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

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Abstract: With the emergence of the Biodur™ S10 plastination process as the gold standard for preservation of biological tissue, similar products and processes have been developed. The North Carolina cold-plastination process is a modification of such. The alteration of the technique is when the chain extender is used: From during impregnation to prior to curing. The NCSX and XI polymers and additives (catalyst, chain extender, cross-linker) yield exceptionally beautiful and aesthetically pleasing plastinated specimens of similar quality to Biodur™ specimens. The North Carolina Cold-temperature technique is used for whole cadavers, organs or portions thereof.

Key words: plastination; silicone; polymer; NCSX; NCSXI; NCSI; NCSV; NCSVI

Introduction

Silicone plastination replaces tissue fluid with a curable polymer. Several other silicone polymers and additives, other than the Biodur™ products, have been developed to carry out the silicone plastination process (Henry et al., 2001). The basic ingredients of each process are well known products of the silicone industry (Henry et al., 2002). Principally, methods of placing the silicone into the biological specimen are similar: Using an intermediary solvent (acetone) along with a decrease in pressure to remove the acetone (von Hagens, 1986; Henry and Nel, 1993; Henry, 2004). Removal of the solvent produces a tissue void and the polymer-mix is drawn into the cells. Each of these variants of the cold temperature silicone plastination technique produces high quality durable plastinates.

Chemicals used in this “generic” silicone-plastination process are similar to the ones used in the Biodur™ S10 plastination process:
- Acetone and possibly methylene chloride
- Silicone polymer

- Catalyst to prepare the silicone molecules for elongation and cross-linkage
- Chain extender to cause the silicone molecules to form longer-chain silicone molecules
- Cross-linker to form a 3-D meshwork of the elongated silicone molecules by side to side linkage

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

Materials and methods

The basic steps of plastination are utilized for each plastination technique:

Specimen preparation, dehydration and defatting (degreasing)

Specimen preparation, dehydration and defatting are similar for all plastination methods. However, it is
necessary to prepare high quality dissected specimens to produce excellent plastinates. Please refer to: “The S10/15 Plastination Technique” for the basic, as well as, additional and more in depth information for specimen preparation and cold dehydration.

**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/methylene chloride) in a biological specimen or tissue with a curable polymer. For this to occur, the chemicals must meet similar criteria as the chemicals used in the “Biodur™ S10 Cold-temperature Technique”.

**Impregnation equipment:** Similar to Biodur™ S10 requirements - Vacuum chamber (kettle), Vacuum pump (oil preferred), Vacuum gauge, Vacuum tubing, Needle valves, Manometer, Specimen basket and Deep freezer.

**Preparing the reaction-mixture:** NCSX or NCSXI polymer is thoroughly mixed with NCSIII (Catalyst) at 100:20-30 to prepare the impregnation reaction-mixture. The reaction-mixture is stored lower than -25°C and used for impregnation in a -15°C deep freezer. The impregnation-mix is not stable at room temperature and will become too viscous over a 6-10 month interval if stored and/or used at room temperature continually.

**Adjusting the vacuum:** Pressure is generally lowered by closing the air intake valves which are located in line between the vacuum kettle and pump. Speed of lowering the pressure in the vacuum chamber is slow and comparable to the Biodur™ S10 process. The NCSX and XI polymers have a lower viscosity than Biodur™ S10 and hence, impregnation may be faster. The impregnation-mixture is reactive and therefore needs to be kept at cold temperature. When monitoring bubble formation, a slow boil is recommended. As with any plastination procedure, if in doubt, reduce pressure slower, to prevent incomplete impregnation which will result in shrinkage.

**Impregnation regimen:** Similar to the Biodur™ S10 Cold-temperature technique (Table 1).

**Rule:** If bubbles are actively rising to the top of the polymer and bursting, do not decrease pressure! It is better to decrease pressure too slow rather than too fast.

Once bubbles are active, solvent vapor pressure has been reached. More pressure decrease is not recommended until bubbling activity slows or nearly ceases. Bubbles should rise slowly but continually as simmering water, not as rapid boiling water. Acetone removal and hence polymer impregnation at cold temperature will likely take 3 to 5 weeks depending on volume of specimens and pump speed. **If** bubbles cease to rise and the pressure is >3mm Hg, close the needle valve to decrease the pressure until active bubbles start to rise again. Usually it is necessary to lower pressure only 1mm to resume active bubble production (vaporization of solvent); but it may take a few minutes before bubble production is observed.

Evacuation of acetone/solvent too quickly will result in incomplete impregnation of the polymer-mix into the specimen and result in shrinkage.

| Day 1 | Load specimens. Allow to equilibrate over night. |
| Day 2 | Start pump, Decrease pressure to: 18cm/7in Hg, by closing the needle valves incrementally. |
| Day 3 | Decrease pressure to: 8cm/3in Hg, by closing the needle valve incrementally. |
| Day 4 | Bubbles form but do not continually rise. Decrease pressure to: 5cm/2in Hg, by closing the needle valve incrementally. |
| Day 5 | If bubbles actively rise to the surface and burst, do not decrease pressure. If no bubbles rising, decrease pressure to: 4cm/1.5in Hg. |
| Day 6 | If bubbles actively rise to the surface and burst, do not decrease pressure. If no bubbles rising, decrease pressure to: 3cm/1in Hg. |
| Day 7 - Day X | Likely active bubbles, do not change pressure! When bubble formation slows dramatically or ceases, decrease pressure 1cm Hg. |

**Table 1.** NCSX or NCSXI/NCSIII - Silicone/catalyst impregnation schedule.

Impregnation at -15°C is complete when needle valves are closed and no more acetone bubbles appear at the surface of the reaction-mixture for several hours and/or near zero pressure has been maintained for a day. **Specimen removal:** Similar to Biodur™ S10 Cold-technique. Drain excess surface polymer back into the impregnation kettle.

