

A treatment protocol for the consolidation of flaking and friable media in illuminated manuscripts

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The **Weissman Consolidation Protocol** is a manual that has evolved over many years during the regular conservation treatment of illuminated manuscripts. Working alongside curators, the book and paper conservators of the Weissman Preservation Center, Harvard Library, have discussed goals and researched trends in media consolidation to create this manual. The resulting protocol became an indispensable guide for staff when undertaking the treatment of over 100 illuminated manuscripts for the recent exhibition, *Beyond Words* (2016-17).

This manual describes specific procedures pertaining to equipment and policies at the Weissman Preservation Center. The general procedures, however, can be adopted by any other conservation laboratory.

CONTENTS

1. STANDARDS OF PRACTICE	page 1
2. PREPARATIONS	3
3. TREATMENT SET-UP	4
4. ADHESIVES	5
5. TREATMENT (1)	7
6. TREATMENT (2)	9
7. TREATMENT DOCUMENTATION	9
8. STAFF TIME COMMITMENT	11
APPENDICES	12-16

1. STANDARDS OF PRACTICE

Principles of standardization of treatment

- The purpose of establishing standards of practice is to integrate uniformity in treatment procedures AND judgment.
- A standardized approach goes beyond procedure and aligns judgment with decision-making.
- Standards set in the form of a protocol encourage all conservators involved to work in a uniform treatment style. The result is that the entire manuscript or collection has the appearance of being treated by one person.
- With a protocol in place, higher-quality work will be easier to maintain, especially on longer projects.
- Best practices are achieved through collective understanding and cooperation. Lots of discussion is necessary, as well as open-mindedness, observation sharing, and letting go of ego.
- A team approach is used to ensure the development and execution of the best quality standards of practice.

Team members and participation

- All consolidation treatment involves a team approach.
- A team consists of two or more conservators. A minimum of two is necessary to validate that the treatment protocol was followed. For larger projects, a team of four or five members is typical.
- One member of the team is designated the lead on each project and coordinates all aspects of the project including: preparing the treatment proposal and photo documentation, treatment, moderating meetings about treatment effectiveness, writing the treatment report, and checking that the photo documentation is complete. The team leader does not necessarily perform all of these tasks, but ensures that all tasks are completed.
- Use of a team improves the quality of treatment, provides efficiency of workflow and reduces the amount of time any one individual spends to complete the project.

Approach to treatment

- All teams follow the same protocol in judgment, decision-making, and procedure.
- Suggested modifications in the protocol are discussed with the team leader. Changes in approach and procedures are not made independently.
- Deviations or modifications to the protocol related to a specific manuscript should be actively communicated to all team members. Merely noting protocol modifications in the work binder may not be sufficient to ensure that all team members adopt changes promptly.
- Deviations or modifications to the protocol that affect all consolidation work should be documented in this written protocol and require notification of all Weissman staff who commonly participate in consolidation work.

January 2018

Key procedural elements of the protocol

- *Consistent procedures.* The use of the same magnification (15x) and tools to judge stability or friability of media.
- Uniform judgment parameters. The decision to treat is based on the actual detection of loose or friable media. **Consolidation is not considered a proactive measure**. Media that is cracked or looks loose or friable but tests stable as determined by the testing protocol is not preemptively treated.
- *Quality control.* One conservator treats a given illumination and a second reviews the work to check treatment success. This system ensures that areas are not overlooked, that the consolidation is effective, and that there is no change in media appearance.
- Open and frequent communication. The treatment binder, which contains the treatment record and printed out paper copies of the folios being treated, serves as the written document to record day-to day observations, treatment progress, cautions, concerns, and insights into artists' technique. In this way, all team members are informed equally.

2. PREPARATIONS

Treatment proposal: estimating consolidation treatment time

- Estimating treatment time should start with calculating the area of the illumination in square centimeters (cm²), deciding on the degree of flaking media (see following), then multiplying the area by the selected time per cm².
- Using the Weissman Consolidation Protocol, consolidation takes 2 minutes/cm² for minor flaking, 3 minutes/cm² for moderate flaking, and 4.5 minutes/cm² for areas that are flaking throughout. For general estimating purposes, 3 minutes/cm² is reasonable.
- Additional time is added for documentation, structural work, and large-format, heavy, or unusual items.

