Chapter 1

Titanium Alloys in Orthopaedics

Wilson Wang and Chye Khoon Poh

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/55353

1. Introduction

Metallic implants are commonly used in the orthopedic field. Despite the large number of metallic medical devices in use today, they are predominantly make up of only a few metals. Metallic alloys such as titanium continue to be one of the most important components used in orthopaedic implant devices due to favorable properties of high strength, rigidity, fracture toughness and their reliable mechanical performance as replacement for hard tissues. Orthopaedic implants are medical devices used for the treatment of musculoskeletal diseases and may consist of a single type of biomaterial or comprise a number of different biomaterials working together in modular parts. Prime examples of titanium implants used in orthopaedics would include prosthetic hip and knee replacements for various types of arthritis affecting these joints, spinal fusion instruments for stabilizing degenerate and unstable vertebral segments, and fracture fixation devices of various types such as plates, screws and intramedullary rods. Although titanium based implants are typically expected to last ten years or more, however longevity is not assured and the lack of integration into the bone for long-term survival often occurs and leads to implant failure. Revision surgery to address such failure involves increased risk, complications and costs. The main reason for the failure of these implants is aseptic loosening which accounts for 60 to 70% of the cases for revision surgery. The success of implants is dependent on firm bonding or fixation of implant biomaterial to bone, for optimal function and lastingness. Therefore one of the key challenges in bone healing and regeneration is the engineering of an implant that incorporates osseointegration with enhanced bioactivity and improved implant-host interactions so as to reduce biological related implant failure.

1.1. Development of titanium alloys for use in orthopaedics

Titanium alloys, originally used for aeronautics, garnered attention from the biomedical field, due to their biocompatibility, low modulus of elasticity, and good corrosion resistance.

Nonetheless, it was the osseointegration phenomenon due to the presence of a naturally formed oxide layer on the titanium surface that sparked development of titanium for use in orthopaedics [1]. Titanium alloys are often used in non-weight-bearing surface components such as femoral necks and stems (Figure 1), as they have lower modulus of elasticity resulting in less stress shielding of bone [2]. Nonetheless the osseointegrative bioactivity is still often not sufficient to attain true adhesion between the implant and bone, which may ultimately lead to mechanical instability and implant failure [3].

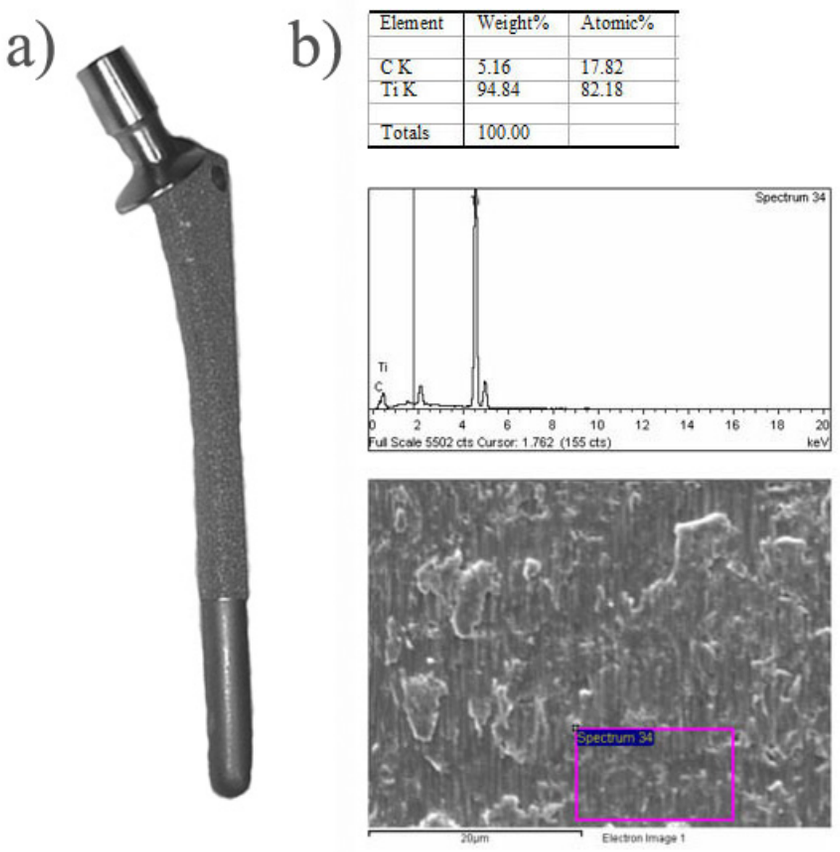

Figure 1. a) Titanium stem and (b) surface elemental analysis.

