Vascular anatomical basis of perforator skin flaps

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Introduction

Musculocutaneous perforator flaps have become a standard technique among surgeons around the world. The earliest work by the pioneers of perforator flaps was done without the benefit of the detailed vascular anatomy required and flaps were developed on a case by case basis. However, as more perforator flaps have been described, the understanding of the vascular anatomy has improved. Currently numerous perforator flaps have been well documented, however, there are probably still more potential flaps available with the rapid development and application of perforator flaps in Plastic Surgery, there has been renewed interest in the vascular anatomical basis of current and potential perforator flaps. Therefore the goal of this article is to review the historical development of the investigation of normal vascular anatomy of the human body, and to outline our current angiographic and computer imaging analysis techniques used to provide high quality angiograms of the human skin vasculature.

Historical Perspective of Vascular Injection Studies:

Jean Riolan (1580-1657) first injected colored dyes to demonstrate the branching of the vasculature, (1). These liquid media or gels such as latex, Berlin blue, and india ink or ink-gelatin mixtures contain fine particles that, when they are injected, fill the vasculature and facilitate dissection. They provide visualization of the actual vascular territory of a specific vessel. Gelatin can be added to the mixture to provide rigidity to the vessels and facilitate dissection [2, 3]. One of the possible problems with ink injection is vascular overinjection resulting in spillage of the dye into adjacent vascular territories. It is difficult to predict the exact quantity of dye necessary to fill a specific vascular territory. Due to the limitations of dye injection, they can be useful in identifying the cutaneous territories of flaps. However, the data acquired in this manner, should probably be regarded as somewhat imprecise.

Review of Angiographic Techniques:

Ink-gelatin mixtures or colored latex gels facilitate dissection by coloring the vessels (Fig. 1). Latex allows visualization of the blood vessels but will not provide the visual assessment of the skin territory which is better demonstrated with ink injection.

Corrosion studies have been used to extensively define the vascular architecture of tissues. The vascular corrosion casts are created using injections of polyester resin and synthetic glass material such as acrylonitrile butradiene sturene and chlorinated polyvinyl chloride. These media can also provide excellent quality specimens for scanning electron microscopy. However these injected specimens are not satisfactory for dissection and are not radioopaque. Therefore, corrosion studies are often used in combination with other forms of vascular investigation to define the vascular anatomy.

Roentgen discovered x-rays in 1895, and the first angiogram was produced soon after by Haschek (4) in 1896 by injecting chalk into the arteries of the human cadaveric hand. Strontium bromide was first used to produce a femoral angiogram in a patient in 1923 (5). Other radioopaque materials such as calcium sulphate (6, 7), mercury (8), barium (9), bismuth (10), colloidal silver (11), lead oxide (12), lead chromate ($PbCrO_4$) (13), vermilion (HgS or mercuric sulfide) (14), sodium bromide (15), and iodized oils (16) have been used for angiography. The most useful angiographic methods have been with the two injectates, barium sulphate and lead oxide.

Barium sulphate was first described as a radiographic contrast agent in 1920 (9, 17). The barium sulphate injection technique includes flushing out intravascular blood and mixing the sulphate with gelatin or latex for subsequent dissection. Although barium sulphate has been used intermittently and has provided some good results, it was soon replaced by the gold standard lead oxide as a contrast agent for the study of very fine vascular network such as those found in the integument (18-20). Barium sulphate has been used to produce high quality angiography using mammographic techniques (21) however this technique is limited to fairly small tissue samples so that the specimens can fit within the mammography unit (22).

Jamin and Merkel described the use of lead oxide and gelatin injection technique in 1907. Salmon modified the lead oxide injection technique and used it extensively to study skin and muscle vascular anatomy (18, 19). Rees and Taylor reevaluated Salmon's work and proposed a simplified lead-oxide injection technique (20-24-26). The lead oxide gelatin injection technique is useful because of the very dense radioopacity of lead combined with the bright orange color which facilitates dissection of vascular structures. It is a reliable inexpensive simple technique to produce excellent angiographic results.

