

Tooth autotransplantations – lessons from animal models: a review

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ABSTRACT: Autotransplantation of teeth is the most natural technique to for replacing missing teeth in exposed parts of the dental arch. Reports from human patients indicate great progress toward successful transplantations. However, complications such as inflammation and ankylosis still occur. To understand regenerative processes after autotransplantations, several animal models have been used (monkeys, rodents, rabbits, cats and dogs) and histological/molecular methods have been established. This review aims to summarise knowledge from animal models and discuss their advantages or disadvantages with respect to possible usage in research.

Keywords: revascularisation; reinnervation; ankyloses; mouse; cat; dog; rabbit

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1. Introduction

For centuries, missing teeth have been replaced in order to improve chewing and for the aesthetic functions of the dental arch. Recent implantology and prosthetic dentistry offers several options, all of which have numerous disadvantages, making it necessary to look for new techniques.

Stem cell-based tooth engineering (Ohazama et al. 2004) may be the future of molecular dentistry; however, the most natural technique that is already widely applied is autogenous transplantation (autotransplantation) of teeth. Autotransplantation represents the surgical movement of a vital or endodontically-treated tooth from its original location in the jaw to another site. This technique dates

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back to 1950 when the first reports emerged (Apfel 1950; Miller 1951; Hale 1954) and was later further developed (Conklin 1969). Gradually, autogenous tooth transplantation has emerged as a suitable treatment method with a reported success rate from 79% (Andreasen et al. 1990a, 1990b, 1990c, 1990d) to 98% (Jonsson and Sigurdsson 2004). However, there are still many complications, which may cause failure of the treatment. The most widespread difficulties include root replacement resorption, root inflammatory resorption, marginal or apical periodontitis and trauma. To investigate processes accompanying autotransplantation and the following post-surgery period in detail, animal models were established to analyse dynamic changes at the tissue, cellular and molecular levels. Several animal species, such as mice, rats, cats, dogs and monkeys, have been used to follow biological processes accompanying tooth autotransplantation.

2. Animal models

2.1. Monkeys

The best model for human dentistry appears to be the monkey. The vervet monkey, the species used in most studies, belongs to the old world monkey family. This animal has the same dental formula as humans (2123/2123) and a close resemblance in root shape and periodontium formation (Figure 1). However, due to ethical and monetary aspects related to the long gestational period, housing, care and handling of these animals, studies using these species are rather limited (Wolfe-Coote 2005). Nevertheless, several investigations focused on pulpal healing and periodontal regeneration were performed in monkeys

(Andreasen 1981a; Kristerson and Andreasen 1984; Schwartz and Andreasen 1988, 2002a; Groisman et al. 1989; Schendel et al. 1990).

2.2. Rodents

The most common laboratory animals used in autotransplantation research are mice and rats, which hold the advantages of a short gestation period, numerous offspring, low housing costs and easy handling. Despite the monophyodont dentition and reduced dental formula (1003/1003) together with hypsodont incisors (Figure 2), toothless diastema and enamel-free areas on molar surfaces, numerous findings can be successfully extrapolated from rodents to humans (Fleischmannova et al. 2008). Various parameters of successful autotransplantation such as periodontal tissue regeneration have already been investigated in the mouse (Ogawa et al. 2006; Hasegawa et al. 2007) and rat (Ohshima et al. 2001; Kawasaki et al. 2004; Izumi et al. 2007). Recent reports have addressed cover reparative dentin formation in the dental pulp chamber and pulp regeneration (Rungvechvuttivittaya et al. 1998; Tsukamoto-Tanaka et al. 2006; Hosoya et al. 2012), as these are thought to be the most critical factors for the survival of the transplanted tooth (Ogawa et al. 2006).

2.3. Rabbit

Rabbits have diphyodont dentition with the dental formula 2033/1023 (Figure 3). Their canines are missing, and diastema is present between the incisors and premolars. Hypsodont

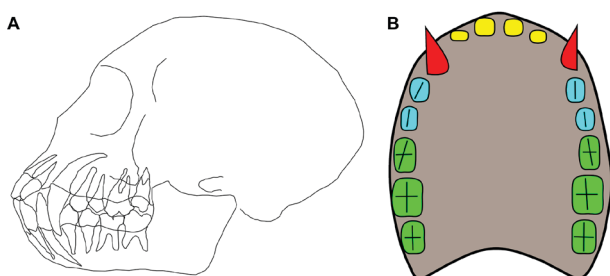


Figure 1. Dental formula of the vervet monkey. (A) Lateral view on the skull; (B) Schematic drawing of the upper jaw. Incisors = yellow, canines = red, premolars = blue, molars = green