The mechanical properties of suitable titanium alloys based on Young's moduli should be similar to that of cortical bone. Cortical bone also termed compact bone is the major and most important constituent of the human skeleton and is crucial for bone functions including organ protection, movement, support etc. Young's moduli of β-type titanium alloys are substantially smaller than those of the α- and (α + β)-type alloys. This has brought on the discovery of harmless low-rigidity Ti alloys such as Ti-13Nb-13Zr, Ti-12Mo-6Zr-2Fe, Ti-15Mo-5Zr-3Al, Ti-15Mo, Ti-35Nb-7Zr-5Ta and Ti-29Nb-13Ta-4.6Zr. Nonetheless there are both advantages and disadvantages of the application of these titanium alloys. These alloys have proved to be effective in preventing bone atropy and enhancing bone remodeling, however the high amount of spring back and low fatigue strength make them undesirable as implant material. Ti-6Al-4V

and commercial purity Ti are currently the most popular materials for implantation purposes. Commercial purity Ti has been tested to be inferior considering tensile strength, while Al and V have been shown to be unsafe. Currently researchers are still trying to develop other grades of alloys, such as Ti-6Al-7Nb and Ti–15Sn–4Nb–2Ta–0.2Pd. The most Ti alloys researched upon are the (α + β)-type alloys for their strength and ductility.

1.2. Bioactivity of titanium alloys

Each manufacturer of titanium implants has his own differing theories on implant designs for specific orthopaedic applications. Generally there are certain guiding principles that will affect the ultimate viability of an implant. The design of the implant has to take into account biomechanical and biological factors that may affect its success. Conformity to native anatomy, material properties and mechanical strength appropriate for the targeted function and environment are some of the considerations that come into play. Despite the benefits and successes of these medical devices, their use is not without risk of adverse effects. Titanium implants generally develop an oxide layer which allows it to integrate with living bone tissue. However, the body can have adverse reactions to titanium like fibrosis and inflammation which may affect its long term functional performance [4]. Success in the application of an orthopaedic implant would depend on various factors and implants may fail due to physiologic reasons such as aseptic loosening.

Generally there are two types of implant-tissue responses [5-7]. The first type is the response of the hosts' tissues to the toxicity of the implanted material. Implanted material may be toxic or release chemicals that could damage the surrounding tissues. The second response which is also the most common is the formation of a nonadherent fibrous capsule between the implant and the hosts' tissues termed fibrosis. This is a natural response to protect the body from a foreign object which may eventually lead to complete fibrous encapsulation [8]. Typically implants are intended to stay fixed in the human body for a long time and bone is expected to grow into the surface of the implant. Unfortunately this does not always happen. Fibrosis referred to as foreign body reaction, develops in response to almost all implanted biomaterials and consists of overlapping phases similar to those in wound healing and tissue repair processes [9]. Despite the biocompatibility of metallic implants used, titanium materials are generally encapsulated by fibrous tissue after implantation into the living body [10]. Cells trapped between the implant and the fibrous capsule also lack general housekeeping tissue functions like removing apoptotic or necrotic cells which can also promote chronic inflammation [11]. Not only that the ECM (extra cellular matrix) secreted by fibroblast is different from the bone matrix formation generated by osteoblast, in the long run this ECM layer may lead to micromotion and the generation of wear particles on the surfaces of the implant [12]. The resulting titanium debris may play a leading role in the initiation of the inflammatory cascade leading to osteolysis [13]. Eventually this causes aseptic loosening as the bonds of the implant to the bone are destroyed by the body's attempts to digest the wear particles. When this occurs the prosthesis becomes loose and the patient may experience instability and pain. Revision surgery to resolve this would entail further costs and morbidities to the patients. For bone tissue, direct osteoblast attachment on metal is important to prevent aseptic loosening of the

