Three-dimensional morphometric analysis of the effects of subperiosteal palatal soft-tissue expansion in growing cats

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Abstract. A prospective longitudinal study in 75 growing cats was conducted to evaluate palatal soft-tissue expansion in cleft lip and palate surgery. In 31 cats, palatal scars were induced by simulated Langenbeck surgery at the age of 8 weeks. At the age of 14 weeks, custom-made tissue expanders were inserted in 61 animals. Tissue expansion was performed by weekly inflation in 33 cats (16 without and 17 with scars) for an 8-week period. The remaining 28 cats (14 without and 14 with scars) served as sham groups. A control group was formed by 14 animals (without scars and without tissue expander). The effects of the experimental interventions were evaluated on a series of dental casts during the inflation period and until 8 weeks after removal of the tissue expander. The results indicate that soft-tissue expansion of the palatal mucoperiosteum is feasible. Until 20 weeks of age, no differences were found between both expansion and sham groups. Thereafter, significant soft-tissue surface-area gain was quantified in relation to the base surface and base diameter of the tissue expander. Iatrogenic side-effects of active tissue expansion consisted of significant transversal growth retardation in the anterior part of the bony palate and dentoalveolar structures. After removal of the tissue expanders, some accelerated growth in the tissue-expansion, scarred-tissue group was seen. It is concluded that palatal soft-tissue expansion is possible in growing cats, with and without the presence of palatal scars; however, this technique, like other kinds of palatal surgery, impairs dentomaxillary growth and development.

Traditional techniques for correction of palatal defects and oronasal communications in cleft lip-palate-alveolus (CLP) patients have been summarized by Millard. Various locoregional pedicled flaps are currently used, such as tadpole flaps, various lingual flaps, buccal musculomucosal flaps, buccal fat pad flaps, and temporal muscle and/or fascial flaps. These flaps do not always yield good results. Complete closure can be achieved in approximately 35–70% of cases at the first attempt, depending on the size and site of the communications. Closure generally becomes more difficult and the techniques become less reliable when several previous attempts at fistula closure have been performed. There are several disadvantages to these various techniques. Most are two-stage procedures and create a donor-site defect, and usually tissue of different origin, texture, and characteristics is used for the repair. For example, lingual flaps may cause temporary impairment of tongue function, as well as speech interference, and sometimes intermaxillary fixation is needed during the healing phase. Free microvascularized flaps, such as the radial forearm fasciocutaneous flap and the rectus abdominis myocutaneous flap...
flap, are hazardous, time-consuming, and bulky and they often cause donor-site morbidity and visible scars.\textsuperscript{7,8,19}

Soft-tissue expansion (TE) has been used in the craniofacial region since 1981.\textsuperscript{20} The use of TE to facilitate the closure of palatal defects in CLP is new and has seldom been reported.\textsuperscript{11,13,35,37}

Quite recently, De Mey et al.\textsuperscript{11} and Abram et al.\textsuperscript{1} advocated the use of a soft-tissue expander to close palatal fistulas. Few studies have reported on the effects of TE on growth and development in growing subjects.\textsuperscript{27,31}

Surgical interventions on the palate always have negative effects on the growth and development of the craniofacial complex.\textsuperscript{6,15,16,25,42,44,45} Cephalometric analysis of maxillary growth after palatal TE showed significant deviations in anteroposterior and vertical growth. The implications of these findings, however, are limited because they were based on two-dimensional (2-D) analysis.\textsuperscript{37} Since TE is a spatial event, three-dimensional (3-D) analysis should give more insight into the positive and/or negative effects of subperiosteal palatal TE. Therefore, a longitudinal animal experiment was performed, including a 3-D morphometric soft- and hard-tissue analysis.

**Material and methods**

Seventy-five male kittens were assigned to five groups:
- I: tissue-expansion group, TEXP (n=16)
- II: sham group, SEXP (n=14)
- III: tissue expansion in scar tissue group, TSCA (n=17)
- IV: sham scar tissue group, SSCA (n=14)
- V: control group, CONT (n=14).

