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Microcorrosion casting of the human respiratory acinus

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Abstract

Microcorrosion casts demonstrating the respiratory acini of human lungs were produced using Tensol Cement No. 70 (a methyl methacrylate mixture). Lung casts were made from foetuses of 19 weeks' gestation to term, a child of 5 years and two adults. Tensol Cement No. 70 was found to infuse the most peripheral airspaces without tissue penetration and could be airdried. Attempts using Batson's medium were hampered by permeation of the airway walls and inability to withstand airdrying. Despite the use of autopsy material, good cell impressions were seen.

At 19 weeks' gestation, the respiratory acinus is simple with only two or three generations of rather tubular respiratory bronchioles. In later intra-uterine development, the number of generations of intra-acinar airways increases and the most peripheral airspaces expand to form initially shallow but later cup-shaped saccules. At all ages, the proximal airways of the acinus have regularly-spaced deep cell impressions suggesting a cuboidal/columnar epithelium while the distal airspaces have less frequent shallow cell impressions suggesting a flattened epithelium.

Measurements of the maximum diameter of the most peripheral airspaces show an approximate doubling of size between 19 weeks' gestation and term and a further doubling by the age of 5 years.

Introduction

The normal structure and pathological changes occurring in many areas of the body can only be appreciated fully by considering the tissue in three dimensions. This is particularly true of lung tissue with its complex branching pattern and variable size and shape of the airspaces. The production of plastic casts to demonstrate the relationship of the larger airways of the lung have been produced for many years. However, similar casts of the terminal airspaces had proved difficult so that most work on foetal lung acinar development had relied on laborious reconstruction methods from serial sections (Boyden, 1965, 1971, 1974; Boyden & Tompsett, 1965).

The first recorded attempt at producing a resin cast of a human foetal lung was made by Boyden & Tompsett (1965) using a foetus of 29 weeks' gestation. They noted that the injection medium (Marco resin) reached as far as the ends of the transitional ducts but attempts to demonstrate structures distal to this resulted in penetration of the alveolar walls. Studies of casts of adult human respiratory acini have succeeded in reaching the terminal airspaces using latex (Pump, 1964), resin (Horsfield et al., 1971), and silicone rubber (Schreider & Raabe, 1981). However, these have not required the higher magnifications possible with the scanning electron microscope (SEM).

This paper presents the method and findings of an SEM study of the development of the respiratory acinus in human foetuses, with lungs from a child and two adults included for comparison.

Materials and Methods

The specimens chosen for scanning electron microscopic examination were from foetuses of gestational ages of 19 (two specimens), 22, 23, 28, 31, 32 (two specimens), 34, 36, 37 (two specimens) and 38 weeks and from a child of 5 years and adults of 23 and 58 years. The term gestational age is used synonymously with "conceptional age"; it is dated from two weeks after the last recorded menstruation and is supported by clinical, ultrasound, and post-mortem assessment of maturation. The foetuses of 19, 22, 23 and 28...
weeks' gestation were obtained from spontaneous abortions. The foetuses of 31, 32 (1), 34, 36, and 38 weeks' gestation were stillborn with evidence of acute anoxic damage. The foetuses of 32 (1) and 37 (1) weeks' gestation had both sustained intraventricular haemorrhages and died within 6 h of delivery. The other foetus of 37 weeks' gestation died in utero after an antepartum haemorrhage. The child's lung was from a 5 year old with a two year history of acute lymphoblastic leukaemia, which may have retarded growth. The adult lungs were from a man of 23 years dying from septicaemia and from a woman of 58 dying from acute myocardial infarction.

All of the subjects had a full postmortem examination supported by histological examination of the tissues. The foetuses were normal except for their immediate cause of death and the child and adults had no evidence of lung disease.

The foetal lungs were removed from the chest soon after death and inflation fixed with 10% formol saline via the trachea or main bronchus. The lungs were inflated using a hand held syringe at no specific pressure to about their normal size as estimated from the size of the thoracic cavity, the main airway was tied and the lungs were kept floating in formol saline.

