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Palatogenesis
Engineering, pathways and pathologies

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Cleft palate represents the second most common birth defect and carries substantial physiologic and social challenges for affected patients, as they often require multiple surgical interventions during their lifetime. A number of genes have been identified to be associated with the cleft palate phenotype, but etiology in the majority of cases remains elusive. In order to better understand cleft palate and both surgical and potential tissue engineering approaches for repair, we have performed an in-depth literature review into cleft palate development in humans and mice, as well as into molecular pathways underlying these pathologic developments. We summarize the multitude of pathways underlying cleft palate development, with the transforming growth factor β superfamily being the most commonly studied. Furthermore, while the majority of cleft palate studies are performed using a mouse model, studies focusing on tissue engineering have also focused heavily on mouse models. A paucity of human randomized controlled studies exists for cleft palate repair, and so far, tissue engineering approaches are limited. In this review, we discuss the development of the palate, explain the basic science behind normal and pathologic palate development in humans as well as mouse models and elaborate on how these studies may lead to future advances in palatal tissue engineering and cleft palate treatments.

Introduction

Cleft lip and palate (CLP) represents the second most common birth defect, with an incidence ranging from 1 in 500 to about 1 in 2,500 births. This suggests that susceptibility genes likely differ between races.[1] Furthermore, environmental risk factors have been identified, making it difficult to isolate a single cause behind CLP. When assessing all cases, annual treatment costs exceed $100 million in direct hospital costs alone in the United States.[2] Taken together, whether one is referring to CLP or isolated cleft palate, these are common birth defects, and they represent an enormous biomedical burden. Affected children with CLP require their first operation as neonates to close the cleft lip; closure of the palate happens during a second operation at approximately 6–12 months of life. Following the initial closure of the cleft palate, these children face continuing challenges with speech, facial growth, dental occlusion and hearing. Affected children often undergo intensive therapy to establish normal speech patterns, but may require further surgery if unsuccessful. In addition to speech, children endure substantial dental problems that will require orthodontic intervention. Not infrequently, affected children have associated midface hypoplasia due to the restraining effect of palatal scarring on the growing maxilla and require orthognathic surgery later in life.

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Development of the primary and secondary palate have been shown to be unique entities on a genetic and embryologic level.[1,3] In the majority (70%) of cleft lip and palate, these deformities occur independent from other craniofacial abnormalities and are called "isolated, non-syndromic cleft lip and palate." Though the large growth of genetics research has uncovered numerous pathways involved with syndromic cleft lip, we lack a solid genetic and molecular understanding of the cause for these isolated cleft cases.

Previous studies indicate that the pathogenesis of cleft palate is multifactorial and likely has both genetic and environmental factors. Much of our knowledge of craniofacial clefting arises from case studies of patients and selected animal models. A number of genes have been identified to be associated with the cleft palate phenotype, but the etiology of the majority of cases remains elusive. In this review, we discuss palatal development, explore the basic science behind normal and pathologic palate development and elaborate on how these findings may lead to future advances in the treatment of cleft palate.

Cleft Palate Development

The facial region of the mammalian embryo originates mainly from the frontonasal prominence (forehead, nose, philtrum and primary palate), the maxillomandibular prominence from the first branchial arch (maxilla, mandible, lateral upper lip and secondary palate) and the lateral nasal prominences. The intermaxillary segment forms when the two medial nasal prominences fuse together at the midline, giving rise to the philtrum of the lip, four incisor teeth and the primary palate of the adult. The secondary palate forms from outgrowths of the maxillary prominences called palatal shelves or palatine processes; these palatal shelves fuse at the midline (**Fig. 1A and B**). The definitive palate is formed following fusion of the primary and secondary

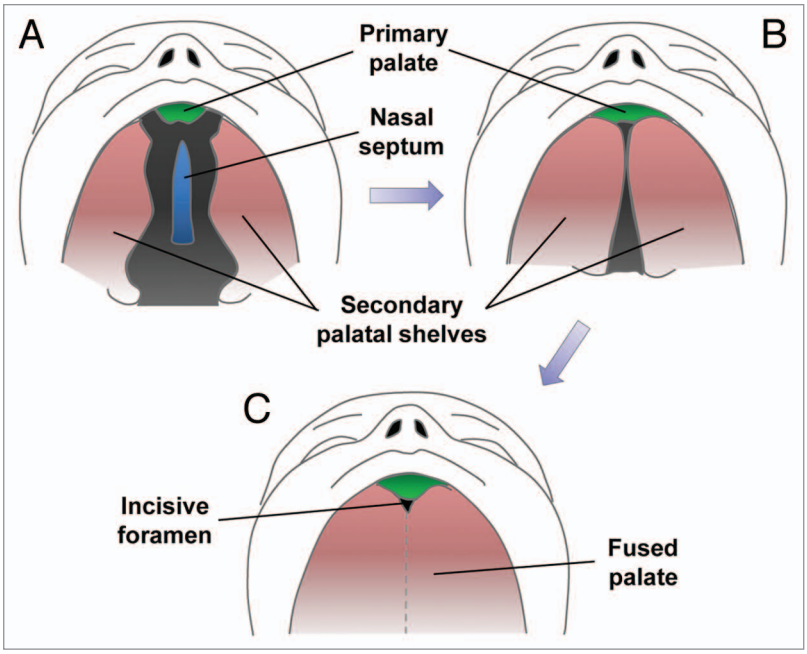

Figure 1. Human palatal development. (A) Week six of human palatal development, with the secondary palate shown vertically on each side of the tongue and a gap between the secondary palate, nasal septum and primary palate. (B) After descent of the tongue, the secondary palatal shelves elevate and orient horizontally, allowing them to come in contact and begin fusing. (C) Fusion of the primary and secondary palate and the nasal septum separating the oropharynx from the nasopharynx. Figure modified from Dixon et al.[1]

palates at the incisive foramen and with the nasal septum above (**Fig. 1C**).

The palate is limited anteriorly by the incisive foramen and extends posteriorly through the structures of the hard palate, soft palate and uvula. The structures anterior to the incisive foramen are collectively referred to as the pre-palatal structures or the primary palate (upper lip and alveolus). The structures posterior to the incisive foramen are called the secondary palate. As is the case for all branchial arches, the first arch that forms the palate contains mesodermal mesenchymal cells, ectoderm-derived neural crest cells, a cranial nerve (trigeminal) and a blood supply (maxillary artery).