metal implant caused by fibroblast layer attachment. Fibrosis can also cause osteoclast-independent bone resorption by fibroblast-like cells. It has been shown that fibroblast-like cells, under pathological conditions, not only enhance but also actively contribute to bone resorption [14]. Successful implant integration into the surrounding tissue is highly dependent on the crucial role of native cells, chiefly osteoblast attaching to the implant surface. Therefore one of the key challenges in orthopaedics is the engineering of an implant with enhanced osseointegration properties to reduce implant failure rates.

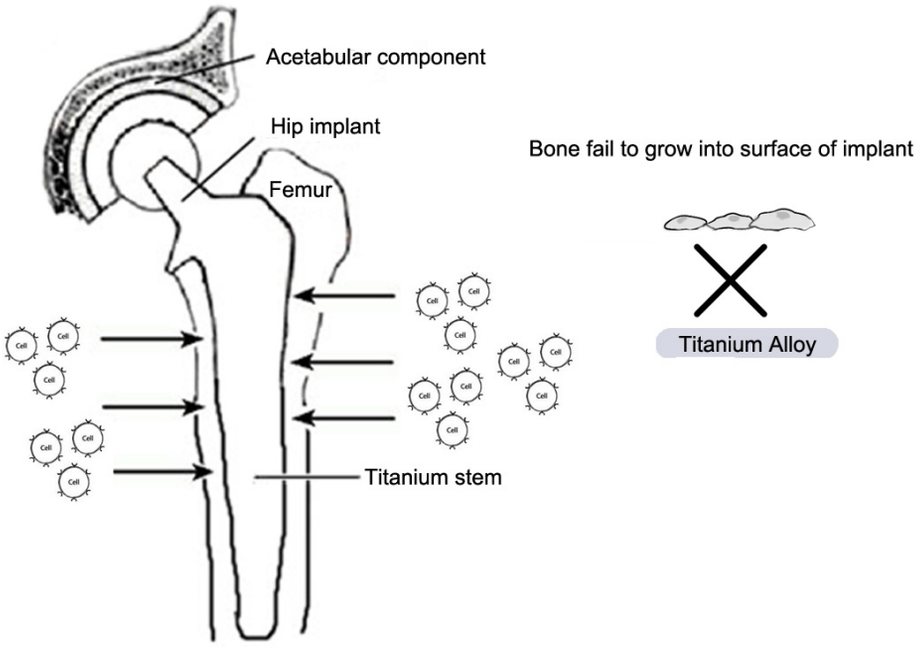

Figure 2. Schematic figure of a hip implant. The femoral neck is the region at risk of compromised vascularity. Arrows indicate area of compromised vascularity where osseiointegration fails to take place.

2. Strategies for conferring enhanced bioactivity to titanium alloys

So far most research efforts have been concentrated on improving the bone-implant interface, with the aim of enhancing bone healing and implant integration via either physical or chemical approaches [15]. The physical approach is focused on the modification of the implant surface morphology and topography using mechanical methods such as machining, acid-etching, plasma spraying, grit-blasting and anodization to improve the microtopography of the surface. The rationale behind this is that an increase in surface roughness of the implant material would provide a higher level of surface energy which would improve bone anchorage, matrix protein adsorption, osteoblasts functions and ultimately osseointegration [16].

The chemical approach is towards the creation of a bioactive implant surface via application of coatings onto the implant layer by biochemical and physicochemical techniques. In bio-

chemical techniques, organic molecules such as growth factors, peptides or enzymes are incorporated to the implant layer to affect specific cellular responses [17]. While in physico-chemical techniques, the incorporation is achieved with inorganic phases such as calcium phosphate which may increase the biochemical interlocking between bone matrix proteins and surface materials thereby enhancing bone-bonding [16]. Many implant modifications may combine both physical and chemical engineering methods. In the following sections we will discuss some of the more popular strategies used to enhance implant integration and bone-bonding.

