INTRODUCTION

Dental implant is an artificial tooth root fixed into the jaws to hold a replacement tooth or bridge (Alghamdi et al., 2013). Titanium is widely used for dental implants because of its biocompatibility, mechanical strength and plasticity for prosthetic design. When titanium is implanted into live bone tissue, it actually integrates with the bone (Naitoh et al., 2010). Bone healing around implants involves the activation of a sequence of osteogenetic, vascular and immunological events that are similar to those occurring during bone healing (Dimitriou et al., 2007). Osseointegration refers to the growth of bone as it incorporates surgically implanted materials (Bougas et al., 2012). In order to enhance bone formation, implants have been coated with bone specific biomolecules (Geng-Sheng et al., 2007). Many kinds of bioactive materials used to coat the surfaces of dental implants (Oida et al., 2002).

Amelogenins is the major organic component in the enamel matrix of developing teeth and plays an important role in enamel biomineralization (Haze et al., 2007). Amelogenins are hydrophobic enamel proteins secreted by ectodermal cells – ameloblasts – during enamel formation. Osteoblasts, odontoblasts and bone marrow stromal cells also express the amelogenin gene (Du et al., 2005a). Amelogenins self-assemble to form a non-soluble protein scaffold in the form of nanospheres which are thought to play a central role in controlling crystal growth and tissue architecture during enamel formation (Du et al., 2005b).

Type I collagen (COL1) is the major organic component of the mineralized bone matrix. By immunohistochemical staining they could detected its expression in bone matrix (Oida et al., 2002). Type I collagen fibers are the most abundant organic constituent and they may be involved in aligning the mineral crystals (Sun et al., 2012).

MATERIAL & METHODS

Materials

1. 80 screw shaped implants, 3.5mm in diameter & a total length of 8mm (threaded part is 5mm & smooth part is 3mm)
2. Amelx (amelogenin) protein (His tag) (ab139212) Abcam UK
3. Anti-collagen-I antibody(ab90395) Abcam UK
4. Detection Kits System (ab 94740) Abcam UK
5. Protein Block 15 Enhancer
6. Naphthol Phosphate
7. Fast Red Chromogen
8. AP-Conjugate
9. Co-factor Enhancer

Methods: Forty healthy male New Zealand rabbits, weighing(2-2.5kg), aged 10-12 weeks were kept in the animal department of (National Center of Drug Control and Research /Iraq)at a constant humidity and temperature of 23°C according to the National Council’s guide for the care of laboratory animals. Eighty machined surface Iraqi implants from commercially pure titanium rod were inserted in...
right tibia of these rabbits. Two Titanium implants were placed in the tibia of each rabbit. The animals were scarified at 1, 2, 4 and 6 weeks after implantation (10 rabbits for each interval). Animals were generally anaesthetized and atraumatic surgical technique was performed to prepare two holes in the tibia, amelogenin coated implant was inserted in one hole and uncoated implant (control) placed in the second one.

All tissue specimens, experimental and controls were fixed in 10% neutral formalin and processed in a routine paraffin blocks after complete decalcification of bone. Each paraffin-embedded specimen had serial sections were prepared as follows: 4 μm thickness sections were mounted on clean glass slides for routine H&E staining procedure from each block of all studied sample. Other 4 sections of 4 μm thickness were mounted on positively charged microscopic slides for immunohistochemical localization of collagen I. The procedure of the IHC assay was carried out in accordance with the manufacturer instructions of Anti-collagen-I antibody (ab90395) Abcam UK and Detection Kits System (ab 94740) Abcam UK.

RESULTS AND DISCUSSION

Successful attachment on artificial surface is prerequisite for inducing new bone formation locally at the site of implantation. Protein-coated surfaces may influence the biocompatibility of implant materials by initiating and supporting osteogenesis (Ogata, 2008).

Collagen (COLL) comprises approximately 90% to 95% of the organic component of bone (Sodek and Cheifitz, 2000). Osteoblasts are known to synthesize and secrete type I collagen and it is a marker of preosteoblasts (Ogata, 2008).

The immunohistochemical staining with coll I monoclonal antibody of 1 week amelogenin-coated implants showed strong positive expression in the osteoblasts, progenitor cells, and osteocytes and in extracellular matrix (figure 1). While the uncoated implant showed weak positive expression in progenitor cells and extracellular matrix (figure 2). This expression then decrease with time for coated groups and increase for the uncoated group, where the coll I expression was negative in osteoblasts, osteocytes and extracellular matrix of 2 weeks amelogenin-coated implant (figure 3). The uncoated implant showed that coll I expression was moderate positive expression in the osteoblasts, osteocytes, progenitor cells and extracellular matrix in the same interval (figure 4).

Furthermore, the localization of The COLL1 was negative in osteoblasts and osteocytes progenitor cell and extracellular matrix in A-coated implant of 4 and 6 weeks postoperatively (figures 5 and 6). The COLL1 localization was moderate positive expression in 4 weeks interval and strongly positive expression in 6 weeks interval in osteoblasts, progenitor cell, osteocytes and extracellular matrix (figure 7 and 8).

These result supported by Du etal, in 2005a, who investigate the gene expression of a differentiation marker of osteoblasts in BMSCs in the presence of amelogenin by use quantitative PCR. The commitment of BMSCs to osteogenic differentiation was demonstrated by the expression of type I collagen which considered as lineage-specific markers of osteoblastic differentiation and it significantly different from control values after 8, 12 and 16 days.

In vitro studies demonstrated that mRNA of collagen type I is expressed during the initial period of proliferation and extracellular-matrix biosynthesis, since it is hypothesized that enhanced expression of osteogenic markers in vitro leads to more and more expeditious bone formation at the bone-
biomaterial interface in vivo (Muller-Mai, 2000, Vogel et al., 2001 and Wilmowsky et al., 2002).

The osteocytes express COLL1 strongly in 1 week interval for experimental groups and in the 6 weeks interval for uncoated group. This agree with Katagiri et al., 2002 and Bonevald, 2007, who said, once differentiated, osteoblasts produce several proteins, such as type I collagen (COL1), osteocalcin (OC), and alkaline phosphatase (ALP), which will generate newly formed bone, and then undergo differentiation under an osteocyte phenotype therefore, COLL1 is a bone marker associated with the differentiation of osteocyte.

Finally the biomimetic coating with amelogenin protein promoting cell adhesion and osteoblast differentiation may have great potential for future dental and biomedical applications.

References

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