Bio-hybrid implant, next generation of bio-engineered implant – A Review.

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Abstract
The lack of periodontal ligament (PDL) support in the implant bone junction is a matter of concern for implant treatment. The direct contact between bone to implant transmit forces directly to bone and reduces its functional efficiency. Several modifications in implant design and implant surface have been tried and tested. Recently bio-engineering and regenerative dentistry has thrown new lights in the modification of dental implant and to eliminate these drawbacks. Stem cell incorporated bio engineered implant also known as bio- hybrid implant has the potential to generate PDL and nerve cells along the implant bone junction. Pluripotent cells present on the bio-hybrid implant surface also have the ability of bone remodeling, wound healing and neuro transmission. This review article highlights recent status in the development of bio-hybrid implants along with its function and advantages.
Keywords: Bio-hybrid implant, ligaplants, bio-engineered PDL, stem cells, regenerative dentistry.

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Introduction

The history of the evolution of dental implants is a rich and fascinating travelogue through time. With advancement of technologies the modern implant treatment has also taken a quantum leap from inserting shell, gold tube to modern era oseointegrated titanium implants.

While dental implants are increasingly becoming the choice of replacement for missing teeth, the complications associated with them are progressively emerging too. Oseointegration is one the key factor for successful outcome of implant treatment is affected by several local and systemic factors.[1] To overcome complication associated with oseointigration several modifications are made that can osseointegrate to bone faster and with a stronger bone-to-implant interface.[2] Along with physical and chemical modification implant surface use of bio mimetic compound is also getting popular as a way to increase bio-functionalization of dental implants.[3]

In spite of all these modifications the absence of periodontal ligament (PDL) attachment around the implant hinders its biological function and efficiency.[4] The periodontal ligament in natural teeth which acts as a viscoelastic "shock absorber" diminishes the magnitude of the occlusal force and distributes stress to the surrounding bone. Absence of PDL reduces the mobility of the implant and directly transfers the stress to the bone implant interface.[5] Absence of proprioceptive fibers in PDL also reduces patients' functional efficiency.

With the advancement of regenerative dentistry, a novel fibrous tooth implant connection using dental follicle stem cells, bioengineered PDL has been developed to address these issues. While ligaplants implants using bioengineered PDL was introduced much earlier, bio-hybrid implant with stem cell technology is newer advancement of this field.

This review provides an insight into available techniques and biomaterials used in bio-hybrid implants with emphasis on the most recent outcomes and future avenues.

Current trends in Bio-engineered PDL Implant

Buser et al. (1990) 1st conceptualized bioengineered PDL and demonstrated PDL of the roots served as a source of cells which could populate the titanium implant surface during healing.[6] Gault et al. in 2010 described the development and clinical application of a tissue-engineered ligament known as "ligaplants".[7] In this process PDL cells obtained from teeth are prepared and cultivated in the bioreactor. Then single layer of these cells secured with biodegradable scaffolds are deposited around the titanium coated implant.[8]

Histologic examination has shown soft-tissue space with dimension and structure similar to PDL space with abundant connective tissue fibers perpendicular to implant surface of ligaplants. Evidence of formation of osteocementum and neo vascularization around bone implant junction also been documented.[9]

Apart from all these advantages ligaplants has shown high degree of implant rejection, limited mobility and no response to noxious stimulus. [8,9]

Bio- hybrid implant

Bio- hybrid implants are implantable electronic arrays which harness stem cells. These implants not only stimulate the growth of damaged or missing tissue but also capable of interacting with nervous tissue and restore neural function (Figure 1).[10] Bio-hybrid eye for the treatment of visual impairment patients and bio- hybrid cochlear implants for hearing loss is quite popular now a day. Osseointegrated bio-hybrid implants not only support mechanical loading but also connected to surrounding via connective tissue which maintains flexibility, mobility.[11] Till now no experimental studies has been conducted on human but animal studies have shown promising results with generation of PDL fibers and nerve interface between bone and implant.