Development of the human hard palate occurs between weeks 5 and 12 of gestation. The lateral palatine processes gradually grow toward the midline, fusing first anteriorly by week 8 and posteriorly as far back as the uvula by week 12. Development of the secondary palate occurs from the lateral palatine processes that grow vertically and obliquely on both sides of the developing tongue (**Fig. 1A**). As the tongue descends, the palatine processes swing upward into a more transverse orientation (**Fig. 1B**). Some instances of cleft palate can be caused by failure of descent of the tongue, as in the case of Pierre Robin Syndrome where children have an underdeveloped mandible and failure of tongue descent, leading to cleft palate formation and respiratory compromise.

In mice, there is a significant amount of similarity in palatal development (**Fig. 2A–D**). Mouse facial development begins at E9.5 (corresponding to week 4 gestation in humans) with the appearance of the frontonasal process, paired maxillary and mandibular processes (late week 4 in humans). These processes have a cranial neural crest mesenchymal core surrounded by ectoderm-derived epithelium.[4] The upper lip develops from the ventral frotonasal process at E10. Subsequently, the nasal placodes invaginate, and the medial and lateral nasal processes form. Growth and apposition of the medial nasal processes with each other and with the maxillary process create the intermaxillary segment, which consists of upper lip, upper two incisors and the primary palate (week 4 in human).[5] By E12.5, the primary palate and upper lip development is complete (week 7 in humans). Secondary palate development starts on E11.5 (early week 7 human gestation). From E12–E14, the palatal shelves enter their active growth phase (human gestation weeks 7–8), at which time the palatal shelves are positioned vertically between the cheeks and lateral to the elevated tongue. At E14.5–E15 (week 9 in humans), the palatal shelves elevate and reorient into a horizontal position above the tongue. Subsequently, the shelves oppose and adhere along their medial edge, creating a transient medial epithelial seam. The palatal shelves then fuse anteriorly with the primary palate at the incisive foramen and dorsally with the nasal septum. The medial epithelial seam disintegrates and fusion of the palatal shelf, primary palate and vomer epithelia allow separation of the oral and nasal cavities, which is necessary for simultaneous breathing and feeding. The hard palate forms from osteogenic differentiation of palatal shelf mesenchymal cells into osteoblasts. Palatal fusion is completed by E15.5, at which point mesenchymal condensation occurs, followed by the osteogenic differentiation of the palatal mesenchyme, leading to formation of the palatine bone in the secondary palate. By E16.5, secondary palate formation is complete (10 weeks in humans).

Abnormal Palate Development/Types of Cleft Palate

Clefts of the palate alone or associated with cleft lip may involve either the primary or secondary palate and frequently both (complete clefts). Those involving the primary palate are associated with clefts of the lip. Palatal clefts may also be unilateral or bilateral. Isolated clefts of the secondary palate (incomplete clefts) occur in the absence of defects in either the lip or the alveolar process (**Fig. 2C and D**). Because palatal fusion occurs in an anterior to posterior direction, clefts of the secondary palate may involve only the soft palate or both the soft and hard palates together.[6] Clinically, clefting in the secondary palate extends anteriorly from the uvula to varying degrees, often involving the hard palate.[7] In complete forms, the cleft can affect the entire secondary palate, reaching the incisive foramen, leaving the nasopharynx in direct communication with the oral cavity. The vomer can thus be seen as a midline structure extending from the base of the skull.

While complete and incomplete clefts of the palate may be readily apparent on physical exam, other, more subtle forms may also exist with variable import with regard to feeding, speech development and ear infections.[8] The submucous cleft palate is defined by the classic triad of a bifid uvula, palatal muscle diastasis and a midline notch in the posterior edge of the bony palate.[8,9] The muscle separation results in a bluish, two-layered mucosal bridge, the zona pellucida, while the midline notch results from an abnormal development of the posterior nasal spine. Though the majority of patients with submucous clefts remain asymptomatic, approximately 15% develop velopharyngeal insufficiency with hypernasal speech.[9] This occurs as the velum is often too short and thin, resulting in limited mobility and easy fatigability. Eventual failure to properly obturate the pharyngeal space develops, particularly after patients undergo adenoidectomy.

All clefts involving the secondary palate (with or without associated cleft lip), including those of the submucous variety, demonstrate abnormal morphology with respect to the levator veli palatini and tensor veli palatini muscles. The levator veli palatini muscles originate from the petrous portion of the temporal bone and medial surface of the auditory tube and normally interdigitate within the central velum to form a sling suspending the soft palate from the base of the skull.[10] The tensor veli palatini originates from the scaphoid fossa and pterygoid plate, coursing around the hamulus to form an aponeurosis in the anterior third of the soft palate.[10] In patients with clefts of the secondary palate, these muscles aberrantly insert into the posterior edge of the hard palate forming Veau's cleft muscle.[11] As the levator veli palatini is oriented sagittaly in an anterior-posterior direction, any attempt at surgical correction must anatomically restore the levator sling to a medial-lateral course for proper vector of pull.[12,13]

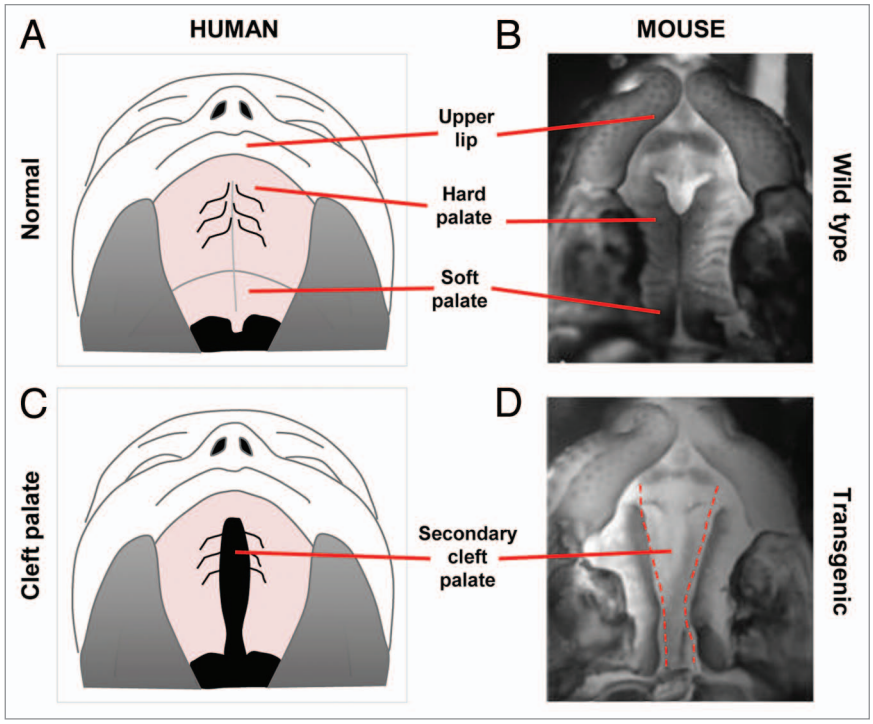

Figure 2. Correlation between human and mouse palates. (A) Normal human upper lip, hard palate and soft palate. (B) Normal mouse upper lip, hard palate and soft palate. (C) Cleft of the secondary palate in human patient. (D) Clefting of secondary palate in transgenic mouse.

