

Custom-built tools for the study of deer antler biology

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1. ABSTRACT

Deer antlers can be developed into multiple novel models to study growth and development of tissues for biomedical research. To facilitate this process, we have invented and further refined five custom-built tools through three decades of antler research. These are: 1. Pedicle growth detector to pinpoint the timing when pedicle growth is initiated, thus stimuli for pedicle and first antler formation can be investigated and identified.

2. Thin periosteum slice cutter to thinly slice (0.2mm or 0.7 mm thick) a whole piece of antlerogenic periosteum (AP) or pedicle periosteum (PP), which facilitates gene delivery into cells resident in these tissues, thus making transgenic antlers possible. 3. The porous periosteum multi-needle punch to effectively loosen the dense AP or PP tissue. This allows most cells of the periosteum to come into direct contact with treating solutions, thus

making artificial manipulation of antler development possible. 4. The intra-dermal pocket maker to cut the thin dermal tissue (less than 2 mm in thickness) of a male deer calf horizontally into two layers to make an intra-dermal pocket. This allows loading of AP tissue intra-dermally to test the theory of "antler stem cell niche" *in vivo*. 5. The sterile periosteum sampling system to allow aseptic collection of the AP, PP or the antler growth centre tissue on farm, thus allowing antler generation, regeneration or rapid growth to be investigated *in vitro*. Overall, we believe the application of contemporary cellular and molecular biological techniques coupled with these custom-built tools would greatly promote the establishment of this unique and novel model for the benefits of biomedical research, and hence human health.

2. INTRODUCTION

Animal models play a critical role in revealing the mechanisms underlying growth, development and pathological processes. Deer antlers are unique mammalian organs that hold the potential to be developed into multiple novel biomedical models. The annual renewal of antlers offers the only opportunity to explore how nature has bestowed full organ regeneration in mammals (1-3). The unprecedented growth rate of antlers (up to 2.7.5 cm per day) provides a rare system where fast cell proliferation is elegantly regulated without becoming cancerous (4-6). The atypical features of antler stem cells, such as expression of key embryonic stem cell markers (i.e. Oct4, Nanog and SOX2) and multi- or pluri-potency, serve as an invaluable model for understanding how embryonic stem cell attributes are retained in the postnatal animal cell populations (7-9). The rapid scar-less healing over a large wound (up to 8-10 cm in diameter) on top of an antler pedicle stump presents a singular case for understanding the mechanism underlying perfect wound healing in adult mammals (6). The remarkable ability of self-differentiation and regulation of antlerogenic periosteum (AP), within which antler stem cells reside, offers a novel system for the exploration of morphogenetic fields in organogenesis in mammals (10-12).

While contemporary cellular and molecular techniques need to be applied to successfully establish the antler model for biomedical research, we found proper methods for antler stem cell tissue sampling, handling and processing are also indispensable. Here, we describe five custom-built tools that have been used to achieve the aforementioned purpose by our group during over three decades of study on antler biology.

3. PEDICLE GROWTH DETECTOR

3.1. Purpose and principle

In the study of deer antler development it is often necessary to know the precise timing when

a pedicle (the permanent bony protuberance from which an antler grows and casts) begins to grow from the frontal crest (future pedicle growth region) of a male deer calf. Regardless of the experience of the operator, it is very difficult to judge by palpation or observation whether a pedicle has begun growing. This is because the size of the frontal crests of deer calves varies considerably in different individuals, and touch alone is not sufficiently sensitive to detect a change in height of a millimetre. Therefore, a practical tool is needed for this purpose, especially if this is a critical parameter, such as investigating the effects of unilateral denervation on deer pedicle initiation (13), or testing whether a hormone (such as testosterone) plays a role in advancing antler development (14). We developed the pedicle growth detector for achieving this purpose (for more details refer to (15)).

The cranial surface area of a future pedicle growth region is reasonably large (25–30 mm in diameter in red deer or sika deer); hence a small change in the height of a slow-growing pedicle will be magnified in its change in volume. If this change can be transduced in shape (large area and short height) to a measurable form (small area and large height), small changes in height of the crest will be detected with reasonable precision. In our design, we chose water as a medium to transduce the shape of the crest or newly formed pedicle tissue. The advantages of using water are: liquid water changes shape readily, water's volume is not sensitive to temperature in operational conditions of 10-30°C, and water has a low viscosity. If the change in volume between consecutive measurements is statistically significant, the pedicle that is measured has begun to develop.

3.2. Construction

Figure 1A describes the construction. For further details see (15).

3.3. Application

Place the detector on the zeroing block. Use a syringe through the rubber stopper to adjust the water level (in blue for easy observation) in the pipette to the zero point (Figure 1B). Restrain the deer either in a hydraulic cradle or through an aesthesia. Completely shave the region around the frontal crest in a 30 mm radius (Figure 1C). Stand on the right side of the deer to measure the left frontal crest/pedicle and the left to measure the right side. Locate the depression on the midline between the orbit and the apex of the crest, and place leg 1 of the detector in the depression (Figure 1D). Align leg 3 of the detector with the external auditory meatus of the opposite ear and rest it on the scalp skin, overlying the hard frontal bone. Tighten the two knurled screws of legs 1 and 3 with leg 2 just touching the scalp (the heights of legs 1 and 3 must remain secure for subsequent readings, as these