Oshima M et a.l (2014) developed 1st bio hybrid dental implant.[11] They used HA coated implant enveloped with embryonic day (ED) 18.5 tooth germ-derived dental follicle tissue (ED18.5-DF) and transplanted into an adult mouse. Nakajima K et al. (2016) used HA & platinum coated titanium implant and enveloped it with rat PDL cells to develop a bio-hybrid implant.[12] Lee D-J et al. (2017) used HA coated titanium implant and enveloped it with different cell type like immortalized human periodontal ligament cells (ihPDLs), immortalized human cementoblasts (ihCEMs), human umbilical vein endothelial cells (HUVECs) in multilayer formation.[13]

A. Stem cell used in bio-hybrid implants:

Stem cells used in bio-hybrid implants can be obtained from extra oral tissues or oral tissues such as craniofacial bone, dental pulp, PDL, dental follicle, apical papilla, oral mucosa, gingiva, and periosteum.[14] The dental stem cells (DSCs) are post-natal stem cells that have mesenchymal stem cells (MSC) like properties including the self-renewal and multilineage differentiation potential.[15]

1. ***Dental pulp stem cells (DPSCs)***
   These are multi potential cells with typical fibroblast-like morphology and dentinogenic, osteogenic, neurogenic, chondrogenic, and myogenic differentiation

potential.[16,17] The human-DPSC cultures contain multipotent neural crest stem cell (NCSC) which can differentiate into neural crest cells . These cells express several bio markers like STRO-1, CD146, OCT4 which are mesenchymal and bone-marrow stem cell markers.[15]

2. ***Periodontal ligament stem cells(PDLSCs)***
These cells too show morphology similar to fibroblasts and can be differentiated into cementoblasts and osteoblasts cells.[15] The rate of proliferation of these cells are higher than DPSCs. STRO-1, CD146, and tendon-specific transcription factor are the biological cues expressed by these cells.[18]

3. ***Stem cells from human exfoliated deciduous teeth (SHED)***
This mesenchymal progenitor stem cell was isolated by Muira et al (2003) from exfoliated deciduous teeth.[19] The rate of proliferation and CFU of SHED is higher than DPSCs with early expression of biological markers like STRO-1 and CD146.[15]

4. ***Tooth germ progenitor cells (TGPCs)***
These are dental mesenchymal cells obtained from 3rd molar. TGPC express different bio markers like STRO-1 and CDs. They help in the expression of pluripotency-associated genes like nanog, oct4, sox2.[20] TGPCs have multilineage differentiation capacity to differentiate into adipocytes, osteoblasts/odontoblasts, osteocytes, and neurons.[15]

5. ***Dental follicle progenitor cells (DFPCs)***
These cells are ectomesenchymal in origin and extracted from follicle of human third molars.[15] These cells express several bio markars like Notch1, STRO-1, and nestin.[21] STRO-1-positive dentalfollicle stem cells (DFSCs) can differentiate into cementoblasts and can form cementum.[22]

6. ***Gingival mesenchymal stem cells (GMSCs)***
GMSCs obtained from gingival tissue and retro molar tissue exhibit clonogenicity, self-renewal, and multipotent differentiation capacity, and immunomodulatory properties.[15,23] GMSCs express CDs and display positive signals for Oct4, Sox2, Nanog, Nestin, SSEA-4, and Stro-1. The main features of this type of cells are tissue regeneration and wound healing caused by increase telomerase activity.[24]

7. ***Alveolar bone-derived mesenchymal stem cells (ABMSCs)***
Matsubara et al. (2005) isolated these spindle shaped fibroblasts like stem cells.[25] Similar to other stem cells these cells also have osteoblastic, chondrogenic and adipogenic differentiation potentials.[15] These cells express the surface markers CD73, CD90, CD105, STRO-1and high level of alkaline phosphatase.[5,15]

8. ***Adipose-derived stem cells (ADSCs)***
ADSCs have the same properties that of DSCs but the only advantage of these cells are they can be in higher amount easily.[26] They can be differentiate into chondrocytes,

osteocytes, or myocytes and can be used areas with boney defect.[27]

Other than these cells other verities of stem cells are also there like stem cells from the apical part of the human dental papilla (SCAP), Bone marrow-derived mesenchymal stem cells (BMSCs), Blood-derived stem cells.[17]

B. Structure of bio-hybrid implant:

Implants functionalized with bio-engineered PDL and stem cells made up of 3 components – implant body, scaffold and stem cells. The function of the scaffold around the implant is to harbor stem cells and to regulate the action of the bio markers. Different materials used as scaffolds are as followed-

1. *Natural Polymers-*
   3D-printed natural polymers like chitosan, collagen most commonly used as scaffolds.

