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REVIEW ARTICLE

The masks of Lorenzo Tenchini: their anatomy and surgical/bioengineering clues*

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Abstract

An academic, anatomist, and Lombrosian psychiatrist active at the University of Parma in Italy at the end of the 19th century, Lorenzo Tenchini produced ceroplastic-like masks that are unique in the anatomical Western context. These were prepared from 1885 to 1893 with the aim of 'cataloguing' the behaviour of prison inmates and psychiatric patients based on their facial surface anatomy. Due to the lack of any reference to the procedure used to prepare the masks, studies were undertaken by our group using X-ray scans, infrared spectroscopy, bioptic sampling, and microscopy analysis of the mask constituents. Results showed that the masks were stratified structures including plaster, cotton gauze/human epidermis, and wax, leading to a fabrication procedure reminiscent of 'additive layer manufacturing'. Differences in the depths of these layers were observed in relation to the facial contours, suggesting an attempt to reproduce, at least partially, the three-dimensional features of the facial soft tissues. We conclude the Tenchini masks are the first historical antecedent of the experimental method for face reconstruction used in the early 2000s to test the feasibility of transferring a complete strip of face and scalp from a deceased donor to a living recipient, in preparation for a complete face transplant. In addition, the layering procedure adopted conceptually mimics that developed only

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in the late 20th century for computer-aided rapid prototyping, and recently applied to bioengineering with biomaterials for a number of human structures including parts of the skull and face. Finally, the masks are a relevant example of mixed ceroplastic-cutaneous preparations in the history of anatomical research for clinical purposes.
Key words: additive layer manufacturing; bioengineering; ceroplastics; face transplant; facial reconstruction; surface anatomy.

Introduction

Lorenzo Tenchini was Professor of Human Anatomy at Parma University from 1881 to the day of his sudden death in 1906 (Fig. 1A). He was born in Brescia in 1852, graduated in Medicine and Surgery at the University of Pavia in 1876, and there pursued an academic career, following Giovanni Zoja at the Institute of Human Anatomy, where he focused on macroscopic anatomy, with particular interest in the central nervous system and its correlation with the psychic function in both normal and pathological conditions (Toni et al. 2016a). During his graduate studies he was particularly influenced by one of his teachers, Cesare Lombroso (Montaldo, 2016), who had established the Forensic Medicine and Experimental Psychiatry Laboratory at Pavia University (later also at Turin University) where Tenchini carried out his very first anatomical clinical study on a criminal subject in 1877. Once appointed in Parma, Tenchini started investigating the correlation between the anatomical organization of the brain and behaviour in psychopaths and inmates (Toni et al. 2016a). Following Lombroso's evolutionist perspective, between 1885 and 1895 Tenchini published his most complete work, *Cervelli di delinquenti* (Brains of Criminals), in four successive volumes (Fig. 1B). In these, he summed up all possible correlations between the macroscopic morphology of the brain hemispheres (Fig. 1C) studied with the Gacomini method (Giacobini et al. 2016) and the personal and judicial history of the deceased inmates, whose bodies he had previously dissected as a consequence of their death in prison of natural causes (usually infectious diseases such as tuberculosis). His attempt was definitely too preliminary and inconclusive due to obvious technological constraints of the time, and only recently are we cautiously approaching some possible insight to such a controversial and socially 'sensitive' topic through the aid of modern *in vivo* imaging techniques (Churchland, 2011; Raine, 2013).

However, Tenchini not only removed and studied the brain of these subjects, he also preserved their skulls in order to carry out anthropometric investigations. In addition, for educational purposes he prepared a number of viscera, trunks, arms and legs of those bodies, using dehydration combined with intravascular injection of coloured resins. All these items are currently stored in the Museum of Biomedicine - BIOMED at Parma University, Italy, accounting for over 350 anatomical samples still in use for teaching in Medicine and Surgery, primarily concepts and procedures in clinical anatomy (Toni & Ruggeri, 1988; Toni et al. 1996; Toni, 1999, 2003, 2008) and recently used as a source for research and scientific disclosure into applied anthropomorphic biomedical robotics (see at http://lafabricadeicorpi.unipr.it/index.php). Similarly, there are around 400 skulls (see at www.anfamedmuseo.unipr.it), part of the anatomical specimens for both academic teaching and forensic research (Guareschi et al. 2016; Donato et al. 2019).

