Background: The placement of the superficial cervical plexus block has been the subject of controversy. Although the investing cervical fascia has been considered as an impenetrable barrier, clinically, the placement of the block deep or superficial to the fascia provides the same effective anesthesia. The underlying mechanism is unclear. The aim of this study was to investigate the three-dimensional organization of connective tissues in the anterior region of the neck.

Methods: Using a combination of dissection, E12 sheet plastination, and confocal microscopy, fascial structures in the anterior cervical triangle were examined in 10 adult human cadavers.

Results: In the upper cervical region, the fascia of strap muscles in the middle and the fasciae of the submandibular glands on both sides formed a dumbbell-like fascia sheet that had free lateral margins and did not continue with the sternocleidomastoid fascia. In the lower cervical region, no single connective tissue sheet extended directly between the sternocleidomastoid muscles. The fascial structure deep to platysma in the anterior cervical triangle comprised the strap fascia.

Conclusions: This study provides anatomical evidence to indicate that the so-called investing cervical fascia does not exist in the anterior triangle of the neck. Taking the previous reports together, the authors’ findings strongly suggest that potential spaces in the neck are directly continuous with the subcutaneous tissue.

REGIONAL anesthetic block of the cervical plexus is an increasingly available alternative for some surgery of the neck, particularly for carotid endarterectomy, because of the possible overall lower incidence of morbidity and mortality. To successfully apply this technique, understanding the detailed configuration of the deep cervical fascia (DCF) is essential because the DCF may serve as an impenetrable barrier to guide and localize the anesthetic agent. However, our knowledge of the anatomy of the DCF is still very limited, which has caused, for example, some confusion about how to perform the anesthetic block, in particular, whether the superficial cervical plexus block should place local anesthetic superficial or deep to the investing layer of the DCF.

Although the anatomy of the DCF is quite complex, its investing or outermost layer is believed to be simple and everyone is agreed on the existence and disposition of this layer. In brief, the investing layer of the DCF is described as a definite, continuous sheet of fibrous tissue that completely encircles the neck. It attaches posteriorly to the cervical spinal processes via the nuchal ligament. It envelops two muscles, the sternocleidomastoid (SCM) and trapezius, and two glands, the submandibular (SG) and parotid. However, several recent reports are not consistent with this general description. For example, a study conducted on serial sections of 10 human fetuses has indicated that the superficial surface of the parotid gland is only covered by subcutaneous tissue. Using the E12 sheet plastination technique, Zhang and Lee demonstrated that the investing layer of the DCF does not exist between the SCM and trapezius muscles, and posteriorly it is unlikely that the investing cervical fascia attaches to the cervical spinous processes because there is no defined nuchal ligament in the human. These findings raise a fundamental question: Does the investing cervical fascia exist? To fully address this question required reexamination of the fascial structures in the anterior cervical triangle that is bordered by the SCMs and the inferior border of the mandible. The aim of this study was to investigate the three-dimensional organization of connective tissues in the anterior triangle of the neck.

Materials and Methods

A total of 10 adult human cadavers (5 men and 5 women, aged 67–89 yr) embalmed with 10% neutral buffered formalin were used in this study. All bodies were donated to the Department of Anatomy and Structural Biology (University of Otago, Dunedin, New Zealand) for teaching and research under the Human Tissue Act 1964.

Seven cadavers were processed as sets of epoxy resin slices (two sagittal, two coronal, and three transverse) using the E12 Sheet Plastination technique. This technique preserves and fixes the cellular constituents of tissue in situ by removing lipids and water and replacing them with a curable polymer resin. The process involves setting the tissue in 20% gelatin at −80°C for 24 h. The frozen specimens were positioned in one of three planes of orientation (coronal, sagittal, or transverse) and cut using a band saw with a 1.6-mm blade set at a thickness of 2.5 mm. The slices were immersed in acetone (86.5–100%) at −25°C over an 8-week period for

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dehydration. They were then degreased at room temperature (18°–24°C) for an additional 4 weeks. The processed sections were polymerized under vacuum pressure with epoxy resin (Biodur, E12/E1/AE10/AE30 [100: 28:20:5], Heidelberg, Germany) at 0°C for 24 h. The polymerized slices were laminated between two 50-µm-thick plastic sheets. This was done to separate and protect the impregnated tissue and allowed curing for 1 week at room temperature or in an oven at 30°–40°C for 3 days to accelerate the process. The translucent plastinations were examined under a Leica MZ8 Stereoscopic Dissecting Microscope (Leica, Heerbrugg, Switzerland) 1.25–5× magnification. Photographs were captured with a Nikon Coolpix 990 Digital Camera (Nikon Corporation, Tokyo, Japan).

