Silicone Plastination of Biological Tissue: Room-temperature Technique
North Carolina Technique and Products

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Abstract: The development of the Biodur™ S10 low-temperature plastination process thirty years ago has sparked development of similar methodologies and products. The Dow/Room temperature-process used silicone chemicals similar to the Biodur™ products. However, Dow changed the order in which the basic Biodur™ S10 plastination chemicals were combined. This combination renders the impregnation-mix non-reactive and hence deep freezers are not needed or used for impregnation. Recently, both the North Carolina and Biodur™ chemicals both have been shown to be compatible for use as a room-temperature format. The North Carolina Room-temperature technique combines the cross-linker and polymer for impregnation, but uses chain extender prior to using the catalyst after impregnation.

Key words: plastination; silicone; polymer; NCSX; NCSXI; NCSIII; NCSV; NCSVII

Introduction

Silicone plastination replaces tissue fluid with a curable polymer. Besides the Biodur™ products, other silicone polymers and chemicals, all well-known in the silicone industry (Henry et al., 2002a), have been developed for use in the plastination process (Henry et al., 2002b). Forced impregnation of silicone polymer into biological specimens is the common thread for all of the generic plastination products. Impregnation uses the same intermediary solvent (acetone) along with a decrease in pressure to extract the acetone (von Hagens, 1979a; 1979b; 1986; von Hagens et al., 1987; Henry, 2004). This resultant tissue void allows the impregnation-mix to be drawn into the specimen. Each generic product produces a durable, high quality plastinated specimen.

Chemicals used in the various “alternate” silicone-plastination processes need to be similar to the Biodur™ S10 plastination technique:
- Acetone
- Methylene chloride
- Silicone polymer
- Cross-linker, enables side to side linkage and formation of a 3-D meshwork to the elongated silicone molecules
- Chain extender, promotes the silicone molecules in formation of longer-chain silicone molecules
- Catalyst, prepares the silicone molecules for elongation and cross-linking

The general steps of silicone plastination are described earlier in this volume. This manuscript will highlight the differences with respect to this alternate process which occurs with room-temperature impregnation and curing of the plastinated specimens.

Materials and methods

The basic steps of plastination are utilized for each plastination process:
Specimen preparation dehydration and defatting
Specimen preparation, dehydration and defatting are the same for all plastination methods. Please refer to:
“The S10/15 cold-temperature plastination technique” for that information. As well, refer to the “Dow room-
temperature technique” for additional information.
North Carolina products for silicone plastination:
NCSX (lowest viscosity): silicone polymer
NCSXI (low viscosity): silicone polymer
NCSIII: catalyst
NCSV: chain extender
NCSVII: cross-linker

Forced impregnation
Replacing the volatile solvent (acetone or methylene chloride) in a specimen with a curable polymer. For this
to happen, the products must meet the same conditions as for the Biodur™ S10 plastination process.
Impregnation equipment: Similar to Biodur™ S10
requirements [vacuum pump (oil preferred) and chamber with see through port and specimen basket, vacuum
gauge, manometer, needle valves]. However, no deep freezer is required or used for impregnation.
Deep freezers are necessary and strongly recommended for dehydration.
Preparing the impregnation-mixture: NCSX or NCSXI
polymer is mixed with NCSVII (Cross-linker) at 100:8
to prepare the impregnation-mixture and stirred
thoroughly. The main difference in this room
temperature methodology is handling of the resultant
reaction-mixture. This mixture is stable (does not
become viscous) when stored and/or used at room
temperature for an indefinite period of time as is the
case with other generic room-temperature polymers.
Adjusting the vacuum: Speed of lowering the pressure
in the vacuum chamber is fast when compared to the
Biodur™ S10 Cold-temperature Technique. The NCSX
and NCSXI polymers have a much lower viscosity than
Biodur™ S10 or Dow™ PR10. The impregnation-
mixture is not reactive, provided it is not exposed to
catalyst. Therefore the polymer-mix remains fluid at
impregnation (room) temperatures. As well, since there
is no increase of viscosity which results from placement
in a cold environment. When monitoring bubble
formation, a rapid boil is recommended. Remember the
plastination principal, if in doubt, decrease pressure
slower, to prevent incomplete impregnation which can
result in shrinkage.
Impregnation regimen
Day 1: The dehydrated and degreased specimens are
removed from the solvent (acetone or methylene
chloride). Excess solvent is drained and the
dehydrated, solvent-filled specimens are placed in the
room-temperature polymer impregnation-mixture.

