

## Biological bone tissue engineering with adipose stem cell combined gene therapy: A future prospect in treating oral peri-implant defects

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### Abstract

Osseointegrated dental implants have become an essential part of modern reconstructive dentistry and are associated with a high success rate and long term beneficial outcomes. Inflammation of peri-implant tissues called periimplantitis threatens the long term success of Dental Implants. However, the establishment of reosseointegration in peri-implant bone defects still remains a clinical challenge. The rise of tissue engineering has provided numerous therapeutic strategies for biomedical application to date. Principles of tissue engineering could be used to restore lost BIC(Bone Implant Contact)in peri-implant defects. Due to the superior osteogenic ability of Adipose-derived stem cell and BMP-2 when combined with the principals of tissue engineering they present a promising alternative for the treatment of oral peri-implant defects. Ex vivo gene therapy could be used to deliver BMP-2 Transgene to overcome the disadvantages associated with BMP-2. This paper presents a detailed description of the merits and demerits of therapeutic strategy- Adipose-derived stem cell combined with ex vivo BMP-2 gene therapy in the regeneration of Oral peri-implant defects.

**Keywords:** Dental implants, Periimplantitis, Tissue engineering, Adipose-derived stem cell, BMP-2, Ex-vivo Gene therapy.

### Introduction

Over in the due course of time, dental care and dentistry have evolved from a primitive form of medicine to modern age treatment, use of preventive dental care and state of the art diagnostics. Dentistry has undergone a revolution with many improvements and changes in the past several centuries. Either from removing caries or replacing missing teeth, modern dentistry aims to restore the function, comfort, aesthetics, speech and health of the patients.<sup>1</sup> With favourable long term outcomes, dental implants have become an essential part of modern reconstructive dentistry. Till date stability of dental implants has been successful in rehabilitating patients suffering from aesthetic, masticatory and economic burdens because of loss of the tooth. However, the maintenance of dental implants is largely threatened by inflammation of peri-implant tissues called periimplantitis. Progressive bone loss leading to irreversible pathological changes as a result of periimplantitis compromises the osseointegration of dental implants.<sup>2,3</sup> Mechanical debridement together with antiseptic and antibiotic therapy known as CIST(Cumulative Interceptive Supportive Therapy) could be used to halt the progressing lesion.<sup>4-6</sup> CIST together with regenerative procedures using Bone grafts or bone substitute materials is often used to repair peri-implant defects. Preclinical evidence suggests the effectiveness in regenerating bone defects using graft substitutes, restablising BIC(Bone Implant Contact) in microbial contaminated implant surface.<sup>7</sup> However, the

efficacy of one technique over others could not be proved yet and no such consensus exists such.

Several recent clinical studies have demonstrated tissue engineering strategies to be alternative to regenerative procedures using either bone grafts or biomaterial-based grafting system.<sup>8</sup> Tissue engineering is a multidisciplinary field that combines principles of engineering, biology and life sciences to develop substitutes or strategies to maintain, restore and improve organ function.<sup>9</sup> Classical approach of tissue engineering involves seeding stem cells into a suitable scaffold with signalling molecules and implantation into the host to induce or conduct tissue growth. This novel method of tissue engineering as a therapeutic Strategy could be used to restore the lost BIC (Bone Implant Contact) in peri-implant defects with the help of transduced osteoprogenitor cell which produce osteoinductive growth factors. Stem cells, signalling molecules together with a biocompatible scaffold form the three fundamental components of tissue engineering.

### Stem Cells

Stem cells are clonogenic in nature with the capacity of self-renewal and with the ability to generate differentiated progenies. Bone marrow-derived mesenchymal stem cells were the first stem cell to be identified by Friedenstein et al. Since then to date they have been considered as the gold standard for stem cell-based therapies. These stem cells have the ability to be adherent, proliferating and capable of

differentiation into progenitor cells of various tissues such as bone, cartilage, muscle, tendon etc. In dentistry, a variety of stem cells have been used for the purpose of tissue engineering. These cells include mesenchymal stem cells derived from the periodontal ligament, Dental Papilla stem cell, SHED, Stems cells from apical papilla. Mesenchymal stem cells could also be derived from skeletal muscles, human adipose tissue, periosteal cells, skin fibroblast etc. These cells together with appropriate scaffold could act as a vehicle for delivery of signalling molecules exerting a paracrine effect, ultimately leading to bone repair around peri-implant defects exerting an autocrine effect. Adipose Tissue stem cells offer a potential candidate in the regeneration of peri-implant defects mediated by means of tissue engineering due to its unique abilities in comparison with stem cells of different origin.