The plastination process results in connective tissue, especially collagen, being endogenously autofluorescent at the 488-nm excitation. Two transverse slices and one sagittal slice from two cadavers were examined using a BioRad confocal laser-scanning microscope (BioRad, Hampstead, United Kingdom). The thickness of the optical section was set up at 107 µm under a 5× objective, and the images were electronically recorded and montaged.

Gross anatomical dissections were performed on three cadavers (two men and one woman, aged 74–81 yr) by a progressive layer-by-layer dissection. Special attention was given to those fascia-like structures in the anterior cervical region and around the SCM. Colored latex was injected under the epimysium of the SCM. Gross anatomical images were recorded with a Nikon Coolpix 990 Digital Camera.

Results

Gross Anatomical Examination

The primary area of the focus in this study involved the investing layer of the DCF in the anterior cervical triangle and fascia around the SCM (SCM fascia). The findings were similar in all three cadavers examined.

After the skin and platysma were dissected free, the fascia-like structure in the anterior cervical triangle was observed that was continuous laterally with the SCM fascia (fig. 1). On the superficial surface, it was difficult to distinguish the SCM fascia from the fascia in the anterior cervical triangle (fig. 1A). To reflect these two fasciae as a single sheet, the use of sharp dissection was required along the medial border of the SCM (fig. 1B). Such sharp dissection created a boundary demarcating these two fasciae because the inner surface of the reflected SCM fascia appeared much more smooth than that of the reflected fascia in the anterior cervical triangle (fig. 1B). On the deep surface of the SCM, the SCM fascia projected or was continuous with the intramuscular septa in the muscle (fig. 1C). Therefore, the use of sharp dissection was required to separate the SCM fascia from the muscle (fig. 1C).

To test whether the SCM fascia bounded a potential space around the SCM and whether this space communicated with spaces in the anterior cervical triangle, pink and blue latex was injected under the right and left SCM fasciae, respectively (fig. 2). This resulted in localized spread of the latex along the superficial surfaces of the SCMs, and the injected latex did not spread into the anterior cervical triangle (fig. 2A). Within each SCM fascia, the spread of the latex was also compartmentalized because after an injection of latex into one head of the SCM, the latex was limited within that head by the intramuscular septa (fig. 2B). The latex spread into the muscle along the intramuscular septum but was not present in the posterior compartment of the muscle (fig. 2C). After transversely bisecting the SCM, a further injection of light-blue latex behind the SCM fascia resulted in spread of the latex along the surface of a strap muscle (e.g., omohyoid muscle), but spread did not extend beyond the muscle (fig. 2C).
In summary, at the gross anatomy level, the investing cervical fascia was demonstrable as a connective tissue sheet extending between the SCMs as well as around each SCM, which is consistent with the traditional description. However, its appearance, thickness, and boundaries varied and were difficult to clearly define.

Examination of the Epoxy Sheet Plastinations

The following findings were based on the observation of the serial E12 epoxy slices with intervals of less than 2 mm. No significant variations were found among the cadavers examined in this study. Because the main structures immediately underneath platysma in the anterior cervical triangle are the infrahyoid muscles and the SGs, the results of this part of the study are presented in two sections: the upper cervical region, which contains the SGs, and the lower cervical region, inferior to the glands.

Upper Cervical Region

In the upper cervical region, the floor of the mouth and the SGs were seen between the SCMs (fig. 3). Deep to platysma, the SG was enveloped by a fascia-like connective tissue layer that was referred to as the SG fascia in this study (fig. 3A). Although some fibers from the deep aspect of the SG fascia seemed continuous with the connective tissue around the hyoid muscles medially and the carotid sheath laterally, no direct fascial connection was found between the SG fascia and SCM fascia (fig. 3A). Instead, a potential space of fatty connective tissue separated the SG fascia from the SCM fascia and platysma (fig. 3A). Compared with the SCM fascia, the SG fascia appeared as a stronger and more regular fascial structure (fig. 3A).

At the level of the inferior margin of the SG, the SG fascia continued medially with the fascia that covered the strap muscles (strap fascia) (fig. 3B), but laterally, inferiorly, and anteriorly, the SG fascia was completely isolated by the fatty connective tissue from the SCM and platysma (figs. 3B and C). Below this level, the strap fasciae of both sides met with each other along the anterior midline and formed a clearly identifiable fascial sheet that covered the deep midline structures (fig. 3B). No other fascia-like structure was found anterior to this midline fascial sheet (fig. 3B).

