# FUTURE ORAL HEALTH CARE THERAPY:

# TOOTH REGENERATION BY TARGETING MOLECULAR THERAPY

An Undergraduate Research Scholars Thesis

by

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# RESEARCH COMPLIANCE CERTIFICATION

Research activities involving the use of human subjects, vertebrate animals, and/or biohazards must be reviewed and approved by the appropriate Texas A&M University regulatory research committee (i.e., IRB, IACUC, IBC) before the activity can commence. This requirement applies to activities conducted at Texas A&M and to activities conducted at non-Texas A&M facilities or institutions. In both cases, students are responsible for working with the relevant Texas A&M research compliance program to ensure and document that all Texas A&M compliance obligations are met before the study begins.

We, Nhu Lai, Stephanie Mai, and Van Nguyen, certify that all research compliance requirements related to this Undergraduate Research Scholars thesis have been addressed with our Research Faculty Advisors prior to the collection of any data used in this final thesis submission.

This project did not require approval from the Texas A&M University Research Compliance & Biosafety office.

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# ABSTRACT


Future Oral Health Care Therapy:
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Tooth agenesis is one of the most common developmental anomalies that affect the number of teeth due to developmental failure, which requires costly dental treatment involving numerous science and public health fields. It can be seen in patients with dental caries, periodontal disease, trauma and more specifically individuals with Down Syndrome. The emergence of regenerative dentistry challenges healthcare professionals to provide a new corrective treatment based on the mechanism of tooth development and biological process of healing and repairing. The studied data supports that the relationship of USAG-1, antagonist to RUNX2 and BMP, has introduced a molecular targeted therapy that assists in regenerating tooth

germs.  BMPs and RUNX2 are transcription factors in stimulating osteoblast differentiation and demonstrate that RUNX2 can regulate BMP signaling independently and on the other hand USAG-1 null mice present with supernumerary tooth formation. The manipulation of USAG-1 can benefit individuals with Down Syndrome. Infections, absence of dentitions, and craniofacial characteristics have led to the difficulty of chewing, swallowing, and high occurrence of pain due to progressive periodontal disease. By taking advantage of the advancement of science and technology, it can promote the future of dentistry by stimulating the third dentition for improvement of patient care and treatment that could prolong the oral health of individuals. However, further research still has to be conducted to investigate the toxicity, teratogenicity, and tumorigenicity of these therapeutic molecules for better results. There are still more ethical concerns that will be brought up to this topic as it is being developed.

# DEDICATION

This research is dedicated to assist people with Tooth Agenesis, especially in the case of Down Syndrome patient who are more prone to have this condition. This promising therapy challenges the dental professionals to address the problem during early stage of development to reduce the cost of multidisciplinary treatment.

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# INTRODUCTION

1. First objective:  The importance of USAG-1 and BMP on the development of tooth regeneration therapy
2. Second objective:  Discuss how gene regulation of USAG-1 can be used to regenerate teeth
3. Third objective:  Discuss the implications for USAG-1 in oral health care.

The aim of this research topic is to present a discussion of tooth regeneration using *Anti-uterine sensitization-associated gene-1 (USAG-1)*. USAG-1 is a bone morphogenic protein (BMP) antagonist that is responsible for tooth morphogenesis. Prior investigations in articles and journals declare missing teeth are commonly through decay or trauma and the most common genetic defect that delivers to the teeth is called ectodermal dysplasia, the mutation in various genes that would be inherited or normal gene that becomes mutated after fertilization. With the signs and symptoms such as abnormal or missing dentition, BMPs could potentially assist with the development of the third dentition. By going towards the idea of regenerating teeth through molecular therapy, it can set the future of dentistry for practitioners to utilize this method to give hope to their patients that are diagnosed with teeth agenesis and encourage teeth to regrow naturally from any defects in order to promote oral health care. This research supports the ADHA research topic Oral Health Care- New therapies and prevention modalities for patients who have congenitally missing teeth. By extending our research, we willcan further investigate how USAG-1 can serve a potential role in serving our patients in the world of dentistry and analyze available sciencethe studies on how it can be clinically applied.