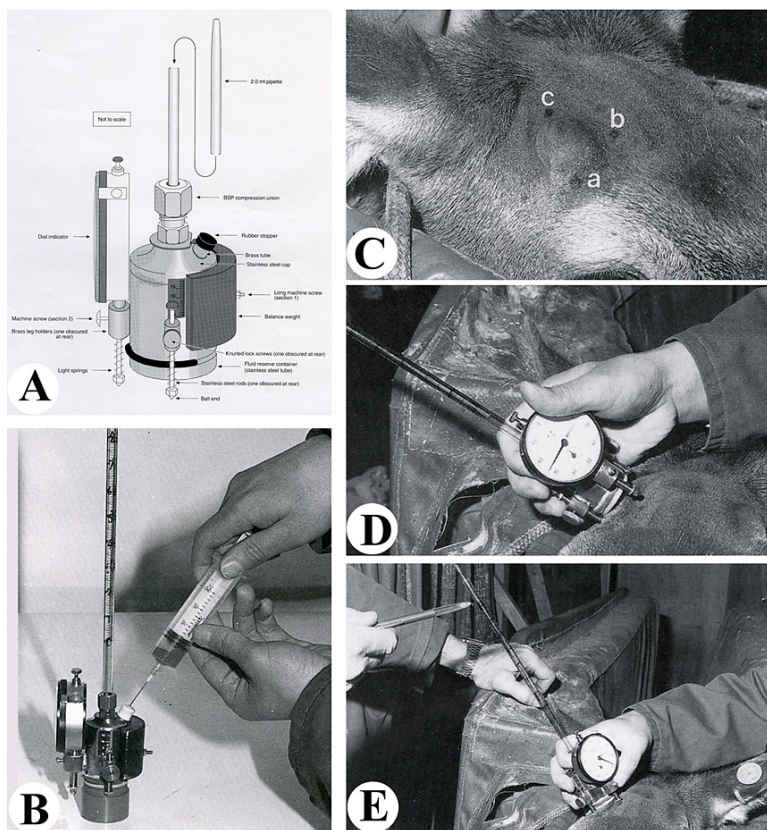


Figure 1. Pedicle growth detector. A, Drawing of the detector. B, Detector on the zeroing block, and adjustment of water level in the pipette by injecting or withdrawing water from the container. C, Three points on the head of a young deer, the labels a, b and c are for legs 1, 2 and 3 of the detector respectively. D, Leg 1 of the detector is placed on the depression on the midline between the orbit and the apex of the crest. E, Three legs of the detector rest on the deer scalp skin, recording the measurement on the dial indicator and the water level position in the pipette.

heights will be suitable for all deer whose crest/pedicle heights are lower than 5 mm). Using legs 1 and 3 as a fulcrum, rotate the detector forward until it is about perpendicular to the head. Record the measurement on the dial indicator so that the same pressure will be applied on the three legs of the detector in subsequent measurements. A second person must record the water level in the pipette (Figure 1E). Mark the point on which each leg stands with a permanent pen or tattoo ink (Figure 1A). Place the detector on the block again to confirm that the water level in the pipette is still at the zero position. Repeat the procedure 3-5 times with the detector placed in the same level position. Record the water level in the pipette each time, calculate the mean and standard deviation, then compare with the previous measurements. The date of pedicle initiation is determined by a statistically significant change in the water level.

3.4. Comments

Our pedicle growth detector can detect a change in pedicle height of 0.6.7 mm. Since palpitation can only detect changes in the crest/pedicle height of at least 5 mm, the sensitivity of the pedicle detector is therefore at least 7.5. times better. However, because

the detector is so sensitive to volume change, the detection precision could be seriously affected by inaccurate use. Therefore it is crucial that the three points on which the detector's three legs stand must be marked accurately and the detector placed precisely each time. Also the hair over the area on which the rubber membrane of the detector rests must be shaved each time when the detector is used.

4. THIN PERIOSTEUM SLICE CUTTER

4.1. Purpose and principle

The identification and functional analysis of molecules that control and regulate antler generation (postnatal organogenesis) and regeneration (full mammalian organ regeneration) is critical to establish antler as a model for biomedical research. Antler development is fully reliant on a piece of periosteum, called antlerogenic periosteum (AP). Deletion of the AP abrogates future antler growth and transplantation of the AP elsewhere on the deer body induces ectopic antler formation (16, 17). Cells resident in the AP express key embryonic stem cell markers and can be induced *in vitro* to differentiate into multiple cell lineages, hence are termed antler stem cells (8). Identifying the

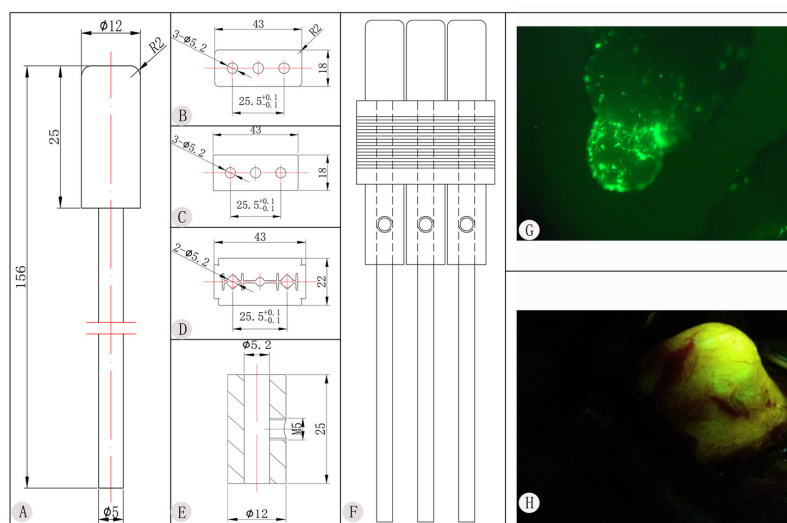


Figure 2. A-F, Diagrams of the components of the thin periosteum slice cutter; G, Green fluorescent protein (GFP)-infected AP tissue for in vivo grafting; H, A small glowing antler bud derived from the grafted GFP-infected AP tissue.

