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Ligaplants - A new ray of hope uncovering the potential of Tissue Engineering

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Abstract

In this modern era, though the fields of regenerative dentistry and tissue engineering have undergone significant advancements, yet its application to the field of implant-dentistry is lacking; in the sense that presently the implants are being placed with the aim of attaining osseointegration without giving consideration to the regeneration of periodontium around the implant. Problems exist with these implants as they lack periodontal ligament. Any inflammation around these implants may cause bone loss than does the inflammation around the natural tooth with periodontal ligament. This can be solved if implants with PDL (periodontal ligament) are developed and can be achieved by Ligaplants which are nothing but a combination of PDL cells with implant biomaterial. Hence, this review article aimed to discuss the benefits of PDL integrated implants over osseointegrated implants. A tissue-engineered periodontal ligament (PDL) around implants would represent an important new therapeutic tool to replace lost teeth. The PDL is the key to tooth anchoring; it connects tooth root and alveolar bone, and it sustains bone formation. Cells were isolated from PDL and cultured in a Bioreactor on titanium pins. Thus,implants with PDL may be installed in the extraction socket of the missing tooth, thereby facilitating the surgical procedure.

Keywords: Implant, Osseointegration, Periodontium, Tissue Engineering, Ligaplant.

Introduction

Dental Implant has become an indispensable part of mainstream Dentistry in the present era of Dental practice, helping Dental surgeons all over to improve the quality of life of the large population of patients. Professor Branemark's work in developing the osseointegrated dental implants constituted the dawn of an era of the "Evidence-Based Dentistry."[1]

Albrektsson et al. [2] later in his study defined osseointegration as the direct contact between living bone and implant at the light microscopic level. This means that the implants are functionally ankylosed to the bone without periodontal ligament support. The survival rate of dental implants is reported to be in excess of 90%. However, failures do occur because any inflammation around them may cause more serious bone loss than do inflammation around natural teeth with PDL. Localized bone loss around osseointegrated implants represents a great clinical challenge.[3].

Many strategies have been experimented to improve the osseointegrative property of the implant for example surface modification to improve the physical, mechanical and chemical characteristics of the implant, modification of shape and design of implant, alteration of surface topography, nanostructured surface coatings or addition of growth factors to implant surface. To overcome these problems, recent scientific research developed an implant with PDL, achieved by combination of the PDL cells with implant biomaterial and named it as LIGAPLANTS. There is very less literature available on Ligaplants.[4] Keeping this in mind we reviewed the properties, procedure of obtaining ligaplants, advantages and disadvantages of the same.

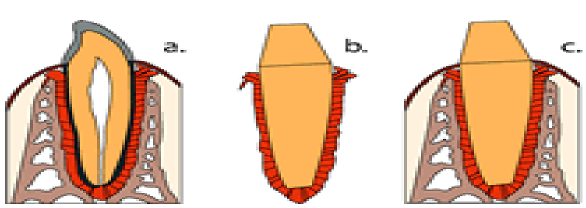

Figure 1: a) Histology of a natural tooth (Periodontal Ligament in red, bone in purple)

b)Ligaplant (biomaterial+livingtissue)

c) Ligaplant re-implanted (The connection to the bone is similar to the one of a natural tooth)

Author and year	Material and Method	Animal/ human study	Conclusion
Gault et al.(2010),[5]	Cells isolated from PDL and cultured in a bioreactor on titanium pins and then implanted in enlarged dental alveolae in dogs and humans	Human and animal study	Ligament-anchored implants,have potential advantages over osseointegrated implants
Rinaldiand Arana Chavez(2010)[6]	Titanium mini-implant placed between the buccal roots of the mandibular first molar of 24 adult rats. Ultrastructural analysis done after 21, 30, 45, 60, 90, and	Animal study	Titanium surface through its well-known biocompatibility exerts an effect on the periodontal ligament to lay down a cementum-like layer on the implant surface

	120 days of implantation		
Lin et al.(2011)[7]	Test site: PDL derived autologous DPCs seeded implants placed in the molar region of the rat model.Control site: Noncellseeded implants placed in the molar region of the rat	Animal study	Suggested the potential to replace missing teeth in humans with dental implants augmented with autologous cell-derived bioengineered periodontal tissues
Kano et al.(2012)[8]	HA–/OCL–, HA+/OCL–, and HA+/OCL+ immediately implanted into extracted tooth sockets with gap remaining PDL of rat molar model and the regeneration of PDL examined histomorphometrically and histologically	Animal study	The remaining PDL tissue around extracted sockets has the ability to regenerate bone and PDL-like tissues gap HA-coated tooth-shaped implants. Occlusal loads to the HA-coated implants may induce regeneration of PD gap Llike tissue in the peri-implant.

