Oral and Craniofacial Reconstruction Using Mesenchymal Stem Cells

Reporting Period: Year 2

The long-term goal of this proposal is to develop stem cell-based treatment for major defects in the orofacial regions. Bisphosphonate related osteonecrosis of the jaw (BRONJ) is a recently described adverse side effect of bisphosphonate therapy, with an estimated 94% of cases reported in the oncologic patients receiving intravenous nitrogen-containing bisphosphonates (BP). Due to the lack of a testable animal model and limited biological tissue specimens, to date, the patho-physiological mechanisms underlying BRONJ remain largely unknown. We have successfully established BRONJ minipig and mouse models treated with oncologic doses of zoledronate (Zometa)/Dexamethasone (Dex) developed BRONJ-like pathological lesions with similar clinical, radiographic, and histological features as described in the human disease. These models will be used to understand mechanism of BRONJ and find appropriate therapeutic approaches for BRONJ. We isolated a new population of stem cells from human orofacial tissue gingiva, a tissue source easily accessible from the oral cavity, namely GMSC, which exhibited clonogenicity, self-renewal, and multipotent differentiation capacities. Most importantly, GMSC were capable of immunomodulatory functions. Cell-based therapy using systemic infusion of GMSC in experimental colitis significantly ameliorated both clinical and histopathological severity of the colonic inflammation, restored the injured gastrointestinal mucosal tissues, reversed diarrhea and weight loss, and suppressed the overall disease activity in mice. The therapeutic effect of hGMSC was mediated, in part, by the suppression of inflammatory infiltrates and inflammatory cytokines/mediators at the colonic sites. GMSC can function as an immunomodulatory and anti-inflammatory component of the immune system in vivo and is a promising cell source for cell-based treatment in experimental inflammatory diseases. In collaboration with investigators in Taiwan, we implanted one type of autologous OMSCs (periodontal ligament progenitors, PDLPs) to treat an orofacial infectious bone defect disease periodontitis. We examined the clinical outcome of three autologous PDLP-treated patients in an effort to provide primary knowledge on the effectiveness of this treatment approach and preliminary clinical evidence for randomized controlled trial in the future. Clinical examination indicated that local implantation of PDLPs may provide therapeutic benefit for the periodontal defects. All treated patients showed no adverse effects during the entire course of follow up. This study demonstrated clinical and experimental evidences supporting a potential efficacy and safety of utilizing autologous PDL cells in the treatment of human periodontitis.

Reporting Period: Year 3

Human orofacial bone-derived mesenchymal stem cells (OMSCs) showed distinct differentiation traits from mesenchymal stem cells (MSCs) derived from long bones, mouse OMSCs have not been isolated due to technical difficulties, which in turn precludes using mouse models to study orofacial diseases. We developed techniques to isolate mouse OMSCs derived from mandibles and verified their MSC characteristics by single colony formation, multi-lineage differentiation, and in vivo tissue regeneration. Activated T-lymphocytes impaired OMSCs via the Fas/Fas ligand pathway, as occur in long bone MSCs. Furthermore, we found that OMSCs are distinct from long bone MSCs with respect to regulating T-lymphocyte survival and proliferation. Our data suggest that OMSCs are a unique population of MSCs and have a role in systemic immunity. Embryologic development and amalgamations of the complex array of bones and cartilage in the craniofacial region have revealed that the molecular mechanisms controlling skeletogenesis in the orofacial bones are quietly unique and different from in the axial and appendicular bones. The discrepancy in bone development between orofacial bones and long axial/appendicular bones give rises to specific diseases in the orofacial bone region, such as periodontitis, cherubism, and hyperparathyroid jaw tumor syndrome, which only affect the jaw bones. Therefore, it is not surprising to find that human OMSCs are distinct from BMSCs in terms of differentiation traits and immunoregulation. MSC mediated bone formation involves in both donor and recipient cells, but only recipient cells contribute to marrow element formation. Our study suggests that both OMSCs and host cells contribute to bone formation in vivo. Ex vivo-expanded BMSCs are capable of suppressing the T-lymphocyte proliferation and activity in vitro, which provides a foundation for using BMSC transplantation to treat T-cell-associated disorders, such as acute graft-versus-host-disease (GvHD) in mice and humans. In addition, we found activated T-lymphocyte induced apoptosis of BMSCs through the Fas/Fasl pathway. Our data suggest that O VX induced T lymphocyte activation may contribute to OMSC damage. Although T lymphocyte activation in O VX condition is a major factor for promoting osteoclast function and inhibiting osteoblast function, we can’t exclude other factors that may also contribute to OMSC deficiency in O VX mice. The immune-modulatory property is related to a high level NO production induced by
IFN+ via enhanced iNOS expression in BMMSCs. In this report, mouse OMSCs showed a stronger suppressive effect on proliferation of anti-CD3 antibody-activated T cells, but only partially inhibited T cell proliferation by anti-IFN+ antibody and the iNOS inhibitor, 1400W. These highly immunosuppressive properties of OMSCs may provide an advantage for tissue engineering in the orofacial region. Surprisingly, mouse OMSCs produced larger amounts of NO than mouse BMMSCs, indicating that OMSCs are more responsive to inflammatory cytokine(s)-induced NO production. We also found that OMSCs were capable of keeping naïve splenocytes including T cell survival more effectively than BMMSCs. Therefore, it is necessary to continue elucidating underlying mechanisms of the interplay between OMSCs and immunity using established various mouse models. Bisphosphonates (BPs) have been used for the clinical treatment of bone diseases with increased bone resorption such as osteoporosis and malignant diseases like multiple myeloma or metastasis to the bone. However, there is increasing evidence associate bisphosphonates treatment with osteonecrosis of the jaws. The detail mechanism of bisphosphonate-related osteonecrosis of the jaws (BRONJ) is unclear and it is very difficult to be treated. In present study, we generated large animal model of BRONJ in miniature pig and treated with allogeneic bone marrow mesenchymal stem cell (BMMSCs) transfusion. Of the 9 miniature pigs received BPs treatment and tooth extraction, 6 pigs disclosed BRONJ with exposed bone. The level of CD4+CD25+ T cells, foxp3+ T cells in the peripheral blood was decreased, while the level of γδ T cells and IL-17 were increased. After MSCs infusion, mucosal and bone healing were achieved, changes in immunity recovered. These findings obtained in a clinically relevant large-animal model of BRONJ provide evidence of the connection of BPs treatment and osteonecrosis of the jaw, as well as the immunity-based mechanism of BRONJ.