molecules that maintain the stemness of antler stem cells and trigger the initiation of antler generation or regeneration is a focus of our research (18).

In vitro and *in vivo* models are needed for verifying the relevancy of these identified candidate molecules, but the ultimate proof would have to be the deer model itself. To establish an *in vivo* deer model, ideally to deliver the genes or their RNAi-constructs of the candidate molecules directly into antler stem cells for evaluating the effects of gain- or loss-of-function of a gene on subsequent antler generation or regeneration. However, due to the location of antler stem cells (underneath skin) and the nature of the tissue (tough fibrous periosteum), accessing these cells for gene delivery *in vivo* is not practical. Furthermore, although the gene- or RNAi-constructs can be readily delivered into singularly cultured antler stem cells, these disaggregated cells can no longer initiate antler generation or regeneration when grafted back to the deer (unpublished data). Interestingly, grafted antler stem cell tissue, even if finely minced, can readily initiate ectopic antler formation (16, 17).

Here we describe a unique tool, a thin periosteum slice cutter, for achieving evenly sliced thin AP or pedicle periosteum (PP, stem cells for antler regeneration) sections just from one cut (Patent No: ZL201420335401.8.). In our previous study, we found that thin sections of antler stem cell tissue coupled with lenti-viral vector delivering system can satisfactorily achieve the purpose of *in vivo* gene delivery (unpublished, see Figure 2).

It is well known that lenti-viral vector can deliver genes into non-dividing cells in tissue, provided the tissue is cut sufficiently thin to allow the

reagents to readily reach majority of the cells. We showed that 0.2. μ m-thick-AP-slices still retain the antlerogenic potential to initiate ectopic antler growth when grafted back autologously and at the same time Lenti-viral vector system can deliver genes to most of cells resident in the AP tissue sections at 0.2. mm thickness (unpublished, see Figure 2). We have successfully grafted green fluorescent protein (GFP)-infected AP tissue slices to initiate antler growth and form a small glowing antler bud (unpublished see Figure 2).

4.2. Construction

The cutter consists of five parts: three sliding rods, a number of double-sided razor blades (e.g. Gillette), a number of partition plates, two stop plates and three sliding sleeves (Figure 3A). These parts are all made of stainless steel, unless otherwise mentioned. A sliding rod consists of 5 mm diameter steel of 131 mm length, plus an end of 12 mm diameter steel of 25 mm length. A stop plate (length 43 mm; width 18 mm; and thickness 5 mm) has three holes drilled in it matching those of the razor blades (see Figure 3A). The partition plates have the same shape as the stop plates, but much thinner (either 0.7 mm or 0.2. mm depending on the purpose). A sliding sleeve (length 25 mm; internal diameter (i.d.) 5.2. mm; and outside diameter (o.d.) 12 mm) has a hole (i.d. 5 mm) drilled in the middle of the shaft for installing a lock screw (Figure 3A). All parts of the cutter can be autoclaved.

For assembly, place the three sliding rods vertically with the thickened ends resting on a flat surface. Firstly slide a stop plate into the three rods, then a razor blade and a partition plate. Repeat

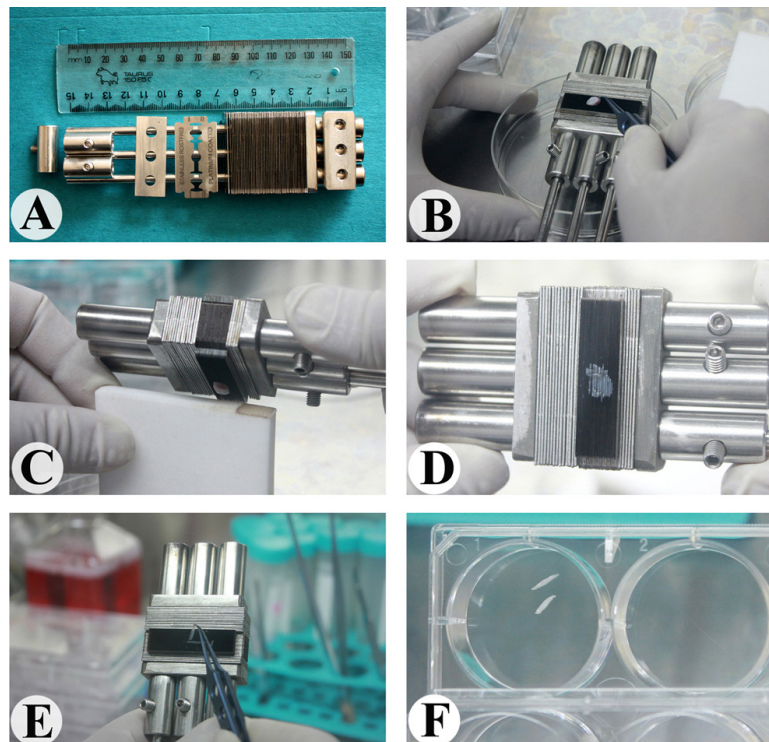


Figure 3. Thin periosteum slice cutter. A, Components for constructing the cutter. B, A piece of periosteum tissue on the razor blades of the cutter. C, The cutter held in a position whereby the attached periosteum faces the Teflon stage. D, The periosteum tissue after cutting. E, A piece of cut thin periosteum slice picked up using a pair of forceps. F, Two pieces of thin periosteum slices in one of the wells of a 6-well-plate.