We have reevaluated the techniques of lead oxide and gelatin angiography in several ways. In an effort to reduce toxicity of the lead in the injectate, we have reduced the amount of lead oxide required to produce excellent angiograms. We assessed the effect of using different types of gel and concluded that a higher quality commercial gel yielded improved results. We have also altered the temperature of the injectate, the lead and the radiographic technique to provide optimal results (27). The purpose of this paper is to outline the results of our studies and to document the lead oxide gelatin injection technique to study the vascular anatomy of the human in an effort to provide a clear anatomical basis for the clinical use of perforator flaps.

Method

The lead oxide and gelatin injection technique:

It is important to inject fresh cadavers as soon as possible after death. At Dalhousie University, the Ethics Committee approves all anatomical projects and the cadavers are available through the Department of Anatomy and Neurobiology Human Donor Program. Cadavers are excluded from study for the following reasons: severe peripheral vascular disease, extensive atrophy or deformity, evidence of widespread metastatic cancer or extensive surgery. The cadaver preparation is carried out in the morgue. The femoral artery and vein are exposed and longitudinal incisions are made inferior to the inguinal ligament. The largest caliber Foley catheter is inserted in the artery proximally and distally and a standard metallic embalming cannula is placed in the femoral vein. The cadaver is warmed prior to injection with lead oxide and gelatin. A solution of 5-10 L of tap water with carbonated saline solution (9% KCL) is warmed to 40°C and then injected under continuous pressure of 140-170 kPa until the venous outflow is clear.

The body is then floated into a warm bath of water at around 40°C. This warms the cadaver to maintain the lead oxide gelatin mixture above its melting point and allows the injectate to circulate throughout the microvasculature without solidifying. It also avoids inadequate injection over dependant pressure points.

The lead oxide gelatin injection solution is prepared with pharmaceutical grade gelatin. The gelatin (5 grams of 300 Bloom phar-

maceutical grade gelatin derived from porcine skin, Sigma G-2500, U.S.A.) are diluted in 100 ml of tap water and heated to 40°C. The red lead oxide (100 grams) are then added to the solution and stirred at regular intervals to avoid sedimentation. The solution is then injected into the femoral artery and continued until the patchy orange color is identified on the extremities and conjunctiva. Thinner cadavers tend to require less injection than more obese cadavers. The average amount of lead oxide gelatin mixture is 20-30 ml/kg. Once the injection is completed, the skin is rinsed and the cadaver is then refrigerated (4°C) or frozen for later dissection.

Perforator identification tecnique:

The cadaver is refrigerated for 24 hours and then the entire cadaver is radiographed and all bony landmarks are labeled with flexible lead wire. Areas of interest are radiographed (Figure 2A) prior to dissection to provide an overview of the vascular anatomy. However, these angiograms tend to be very confusing to analyze due to the overlapping 3-dimensional nature of the multiple vessels. The tissues are then sequentially dissected, photographed and radiographed in order to provide an increasing degree of detail about the area of tissue of interest (Fig. 2, 3). We vary the incisions used to remove the integument to alternate the areas that are disrupted by the dissection approach. It is important to maintain a standardized method of photography, dissection note taking, clipping of vessels and so on, to accurately document the vascular anatomy. The type of data collected includes the type of perforator (musculocutaneous versus septocutaneous, the muscle of origin of the perforator, the main source vessel, the pedicle length, and diameter of the vessel at the deep fascial level. The integument is then removed at the fascial level and unrolled and mounted on cardboard sheets to maintain the exact dimensions (Fig. 2D,E). It is then radiographed and frozen. The deep tissues including muscle and bone of each cadaver area are then radiographed at various stages of the dissection (Fig. 3C,D). This provides information about the main source vessel and pedicle length of each of the perforators.