After a variable fixation time (minimum two days, maximum six months) a plastic injection cast was made by infusing methyl methacrylate mixture (Tensol Cement No. 70 (ICI)) at room temperature. This requires a high infusion pressure which was achieved with a hand held syringe. The infusion was stopped after 5 to 10 minutes, when the plastic was visible beneath the visceral pleura or started leaking from a small cut made in the periphery of the lung. During the infusion and polymerization, the lung remained floating in formol saline or water to prevent distortion of the cast and to allow any leakage of plastic to disperse. This methyl methacrylate mixture has a viscosity of 1.3 Pa s at room temperature (blood plasma viscosity at 25°C = 1.3cPs) and, in industrial use, it shrinks by up to 20% during polymerization and polymerizes over about 12-24 h (manufacturer's data).

All of the casts examined in the SEM were prepared using Tensol Cement No. 70. However, the initial attempts to produce lung casts were made using Batson's medium (Polysciences Inc., Warrington, PA.) both with and without prior formalin fixation. Batson's medium has a viscosity of 2.6 Pa s at 4°C (Hodde & Nowell, 1980) and claimed shrinkage of around 1% (Lametschwandtner et al., 1984). Two casts were attempted with Tensol Cement without formalin fixation.

The lung tissues were digested by suspending the injected lung in concentrated potassium hydroxide solution for about 6 h followed by 18 h continuous washing in tap water. This was repeated until a clear cast was obtained, which took from 3 to 10 days, depending on the size of the lung. The cast was then washed in several changes of distilled water in an ultrasonicator before air drying. Small pieces of the cast from each available lung lobe were dissected off, mounted on stubs, and gold coated in a Polaron E5100 sputter coater. They were examined in a Cambridge Stereoscan Type 96113 Mark 2A and Coates and Welter scanning electron microscope at variable accelerating voltages (15-30 kV).

In most cases one lung was infused with plastic while the other was processed through paraffin wax for examination of haematoxylin and eosin sections. Since most specimens were inflated via the trachea, both the corrosion casted lung and the sectioned lung were inflated to the same extent.

The adult and child lungs were inflation fixed with formol saline through a main bronchus at a continuous pressure of 30 cm H₂O for 3 h. The main bronchus was then tied and the lung floated in fixative for 48 h. Infusion with the casting material was designed to resemble the method used for the foetal lungs as closely as possible. Thus airways were dissected until one was reached with an internal diameter of about 5 mm. This was infused with Tensol Cement with a hand held syringe and then processed in the same way as the foetal lungs.

Measurement of terminal airspace size
A mean terminal airspace diameter was estimated on each specimen by measuring a minimum of 50 terminal airspaces. In irregularly shaped airspaces, the average diameter was used. All of the foetal lung measurements were taken directly from the screen at the same magnification and specimen height using the Cambridge SEM. The microscope had only been calibrated by the manufacturer and so the measurements, although accurate as relative values, may not be totally true in absolute terms.

No terminal airspace measurements were made on three of the specimens because of severe autolytic changes in two cases (19 and 22 weeks) and evidence of intrapulmonary haemorrhage in one case of 37 weeks' gestation.

Results
In most of the casts produced by formalin fixation followed by methacrylate infusion, there was good penetration of the terminal airways but with no permeation of the interstitial tissue. Occasionally the plastic entered the superficial pleural lymphatics so outlining the periphery of the secondary lobules (Fig. 1). This did not cause any problems as it is simple to dissect these lymphatic vessel casts to reveal the underlying lung. Sometimes there were small areas where several adjacent acini supplied by a common airway were unfilled. This was probably due to either air bubbles in the plastic or debris in the airway.

The preliminary attempts to produce lung casts used Batson's medium both with and without prior formalin fixation. In both cases, although the terminal airspaces were visible in small localized areas, most of the lung became a solid mass of plastic due to interstitial tissue permeation. When Tensol Cement No. 70 was employed without formalin fixation, it failed to penetrate beyond small bronchi. This suggests that, although a high pressure was generated in the syringe, the viscosity of the plastic and the resistance encountered in the tubing leading from
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Fig. 1. A light micrograph of a cast of a whole right foetal lung demonstrating good filling of terminal airways, no permeation of interstitial tissue and focal casting of superficial lymphatics (L) (32 weeks' gestation. Bar=0.5cm).

Fig. 2. At 28 weeks' gestation, the terminal airspaces are widely separated by the relatively large amount of interstitial tissue (Bar=35µm).

Fig. 3. Higher magnification of Fig. 2 shows the terminal clusters to have the rather simple appearance of a sac with shallow outpouchings (28 weeks' gestation. Bar=10µm).

Fig. 4. By 32 weeks' gestation, the terminal saccules have larger and more cup-shaped outpouchings (Bar=50µm).