alternating razor blades and partition plates until the width of the assembled blades is sufficient to cover the tissue to be cut. Slide in the second stop plate before putting a sliding sleeve into each rod and fasten the lock screw of each sleeve using an Allan key to ensure the assembled blades and partition plates are held together tightly.

4.3. Application

To cut a piece of periosteum, a separate cutting stage is needed (ideally use Teflon). The stage has length, 5.5. mm; width, 5 mm; and thickness, 5-8 mm (depends on the width of the tissue). Transfer a piece of periosteum (AP or PP) sampled under a sterile condition onto the surface of the blades using a pair of fine tweezers (Figure 3B) and turn the cutter upside down to allow the attached tissue rest on the Teflon stage (Figure 3C). Push the cutter hard downwardly against the stage to let the blades cut through the periosteal tissue. Lift the cutter (Figure 3D) and use fine tweezers to transfer each tissue slice lying in the space between every two blades (Figure 3E) to a 6-well-plate for further treatment (Figure 3F).

4.4. Comments

The number of razor blades and partition plates can be altered according to the width of the

tissue to be cut. The dimension of the Teflon stage can also be adjusted accordingly. Our experience is that the thinner the stage, the easier it is to cut. In addition to preparing tissue for *in vivo* gene delivery, the cutter can also be used for preparing tissue for primary cell culture via enzymatic release of cells from the sampled tissues. Since our cutter can cut tissue blocks into even thin sections (from only one cut), the release of cells would be more consistent across all sampled tissue blocks.

5. POROUS PERIOSTEUM MULTI-NEEDLE PUNCH

5.1. Purpose and principle

An alternative way to increase the efficiency of delivering molecules, RNAi constructs or vectors into AP or PP cells as opposed to cutting tissue into thin slices, is to make a whole piece of periosteum more permeable to solutions and/or reagents. The advantage of this approach over using thin periosteal slices is that antler development can occur in the same season as the AP implantation. Although the delivery efficiency can be higher with the thin-slice-approach, antler growth may only be initiated in the spring of the following year at the earliest. Which way should be selected for the creation of a genetically modified antler depends on the purpose. The best approach to make

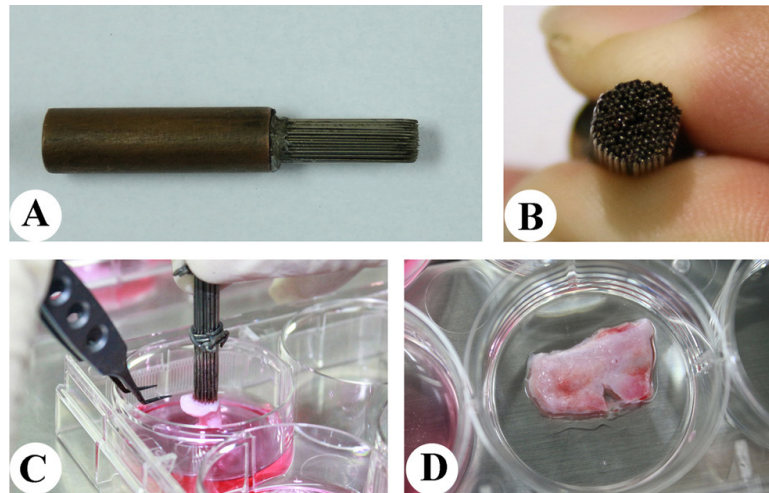


Figure 4. Porous periosteum multi-needle punch. A, A multi-needle punch. B, View of the puncher from the plane of needle points. C, Needle points of a punch rest on a piece of periosteum. D, Hole mark left on a piece of periosteum after punching.

periosteal tissue more permeable is to change its nature from dense to being more porous. Here we describe a simple, but very effective multi-needle punch tool to achieve this purpose (Patent No: ZL201420244482.0.).

5.2. Construction

The punch consists of two parts: a bundle of thin needles (length 56 mm; diameter 0.3.5 mm) and a copper-tube-handle (length 28 mm; diameter 12 mm; wall thickness 2 mm) (Figure 4A). The shape and size of the punch (including the handle) can be altered according to the particular piece of periosteum and/or tissue to be worked with. A bundle of needles, consisting of certain numbers (we used 120), are forcefully inserted into the handle to make them tight, and then welded together at the base with tin solder after making sure that all the needle points are positioned at one plane (Figure 4B). Based on this design, the distance between every two needle points is approximately 0.7. mm (Figure 4B).

5.3. Application

Transfer a piece of periosteum (AP or PP) sampled under sterile condition to a 6-well-plate using a pair of fine tweezers, and place the punch on the periosteum with the needle points resting on it (Figure 4C). Push the punch hard downwardly to allow the needle points to penetrate through the tissue until the needle-points touch the plastic surface of the well. Repeat the process until the entire surface of the periosteum is properly punched (Figure 4D). Add culture media and/or reagents into the well to soak the punched-periosteum.