The radiography of the tissues dissected needs careful planning. To facilitate the radiographic analysis and angiographic process we have developed a table specifying the settings used for each region depending on the thickness, lead oxide content and density of the tissue being examined (Table I). The integument of each area is radiographed as a series of overlapping angiograms using the same settings which will facilitate the later stage of digital processing in combining the radiographic plates.

Data anlysis and presentation

The source vessel is defined as the principal terminal branch of the vascular axis of a region and corresponds to the main artery supplying each angiosome as described by Taylor and Palmer(24). A vascular territory is defined as the total two-dimensional area of integument supplied by one source artery, while a perforator zone is defined as the two-dimensional area of integument supplied by a single perforator.

Scion Image for Windows™ and Microsoft Excel™ software were used to calculate the area from the angiograms of each region. The boundaries of adjacent perforator zones are defined by the presence of choke or reduced-caliber vascular anastomoses. In some cases, true anastomoses (intra-arterial communication with no reduction in diameter) were noted between perforator zones. In these situations, estimations were made regarding the division between zones. Standard deviation was calculated to show the variability in area between cadavers. However, due to anatomical variation in the size of individuals, the area of the zone is dependent on the total surface area of the region and individual.

Results

Over the past five years we have dissected a total of 21 human fresh cadavers after lead oxide injection studies. A total of approximately 7000 radiographs have been reviewed and summarized. We present the summarized results of our anatomical research in the areas of head and neck, upper limb, torso and lower limb regions.

The vascular anatomy of the integument is presented first as a summary of the whole body data followed by individual analyses of each of the four anatomical regions. In each angiogram, the contribution of different vascular territories is overlaid with colour and perforator trunks are labeled with lead beads or clips (Fig.4-7). The quantitative data in this article is based on the results from cadavers that demonstrated complete perfusion of the injectate and thus exhibited the best angiographic detail with regards to vascular territories and perforator zones.

The human integument is supplied by approximately 442 ± 121 perforators greater than 0.5 mm in diameter from 120 source arteries. These vessels are duplicated between sides and thus form the basis of the 60 vascular territories (Table II). Each source vessel provides arterial supply to a vascular territory. The perforators of the particular source vessel may vary in number or size but in general are consistent from individual to individual. Any perforator flap based on the source vessel should be identified with this arterial name to standardize the nomenclature(28).

Of the 442 perforators, approximately 160 passed through loose connective tissue or intramuscular septa (i. e. SC perforators) en route to supplying the skin and approximately 283 emerged from muscle tissue (i.e. musculocutaneous perforators). The proportion of musculocutaneous and septocutaneous perforators does vary from region to region in the body and from individual to individual. However, on average, the musculocutaneous perforators outnumber the septocutaneous perforators in a ratio of 3:2.

The superficial pedicle length of each perforator was measured directly from the original angiograms and an average value was calculated for the corresponding vascular territory. This value estimates the distance between the deep fascial planes (i. e. the plane of integumentary elevation) to the point where the perforator's internal diameter became less than 0.3 mm. The diameter values are average external diameters of the perforators and were measured and recorded directly during the dissections.

Table I: *Radiographic setting of various tissues for vascular studies*

Tissue	kVp	Tissue	kVp	Tissue	kVp
Integument		**Deep Tissue**		**Bone**	
< 1.0 cm (thick)	44	Head	100-110	Skull	65-70
> 1,0 cm	46	Neck	85	Spine	65
		Shoulder & Arm	70-80	Scapula	50-55
Muscle		Elbow & Forearm	65	Rib	50
< 1.0 cm (thick)	44	Hand & Wrist	55-60	Humerus	60
Latissimus Dorsi	46	Thorax	80-90	Hand	55-60
Trapezius	46	Abdomen & Pelvis	90-100	Pelvis	60
Quadriceps	50-55	Thigh & Hip	75	Femur	65
Gastrocnemius	50	Knee & Leg	70	Tibia	60
Gluteus Maximus	50-55	Ankle y foot	65	Fibula	55