Examination

- **Transmitted light (1):** Using the fiber optics at the microscope set-up works well. Hold the leaf at a 60-45 degree angle and visually scan the entire folio (without the aid of the microscope). When the parchment is thin enough, a visual assessment of the condition and location of paint loss is immediately apparent. Transmitted light also clearly shows the correspondence of the design on the opposite side of the leaf, the manner of paint application, and the relative opacity and translucency of the media.
- Transmitted light (2): A fiber-optic light sheet is an excellent light source to use when capturing an image with transmitted light. The thin light sheet is inserted beneath the leaf. Provide protection (interleaving) between the manuscript folio, fragile surfaces, and the verso of the light sheet.
- **Raking light:** Using the fiber-optic lights at the microscope set-up, position the light at a low (raking) angle to assist in examination of the media and to identify the smoother (hair) side and the more nappy (flesh) side of the parchment. Sometimes it is difficult to differentiate the sides of the parchment due to its preparation, but more extreme raking light may help.

January 2018

3. TREATMENT SET-UP

Cradle

- The handling and strapping required to treat bound manuscripts poses a risk to any fragile media. Care should be taken to minimize handling whenever possible.
- Manuscript conditions that affect safe handling and opening need to be considered. For example, gutter cockling poses a significant risk of loss of media near the gutter. The process of consolidation itself may cause damage and loss and needs to be weighed against the need for stabilization.
- The cradle should support the full dimension of the volume covers and the leaf to be treated should be positioned parallel to the table surface. The cradle angle is determined by the ease with which the manuscript can be opened without stressing the binding or causing the leaves to buckle. For ease of maneuvering the microscope and especially to examine and treat illuminations close to the gutter, the cradle angle will need to be 100 degrees or greater.
- When treating a leaf that is near either end of the text block, such that most of the text block is on one side, use card or board or Volara for support beneath or behind the cover to fill the gap and reduce stress to the hinge.

Strapping and interleaving

- Place a sheet of silicone release paper beneath the leaf being consolidated.
- Strap the manuscript in place with strips of polyethylene clamped to the cradle with bulldog clips.
- Strap the folio being treated with one or more narrow strips (1/4") of polyethylene, preferably placed outside of illuminated areas. If the strapping is positioned over a design, placing a strip of silicone release paper beneath the strap is advisable.
- Caution should be used when moving silicone release paper over the surface of media to avoid catching edges of the paper on media.
- To protect the facing folio, use protective interleaving, such as silicone release paper, as well as folder-weight paper. One-inch wide strapping works well for this.
- After the consolidation session has been completed on the selected folio, leave a sheet of silicone release paper between the treated folio and the facing folio overnight as a precaution to prevent any undried adhesive from sticking to the facing folio.
- The day after treatment or at the next work session, remove the interleaving sheets. It is important that interleaving sheets be removed to avoid unwanted stress on the binding.
- It is generally recommended to sequence the consolidation treatment from the back of the manuscript to the front when working on the recto folios, and from the front to the back when treating the rectos. This way, the treated folio is lying flat instead of being flexed to an upright position.

Working magnification and lighting

- Perform treatment at **15x magnification**. It is important that everyone makes judgments based on observations made at a similar magnification.
- At the WPC, one microscope (east) has a digital display that is calibrated and shows the actual magnification. Our second microscope (west) does not have a calibrated zoom dial for the .63x lower objective. The actual magnification on this microscope is the magnification

on the dial multiplied by 0.63. Therefore, the dial positioned at 24x corresponds to an actual magnification of 15x.

- The lighting (fiber optics) is usually configured for raking (45 degrees or lower) light. Either align both of the lights on one side or position one light away.