The animals were housed under normal laboratory conditions in the Central Animal Laboratory, University of Nijmegen, and received a diet consisting of granules, canned meat, and drinking water ad libitum. The cats were weighed every week and before any intervention.

All interventions were performed under standard general anesthesia, induced by 30 mg/kg Nembutal\textsuperscript{17} i.m. (ketamine HCl 100 mg/ml, A.U.V. Cuijk, The Netherlands) with 0.5 ml atropine i.m. (atropine sulfate 0.5 mg/ml), and maintained after intubation with a mixture of O$_2$, N$_2$O, and Ethrane\textsuperscript{18} (enfurane, Abbott BV, Amstelveen, The Netherlands).

A modified Langenbeck operation was performed under sterile conditions in the TSCA and SSCA groups at the age of 8 weeks (Langenbeck, 1861).\textsuperscript{19} For this purpose, the oral cavity and perioral region was disinfected with Betadine\textsuperscript{8} solution (povidone-iodine 10%, Dugra-Pharma BV, Die- men, The Netherlands). A standardized, elliptic, soft-tissue defect was created in the median region of the palate by excising a mucoperiosteal flap. This flap extended from just behind the incisive papilla to the caudal margin of the hard palate. The maximum width of the flap was one-third of the transverse distance between the deciduous first molars. On both sides, relaxation incisions were made adjacent to the posterior teeth. The mucoperiosteum was elevated and the soft-tissue defect was closed in the midline, in one layer, with Vicryl\textsuperscript{16} 5-0 (Ethicon, Johnson & Johnson, Amersfoort, The Netherlands), leaving two areas of denuded bone adjacent to the posterior teeth. Healing was allowed for 6 weeks until the age of 14 weeks.

At 4 weeks of age, custom-made, hemispheric soft-tissue expanders (CUI\textsuperscript{19}, Inamed BV, Breda, The Netherlands) were inserted in groups TEXP, TSCA, SEXP, and SSCA (Fig. 1). The silicone tissue expanders had a diameter of 12 mm at their base and a filling volume of 1 ml.\textsuperscript{21,23,28} They had a filling tube with an incorporated self-sealing port. To introduce the expander, a standardized para-marginal palatal incision from left to right canine was made, creating a submucoperiosteal pocket of approximately 15×15 mm, by elevation of the mucoperiosteum. Both anterior palatal neurovascular bundles were coagulated, while the posterior palatal neurovascular bundles were spared. A 2-mm incision was made on the left buccal side, between the canine and first deciduous molar and the gingiva, and the mucoperiosteum was tunneled to admit the external filling tube. The palatal incision was closed with Vicryl 5-0 sutures.

No surgery was performed in group CONT, but the animals were subjected to the same regimen of recording.

All inserted expanders were initially filled.
with 0.1 ml of a radiopaque solution (Angiograft®; meglumine-amidotrizoate 0.650 g/ml; 306 mg I/ml, Schering AG, Berlin, Germany).

Perioperative antibiotics were administered: preoperatively, 0.5 ml Albipen® 15%, i.m. (ampicillin anhydrite 150 mg/ml, Gist-brocades NV, Delft, The Netherlands), and postoperatively, on days 1 and 3, 1.0 ml Albipen® L.A., i.m. (ampicillin anhydrite 100 mg/ml Gist-brocades NV, Delft, The Netherlands).

The wounds were inspected daily for the first week after surgery or, when indicated, for a longer period. Healing was allowed for 2 weeks until the age of 16 weeks. Thereafter, approximately 0.1 ml Angiograft was added weekly to each tissue expander in the TEXP and TSCA groups with a blunt, 22-gauge needle until the maximum volume was reached at the age of 24 weeks (Fig. 2).

Then, the tissue expanders and the tissue surplus were surgically removed, and the wound in the midline was closed with Vicryl 5-0 sutures. The animals were followed for another 8 weeks, the same regimen of recording of data being used.