Tenchini's masks: a technical conundrum in anatomical ceroplastics

A very peculiar aspect of Tenchini's method of anatomical research was to reproduce the faces of the subjects whose autopsies he had conducted, in the form of masks (Fig. 1D–G). From 1885 to 1893 he probably produced more than 150 of these, but only 46 are currently kept at BIOMED in Parma (see at www.anfamedmuseo.unipr.it), and 30 are on view at the Museum of Criminal Anthropology 'Cesare Lombroso' in Turin, Italy. The masks are best associated with the Lombrosian epistemological view to which Tenchini adhered, an outgrowth of the physiognomical tradition according to which behavioural aberrations could variously be connected to dysmorphic somatic features of the face and skull. Therefore, the masks were developed as a means visually to distinguish different types of altered behaviour and/or crimes, each ascribed to a specific subject. Unfortunately, the real technical procedure used to fabricate these masks has remained a mystery for over 120 years. Tenchini never mentioned it in any of his scientific publications still preserved in the Historical Library of BIOMED in Parma (Toni et al. 2016b), but later authors suggested that the masks were standard ceroplastic artefacts. In particular, it has been claimed that these masks were made entirely of coloured wax, either applied to a plaster cast of the deceased person's face or directly moulded on the face of the body (this is known as *moulage*), and later completed through the insertion of hairs, to make the physiognomic reconstruction more life-like (Toni et al. 2016a).

However, it has been noted that a distinctively empathic and markedly dramatic visual quality characterizes these masks (Burman, 2016), suggesting a peculiar attention to the mimic surface anatomy of the face, including its subcutaneous fibrous (i.e. dermal/fascial) and muscular components. This, combined with the historical evidence that Tenchini had developed a specific procedure to achieve

Fig. 1 (A) Portrait of Lorenzo Tenchini, Oil on canvas, 1911, BIOMED, Parma, Italy. To our knowledge, this is the only image currently available of Tenchini, although dated 5 years after his sudden death at age 54. (B) Frontispiece of an original copy of the book by Tenchini on Criminal's Brains (kept in the Historical Library at BIOMED), and the brain of subject no. 72 (catalogued as 'murderer', whose mask is lacking) prepared using Giacomini's method, as displayed at the International Exposition: 'The Fabric of the Bodies: from Anatomy to Robotics', Palace of the Governor, 14 October–17 December 2017, Parma, Italy (link at http://lafabricadeicorpi.unipr.it/index.php). (C) Mask of subject no. 104 (catalogued as 'thief'). (D) Mask of subject no. 125 (catalogued as 'injurer'). (E) Mask of subject no. 143 (catalogued as 'wilful murderer'). (F) Mask of subject no. 146 (catalogued as 'arsonist). Note the striking empathic tone of the physiognomy. Judicial and autopsy histories of most of the portrayed subjects are recorded in handwritten entries prepared by Tenchini himself and kept in an archive at BIOMED in Parma, Italy. To ensure ethical confidentiality, none of the masks shown here contain biographically sensitive data nor does their exhibition lead to violation of current privacy issues (from Toni et al. 2016a, with permission).

such a result, suggested to us that he must have adopted a hitherto unknown method to assemble these structures. In particular, a short commentary published in the Proceedings of the 2nd International Congress of Criminal Anthropology held in Paris in 1889, and an astonishing statement in a letter sent by Tenchini himself to Lombroso in 1906 (Toni et al. 2016a) led us to the conclusion that Tenchini must have used the facial skin (or even other subcutaneous components) of the cadavers he studied, in a sort of unintentional face transplant (completely unthinkable more than 100 years ago). As maintenance of several aesthetic features of the human face depends on the presence of different depths of the facial soft tissues (see Wilkinson, 2010 for a review on this topic), and this presumption is also at the basis of the recent surgical procedures of face transplantation (Siemionow & Agaoglu, 2006), we hypothesized that Tenchini might have attempted to attain this result on his masks. To ascertain this possibility, a series of investigations on the masks' structure were carried out by our group between 2010 and 2016, and recently brought to the attention of the international scientific community (Toni et al. 2016c; Toni, 2019).