**Curing/Hardening/Cross-linking**
**Equipment for curing:**
- Similar to the Biodur™ S10 technique. However, the chain extender (NCSV) is utilized separately after impregnation, prior to cross-linking.
Closed environment for NCSV application - The same type or same chamber as for cross-linking is used: Air-tight closed container, large enough to contain the specimens.

Aquarium pump or small ventilator (fan, spark free): To volatilize the NCSV or NCSVI.

Desiccant (CaSO₄₅): To remove moisture from the air around the specimen in the curing/chain extension chamber.

Compressed air connection, tubing and connectors: To dilate specimens and/or to evacuate polymer trapped in the organ.

Absorbent paper: To wipe excess polymer-mix from the specimen surface.

Curing, hardening and cross-linking the reaction-mix within the specimen to make the specimen dry is a two-step process. Chain extension of the silicone molecules is completed to end linkage of the silicone molecules. After the impregnated specimens have been drained of their excess polymer and wiped, the chain extender (NCSV) is applied via vaporization of the NCSV in a closed environment for two or three days. During this time, longer silicone molecules are forming. After exposure of NCSV to the specimens, the specimens should remain at room temperature for a few days to allow the maximum chain extension (slow cure). During this time the specimens must be positioned anatomically correct and dilated to assure correct position as chain extension progresses.

Cross-linking or connecting the silicone polymer molecules side to side, thus forming a firm 3-D meshwork of the silicone polymer, is caused by the NCSVI component. This reaction and process is similar to that of Biodur™ S10 cold-technique. The drained and chain lengthened specimens are placed in a closed environment with 10 - 20ml of NCSVI (cross-linker). The NCSVI is volatilized for 5 - 10 minutes which commences the cross-linking of the silicone on the surface of and in the specimen.

**Results**

The NCSX or XI cold-impregnated specimens have the same great qualities of the Biodur™ S10 cold-technique specimens, including clarity of surface detail (Fig. 1). They are dry, durable, free of offensive odors and thin specimens exhibit some flexibility. North Carolina plastinated specimens are the real specimen and not a model (Figs. 1-3). NC cold-temperature specimens are used as teaching aids both in the class room and in clinics. When assembled in a group, they make an impressive library of specimens of normal, exotic and pathological anatomy. Silicone preserved specimens are useful in research, especially to preserve findings.

| Day 1 | Bring specimens to atmospheric pressure and allow to drain in the cold chamber. |
| Day 2 | Bring specimens to room temperature to drain. Return drained impregnation-mix to freezer daily. |
| Day 3 | Allow specimens to drain. Manicure specimen surface and dilate or position anatomically. Apply the chain extender NCSV via vaporization. |
| Day 4 | Expose specimens to NCSV chain extender and manicure surface. |
| Day 5 | Allow specimens to slow cure for days to several weeks. |
| Day 6 or longer | Expose specimens to gas cure NCSVI and manicure surface. |
| Day 7 or X + 1 | Surface will be nearly dry. Manicure the surface and expose to NCSVI. |
| Day 8 or X + 2 | Likely surface will be nearly dry. Manicure the surface and expose to NCSVI. Leave in gas chamber or enclose in plastic bag or container to assure curing to the depths of the specimen. |
| Day 9 or X + 3 | Likely surface will be dry. Leave in gas chamber or enclose in plastic bag or container to assure curing into the depths of the specimen. |
| Day 10 or X + 4 | Use specimen as desired or leave contained in the closed environment. |

**Table 2.** Chain extension and curing schedule for NCSX or NCSXI silicone polymer.

**Discussion**

NCSX or NCSXI cold (Figs. 1-3) or ambient-temperature (Fig. 4) impregnated specimens have the great qualities of specimens produced by the Biodur™ S10 ambient and cold-techniques, as well as superb clarity of surface detail (Smoldak et al., 2005). They are dry, durable and free of offensive odors. As well, flexibility is inversely proportional to specimen thickness. Both cold and ambient-temperature specimens have been used as teaching aids both in the class room and the clinical setting (Latorre et al., 2001). Also, specimens have been used to compile a library of specimens for normal, exotic and pathological anatomy (Henry, 2005a; Sakamoto et al., 2006) and in research.

It should be understood that in cold, impregnation, using a deep pool of polymer >40cm, makes it difficult
Figure 1. Pathological specimen: Cat kidney - Lymphosarcoma metastasis. North Carolina Cold-temperature technique.

Figure 2. Camel liver, parietal surface. North Carolina Cold-temperature technique.

Figure 3. Camel liver, visceral surface. North Carolina Cold-temperature technique.

Figure 4. Dog heart auricular surface. North Carolina Ambient-temperature technique.
to get the pressure low enough for the solvent (acetone) to vaporize from the bottom specimens (Henry and Thompson, 1992; Henry, 2005b). The departing bubbles have to overcome the weight of the overlying polymer (von Horst, 2005). Therefore impregnation should be monitored closely.

The NCSX or XI polymer and NCSIII catalyst may also be used for impregnation at ambient-temperature. The protocol is similar to that of the “Biodur® S10 ambient-temperature technique” (von Hagens, 1986). Impregnation can be completed in one week. The same precaution with the polymer impregnation-mixture must be adhered to. After impregnation, the mix must be returned to at least a minimum of a -25°C freezer to minimize thickening of the impregnation reaction-mix.

Literature cited

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