Molecular Genetics of Cleft Palate

Gaining a solid understanding of the molecular causes of cleft palate has been complicated due to the plethora of factors that can, when mutated, result in various forms of clefting. There has been a large number of studies on a variety of pathways addressing palatal development. We performed a literature search of PubMed as an attempt to break down this plethora of pathways investigated. The following search terms were used in combination to identify appropriate studies: Cleft palate, palatogenesis, gene signaling and animal studies. The search was limited to studies published in English from 2000–2011 and included both human and animal studies (**Tables 1 and 2**). Extracellular signaling factors were the most commonly studied, with the transforming growth factor beta (TGFβ) superfamily as the subject of the majority of these studies (**Table 2**). To assess animal models currently being used to study CLP, a literature search of PubMed was performed using the following search terms: Cleft palate, palatogenesis, repair, gene signaling and animal studies. The search was limited to studies published in English from 2000 to 2011. Studies were excluded if the full text was inaccessible, or if the animal model was not clearly identified in the methods section. Clearly, the majority of studies used mice as the primary animal model (**Table 3**). Most in vivo models are of two varieties: transgenic mice with cleft phenotype and a teratogen-induced cleft palate.

Although advances have been made in identifying the genetic causes for some syndromic forms of cleft palate, etiology of the more common non-syndromic forms remains poorly characterized.[1] Using various experimental approaches, researchers are now uncovering the molecules and cellular processes that can go awry in cases of palatal clefting.[1] Exploring each pathway in detail is beyond the scope of this review; however, herein, we review those pathways most commonly found to be aberrant in the onset of cleft palate.

PDGF signaling. Platelet-derived growth factor (PDGF) and its receptors (PDGFRα and -β) have specific roles in promoting tissue-tissue interactions to control cell migration, proliferation and survival during embryonic development.[14] Deletion of *Pdgfr*α in the neural crest leads to defects in palatal fusion, nasal septation and abnormal development of several facial bones and cartilage structures in mouse models.[14] Deletion of *Pdgfa* and *Pdgfc* also produce severe craniofacial phenotypes. *Pdgfc*-null

Table 1. Genetic pathways involved in cleft lip and palate

Article No.	PMID	Genetic Pathway (wnt, shh, BMP)	Evidence
1	12223417	Fgf8	1C
2	15572143	Fgf10	1C
3	21185284	Wnt	1E
4	19631205	BMP, FGF, wnt	1A, C, E
5	15102710	BMP	1A
6	16990542	Sumo1	3
7	17846996	TBX22/Sumo1	3
8	17089422	PVRL1	4
9	12520078	EGFR	1A
10	14728799	Msx homeobox	1A
11	17601559	Wnt	1E
12	14755462	Wnt	1E
13	11359935	TGFβ	1A
14	12019011	TGFβ	1A
15	16607638	Jag2-Notch1	6
16	18413325	Wnt	1E
17	14756664	BMP, Shh	1A, D
18	14645125	MES degeneration	4
19	15361870	PDGF	1B
20	15203181	GABA	6
21	14729481	Smad2-dependent Alk-5-driven by TGFβ3	1A, 2
22	16955138	Insig 1,2 cholesterol pathway	7
23	18161794	Nat2	8
24	15831593	CDH1/E-cadherin	4
25	12376112	TGF/β3	1A
26	10932185	Ryk (RTK)	8
27	17041601	IRF	2
28	18452349	GABRB3	6
29	17849453	GAD 67	6
30	12975342	Tgf βr2	1A
31	12807959	MSX1	1A
32	16284941	Meox-2	6
33	16998816	Wnt9b	1E
34	9215640	TGFβ3	1A
35	15103710	GAD67	6
36	2416245	HA and CS	5
37	12219090	IRF6	2
38	18001154	Activin signaling	1A
39	16496313	Wnt3/Wnt9b	1E
40	11420059	Col11a1	5
41	18191119	Wnt11 and Fgfr1b	1C, 1E
42	11391199	Fgfr 1/2	1C
43	17377962	SATB2	3
44	15716346	Bmp	1A
45	15731757	TGFβR1/2	1A
46	10716617	Bmp	1A
47	10753521	TGFβ3	1A
48	12673280	Col11A2	5
49	15870292	Mn1	3
50	15721140	Notch/jagged2	6
51	16723652	MSX1	1A
52	10853828	Desmosomal DSC2	4
53	17376812	Snail, slug	6
54	15275855	TGFβ3	1A
55	17452626	TGfβ3/ smad	1A, 2
56	17442359	Retinoic acid	6
57	16880535	Crk	4
58	15199404	Fgf/shh	1C, 1D
59	16168717	Shh	1D
60	17360555	Fgf	1C
61	17097601	Pax9/shh in TGFβ3 regulation	1D, 1A
62	15562585	Type II collagen	5
63	12203729	Sim2 in HA synthesis	5
64	10932188	PVRL1, nectin-1	4
65	10731669	$p57^{Kip2}$, CDK inhib.	8
66	12490557	PDGFRalpha	1B
67	12854656	Folbp1, Shh	1A
68	15028671	Mnt	2
69	12068957	TGFβ3	1A
70	10742093	Msx1	1A
71	16109396	MES cells/Shh	1D
72	12651933	MSX1TGFβ3	1A
73	16280635	DHCR7, cholesterol	7
74	12812790	Foxf2	2
75	15888125	TGFβ/Smad 3/dishevelled	1A, 1E, 2
76	17693063	FGF/Spry2/Shh	1C, 1D
77	17941048	PDGF-C	1B
78	15543606	PDGFR-alpha	1B
79	16780827	Irf6/TGFβ	1A, 2
80	16141225	Shox2, Fgf	1C
81	17652350	PDS5B	6
82	12163415	Msx1/Bmp	1A
83	11165488	Fgf	1C
84	15939375	IRF6/Smad	2
85	11559848	TBX22	3
86	12412015	Tbx22	3
87	15118109	Tbx10	3
88	12165566	TTF-2	2
89	19092777	PDGF-C	1B
90	12815624	TGFβ3	1A
91	12627230	FGFR1	1C