Submerge the specimens immediately to prevent solvent evaporation from their surface and hence,
drying. The port (glass) on the vacuum chamber is
closed and the specimens allowed to accommodate/equilibrate in the polymer-mix overnight.

Day 2: The vacuum pump is warmed briefly. Close the
needle valves and lower pressure (apply vacuum) to
seal the chamber. Lower the pressure until bubble
formation becomes rapid (decrease to around 30-25cm/13.5-13in Hg pressure). When a rapid boil is
achieved, maintain this pressure by opening the
vacuum adjustment valves slowly until pressure is
stabilized. The vapor pressure of acetone at room
temperature (+25°C) is 22cm/10in Hg and MeCl is
43cm/17in Hg. Continue to monitor the pressure and
decrease the pressure as needed to maintain a rapid
boil by closing the needle valves incrementally.
Likely adjustment will be every one half hour for the
first three or four hours.

Day 3: Continue to monitor bubble production (solvent
extraction) and keep a rapid boil by decreasing
pressure (closing the valves) as needed.

Day 4a: Small specimens - Impregnation will be
complete as noted by cessation or reduction in number
of 1-2cm size bubbles forming and/or reaching <1cm
Hg of pressure. Note: When pressure goes below 4 or
5 mm Hg, larger bubbles will rise and burst if the
chamber is tilted or shaken. These are not likely the
solvent (acetone) but likely water vapor or the cross-
linker, NCSVII vaporizing. Vacuum should be turned
off when this occurs. Turn off pump and bring
specimens to atmospheric pressure. Allow the
impregnated specimens to sit in the polymer-mix over
night at atmospheric pressure.

Day 5: Proceed to Step 4 - “Curing”.

Day 4b: Large specimens or a large quantity of
specimens. Continue to monitor solvent extraction by
watching bubble formation and by reading and
adjusting pressure to maintain a rapid boil (extraction
of the solvent).

Day 5 or plus X: Continue to monitor solvent extraction
(watch bubble formation) and maintain a rapid boil. If
impregnation is completed, as noted by cessation or
reduction of 1-2 cm size bubble formation and/or
reaching 5mm Hg of pressure, turn off pump and
bring specimens to atmospheric pressure (see note in
Day 4a). Proceed to Step 4 - “Curing”.

Rule: If 1-2 cm bubbles are actively rising to the top of
the polymer and bursting, impregnation is not finished!
Impregnation will be complete when both needle valves
are closed, pressure has reached <5mm Hg and/or 1-
2cm diameter bubble production is greatly diminished.
Note, once the 1-2cm bubbles have subsided, larger 4-5
cm bubbles will explode to the top if you shake the vacuum chamber. These are likely not acetone bubbles but either water vapor or cross-linker (NCSVI) vaporizing. Nearly complete evacuation of acetone/solvent is necessary to avoid incomplete impregnation of the specimen with the polymer-mix which may lead to shrinkage.

Day 1
Load specimens and allow to equilibrate over night.

Day 2
Start pump: Decrease pressure until rapid boil is produced (around 30 to 25cm/13.5 to 13in Hg pressure). Maintain rapid boil - Decrease pressure (incrementally, close needle valves) as needed.

Day 3
Maintain rapid boil - Decrease pressure (close needle valve incrementally).

Day 4a
Small specimens: Maintain rapid boil until boiling ceases (4-5mm Hg) or when 1-2cm bubbles cease to form, turn off pump, return to atmosphere, allow specimens to equilibrate overnight and proceed to Step 4: Curing, the next day.

Day 4b
Large specimens: Maintain rapid boil – Decrease pressure as necessary.

Day 5 + X
Maintain rapid boil until 1-2cm bubbles cease and/or 5mm Hg pressure is reached. Turn off pump, return to atmosphere and proceed to Step 4 - Curing.