Adipose tissue stem cells are multipotent in nature with the capacity to differentiate into cells of multiple tissue lineage including osteogenic cells.<sup>10,11</sup> Easy access to adipose tissue stem cells than stem cell of bone marrow origin. Abundant volume of stem cells can be extracted by Liposuction, which is relatively an easy surgical procedure with low risk of complication and associated donor site morbidity whereas extraction of stem cells from bone marrow is severely painful and a difficult procedure with potential unpredictable drawbacks.<sup>12</sup> Mesenchymal stem cells isolated from adipose tissue showed higher proliferation rates with shorter generation time than Bone marrow stem cells.<sup>13</sup> Adipose tissue stem cell expresses mesenchymal stem cell markers-CD44, CD73, CD90 and CD105 and are negative for hematopoietic stem cell marker like CD45, CD11b, CD19 and HLA-DR.<sup>13</sup> If adipose stem cells are transduced with growth factor producing genes by Ex vivo gene therapy using viral vectors then a high transduction efficiency could be achieved with an increased level of production of growth factors.<sup>13</sup> Adipose tissue stem cells express CD31 which is an endothelial cell marker, thus adipose stem cells have angiogenic abilities.<sup>14</sup> Adipose stem cell are highly biocompatible which is mainly due to its immunosuppressive, immunomodulatory and anti-inflammatory properties.<sup>14,15</sup> These stem cells lack both MHC I and MHC II, directly or indirectly modulate T Cell and B cell suppression and express various inflammatory cytokines. For these reasons, adipose tissue stem cells have been widely used in periodontal regeneration.<sup>16,17</sup>

### Signalling Molecules

Signalling molecules are proteins, a part of the complex system acting locally or systemically to govern the growth and function of cells. A variety of signalling molecules have

been described in the literature regulating various cellular activities in our body including mitogenesis, chemotaxis, angiogenesis etc. For the purpose of Tissue Engineering growth factors and morphogens are used as signalling molecules which act by altering cellular phenotype leading to differentiation of stem cells to the cells of interest to be engineered called the process of osteoinduction. These molecules include Bone morphogenic protein BMP-2(OP-2), BMP-3(Osteogenin), BMP-7(OP-1), Platelet-Derived Growth Factors, Insulin-Like Growth Factors, Transforming Growth Factors, Fibroblast Growth Factor, Enamel Matrix Derivative. Bone Morphogenic Protein are members of Transforming Growth Factor- $\beta$  super family. These Bone Morphogenic Proteins play an important role as signalling molecules in chemotactic proliferation and differentiation of osteoprogenitor cells ultimately inducing bone formation. Bone Morphogenic Protein 2(BMP-2) possesses strong osteoinductive activity and is among the most extensively researched signalling molecule. Rh BMP-2 is FDA approved and is known to up-regulate bone healing process thus assisting in bone regeneration.<sup>18</sup> Rh BMP-2 is mainly responsible for differentiation and maturation of cells<sup>19</sup> whereas other signalling molecules like platelet-derived growth factors are mitogenic in nature and enhances angiogenesis.<sup>20</sup> Use of BMP-2 is known to increase osseointegration around dental implants with peri-implant defects.<sup>21</sup> However, the use of BMP-2 is associated with shorter half life, localized action and rapid local clearance.<sup>22</sup> Therefore, a superior physiological dosage is required to produce effective results and to maintain therapeutic levels. But this high concentration of BMP-2 leads to seroma formation, immature trabecular pattern with inferior BIC (Bone Implant Contact).<sup>23,24</sup> Adipogenesis, cyst formation, ectopic bone formation, osteolysis and stimulation of cancer cells are among the side effects associated with the use of BMP-2.<sup>25</sup>

To overcome the clinical side effects associated with BMP-2, BMP-2 gene delivery could be used as an alternative form of delivering BMP-2 protein to the site of the defect. Gene therapy has been used exogenously to produce BMP-2 protein for prolonged period either by using direct or indirect gene transfer techniques.<sup>26</sup>

Gene therapy involves a process in which gene for the protein is transferred or transduced into an appropriate host cell. BMP-2 transgene could be transduced to Adipose-derived stem cells for high-quality results thereby overexpressing BMP-2. Vectors for transfer of genes can be viral or non-viral either in an ex-vivo or in-vivo manner. The virus is having a natural ability to infect and can translocate their own DNA to host cells. Virulence and

Pathogenicity of these viruses are generally eliminated and are modified with target genes to form viral vectors. Commonly used viral vectors include vaccine virus, herpes simplex virus, measles virus, poxvirus, lentivirus,<sup>27</sup> retrovirus,<sup>28</sup> adenovirus and adeno associated virus<sup>29</sup>. The use of viral vectors mostly Adeno associated virus has been associated with high transduction efficiency and high levels of transgene expression than non-viral vectors. However, the disadvantages of the use of viral vectors include-immunogenicity of the virus can lead to an immune response in the host, expensive and complex to process, safety issues including intentional mutation, life-threatening diseases, risk of cancer development etc.