Table 1 (continued). Genetic pathways involved in cleft lip and palate

Article No.	PMID	Genetic Pathway (wnt, shh, BMP)	Evidence
92	18264099	PDGF	1B
93	17066417	PDGF-C/RA	1B, 6
94	18278815	IRF6	2
95	19401770	IRF6	2
96	12412014	IP 3/TGF	1A
97	19282774	IRF6	2
98	19036739	IRF6	2
99	18948418	TBx22	3
100	16405370	PTCH	1D
101	14729838	TBX22	3
102	18431835	TGFβ3	1A
103	18423521	TFAP2A	9
104	19281781	TGFβ1/3	1A
105	16641627	PVRL1/Jag2	4, 6
106	14630905	MSX1	1A
107	18836445	AP-2/IRF6	2
108	11559849	PVRL1	4
109	17868388	TBX22	3
110	19249007	BMP4	1A
111	12514106	Fgfr1	1C
112	16563169	IRF6, LHX6, LHX7	2
113	19341725	Hand2	1A
114	12571099	Hand2	1A
115	12163415	Msx1à BMP, shh	1A, 1D
116	15317890	IRF6	2
117	12374769	TBX22	3
118	20652317	IRF6	2
119	21295280	FAF1	6
120	21195053	Sprouty-2/FGF	1C
121	21061289	ATRA/BMP	1A
122	21058326	Chlorcyclizine, H1 antag, Wnt, FGF, BMP	1A, 1C,1E
123	21041365	ADAMTS9/20	9
124	21034733	BMP	1A
125	20967564	TGFB3	1A
126	20945347	TGFB3	1A
127	20940229	Fz	1E
128	20920724	FGF10	1C
129	20809987	TGFB3	1A
130	20727875	BMP/Noggin	1A
131	20616530	Twist1, Snai1 and Runx2	6
132	20506229	JARID2	9
133	20451169	RBM10	9
134	20424327	P63/IRF6	2
135	20424318	P63/IRF6	2
136	20196077	IRF6	2
137	20165883	RECK, MMP2/3/9	5
138	20133659	FGFR2	1C
139	20007998	PRDM16/TGFβ	1A
140	19934017	Msx1/Shh	1A, 1D
141	19782673	Shh	1D
142	19769959	TBX2/3	3
143	19676060	p63	3
144	19653318	Hoxa2/msx1/BMP	1A
145	19648291	Tbx22	3
146	19598129	Wnt	1E
147	19415673	TGFβ3	1A
148	19394325	Shh	1D
149	19388084	Smad2/3, RA	2, 6
150	19097194	Pkdcc	8
151	18948417	Wnt	1E
152	18816854	Gli3	2
153	18697225	TGFβ/twist	1A
154	18694570	TGFβ/Smad	1A, 2
155	18483623	Pax3/BMP	1A
156	18470539	Zfhx1a/TGFβ	1A
157	18437907	RA/BMP	6, 1A
158	17967447	TGFβ	1A
159	17927973	Menin, Pax3, Wnt	6, 1D
160	17586487	Tshz1	2
161	17520166	Eh (hairy ears) Hoxc5	9
162	17337617	MYH9	6
163	17293880	GSK3β glycogen	5
164	17028893	Tbx3/BMP4	3, 1A
165	17022082	CBFB	6
166	16761282	Runx3/TGFβ/BMP	1A
167	16751186	Fidgetin/ATPase, AKAP95	4
168	16546851	Sox9	6
169	16410076	atRA/TGFb/Smad	1A, 2
170	16330189	Msx/BMP	1A
171	16313756	BMP	1A
172	16199551	PHF8	2
173	16028273	FGF twist, snail/BMP ID1	1C, 1A, 6
174	16026545	TGFβ2	1A
175	15914589	twist, snail and E-cadherin	6, 4
176	15649471	Smad/TGFβ	1A, 2
177	15282310	Sall3	2
178	15175245	Osr2, Pax9, TGFβ3	1A
179	15162512	Fgf8	1C
180	14761683	PKA pathway	8
181	14702176	iNOS, BMP	1A
182	14691138	TGFβ	1A

Table 1 (continued). Genetic pathways involved in cleft lip and palate

Article No.	PMID	Genetic Pathway (wnt, shh, BMP)	Evidence
183	14643678	Zfhep	6
184	12666199	Decorin, biglycan	5
185	12666195	Tbx22	3
186	12606284	GABA(a) β3	6
187	12388835	PKA	8
188	12204278	Tbx22	3
189	12203737	Titf2/foxe, TTF2 fork	2
190	12175701	CREB, phosphotase	8
191	12086327	Lhx6	2
192	11969262	RA/BMP	1A, 6
193	11795938	EGFR	1A
194	11669453	TGFβ/RA	1A
195	11354513	CREB, cAMP	8
196	11330859	Twist, Fgfr	1C
197	10789828	Hoxa-2	1A
198	10669089	TSP-2/TGFβ	1A
199	21246652	Ephrins	8
200	20333300	GABA, GAD1, Viaat	6
201	20213699	Shh	1D
202	19235875	Fgfr2	1C
203	18176224	Fgfr2	1C
204	16712891	MAPK	8
205	15543606	PDGFRα	1B
206	12011971	TGFβ, RhoA	1A
207	11238884	Dlg, SAP97	6

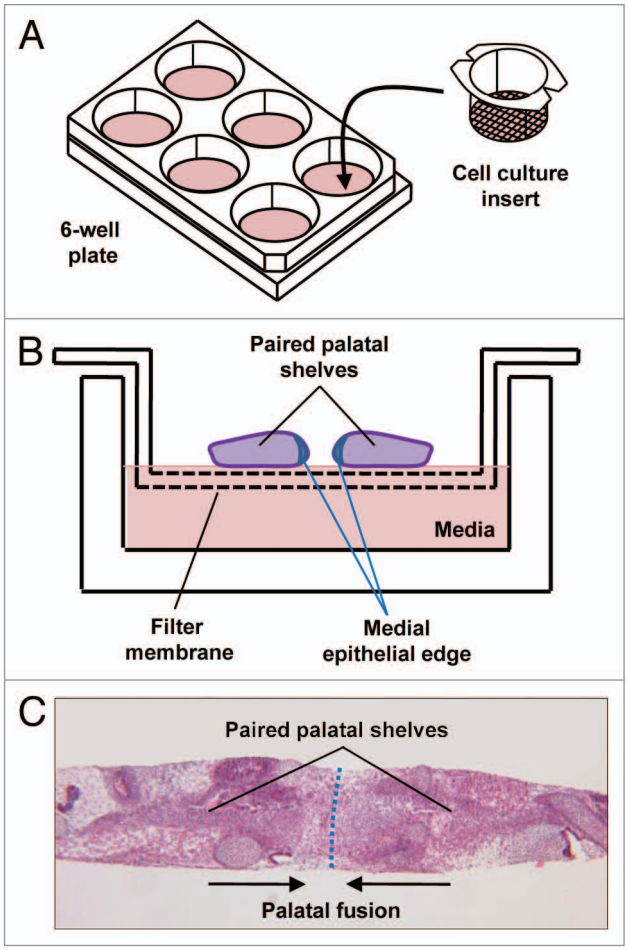

Figure 3. Palate cultures. (A) Schematic of palate culture using 6-well plate and insert with 0.4 μM pores allowing cytokines but not cells to pass through. (B) Schematic of paired palatal shelves in palate culture. (C) H&E stain of palatal fusion after 72 h of 2 palatal shelves in culture while in contact with each other.

