SPI Chem™ Wet Surface Replica Kit

Complete Instructions: All sections on one page

Introduction

The SPI Replica Kit for light microscopy (LM) and scanning electron microscopy (SEM), which we call the "Wet Replica Kit" was designed for those instances where a sample needs to be examined by SEM but for one or more reasons, the sample is not available (or in a suitable form) to be cut up and placed in the vacuum of the typical SEM. Some examples for which the Wet Replica kit was developed are the following:

a) A surface that cannot be dehydrated (so that it is vacuum compatible) by dehydration prior to examination, such as live human skin, plant leaf or fruit surfaces, dental surfaces such as teeth and mucousal membranes, or "wet" paper or drying paint,

b) Samples that are literally too large to fit (nondestructively) into the specimen chamber of the typical SEM,

c) Before/after studies of samples that absolutely cannot be cut up such as large industrial rollers or platens, perhaps operating at elevated temperatures, where specific areas are to be replicated as a function of time. The Wet Replica Kit system can be cured at temperatures above room temperature so time can be saved by not having to cool down the surfaces of interest to room temperature.

In all of the above cases, there is the further potential to follow the same identical area, as a function of time, in order to reveal the dynamics of change that might be occurring on a sample surface. For example, the longevity of a skin moisturizer can be followed by making replicas as a function of time. Comparisons with competitive products can be accurately made by running the same experiment on the other side of the face, hand, etc. The methodology described here is chiefly intended for preparation of samples for scanning electron microscopy, but samples made using these techniques are also fully suitable for examination using light microscopy techniques. The positive replicas can also be used for profilimetry experiments, but care must be taken that the profilimeter stylus does not dig into the relatively soft polymer of the replica.

General Methodology

Once the area to be studied is selected, the white replicating resin is mixed with an appropriate amount of the "catalyst" (see below under "mixing instructions"), and the now curing resin system is spread onto the surface to be replicated. If the replicating material has been stored to keep the components fresh, and the catalyzed resin is prepared according to these instructions, after about 20-30 seconds on the surface, the resin will have polymerized to the degree that if the test surface is in fact human skin, then changes to the outer skin surface as a result of the occlusivity of the resin itself will not be manifest in the replica.

This replica that is produced directly from the surface of interest is called the "negative" replica, "negative" because whatever goes "down" in reality, goes "up" in the replica, and vice versa. For example, a whisker coming out of a man's beard goes "up" but in the replica, it is manifest as a "hole" (e.g. goes "down").

The white powder for converting the negative to a positive replica (one that exactly replicates the original surface) can be used to replicate the replica, thereby generating a high resolution "positive" replica of the surface under study. Instructions for making the positive replicas follow below. The positive replica has an indefinite shelf life, but the negative replica may deteriorate with time, so conversion of the negative replicas to positive replicas should be done within a few days following replication.

Making the negative replicas

The area to be replicated should be selected, ideally a circular area not more than 20 mm (about 0.8 ") in diameter. If the surface need not be treated 100% non-destructively, then using a SPI # 05022-AB Diamond Scribe, an arrow or some other fiduciary mark can be applied next to the specific area of interest so that when later replicas are made from the same identical area, these identical areas can be readily located in the SEM using such landmarks.

While the optimum cure time is dependent on the quantity of catalyst used, and also the temperature, typically, several drops of catalyst in a volume of 5-10 ml of resin would result in a cure time of roughly 20-30 seconds to partial completion (no changes in the surface being replicated will be manifest in the replica) to about two to four minutes until the replica is really cured (suitable for removal and storage for conversion to a positive). We like to wait for at least 5 minutes just to make sure that the replica is not removed too soon; waiting longer will not harm the replica, although it may annoy live subjects.

To obtain maximum working time in situations where the initial curing time is not of importance, such as inert surfaces like shoes or painted metal, as little as one drop of catalyst may be used, extending the curing time to several minutes. We recommend, however, that no more than 10 ml of resin be prepared at one time because of the difficulty of intimately mixing the two ingredients without incorporating air bubbles.

The best results are obtained by literally rubbing or massaging the curing system onto the surface being replicated (both to ensure optimum mixing of the two ingredients and to reduce the possibility of the presence of air bubbles) a second layer of resin is recommended in order to give the entire negative replica more "body", which is needed to make the negative more durable for the positive replica step.