Media stability testing tool and procedure

- Identification of unstable media requiring treatment is based on a uniform testing procedure and tool.
- **The testing tool is a dental absorbent point inserted in a pin vise**. Using the absorbent point, lightly touch to test for loose and/or friable media. An xx-fine point (available from a variety of suppliers) works well.
- Gently stroke, touch, or tap the media with the paper point to identify loose flakes or friable media.
- Loose flakes can be identified by shadows that increase or decrease as the point gently presses on flakes of media. Loose areas are also identified when areas are dislodged (sometimes unintentionally) by the testing procedure.
- Friable media can be observed as offset to a facing page, as powdery, fly-away particles, or as a granular "sugar-like" surface with loose pigment agglomerates.
- The decision of when to treat a specific area is based on actual observation of loose or friable media. **Consolidation treatment is not a proactive measure**. Media that is cracked but stable, as determined by the testing protocol, is not treated.
- Replace the point at each working session or more frequently if tip becomes too soft.

4. ADHESIVES

Aqueous

- In general, the conservators at the WPC prefer to use consolidation adhesives in an aqueous system.
- The adhesives currently in use are bovine gelatin (Acros Organics gelatin, type B [alkaline hydrolyzed tissue] from bovine skin, catalog #61225), and fish gelatin (high molecular weight [HMW] fish gelatin, Norland Products).
- The exact properties of gelatin and other natural adhesives may change significantly from batch to batch. Color, exact bloom strength, ash content, etc., will vary. Newly purchased batches of adhesive should be evaluated upon receipt to determine if they are suitable. In particular, changes in bloom strength of gelatin may require adjustment to the concentration of gelatin in consolidation solutions.
- The project leader determines the adhesive and concentration to use for the specific manuscript being treated.
- Switching to a different consolidation adhesive or change of adhesive concentration is made in consultation with the project leader and team members.
- When greater flow or strength is required, HMW fish gelatin has been effective.
- Other adhesives may be tried when the standard application is insufficient to hold the loose media or is incompatible with the particular media or situation.
- Other aqueous adhesives may include isinglass and funori. Non-aqueous adhesives may include Klucel G and Aquazol.

January 2018

Bovine gelatin

- Acros Organics gelatin, type B (alkaline hydrolyzed tissue), from bovine skin, catalog #61225. Bovine gelatin is granular and amber in color. This gelatin has low bloom strength, estimated at around 100 by the supplier.
- Generally, a 1.5% stock solution is prepared.
- Stock solutions can be further diluted with up to 30% ethanol to improve flow.
- When altering a stock solution, transfer the quantity for use to a separate vial. Discard any altered solution after use. Avoid altering the entire stock solution.
- Gelatin swells in cold water but will not dissolve. Gelatin is insoluble in ethanol but solutions can incorporate approximately 30% alcohol.
- The temperature of the prepared gelatin is maintained at about 37-40°C (about 100-105°F) for use. Keep the solution in a mini Erlenmeyer flask set in a bain-marie of warm deionized water. At the WPC, we make a snug basket from a rigid plastic mesh material to elevate the flask in the bain-marie. The bain-marie is heated with a cup warmer plugged into a rheostat set at 120v and 70% output to keep the gelatin solution at the desired temperature.
- The gelatin sets by cooling.

Fish gelatin

- High molecular weight (HMW) fish gelatin, Norland Products, is sold as cream-colored flakes.
- Generally, a 1 to 1.5% stock solution is prepared.
- Stock solutions may be further diluted with up to 40% ethanol to improve flow.
- If you alter a stock solution, transfer the quantity for use to a separate vial. Discard the altered solution after use. Avoid altering the entire stock solution.
- A 6% solution of fish gelatin has been used to secure metal leaf.
- Fish gelatin is prepared in water and heated to swell and dissolve the flakes, as with the preparation of bovine gelatin.
- Fish gelatin is typically used at room temperature instead of slightly warm, as is the case with bovine gelatin.
- Fish gelatin sets by evaporation and takes longer than bovine gelatin, which sets as it cools. Because of the longer set time, paint flakes may need slight pressure and longer drying times to encourage adhesion to the substrate and may also improve flow into porous, granular paint matrices.