# 1. THE IMPORTANCE OF USAG-1 AND BMP ON THE DEVELOPMENT OF TOOTH REGENERATION THERAPY

Tooth anomalies are reported in 1% of the population, such as the increase or decrease of teeth congenitally[1]. Tooth agenesis is one of the most common developmental anomalies that affect the number of teeth due to developmental failure. The prevalence of tooth agenesis in non-syndromic form ranges from 1.6% to 9.6% in different geographic areas and rate[2]. In most of cases, the most frequent missing teeth are maxillary laterals and the mandibular second premolars[3]. Congenitally missing teeth (CMT) or hypodontia is the most common multifactorial anomaly requiring costly dental treatment involving numerous science and public health fields such as orthodontics, pediatric dentistry, prosthodontics, periodontics, and maxillofacial surgery[3]. In fact, the treatment varies in some countries from $3000 to $15000 for minor prosthodontic interventions like a fixed partial denture in mild cases with only one or two missing permanent teeth or can be up to $60,000 for comprehensive interdisciplinary treatments[3]. Although asymptomatic, this condition may lead to clinical problems, including non-eruption a series of teeth, compromised esthetics, affecting patient's self-esteem, causing difficulty in speech and mastication, and poor quality of life[3]. Moreover, patients with missing teeth may suffer from complications such as malocclusion, mastication problems, periodontal problems because of excessive occlusal force, post-eruptive tooth breakdown, and increased susceptibility to caries[3]. These negative effects to both esthetic and function may require challenging multidisciplinary treatment. Therefore, addressing the problem during the early stage of development, when a primary tooth is congenitally absent, its permanent counterpart might avoid functional, esthetic, and occlusal problems.

In hope of a promising approach for tooth regeneration therapy, the usage of Anti-USAG-1 (Uterine sensitization-associated gene-1) and its interference with BMP signaling will furthermore assist in accelerating tooth development. Looking at it from another perspective, a limitation of USAG-1 will heighten BMP signaling, leading to supernumerary teeth development. Bone morphogenetic proteins (BMPs) are highly conserved signaling molecules that are part of the transforming growth factor (TGF)-beta superfamily[4]. This protein functions in the patterning and morphogenesis of many organs, including the dentition's development, plays an important role in the specification and patterning of the early embryo and regulates apoptosis in many developmental processes[4]. On the other hand, Uterine Sensitization-associated gene-1 (USAG-1) is abundantly expressed in the kidney and functions as a BMP antagonist that suppresses teeth development. It has been shown that mice lacking USAG-1 are resistant to kidney injury and that USAG-1 is the primary negative regulator of BMP in the adult kidney[5]. In addition, USAG-1 controls the number of teeth by inhibiting the development of potential tooth germs in mice, anti-USAG-1 antibody administration is a possible treatment for tooth regeneration therapy[5]. The usage of USAG-1 neutralizing antibody antagonist the function of BMP, a natural teeth developing molecule which could generate a whole new tooth in ferrets[1].

USAG-1 and BMP-7 were expressed within odontogenic epithelium and mesenchyme during the late bud and early cap stages of tooth development[4]. USAG-1 is suggested to inhibit Wnt and BMP signals via direct binding to BMP and the Wnt coreceptor LRP5/6[1]. To investigate whether inhibition of USAG-1 function rescues congenital tooth agenesis, the researchers from Kyoto University Japan purified five mouse USAG-1 monoclonal antibodies (#12, #16, #37, #48, and #57) using a bioactive human USAG-1 recombinant protein derived from *Escherichia coli* as an antigen and *USAG-1*−/− mice[1]. These five antibodies were categorized into three

different classes, based on their interfering abilities of the binding to both BMP and Wnt (#57), BMP (#12 and #37), or Wnt (#16 and #48). Each USAG-1 antibody was systemically administered to *EDA1* pregnant mice. The results showed that USAG-1–neutralizing antibodies #16, #37, #48, and #57 reverse molar hypodontia in the mandible of *EDA1*−/− mice compared with control. USAG-1‑neutralizing antibody #37 arrests hypodontia at a high rate and in a dose-dependent manner. In addition, USAG-1‑neutralizing antibodies #12, #16, #37, and #57 showed the forming of supernumerary teeth in the maxillary incisor, mandibular incisor, or molar of *EDA1* KO/hetero mice. Unexpectedly, USAG-1–neutralizing antibody #57 induced the formation of supernumerary teeth in the maxillary incisor, mandibular incisor, or molar of wild-type mice at a high rate and a dose-dependent manner[1]. However, fused molars were observed instead of supernumerary teeth in the maxillary molar region. The experiment confirmed that all antibodies could bind the mouse and human USAG-1 recombinant proteins, although #16 and #48 showed low affinity.

These results indicate that BMP signaling is essential for determining the number of teeth in mice. Furthermore, a single systemic administration of a neutralizing antibody can generate a whole tooth[1]. The analyses of mouse models show the benefits of monoclonal antibodies to successfully approach tooth regeneration and provide a new corrective treatment for a clinical problem that can currently only be resolved with implants and other artificial. This study tested the hypothesis of using a monoclonal anti-USAG-1 antibody to induce the generation and recovery of tooth development in mice.