Experimental studies on the structure of Tenchini's masks

In a first step, stratigraphic analysis using computed tomography (CT) scans revealed that the facial wall of the masks was arranged in three layers, the deepest of which was a high-density base applied directly onto the wooden support to which each mask was attached. This layer, compatible with an inorganic mineral compound such as plaster, was covered by an unbroken layer of a mostly radiopaque material with densitometric values matching those of an organic tissue, such as cotton gauze or human skin, in turn covered by a top layer of varying thickness made with an oily paste distinctly resembling wax (Fig. 2A,B). CT scanning did not reveal whether the latter two layers (organic + oily paste) reproduced the fine morphology of dermal/subdermal structures including mimic muscles; however, it was apparent that their assemblage gave rise to changes in depth reminiscent of those proper to the facial soft tissues in the human body, currently used as references for facial reconstruction (see Wilkinson, 2010 for a review).

In a second step, macroscopic analysis of the facial wall confirmed the presence of a stratified structure compatible with plaster and wax (Fig. 2C), the latter assembled from a both superficial and intermediate coloured material easily melting at 60 °C, in some instances containing a thin and reticulated material resembling natural fibers like cotton or hemp (Fig. 2D). In a third step, Fourier-transform infrared spectroscopy (FTIR) confirmed that the chemical nature of this substance was consistent with linen oil, sealing wax, and beeswax (Fig. 2E), whereas the deepest layer was made of bihydrated chalk, all compounds largely used in classical anatomical ceroplastics during the 19th century (Gabbriellini et al. 2016). Finally, to determine the still unknown nature of the intermediate layer, serial biopsies of the face wall at different depths and positions were obtained (Fig. 2F,G), and samples either resin-embedded and cut as two thickness (1 μm and 70–90 nm) sections for eventual analysis with light (LM) and transmission electron (TEM) microscopy or critically dried and gold-coated for scanning electron microscopy (SEM). As a control, samples of dehydrated skin from an anatomical specimen of the BIOMED collection not belonging to the same subjects used to prepare the masks were collected (Fig. 2H). Specifically, a classic anatomical preparation consisting of a human trunk with the head and shoulders was selected among the numerous available trunks at BIOMED, and supposed to be prepared by Tenchini following a standard methodology for macroscopic specimens. Skin biopsies were performed at four different locations including the front, cheek, neck, and shoulder and processed by LM for comparisons with the mask's intermediate layer.

At LM levels, the intermediate layer appeared to be composed of gauze very similar to thick cotton, spread on both sides with a waxy paste as would happen if the cotton tissue had been soaked in a bath of liquid wax. In some instances, the layer of wax in direct contact with the superficial side of the cotton tissue exhibited strips of cellular fragments morphologically consistent with skin bacteria and degenerated epithelial cells (Fig. 3A–E). Comparison of this altered epithelial layer with the skin of the anatomical preparation chosen as a control (see above) confirmed that the cellular debris covering the cotton was sclerotic and partly necrotic epidermis (Fig. 3B,G,H). Support for this view was achieved by TEM and SEM analysis showing that the sclerotic and necrotic epidermis in the mask's intermediate layer was colonized by bacteria and microorganismic spores classically known as residents of the human skin (Fig. 3C,D). As such, it was apparent that human epidermis had been deposited on top of the waxy cotton as an additional thickness to the masks' final preparation. Further confirmation of this view was provided by the presence of erythrocytes still entrapped in the hairs of the intermediate cotton/hemp layer (Fig. 3F), suggesting that blood cells leaked out of dermal blood vessels at the time the epidermis was transferred onto the waxy cotton, and remained segregated underneath within its fibers. All this histological evidence was consistent with those previously reported for epidermis, resident bacteria, and red blood cells in microscopic analysis of dehydrated and mummified specimens from anthropological/anatomical sources (Zimmerman, 1973; Hino et al. 1982; Chang et al. 2006; Janko et al. 2012). In contrast, no structures histologically compatible with dermal connective tissue, and hypodermal structures (vessels, nerves, exocrine glands, fat lobules, neural corpuscles, hair follicles, fasciae, mimic muscles) were identified in the masks analysed, although in a preliminary study we had suggested that fragments of these structures (hairs, collagen fibers, muscular tissue) might have been harboured in the intermediate layer (Toni et al. 2016a).