Hence, in the upper cervical region, the fascial sheet between the SCMs was dumbbell-like, with the strap fascia in the middle and SG fasciae on both sides (fig. 3B). This fascia sheet had free lateral margins and did not continue with the SCM fascia (figs. 3A and B). At the inferior margin of the SG, this fascial sheet continued only with the strap fascia as the inferior pole of the SG fascia floated in the fatty connective tissue (fig. 3C).

Lower Cervical Region

At the level of the upper border of the thyroid cartilage (fig. 4A), the basic pattern of the fascial arrangement was similar to that in the upper cervical region except for the disappearance of the SG fascia. The strap fascia was strong and very well defined, particularly its anterior and lateral parts. Again, no direct connection was found...
between the strap fascia and SCM fascia, although both fasciae seemed to fuse with the carotid sheath. The fatty connective tissue mass between the two fasciae was superficially sealed from the subcutaneous tissue by platysma. Fascicles of platysma formed a multilayered and sometimes discontinuous muscular layer (figs. 4A and B). Some fascicles of platysma contained both muscular and aponeurotic fibers (fig. 4A).

Below the lower border of the thyroid cartilage (fig. 4B), the area of fatty tissue between the SCM fascia and strap fascia became wider. The SCM fascia was well defined in the lower cervical region, whereas the lateral part of the strap fascia became less clear because the omohyoid muscle extended laterally and the strap fascia and the muscle fibers overlapped with each other. Superficially, the multilayered platysma covered both SCM fascia and the fatty connective tissue mass between them. Within some fascicles of platysma, both muscular fibers and aponeuroses coexisted in a same fascicle. On transverse sections, sometimes, a single sheet of the connective tissue was found between platysma and the SCM, the fatty mass, or the infrahyoid muscles (fig. 4B).

To reveal whether the connective tissue sheet underneath platysma was the medial extension of the SCM fascia, plastinated slices were examined under the confocal microscope (fig. 4C). With the use of confocal microscopy, it became apparent that this connective tissue sheet was not a continuous single structure. It was composed of several small connective tissue sheets and the ends of each small sheet overlapped with that of the adjacent sheets, appearing as a single sheet. Some small connective tissue sheets were continuous with the connective tissue fibers in the fatty tissue mass, which was very similar to the appearance of the skin ligaments in the subcutaneous tissue. On the superficial surface of the SCM, these small connective sheets were physically very close to the SCM fascia, but no direct connection was found between the sheets and the SCM fascia. Tracing these small connective tissue sheets in the adjacent slices indicated that they were the inferior extensions of the aponeuroses of platysma fascicles. On the transverse sections, however, the tracing was difficult because of the greatly variable appearance of each small sheet within a very short distance (fig. 4D). The direct continuities between the muscular and aponeurotic fibers of platysma and between the aponeurotic fibers of platysma and the skin ligaments were well demonstrated on the sagittal sections (fig. 5). As shown in figure 5, platysma hung down in front of the infrahyoid muscles and gradually became aponeurotic (fig. 5A). It then fanned out and disappeared in the subcutaneous tissue as the skin ligaments (fig. 5B).

Along the anterior midline, there were a number of connective tissue layers (fig. 5). They were the medial extension of the strap fasciae (fig. 4D), the aponeuroses...
of platysma fascicles (fig. 5), the skin ligaments in the subcutaneous tissue (fig. 5), or the fibrous connection of the periosteum of the clavicles and sternum (fig. 4D), but none of them were derived from the SCM fascia (fig. 4D).

In summary, at the macro-microscopic level, no single connective tissue sheet extended directly between the SCM fasciae. The fascial structure deep to platysma in the anterior cervical triangle comprised the strap fascia that continued laterally with the SG fascia but not the SCM fasciae.

Discussion

This study provides clear evidence to indicate that a direct fascial linkage does not exist between the SCMs, and thus there is no so-called investing layer of the DCF in the anterior triangle of the neck. Taking the previous reports\textsuperscript{15–18} together, our findings strongly suggest that the deep cervical structures (e.g., nerves, vessels, viscera) are not fully enveloped by a single investing layer of the DCF. The structures outside the prevertebral layer of the DCF are mainly compartmentalized and protected by the strap, SCM, and trapezius muscles, which each have their own well-defined fascial sheet. Potential spaces of fatty connective tissue around those structures outside the prevertebral fascia communicate with each other and are directly continuous with the subcutaneous tissue \textit{via} the potential channels of fatty tissue between the muscles\textsuperscript{15} (fig. 4).