**Reporting Period:** Year 4

The long-term goal of this proposed study is to explore a new stem cell-based treatment for major defects in the orofacial regions. Bisphosphonate related osteonecrosis of the jaw (BRONJ) is a recently described adverse side effect of bisphosphonate therapy, with an estimated 94% of cases reported in the oncologic patients receiving intravenous nitrogen-containing bisphosphonates (BP). Due to the lack of a testable animal model and limited biological tissue specimens, to date, the patho-physiological mechanisms underlying BRONJ remain largely unknown. Previously we established BRONJ mouse model and found regulatory T cells can prevent BRONJ in mouse model. Recently, we have established BRONJ pre-clinical model in minipigs and confirmed that regulatory T cells and Th17 cells contribute to the occurrence of BRONJ. In order to further characterized cell-based therapy for orofacial defects, we generated radiation-induced jaw bone necrosis model in minipigs and use mesenchymal stem cell (MSC) implantation to cure the necrosis, suggesting a potentiality of using cell-based therapy for jaw bone regeneration. To further understanding mechanism by which MSCs are capable of regenerating orofacial bones, we showed that MSC-based bone regeneration inhibited by recipient T cells via IFN-gamma and TNF-alpha. Local aspirin treatment can block T cell activity and, therefore, improve MSC-based orofacial bone regeneration. Moreover, we demonstrated that ERK signaling pathway controls orofacial MSC-mediated bone regeneration. ERK1/2 inhibitor treatment rescued bFGF-induced osteogenic differentiation deficiency. Finally, we showed that vitamin C treatment improved capacity of orofacial MSC-mediated orofacial bone regeneration in minipigs through up-regulation of telomerase activity.

**Reporting Period:** Year 5

The goal of this grant proposal is to characterize orofacial mesenchymal stem cells and determine the feasibility of reconstructing the orofacial defects caused by a variety of diseases such as osteonecrosis of the jaw using mesenchymal stem cells. Our study focuses on mesenchymal stem cell characterization, disease model generation, and mesenchymal stem cell-based orofacial bone regeneration in large animal model. • We identified that Erk1/2 signaling regulate both MSC-mediated bone regeneration and immunomodulation (manuscript in preparation). • We showed that MSC-based immunotherapy involves in coupling via FasL/Fas to induce T cell apoptosis (Akiyama et al., Cell Stem Cell 2012). • We have established jaw osteoradionecrosis (ORN) pre-clinical model and shown that mesenchymal stem cell-based implantation can cure ORN in minipigs (Xu et al., Cell Transplantation 2012). • We continue to characterize effectiveness of mesenchymal stem cell-based therapy for bisphosphonate-associated osteonecrosis of the jaw (BRONJ) in pre-clinical model (manuscript in preparation) and confirmed some clinical phenotypes in BRONJ patients (Patel et al., Oral Diseases 2012).

