

## COMPARATIVE EXPERIMENTAL STUDY ON GOAT LUNG FOR PREPARATION OF LUMINAL CASTING MODEL WITH USING OF TWO DIFFERENT METHODOLOGY

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### ABSTRACT

Dry 3D model is more convenient to study anatomical features of organ and luminal casting preparation is best to get bronchopulmonary tree of lungs. The comparison case study was designed in P.G dept of Anatomy(Sharir Rachana), National Institute of Ayurveda, Jaipur (NIA) on 4 samples of goat lungs to get their luminal casting where two variety,(general purpose and Germany one)of silicon polymer for filling in lumens and two different methods chemical and boiling were used to disintegrated the surrounding tissue over bronchioles. Simultaneously all the steps of casting were done on 4 samples in two group A and B where each one group having two variety of A (A<sub>GP</sub>& A<sub>G</sub>) & B(B<sub>GP</sub>& B<sub>G</sub>) that are based upon variety of used polymer. For A group KOH water based solution and for B group boiling method of disintegration was used. In result, general purpose silicon with boiling method is appropriate for getting the luminal cast of lung.

**Keywords:** Goat Lung , Luminal casting, silicon polymer, preparatory chemical and boiling method

**INTRODUCTION:** In-situ study of bodily parts involves physical and virtual dissection but microtubular connections and canalicular elements of an organ even though cannot be seen without helping of any other chemical adds. Anatomical internal features of an organ having with tubular channels like as minor and major calyx of kidney, ventricle of brain, trachea bronchial tree of lungs, vascular segments can be studied by taking a cast of the same using of luminal casting etc. Dry specimen like ear ossicle bones like hyoid, which are delicate and difficult to handle can be embedded with transparent silicon also as variety of model. Plastination is also other method for the preservation of perishable biological specimen. In-vivo study of whole canalicular channels of an organ in its 3D form is quite difficult with wet formalin preserved specimen. Due to inconveniency in handling of formalin

preserved models, different dry technique of model preparations are quite popular now a days such as casting, plastination, acrylic sheet slides model, plaster of paris model but selection of model technique is again depends upon anatomy of an organ. Based upon anatomical features of any bodily part, selection of any specific model technique can be selected. Following criteria should be checked before to select the way of any model preparation.

1. Flexibility of model
2. Total Costing
3. Maximum saving of anatomical feature on both aspect externally and internally
4. Durability
5. Visibility
6. Duration of making process

Luminal casting is one of the technique through which whole canalicular segments are molded as harden structure by sustain there in-situ connections of channels as

they are in their living status. Bronchopulmonary tree is one of canalicular airways in body and divides more at distal in various of pattern till reaching of alveoli wall. These micro channels can be studied in proper way by using this luminal casting technique of model preparation. This model technique is to preserve the intra organ tubular connection using with hardener polymer that may be silicon, RTV -615(transparent variety silicone), epoxy resin etc. Basic physical property of any hardener that must be selected for casting purpose, is the viscosity, to reach maximum at distal level of channel. After attaining its settle time to hard, further hardening carry forward with dissolving of surrounding epithelial tissue of organ. This disintegration of tissue can be applied by both physical and chemical methods but during the process of disintegration, hardening of polymer must be enhanced without effecting its chemical integrity irrespective to mode of disintegration of surrounding tissue. For this sake, temperature and safest corrosive material can be taken. As result dry 3D model of bronchopulmonary tree can be achieved but the texture of model depends upon the used polymer and method of disintegration. Various kinds of polymer can be used that lead to be different appearance of bronchopulmonary tree in its visibility and consistency of material also. To draw any conclusive point two comparative case study has been done based upon the use of polymer and mode of disintegration of tissue focusing to achieve the most distal canalicular bronchopulmonary segment by using the goat lung in P.G Dept. of Anatomy (*Sharir Rachana*)at National institute of Ayurveda. Based upon both criteria maximum

homogenous nature with human lung and previously available studies, goat lung has been used as constant factor in both case study. Every time goat lung has been chosen with criteria of weight and duration of time after death time.