mA=100, Sec. = 3/20

Head and neck cutaneous vascular anatomy

The head and neck has approximately 25 perforators (>0.5 mm) per side of the body (Fig. 4). The cutaneous vessels of 10 source arteries comprising the vascular territories in this region supply the integument of the head and neck. In the skin of the head, the arteries interconnect to form a rich network. The primary blood supply to the integument of the face and scalp is from large cutaneous branches of the external and internal carotid arteries. The large caliber and superficial nature of the vessels in this region can be attributed to the overlay of the facial and scalp skin on the bony skeleton of the head. Branches of the external carotid system supply most of the head skin, with the exception of a mask-shaped area that surrounds the eyes and covers the central forehead and upper two thirds of the nose. Arteries to this region arise from the ophthalmic branch of the internal carotid system. In contrast, the longitudinal muscle structure of the neck allows for smaller more numerous musculocutaneous perforators to supply the skin in this region. Perforators from the internal and external carotid arteries and rami of the thyrocervical trunk (transverse cervical, supraclavicular, suprascapular, dorsal scapular arteries) supply the integument of the neck. An angiogram of the vascular territories of the integument of the head and neck region is shown in Figure 4.

Upper limb cutaneous vascular anatomy

The upper extremity is commonly involved in severe soft tissue injuries requiring coverage by a regional pedicled flap or microvascular free tissue transfer. The integument of the upper extremity constitutes approximately 10% of the total surface area of the body. An average of 48 ± 19 perforators from 15 vascular territories supplied the integument of the upper extremity. Septocutaneous arteries predominate in the shoulder, elbow, distal forearm and hand regions. Musculocutaneous perforators are more numerous in the upper arm, and proximal forearm. The average perforator size in the upper extremity was approximately 0.7 ± 0.2 mm in diameter, and supplied an average area of 35 cm^2. An overview of the vascular territories of the upper extremity is depicted in Figure 5.

Torso cutaneous vascular anatomy

The integument of the torso is used extensively in reconstructive surgery for flap harvest. Large vascular perforators from 17 source arteries supply the various donor sites of the trunk. The majority of these perforators are musculocutaneous, originating from the primary blood supply of the broad superficial muscles in this region. Several large septocutaneous perforators arise from the perimeter of these muscles, and from near the joint creases of the extremities where the skin is tethered to underlying connective tissue. The large septocutaneous perforators are easily distinguishable in angiograms of the integument because they frequently have a larger diameter and travel greater distances, thus supplying large vascular territories.

The integument of the trunk covers approximately 30% of the surface area of the body. An average of 122 ±48 perforators from 17 vascular territories supplies the integument. The ratio of musculocutaneous to septocutaneous perforators is 4:1 (Fig. 6). The average diameter and area supplied by a single perforator from the torso region are approximately 0.7 ±0.2 mm and 40 ±15 cm2, respectively.

Lower limb cutaneous vascular anatomy

The lower extremity is the largest donor site for perforator flap harvest in the body. It accounts for 46% of the total body surface area of the integument (thigh 21%; leg 13%; buttock 5%; foot 7%). This region is very important donor site for perforator skin flaps. In general the region is not yet completely explored in terms of the possible perforator flap donor site available.

The lower extremity, particularly the thigh, appears to have the greatest potential for the development of new or modified perforator flap harvest. An average of 93 ± 26 perforators from 21 vascular territories supplied the integument of the lower extremity. Musculocu-

Table II. *Summary of quantitative data for the distribution of cutaneous vascular territories and their perforators in the four regions of the body from a series of five fresh cadaver dissections (n=10) injected with a modified lead oxide and gelatin procedure. The vascular territories, average number of perforators, superficial pedicle length, average diameter at the level of the deep fascia, and ratio of musculocutaneous to septocutaneous perforators are presented according to region. Number of vascular territories corresponds to one half of the body.*