Preparation and storage

- Weigh out the dry adhesive and add deionized water to the desired concentration.
- Briefly stir with a glass rod to disperse the gelatin.
- Dissolve by heating in a bain-marie on a magnetized hot plate. At the WPC, we use a small Erlenmeyer flask set in a snug-fitting basket made from a rigid plastic mesh set into a Pyrex dish filled with warm deionized water. Place a small, Teflon-coated magnetic stir bar in the flask. Alternatively, you can stir the solution with a glass rod intermittently. Set the temperature slightly above 40°C and the speed to about 500 rpm.
- Remove from heat when you do not see density changes in the solution. There should be no transparent swollen granules when the flask is held up to the light and swirled. This takes about 20 minutes.

- Remove the stir bar. Seal the flask with laboratory sealing film (Parafilm M), label the flask with contents, concentration, date, and preparer's initials, and store refrigerated.
- Gelatin is made fresh weekly.
- Gelatin should be stored in a refrigerator overnight. Gelatin inadvertently left out overnight should be discarded.
- It is advisable to make fresh gelatin when the prepared gelatin starts to separate or does not feel tacky when rubbed between fingers.
- See Appendix 3 for a detailed description of the preparation of bovine gelatin.
- See Appendix 4 for a detailed description of the preparation of fish gelatin.

Non-aqueous adhesives

- Dilute solutions of Klucel G in ethanol may be used for media that reacts poorly to moisture. Glossy or glazed media can be problematic where the addition of moisture will cause expansion of the media, which in turn can cause loss, etc.
- Klucel G solutions in ethanol evaporate rapidly, causing the adhesive to thicken and become stringy. Therefore, monitoring the consistency of the dilution is critical. New stock solutions should be prepared weekly.
- The application of Klucel G is physically difficult to control, as the adhesive often becomes stringy and forms globs as the ethanol evaporates, even on the brush. Once a film of Klucel G is on the paint surface, it is difficult to re-solubilize and reduce adhesive residue.

5. TREATMENT (1): Adhesive application

Brush

- There are many subtle variations in the brush application of the gelatin. **Recommended brush size is 3/0 to 5/0.**
- The concentration, viscosity, and surface tension of the gelatin (and other consolidation adhesives) are interrelated and this matters with regard to flow and ultimately the degree of penetration of the consolidation adhesive.
- Make a test application to the media and observe the absorption rate. Examine for gloss once dry.
- One method is to first wet the area for treatment with ethanol, then apply gelatin to the same area. The ethanol helps the adhesive flow along cracks and beneath flakes. Be aware that the adhesive can travel farther than desired.
- Use caution in applying ethanol. Loose particles and flakes may be lifted and moved by the rapid flow of ethanol.
- Another method is to apply gelatin directly without ethanol to achieve a slower rate of penetration.
- Avoid use of ethanol when penetration into the parchment or paper is a risk.
- A variation can be to wait longer between the application of ethanol and the application of gelatin.
- The stock solution may also be further diluted with ethanol. This is especially effective with very powdery media.
- If there are tented flakes or loose particles that require weight or light pressure to set down, wait about ten seconds after the adhesive application and apply slight pressure through

silicone release Mylar or silicone release paper using a soft, narrow Delrin spatula or finger and/or let the area dry under a mini weight.

- Repeated applications of adhesive on difficult areas may be needed. Allow ample drying time between applications in order to reduce the chance of penetration of the parchment support and possible staining on the verso.
- In the event of a stray droplet or over-application of adhesive, the paper points used for testing are highly absorbent and may also be used to rapidly wick up adhesive. A paper point that has been gently curved may be used to absorb along the bottom of the curve rather than at the point. Paper points used to absorb adhesive should be discarded and replaced; do not use for further media testing.
- Allow the adhesive to dry thoroughly for at least 12 hours after application before assessing the final success of treatment. Residual moisture may leave pigments, adhesive, and parchment more flexible over shorter periods of time and make it difficult to identify remaining issues.