neonates have a complete cleft of the secondary palate, accompanied by failure of the palatal bones to extend across the roof of the oronasal cavity. Compound deletions lead to more severe phenotypes, for example, *Pdgfc*/*Pdgfa* double-knockout embryos develop a cleft face with cranial bone defects.[15]

Wingless type (Wnt) protein signaling. Wnt signaling regulates numerous developmental processes, including cell proliferation, differentiation and survival.[16] Wnt signaling also plays an important role in the generation and migration of neural crest cells and the development and patterning of the embryonic face in various species.[17-19] Several members of the Wnt family, including ligands, receptors and co-receptors are expressed in the developing facial prominences.[20-22] Wnt pathway activity is specifically localized to facial epithelia and underlying mesenchyme in the lateral nasal, maxillary and mandibular prominences.[17] The hypothesized role for Wnt activity in the facial prominences varies within different tissues. In neural crest mesenchyme, Wnts promote proliferation, thus promoting the growth of the maxillary prominences that come together to form the palate.[17] In the facial epithelium, expression of multiple Wnts is essential for the fusion of facial prominences.[22-24] Onset of clefting is linked to disruptions in various Wnt genes,[25-27] and perturbations of the pathway produce mild to severe facial clefting in various animal models[17,24] as well as in humans.[28] For example, mutation of *Wnt9b* in mice leads to cleft lip and palate and the A/Wysn strain of mice, which have an insertional mutation near the *Wnt9b* locus, have an increased incidence of spontaneous cleft lip and palate.[29] Furthermore, aberrant expression of the low density lipoprotein receptor-related protein 6, *Lrp6*, a Wnt pathway co-receptor, also results in cleft lip and palate.[24]

Our laboratory has studied cleft palates present in both the Indian Hedghehog-null mutant as well as the GSK-3β-null mutant. In both of these models, we noted an obvious cleft in the secondary hard palate, which we believe is caused by dysregulated Wnt and Hedgehog signaling. Previous studies have shown that increase in canonical *Wnt*[30] or decrease in *Hedgehog* signaling[31] result in inhibited ossification. These reports suggest that increased *Wnt* signaling, such as that observed in the GSK-3β-null embryo, act to inhibit the ossification program already in place.[32]

BMP signaling. Bone Morphogenic Protein (BMP) signaling participates in the induction, formation, determination and migration of the cranial neural crest cells which give rise to most

Table 2. Summary of molecular pathways

	Pathway Studied	Number of Studies	Percent of Studies
Extracellular signaling factors	TGFβ superfamily: TGFα, TGFβ, EGFR, Msx1, BMP, Activns, Hand2	71	**30.1%**
	Platelet derived growth factor: PDGF-C	8	**3.4%**
	Fibroblast growth factor: FGF	21	**8.9%**
	Sonic Hedgehog: Shh, PTCH, SMO, CBP	13	**5.5%**
	WNT: Wnt, Dv, Fz	13	**5.5%**
	Total:	126	**53.4%**
Transcription Factors	FFAP2a, AP-2a, IRF6, FOXE1, TTF-2, GLI, SMAD1, Mnt, Phf8	31	**13.1%**
T-box Transcription Factors/Protein Modification	TBX22, TBX1, TBX10, Mn1, SUMO, p63, SATB2	17	**7.2%**
Cell adhesion molecules	PVRL1, Nectin, Integrin, E-cadherin, Desmosomal protein, Crk	10	**4.2%**
Extracellular Matrix	Collagen, GAG, CSPG, HA, MMP	8	**3.4%**
Other Factors	Jag, Notch, GABA, RA, Snai1, Snai2, Runx2, Meox, Pax1, Pax3, PDS5B, FAF, Sox9, MYH9, Zfhep, Dlg	27	**11.4%**
Cholesterol Pathway	Sterol, Insig 1, Insig 2	2	**0.85%**
Kinase Cascades	NAT2, MAPK, CREB, PKA, camp, Eph, RTK, $p57^{Kip2}$	10	**4.2%**
Genes	JARID2, ADAMTS9/20, TFAP2A, RBM10, Hoxc5	5	**2.1%**

of the craniofacial structures. Subsequently, it is also important for patterning and formation of facial primordia. In mice, loss-of-function type I BMP receptor (Bmpr1a) mutations in the craniofacial primordia results in cleft lip and palate. Interestingly, deficiency of Bmp4 ligand alone resulted in cleft lip only.[33] Furthermore, downstream targets within the BMP pathway also have a link to cleft palate. Mutations in the mouse homeobox gene Msx1 results in cleft palate, and this represents a potential model on which to study cleft palate development.[34]

TGFβ3. TGFβ3 is a member of the TGFβ superfamily and is expressed by medial edge epithelial (MEE) cells just prior to fusion of the palatal shelves. TGFβ3 is required for palatal shelf fusion,[35,36] as evidenced by homozygous null TGFβ3 newborns, which exhibit a cleft secondary palate.[37] Furthermore, administration of anti-TGFβ3 antibodies prevents fusion of the palatal shelves.[38] Data suggest the role for TGFβ3 in palatogenesis relates to regulation of the breakdown of epithelia that lie between the palatal shelves. In the TGFβ3-null mice, the palatal shelves appear to approximate and adhere, but the epithelial seam remains, thus preventing fusion. Further support for the role of TGFβ3 during palatal fusion comes from biochemical approaches.