Region	Number of Vascular Territories	Average Number of Perf.	Superf. pedicle Length (cm)	Diameter (mm)	MC:SC
Whole Body	**60‡**	**442**	**33**	**0.7**	**3:2**
Head and Neck	**10**	**20**	**37**	**0.9**	**1:3**
Scalp	4	7	49	1.1	1:4
Face	4	5	38	0.9	1:4
Neck	2	8	29	0.7	3:2
Upper Extremity	**15**	**48**	**33**	**0.7**	**2:3**
Shoulder and Arm	7	22	38	0.8	2:3
Elbow and Forearm	5	24	25	0.5	1:1
Wrist and Hand	3	3*	44	1.3	1:4
Trunk†	**16**	**61**	**32**	**0.7**	**4:1**
Chest	4	10	35	1.0	4:1
Abdomen	7	20	30	0.7	4:1
Upper Back	5	24	31	0.8	4:1
Lumbar Region	1	6	27	0.7	1:2
Lower Extremity	**21**	**92**	**33**	**0.7**	**1:1**
Gluteal Region	3	21	24	0.6	9:1
Hip and Thigh	5	34	35	0.7	3:2
Knee and Leg	8	30	36	0.7	1:1
Ankle and Foot	5	6*	29	0.8	1:4

*These values were calculated under the assumption that the integument of the hands and feet are supplied by only a few large direct cutaneous perforators from their respective arterial arches.

†The summary data for the Trunk excludes the external genitalia and perineum.

‡The total number of vascular territories is not a sum of the number of regional territories due to the presence of shared territories that span the regional devisions.

taneous perforators were in equal proportion to septocutaneous perforators (1:1). The average diameter and area supplied by a single perforator was approximately 0.7 ± 0.3 mm and 47 ± 24 cm^2 respectively in the lower extremity. An overview of the vascular anatomy of the integument of the lower extremity is showed in Figure 7.

Discussion

The overall goal of these anatomical research studies has been to evaluate the cutaneous vasculature in order to more precisely develop perforator flaps. We have documented the perforators which supply the integument in terms of the source vessel, diameter and length. This information is needed to standardize the nomenclature and allow further description of novel and useful perforator skin flaps (28).

The study of anatomical details of the vasculature has been aided by the use of radio-opaque contrast materials. In particular lead oxide has been in use as an injectable contrast material for studying the vascular anatomy of the human body since its first reported by Jamin and Merkel in 1907(12, 23). However, lead oxide is a heavy metal and tends to precipitate out of aqueous solutions. Rees and Taylor(20) recommended a lead oxide-gelatin mixture as a perfusate. This preparation has been routinely used in many different studies by different investigators since 1986. The current work focused on decreasing the amount of toxic exposure to lead oxide by determining the smallest required quantity needed to produce excellent angiograms. We have also tested the effects of using different types of gel, different concentrations of gelatin, varying temperatures and lead oxide dosages, and radiography(27).

Gelatin is a protein and in aqueous solutions is a hydrophilic colloid. These macromolecules can form a three-dimensional network. If water is added to fill up the space between the networks, the complex swells and forms a gel. However, gelatin is only partially soluble in cold water. Upon heating to about 40°C, any gelatin that has been allowed to hydrate for about 30 minutes melts to give a uniform solution(29).

Gelatin is available in different gel strengths and particle sizes allowing it to be individually selected to suit different applications and processing requirements(30). In general one can say that the lower the mean molecular weight of a gelatin, the lower the gel strength and viscosity of its solution. Industrial gelatin has more gel strength and viscosity and pharmaceutical gelatin with Bloom strength of 300 is used in the manufacturing of both hard and soft pill capsules. The role of gelatin in the injection protocol is to keep the lead oxide evenly distributed within the vessel system and prevent spillage into the tissues during dissection. The amount of gelatin used should be properly controlled. If inadequate gelatin is used to form a gel, the injectant will not set during refrigeration and the lead oxide will not be evenly distributed. Conversely, if too much gelatin is used, the gel will be too thick and set too quickly to reach the small vessels. In general, the gelatin concentration in the injectant should not fall below 5% or it will be too dilute to agglomerate(31).