5.4. Comments

Theoretically, cell damage in antler stem cell tissue should be negligible through this type of

punching as the size of each periosteal cell is around 10 μm (19) and the distance between any two needle points is around 700 μm . The punched-periosteum should still possess sufficient number of viable cells for initiating antler growth. Thus far we have tried this approach for the investigation of the relationship between AP cell polarisation and antler morphology. Repeated punching on a piece of AP using our multi-needle punch did not impair the antlerogenic potential for the AP, which initiated the antler growth in the same year after grafting autologously (Figure 5).

6. INTRA-DERMAL POCKET MAKER

6.1. Purpose and principle

Although both antler generation and regeneration are stem cell-based processes, to initiate these processes antler stem cells must interact with their niche cell types, i.e. the enveloping skin cells (20–22). The underlying mechanism of these interactions is thus far not well understood, however, dermal papilla cells in the hair follicles may mediate the process (Goss, '87; Li *et al.*, 2008, 2009). One of the most effective ways to confirm this involvement is to by-pass the interposed tissue layers between the two interactive tissue types (i.e. subcutaneous connective tissue and the inner dermis) to deliver antler stem cell tissue directly underneath the hair follicles. The outcome of subsequent antler generation is then compared to subcutaneously transplanted approach. If hair follicles were to mediate these interactions, less quantity of antler stem cell tissue would be needed to successfully initiate ectopic antler generation. This is because the intradermal transplantation approach no longer requires the implanted AP to build up sufficient tissue mass for creating the close contact with overlying skin, which is required for the subcutaneous implantation

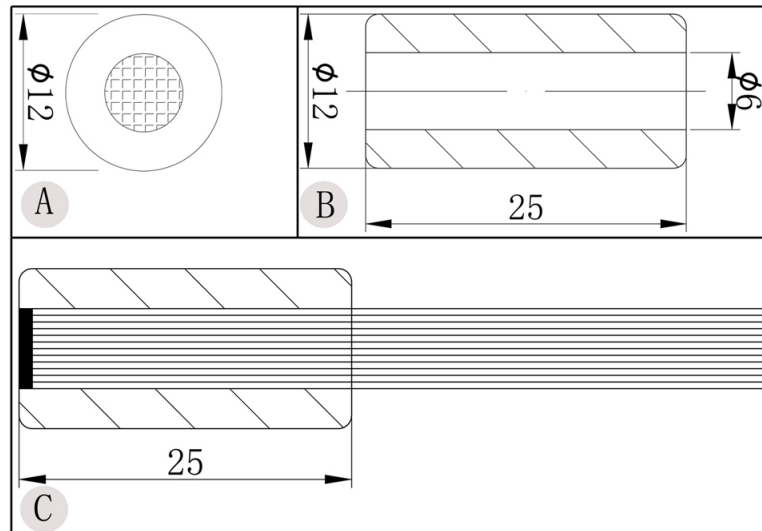


Figure 5. Diagrams of the porous periosteum multi-needle punch. A, Top view of the punch; B, Lateral view of the punch handle; C, Lateral view of the whole punch.

approach (23). Only one fourth (unpublished data: 1/6) of the AP tissue mass was required to initiate ectopic antler growth when being delivered directly underneath the hair follicles compared to the case of subcutaneous transplantation (17). To deliver antler stem cell tissue directly underneath the hair follicles an intradermal pocket in the scalp skin needs to be created. Since the scalp skin of a yearling stag is very thin (around 2 mm) and touch, it would be almost impossible to precisely cut an intradermal pocket without damaging hair follicles or puncturing the pocket simply by using a handheld scalpel. Therefore, we designed a custom-built intradermal pocket maker (Patent No: ZL201410281710.6.).

6.2. Construction

The pocket maker consists of two main parts: a base and a handle (Figure 6A). For the base: 1) one stainless steel sheet: width, 40 mm; length, 103 mm; thickness, 1.3. mm; with the rounded corners at one end and a centrally located slot (Figure 6A). The slot is 5 mm wide and 50 mm long, and located at the rounded-corner-side (8.1.2 mm to the end). 2) Two stainless steel clamps, each of which consists of five parts: frame, supporter, upper grip, lower grip and tightness adjuster. The frame is a piece of angled stainless steel: width, 16.0.0 mm (each side, 8 mm wide); length, 70.0.0 mm; and thickness, 1.3. mm. The supporter consists of two stainless steel tubes: i.d., 2.7.0 mm; o.d., 6.3.5 mm; and height, 10.2.0 mm. The upper grip: width, 6.2.0 mm; length, 50.3.0 mm; and thickness, 4.2.0 mm. The lower grip: width, 6.2.0 mm; length, 50.3.0 mm; and thickness, 0.7.0 mm. The tightness adjuster consists of two knurled lock screws and a light spring. The knurled lock screw: diameter, 3.0.0 mm; length, 10.5.0 mm. The light spring: diameter, 5.7.5; and length, 12.0.0.