The syringe resulted in only low pressures being transmitted to the lung airways. This would also explain why lungs never increased in size during plastic infusion after formalin fixation. No attempt was made to monitor the syringe pressure during the plastic infusion because of this expected discrepancy between syringe and small airway pressures.

The choice of Tensol Cement No. 70 was made principally because of its high viscosity. However, it was found to have the advantage of being strong enough to withstand air drying and was easy to coat with gold. A minor disadvantage was that, although strong, it was brittle and had to be snapped rather than cut which made dissection slightly difficult. Batson's plastic was found to be brittle in the larger airways but liable to collapse in the smaller airways if air dried and it could also penetrate the tissues.

The plastic casts were reasonably stable under the electron beam at accelerating voltages between 15-30 kV at lower magnifications. However, if a terminal airspace was viewed for 2 minutes or more at magnifications greater than
The cast initially showed surface crack artefact which, in some cases, progressed over a few minutes to distort the cast in small areas. The rest of the cast would not be damaged.

Cell impressions were frequently clearly visible on the casts despite the potential problems of autolysis when using autopsy material. The distinction between cuboidal or columnar epithelium could often be deduced from the density and depth of the cell impressions. High epithelium produced fairly regularly spaced deep impressions (Figs. 2 & 3) while low epithelium made shallow irregular indentations (Figs. 4 & 5). Smooth dome-shaped endings with no cell impressions were interpreted as incomplete filling with plastic (Fig. 6).

The identification of the different airways in these casts is dependent on their shape, distance from the most peripheral airway and type of epithelial lining. The interpretation of cell impressions as an aid for identifying terminal and respiratory bronchioles and alveolar ducts and sacs is complicated by the changes in the epithelial lining which occur during lung development. At all ages, the terminal bronchiole, which is the airway supplying each respiratory acinus, is lined by high epithelium (columnar or cuboidal, ciliated and non-ciliated) while the most peripheral airways are lined by flattened epithelium. The number of generations of airways within the acinus increases during development. Thus, during the canalicular period (13th–25th week of gestation), there are up to three generations of airways termed respiratory bronchioles lined by high epithelium proximally and flattened epithelium distally. In the terminal sac period (26th week of gestation to term) one or two generations of short straight transitional ducts are added leading to several additional generations of saccules. In the adult, these structures are represented by the alveolar ducts and alveolar sacs. All of these additional airways are lined by flat epithelium (Boyd, 1965; 1967, 1969, 1971, 1974; Hislop & Reid, 1974). The changes in the respiratory acinus which occur after 19 weeks’ gestation have been described and illustrated in an earlier paper (Dilly, 1984). At 19 weeks’ gestation, the acinus has a simple structure with only two or three generations of respiratory bronchioles most commonly showing dichotomous branching although occasional trichotomous branches are seen. Regular deep cell indentations are seen on the cast up to about 50µm from the periphery while beyond this point the cell impressions are more widely spaced and shallower. This suggests a cuboidal or columnar epithelium lining the proximal respiratory bronchioles with a flattening of the epithelium at the periphery in agreement with histological studies (Hislop & Reid, 1974). The airspaces are visible without dissection at this age because of the large amount of interstitial tissue.

Two months later the number of generations of airways with deep cell impressions has increased and the most peripheral airspaces have expanded to form saccules. These are initially shallow with wide mouths (Figs. 2 & 3) but, during the rest of intra-uterine development, become larger and more rounded (Fig. 4). By term, transitional ducts link terminal respiratory bronchioles to the saccules. The saccules form a complex cluster of irregularly shaped pouches with shallow cell indentations (Fig. 5). The transitional duct is only present during intra-uterine and neonatal development after which it is represented by the alveolar ducts.
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Table 1: Changes in terminal airspace diameter with age

<table>
<thead>
<tr>
<th>Age</th>
<th>Terminal airspace diameter (µm)</th>
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<tbody>
<tr>
<td></td>
<td>Mean</td>
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<tr>
<td>Weeks in utero</td>
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<td>19</td>
<td>26</td>
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<td>37</td>
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<td>38</td>
<td>61</td>
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<tr>
<td>Years post natal</td>
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<tr>
<td>5</td>
<td>137</td>
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<td>23</td>
<td>199</td>
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<tr>
<td>58</td>
<td>291</td>
</tr>
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</table>

Regression line: $y=1.8x-1.18$; these relate to the intrauterine period only

Both transitional and alveolar ducts were observed to have only shallow indentations.