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Instructions for Use: Inspection of negative replicas

Inspection is done using a stereo zoom light microscope with optics up to 70X. While even the best of "masters" making replicas do on occasionally end up with a small area containing a few "bubbles", one should find areas that are devoid of such artifacts on all replicas of a series so that when the SEM work is actually done, an area can be selected which contains no artifacts in any replica in the series. If such an area can not be found, then we would recommend going back and making a new set of negative replicas rather than putting good time after bad and trying to resurrect good results from a basically bad results situation.

Making the Positive Replicas

We recommend the use of a hot plate, aluminum foil covered. Then onto roughly 2" squares of aluminum foil, each positive, one at a time, is put up side-up (the replica surface is the "up" side) and heated to a temperature of about 130 C. Some of the white replica powder can be placed on the now becoming hot surface. With a PTFE-coated spatula, the molten polymer is literally pressed into the white negative replica to ensure intimate contact and thorough wetting without air bubbles. Additional powder can be added until the positive replica is at least 1 mm thick. There may be some odor associated with the melting polymer, and we recommend that this step be carried out under a properly operating laboratory hood. Evolution of smoke indicates that the temperature of the hot plate is excessive.

For surfaces which are especially rough and/or with "holes" (for example, facial replicas showing whiskers), we recommend a next step of heating in a vacuum for a few minutes to pull out any entrapped air bubbles still in the negative.

The next step is to press into the molten polymer a 1" round aluminum mount (we recommend SPI #01510 1" round mounts which will fit into any universal metallographic mount holder). Pick up the whole assembly up with an ordinary pliers and plunge the entire mount and positive replica into an ice water bath.

After a few minutes for equilibration and cooling, the mount is retrieved from the ice water bath, and the remaining negative replica carefully and slowly removed from the

positive replica, which should remain attached to the aluminum mount. If the positive replica should come off at this point, we would recommend re-attaching the replica using SPI #05010-AB 5-Minute Epoxy.

Metallization of the positive replicas

We recommend sputter coating with gold, typically a bit more than the "normal" 10 to 20 nm, in order to impart maximum reflectivity when viewed by stereo zoom light microscope. Since one never looks at these positives at magnifications greater than about 700X (structure from the replicating system starts to be resolved), this additional thickness of gold coating has no deleterious effect.

Gold palladium is an acceptable metallization as well, however gold is to be preferred because of the shorter sputtering times, which reduce the exposure to radiant heat of the polymer of the positive replica.

Inspection of positive replicas for quality

The same stereo zoom microscope should be used to examine the area that was tentatively selected for examination by SEM. Make sure that the chosen area is devoid of artifacts including air bubbles. If one or more of the positive replicas shows artifacts in the positives but not in the negatives, quite possibly one can make a second positive that could be better than the first.

Special hints and tips

1. Never forget that the making of these replicas is almost more of an art than a science. Be patient. Practice, including replica conversion and microscopy, before working on unique samples or extensive protocols. It could take some time before one developed the "art" of making both outstanding "negatives" and, also, outstanding "positive" replicas.

2. Some "as presented" surfaces are not "clean". For example, take a dry skin subject with dry flaky skin on the legs. While the negative replication process is

described as being non-destructive, obviously, skin which is just on the verge of flaking off is indeed going to be picked up and in fact removed on the negative replica. This "flake" of skin has to be removed because it is going to interfere with the making of a good positive replica.

We have found that the typical positive replica is highly efficient in terms of cleaning off such debris. Therefore for such surfaces, such as for dry skin studies, we find that best microscopy results are obtained not from the first positive replica that is made but from the second.

3. The following is the earliest published literature reference using the SPI #01090-AB Wet Replica Kit.

   C.A. Garber, et al., "Characterizing Cosmetic Effects and Skin Morphology by Scanning Electron Microscopy", J. Soc. Cosmet. Chemists, 27, 509-531 (November 1976).

4. The "catalyst" will react very easily with oxygen, resulting in a loss of catalytic activity. We recommend dispensing the catalyst with a something "better" than an ordinary eye dropper, such as a small Pasteur pipette and a rubber bulb. The container holding the catalyst should be opened for the briefest possible time interval. Once the catalyst is withdrawn, the container should again be tightly stoppered.