The handle consists of three stainless steel strips and two knurled lock screws. The strips: width, 13.0.0 mm; and thickness, 1.3. mm. Strips 1 and 3: length, 17.5.0 mm; Strip 2: length, 10.3.5 mm. Strip 2 has a tapered end (Figure 6A). Screw 1: height, 10.6.0 mm; i.d., 2.3.5 mm; and o.d., 6.5. mm. Screw 2: height, 10.6. mm; i.d., 2.3.5 mm; and o.d., 5.1.0 mm.

The handle is attached to the base by a screw (3mm in diameter), which protruded through a centrally located slot (5mm wide) in the base (Figure 6A). This design can facilitate free lateral movement of the scalpel blade, whereas its movement in the longitudinal direction is guided by the slot and its height above the base is fixed at a distance (1mm) that ensures the cut is made below the level of hair follicles (Figure 7).

6.3. Application

To create an intradermal pocket for autologous AP tissue implantation, a 45mm long skin incision (Figure 6B) is positioned centrally and cut coronally using a handheld scalpel along the line drawn between the anterior points of the two frontal crests of a male deer calf. This is where the skin is found to be the thickest (around 2.0. mm) on a deer's head. A 20 mm wide and 1–2mm deep incision is made on the freshly cut dermis surface (Figure 6C) at the rostral side to provide guidance for the blade that is attached to the device. The base of the device is inserted rostrally under the skin through the 45mm long skin incision and the skin is then tightly clamped when the tip of the device-attached blade is properly inserted into the 1–2mm deep guidance-dermis-incision (Figure 6D). The depth of the guidance-incision is then extended rostrally to create an intradermal pocket by laterally

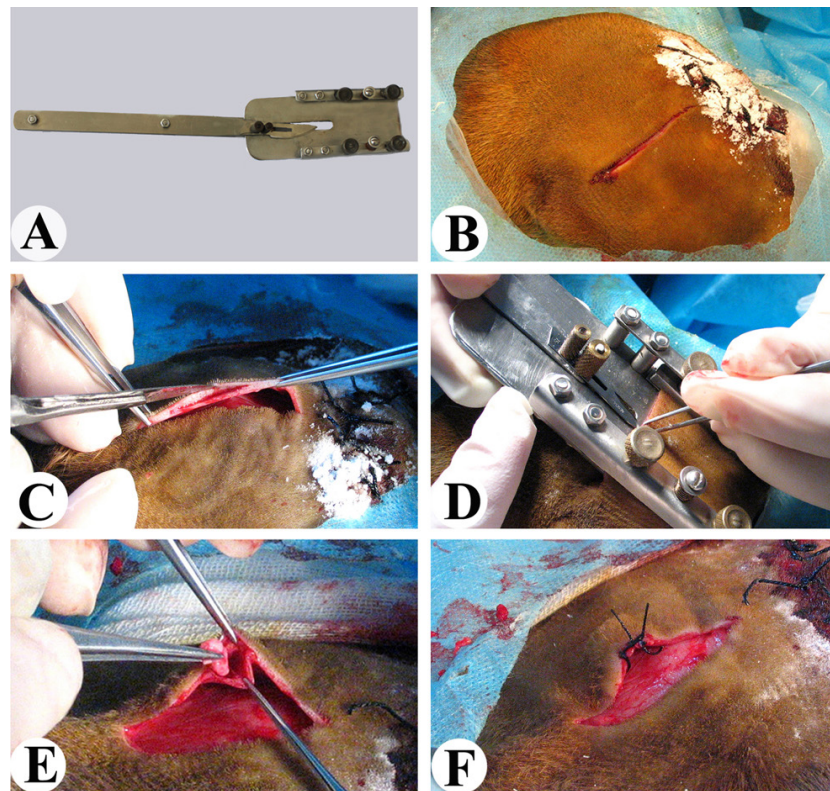


Figure 6. Intra-dermal pocket maker. A, A pocket maker. B, a 45 mm long skin incision cut between the anterior points of the two frontal crests. C, A 20 mm wide and 1–2mm deep guidance-incision on the freshly cut skin surface. D, Insertion of anterior part of the maker underneath of skin through the 45 mm incision, and blade attached to the maker into the 20 mm skin incision. E, Loading of the minced periosteum tissue into an intra-dermal pocket. F, Suture of an intra-dermal pocket after periosteum tissue loading.

moving the handle between the 10 and 2 o'clock positions about the central pivot axis of the blade (Figure 6D). By this means, an intradermal pocket, approximately 10–15mm deep is created. The mouth of each intradermal pocket is opened using two pairs of fine forceps to facilitate the loading of the pellet of minced AP into the pocket (Figure 6E). A suture is then used to seal the opening of each intradermal pocket (Figure 6G).

6.4. Comments

To make a perfect intradermal pocket, the scalp skin must be held flat and tightly extended with the two parallel metal clamps on the device, and the guidance-dermis-incision must be made before using the device.

7. STERILE PERIOSTEUM SAMPLING SYSTEM

7.1. Purpose and principle

The sampling of the AP or PP for *in vitro* (19) studies must be carried out under sterile condition and preferably not involve slaughter of the deer. Practically it is very difficult to carry out AP and PP removal under sterile condition on deer farms. To overcome

this problem, we have designed a portable glass box (Patent No:ZL201420244472.7.) This box, in conjunction with commensurate metal stand and lifting platform, has been successfully used by our group to biopsy the AP or PP tissues for *in vitro* culture without having the deer slaughtered.