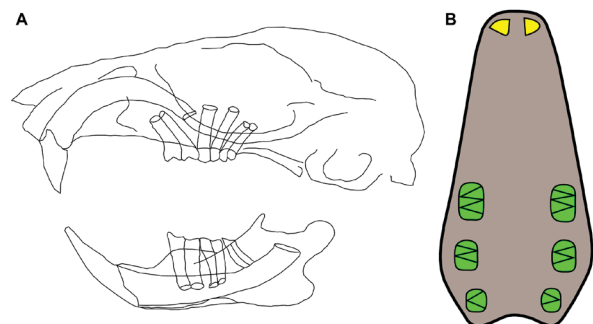


Figure 2. Dental formula of the mouse. (A) Lateral view on the skull; (B) Schematic drawing of the upper jaw. Incisors = yellow, molars = green

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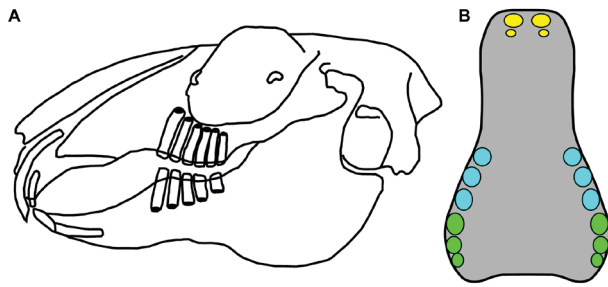


Figure 3. Dental formula of the rabbit. (A) Lateral view on the skull; (B) Schematic drawing of the upper jaw. Incisors = yellow, premolars = blue, molars = green

incisors are abraded continuously; the occlusal cusp surfaces of the molars and premolar teeth have enamel folds or ridges formed by enamel; the troughs consist of dentin and cementum. The shapes of the occlusal aspect of these teeth are very close to square. All teeth are rooted, as in the mouse.

Rabbits have a quite short gestation period of 31 days. Rabbit teeth are similar to rodent teeth with respect to the presence of incisors and molars, where the first molar is the largest. However, rabbits are even more similar to humans with regard to the presence of premolars and the fact that they are also diphyodont. Rabbits have a more spacious oral cavity than mice, thus allowing comfortable manipulation. Previously, research on rabbits confirmed the influence of surgical trauma and periodontal healing on the success of dental autotransplantations (Birman and Araujo 1975; Mirzabagi 1978). However, rabbits have not been employed in recent tooth autotransplantation research.

2.4. Cat

Cats have normodont dentition with incisors, canines, premolars and molars similar to humans; their dental formula is 3131/3121 (Figure 4). Moreover, the dentition is diphyodont and the length of their gravidity, about 58 days, is acceptable for research. On the other hand, the shape of lateral teeth is different than in humans as no feline teeth, including the molars, have grinding surfaces. The upper molar and the first upper premolar are the only lateral teeth with one root; the others have two roots, while the third upper premolar has three roots. Therefore, atraumatic extraction of these teeth is difficult. In particular, the process

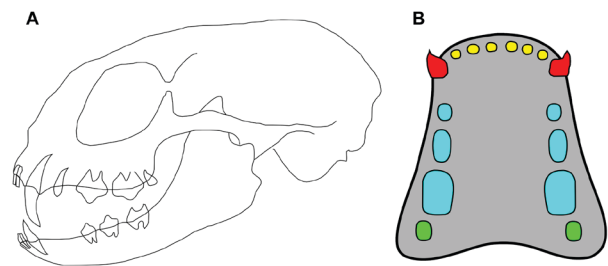


Figure 4. Dental formula of the cat. (A) Lateral view on the skull; (B) Schematic drawing of the upper jaw. Incisors = yellow, canines = red, premolars = blue, molars = green

of reinnervation was investigated in cats (Robinson 1983; Holland and Robinson 1987a, 1987b; Holland et al. 1991).

2.5. Dog

Dogs appear to be a suitable model for dental research because of their dental formula (3142/3143), diphyodont dentition and their gestation period of 63 days (Figure 5). The shape of the teeth is also not the same as in humans, but dogs, unlike cats, have grinding surfaces on their molar teeth. The teeth that are mostly utilised for dental research are the incisors, first premolars and third lower molars, which possess only one root. Canines have just one long root, while the other teeth have two or three roots. However, as in the cat and monkey, the exploitation of dogs has ethical limitations in research. Nevertheless, some new insights have been obtained from experiments in dogs, particularly related to tooth revascularisation and healing processes (Claus et al. 2004; Ferreira et al. 2010; Marques-Ferreira et al. 2011). Dogs were also successfully used for autotransplantation protocol testing and optimisation (Ferreira et al. 2010;