Thirteen cats had to be excluded from the study due to technical failures. Sixty-two cats could be followed until the age of 20 weeks, and 52 cats were followed for the entire inflation period up to 24 weeks of age. Forty were studied during a period of 8 weeks after removal of the tissue expander.

Dental casts were made every 2 weeks by means of an alginate impression (CA37® Superior Pink, Cavex Holland BV, Haarlem, The Netherlands) in individually designed impression trays (Fig. 3). The casts were analyzed and digitized with a standard reflex microscope (Reflex Measurement Ltd, Somerset, UK). Four observers were involved, independently identifying the following 10 defined points with a magnification of 10 times (Fig. 4):
- 1 = most posterior point of the rugae in the midline
- 2 and 10 = posterior intersection, marginal gingiva and third deciduous molar (incuspid third premolar) on the right and left sides, respectively
- 3 and 9 = anterior intersection, marginal gingiva and second deciduous molar (second premolar) on the right and left sides, respectively
- 4 and 8 = posterior intersection, marginal gingiva and canine on the right and left sides, respectively
- 5 and 7 = posterior intersection, marginal gingiva and third incisor on the right and left sides, respectively
- 6 = anterior intersection, interdental papilla and central incisors in the midline.

On the dental cast models, a virtual 2.0-mm mesh grid was generated, and the x, y, and z coordinates of each nodal point were determined. In this way, a 3-D field of points was created which was limited by the 10 defined points. A virtual 2-D area was also denominated by these points. The following surfaces, distances, and angles were calculated (Fig. 5):
- 2-D and 3-D surface areas
- transverse distance from the highest point of registration, 3-D minus 2-D calculation
- sagittal distance from the highest point of registration, 3-D minus 2-D calculation
- 1–6 = anteroposterior distance (APD)
- 2–10 = posterior transversal distance (PTD)
- 3–9 = middle transversal distance (MTD)
- 4–8 = anterior transversal distance (ATD)
- <2–3–4 = right lateral angle (RLA)
- <8–9–10 = left lateral angle (LLA).

The error of the procedures was determined by repeated measurements of 304 models of different cats at different ages.

**Statistical procedures**

Means and standard deviations were calculated for the increments of all variables in all groups. Comparison between the groups was
carried out by one-way ANOVA and analysis of covariance; if significant differences between groups were found, Tukey’s multiple comparisons test was used for evaluation of these differences. Analysis was performed for the period of 14–20 weeks of age for 62 cats and for the period of 14–24 weeks of age for 52 cats. In 40 cats, the period of 8 weeks after removal of the tissue expanders was evaluated. Intraobserver and interobserver variability tests were performed to check the accuracy and reproducibility of the procedures.

Results

Error analysis

The duplicate measurements at different ages showed a mean total error of the anterior, middle, and posterior transversal distance measurements of maximally 0.2 mm. The mean total error of the angle measurements was 1.6. Surface area calculations showed mean total errors of 15 mm² for 2-D surfaces, 25 mm² for 3-D surfaces, and 17 mm² for 3-D – 2-D differences.

On the basis of these results, the accuracy and reproducibility of the methods were considered to be acceptable.

Effects of the Interventions

Mutual comparison of all groups by one-way ANOVA at the age of 20 weeks revealed that none of the 2-D parameters showed a significant difference between the comparable scarred and nonscarred groups.

At the age of 24 weeks, the anterior transversal distance (ATD) and the left lateral angle (LLA) showed significant differences between the groups (Table 1a). In groups TSCA and SSCA, ATD was significantly smaller than in group SEXP. LLA was smaller in group CONT than in groups TEXP, TSCA, and SSCA (Table 1a).

During the whole expansion period of 10 weeks after insertion of the tissue expander (Table 1b), significant differences were observed for the increments of the variables ATD and LLA. In the TSCA group, there was a smaller increment of ATD than in the CONT and SEXP groups. LLA diminished significantly in the CONT and SEXP groups, whereas it increased in the TEXP group (Table 1b).