# 2. GENE REGULATION OF USAG-1 AND HOW IT CAN BE USED TO REGENERATE TEETH

In a study about "Development of tooth regenerative medicine by controlling the number of teeth using targeted molecular therapy", Takahashi et al, the authors group's study has run experiments in double null USAG-1 and RUNX2 mice to investigate the potential relationship between these two genes in regulating tooth germ formation. Three phenomena were observed. In fact, their group has observed three phenomenon which were: the prevalence of supernumerary teeth was lower than in USAG-1 null mice, tooth development progressed further compared than in RUNX2 null mice, and the frequency of molar lingual buds was lower than in RUNX2 null mice. According to the group's results, USAG-1 is BMP antagonist[8]. The results of this study. According to the study, this indicated that USAG-1 regulates local BMP activities via its functions as a BMP antagonist and resulting in the induction of apoptosis and destruction of the rudimentary incisors. In the condition of lacking USAG-1 is not present, the cell apoptosis of BMP can be prevented and lead to the successive development of rudimentary maxillary incisors in RUNX2 null mice. These results have demonstrated the abrogating USAG-1 expression partially rescued the hypoplastic and poorly differentiated molar and incisor phenotypes in RUNX2 null mice.

The new discovery about the relationship of RUNX2 and USAG-1 has helped scientists develop molecular targeted therapy that can help patients with congenital tooth agenesis by regenerating tooth germs[7]. In another study, when USAG-1 was inhibited in RUNX2-deficient mice, tooth regeneration happened[9]. Thus, it means depression of USAG-1 can be a new method to help with tooth regeneration[2]. RUNX2-null mice were demonstrated that RUNX2 can regulate

BMP signaling independently and that on the other hand USAG-1 null mice present with supernumerary tooth formation. However, it is still a hypothesis until a new study was done to examine if the local application of topical treatment to inhibit USAG-1 will have the potential to rescue teeth in RUNX-null mice. In the past, implants or dentures were an alternative method to restore tooth structure. Further investigation in regenerating tooth structure by using targeted molecular therapy on USAG-1 has given patients more options for missing teeth. One of the studies about this topic was the study with Mishima's group using topical inhibition on USAG-1 can help to recuse tooth germs in RUNX2-null mice has played an important part in proving this hypothesis as Abrogating USAG-1 can regenerate the whole tooth structure. These scientists have discovered that supernumerary teeth can be formed in a mouse model by crossing RUNX2 null mice with USAG-1 null mice[9]. In recent years, there are many new targeted molecular therapies such as antisense RNAs, small-interfering (si)RNA or RNA aptamers have been developed. One of these methods is that Stealth siRNAs is a method that has demonstrated were the effective into targeting USAG-1 expression in dental epithelial stem cell[9]. By using this new target therapy, the study group tested the hypothesis that topical inhibition of USAG-1 can help rescue arrested tooth formation in RUNX2 null mice. In this study, two USAG-1 siRNAs (#903 ad #304) were used to apply in mHAT9d cells combined with a cationic gelatin sheet. The result came out as there was teeth formation after the local siRNA administration even though the positions and shapes of the tooth germs developing inside the capsules were not typical[9].

After studying and applying experiments on mice to understand how Runx2 and Usage-1 interact with each other, the research team had successful proof that Usage-1 Stealth siRNA can help regenerate tooth germs in Runx2-null mice. On the next experiencing level, molecularly targeted therapy can be suggested such as topical application of cationized gelatin

hydrogels to target the specific Usage-1 to rescue tooth formation or regenerate it in congenital tooth agenesis mice. The next step is to develop this topical application of candidate molecules to help regenerate the whole tooth germ in a favorable environment instead of partially rescuing the tooth development. As indicated above, the arrested tooth development was rescued in RUNX2-null and USAG-1-null mice and demonstrated supernumerary teeth in these mice. Using targeted molecular therapy as local application of monoclonal neutralizing antibody/siRNA with cationized gelatin on USAG-1 seems a potential way to help regenerate tooth formation in congenital missing patients[7]. This method of siRNAs shows higher efficiency knockdown of target mRNA expression, higher specificity, greater stability, and less cellular toxicity[9]. This new figuring has opened a new method to help patients with congenital tooth agenesis. This new molecular therapy can help patients generate teeth by developing this new targeted molecular therapy working toward benefit in human medicine. However, further research still has to be conducted to investigate the toxicity, teratogenicity, and tumorigenicity of these therapeutic molecules for better results[7].

## 3. DISCUSS THE IMPLICATION OF USAG-1 IN ORAL HEALTH

In hoping for a promising approach for tooth regeneration therapy, the usage of Anti-USAG-1 and its interference with BMP signaling can furthermore assist in accelerating tooth development. Looking at it from another perspective, it has been demonstrated than an inadequate amount of USAG-1 will heighten BMP signaling demonstrating the development of supernumerary teeth. With the important finding and understanding with consideration of either blocking or eliminating USAG-1, we can see a reassuring pathway for tooth regeneration therapy.