Brush application technique tips

- Dealing with loss of a flake: When removing the clear silicone release Mylar after applying gelatin followed by light weight or pressure, you may find that the flakes or particles are coming off with the Mylar instead of remaining nicely in place. If this occurs, do not panic. Instead, stop immediately and carefully put the Mylar (to which the pigment has adhered) back into place. Next, slowly withdraw the Mylar from the area while at the same time using the Delrin tool or your finger to apply gentle pressure on the area of detachment. This enables you to slide the release paper away while keeping the pigment in place. Allow the area to dry and then re-treat.
- As a reminder, one usually withdraws and lifts away the release sheet slowly and at a low angle to prevent these kinds of problems.
- To help keep track of your working area, use two narrow strips of silicon release paper placed across the illumination at the top and bottom of the microscope field of view. The strips, which can be moved as your work progresses to the adjacent area, can be especially helpful in keeping you on task when the design is fairly repetitive.
- Caution should be used when moving silicone release paper over the surface of media to avoid catching edges of the paper on media.

Mist

- Misting using a nebulizer with dilute (.75% to .5%) gelatin on broad areas of uniform granular, friable, or powdery pigment works well with some manuscripts.
- The use of punched or tailored masks to direct the mist to specified locations and block the mist from unwanted areas prevents excessive adhesive from coming in contact with the media. Masks of black paper or board are helpful in visualizing the mist application.
- Mist application relies on penetration through the media matrix and/or the support surface. The addition of small amounts of ethanol to the gelatin solution may aid penetration, particularly on media that is slightly hydrophobic.
- When the areas needing treatment are very small, isolated, or when there are significant areas of loss exposing the parchment, controlling the mist to select areas can be difficult.
- Multiple light applications are preferable to heavier applications.

- Over-wetting areas with misting is a risk because the solution is less viscous. It is important not to linger in one area. This is a problem with some colorants and with thinner parchment, at times causing distortion and penetration of color to the opposite side.

6. TREATMENT (2): Double checking

- Checking the success of the treatment is performed by a conservator other than the person(s) who performed the initial treatment.
- Checking involves reviewing the folio both for unstable media that was missed and for treated media that still requires additional attention.
- At least one full day should be allowed after treatment to ensure that adhesives have set and dried fully.
- The checking procedure is identical to the initial testing process described above. The treatment is deemed successful if there is no movement of the media as indicated by no change in shadow, friable media is no longer dislodged, and there is no visible change to the media surface from treatment.
- The evaluation, treatment, and checking steps are repeated as necessary with successive rounds concentrating on problematic areas to ensure success.
- Repeated treatment in an effort to stabilize media may see diminishing returns and potentially increase the risk of staining, translucency, etc., from over-application of adhesive. Repeated failure to stabilize an area may indicate a need to re-evaluate the adhesive concentration and/or the adhesive type, application technique, etc.
- Media issues, particularly those that are spread throughout a manuscript that cannot be satisfactorily stabilized in two or three attempts, require re-assessment of not only the technique and materials but also of the appropriateness of any treatment. Consultation with curators is necessary to determine the need to limit or end treatment of any manuscript. Curators should be encouraged to limit access and use of partially treated or untreated manuscripts.

7. TREATMENT DOCUMENTATION

Image capture

- Take Before Treatment (BT) images using the normal set-up in the photo studio. Add a 1" square of white card to serve as the legend for the adhesive type to be added later in Photoshop.
- Image all rectos, then all versos in order to reduce handing. Remember to change the photo file number order to match the folio sequence.
- When the manuscript is small and opens easily, capture full opening in one image.
- At the WPC, remember to follow file-naming protocol for our Conservation Records Network (ACORN), the Weissman Preservation Center database, and for the Digital Repository System (DRS).)
- Convert and save images as TIFF files.
- Store images on the H drive. H:\Preservation\Conservation\Digital Documentation Workspace\Consolidation projects.
- Create a folder titled with the ACORN #/manuscript Call number. Create sub folders for the BT and During Treatment (DT) images.