Ligaplants: Implants with Periodontal ligament are placed in the extraction socket of the missing tooth, thereby facilitating the surgical procedure. Natural implant anchoring might also be compatible with further growth and development of the alveolar bone housing, and it may allow tooth movements during orthodontic therapy. Ligaplants have the capacity to induce the formation of the new bone, when placed in sites associated with large periodontal defects.[9, 10]

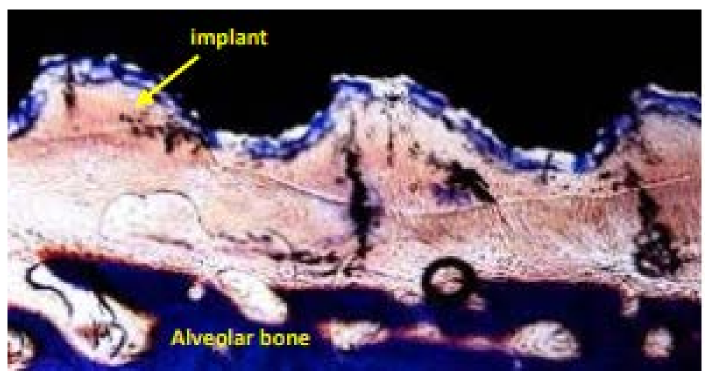

Fig. 2: Ligaplants- Alveolar bone housing implant

Properties of Ligaplants

1. It acts like a shock absorber, also gives the tooth some movement in the socket.
2. It also provides proprioception.
3. The PDL also has an important interaction with the adjacent bone, playing the role of the periosteum, at the bone side facing the root.
4. It is also a home to vital cells such as osteoblasts, osteoclasts, fibroblasts, cementoblasts, and most

importantly undifferentiated mesenchymal stem cells which are osteoconductive in nature.

5. These cells are all important in the dynamic relationship between the tooth and the bone.[11]

Procedure of obtaining Ligaplants: Transplantation of tooth with double PDL stimulation is one of the best examples of its healing capacity. The donor tooth is extracted and immediately replanted in its original alveolus, 14 days before transplantation. Cell proliferation and differentiation is seen as this Deliberate trauma triggers a healing process within the PDL. The transplantation of the tooth can be performed with millions of cells full activity attached to its root by new Sharpey's fibres after 14 days, when the cell culture reaches its peak of activity.[12]

A similar cell culture around an artificial root using Tissue Engineering techniques are now used. To obtain ligaplants there are 3 steps:

1) **Temperature responsive culture dishes preparation:** On-to polystyrene culture dishes, N-isopropylacylamide monomer in 2-propanaol solution is spread. Then these dishes are subjected to electron beam irradiation with an Area Beam Electron Processing System.

The dishes are then rinsed with cold water to remove ungrafted monomer and then sterilized with ethylene oxide.1,3 2) [12,10]

2) **Cell culture and cells:** From an extracted tooth human periodontal ligament cells are isolated. From the middle third of the root, periodontal tissue is scraped with a scalpel blade after extraction.

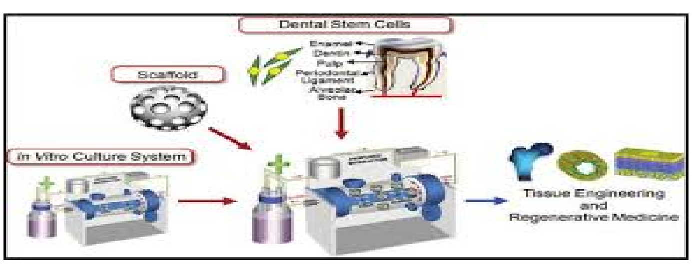

Fig. 3: Cell culture and cells.

The harvested tissue is placed into culture dishes containing Dulbecco's modified Eagle's minimal essential medium, supplemented with 10% fetal bovine serum and 100units/mL of penicillin-streptomycin. Then,in a humidified atmosphere of 5% CO_2 at 37°C for 48 hours those outgrowth cells are cultured to allow attachment of the cells to the dishes. The debris is eliminated by washing the dishes and the medium has to be changed three times per week.