FOXE1 (Forkhead box protein E1). FOXE1 is a forkhead-containing transcription factor that is involved in embryonic pattern formation. The FOXE1 gene is expressed at the point of fusion between maxillary and nasal processes during palatogenesis.[39] Positional cloning and candidate gene sequencing show a correlation between mutations in FOXE1 and the occurrence of cleft lip and palate.[39] FOXE1 is expressed in the secondary palate epithelium of both mice[40] and human embryos.[41] Furthermore, mice with a null mutation in FOXE1 have cleft palates.[42]

Irf (Interferon regulatory factor). Irf6 is a member of a large family of transcription factors that bind to specific DNA sequences and regulate gene expression. In mice, disruption of this gene results in clefting.[43,44] In humans, mutations in IRF6 have been shown to cause Van Der Woude syndrome and popliteal ptyergium syndrome, two disorders that are characterized by the presence of a cleft palate.[45] Furthermore, variations in IRF6 have been found to increase the risk for isolated cleft lip and palate.[46] *Irf6* mutant mice exhibit a hyper-proliferative epidermis that fails to undergo terminal differentiation, causing epithelial adhesions that occlude the oral cavity and result in cleft palate.[43,44] Taken together, these data suggest Irf6 mutations may result in defective elevation of palatine shelves, secondary to inappropriate adhesions with oral epithelium.

Table 3. Summary of animal models used for palate study

Animal Type	% of studies
Mouse	156/166 = 94.0%
Chicken	4/166 = 2.4%
Zebrafish	3/166 = 1.8%
Rat	3/166 = 1.8%

VAX1. *VAX1* is a member of the Emx/Not gene family and encodes a transcriptional regulator with a DNA-binding homeobox domain. Single nucleotide polymorphisms in *VAX1* have been found to be overrepresented in patients with cleft lip and palate, suggesting that variants in *VAX1* itself may contribute to development of clefting (reviewed in Dixon et al. 2011). Mouse knockouts for *Vax1* show cleft palates, and this gene is expressed widely in developing craniofacial structures.[47] Therefore, variants in *VAX1* are strong candidates in the etiopathogenesis of cleft lip and palate.

Teratogen-induced cleft palate. Along with pathway manipulation, scientists have also exposed genetically susceptible mouse strains such as C57B/L in utero to teratogens such as phenytoin, corticosteroids and retinoic acid.[48] Such manipulations are thought to create a cleft due to alterations in mucopolysaccharides and glycosaminoglycans in the developing mesenchyme.

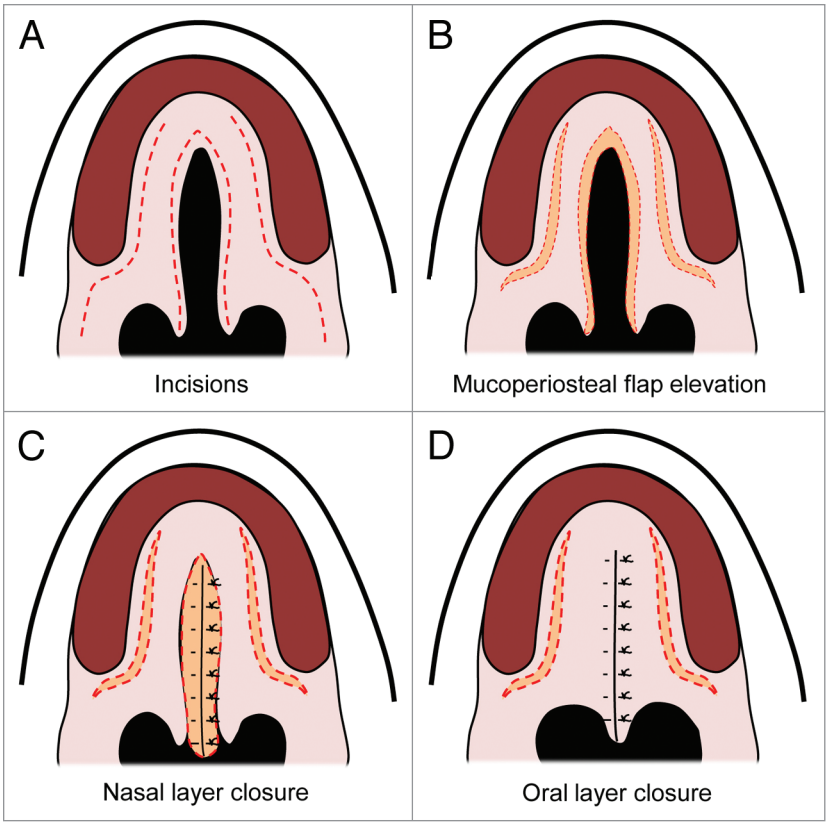

Figure 4. Von Langenbeck palatal repair. (A) Secondary cleft palate palate with dotted red lines demonstrating incisions. (B) Mucuoperiosteal flap elevation with orange demonstrating opening of the incisions. (C) Midline nasal closure of the defect with the nasal layer in orange. (D) Closure of the midline incision with the oral mucosa over the nasal layer closure.

Alternative models. Though transgenic models offer significant insight, it is difficult to manipulate the development of these mice in utero. Thus, palate organ cultures represent another promising methodology that allows direct manipulation of the palatal mesenchyme. In vitro chick palate models have been previously reported, and our laboratory has utilized a similar in vitro model using palates from E13.5 mice (**Fig. 3**). In our model, palate cultures are maintained for up to 96 h, and fusion is seen as early as 72 h. This model allows us to assess the critical distance needed for palates to be separated before clefting occurs. In addition, effects of various protein ligands on palatal gene expression can be studied.[48,49] The disadvantage, of course, is that in vitro conditions do not completely mimic in vivo correlates, and thus palate cultures are better for analyzing pathways than morphogenesis.

Clinical Implications and Potential Therapies

While research continues on the molecular genetics of cleft formation, surgery remains the mainstay for treatment of palatal defects. The first report of a cleft palate repair is attributed to LeMonnier, who incised the cleft edges and placed sutures leading to suppuration and then healing across the defect.[6,50] Von Langenbeck later introduced the use of mucoperiosteal flaps to close clefts involving the hard palate (**Fig. 4**).[9,50]

A literature search of PubMed was performed to assess practice patterns of current surgical repair of cleft palates. The following search terms were used in combination to identify appropriate studies: Cleft palate, surgery, treatment and repair. The search for repair type was limited to studies published in English from 2000 to 2011 with a focus on patients within the age group 0–23 mo. Studies were excluded if the full text was inaccessible or surgical repair type was not clearly identified in the methods section. We found a wide variety of approaches used with a paucity of randomized controlled studies for any technique (**Tables 4 and 5**).