At 5 years of age, the terminal airspaces closely resemble those of the adult lung although they are smaller. In both there are deep cup-shaped alveoli separated by narrow clefts of interstitial tissue and alveoli open directly into alveolar ducts. None of the airways distal to the terminal bronchioles have regular deep cell impressions (Fig. 6).

The measurements of the diameter of the most peripheral airspaces are presented in Table 1. These are obviously influenced by the extent of inflation with formalin and shrinkage of the plastic during polymerization but do illustrate the relative changes in size. There is a clear increase in terminal airspace diameter from 19 weeks' gestation to term with an approximate doubling of size during this period. By the age of 5 years, the size has more than doubled again but is still smaller than in the adult.

Discussion

This study has aimed to produce microcorrosion casts of human autopsy lungs suitable for examination in the SEM. The problems of casting lung airways are rather different to casting blood vessels. First, it is more difficult to simulate physiological conditions in the lung as the lung normally inflates by negative pressures and the enlargement of the airspaces is influenced by the air/water boundary and the role of surfactant. In blood vessels it is at least theoretically possible to use a perfusion pressure and perfusate comparable with that in life. Second, the airways are blind-ending with no free run-off for the casting material so greatly increasing the potential problem of extravasation.

A two-step approach has been adopted in this study so that these two problems could be tackled separately. The first step was to fix the airways in an inflated position using formalin and the second was to infuse the airways with plastic.

Formalin fixation of the specimens before casting provided numerous advantages. Most importantly, it allowed good control over the amount the lung is inflated. This is especially relevant when the results are to be compared with other studies, since the most common method for preparing human autopsy lungs involves formalin liquid or vapour fixation. Other authors who have measured terminal airway size have varied slightly in their inflation technique. Dunnill (1962) and Boyd & Tompsett (1965) inflated the lungs to approximately the size of the thoracic cavity but used no specific inflation pressure while Davies & Reid (1970) used an inflation pressure of 75cm of water and Langston & Thurlbeck (1982) a pressure of 25cm of formalin. The use of a set inflation pressure for foetal lung studies is at best arbitrary and may be misleading since the compliance of the terminal airspace walls probably changes during development as the amount of interstitial tissue decreases.

Since the airways were fixed open by formalin fixation, it had the advantage of making it easier to recognize any failure of the plastic to reach the terminal airspaces. These appeared as smoothly rounded, dome-shaped ends with no cell impressions. If prior formalin fixation is not used, then the plastic will be imprinted by the cells of the non-expanded airway making it harder to recognize poorly infused regions.

If both lungs are inflation fixed but only one lung is infused with plastic, it enables the non-casted lung to be assessed for any pathological or autolytic changes which might influence the interpretation of the cast. This is particularly important when using human tissue where the precise cause of death is only determined at autopsy. Finally, since fixed lungs can be stored almost indefinitely, it greatly increases the number of specimens which can be used and allows the collection of pathological material.

The only disadvantage of fixation before casting is that it is said to be more difficult to digest the tissue afterwards and to remove all the debris (Hodde & Newell, 1980). In this study there was no problem in digesting the lung tissue to produce a macroscopically clean cast although there was often microscopical debris left.

It has always been a problem to infuse precisely the right amount of casting material to reach the terminal airspaces, but not so much that it penetrates the tissue. One approach is
to calculate the required volume from physiological data. Schreider & Raabe (1981) produced silicone rubber casts of two adult lungs by infusing a set volume of material with the lungs still within the thoracic cage. They also made small incisions through the chest wall to release the negative pressure, aiming to reproduce the state of the airways at the end of inspiration. However, not all alveoli were fully expanded and only the best areas could be evaluated.