2.1. Inorganic coatings

Calcium phosphate coating has been widely used in the orthopaedic field due to their similarity with the mineral phase of bone [18] and are known for their bioactive properties which are beneficial in bone-bonding [19]. As calcium phosphate generally lacks the mechanical strength for use as bulk materials under loading conditions, they are often coated onto the surface of metallic implants. There are several studies published which have shown the favorable use of calcium phosphate coatings in increasing the biocompatibility of bone-implant interface, implant anchorage and integration [20]. The calcium phosphate layer functions as a physiological transition between the implant surface and the hosts' tissues which guides bone formation along the implant surface and the surrounding tissues. One of the most successful method for the application of calcium phosphate coatings is via the plasma-spraying method due to its advantage of extensive coating capability and high deposition rate. However despite numerous findings [21] that report the beneficial osteoinductive properties of plasma-sprayed calcium phosphate coatings, there are still some concerns regarding its use. Plasma-sprayed coatings are not uniform and there is poor control over thickness and surface topography, which may result in implant inflammation when particles are released from these heterogeneous coatings. To overcome these drawbacks, various other deposition strategies have been developed and employed such as biomimetic, electrophoretic and electrospray deposition etc. However care should be taken when comparing the efficacy of each of these methods which would require a comprehensive evaluation of both biological response and clinical performance. Although calcium phosphate coatings have been shown to be beneficial in enhancing bone-bonding, there is still no general consensus on the use of calcium phosphate coating systems. The main problems include large variation in the quality of calcium phosphate coatings, even between different batches and market forces which offer other cheaper alternatives [22].

2.2. Organic coatings

Surface modification of implant materials with growth factors and peptides is gaining popularity in the recent years [23, 24]. Various therapeutic biomolecules of interest can be immobilized onto implant surfaces to enhance the bone-implant interface interactions. Currently more popular approaches would include the immobilization of bone growth factors such as bone morphogenetic proteins (BMPs) to enhance osteogenesis and the deposition of peptide sequences to induce specific cellular functions. Growth factors

immobilized on orthopaedic devices have been reported to enhance osteoblastic activity and favor implant integration [25]. The most commonly used growth factors in orthopaedics are members of the transforming growth factor beta (TGF-β) superfamily including the BMP family, especially BMP2 and BMP7. Growth factors may be physically adsorbed or covalently grafted onto the implant surface and various studies have shown that the loading of implant with these factors can enhance interactions at the bone-implant interface and aid the remodeling process ultimately improving implant integration [26-28]. However critical factors in the successful use of growth factors in orthopaedic devices are the optimum dosage, exposure period and release kinetics, all have to be considered carefully to avoid the detrimental effects associated with growth factor use such as high initial burst rate, ectopic bone formation and short half-life. More recently, peptide sequences with the ability to target specific osteogenic cellular functions of differentiation and mineralization have been developed [29, 30]. These short functional fragments derived from the original protein have increased shelf life, can be synthetically produced and are more resistant to denaturizing effects. Their usage would provide significant clinical benefits over the use of conventional proteins. They can be linked to the implant surface to provide biological cues for bone formation. Additionally other peptide sequences in use include the RGD, YIGSR, IKVAV and KRSR which have been used to improve cellular adhesion and bone matrix formation [31-33].

2.3. Organic–inorganic composite coatings

Research in the recent years have concentrated on the development of bioactive composite coatings which mimics the structure of the bone tissue. These composite coatings would combine calcium phosphate with growth factors, peptides, antibodies etc. to enhance interactions at the bone-implant interface. However due to the fact that often high temperature or non-physiological conditions are needed in the preparation of calcium phosphate coatings, only physical adsorption is employed in deposition of the biomolecules on the implant surface [34, 35]. However with physical adsorption techniques, initial high burst rate is often observed, which is not desired [36]. Therefore coating techniques that create a gentle sustained release kinetics are preferred. A recently published paper have shown that calcium phosphate coating combining slow release of antibiotics, aids in early success at recruitment of bone cells [37]. Many other studies have shown that depositing BMP2 and TGF-β onto the implant surface would greatly enhance bone-bonding at the bone-implant interface [25, 34]. The biological efficacy of orthopaedic implants can be improved greatly by both physical and chemical modifications. The use of a wide multitude of engineering techniques in the manipulation of surface topography, morphology and incorporating the use of various inorganic and organic components would directly influence the response in the local bone-implant interface and the apposition of new bone. With the development of new techniques and strategies on composite coatings to better mimic the human bone structure this would result in a new generation of orthopaedic implants with improved implant integration and bone healing.