Gelatin forms thermally reversible gels with water, and the gel melting temperature (<35°C) is below body temperature(30), Making use of its thermally reversible properties, our protocol allows for re-injection if the initial first injection procedure fails. After immersion in a warm water bath of greater that 40°C for several hours, warm water (40°C) can be used to flush the remaining lead oxide and gelatin injectant from the under-perfused extremity and re-injection of a fresh mixture can proceed.

Thermal treatment or pH change of the cadaver can denature the proteins of the gelatin complex causing them to lose their functionality. Acidification to about pH 4 and warming to 50°C is known to denature some types of gelatin. Other studies have previously reported that water bath temperatures of 50°C are not to be exceeded to prevent gelatin denaturation(20, 30, 32). On the other hand, water temperature over 60°C can damages the capillary vessel to leak, induce skin burn and sloughing also.

Table III. *Summary of quantitative data for the distribution of arterial perforators in the human integument in a series of five fresh cadavers (n=10) using the modified lead oxide and gelatin injection procedure. The average total area, area per vascular territory, and area per perforator are listed by anatomical region. The vascular density of the integument was calculated as the average number of perforators in a 10 cm x 10 cm (100 cm^2) area. The surface area of the integument of each sub-region is presented as a percentage of the larger anatomical region, whole body and half body (divided mid-sagittally).*

	Area (cm^2)	Area/ Territory (cm^2)	Area/ Perf. (cm^2)	Vascular Density (p/100 cm^2)	Percent Area of Region (%)	Percent Area of Whole Body (%)	Percent Area of Half Body (%)
TOTAL BODY	**16144**	**-**	**36**	**3**	**-**	**100**	**50**
Head and Neck	**809**	**640**	**32**	**2**	**100**	**5**	**10**
Scalp	325	308	44	2	40	2	4
Face	201	95	19	2	25	1	3
Neck	284	304	38	3	35	2	4
Upper Extremity	**1670**	**1670**	**35**	**3**	**100**	**10**	**21**
Shoulder and Arm	734	734	33	3	44	5	9
Elbow and Forearm	565	565	24	4	34	3	7
Wrist and Hand	372	372	124	1*	22	2	5
Trunk	**2217**	**230**	**40**	**3**	**100**	**14**	**28**
Chest	520	225	57	2	23	3	6
Abdomen	819	160	42	2	37	5	10
Upper Back	892	229	40	3	32	6	11
Lumbar Region	157	157	29	4	7	1	2
Lower Extremity	**3376**	**247**	**36**	**3**	**100**	**21**	**42**
Gluteal Region	419	211	19	5	12	2.5	5
Hip and Thigh	1408	326	39	2	42	9	18
Knee and Leg	1149	208	38	3	34	7	14
Ankle and Foot	399	77	68	2*	12	2.5	5

*These values were calculated assuming the hands and feet are supplied by very few direct cutaneous perforators, and therefore reflect a lower than expected vascular density.

†The summary data for the Trunk excludes the external genitalia and perineum.

Our modification of the lead oxide injection technique is a simple method to produce high quality angiograms. The objectives of this study were to improve the lead oxide-gelatin injection technique by addressing: 1) Decrease toxicity of lead oxide by the amount of lead oxide has been reduced from 200 g to 100 g and vaporous decrease of the lead oxide by warming the injectate from 50°C to 40°C 2) Precipitation of lead oxide was decreased with the use of industrial strength gelatin (5%). Floating the cadaver in a warm water bath allowed the injectate to perfuse blood vessels over pressure points and decreased the number of unfilled vessels.

Although angiography can define the precise course of arteries and their interconnections with adjacent vessels, it has the major limitation of superimposition of the vessels because all three-dimensional anatomy is compressed into two dimensions. Thus it can be difficult to determine three-dimensional positions and the relationships of vessels to each other or to other structures.