Tenchini's masks are the first example in the history of anatomy of a layered, three-dimensional (3D) reproduction of the facial wall mimicking that of simulated facial transplants on an inorganic support

Our investigation on Tenchini's masks has disclosed a complex and stratified organization of their facial structure, completely unexpected but surprisingly coherent with the stratified organization of the cutaneous and muscular wall of the human face (Fig. 4A,B). In particular, a series of at least six different layers of material were found: (1) a supporting plaster reliably reproducing the contour of the facial bones (likely obtained by a negative casting procedure with liquid chalk directly deposited and solidified on the surface of the cadaveric face), ultimately covered by (2) two alternating layers of wax separated (3) by a network/gauze of natural fibers (either cotton or hemp). Such a preparation could easily be achieved by soaking the vegetal tissue in a bath of liquid wax. On top of the wax-embedded gauze is (4) a layer of human epidermis, as deduced by the presence of degenerated epithelial cells and bacteria typically resident on the human skin. Whether this epidermis was treated with fixatives or specifically dehydrated is not known but, judging from the not properly preserved degree of conservation and bacterial growth, we raise the possibility that it was applied to the surface of the wax-

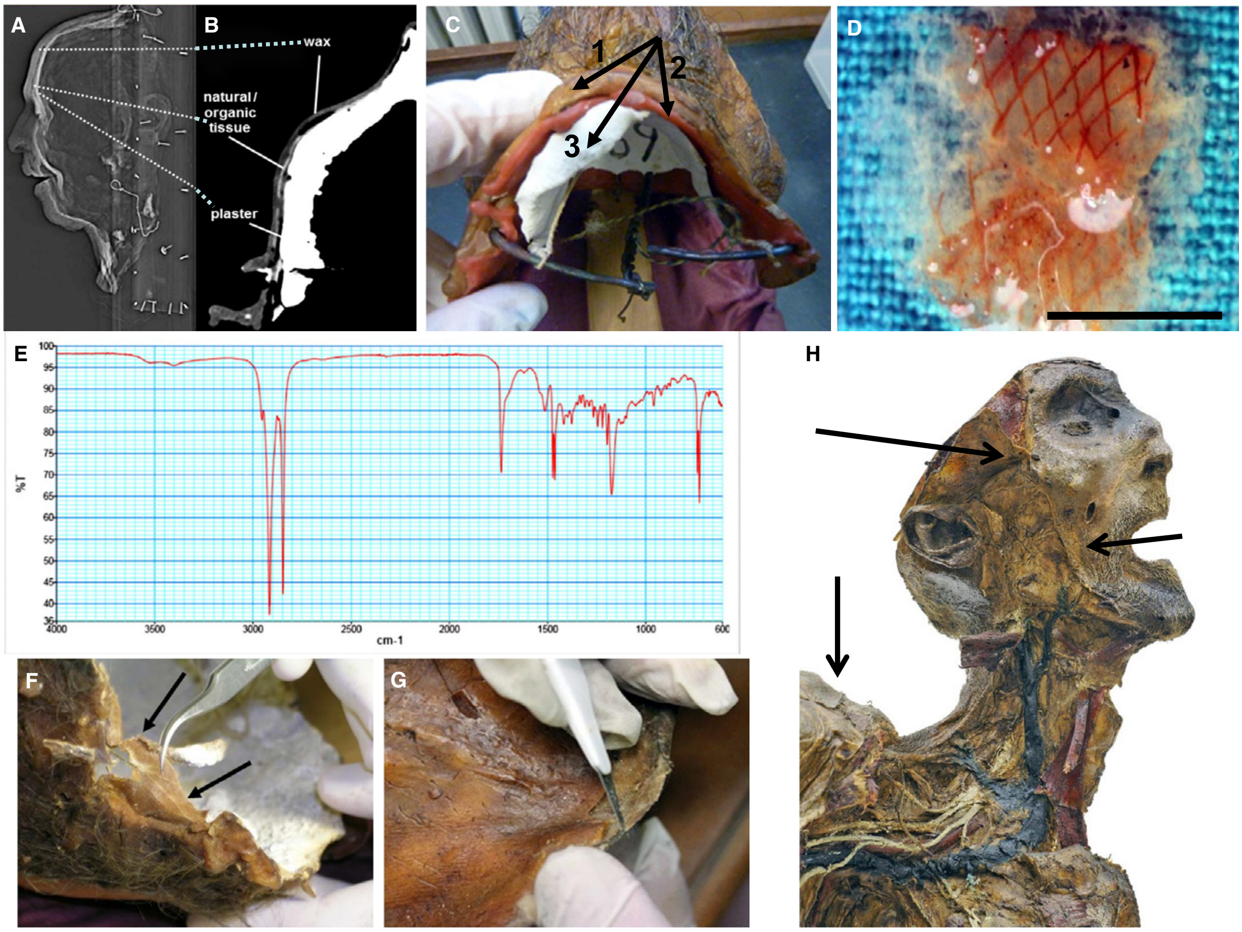

Fig. 2 (A) Computed tomography sagittal section of the mask from subject no. 143 (T1-weighted image, CS = 0.5, Somatom Emotion 6, Siemens, courtesy of Prof. Cristina Rossi, Radiology Center, Parma University Hospital, Parma, Italy). Note three layers with different levels of radiopacity. (B) Grey densitometry revealed wax on the surface, cotton/hemp or human skin at the intermediate level, and plaster at the bottom (partly modified from Toni et al. 2016a, with permission). (C) Macroscopically, the stratification corresponded to (1,2) superficial and intermediate layers rich in wax and (3) a deep layer of plaster (mask of subject no. 69). (D) Temperature-dependent melting (60 °C) of wax fragments from