Overall, the most widely used techniques include Von Langenbeck's, the Vaeu-Wardill-Kilner and the two-flap repair described by Bardach.[6,51] While many modifications of each exist, the main principles across all cleft palate repairs include tension-free closure of the oral and nasal layers, dissection of muscles from the posterior edge of the hard palate and construction of a horizontally oriented palatal sling to restore normal velar function.[51]

Repair of the cleft palate begins with an incision along the cleft margin at the junction between oral and nasal mucosa. The incision is carried anteriorly along the gingiva, allowing elevation of mucoperiosteal flaps off the hard palate.[10,51] With exposure of the greater palatine neurovascular bundle, mobilization can be performed by gentle stretching using scissors. The tendon of the tensor veli palatini can also be divided medial to the hamulus to facilitate medialization of the levator muscle.[9] The nasal mucoperiosteum is then widely mobilized from the undersurface of the hard palate. Posteriorly, an intravelar veloplasty is typically performed, with separation of the oral, muscle and nasal linings and release of the muscles from their abnormal attachment to the posterior edge of the bony palate. Closure of the defect is performed in three layers (nasal mucosa, velar muscle and oral mucosa), with horizontal reorientation of the levator veli palatini establishing proper orientation of the sling. In the region of the hard palate, a two-layer repair is performed, with the nasal layer sometimes requiring a vomerine flap.[6]

In patients with either clefting of the soft palate or a submucous cleft, a Furlow palatoplasty can also be performed. Double opposing z-plasties are fashioned on the velum, with release of the levator muscle from the posterior edge of the hard palate.[52] Transposition of the flaps yields retropositioning of the muscle to a more medial-lateral position.[53] With this technique, simultaneous palatal lengthening and reconstruction of the levator sling is established along with additional narrowing of the nasopharyngeal aperture.[11] Velopharyngeal competence allowing development of normal speech is one of the most critical outcomes in cleft surgery, and the Furlow technique has been associated with some of the lowest rates of persistent velopharyngeal insufficiency following primary repair.[52-55]

Table 4. Procedure used for palate repair

Article No.	PMID	Repair Type	Level Evidence
1	11879069	Furlow double opposing Z-plasty	2
2	18827646	Bardach two-flap	3
3	16816695	Bardach two-flap	4
4	19443049	Millard and two-flap	3
5	12172182	Two-flap	4
6	15145666	Furlow	4
7	18594410	Furlow w/vs. w/out islandization	3
8	16799309	Furlow	4
9	16154317	Vomer flap with push back	3
10	18991170	Secondary gingivoalveoloplasty	3
11	12733955	Furlow	4
12	12142649	Modified Langenbeck, Ernst, Veau and Axhausen technique	3
13	14697077	Wave-line technique	2
14	18177196	Square-flap method	4
15	14663803	Musculomucosal buccal flap	4
16	17105319	Mucoso-periosteal flap palatoplasty	4
17	17105319	Marginal musculo-mucosal flap	2
18	16876078	Modified z-plasty	5
19	16077326	Two-flap, V-Y, and dorrance with new adhesive	5
20	11711920	Von Langenbeck, Wardill-Kilner,	4
21	16929186	Furlow	3
22	10029477	Furlow	4
23	15988251	Veau-Wardill-Kilner	4
24	15213528	Furlow	4
25	19165004	Modified 2 flap	2
26	10626963	Push-back palatoplasty	5
27	10839414	Modified Furlow	3
28	15896890	Furlow	4
29	16681406	Vomerine flap and Von Langenbeck incisions	2
30	20842387	Vomer flap	2
31	20413269	Distraction osteogenesis (DO) vs. conventional orthognathic (CO)	2
32	20335871	Millard, Pfeifer, Afroze	1
33	20299247	CO vs. DO	2?
34	20227244	Collagen membrane	4
35	20163243	Collagen matrix w/rhBMP-2	2
36	20048619	No tensor transection, tensor transection alone and tensor tenopexy	3
37	19816334	Tennison, periosteoplasy, pushback	4
38	17652050	Von Langenbeck and Furlow	3
39	17440366	Wardill-Kilner vs. Kriens	2
40	17328643	Vomerine and Von Langenbeck	2
41	17105327	Von Langenbeck	1
42	15861051	Vomer, pushback, Von Langenbeck	3
43	14577817	Millard, Von Langenbeck	2
44	12498603	Modified Von Langenbeck	2
45	11141029	Millard, Von Langenbeck	2

Level 1, Prospective multi-center double blinded randomized control study; Level 2, Prospective randomized control study; Level 3, Retrospective analysis, case control study or systematic review of studies; Level 4, Case series; Level 5, Expert opinion, case report or clinical example.

Table 4 (continued). Procedure used for palate repair

Article No.	PMID	Repair Type	Level Evidence
46	12846601	Zurich, Von Langenbeck	2
47	12846598	Millard, Von Langenbeck	2

Level 1, Prospective multi-center double blinded randomized control study; Level 2, Prospective randomized control study; Level 3, Retrospective analysis, case control study or systematic review of studies; Level 4, Case series; Level 5, Expert opinion, case report or clinical example.

Table 5. Summary of procedures used for palate repair

Level	% of studies at that level
1	2/47 = 4.3%
2	16/47 = 34.0%
3	11/47 = 23.4%
4	15/47 = 31.9%
5	3/47 = 6.4%

While techniques for cleft palate repair have become well-established, postoperative development of oronasal fistulas still remains a significant problem. Reports have noted an incidence ranging from 11% to 23%, with the most likely site being the junction of the hard and soft palates.[56-59] Depending on size, fistulas may contribute to hypernasal speech, nasal regurgitation and food trapping. Several retrospective studies have identified the extent of cleft to be a significant factor, as patients with bilateral clefts were found to have a 2- to 3-fold higher incidence of postoperative fistula development compared with unilateral clefts.[51,60] Operator experience has also been shown to play a role.[58,60] Recent investigations have evaluated the utility of the buccal fat pad as adjunctive tissue for use in both primary palatal cleft repair and treatment of postoperative fistulas. Used in a pedicled fashion with overlying mucosa, the buccal fat pad has been shown to successfully treat wide oroantral and oronasal clefts.[61,62] More recently, buccal fat has also been employed to cover laterally exposed bone adjacent to gingival mucosa following medialization of the mucoperiosteal flaps.[63] As this was found to re-epithelialize within two weeks, use of the buccal fat pad may result in an eventual reduction of palatal scarring, which may limit subsequent growth restriction of the maxilla. And in similar fashion to AlloDerm, the buccal fat pad has also been shown to be effective as an interpositional layer between oral and nasal lining in the repair of postoperative fistulas.[63] Fuimora et al. have even evaluated the utility of combining the two, with the successful treatment of oronasal fistulas using pedicled buccal fat covered with lyophilized dermis in adult patients.[64]

Palatal Tissue Engineering

The use of autogenous grafted material is now the standard of care, but tissue engineering is an attractive alternative that could greatly reduce the morbidity of surgery and potentially enhance the healing process. An exhaustive literature search only yielded 36 non-review studies discussing tissue engineering of the palate (**Table 6**). Of these 36 studies, 20 discussed engineering of the mucosa (**Table 7**). Regarding mucosal repair, cultured epithelial grafts, dermal substitutes and a combination of the two, called mucosa equivalents, are commonly used to provide extra tissue and aide in wound healing after cleft palate repair. Cultured epithelial grafts can provide coverage for large areas while being derived from only a small biopsy, but they are prone to infection and fail to reduce scarring or contraction in full-thickness wounds due to absence of a dermal component.[65] Such grafts can be either allogenic or autologous. Allogenic grafts have the advantage of being readily available but have a low take rate and are generally only used for temporary coverage, while autologous grafts take require extra time to culture but have a higher take rate.