- BT files are copied and re-labeled as DT images. The DT files are annotated in Photoshop to record the consolidation work.
- The completed photo documentation includes BT files, DT files (actually marked-up BT files), and After Treatment (AT) files.
- When the manuscript is to be imaged by Imaging Services after treatment, the WPC does not take AT images of the consolidation work in order to avoid unnecessary handling of the manuscript.

Work binder

- Create a work binder for each manuscript. Multiple projects may share the same binder separated by notebook dividers.
- The binder contains at minimum: a time record sheet, the Treatment Proposal (ACORN), and print-outs in black and white of the BT images of each page being examined and/or treated for consolidation.
- If working at the WPC, follow instructions on printing the black and white images for the binder (see Appendix 1).
- The binder may also contain additional information about the manuscript such as published information on the artist or the manuscript itself. Additional material is included at the discretion of the team working on the manuscript. Additional material is intended to be summary information helpful to the treatment and understanding of the particular manuscript.

Annotating the digital image

- At the WPC, the DT images (which begin as copies of the BT capture) are annotated in Photoshop to indicate, as accurately as possible, the locations treated on each folio. Currently, the images are marked up on the background layer and flattened.
- Enlarge the image to 100-200% magnification for ease and accuracy of marking.
- Each adhesive used in treatment is color-coded differently. Acros gelatin is typically designated by the color magenta (R/G/B: 225/0/255). See Appendix 2 for other adhesives and possible color legends.
- Use the "brush tool" (not brush mixer) set at 6 pixels and 75% opacity to draw a line or dots corresponding to the location in the image where adhesive was brush applied. Give the line or dot have a hard edge (compared to a gradation).
- Use the "Lasso" tool (or draw around an area) and fill using the "paint bucket" at 50% opacity in areas where the adhesive was broadly applied. Tolerance is set at 100.
- Use a patterned fill of stripes for mist application.
- Layered TIFF files may be used to document adhesive application without altering the original image. However, it should be noted that layered files are significantly larger in size and add to the complexity of the file itself as well as to the documentation process. Moreover, long-term storage may be more costly and the functionality of layered files may not be guaranteed over time.

Marking up the paper copy

- The printed out paper copy of the image is the primary platform for day-to-day communication between team members.
- The margins are utilized to capture important information pertaining to treatment.

- Date and initial the paper copy of the folio being treated.
- Mark up the paper copy, roughly duplicating the digital record, with a red marker in the areas treated. The mark-up is not as precise or as complete as the digital record. When the illumination is large and the printed copy is small, marking the treated areas on the copy is not always feasible. Sometimes, the printed paper copy is enlarged for ease of marking.
- If treatment of the entire page was not completed during the work session, clearly indicate the portion treated so the next person knows what portion still needs to be treated.
- For complicated folios requiring re-treatment, the use of Mylar overlays to record subsequent treatment is helpful.
- If no consolidation treatment was necessary on the entire folio, write "no treatment" in the margin and draw a diagonal line across the bottom corner (if appropriate) as described below.
- Once the entire folio has been treated, draw a line diagonally across a bottom corner, preferably the bottom right. Write "ready for check" above the line, along with the date and your initials. This indicates that the folio is ready to be checked.

Documenting the double checking system

- The diagonal line with the date and conservator's initials is critical because it communicates to the next team member that the folio is now in queue to be checked for media stability.
- Areas requiring re-treatment are marked on the printed paper copy with a different colored pencil lead (often blue or yellow) along with comments. In this way, problematic areas are clearly communicated to the team.
- The checking step is a key feature of the protocol. Since all consolidation work is performed with magnification, there is no other way to verify treatment success except through the microscope.
- The checking step ensures that we have performed the best job possible.

Finalizing the digital and paper record

- After all treatment is completed, a final treatment report is written that includes specific tasks performed, materials used, and the names of the conservators who worked on the manuscript.
- At the WPC, the report and all the images (before and after treatment as well as the marked-up images in Photoshop) are archived in ACORN, the WPC's treatment record database, and uploaded into a digital repository for permanent record storage.
- The paper records are archived in a paper record file.