layers (1,2) of the mask from subject no. 153. The fragments contained different pigments, and a fiber network likely of vegetal origin (bar = 12 mm). (E) FTIR spectrum of layers (1,2) from the mask of subject no. 69. Peaks are compatible with pigmented beeswax. In other masks (e.g. subjects nos 149 and 155) linseed oil and sealing wax were recognized. (F) Bioptic sample of the intermediate layer (arrows) from the mask of subject no. 143. The material is similar to parchment. (G) Bioptic sample of the intermediate layer (arrows) from the mask of subject no. 122, at the level of the neck. Note that it is covered by reddish-pigmented wax. (H) Dehydrated anatomical specimen from the Tenchini collection at BIOMED, in Parma, Italy, whose facial and shoulder skin (arrows point to the areas of sampling) was used as a control for comparison with the material of the intermediate layer found in the masks.

coved gauze in the absence of any preservation method. Finally, this epidermal layer was (5) superficially protected by a third layer of pigmented wax offering support for the 'artistic' implant of natural hairs (possibly those of the original cadaver) to reproduce the adnexal physiognomy of the subject studied. Remarkably, this meticulous layered organization and its final thickness effectively recapitulate the succession of natural cutaneous, connective dermal, hypodermal and muscular components of the cadaveric face, likely contributing to the hyper-realistic note historically characterizing the expression of the masks.

Current methodologies for facial reconstruction imply use of codified standards for depths of facial soft tissues at different bony landmarks on the face skeleton. These different thicknesses are applied either manually, following an 'artistic-like' modelling (Wilkinson, 2010), or virtually, as a result of computer-based algorithms taking into account a number of skull images at different projections, and implementing them with anthropometric variables such as the supposed geometry of the facial profile and body mass index of the subject (Claes et al. 2006, 2010). The layering procedure adopted by Tenchini shared elements of these