Physiological data is not available for the stages of foetal development investigated in this study and so infusing a calculated volume was not possible. An alternative approach was tried of reducing the likelihood of tissue penetration. Matsusaka & Fujibashi (1974) suggest that pre-fixation of blood vessels reduces extravasation and so here was another reason for prefixing the lungs. A thicker casting material was felt less likely to leak out of the airways and so only Batson's medium and Tensol Cement No. 70 were used because they were known to have high viscosities. A recommended property of the injection media for casting vessels (criterion 1 in Hodde & Nowell, 1980) is that it should have a sufficiently low viscosity to allow filling of tubules of less than 5µm but this is obviously unnecessary when casting airways. It is interesting that the Tensol cement rarely penetrated airway walls while Batson's medium commonly did. Obviously it is not only viscosity which influences tissue penetration in agreement with comments by Lametschwandtner et al. (1984). Most of the increase in volume of the respiratory acinus which occurs with age is produced by an increase in the number of alveoli (Dunnill, 1962). However, as demonstrated in this and previous studies (see below), there is also an increase in terminal airspace size. The problem of what is the appropriate amount of inflation applies to all studies, but the three dimensional image of corrosion casts is more suitable for measuring maximum widths than the two dimensional cross-section which will cut rarely through the widest point. Langston & Thurlbeck (1982) found a wide range of mean "airspace" sizes measured in 26 foetuses from 19 weeks' gestation to term. The means ranged from 28µm to 128µm with a trend towards larger mean airspace diameter with increasing gestation (correlation coefficient=0.67). Alveolar size at birth has been reported as 150µm (Dunnill, 1962) and 80µm (Thurlbeck, 1982) with the range in a 2 day infant being from 40-120µm (Boyden & Tompsett, 1965). In prepubertal children, the reported sizes are 230µm (age 4 & 8 years (Dunnill, 1962)), and 90-95µm (3-6 years (Thurlbeck, 1982)). Adult alveolar measurements have been made on sectioned material (280µm (Dunnill, 1962)) and from injection replicas (250µm (Schreider & Raabe, 1981)); 212µm length, 205µm width (Pump, 1964)).

The alveolar measurements presented in this paper are similar to these values (other than Dunnill's) although having a tendency to be slightly smaller.

SEM studies of animal lung tissue have found latex and cementex are useful airway injection media and have used these to demonstrate the relationship between the airways and small blood vessels by double infusion techniques (Nowell et al., 1972). The cells surrounding the airways have been visualized by delicate removal of connective tissue using trypsin and ultrasonication (in Lametschwandtner et al., 1984). Although human autopsy material will always suffer from some degree of autolysis, the present microcorrosion cast study has demonstrated that airway detail is reasonably preserved and that it is feasible to use this technique on pathological tissue. Double infusion techniques and enzymatic digestion of human tissue would be an interesting future development.

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References


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Discussion with Reviewers

D. Schraufnagel: How soon after death were the specimens obtained? What changes occur in the casts or casting procedures with increased time from death to fixation?

Author: Autopsies were generally performed within 24 hours of delivery or death but always within 48 hours. In the case of stillbirths, it was not known when death had occurred prior to delivery. For this reason, it was extremely useful to cast only one lung so that the other lung could be sectioned and assessed for autolytic changes. Clear cell impressions were easier to identify on the casts produced from well preserved lungs. There was no obvious relationship between post mortem epithelial autolysis and leakage of the infused plastic.

D. Schraufnagel: We have casted the alveoli of rats with Mercox with satisfactory results. However, a problem that we had is that alveoli are so tenuously connected that when we sliced the peripheral lung the alveoli crumbled away. Did you encounter a similar problem?

Author: Yes, the alveoli did crumble easily. I did not slice the peripheral lung but would deliberately crumble some airways in order to gain access to an adjacent terminal bronchiole or more proximal airway. This could then be cut with fine scissors and one or more acini lifted out without damaging the alveoli.

A. Johansson: Is it possible to give values of the size of terminal airspaces as inflation of the lungs at fixation should be rather arbitrary and as the number of foetuses of each gestational age as well as number of autopsies are small?

Author: The arbitrary and probably variable inflation of the lungs is an unavoidable problem which applies to any morphometric study of the respiratory system. The values for the sizes of the terminal airspaces were included because, although only 10 foetal cases were assessed, a significant relationship was found with gestational age. The number of cases cannot compare with the excellent prenatal series of 26 cases by Langston and Thurlbeck (1982) and Thurlbeck's (1982) postnatal series of 56 cases. However, other studies of the respiratory acinus have only limited material. Dunnill (1962) measured the alveolar size of one case at birth, 7 cases between birth and 2 years and 1 case at 4 years and one at 8 years. Boyden and Tomsett's (1965) measurements relate to a single 2 day old infant and the two adult studies using corrosion casts have been based on only one (Schreider and Raabe, 1981) and 2 cases (Pump, 1964). (Schreider and Raabe produced two corrosion casts but only made measurements on one.)