#### Material

**1.Goat lung:** Average weight has been selected i.e 400-500gm with 20-25 available of tracheal rings which has been used within 2 hrs after its cut. This branching pattern similarity between the two species makes the goat lung an ideal experimental model to study human airway diseases and effect of medication on diseases like asthma (Els N. Meeusen et al, 2009). Anatomical similarities can be taken here as constant feature with this study.

**2. Polymer :** silicon general purpose and translucent Germany made available in hardener shop in market. For this study two qualities of silicon are used i.e 1. GP silicon sealant(Dr. Fixit 501 ) 2. Germany made(Dow Corning® 995 Silicone Structural Adhesive) silicon sealant. Various authors have described the use of different materials to prepare corrosion casts of the bronchial tree, for example 12% warm gelatin solution GP silicone sealant , Dr. Fixit 501 silicon sealant (Tompsett, 1970; Menaka, 2007; Prasad, 2009; Casteleyn et al., 2009)<sup>i,[6]</sup>and Dow Corning® 995 Silicone Structural Adhesive silicone sealant prepared by Germany based with comparative higher consistency and more opaque gelatin solution<sup>[7]</sup>. Since there is a continuous decrease in the luminal size as the tree divides, the material must be able to reach the terminal end up to the level of the alveoli.<sup>ii [9],[10]</sup>

3. Dissolving agent : Potassium hydroxide pellets and boiled tap water for high tem.

Methods: For both study following steps has been used as commonly and both studies carried out under the temp. of 38<sup>0</sup>-40<sup>0</sup> C with 60% humidity.

Step 1 : Preparation of specimen : A. Washing B. Drying

fresh goat lung has been collected from nearby college area slaughter house. After the cutting of animal, within in two hr fresh specimen has been taken into

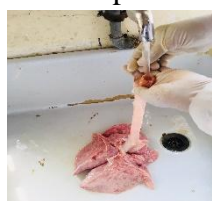


Fig. 1. Preparation of specimen by washing through tap water.

B. for 8-10 min, washed specimen has been hanged with no pressure hands so that gravity method of drainage can works here. this process of drying has been used only for proper drainage of tap water. No externally blowing elements and high tem. has been used.2

Step 2. Cutting and tying

Among whole tracheal length, one third has been kept with specimen. Length measured with counting of tracheal rings here in these both studies. For this purpose, 22-25 tracheal rings have been kept for final specimen, upper cut end is tie up with silicon gun by inserting the nosel within lumen of trachea and tie up with cotton thread at base ring of gun.

Step 3 . Filling Approaching towards the distal end of bronchioles is main purpose of filling in which gravity and pressure technique both can be used together. Rhythmical pressure and gentle squeezing towards the downward direction with pressing of lumen of trachea firstly is to be done and after that downward inclination of polymer within each one bronchiole should be checked one by one. Application

dissection hall of department and kept under the running tap water for 10 min. after flushing of all blood clots on its external surface, washing has been done internally by mild flow of tap water has been permitted into trachea. Drainage has been carried out by both gravity and squeezing with gentle pressure with both hands over lungs external surface together. 8-10 times washing has been done till minimal of blood tinged water coming out with minimal of froth.



Fig. 2 showing Squeezing and gravity method to remove the blood tinged

of gentle pressure especially keep in mind to protect the inner wall of tissue. Gentle pressure is performed in succeeding downwards direction gradually towards distal level. As more as silicon filled with in tubules of organ they palpate as flexible hardening due to consistency of polymer and more over proximal side, opaque appearance of secondary bronchiole can be seen. As gentle pressure reaches more distally at each lobe, tis flexible hardening will give confirmation of polymer filling. This process will take 40-50 min average with proper filling at more distal point.

Step 4 : Settle down period

This step is depends upon the chemical properties of silicon. More viscous polymer is take more time settle properly with the lumen of bronchioles specially more at proximal level due to their widen area. High tem. with low humidity is appropriate with silicon polymer but duration is still subject of variation. Minimum 2-3 hrs are required as found in this study. After completing of step 3 all specimen have been kept under 45-48<sup>0</sup> c

tem. for 3 hrs. over dry cotton cloth surface area.