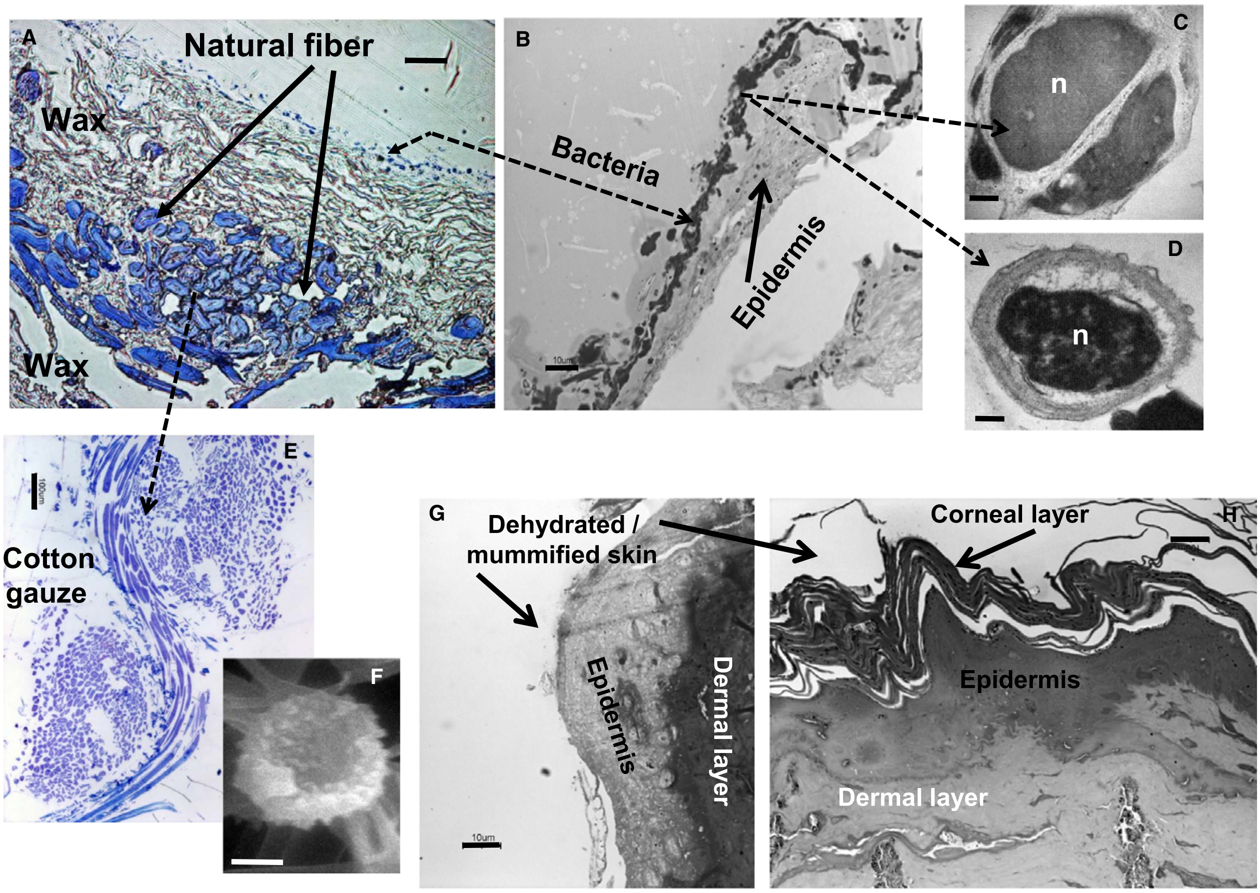

Fig. 3 (A) LM image of an Epon 812-embedded, 1-μm section of a fragment of the intermediate layer (transverse cut through the thickness) from the mask of subject no. 149 (toluidine blue staining). Note the presence of the stained fibrous material, covered on both sides by unstained wax. On top of the superficial layer of wax, a thin line of blue nuclei and cell fragments from bacteria and microorganismic spores is visible. (B) LM section analogous to that in (A), depicting the same line of bacterial cells covering a strip of compact, sclerotic, and partly degenerated epithelial tissue containing scattered cell nuclei compatible with epidermis without *stratum corneum*. (C) TEM image of an Epon 812-embedded, 70–90 nm section (high contrast preparation as described in Toni et al. 1990), of the same fragment shown in (A) and (B), depicting *Staphylococcus aureus*, a common saprofyte of the human skin. (D) TEM image of the same section as in (C), showing a microorganismic spore typical of the human skin. (E) LM control image of standard cotton gauze, prepared as the material fragment shown in (A). Note that the structure of natural fibers recapitulates that of the stained fibrous material in (A), indicating that both are cotton or hemp. (F) SEM image of a cell with a typical biconcave morphology compatible with a human erythrocyte (sample prepared and analysed as described in Zamparelli et al. 2014). This suggests that dermal fragments including blood cells were accidentally transferred to the masks during the 'transplant' of the cadaveric epidermis. (G,H) LM images of Epon 812-embedded, 1-μm sections of dehydrated skin (face and shoulder, respectively) from an anatomical specimen of Tenchini's collection used as internal control (toluidine blue staining). Note the striking similarity of the facial epidermis between the samples (G) and (B). Scale bars: (A,B,G, H) 10 μm; (E) 100 μm; (F) 1 μm; (C,D) 0.1 μm.