These findings provide an anatomical basis for a recent debate about the placement of superficial cervical plexus block\textsuperscript{8,9} The classic technique of superficial cervical plexus block is to inject the agent in the subcutaneous plane along the posterior border of the SCM in the cranial and caudal direction from the midpoint of the posterior border of the SCM.\textsuperscript{6} Some people also suggest placing the superficial cervical plexus block under the so-called investing fascia.\textsuperscript{8,22} Interestingly, clinical reports were similar, confirming that both placements of the superficial cervical plexus block can provide effective anesthesia.\textsuperscript{6,22} The anatomical evidence from this study, as well as the previous report,\textsuperscript{15} strongly support those clinical reports because the investing cervical fas-

\footnotesize{\textsuperscript{966} NASH ET AL.}

\textsuperscript{15} Anesthesiology, V 103, No 5, Nov 2005

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cia does not exist and deep cervical spaces continue directly with the subcutaneous tissue via the potential spaces of fatty tissue around the SCMs, thereby allowing unimpeded spread of injected local anesthetic. However, care should be taken while performing superficial cervical plexus block. First, our results do not support the suggestion that the injection should be made into the body of the SCM. As shown in figures 2–4, the SCM has its own well-defined fascial sheet that continues with the intramuscular septa between the muscular fascicles. This connective tissue sheet is strong and most likely impenetrable. Delivering the local anesthetic within this sheet may be very localized and may only affect those branches of the cervical plexus that closely run on the surface of the muscle. Similarly, the placement of the block should be kept slightly away from any muscular structure in the region, including the trapezius, scalenus, and strap (particularly the omohyoid) muscles because each of these muscles has a similar connective tissue sheet to that of the SCM. Finally, it is still unclear whether superficial cervical plexus block can affect the roots of the plexus that are covered by the prevertebral fascia, although it has been suggested that the injectate may be able to penetrate the deep fascia via the “pores” at which the nerves pierce the fascia. In fact, the configuration of the prevertebral layer of the DCF may be much more complicated than that described in conventional anatomical textbooks. A previous study has indicated that in the dorsal cervical region, the prevertebral fascia is closely associated with each individual deep cervical muscle, and its continuity shows some regional variations. Our preliminary observation suggests that a similar pattern to the prevertebral fascia may exist in the anterior cervical region.

The conclusion that the investing cervical fascia does not exist challenges our traditional belief that is largely based on an original and remarkable report from Grodinsky and Holyoke in 1938. In Grodinsky and Holyoke’s study, 75 adult cadavers and 5 fetuses were examined at the gross anatomical level using dissection and gelatin injection methods. They described the investing layer of the DCF as “a definite sheet of fibrous tissue completely encircling the neck.” However, they did notice that the investing fascia fused with the strap fascia in both anterior and posterior cervical triangles. A similar appearance was observed in this study at the gross anatomy level. However, when examined at the macro-microscopic and microscopic levels, this continuity of the investing fascia disappeared. As shown in figures 3 and 4, a clear gap filled with fatty connective tissue can be seen between the strap and SCM fasciae. Such a gap is difficult to demonstrate at the gross anatomical level.

The study of the configuration of connective tissue in the cadaver is complicated by the fact that great difficulties exist in dissecting out the fasciae. Under a dissecting microscope, one may be able to trace the aponeurotic or tendon fibers of a muscle, but it is almost impossible to distinguish between the membranous (or fibrous) part of the subcutaneous tissue, deep fascia, epimysium, and epitendineum. Although histologic examination may be able to overcome the problem, the application of this method is greatly limited by the size of sample areas.

By definition, the deep fascia is a thin fibrous membrane, devoid of fat. Practically, it is not always easy to dissect and identify the demarcation between the subcutaneous tissue and the deep fascia or between the deep fascia and the epimysium or epitendineum. This is particularly true at some sites where the deep fascia is looser in texture and more irregularly arranged because there is no absolute criterion determining what “looser” and “firmer” mean. Therefore, arguments as to the existence of a certain deep fascia may be largely a question of semantics. Nevertheless, we believe that the E12 sheet plastination used in the current study and previous studies is an appropriate technology to trace the configuration of dense and loose connective tissues because it...
retains all of the structures in their “living” position, while different types of tissues (fatty and fibrous connective tissue and muscular tissue) are still clearly distinguishable.

In conclusion, evidence presented here as well as in our other studies\textsuperscript{15–18} clearly indicate that the investing cervical fascia does not exist in humans as generally believed previously. This finding will not only benefit anesthesiologists who perform cervical plexus blocks, but will also provide anatomical basis for surgeons to design an endoscopic approach to the structures in the anterior and lateral cervical triangle regions, such as thyroid gland, carotid sheath, deep cervical lymph nodes, and retropharyngeal and parapharyngeal spaces.

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