8. STAFF TIME COMMITMENT

Team commitment for large projects is one or two 3- to 4-hour session(s) per week.
Depending on deadlines, additional sessions may be necessary. This allows for two convenient shifts of consolidation work by different conservators per day and reduces staff fatigue.

APPENDIX 1

PHOTO DOC Printing Paper Copies of Images for the Work Binder

- Open Adobe Bridge.
- Open the folder where your images are stored. Note that the image files need to be TIFFs.
- Along the upper right edge of the screen, select "OUTPUT".
- Make sure that "PDF" is selected and that the template shows "Custom". This will print the images in the format needed for the consolidation binder (1 column, 1 row, with adequate margins for making notes).
- Select all images you want to print.
- When "View PDF After Save" is selected, a PDF of your files will automatically open.
- Select "Save". You will be prompted to choose a location to save a PDF document.
- Print as needed (you may need to manually select duplex printing, etc.).

APPENDIX 2

Color Legend in Photoshop

It is important that each type of adhesive used on each folio be designated by a different color and that the legend in the image indicates the color coding. If no treatment was required, "No Treatment" in black is entered on the color legend in the image.

When designating a color to mark up the images, set the color selection in Photoshop to "web" and select standard colors from the color bar or type in the specific RGB values.

Typical colors used:

Acros Gelatin: Magenta (R/G/B: 255/0/255)

HMM Fish Gelatin: Yellow (255/255/0)

Other adhesives and suggested colors include:

Klucel G: Green (0/255/0)

Aquazol: Orange (255/102/0)

Isinglass: Cyan (0/225/255)

Funori: Green (0/255/0)

APPENDIX 3

Preparation of Bovine Gelatin for Media Consolidation

ADHESIVE: Acros Organics gelatin, type B (alkaline hydrolyzed tissue), from bovine skin, catalog #61225, with a bloom strength of ~100

To prepare a 1.5% solution:

- 1) Transfer 0.3 g of Acros Organics gelatin into a 25-mL Erlenmeyer flask.
- 2) Fill the flask with cold or room-temperature deionized water up to the 20-mL mark.
- 3) Briefly stir with a glass rod to disperse the gelatin.
- 4) Heat in a bain-marie on a magnetized hot plate (the bain-marie consists of a small Pyrex bowl and a square, open-framed plastic jig). Elevate the flask in the jig so the bottom of the flask is surrounded by warm water.* Place a small Teflon-coated magnet in the flask. Alternatively, you can stir the solution with a glass rod intermittently. Set the temperature slightly above 40°C and the speed to ~500 rpm.
- 5) Remove from heat when you no longer observe density changes in the solution. There should be no transparent swollen granules when the flask is held up to the light and swirled. This takes ~20 minutes.**
- 6) Remove the magnet.*** Seal the flask with laboratory sealing film (Parafilm M) when not in use. Store refrigerated. Label the container with the contents, concentration, date, and preparator's initials.

*Non-deionized water may be used in the bath during solution preparation; however, **deionized water must be used while consolidating.**

** Use of hot deionized water or hot non-deionized water in the bath will expedite preparation. ***The Teflon-coated magnet can be easily guided out of the flask by placing a stronger magnet on the outside.

VARIATION: A drop or two of ethanol may be added directly to the gelatin solution to improve flow and wetting. Although not currently part of the standard recipe, this practice was common in the past and may be considered if a specific manuscript requires.

EXPIRATION: Make a fresh solution when the current one ceases to gel, loses tack, or separates. Keep no longer than one week.

MAINTAINING SOLUTION WHILE WORKING: Keep the solution in an Erlenmeyer flask in a bain-marie of warm deionized water. Elevate the flask in the square, open-framed plastic jig. Set the rheostat at 120v and 70% full output to keep the gelatin solution at ~100-105°F.