Step 5 : Disintegration

A **chemical method** : KOH potassium hydroxide has been selected for the purpose of tissue dissolving. It is corrosive, strong base and easily soluble in water, glycerol and alcohol. For this study two different concentration of KOH water based solution (sol<sup>n</sup>) with 10% and 20 % concentration(con<sup>c</sup>) has been used for sample A. firstly sample has been kept in 20% con<sup>c</sup> sol<sup>n</sup> for 5 hours(hrs) and then after it has been put into 10% sol<sup>n</sup> next for 10 hrs. then simple tap water washing procedure has been done to removing the slough tissue. These steps were repeated for three time to total removing of surrounding tissue.

B. Boiling method : Boiled tap water in open stainless steel container with capacity of 10 lt. with dimension of 24 inch height and 15 inch width were used for this procedure. Continuous red filament heat of light heater was used. Instead of continuous boiling, segmented boiling procedure had been adopted along with drainage under tap water with brushing for 15-20 min after every 3 hours of boiling. Three rounds of same above, total 9 hrs. of boiling, all surrounding gets convert into small pieces and appears as stuck between the channels of bronchiole which needs only tap water washing. During washing process, soft bristle 2 inch wall paint brush were used to remove small pieces of tissue embedded between the bronchioles every time. This brushing method should be done very gently without making harm to silicon filled bronchioles. Although due to high temperature. elasticity and hardening doesn't get effect so that with brushing and tap water drainage method, easily slough

can be remove easily without making harm to smallest distal bronchioles.<sup>[8]</sup>

Step 6 : Bleaching : (if required) yellowish tinged of polymer may get converted into whitish with using of any simple bleaching technique. Here for this study hydrogen peroxide reagent (topical solution USP) has been used for this same with 3.5% w/v con<sup>c</sup> and for 10 hrs duration sample were put into solution.

Step 7 : drying : After getting brush like bronchopulmonary tree, drying with hair dryer was used and specimen kept under the jar.

### **OBSERVATION AND DISCUSSION**

For this study simultaneously 2 goat lungs had been filled with general purpose(GP) silicon polymer with naming of sample A<sub>GP</sub> and B<sub>GP</sub> and remaining 2 with Germany silicon polymer with naming of sample A<sub>G</sub> & B<sub>G</sub>. Among all above mentioned steps, step 1to3 applied equally to both. Difference in filling timing and distal approaching in bronchioles were observed which shown in graph 1 and pic.1 respectively. Step 5, total duration in settle down period with both variety also was found differently as shown in graph 2 which is lesser for GP polymer. Next for step 6, Both A sample (A<sub>GP</sub> & A<sub>G</sub>) were kept into KOH solution simultaneously as same as above mentioned and difference were observed. Although KOH solutions are severe irritants to skin and other tissue.<sup>iii</sup>but it commonly referred as "chemical cremation agents" as it hastens the decomposition of soft tissues, for both animal and human tissue, to leave behind only the bones and other hard elements. Entomologists wishing to study the fine structure of insect anatomy may use a 10% aqueous solution of KOH to apply this process<sup>[5],[6]</sup> . For both A group same

timing for disintegration were observed. Effect of different concentration of KOH were not found on the integrity of Polymer. As no any pieces of separated bronchioles were found mixed with pieces of slough at base of immersing sol<sup>n</sup>. Next both B sample were put into boiled water and disintegration of tissue were done with method B of above. Again duration of boiling hours and washing for both B(B<sub>GP</sub>& B<sub>G</sub>) sample were remained same. Effect of used variety of polymer with boiling method didn't observed here in this study as shown in Fig 2. In final appearance of both group of samples as shown in Fig.3 where difference was observed. In both B samples more distal bronchioles were found in comparison of both A samples. Total duration of disintegration with both group were observed same. In additional Step 6 of bleaching were used for both B sample due yellowish appearance of polymer after hours of boiling but no such kind color changing phenomena were observed with both A sample where KOH were used.