Table 1: Impregnation schedule for NCSX or NCSXI (polymer)/NCSVI (cross-linker). North Carolina room-temperature silicone technique.

Specimen removal and drainage of surface polymer impregnation-mix: Follow the S10 protocol. Since the impregnation-mixture is stable, drain specimens into the room-temperature plastination chamber.

Curing (hardening or cross-linking)
Equipment for curing:
- Absorbent paper to wipe excess polymer-mix from the specimen.
- Closed environment for NCSV application via vaporization.
- Gloved finger, paint brush or mist bottle to apply NCSIII (cross-linker) to the specimen. Do not reintroduce gloved finger or brush into stock reservoir of catalyst if specimen has been touched. The stock solution will be contaminated with cross-linker and/or polymer and at first become viscous and eventually solidify.
- Foil (plastic wrap) to seal the specimen in an airtight environment and keep the Catalyst (NCSIII) next to the impregnated specimen.
- NCSV - chain extender and NCSIII - catalyst

Curing of the impregnation-mixture within the specimen is a three-step process:
Drain: Drain the excess impregnation polymer-mix from the specimens.
Chain elongation: Chain elongation of the silicone polymer molecules by end to end alignment of the molecules. Chain elongation occurs as the NCSV vapor is applied to the surface of the specimen. NCSV is vaporized in an enclosed chamber by using an aquarium pump or ventilator for a few minutes once a day for one to three days. This process is similar to the cross-linking application in the Biodur™ S10 Cold- or Ambient-temperature technique.

Day 1
Bring specimens to atmospheric pressure and allow to drain into chamber.

Day 2
Empty polymer-mix from hollow organs. Place specimens on absorbent toweling to drain polymer-mix. Wipe excess polymer from the surface.

Days 3 and 4
Manicure specimen surface, dilate and/or position anatomically. Chain extend: Vaporize the NCSV in an enclosed environment.

Day 5
Wipe excess polymer-mix from surface of specimens. Apply NCSIII to specimen surface and wrap in foil/plastic wrap.

Day 6
Unwrap specimen and examine curing rate. If necessary, apply more NCSIII and rewrap with foil. If the curing is complete, specimen is ready to use.

Day 7
Unwrap specimen and examine curing rate. If necessary, apply more NCSIII. If curing is complete, specimen is ready to use.

Day 8
Use specimen as desired.

Table 2. Curing schedule for NCSX or NCSXI. North Carolina room-temperature silicone technique.

Catalyzing and cross-linking: Catalyzing and cross-linking of the silicone polymer molecules. This reaction occurs when the catalyst (NCSIII) is applied to the surface of the impregnated specimen. It enables the NCSX or XI molecules in the impregnation-mix to react with the NCSVI which is in the impregnated specimen. Cross-linkage changes the polymer from a liquid to a solid. Mist or wipe NCSIII onto the surface of the specimen, wrap specimen with foil and leave over night. Wrapping in foil (plastic wrap) is necessary to facilitate cross-linking.
Figure 1. Lateral view of feline left thoracic limb - North Carolina Room-temperature silicone technique.

Figure 2. Bowfin fish - North Carolina Room-temperature silicone technique.

Figure 3. Auricular surface of canine heart - North Carolina Room-temperature silicone technique.

Figure 4. Dorsal view of prosected equine brain stem - North Carolina Room-temperature silicone technique.
Results
The NCSX or XI impregnated and cured specimens are dry and durable. Thin specimens exhibit some flexibility. The room-temperature plastinates are not models but real specimens (Figs. 1, 2, 3, 4). They make excellent teaching and public relation aids.

Discussion
As with all plastination processes, the room-temperature plastination produces real specimens and not models. The specimens are dry and odorless. Only time will reveal if the NCSX develops a less than transparent surface which conceals intricate surface cellular detail as seen with the Dow®/Corcoran chemicals (Henry et al., 2004; Smolukla et al., 2005a; 2005b). Room-temperature technique specimens are used as teaching aids (Latorre et al., 2001). They have been used to compile a library of specimens for normal, exotic (Fig. 2) and pathological anatomy (Henry, 2005) and is useful in research (Raoof, 2001).

Literature cited

Product distribution:
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