A MODEL FOR MUSCLE REGENERATION IN THE SOFT PALATE

Paola L. Carvajal Monroy, Johannes W. Von den Hoff, Frank A.D.T.G. Wagener, Anne Marie Kuijpers-Jagtman
Department Orthodontics and Craniofacial Biology, Radboud University Nijmegen Medical Centre. The Netherlands.

Introduction

Children with a cleft in the soft palate have difficulties with speech, swallowing, and sucking. These patients are unable to separate the nasal from the oral cavity; a phenomenon known as velopharyngeal dysfunction. Surgical repair is required in order to close the defect and to reconstruct the muscle sling of the m. levator veli palatini, the major muscle of the soft palate. It ensures optimal function and normal speech development. However, about 10% to 30% of the treated patients still have inadequate velopharyngeal function. Scar formation and defective regeneration hamper the functional recovery of the muscles after cleft palate repair. Therefore, the aim of this study is to investigate the anatomy and histology of the soft palate in rats, and to establish an in vivo model for muscle regeneration after surgical injury.

Material and Methods

Approval of the research protocol was obtained from the local Board for Animal Experiments in accordance with Dutch laws and regulations (RU-DEC 2011-125). 14 male adult Sprague Dawley rats were used. The animals were divided into three groups: Group 1 (n = 4) and 2 (n = 2) to investigate the histology of the soft palate respectively, and group 3 (n = 8) for surgical wounding of the soft palate. In the last group, two animals were used as control, and the wound area was evaluated after 7 and 56 days using (immuno)histochemistry for muscle markers (AZAN staining. Pax 7, MyoD, MyoG, and ASMA).

Results

Our study shows that the anatomy and histology of the soft palate muscles of the rat are largely comparable to those of humans. Two main areas were identified in the soft palate, an anterior and a posterior area. The anterior area is characterized by a thick layer of salivary glands, which is covered by a layer of oral (bottom) and nasal mucosa (top). The oral surface is covered by a keratinized stratified squamous epithelium, while the nasal surface is covered by a pseudostratified ciliated columnar epithelium. The oral mucosa contains a thick collagenous submucosa (Figure 1).

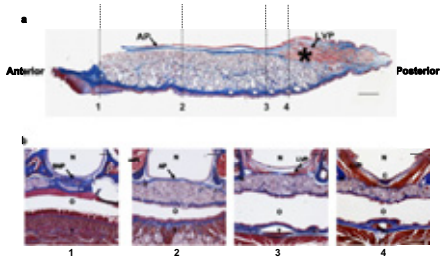

Figure 1. Histology of the soft palate.
Paraffin sections were stained with AZAN. (A) Midsagittal section of the soft palate. The anterior two thirds of the soft palate mainly contain salivary glands. The posterior third of the soft palate contains an additional layer of muscle tissue. (B) Coronal sections of the soft palate. The dotted lines in figure A indicate the position of the coronal sections. N: nasal cavity, O: oral cavity, PA: palatine aponeurosis, SG: salivary glands, PNS: posterior nasal spine, LVP: levator veli palatini muscle, H: pterygoid hamulus, T: Tongue. The bar represents 500 µm.

All animals survived surgery and showed no weight loss. After seven days, all wounds showed complete clinical healing. After 7 days, granulation tissue and initial regeneration of muscle fibers and salivary glands was observed. After 56 days, more collagen was present and only few myofibers had regenerated. Myofibroblasts were not present in the muscle tissue of the controls or at 56 days after wounding but only in blood vessels and salivary glands (Figure 2).

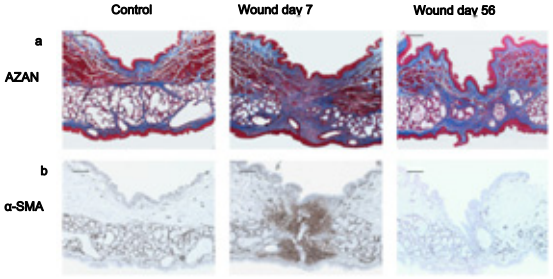

Figure 2. Regeneration of the soft palate after wounding.
Control and wound tissues from the soft palate were stained with AZAN, and with antibodies against myofibroblasts (ASMA). (A) AZAN staining. Connective tissue is stained blue, muscle tissue red. Black arrows indicate muscle fibers, white arrows indicate initial regeneration of salivary glands. SG: Salivary glands. (B) ASMA (Brown). The bar represents 200 µm.

At 7 days the wounds showed large numbers of myofibroblasts, and more activated SatCs (Pax7-MyoD- and MyoG-positieve) in regenerating muscle fibers than in control muscles. At 56 days no myofibroblasts were present anymore (figure 3).

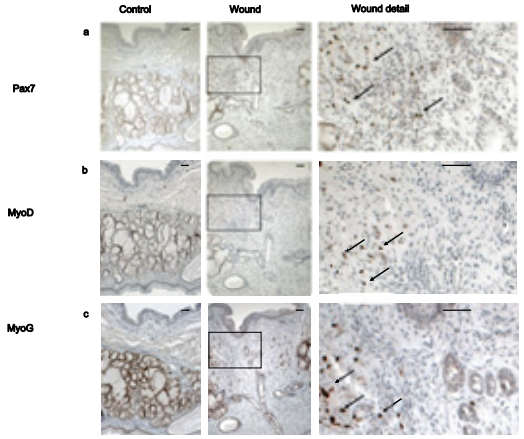

Figure 3. Satellite cells and myofibers after wounding.
(A) Satellite cells (arrows) express the transcription factor Pax7. (B) The myogenic determination factor 1 (MyoD, arrows) is expressed during satellite cell proliferation. (C) Differentiation is marked by a decline in Pax 7 expression, and the induction of myogenin (MyoG, arrows). Pax7-, MyoD-, and MyoG positive cells are stained brown. The bar represents 50 µm.

Conclusion

We show that this model is suitable to study muscle regeneration in the rat soft palate, and allows the development of novel adjuvant strategies to promote muscle regeneration after surgery. This will offer new perspectives for the treatment of CLP patients, and for various other conditions in which the regeneration of head muscles is compromised.