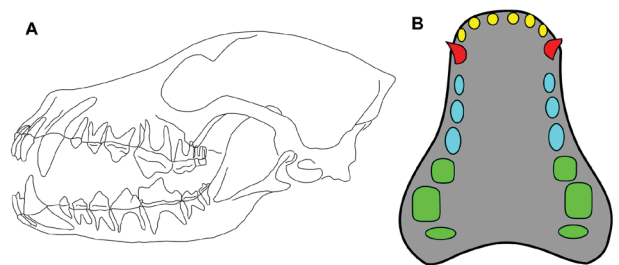


Figure 5. Dental formula of the dog. (A) Lateral view on the skull; (B) Schematic drawing of the upper jaw. Incisors = yellow, canines = red, premolars = blue, molars = green

Ferreira et al. 2012). Moreover, several case studies from pet medicine have provided new insights applicable for human autotransplantation techniques (Sun et al. 2011; Lu et al. 2013).

3. Investigated parameters

3.1. Revascularisation

Dental pulp vitality is dependent on the nutrition delivered via blood vessels; therefore, the success of transplantation depends on revascularisation of the tooth (Ferreira et al. 2010). The formation of new blood vessels in immature or mature dog teeth after autotransplantation was investigated in several studies (Skoglund et al. 1978; Skoglund 1981; Laureys et al. 2001). However, anastomoses with original pulp vessels were discovered in only a few teeth (Skoglund et al. 1978). Insufficient nutrition causes necrosis followed by inflammation of the periodontal ligaments and root resorption (Andreasen 1981a).

Revascularisation was also found in teeth that had undergone a pulpectomy. In single-rooted teeth, the pulp was removed from the chamber from the apical side by a nerve broach immediately after tooth extraction, and then the tooth was autotransplanted. The studies showed that revascularisation started seven to 10 days after surgery (Laureys et al. 2001). Total revascularisation was observed within 18 to 40 days (Claus et al. 2004). Moreover, sub-dentinal capillary plexus missing in teeth with the original pulp was re-established in pulpectomized teeth (Skoglund 1981).

It was suggested that pulpal necrosis is the main cause of periapical inflammation (Andreasen 1981a); when the original pulp is extracted, the danger of necrosis is minimised (Claus et al. 2004). Furthermore, the minimum diameter of the apical foramen was shown to be critical for pulp regeneration (Claus et al. 2004). Despite the fact that no minimal width of the foramen was determined in later studies (Laureys 2013), the diameters of foramina in the most successful cases ranged between 0.32 and 0.65 mm (Laureys et al. 2013).

3.2. Reinnervation

Another important factor for establishment of a vital tooth after transplantation is reinnervation.

The formation of new nerve connections is a slow process with the maximum speed of 5 mm per week in humans (Al-Majed et al. 2000). Moreover, the initiatory delay and terminal arrest in connection formation between axons and receptors/effectors has to be taken into account (Byers et al. 2003). Reinnervation success was evaluated using microscopy or after electrophysiological stimulation. The results range from 11% of reinnervated autotransplanted teeth (Altonen et al. 1978) to 84% (Robinson 1983) or even 89% (Ohman 1965).

The stimulation of reinnervation using bipolar electrical impulses was tested on cats. Electrical stimulation evoked a jaw-opening reflex nine to 24 weeks after surgery. Moreover, the reflex had a raised threshold and increased latency (Robinson 1983). On the other hand, histological analysis revealed that the quality of the nerve supply was not as good as in control teeth. The number of axons was reduced, they were located mainly near to the apices of the teeth and their width was smaller (Holland and Robinson 1987b).

Pulpal reinnervation was also analysed in vervet monkeys. Lateral incisors were autotransplanted to the collateral side, animals were euthanised one, two or four months after the surgery and histological specimens were examined. S-100 monoclonal antibody staining revealed nerve fibre formation in the pulp chamber starting one month after transplantation. At four months, a large number of nerve bundles were seen throughout the whole pulp (Schendel et al. 1990).

3.3. Reparative reactions

External natural as well as artificial stimuli acting on the dental pulp following tooth transplantation cause reparative reactions (Hosoya et al. 2012). Two types of extensively produced mineralised tissue were found in investigated pulp chambers – bone-like tissue and tertiary (reparative) dentin, which can be present in small amounts physiologically in the pulp chamber (Tsukamoto-Tanaka et al. 2006; Hosoya et al. 2012).

The influence of an extended surgery time as well as the occlusal force on pulpal healing in replanted mouse molars was found to be important (Hasegawa et al. 2007). The production of new tertiary dentin started within five to seven post-operative days, while the bone-like tissue appeared

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at Day 14. Prolonged operation time increased the formation of bone-like tissue and expansion of the inflammatory reaction (Hasegawa et al. 2007). Notably, non-occluded teeth after autotransplantation indicated a higher degree of tertiary dentin production (Hasegawa et al. 2007).