Analysis of covariance showed that scarring itself was not decisive for the 3-D parameters, surface area, and transversal and sagittal distance. This means that pooling of the groups TEXP and TSCA, as well as of the groups SEXP and SSCA, was allowed, resulting in a group TE, consisting of 29 (20) cats, and a group SH, consisting of 22 (17) animals. The three groups TE, SH, and CO (controls, n=14) were mutually compared by one-way ANOVA, and significant differences, if any, were subsequently explored by means of Tukey’s multiple comparisons test (Tables 2a and b).
Table 3. Increments in first 4 weeks after removal of tissue expander

<table>
<thead>
<tr>
<th>Group n=41</th>
<th>I TEXP n=10</th>
<th>II SEXP n=7</th>
<th>III TSCA n=6</th>
<th>IV SSCA n=6</th>
<th>V CONT n=12</th>
<th>Pooled SD</th>
<th>Intergroup differences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variable</td>
<td>Mean</td>
<td>Mean</td>
<td>Mean</td>
<td>Mean</td>
<td>Mean</td>
<td>P&lt;0.05</td>
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<tr>
<td>2-10 PTD</td>
<td>0.48</td>
<td>0.56</td>
<td>0.94</td>
<td>0.82</td>
<td>0.56</td>
<td>0.32</td>
<td>I, V&lt;II</td>
</tr>
<tr>
<td>3-9 MTD</td>
<td>-0.74</td>
<td>0.42</td>
<td>1.03</td>
<td>0.20</td>
<td>0.50</td>
<td>0.76</td>
<td>I&lt;II, III, V</td>
</tr>
<tr>
<td>4-8 ATD</td>
<td>0.55</td>
<td>0.45</td>
<td>1.74</td>
<td>0.98</td>
<td>0.46</td>
<td>0.46</td>
<td>I, II, IV, V&lt;III</td>
</tr>
<tr>
<td>&lt;2-3-4 RLA</td>
<td>-5.04</td>
<td>-0.45</td>
<td>2.86</td>
<td>-3.40</td>
<td>1.38</td>
<td>3.13</td>
<td>I&lt;II, III, V&lt;IV&lt;III</td>
</tr>
<tr>
<td>&lt;10-9-8 LLA</td>
<td>-4.00</td>
<td>1.01</td>
<td>1.42</td>
<td>0.47</td>
<td>0.30</td>
<td>5.30</td>
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Values in millimeters and degrees, respectively, per month.

Table 4. Increments in last 4 weeks after removal of tissue expander

<table>
<thead>
<tr>
<th>Group n=41</th>
<th>I TEXP n=10</th>
<th>II SEXP n=7</th>
<th>III TSCA n=6</th>
<th>IV SSCA n=6</th>
<th>V CONT n=12</th>
<th>Pooled SD</th>
<th>Intergroup differences</th>
</tr>
</thead>
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<tr>
<td>Variable</td>
<td>Mean</td>
<td>Mean</td>
<td>Mean</td>
<td>Mean</td>
<td>Mean</td>
<td>P&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>2-10 PTD</td>
<td>0.48</td>
<td>0.25</td>
<td>0.71</td>
<td>0.38</td>
<td>0.45</td>
<td>0.30</td>
<td>-</td>
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<tr>
<td>3-9 MTD</td>
<td>0.28</td>
<td>0.37</td>
<td>0.78</td>
<td>0.71</td>
<td>0.55</td>
<td>0.32</td>
<td>I&lt;III</td>
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<tr>
<td>4-8 ATD</td>
<td>0.13</td>
<td>-0.02</td>
<td>0.60</td>
<td>0.18</td>
<td>0.25</td>
<td>0.24</td>
<td>I, II, IV, V&lt;III</td>
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<tr>
<td>&lt;2-3-4 RLA</td>
<td>0.56</td>
<td>2.26</td>
<td>1.94</td>
<td>3.05</td>
<td>2.14</td>
<td>2.18</td>
<td>-</td>
</tr>
<tr>
<td>&lt;10-9-8 LLA</td>
<td>0.34</td>
<td>2.07</td>
<td>2.29</td>
<td>3.20</td>
<td>1.38</td>
<td>2.62</td>
<td>-</td>
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Values in millimeters and degrees, respectively, per month.