Owing to a stable and safe delivery and because of inherent potential disadvantage associated with in vivo gene therapy, Ex-vivo gene therapy methods are preferred. Though in vivo gene therapy is faster, simpler, less expensive with the risk of contamination being minimal. However, this process is associated with a less transduction efficiency, less protein formation, transduction in non-target sites, high amount of antibodies to both vectors and growth factors and more viral titre attained.<sup>27</sup> Ex-vivo gene therapy is a process in which target cells are cultured and genetically manipulated invitro followed by implantation into the osseous defect. Though it is an invasive method with an increased requirement of resources and cost. Ex-vivo gene therapy allows for genetic manipulation of desired cells and measurement of transduction efficiency.<sup>30</sup> Prolong expression of the transgene with increased quality of bone healing can be achieved. Overall there a decreased margin of a safety concern than in vivo gene therapy.<sup>31</sup>

In a study by Hsu et al (2008) where mesenchymal stem cells from human adipose tissue were transduced with BMP-2 transgene by adenovirus induced bone formation enhancing spinal fusion in the rat model.<sup>32</sup> Dragoo et al (2005) and Peterson et al (2005) used human adipose-derived stem cells and transduced them with BMP-2 transgene by using adenovirus to produce tissue-engineered bone in rat models.<sup>33,34</sup>

## Scaffold

A scaffold is a temporary framework in which stem cells are allowed to proliferate and differentiate to generate desired tissue. The main function of scaffold includes cell attachment and migration with a sustained release of growth factors. A scaffold should be biocompatible and enable the influx of oxygen. It should possess adequate mechanical resistance and should be porous enough for the diffusion of nutrients and allow the ingrowth of tissues and vessels. The scaffold undergoes resorptions at a rate compatible to the

that of new tissue formation and provides a template for tissue regeneration.

A huge part of literature supports the increased osteogenic potential with superior quality of bone regeneration by BMP-2 transduced Adipose-derived stem cell. Chen et al (2010) transinfected Adipose-derived stem cell with Rh BMP-2 plasmid and implanted them in ulnar bone defects in minipigs. He observed a better treatment effect on bone defect and accelerated bone formation than matched controls.<sup>35</sup> Lee et al (2010) by using a bicistronic vector transduced Adipose-derived stem cell with BMP-2 genes and found increased alkaline phosphatase activity and increased bone mineralization in Comparison to matched controls.<sup>36</sup>

Because of the abilities of adipose-derived stem cell transduced with BMP-2 transgene by ex vivo gene therapy when implanted within a peri-implant defect in periimplantitis could also produce desired results. Shanbhag et al (2018) in a systematic review and meta-analysis addressed the novel role of Bone tissue Engineering in in-vivo regeneration of oral peri-implant defects.<sup>37</sup> The first study to adapt BMP-2 transduced adipose-derived stem cell in treatment of peri-implant defects was by Lianyi Xu et al(2016). Ligature induced periimplantitis models in beagle dogs were treated with BMP-2 transduced adipose-derived stem cell showed excellent results. The author observed a decrease in probing depth and clinical attachment level thus suggesting a reduced inflammation of soft tissues. Increased alkaline phosphatase activity depicted strong osteogenic ability of BMP-2 transduced adipose-derived stem cell. A stronger denser and compact bone was formed with increased Bone Implant Contact. The author concluded a high percentage of re osseointegration with an increased bone fill and a high mineralization apposition rate.<sup>38</sup> However further these kinds of researches are needed to be carried with this novel method of using BMP-2 transduced adipose-derived stem cell in the correction of periimplantitis.

Some obstacles are still faced to date in applying the principles of tissue engineering strategies in treating oral Perimplant defects. These include limitations such as the stem cells are only assessed in vitro and further research and experimentation in vivo are needed to appreciate the osteogenic potential and bone inducing capacity of BMP-2 transduced adipose-derived stem cell. Human trails and studies are needed to be conducted before firm conclusions can be drawn. Excluding age and sex, no correlation between BMP-2 production and other factors like diabetes, hypertension or effect of other systemic diseases are studied till date. Optimal seeding density of stem cells and other

protocols are yet to be standardized. Regulatory obligations and safety issues regarding the use of viral vectors are also needed to be addressed. Tissue engineering is a costly procedure and limitation of financial support becomes a major obstacle to tackle. There are a large heterogeneity and variations among the studies in regards to stem cells, biomaterials, scaffold including methodological factors thus a definite inference couldn't be drawn.

## Conclusion

In the modern age, dental implants without a doubt has become a boon to dentistry. Its development and use are considered as one of the biggest advances in dentistry over the last few decades. It has become a popular and effective way to replace missing teeth thus rehabilitating patients both physically and mentally. With a huge number of the population opting to get a dental implant done for replacing missing teeth the incidence of Perimplantitis is also increasing respectively. Application of Tissue Engineering Strategies by using Viral BMP-2 transduced adipose-derived stem cell with a superior osteogenic potential seems to be a promising treatment modality in regeneration therapy of peri-implant defects and periimplantitis. It would take time for this novel and promising technology to reach from bench to bedside that is from research to dental clinics. Despite limitations, history has always thought us that most of the imaginative, unconventional and groundbreaking technologies become more affordable as they become popular. Thus, it would be interesting to see the scope of tissue engineering in field of implant dentistry.

## Source of Funding

None.

## Conflict of Interest

None.

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**How to cite this article:** Mohammad A, Ali AR. Biological bone tissue engineering with adipose stem cell combined gene therapy: A future prospect in treating oral peri-implant defects. *IP Indian J Anat Surg Head Neck Brain.* 2020;6(1):1-5.