3. Osseointegration of the implants

The clinical strategies to manage musculoskeletal defects would center around three components: cells, structure and growth factors. For the design of implant materials, cells and proteins at the implant interface plays a critical role [38]. The utilization of biosignal proteins such as growth factors for development of bioactive implant materials holds great potential. Especially due to the scarcity of stem cells in the body, materials which regulates cellular functions such as adhesion, growth and differentiation are desired.

One of the most important process in determining the success of an orthopaedic implant is osseointegration. Osseointegration is defined as the formation of a direct structural and functional connection between the living bone and the surface of a implant [39, 40]. An implant is considered osseointegrated if there is no progressive relative movement between the implant and the bone it has direct contact with [40]. Under ideal conditions, implants could permanently become incorporated within the bone and persist under all normal conditions of loading, that is the two could not be separated without fracture. Vascularization which is the provision of blood supply is a critical component for the process of osseointegration. The differentiation of osteogenic cells is highly dependent on tissue vascularity and ossification is closely linked to the vascularization of differentiating tissue [40]. Therefore the success of tissue healing, regeneration and integration lies in the key process of revascularization which is crucial in improving the successful integration of implants [41, 42].

Bone healing around implants involves a cascade of cellular and biological events that take place at the bone-implant interface until finally the entire surface of the implant is covered by newly formed bone. This cascade of biological events is regulated by differentiation of cells stimulated by growth factors secreted at the bone-implant interface [40]. There has been considerable interest in modifying implant surfaces with growth factors to improve their cell functions and tissue integration capacity at the bone-implant interface. Enhanced cell functions and cell substrate interactions have been demonstrated with growth factors immobilized onto implant materials [26-28]. One of the more important growth factors for stimulating neovascularization (i.e. formation of new blood vessels) in target areas [43] would be angiogenic growth factors, crucial in improving the successful integration of implants both *in vitro* and *in vivo* [41, 42]. Of these angiogenic factors, vascular endothelial growth factor (VEGF) is the most potent and widely used key regulator of neovascularization [43, 44]. VEGF is a crucial factor in not only angiogenesis regulation but also in osteoblast [45] and osteoclast function [46-48] during bone repair. VEGF acts directly on osteoblasts, promoting cell functions such as proliferation, migration and differentiation [49, 50]. In addition, VEGF also indirectly affect osteoblasts via its influences on endothelial cells [51, 52]. VEGF is known to induce endothelial cells in surrounding tissues to migrate, proliferate and form tubular structures [53] and is an essential survival factor for endothelial cells [51] and new vessel formation [54]. Endothelial cells are needed to provide complex interactive communication networks in bone for gap junction communication with osteoblasts crucial to their formation from osteoprogenitors [55]. Furthermore VEGF stimulates endothelial cells in the production of beneficial bone forming

factors acting on osteoblasts [50]. In all, the effects of VEGF on osteoblasts, osteoclasts and endothelial cells may synergistically act to enhance bone formation.

3.1. Fixation of titanium implants

The fixation of prosthetic components to the bone can be done with or without bone cement. In the cemented technique polymethylmethacrylate (PMMA) is used to "glue" the metal to the bone. In direct biological fixation, precise bone cuts are required to achieve maximum contact between metal and bone. The advantage of cement fixation is that the prosthetic components are instantly fixed, allowing movement immediately after surgery. However in the instances where revision surgery is required, it is extremely difficult to chip out all the cement during implant replacement. Cement fixation is usually employed on elderly patients over sixty-five where their bone stock is more osteoporotic with less likelihood of growing into the prosthesis and chances of revision is lower due to less demands on the implant and shorter remaining life expectancy compared to younger patients. Direct biological fixation is generally used for young patients due to better bone stock and ingrowth potential. The disadvantage of biological fixation is that it can take weeks or months to be fully complete during which weight bearing activity is restricted. However the final fixation achieved is more natural with complete incorporation of implant within the bone in ideal situations. Furthermore in case of young patients the chances for future revision surgery is higher and it would be easier to revise a cementless prosthesis without the need for cement removal. Another problem perceived was that cementless titanium stems have been reported to be more resistant to osteolysis and mechanical failure compared to similar cemented titanium stems [56]. The features of titanium that are detrimental to the cement environment seems to have no effects in the cementless environment and may in fact be beneficial leading to differences in performance of the two techniques. Therefore the enhancement of the bone implant interface especially in direct biological fixation with titanium implants would be extremely useful. This would greatly reduce the lag period in which osseointegration occurs between the prosthesis and the patient's bone.