Human periodontal ligament cells are placed on temperature-responsive culture dishes (35 mm in diameter) at a cell density of $1x10^5$ and cultured at 37°C supplemented with 50mg/mL ascorbic acid 2-phosphate,10nM dexamethasone and 10nM ßglycerophosphate that function as an osteodifferentiation medium to harvest the cell sheet.[12,10]

3) PDL cells culturing in a bioreactor: A hydroxyappatite (HAP) coated titanium pin, is placed in a hollow plastic cylinder leaving a gap of 3mm around the pin. Through the gap culture medium is continuously pumped. Single cells suspension, obtained from human, is seeded first into plastic vessels under a flow of growth medium for 18 days. [12,10]

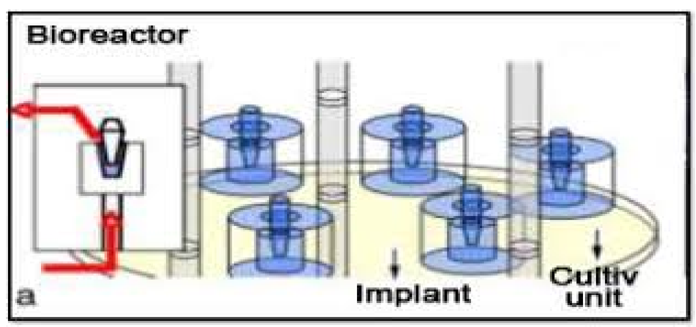

Fig. 4: Bioreactor

Osseo integration versus Periodontally Integrated Integration

PDL permits micro movements and acts as a shock absorber which causes qualitative difference in force distribution between implant supported prostheses and natural teeth abutments.

In osseointegrated implants, no fibrous capsule was found. The interfacial layer at the titanium- bone interface is rich in noncollagenous proteins as well as certain plasma proteins. The plasticity and biological remodeling possessed by the natural tooth is lacking in osseointegrated implants as they exhibit a rigid bone-implant interface, and this is responsible for decreased amount of mobility under functional loading and the transfer of excessive stresses to the surrounding bone that results in marginal bone resorption.[13]

On the other hand, PDL integrated dental implants help in formation of new cementum on the implant surface along with complete development of periodontal attachment that includes Sharpey's fibers and PDL fibers. This allows for bone remodeling and permits curative orthodontic movements of malpositioned dental implants.[13]

Osseo integrated Implants versus Periodontally Integrated Implants (Ligaplants)

Osteintegrated Implants	Ligaplants
Localized bone loss- Excessive stress that accumulate at the crestal region of the implants leads to bone loss at this region.[14]	Ligaplants dissipates these forces. [15]
Diminished ability of dental implants to adapt to occusal trauma can be attributed to this lack of periodontal proprioceptive mechanism.[16]	Sensitive proprioceptive mechanism and is therefore capable of detecting and responding to a wide range of forces applied to the teeth.
Connecting teeth to osseointegrated implants presents a biomechanical challenge due to the differential support and mobility provided by the implant and the tooth.[17]	When tooth- implant supported restorations would be fabricated using support from periodontio integrated implants higher success rates can be expected due to similar resilience of tissues supporting teeth and implants.
Contraindicated in growing patients.	Successfull placement implants in patients undergoing craniofacial/skeletal growth process.
Behave as an ankylosed element.[18]	Move them orthodontically.
The tissues around implants are more susceptible to plaque-associated infections that spread into the alveolar bone, primarily due to the lack of a periodontal ligament, making them more prone to bone loss.[19]	Provide better defensive capacity also enhance repair and regeneration of bone defects in their vicinity.

Models for cell based engineering of tooth and implant supporting tissue constructs.

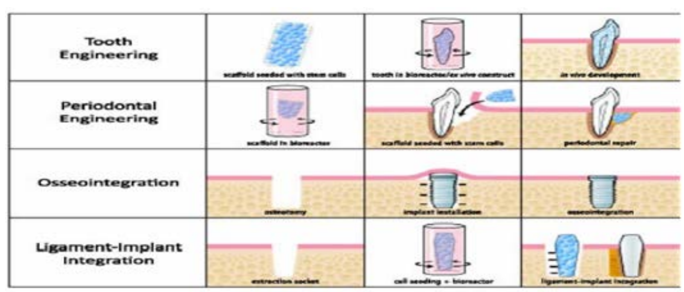

Fig. 3: Stem cell-based therapies in the bioengineering of teeth, periodontium and alveolar bone structures. In the situation of the formation of a tooth–implant interface,

periodontal ligament stem cells offer the potential to form tooth–ligament–bone interfacial complexes.