Table 5. Increments 8 weeks after removal of tissue expander

<table>
<thead>
<tr>
<th>Group n=41</th>
<th>I TEXP n=10</th>
<th>II SEXP n=7</th>
<th>III TSCA n=6</th>
<th>IV SSCA n=6</th>
<th>V CONT n=12</th>
<th>Pooled SD</th>
<th>Intergroup differences</th>
</tr>
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<tbody>
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<td>Variable</td>
<td>Mean</td>
<td>Mean</td>
<td>Mean</td>
<td>Mean</td>
<td>Mean</td>
<td>P&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>2-10 PTD</td>
<td>0.48</td>
<td>0.40</td>
<td>0.86</td>
<td>0.59</td>
<td>0.50</td>
<td>0.19</td>
<td>I, II, V&lt;III</td>
</tr>
<tr>
<td>3-9 MTD</td>
<td>-0.23</td>
<td>0.39</td>
<td>0.90</td>
<td>0.45</td>
<td>0.52</td>
<td>0.39</td>
<td>I&lt;II, III, IV, V</td>
</tr>
<tr>
<td>4-8 ATD</td>
<td>0.34</td>
<td>0.22</td>
<td>1.16</td>
<td>0.58</td>
<td>0.36</td>
<td>0.25</td>
<td>I, II, IV, V&lt;III</td>
</tr>
<tr>
<td>&lt;2-3-4 RLA</td>
<td>-2.22</td>
<td>0.90</td>
<td>2.40</td>
<td>-0.17</td>
<td>1.75</td>
<td>1.97</td>
<td>I&lt;II, III, V</td>
</tr>
<tr>
<td>&lt;10-9-8 LLA</td>
<td>-1.81</td>
<td>1.53</td>
<td>1.76</td>
<td>1.82</td>
<td>0.83</td>
<td>2.68</td>
<td>-</td>
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</table>

Values in millimeters and degrees, respectively, per month.

Discussion

The present study aimed to evaluate the possibilities and effects of tissue expansion in the palatal mucoperiosteum in growing cats, with and without pre-existing scars, using a hemispheric tissue expander 12 mm in diameter and 6 mm in height with an external filling port.

Palatal TE may interfere with food intake by mechanical hindrance and expansion-related discomfort, which is reported to occur during and shortly after inflation\textsuperscript{19}. To monitor this possible effect, the weight gain was carefully recorded on a weekly basis, and no detrimental effects were found.

Complications of TE in the head and neck area were reported first by Manders et al.\textsuperscript{23} in 1984, and later by Antonshyn et al.\textsuperscript{24}. Complication rates vary from 0 to 69\%, depending on surgeon, time, technique, and anatomic site. Most authors report high complication rates early in their experience, with a subsequent decrease. Complication rates in the present study varied from 20 to 25\%, depending on the group and the initial filling of the expander. Erosions, necrosis of the soft tissues, leakage, and extrusions of the expander were the most common complications and were seen mainly in the scarred-tissue groups.
In order to check the validity of the TE technique in scarred tissue, as is usually present in CLP situations, we performed a modified Langenbeck operation to induce palatal scars. The dentomaxillary anatomy and the effects of simulated Langenbeck surgery in dogs are well documented, and are assumed to be virtually the same for cats. The most prominent growth disturbances due to this type of surgery were found in the transverse dental arch measures. The present study revealed no significant influence of the simulated Langenbeck surgery on the parameters in the transversal plane until the age of 20 weeks. As a consequence, the Langenbeck and non-Langenbeck groups were pooled for that period. Initially, there were only a few significant differences in the transversal distances. However, in time, the differences became significant in the anterior palatal region, indicating a continuous influence of the combination of modified Langenbeck operation and TE.