3.2. Surface functionalization by growth factors immobilization

One promising way to incorporate growth factors usage with implant materials would be by surface functionalization of growth factors. Soluble growth factors work by binding with cognate receptors on cells to form complexes which would result in autophosphorylation of the cytoplasmic domains of the receptors and this phosphorylation activates intracellular signal transduction. The formed complexes are then aggregated and internalized into the cells by both clathrin-dependent and clathrin-independent mechanisms which leads to the recycling of the receptors for degradatory down-regulation [57]. Similarly immobilized growth factors work by forming complexes with the cell surface receptors, however the signal transduction is expected to last longer than soluble growth factors due to the inhibition of the internalization process. Multivalency is another important phenomenon responsible for this prolonged enhanced mitogenic effect. Multivalent ligands interact and bind avidly to multiple

surface cell receptors through several binding modes. This enhances the formation of ligand-receptor complexes which are critical for signal transduction and the multivalent ligands are able to stabilize and prevent lateral diffusion of the formed complexes leading to the prolonged effect. Figure 3 shows the interactions of cells with the different forms of growth factor and the enhanced mitogenic effects.

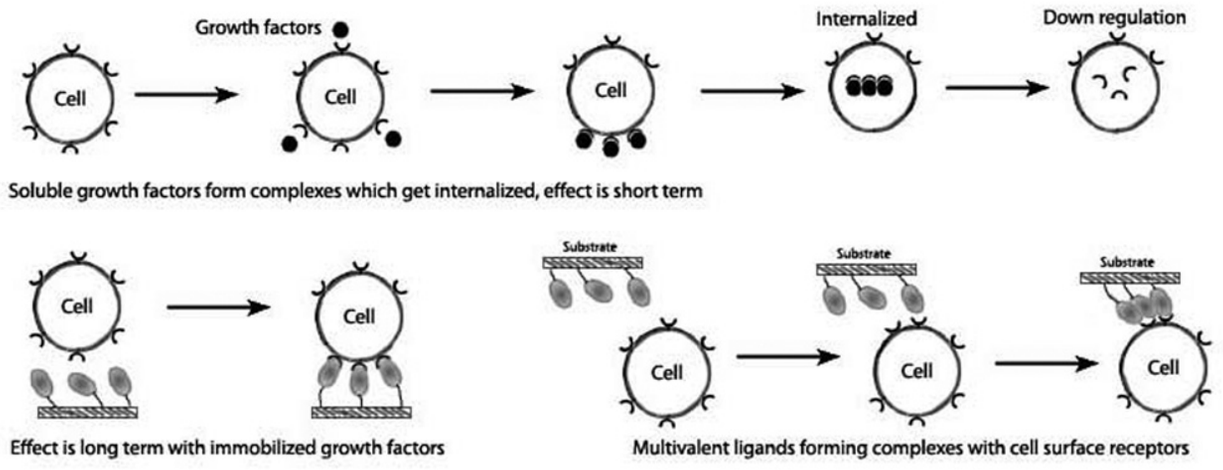

Figure 3. Effects of soluble growth factors compared to immobilized growth factors.