2. *Synthetic Polymers-*
   Different synthetic bio-degradable polymers like poly(ethylene glycol)-b-poly(l-lactide-co-"-caprolactone) (PELCL), poly(lactic-co-glycolic) acid (PLGA) , poly(caprolactone-co-ethyl ethylene phosphate) (PCLEEP) are used for fabrication of scaffolds. The verifying porosity (i.e., pore size of 150–500 nm) of these polymers helps to control release of cells and biological cues.[28]

3. *Bio-ceramics-*
   Hydroxyapatite nanoparticles, injectable mesoporous silica nanoparticles, bioactive glass nanoclusters are often used with polymers to fabricate scaffolds.[28]

C. Fabrication of bio-hybrid implant

1. Stem cells are extirpated from desired sites and are cultured in different culture medium like (Dulbecco's minimum Eagle's medium with 10% foetal bovine serum and 1% penicillin/streptomycin solution, Minimum essential medium alpha, endothelial growth media) with the help of bio degradable scaffold for stipulated days.[11,13]
2. After harvesting different stem cells, cell sheets are detached from cultured medium. Each isolated tissues were wrapped around HA coated implant surface of approximately 50 mm in diameter.[11]
3. Then these implants are placed onto a cell culture insert for 24 hours before transplantation. (Figure 2)

Discussion:

The main difference between the bio-hybrid implant and the ligaplants is that three dimensional cultured stem cells are used in bio-hybrid implants over two dimensional PDL cells from extracted teeth. The bio-hybrid implant not only helps to grow cementum, PDL, alveolar bone around implant but also helps to protect the implant from noxious stimuli generated by occlusal forces. Pluripotent stem cells in the cell sheet of bio-hybrid implant surface contains Hertwig's epithelial root sheath and epithelial cell rests of Malassez which secrets Wnt signaling and regulates formation of cementoblasts.[29] The newly formed nerve cells at implant bone junction create microTENNS like structures. Neural bodies of the nerve cells are situated at one end of collagen hydrogel scaffold structure and their axonal projections grow unidirectionally toward the target tissue which forms a neural network that could act as a replacement for CNS reconstruction (Figure 3). [30,31] Oshima

M et al. (2014) and Nakajima K et al. (2016) detected Anti-neurofilament (NF)-immunoreactive nerve fibers and expression of c-Fos immunoreactive neuron in the PDL. The level of expression of these factors is equal to natural tooth which can be observed following noxious stimulation following 2 hours after orthodontic treatment.[11,12] Functional analysis of bio-hybrid implant shows movement of implant similar to natural teeth with successful bone remodeling through generation of Colony-stimulating factor-1 (Csf-1) and osteocalcin (Ocn) on the compression and tension sides respectively.[13]

More over bio-hybrid implants act as a reservoir of immune cells which helps to bone remodeling and regeneration of bone defect.[11] Bio-hybrid implants provides platform onto which cells are seeded and also incorporate molecular factors (growth factors, guidance molecules, VEGF-alpha, insulin-like growth factor 1 etc) to stimulate and guide outgrowth of cells. These factors together known as secretomes enhance the migration, proliferation, and expression of osteogenic marker genes like RUNX2, SMAD5, RANKL.[32] Whether fixed to the implant surface or delivered via microfluidic channels, these molecular factors offer an advantage over traditional cell transplantation by facilitating integration of cells to the intended targets.[10] Tissue non-specific alkaline phosphatase (TN-ALP), ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENNP1), integrin binding sialoprotein are among such factors which regulates phosphate level and mineralization of hard tissues.[11]

These factors also work in epigenetic mechanism which includes DNA methylation and histone modifications. During osteoblastic differentiation of stem cells increased methylation of the promoter of LIN28 facilitates osteogenesis by downregulation of Lin28 gene.[33] DNA methyltransferases secreted by stem cells molecules are responsible for DNA methylation. This causes more condensed DNA structure, transcriptional repression, and gene silencing and regulates genes responsible for bone metabolism and expression of several factors like alkaline phosphatase, sclerosin, osteoprotegerin.[19] Histone acetyl transferase, histone de-acetylases are the enzymes responsible for histone modification which in turn causes osteoblast differentiation influenced by the action of osteocalcin and osteocalcin promoters.[34]

Conclusion:

The concept of bioengineered PDL generated by stem cell has the potential to restore missing natural teeth using oral implants. This review provides an overview regarding different aspect of bio-hybrid implants. However, several questions like strength and longevity of the PDL attachment, functional efficiency of connective tissue is still not answered. Further human trials and long term clinical trials are needed to determine the scope of bio-hybrid implants.