TE caused a significant increase in the palatal soft-tissue surface area, as was expected. It yields a temporary tissue gain of approximately 85% of the base surface of a hemispheric tissue expander after 8 weeks of active expansion. In the literature, tissue gain was initially related to the base dimensions of the tissue expander, and authors recommend that the expander base area be 1.5 times the size of the defect to be closed. There is a wide range of percentages of area increase, varying from 12.5 to 110%, based on either estimation, measuring, or calculation. Theoretically, a 100% area gain can be reached if a hemispheric tissue expander is used (surface of hemisphere minus surface of circle, divided by the surface of the circle: 24π − πR2/2 = 1). Van Rappard et al. and Shively used different mathematical formulas for the expected gain, and concluded that area gain is a function only of the height of the expander and not of its base diameter. In vivo, Van Rappard was able to realize a surface area increase of 25−38% of the mathematically expected area gain (depending on the shape and height of the tissue expander). The findings in this study for soft-tissue gain in transversal and sagittal directions (36% and 32%, respectively) are rather favorable, corresponding to data found by Nordström & Devine (1985) (39% and 27%) and VanderKolk et al. (1987) (45% and 30%)..

Palatal surgery and TE cause some adverse side-effects in the bony palatal and dentoalveolar structures. The anterior transversal distance increment was significantly smaller in the TE in scarred tissue situation than in the sham expansion and control groups, if the whole expansion period was considered. However, after removal of the tissue expander, significantly increased growth of almost all parameters took place in the TSCA group.

The differences between the left and right lateral angles may be related to the fact that the filling tube was located on the left side in all cases. An explanation may be found in the formation of scar tissue after the small buccal incision and tunneling of the mucoperiosteum, with possible deterioration of the lateral growth at one side. In the first 4 weeks after removal of the tissue expanders, nearly all parameters in the experimental expansion without scar group were smaller than in the expansion with scar group, and some were less than the control values. The effects after removal of the tissue expander were less pronounced in the second period of 4 weeks than in the first. In general, the growth increments of the cats decreased with age. However, significant differences in increments remained over the total period of 8 weeks, indicating a prolonged effect.

It can be concluded that TE of the palatal mucoperiosteum is quite possible in young cats, with and without scars of the palatal tissues. The soft-tissue gain, as quantified by this method of morphometric analysis, was related to the base surface and base diameter of the tissue expander, being 85%, 36%, and 32% for surface area increase, and transversal and sagittal distance increments, respectively. The effects on growth and development comprised mainly retardation of transversal growth of the dentoalveolar structures at the anterior palatal level, probably caused by traction of the soft tissues overlying the superimpositionally located tissue expander. In the TSCA group, these effects were temporary, since there was accelerated growth in the area after removal of the tissue expander.

Future histomorphologic analysis will probably afford more insight into tissue reactions, and cellular and intercellular matrix responses to tissue expansion, allowing us to explain the cephalometrically and morphometrically detected phenomena.

Acknowledgements — We are indebted to CUI8, Carpinteria, USA, and Inamed BV, Breda, The Netherlands, for production and distribution of the custom-made tissue expanders; to Jan A. D. Laverman for producing the cast models; to Judith M. C. Spijkers, George F. Robinson, and Eefje E. Westering for analysis and digitization of the cast models with the reflex microscope; to Servaas J. A. M. Nottet for programming and data acquisition; to Theo H. M. Arts, Fred H. G. Philippsen, Ton J. Peters, and Albert M. Peters for animal care; to Petra van Veelen for typographic assistance; and to Eric N. Robertson for correcting the English text.

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VAN DAMME PHA, MATLIA JC, KUIJPERS-JAGTMAN AM, FREEDHOFER HPM, VAN ’T HOF MA, SHUENERS JMC. Two-dimensional cephalometric analysis of the effects of subperiosteal palatal soft tissue expansion in growing cats. Submitted.


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