In order to effectively derive the effect from immobilized growth factors, strategies have to be developed that can optimize the structure to elicit the desired biological response. One of the problems encountered with implant materials for surface functionalizaton is the lack of suitable chemical groups on the surface. For more versatility and applicability, the concentrations of the OH group and other reactive groups such as amino or carboxyl groups have to be increased. The initial organic layer immobilized on the implant materials can then be used as a tether for biomolecular components used to mediate cell attachment. Another issue which merits investigation is the control of the retention and/or release of the biomolecules from the implant surface. The easiest and most common method employed for delivery of biomolecules is physical adsorption, which unfortunately provides little control over the delivery and orientation of the biomolecules. Bonding of the biomolecules and use of coatings incorporating them would be alternative methods of delivery to the bone-implant interface. Regardless, the preferred and chosen immobilization technique would depend on the specific working mechanism of the biomolecules. Given the above scenario, surface functionalization of biomaterials in order to enhance biocompatibility and promote osseointegration has great potential in addressing the problems of prosthetic joint implant longevity and survival.

Immobilization techniques are broadly classified into four categories, namely a) physical adsorption (via van der Waals or electrostatic interactions), b) physical entrapment (use of barrier systems), c) cross-linking and d) covalent binding. The choice of the technique would depend on the nature of the bioactive factors, substrates and its application. It will not be possible to have a universal means of immobilization, however developing a viable method-

ology which can provide for a facile, secure immobilization with good interactions for orthopaedic implants is vital.

3.2.1. Physical adsorption

This is the simplest of all the techniques available and does not alter the activity of the bioactive factors. Physical adsorption techniques are mainly based on ionic and hydrophobic interactions. If the bioactive factors are immobilized via ionic interactions, adsorption and desorption of the factors will depend on the basicity of the ion exchanger. A reversible dynamic equilibrium is achieved between the adsorbed factors and substrates which is affected by the pH as well as ionic strength of the surrounding medium. Hydrophobic interactions offer slightly higher stability with less loss of the factors from the surface of the substrates. Although physical adsorption systems are simple to perform and do not require extensive treatment to the bioactive factors and substrates used however there are certain drawbacks. These systems suffer from low surface loading and biomolecules may desorbed from the surface in an uncontrolled manner.

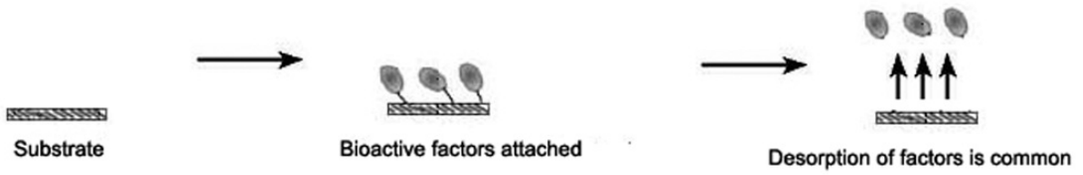

Figure 4. Schematic diagram showing physical adsorption system with proteins.

3.2.2. Physical entrapment

This method is employed with barriers including natural polymers like gelatin, agar and alginate entrapment systems. Other synthetic polymers employed include resins, polyurethane prepolymers etc. Some of the major limitations of the entrapment system is the diffusional problem where there is possible slow leakage during continuous use due to the small molecular size of bioactive factors, and steric hindrance which may affect the reactivity of the factors. Recent development of hydrogels and water soluble polymers attempt to overcome these drawbacks and have attracted much attention from the biomedical field.

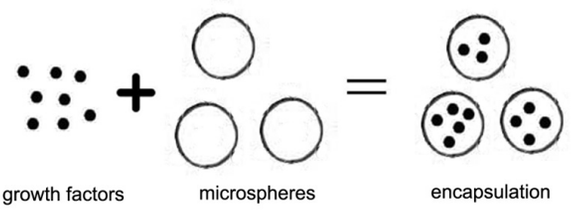

Figure 5. Schematic diagram showing barrier system with proteins.

http://dx.doi.org/10.5772/55353

3.2.3. Cross-linking

Bioactive factors can also be immobilized through chemical cross-linking via homo- as well as heterobifunctional cross-linking agents. Among these glutaraldehyde cross-linking are the most popular due to its low cost, high efficiency and stability [58-60]. Glutaraldehyde is often used as an amine reactive homobifunctional crosslinker for biochemistry applications.