Precaution When Preparing Ligaplants

A cushion of sufficient thickness favours the formation of PDL and on the other, the prolonged cell culturing may favour the appearance of non-PDL cell types, In order to favour the appearance of non- PDL cell types. In order to preserve the cell differentiate state and to obtain adequate cell stimulation, the bioreactor has been constructed with the aim to resemble the PDL situation during cell growth and surrounding hollow cylinder. It is thereby anticipated that the PDL phenotype would be favoured implicating a tight attachment of cells to the implant. So the preparation of the ligaplants should have minute mechanical movements of the medium flow and space between the implants and the culture treatment should be optimal to obtain the successful ligaplants which brings big improvements to the implant system.[20]

Advantages of Ligaplants

1. It alleviate problems like gingival recession and bone defects of missing tooth.
2. Mimics natural insertion of natural tooth roots in alveolar process.
3. Ligaplants become firmly integrated without interlocking and without direct Bone contact, despite the initial fitting being loose in order to spare PDL cell cushion.
4. Bone formation is induced and movements of ligaplants inside the bone Suggesting suggesting an intact communication between bone and implant surface.[21,22]

Disadvantages of Ligaplants

1. If proper caution (temperature, cells used for culturing, duration etc..) is not taken while culturing of ligaplants, it may develop non periodontal cells which may lead to failure of ligaplant.
2. Cost is high because of limited facilities and labour. Host acceptance is unpredictable which may result in failure of implant.
3. The factors affecting the host to accept the implant or the growth of PDL in the socket is unpredictable, which may result in failure of implant.[23]

Clinical importance of ligaplants

For reconstruction and regeneration, the important elements required are as follows:

- Matrix or a scaffold
- Signaling molecules
- Cells.

Tissues prepared in laboratory are cultivated with *In vitro* technique. The cells are cultured on the biodegradable scaffolds or matrix with the help of signaling molecules, following which they are transplanted into the body. Whereas, when all the cultivated vital elements are placed in a tissue defect and undergoes a natural healing process in the body giving rise to regeneration, it is called as *in vivo* technique.

It induces intrinsic healing activity at the site of tissue defect using the three elements. This can be done by both *in vitro* and *in* vivo[24]

Evidence Based Studies on Ligaplants

Nyman et al.1982 suggested that the cells of the periodontal ligament possess the ability to reestablish connective tissue attachment. Nunez et al (2012)further validated the regenerative potential of periodontal ligament derived cells in a proof of principle study. Several in vivo experiments have demonstrated the formation of cementum –like tissue with an intervening periodontal ligament, when the dental implants were placed in proximity to tooth roots. Mechanism of this phenomenon appeared to be due to migration of cementoblast and PDL fibroblast precursor cells towards dental implants due to contact or proximity of the tooth related cell populations to those implants.

Risk Factors of Ligaplants

The development of PDL for the generation of PDL depends majorly on site signaling, which is largely mediated by anatomic code and homeogene-coded transcription factors. These homeoproteins are quintessential for the synthesis of cell surface and signaling components. The factors affecting the growth of PDL in the desired site are often unpredictable, and hence, it becomes a major risk factor for the treatment results to be obtained.[25]

Success of Ligaplant

The development of a regenerative PDL depends on site-specific signaling, which in turn is mediated by an anatomic code, written in expression patterns of Homeogene-coded transcription factors. Hence, the Homeoproteins influence the synthesis of cell surface and signaling components, and signals from the cell surface feedback to modulate Homeogene expression, whereby cell identities are established according to the anatomic site and tissue type Homeogene Msx2 has in fact been implicated in the singregation of mineralized bone versus non-mineralized PDL[26]. For the inhibition of mineral formation of PDL, a role of asporin (an SLRP protein that is present in the extracellular matrix) has been introduced.[27, 28]

Conclusion

Although it has been revealed that generating a periodontal-like tissue around implants is possible, still a predictable and feasible method for producing dental implants with periodontal-like ligament has not been innovated. A major concern being the rational application of stem cell based tissue-engineering technology in clinical practice. Besides, the costs and time required from a practical standpoint for such tissue engineering applications is significant. Yet, this revolutionary approach to develop periodontio-integrated implants; however, opens up exciting possibilities for both Periodontologists and Oral implantologists and offers many interesting possibilities of utilizing ready-made, off-the-shelf biological tooth replacements that could be delivered to serve as hybrid-material-living oral implants.[30]

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