**Reporting Period:** NCE

The purpose of this grant proposal is to characterize orofacial mesenchymal stem cells and determine the feasibility of reconstructing the orofacial defects caused by a variety of diseases such as osteonecrosis of the jaw using mesenchymal stem cells. Our study focuses on mesenchymal stem cell characterization, disease model generation, and mesenchymal stem cell-based
tissue regeneration in small and large animal models. • We identified that Erk1/2 respectively regulate MSC-based immunomodulation and osteogenic differentiation. • We showed that MSC-based immunotherapy involves in coupling via FasL/Fas to induce T cell apoptosis. • We have established jaw osteoradionecrosis (ORN) pre-clinical model and shown that mesenchymal stem cell-based implantation can cure ORN in minipigs. • We continue to characterize effectiveness of mesenchymal stem cell-based therapy for bisphosphonate-associated osteonecrosis of the jaw (BRONJ) in pre-clinical model (manuscript in preparation) and confirmed some clinical phenotypes in BRONJ patients. • We found that inflammation environment inhibits MSC-based bone formation via TNF Alpha and IFN-Gamma.

Oral and Craniofacial Reconstruction Using Mesenchymal Stem Cells

Grant Type: New Faculty I
Grant Number: RN1-00572

Project Objective: The objective of this grant proposal is to characterize orofacial mesenchymal stem cells and determine the feasibility of reconstructing the orofacial defects caused by a variety of diseases such as osteonecrosis of the jaw using mesenchymal stem cells. The study focuses on mesenchymal stem cell characterization, disease model generation, and mesenchymal stem cell-based tissue regeneration in small and large animal models.

Investigator:

Name: Songtao Shi
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Disease Focus: Bone or Cartilage Disease
Human Stem Cell Use: Adult Stem Cell
Award Value: $3,242,651
Status: Closed
Application Title: Oral and Craniofacial Reconstruction Using Mesenchymal Stem Cells
Public Abstract: The overall goal of this proposal is to explore a new stem cell-based treatment for major defects in the orofacial regions resulted from burns, physical injuries, genetic diseases, cancers, infectious diseases, and recently, bisphosphonate-associated osteonecrosis of the jaw (BONJ), using the patient’s own stem cells obtained from the oral cavity known as orofacial mesenchymal stem cells (OMSCs).

The standard surgical reconstruction of orofacial defects relies on different sources of bone grafts harvested from distant anatomical site of the same patient or other donors. However, those approaches are associated with higher morbidity and unpredictable clinical outcomes. Evidences have shown that bone marrow mesenchymal stem cells (BMMSCs) could be a promising alternative for bone reconstruction but not in the orofacial region. These clinical results may be due, in part, to the fact that orofacial and long bones are derived from different cell origins, termed as neural crest cells and mesoderm, respectively. In addition, OMSCs are readily accessible from the oral cavity and can be easily expanded for cell-based therapies due to their inherently high proliferative capability. These evidences suggest that neural crest cell-associated OMSCs might be a superior cell source for orofacial bone regeneration as compared to BMMSCs.

In this study we will compare human OMSCs and BMMSCs in terms of stem cell characteristics and will test their tissue regeneration capacities in the restoration of orofacial defects including the recently drug-induced bone necrosis defects caused by the commonly used drug, bisphosphonate in our established animal models. Our laboratories have recently demonstrated feasibility of using BMMSCs to partially repair craniofacial defects in mouse models. In this proposed study, we will use OMSCs as a model system to determine whether and how individual OMSCs can be utilized as a novel cell therapy for orofacial tissue regeneration. We anticipate that the patient’s own OMSCs will be capable of forming orofacial tissues and will highlight future clinical treatments for orofacial defects.

Statement of Benefit to California: There is a great clinical demand for developing more optimized approaches to repair facial defects caused by burns, trauma, genetic anomalies, cancers, and recently, the devastating drug-induced osteonecrosis of the jaw associated with the commonly used drug, bisphosphonate (BONJ). Current therapeutic approaches are deficient in supplying appropriate tissues for major facial reconstruction. By generating an optimal supply of human orofacial mesenchymal stem cells (OMSCs) for stem cell-based therapy, we hope to circumvent the limited tissue resource and provide a more superior cell source for future facial tissue regeneration. More importantly, Californians who are head and neck cancer survivors, or suffer esthetic and functionally debilitating orofacial defects will benefit from the advances in stem cell biology and its clinical applications, specifically in the field of orofacial reconstruction. In this proposal, we will expand current knowledge of stem cell biology of OMSC and test the feasibility of utilizing these autologous stem cells in the treatment of diseases such as BONJ. The novel approach in the reconstruction of the orofacial defects using OMSC-based therapy will replace standard paradigm of treatment which involves multiple surgeries, lengthy operating time, cost, and morbidity to the patients. The success of this proposal will not only benefit the people of California, but will have high impact on the state economy by reducing the medical cost and overall financial burden on the State of California Health Insurance.

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