionary/symmetric>
IPA: /sɪ'met.rɪk/
Phonetic Spelling: sih·met·rik
20. Excised
Pronunciation link: <https://www.merriam-webster.com/dictionary/excise>
IPA: /ɪk'saɪzd/
Phonetic Spelling: ik·syzd