**RESULT:** Due to difference in viscosity of both variety, duration of filling and distal approaching was found differently. GP purpose sealant were reached more distal at bronchioles although gentle

squeezing had been applied same for all to excluded factor of variant. Settle down polymer timing in channels also depends upon the viscosity of polymer. More opaque is less viscous have lesser period of making hard after exposing externally. Higher concentration of KOH sol<sup>n</sup> doesn't lead to any effect on silicon polymer irrespective to changing in its variety. Due to corrosive nature<sup>iv</sup>, newly and fresh prepared sol<sup>n</sup> shows higher activity of disintegration and after that it gets slower. This slower rate may produce extra secondary purification in tissue. amount of solution depends upon the size of sample also. It should be merged with 2 times more than sample volume. Costs also matter in case of KOH when repeatedly changing of solutions are taken. This may save the total timing of disintegration also. For the boiling method, higher temperature also harden the polymer safely without distracting in its integrity but bleaching also need to be add to remove the yellowish tinged after repeated hrs of boiling in high tem. Whether KOH sol<sup>n</sup> doesn't effects the color and integrity of silicon polymer. Selection of bleaching agent shouldn't be used at the cost of integrity of polymer.

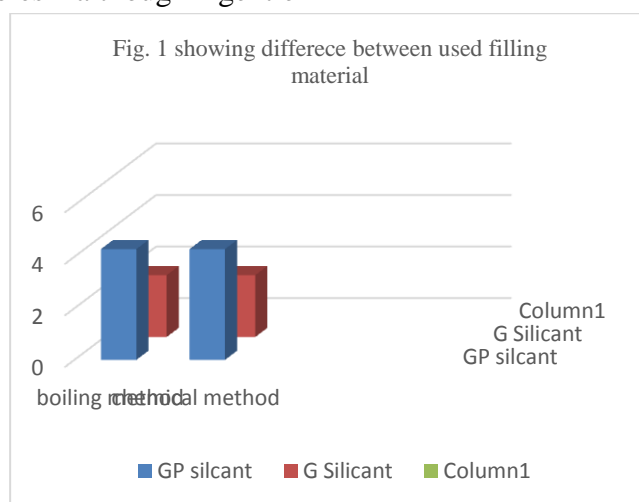
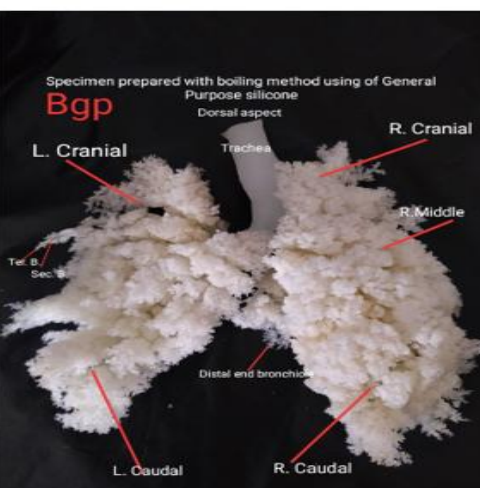
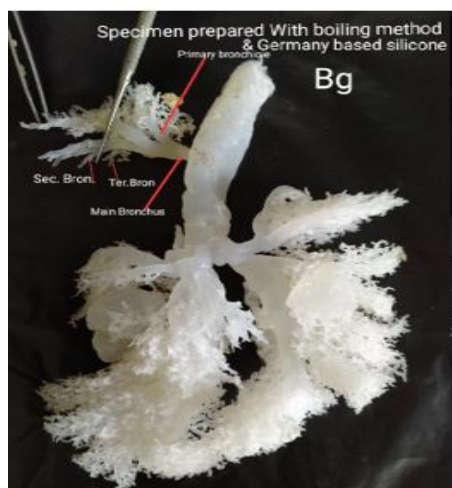
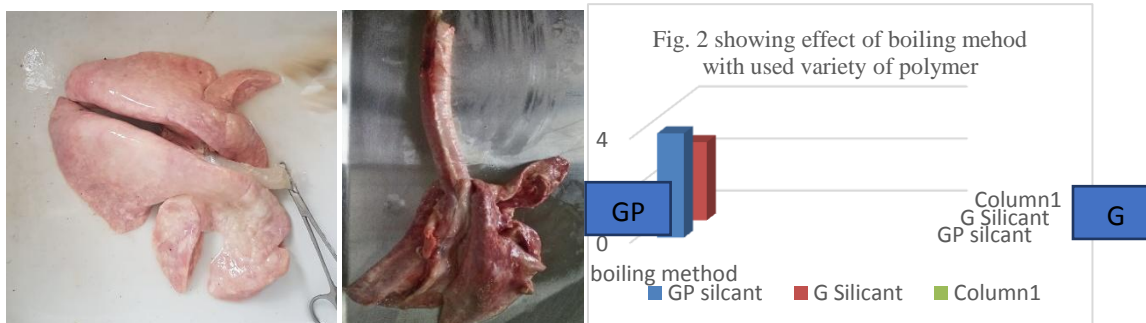