Dermal substitutes made from polymers, purified collagen or de-epidermized dermis (DED or AlloDerm) provide additional physical support that is often lacking in epithelial grafts. However, some require a secondary procedure to apply a split-thickness skin graft or cultured epithelia. In recent years, repair of palatal fistulas have begun to employ acellularized dermal matrix (AlloDerm) with promising results. Using AlloDerm as an interpositional layer between nasal and oral mucoperiosteum has been shown to significantly reduce fistula recurrence rates in multiple series.[66,67] Furthermore, recent studies have also demonstrated the utility of AlloDerm as an adjunctive measure in the primary repair of wide clefts. In a series of seven patients with palatal clefts wider than 15 mm, placement of AlloDerm between the muscle and oral lining was found to result in the development of no postoperative fistulas.[68] Even in two patients with oral dehiscence and exposure of the AlloDerm, uneventful healing proceeded, with remucosalization occurring over a 4-week follow-up.[68]

Cultured mucosa equivalents provide an epithelial and dermal substitute in a one-step process, which seem to be the optimal replacement for mucosa, since this provides material for repair with properties closest to the original tissue. However, long-term evaluation of their clinical efficacy is still lacking. Tissue engineering using epithelial and mesenchymal stem cells could greatly enhance these options for repair once characterization and isolation of true stem cells can be routinely achieved.

The other tissue discussed in 20 of the 36 palatal tissue engineering articles was palatal bone (**Table 7**). In cleft palate repair, one of the major challenges lies in reconstructing the bony hard palatal and alveolar defects. Surgical repair with autogenous bone grafts is the current standard of care. Bone is most commonly harvested from the iliac crest but can be taken from the rib, tibia, calvarium or mandibular symphysis. This often requires multiple operations and extensive healing time and is associated with high donor site morbidity, including postoperative pain, altered sensation, scarring and infection. In addition, bone graft harvest ultimately yields a very limited quantity of bone for reconstruction.

Table 6. Recent studies of palatal tissue engineering

Article no.	PMID	Tissue Engineered	Experimental model	Review?	Species
1		**bone/mucosa**	**mouse/rat/human/in vitro**		
2	21465220	bone	ALL	review	ALL
3	21282641	mucosa	mouse		ALL
4	20863943	bone	human		ALL
5	20676486	bone	rat		ALL
6	20646804	bone	in vitro (rat bone marrow)		ALL
7	20524191	mucosa	human		ALL
8	20078790	mucosa	ALL	review	ALL
9	19615638	bone	human		ALL
10	19473448	mucosa	rat		ALL
11	18834238	mucosa	human		Dog
12	19387150	bone	in vitro		Dog
13	19184287	bone	rat		Dog
14	19043190	bone	human		human
15	19018060	both	human	review	human
16	18774917	both	ALL	review	human
17	18650554	mucosa	in vitro		human
18	18520371	mucosa	rat		human
19	18427300	both	ALL	review	human
20	18427291	both	ALL	review	human
21	18319673	both	ALL	review	human
22	18269029	mucosa	rat		human
23	18206405	bone	human		human
24	18093570	mucosa	rat		human
25	18041711	mucosa	in vitro		human
26	18022477	both	ALL	review	human
27	17971350	mucosa	Dog		in vitro
28	17764402	mucosa	Dog		in vitro
29	17667684	bone	human		in vitro
30	17465023	mucosa	in vitro		in vitro
31	17393689	bone	Dog		in vitro
32	17346560	both	ALL	review	in vitro
33	16525276	bone	rat		in vitro
34	15948680	mucosa	human		in vitro
35	15927149	bone	human		mouse
36	15894371	mucosa	in vitro		mouse
37	15348479	bone	rat		rat
38	14619596	mucosa	rat		rat
39	14513576	mucosa	rat		rat
40	12544232	bone	mouse		rat
41	12109699	mucosa	in vitro		rat
42	11896993	mucosa	in vitro		rat
43	11213985	mucosa	human		rat
44	11203575	bone	human		rat
45	10845305	both	ALL	review	rat
46	9821920	mucosa	human		rat

Table 7. Summary of tissues described in tissue engineering of the palate

Mucosa = 20/36	0.555 = 55.5%
Bone = 16/36	0.44 = 44%

Furthermore, this bone often does not fully integrate into the host site and can undergo some resorption. Bony repair needs to be very strong to support tooth eruption and to withstand physical stress from muscles of mastication. There are also allogeneic and synthetic material available for grafting, and while these solve the problem of donor site morbidity, there is still the risk of infection, elicitation of an immune response and problems with structural integrity and contour.[69]

The use of tissue engineering could avoid many disadvantages of autogenous grafting, such as donor site morbidity, and could potentially decrease the number of surgeries needed while providing improved outcomes. Only a limited number of studies currently exist exploring palatal tissue engineering, and of these studies, only 36% pertain to human subjects, making it an attractive avenue for future research endeavors (**Table 8**).

Table 8. Summary of animal models in studies discussing tissue engineering of the palate

Dog = 8.3%
Human = 36%
In Vitro = 22%
Mouse = 5.5%
Rat = 27.8%

Conclusion

Craniofacial clefts, specifically clefting of the lip and palate, remain a significant biomedical burden. Therefore, understanding normal palate development as well as aberrant pathways involved in abnormal palate development is crucial to allow us to better develop therapeutic modalities to treat these patients. Clearly, despite an improvement in surgical outcomes, there is a paucity of randomized controlled studies (level I data), leading surgeons to depend on clinical reports and non-randomized trials, which are often misleading. The current gold standard for treating cleft palate patients involves a team approach between plastic surgeons, pediatricians, otolaryngologists, speech pathologists, orthodontists and geneticists, underscoring the myriad of complications caused by CLP beyond just the tissue deficit. Thus, identifying the major pathways involved and manipulating those pathways prior to birth would represent a monumental step to prevent the primary and many secondary complications caused by CLP. Tissue engineering approaches also remain an exciting and potentially profitable direction of investigation as the field of regenerative medicine advances.

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