The concept to keep sterile for periosteal tissues during sampling is straight forward, i.e. to create an isolating layer that can effectively separate the surgical site from the environment and at the same time allow access for the operator's hands and necessary surgical equipment. We found that covering the operated site by a longitudinal glass box could effectively lower down the contamination rate (essentially none provided the sterilisation in the box and the operated site is properly done). The glass box is simple to build, and can be easily carried around on a deer farm.

7.2. Construction

The system consists of three independent components: a glass box (Figure 8A and 8B), a metal stand (Figure 8A) and a lifting platform (Figure 8C). The glass box is rectangular in shape (length, 80 cm; width, 40; the height, 40 cm; and thickness of glass sheet, 9 mm), and consists of six pieces of glass.

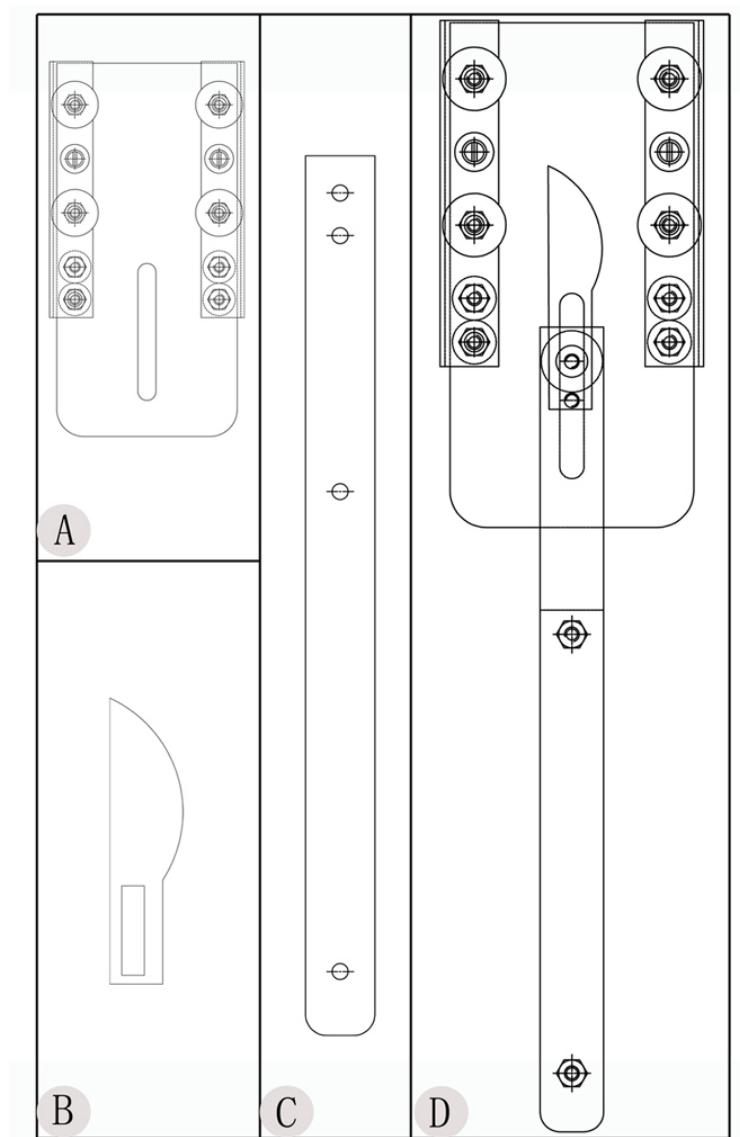


Figure 7. Diagrams of the sterile periosteum sampling system. A, Front view of the glass box; B, Back view of the metal stand; C, Lateral view of the glass box; D, Lateral view of the metal stand; E, Lateral view of the custom-built curved head bracket; F, Front view of the custom-built curved head bracket.

Piece 1 (length, 80 cm; and height, 38.2 cm) has two archways (radius, 5 cm; height, 8 cm) for an operator's two hands to access the surgical site from outside the box (front piece); Piece 2 is the same to Piece 1, but no archway (back piece); Piece 3 (height and length, 38.2 cm) has a hole (diameter, 11 cm) for delivering surgical items into the box (left piece); Piece 4 is the same to Piece 3, but no hole (right piece); Piece 5 has a hole (diameter, 10 cm) for the exposure of surgical site of a deer head to inside of the box (bottom piece); Piece 6 is the same to Piece 5, but no hole (top piece). These six pieces of glass are glued together with silicone gel according to the design, and reinforced using an angle stainless steel frame. Every two stainless steel handles are welded onto each angle steel piece at the upper left and right sides (Figure 8B). A UV light bulb is

installed inside the angle steel piece at the upper back side for sterilization purpose, and wire of the bulb goes out through a hole drilled in Piece 5 (Figure 8A). The archways and holes are sealed with Velcro strips and corresponding sized cloth (Figure 8B).

The stand (Figure 8A) is made up of 14 pieces of square metal tube (length, 3 cm; thickness of metal sheet, 2 mm): four leg tubes for supporting the glass box (length, 95 cm); four top tubes for holding the box (length, 90 cm; width, 40 cm), but the central part (30 cm) of the one at the back is removed to clear the way for a deer's neck (Figure 8E); four extra tubes for stabilizing the four leg tubes (length, 90 cm; width, 40 cm); two short tubes for bracing the separated back tube (Figure 8E). All tubes are welded together according to the design (Figure 9).