January 2018

APPENDIX 4

Preparation of Fish Gelatin for Media Consolidation

ADHESIVE: High molecular weight fish gelatin (Norland Products)

To prepare a 1.0-1.5% solution:

- 1) Transfer either 0.2 or 0.3 g for 1% or 1.5 % solution respectively to a 25-mL Erlenmeyer flask.
- 2) Fill the flask with cold or room-temperature deionized water up to the 20-mL mark, or measure 20 ml using a graduated cylinder.
- 3) Briefly stir with a glass rod to disperse the gelatin.
- 4) Heat in a bain-marie on a magnetized hot plate (the bain-marie consists of a small Pyrex bowl and a square, open-framed plastic jig). Elevate the flask in the jig so the bottom of the flask is surrounded by warm water.* Place a small Teflon-coated magnet in the flask. Alternatively, you can stir the solution with a glass rod intermittently. Set the temperature slightly above 40°C and the speed to ~500 rpm.
- Remove from heat when you no longer observe density changes in the solution. There should be no transparent swollen granules when the flask is held up to the light and swirled. This takes ~20 minutes.**
- 6) Remove the magnet.*** Seal the flask with laboratory sealing film (Parafilm M) when not in use. Store refrigerated. Label the container with the contents, concentration, date, and your initials.

*Non-deionized water may be used in the bath during solution preparation; however, deionized water must be used while consolidating.

** Use of hot deionized water or hot non-deionized water in the bath will expedite preparation. ***The Teflon-coated magnet can be easily guided out of the flask by placing a stronger magnet on the outside.

VARIATION: A drop or two of ethanol may be added directly to the gelatin solution to improve flow and wetting. Although not currently part of the standard recipe, this practice was common in the past and may be considered if a specific manuscript requires.

EXPIRATION: Make a fresh solution when the current one ceases to gel, loses tack, or separates. Keep no longer than one week.

MAINTAINING SOLUTION WHILE WORKING: In general, the solution is used at room temperature. If desired, however, the solution can be warmed to increase fluidity. This procedure is similar to that of keeping a bovine gelatin solution in an Erlenmeyer flask in a bain-marie of warm, deionized water. Elevate the flask in the square, open-framed plastic jig. Warm the bain-marie on a cup warmer plugged into a rheostat to maintain the desired temperature. The rheostat set at 120v and 70% full output will keep the gelatin solution at ~100-105°F.

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APPENDIX 5

Supplies and Tools

Microscope

Leica MZ16 microscope – Leica Microsystems <u>http://www.leica-microsystems.com</u> .63 Objective, 10x eyepieces, Ergo tube 10 deg. to 50 deg., Motor focus with inclinable column, motor focus footswitch and manual bench-top control knobs

Schott, KL 1500 LCD http://www.us.schott.com/lightingimaging/english/microscopy/products.html

Consolidation tools

Paper points

Kerr Endodontics Absorbent Points, XX-Fine (16215) - Sourced from Darby Dental <u>https://www.darbydental.com/scripts/ProdPage.aspx?grp=8540198</u>

Brushes

Brush options come and go frequently; these are our current favorites: Escoda Perla synthetic 5/0 and 4/0, round, short handle <u>http://www.dickblick.com/products/escoda-perla-toray-white-synthetic-round/</u> Escoda Barroco Toray gold 5/0 and 4/0, round, short handle <u>http://www.dickblick.com/products/escoda-barroco-toray-gold-synthetic/</u> Raphael Kolinsky 5/0 and 4/0 (Note: these brushes may be difficult to locate)

Nebulizer

Pari LC Plus nebulizer Devilbiss Traveler compressor

Delrin micro spatula – Hand shaped in-house

Delrin – 1/8" x 1" wide, sourced from McMaster-Carr http://www.mcmaster.com/#8739K11

Adhesives

Acros Gelatin type B (#61225-5000) – Sourced from VWR Scientific https://us.vwr.com/store/product/18604377/gelatin-type-b-laboratory-grade

High Molecular Weight Fish Gelatin - Norland Products https://www.norlandprod.com/