The limitations of the lead oxide and gelatin injection technique include the inability to prevent bursting of small capillaries when excessive pressure is applied during injection, inability to reverse postmortem degradation of the vascular system, and inability to completely inject areas of unrecognized pre-mortem pressure sores. Also, overfilling of the arterial system can lead to venous filling, sometimes seen in the superficial venous system of the extremities. Lead oxide is also a toxic substance that requires the operator to wear mask and gloves during manipulation. A special facility for disposal of lead is also necessary.

Data processing

While it is crucial to create excellent angiograms, the data processing which follows is probably more time consuming and important to the vascular anatomical research. Scion Image is an image processing and analysis program which can acquire, display, edit, enhance, analyze and animate images. Scion Image for Windows can be used to measure area, mean, centroid, perimeter, etc. of user defined regions of interest. It also performs automated particle analysis and provides tools for measuring path lengths and angles. Spatial calibration is supported to provide real world area and length measurements. Results can be printed, exported to text files, or copied to the Clipboard.

We have measured the diameter, length, origin and course of each perforator in order to define its use in an operative procedure. The results were entered into Excel spreadsheets in order to calculate area, count, average, standard deviation which will automatically be updated when further data is entered.

Adobe PhotoShop is a bitmap graphic editor the file format, image size, and color modes are then set according to the requirements for publication. For example, figures submitted for publication in the Plastic and Reconstructive Surgery journal must meet the following criteria:

• Be saved in Tag Image File Format (TIFF), Encapsulated PostScript (EPS), or PowerPoint (PPT) format.

• Have a minimum width of 40 picas (that is, two “landscapes” or three “portraits” across), 30 picas (two “portraits” across), or 19 picas (for a figure that will be placed in one column). There are 12 points in a pica and 6 picas in an inch; therefore 30 picas equal 5 inches.

• Be in the color mode cyan-magenta-yellow-black (CMYK) if they are produced digitally.

TIFF is a bitmap file format for images that is based on a simple 32-bit uncompressed image that is used to exchange images between application programs. CMYK is a color model in which all colors are described as a percentage mixture of the colors cyan, magenta, yellow, and black. HSB and RGB are also common color models. Although all color and tonal corrections may be performed in either CMYK or RGB color modes, the mode should be carefully selected. Whenever possible, multiple conversions between modes should be avoided, because color values are rounded and lost with each conversion. If an image must be converted from one mode to another, most tonal and color corrections should be performed in RGB mode and CMYK mode can be used for fine-tuning. Advantages of working in RGB mode include: improved performance and saved memory as a result of working with fewer channels; enhanced device independence, because color spaces do not depend on ink so that corrections are preserved regardless of the monitor, computer, or output device used; and preservation of more colors after adjustments, because the gamut of RGB spaces is much larger than that of CMYK spaces.

Conclusions

To obtain excellent radiographs and scientific data that demonstrate the intricate details of vascular anatomy, while facilitating laboratory dissection and image processing, we suggest the following recipe and injection sequence:

1. Preparation of injectant: 5% gelatin (300 Bloom, 40°C) and lead oxide (100% w/v).
2. Pre-injection of a carbonated 9% KCl saline solution (40°C) to remove excess systemic blood, clean the vessels and warm the body.
3. Float cadaver in a warm water bath (40°C) to alleviate any pressure points that may be otherwise under-injected.
4. Injection of the lead oxide-gelatin suspension using approximately 25ml-30ml/kg.
5. The cadaver is refrigerated for dissecting next day.
6. Whole cadaver is radiographed to plan a dissection approach.
7. Landmark, photography, dissects and x-ray layer-by-layer.
8. Image processing by PhotoShop.
9. Data analysis and presentation by Scion Image and Excel.

This simple and inexpensive angiographic and computer imaging technique is clearly defined and yields excellent resolution of the vascular architecture. The intricate details captured by this technique can demonstrate circulatory patterns within the integument, muscle, bone, periosteum, tendon and nerve and can therefore improve our understanding of clinically related vascular anatomy. This information will assist the surgeon in choosing and designing perforator skin flaps in reconstructive surgery.

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