principles, although there is no evidence that he moulded the materials replacing the facial soft tissues in a way specifically to reproduce the morphology of the dermal, subdermal, and muscular parts of the face. However, using CT we recorded differences in depth of the covering materials at different levels on the facial contour (Toni, 2019), suggesting an intent to provide a surface morphology consistent with that of the original facial thickness (Claes et al. 2006; Wilkinson, 2010).

Thus, the unique technical procedure adopted by Tenchini to reproduce the surface anatomy of the human face exhibits similarities with that recently developed to test the feasibility of transferring a complete strip of face and scalp from a deceased donor to a living recipient in the attempt to reconstruct surgically a disfigured face using a face transplant (Siemionow & Agaoglu, 2006). Indeed, with this method the whole facial skin (epidermis + dermis), hypodermal structures (vessels, nerves, cutaneous adnexa), and mimic muscles of the deceased donor are removed from his cadaver, and then grafted onto a glass or synthetic 3D resin model of the recipient's face. In this manner, once transferred onto the synthetic facial contour, the thickness of the donors' facial soft tissues is maintained, ensuring a robust cosmetic result. In particular, this approach has

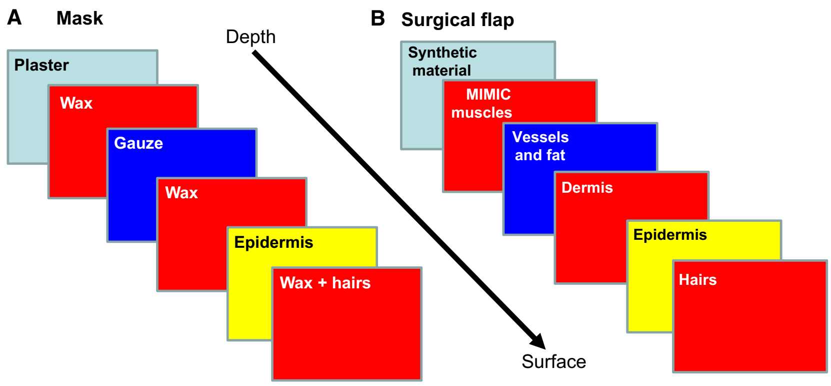

Fig. 4 (A) Schematic of the layered organization of the facial wall in the masks compared with (B), the layered anatomical organization of the surgical flap (epidermis + dermis + hypodermal structures) used for experimental transfer onto a synthetic 3D resin model of the recipient's face in preparation for a real face transplant (see Siemionow & Agaoglu, 2006; Siemionow, 2015). Note the similarity in the 3D organization of this layering between that of the masks and human face and frontal scalp, in a sort of 'recapitulation' for reconstructive purposes.

revealed that the morphology of the facial skeleton and the integrity of the mimic muscles, including their depths, are the essential precondition for preserving the expressive traits of the reconstructed face. This evidence is in accord with the current techniques of facial reconstruction onto the skull or skull replica requiring that the facial musculature is modelled before applying the skin to depict the final facial appearance (Wilkinson, 2004). Therefore, the combined transplanting of the missing subcutaneous parts and muscle tissue along with the skin and attached vascular–nervous peduncles, has become the key step for surgically reconstructing *in vivo* a face in a suitable way.