Figure 8. Sterile periosteum sampling system. A, A rectangular glass box and top part of a metal stand. B, Photo of partial glass box to show the attached UV light, carrying handles, and hole sealing Velcro strips and cloth. C, A movable hydraulic lifting platform attached with a custom-built curved head bracket. D, A shaved, sterilized and sterile cloth covered surgical area (incipient pedicle). E, The whole system in place with the emphasis on the interface between margin of the surgical site and edge of the bottom hole of the glass box. F, A bony incipient pedicle exposed after surgically opening the covering skin inside the glass box.

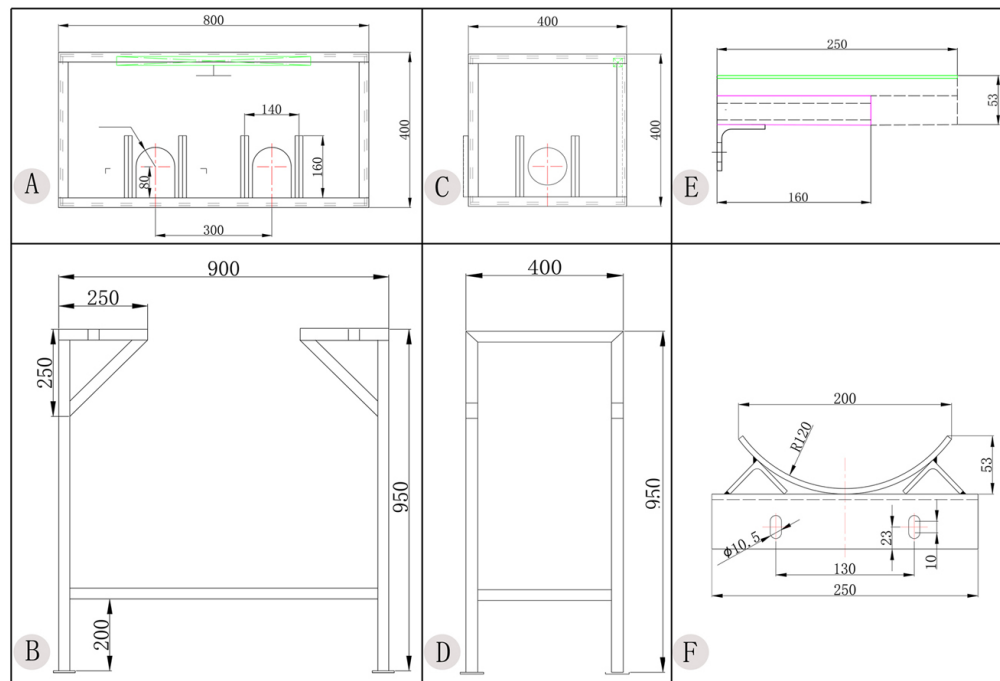


Figure 9. Diagrams of the components of the Intra-dermal pocket maker. A, Base of the maker; B, A scalpel blade; C, Handle of the maker; D, Top view of the maker.

The lifting platform is constructed using a commercially available movable mini-hydraulic lifting platform (TF50; Zhejiang Noblelift Equipment Joint Stock Co. Ltd, China) and a custom-built curved head bracket (Figure 8C). The platform (area, 850 cm x 500 cm) can lift up to 500 kg and the maximum lifting distance is 595 cm. The custom-built curved head bracket consists of 4 metal pieces: 1 piece of curved metal sheet (length, 25 cm; width, 20 cm; depth of the curve, 3.4 cm; thickness of the sheet, 5 mm); 1 long piece of angle iron (length, 25 cm) with two holes being drilled on one surface (distance between the two holes, 12 cm); and 2 short pieces of angle iron (length, 16 cm). These 4 pieces are welded together according to the design (Figure 9F).

7.3. Application

When the system is used for periosteum biopsy, a deer under general anaesthesia is transferred to the lifting platform with its head resting on the bracket. The operated site is thoroughly shaved, cleaned and sterilised with alcohol and iodine tincture (Figure 8D). The glass box resting on the metal stand has been sterilised by the UV light installed in the box. The movable platform is then pushed underneath the metal stand and stopped at the point where the operated site of the deer head is aligned with the hole of Piece 5 glass (the bottom piece) of the box. The platform is then lifted up to the level that allows the operated site (frontal crest region or pedicle region) to directly enter the box through the bottom hole and the platform position is adjusted to centralise the operated site in the hole. Finally the platform is gently lifted up again to let the margin of the surgical site and edge of the glass hole come together closely to seal the hole (Figure 8E). Sterilised gauze is normally used to perfectly seal any possible gaps between the surgical site and the edge of the glass hole before starting the surgery (Figure 8F).

7.4. Comments

A larger sized lifting platform would need to be used if mature stags are to be operated using this system (700 kg or even bigger). The diameter of the bottom hole of Piece 5 glass should be altered according to the size of surgical site. However, this change should not be too much in order to maintain the seal at the margin of the surgical site.

8. CONCLUSION

Deer antlers are potential novel models for biomedical research, but the process to develop these models to the stage that can be readily used by the biomedical field is slow. One of the main reasons for this current situation is undeniably the lacking of some of key proper tools to assist the model development. Over three decades of research on antler biology and biomedical model development we have encountered

these problems and developed tools to successfully solve each of them. We believe that if our five custom-built tools reported here are adopted by researchers who work in this fascinating field, it would greatly help for the development of these deer antler models for biomedical research.

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