Fig. 4 Sample view after filling General purpose silicon & Germany sealant.



**CONCLUSION:** Less opaque and more viscous polymer is best for luminal casting for lungs. GP is appropriate for this as it easily reaches to distal end of bronchioles very easily rather than Germany based silicon because of less viscose propriety. Due to reduce in viscosity distal ends of bronchioles are not obtained in required

model. Use of boiling method or high temp. properly fixes the polymer in lumen so proper branching pattern well appears in comparison of chemical method emerging heat is not works as perfectly tool of fixation so distal branches may get affected. Higher con<sup>c</sup> of solution may able to work better which is not happen

properly with 20% con<sup>c</sup> sol<sup>n</sup>. Increase concentration of KOH more hastens the decomposition process. Although amount and frequency of required solution depends upon the amount of animal tissue also. Safest and cheapest method to get this type of casting is boiling method of

disintegration which is good but total duration of procedure is more comparison to KOH chemical method. Variation in con<sup>n</sup> of KOH water-based solution doesn't effects the integrity and color of silicon polymer but at the level of safety and cost not appropriate as first choice.

## REFERENCES:

- <sup>i</sup>HenryRW.2008:Tracheobronchial cast preparation .J Int. Soc. Plastination 23:30-39, V. Ramkrishna, V. Leelavathy, Corrosion Cast of Bronchopulmonary Segments, the journal of plastination vol.29.1/jp\_vol.29.1\_july17
- <sup>ii</sup>R. Ananya, S. Sangeetha , M. Karthick Ganesh Study of bronchial pattern of goat lungs by corrosion casting, Drug Invention TodayVol(issue):11:2637-2639
- <sup>iii</sup> Venkatesh G Kamath, Radhakrishna K shetty, Muhammed Asif, Ramakrishna A. A COMPARATIVE STUDY OF TRACHEO BRONCHIAL PATTERN USING LUMINAL PLASTINATION. Int J Anat Res 2013;03:161-64.
- <sup>iv</sup> <https://www.mdpi.com/1996-1944/12/17/2796/pdf-vor>
5. Thomas Eisner (2003). *For the Love of Insects*. Harvard University Press. p. 71.
6. Prasad RV. 2009: Silicone bronchial tree corrosion cast of domestic animals. Abstract in 5th meeting of the Young Generation of Veterinary Anatomists held at Dept of Pathobiology, Faculty of Veterinary Medicine, Utrecht University, Netherlands, May 21-22.
7. <http://www.crlaurence.com/DataSheets/SpecData/995silicone.pdf>
8. Rakesh Narayanan V. 2015: Preparation of low cost bronchopulmonary airway cast. J Anat Soc India 64: 162-165.
9. Menaka R. et al 2015 : Luminal Corrosion Cast of Tracheo-Bronchial Tree of Equine Lung , Veterinary Research International | July-September, 2015 | Vol 3 | Issue 3|Pages 68-70
10. Menaka R, Kelawala, NH and Vyas KN (2015). Plastination technique represents a life in biological specimens—An overview. Veterinary Research International, 3(2): 20-23
11. Michael L, Themistocles C, Loannis KT, Panagiotis K and Theodosios D (2005). Topographic anatomy of bronchial arteries in the pig: A corrosion cast study. Journal of Anatomy, 207: 427-432.
- 12.Parashuram R, Dakshayani KR and Arasi KS (2010). Silicone gel luminal cast of trachea bronchial tree. Anatomica Karnataka, 4(3): 67-72.

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