In the case of Tenchini's masks, two elements are consistent with the experimental simulation described above. First, the fabrication of a plaster cast directly onto the cadaveric face, to reproduce in detail the surface morphology of the facial skeleton. Secondly, the substitution of the dermal/subcutaneous tissue and mimic muscles with precisely alternated layers of waxy material and fibrous cotton/hemp tissue, collectively adapting to some of the natural changes in depth of the native soft and muscular tissues, along with the entire facial and scalp epidermis likely coming from the subject portrayed. Therefore, we believe that the 3D layered approach to the facial and scalp wall pursued by Tenchini represents an exceptional antecedent in the history of reproductions of human anatomical structures of the face (Toni et al. 2019), and might have played a significant role in the empathic quality of the masks' faces.

In addition, after more than 100 years it confirms the statement on the masks' construction made by Tenchini himself to Lombroso in 1906 when he wrote to him that 'my method enables the preservation of the epidermis'. In particular, as we have previously reported in detail (Toni et al. 2016a), Tenchini received a request for masks from Lombroso following their exposition in Turin at the 6th International Congress of Criminal Anthropology; in that very year. Tenchini, however, stresses that he cannot immediately meet this request because those artefacts were unique as a consequence of the peculiar technique he used to provide their facial expressions. Indeed, he explained that preservation and transfer of the epidermis of the original cadavers was pursued, and as such a single mask could not be serially reproduced, as commonly done with standard ceroplastics. For this purpose, Tenchini possibly also introduced an original method for separation of the human epidermis from the dermis, though still unknown to us. The only antecedent we are aware is that of mechanical suction by dry cupping described by Paul Gerson Unna in 1878, perfected in the present time in dermatological research. However, all other methods introduced in the scientific literature are effective only from 1925 and 1941–42, including use of acetic acid, trypsin, heat, hypertonic salt solutions, and various chemicals (including alkali and neutral salts) (for a review see Zou & Maibach, 2018). Studies by our group are in progress to shed light on this remaining obscure technical aspect (Toni, 2019).

Finally, it seems valuable to note that the method of fabrication applied by Tenchini to his face artefacts has some unexpected similarities with the method of progressive stratification with biomaterials known as additive layer manufacturing, recently used in 3D reconstruction of facial, skull, and scalp defects (Siemionow, 2015; Simonacci et al. 2017), and for a vast array of other medical applications including anatomical-surgical teaching (Ratinam et al. 2019).

Conclusions

From the perspective of contemporary anatomy and primarily of its clinical applications, Lorenzo Tenchini unexpectedly comes across as a forerunner of some major achievements. In particular, we believe that he anticipated (though unintentionally) an experimental *en bloc* transplant of a complex anatomical structure such as the facial epidermis onto a 3D surrogate of the dermal/hypodermal layers mimicked by natural fibers and waxes of different composition and thickness. In addition, he developed a method of additive layer fabrication that has strong analogies with the technical bases of the modern 3D bioprinting. Collectively, the scientific legacy on this forgotten Italian anatomist of the 19th century offers a 'glimpse back to the future' for scientific horizons which the human anatomical sciences are opening up in the 21st century.

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Conflict of interest

All authors confirm that they have no conflicts of interest to declare.

Authors' contributions

Drs Fulvio Barbaro, Elia Consolini, Giulia Toscani: anatomical, structural, light microscopic, analytical, and historical/artistic evaluations of the mask components and dehydrated anatomical samples of the Tenchini collection at BIOMED. Dr Nicoletta Zini: transmission electron microscopic studies of the mask components, and dehydrated anatomical specimens. Mr Davide Dallatana: technical supervision, bioptic sampling, and SEM analysis of the materials. Drs Pietro Setti and Salvatore Mosca: anatomical study of the consistency of the layering procedure in the mask preparation, and comparison with the native anatomical-surgical organization of the cadaveric face. Dr Giusy Di Conza: updating and revision of concepts and ideas. Dr Elena Bassi: acquisition of data, data analysis, contribution to concept/design. Drs Enrico Quarantini and Marco Quarantini: preliminary evaluation of the masks' organization, primarily at the level of the lower face quadrants (oral components) through advanced imaging techniques (VistaCam Durr Dental Apparatus). Prof. Edoardo Raposio: surgical relevance of the concept/design. Dr Marina Gorreri; contribution to the concept/design, primarily in the frame of the academic third mission of BIOMED. Prof. Alessandro Porro: historical and social relevance of the concept/design. Prof. Roberto Toni, organization and presentation of data, vision of the concept/design, critical revision of the manuscript.

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