3.4. Tooth attachment – ankylosis

Ankylosis or replacement root resorptions are other causes of tooth loss after autotransplantations. Roots are progressively replaced by fibrovascular and bone tissues (Katayama et al. 2006). Therefore, it is necessary to reactivate periodontal ligament formation of the autotransplanted tooth for successful treatment (Andresen 1981a). A short operation time and a traumatic extraction of the tooth are the most important factors when trying to eliminate the incidence of ankylosis (Cohen et al. 1995).

Orthodontic loading time influences the periodontal healing and root resorption (Sun et al. 2011; Lu et al. 2013) as was found in beagle dogs. Their teeth were extracted, autotransplanted and half of them were orthodontically loaded at different time points and using different time courses. Changes in the periodontium were evaluated by measurement of the pocket depth, histomorphometric analyses and by the expression of alkaline phosphatase (ALP) and basis fibroblast growth factor (bFGF). The incidence of ankylosis in orthodontically loaded teeth was lower than in teeth without the load. The best results were found in the group where autotransplanted teeth were loaded by orthodontic treatment that was started four weeks after surgery with a duration of two weeks (Lu et al. 2013).

3.5. Inflammatory root resorption

One of the main reasons for the failure of autotransplantation treatment is inflammatory root resorption. The inflammation is most frequently caused by necrotic pulp tissue (Schwartz and Andreasen 2002a; Claus et al. 2004; Azevedo et al. 2007). Therefore, endodontic treatment was suggested to reduce the risk (Andreasen 1981b; Claus et al. 2004; Azevedo et al. 2007). This was confirmed in vervet monkeys where the endodon-

tic treatment almost completely reduced the inflammatory resorption (Schwartz and Andreasen 1988; Schwartz and Andreasen 2002a). Additional observations suggest that root canal therapy can be postponed for up to 40 days after transplantation as there were no differences in the presence of the root inflammatory resorption at seven and 40 days (Azevedo et al. 2007).

3.6. One vs. two stage approaches

Autotransplantations usually proceed as a one-stage technique; the tooth is extracted and immediately transplanted (Marques et al. 2010). When there is a reason for delayed transplantation after the extraction, e. g. due to orthodontic treatment to establish the space of the recipient alveolar bed or in patients with clefts, the tooth can be cryopreserved (Schwartz and Rank 1986; Laureys et al. 2001). The cryopreservation of teeth with pulpectomy was shown to have no impact on revascularisation after autotransplantations in dogs (Laureys et al. 2001).

The two-stage surgery technique covers preparation of the alveolar recipient bed in the first step followed by delayed (five to seven days) transplantation (Nethander et al. 2003; Marques et al. 2010). This approach allows the healing of the alveolar bed before tooth positioning and should provide for more sufficient nutrition and curing of the transplanted tooth (Ferreira et al. 2010; Ferreira et al. 2012). The efficiency of both approaches on dental and periodontal healing was tested in incisors and premolars from beagle dogs. Half of the teeth were autotransplanted immediately after extraction; the second half was transplanted following alveolar bed healing. However, the histological and immunohistochemical evaluation did not reveal any differences in revascularisation (Ferreira et al. 2010), periodontal regeneration (Nethander et al. 2003) or in the thickness of the dentin formed in transplanted teeth (Ferreira et al. 2012) when comparing the two approaches.

4. Perspectives

The greatest advantage of tooth autotransplantation is that the organism's own organ (tooth) is used for replacement. The disadvantage is that autotransplantation does not generate a new tooth

but just fills in the gap in a more exposed part of the dental arch. To search for other options, stem cell-based tissue engineering (Ohazama et al. 2004), allotransplantations or even xenotransplantations have been tested using animal models (Groisman et al. 1989; Schwartz et al. 1990; Schwartz and Andreasen 2002b; Kim et al. 2006; Takamori et al. 2008; Mutoh et al. 2011; Saito et al., 2011).

Animals have been widely used to help understand cellular communication between dental and surrounding tissues. Numerous animal studies have served as a useful basis for optimising surgical protocols and for the prevention of post-surgery complications. They have provided new insights regarding the use of orthodontic force (Lu et al. 2013) or the postponing of endodontic treatment (Azevedo et al. 2007) to increase the success of autotransplantation. Studies regarding cryopreservation during the autotransplantation process have shown new possibilities for patients with cleft lips or palates (Laureys et al. 2001). Unfortunately, the most frequently analysed animals exhibit different dental formulas to humans, except for monkeys. Moreover, the speed of the healing process in bones can be different, e.g. it is twice as fast in dogs than in humans (Huebsch and Hansen 1969), good oral hygiene cannot be guaranteed in animal models (Lu et al. 2013) and their subjective feelings cannot be investigated. However, beyond these handicaps, research on animals yields fundamental insights that can be applicable to human dentistry.

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