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Figures

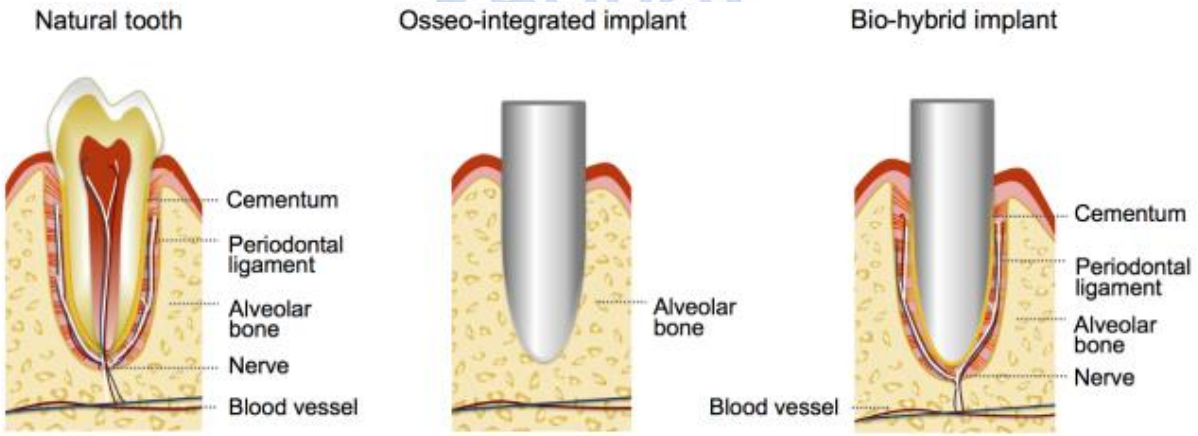

Figure 1

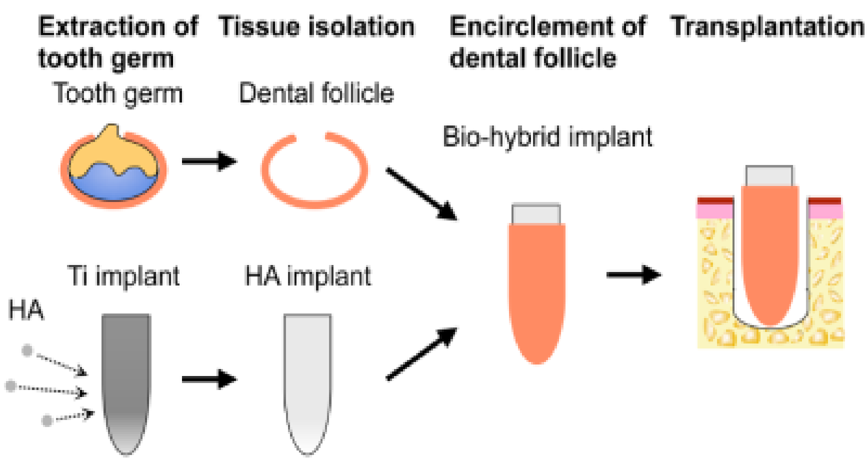

Figure 2

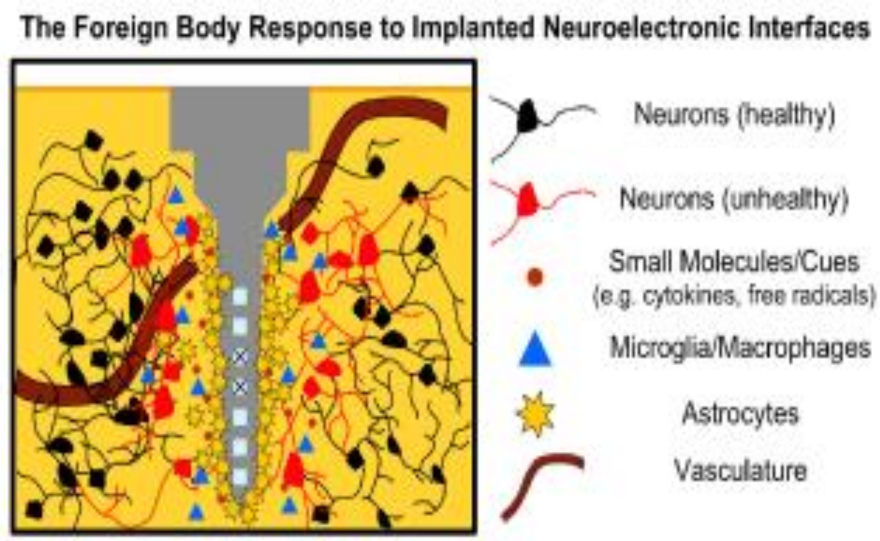
The Foreign Body Response to Implanted Neuroelectronic Interfaces
Neurons (healthy)
Neurons (unhealthy)
Small Molecules/Cues
(e.g. cytokines, free radicals)
Microglia/Macrophages
Astrocytes
Vasculature

Figure 3