5. The catalyst should not be put in contact with any part of the human body. It could be absorbed fairly readily with completely unknown consequences. However, impressions or what we call "negative replicas" can be made of human skin so long as a) the few drops of catalyst used in the resin are first mixed well for 5-10 seconds BEFORE being applied to the skin site and b) the resin is in a sense "massaged into" the skin, that is by the time the curing system actually is applied to the skin surface, the catalyst no longer exists in its original form and is already becoming a part of the resin as part of the curing reaction. There should not be any such thing as a free molecule of catalyst at this point.

   Since approximately 1972, members of the technical staff of Structure Probe ,

Inc. have applied this replica system to literally hundreds of human subjects as well as numerous other surfaces. Sites studied have included faces, shoulders, dorsal side (e.g. back) of the hands, lips, fingernails, legs, and even the buttocks (syringe needle puncture studies). We have successfully used the system in the oral cavity to follow the long term changes of margins and also to replicate the mucousal lining of the mouth to study the effect of mouth washes. Never in all of these instances of use of clinical subjects have we ever found any subject who has reacted in any way to the application of the replicating material.

While this has been our experience, we can not project to all subjects. We do not know to what degree there could be "reactors" in the general population and therefore like with any system being used for research purposes, the appropriate levels of caution should be observed. And in the event of even the slightest indication of any kind of reaction starting to occur, use of that human subject should be discontinued at once.

6. We have noted that when replicas are being made on many surfaces (many clinical subjects, for example) in the course of a day, the person actually making the replicas may, on occasion, develop some kind of apparent sensitivity, in the form of a slowly developing dermatitis. This is particularly noticed toward the end of the day. We have not found any kind of precaution that will keep this from happening in some persons. However, we do know that the likelihood of this happening can be greatly reduced by a) making sure that there is good airflow in the room where the replicas are being made to prevent the buildup of any kind of concentration of volatiles emanating from either the resin (which we don't think is possible) or the catalyst (more likely), and b) washing the hands with soap and water every 30-60 minutes and being especially careful to pull out from under the fingernails remains of cured silicone polymer.

   Because there is a vigorous mixing process, and always the possibility that something could splash into one's eye, we recommend the use of safety glasses at all times when replicas are being made.

7. If "hot" surfaces are being replicated, for example, the "hot" rollers in an industrial rolling plant, while there is obviously some upper limit the system can tolerate, reducing the amount of catalyst used will reduce the reaction rate so that the cure does not occur too fast.

Design of experimentss

What is provided as SPI #01090-AB is the kit of the three raw ingredients and this over view of how the kit can and should be used. Members of the technical staff of Structure Probe, Inc.'s laboratories have had almost thirty years of experience using this (and predecessor) systems for the production of replicas from wet surfaces, especially human skin. In order for a successful project there are two important considerations: First, one must know how to make the replicas. Good replica making comes with experience and practice. Perhaps even more important, however, is the protocol for actually doing a project.

For example, if the objective is to follow a patch of dry human skin around the eyes on the face, as a function of time after application of a "moisturizer", questions that must be answered would include a) what kind of controls should be used, b) how often should replicas be made, c) how many subjects should be studied to obtain statistically meaningful results, plus d) other questions that would be goal specific.

Another type of study might be to study the efficacy of a moisturizing soap and its effect on dry chapped hands and again, the same kinds of questions must be answered in order that the right kind of clinical protocol be first established.

The same follows though for one doing wear studies on floor tile, ageing studies on automobile paint systems, or even toilet bowl cleaners. The making of the replicas is one thing, the setting up of the optimum protocol is an entirely different issue.

For help and assistance in the establishment of the right protocol, you might want to consider taking advantage of the many years of experience of the technical staff of Structure Probe. Have them spend a day with you, let them show you their "art" in the making of negatives (and positives), and let them hear your technical objectives and then let them rely on their years of experience to propose the optimum protocol. The charge would be for one day of their time plus reasonable (e.g. coach) travel from West Chester, PA to the designated location and return including hotel and other associated travel expenses.