This approach is ideal for congenital tooth agenesis and genes that cause congenital tooth agenesis. This genetic defect is composed of genes such as RUNX2, EDA, MSX1, PAX9, AXIN2, and ENT10A that can be mutated and be used as a biomarker and a neutralizing anti-USAG-1 or USAG-1 siRNA as the molecularly targeted drug. Tooth agenesis is the occurrence of missing one or more teeth and is considered the "phenotype feature of conditions such as ectodermal dysplasia, cleft lip, cleft palate, Down syndrome, and Van Ver Woude syndrome"[13]. It can be diagnosed in people with Down syndrome and children sometime in the first ten years of their life. Individuals with Down syndrome are more prone to develop tooth anomalies, periodontal disease, present congenitally missing teeth, and encounter delayed eruption than the general population[12]. Studies of missing teeth and malocclusion are well documented, it is also well acknowledged that there is a possible correlation between congenital missing permanent teeth and "ectodermal dysplasia, local inflammation that damages the tooth germ, or other medical infections"[11]. In addition, children and adults with Down syndrome have been seen with malocclusion, due to the missing teeth, delayed eruptions, and their craniofacial morphology

characteristics such as prominent epicanthic folds, hypoplastic maxilla, crossbites, and microcephalic[14]. With infections, absence of dentitions, and craniofacial characteristics, these conditions have led to the difficulty of chewing, swallowing, and high occurrence of experiencing pain due to rapid and destructive periodontal disease. The obstacles lead to poor oral health and proper care can only offer a limited amount of care to prevent the progression of the active disease.

A greater understanding of the important findings in understanding the manipulation of USAG-1 can benefit individuals with Down syndrome especially if it can assist those who have been diagnosed with teeth agenesis or have delayed teeth eruption. In addition, stimulating the third dentition might prove an important area for the new coming in future oral health care therapy. By taking advantage of the advancement of science and technology, it can promote the future of dentistry for improving patient care and offering treatment that could prolong the oral health of individuals with Down syndrome and other disabilities that induce tooth agenesis. However, a challenging problem that arises in this domain is the insufficient number of studies on human subjects and is generally not agreed to be tested on human subjects. The majority of studies have been conducted on mice and the results reveal that the stimulation of the third dentition is possible.

For the purpose of further research and experiment, it is wanting to be used to give the future of oral health care to promote the potential of developing a new therapy and prevention. Although there are immense concerns about the "sharp ethical and political controversies"[3] about studies on human embryonic stem cells, the manipulation and administration of anti-USAG-1 is done on non-embryonic stem cells. However, the concern still continues on with "cost and safety of the cell source and concerns regarding the potential for contamination or

tumorigenicity,"[10]thus it had not reached the optima readiness for clinical applications. In the future, we hope to see more studies and results that will reach that readiness and apply it clinically.

# CONCLUSION

Research has demonstrated the BMP antagonists possibly influence the activity of BMP in kidney diseases, and teeth development is discussed. Uterine sensitization-associated gene-1 (USAG-1), a novel BMP antagonist abundantly expressed in the kidney, is the main obstructive regulatory element of BMP-7 and that mice lacking USAG-1 (USAG-1(-/-) mice) are resistant to kidney injuries[6]. BMP-7, referred to as osteogenic protein-1, was initially identified as a potent osteogenic factor purified from bone[6]. The interaction of USAG-1 with both BMP and Wnt results in several of the antibodies leading to poor mice's birth and survival rates, affirming the importance of both BMP and Wnt on whole-body growth. However, one promising antibody disrupted the interaction of USAG-1 with BMP only[5]. Anti-USAG-1 antibody administration is a promising approach for tooth regeneration therapy. Although extensive research has been done for generating teeth in Congenital missing teeth cases using tissue engineering techniques, none of the available treatments are clinically applicable due to cost and safety. Although it is considered necessary to generate a new original tooth germ, the presence of rudimental tooth primordia was observed in the investigation. USAG-1 inhibits the growth of tooth primordia. Besides, congenital tooth agenesis associated with various genetic abnormalities is caused by arrested tooth development. For this reason, the conventional tissue engineering approach is not suitable for tooth regeneration. The study outcomes show that cell-free molecular therapy targeting USAG-1 is effective in treating a wide range of congenital tooth agenesis and the induction of third dentition. The impression for this topic is that the new therapy to regenerate teeth can be a huge discovery in helping patients who have missing teeth. Now not only implants or restorative treatments are the only options to help patients gain their tooth structure but

patients can have biological tooth structure. This research has opened new hope for patients with missing teeth due to caries, periodontal disease, or even congenital ectodermal dysplasia. Further research However, this research is still being developed so further research will have to be conducted more is needed and clinical applications are needed for greater awareness need to be developed to have this more understanding about this new method and the application to restorative care. There are still more ethical concerns that will be brought up to this topic as it is being developed.

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