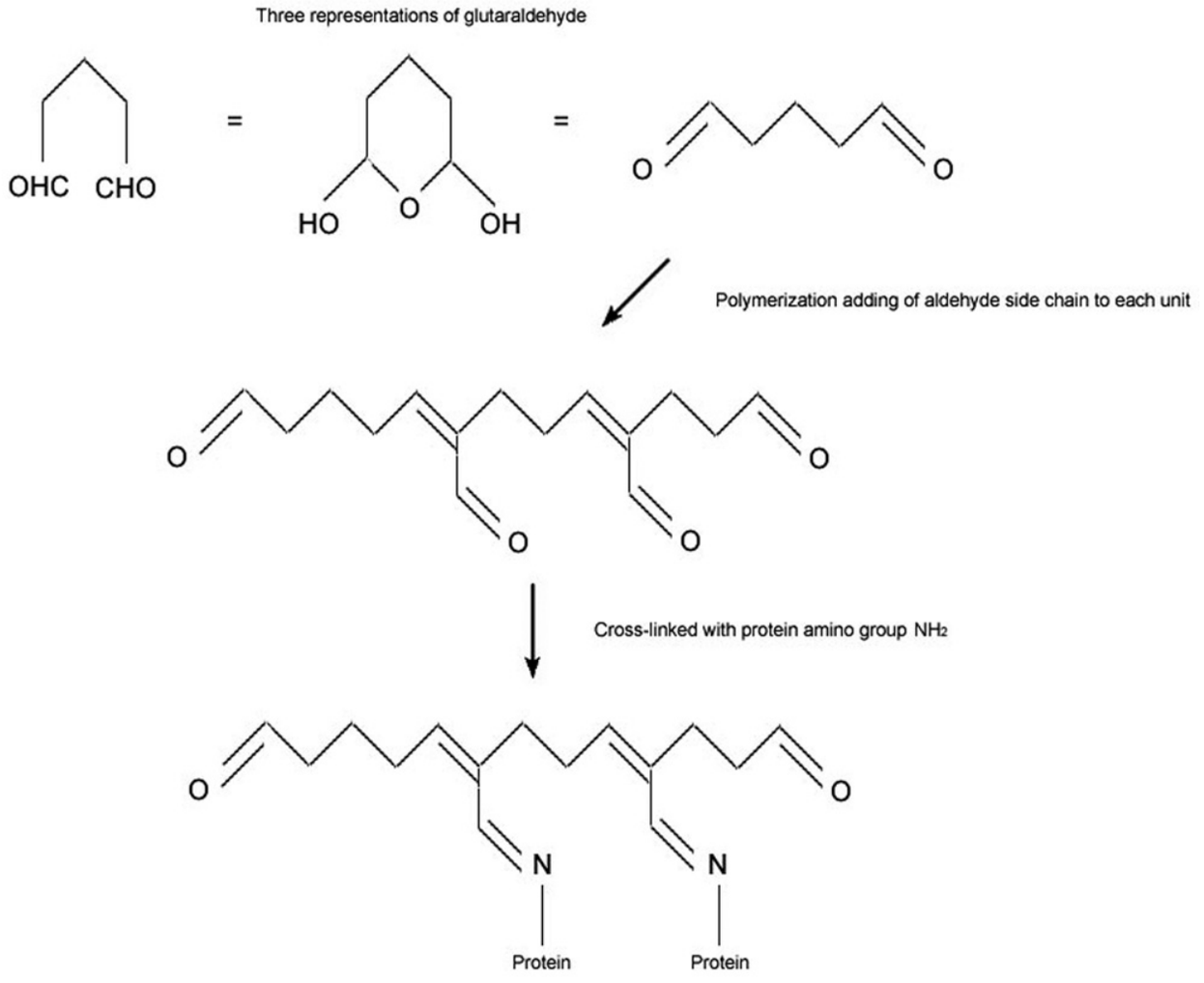

Figure 6. Schematic diagram showing glutaraldehyde cross-linking with proteins.

3.2.4. Covalent binding

Covalent binding is another technique used for the immobilization of bioactive molecules. The functional groups investigated are usually the carboxyl, amino and phenolic group of tyrosine. Bioactive factors are covalently linked through functional groups in the factors not essential for the bioactivity. The covalent binding should be optimized so as to protect the active site and not alter its conformational flexibility.

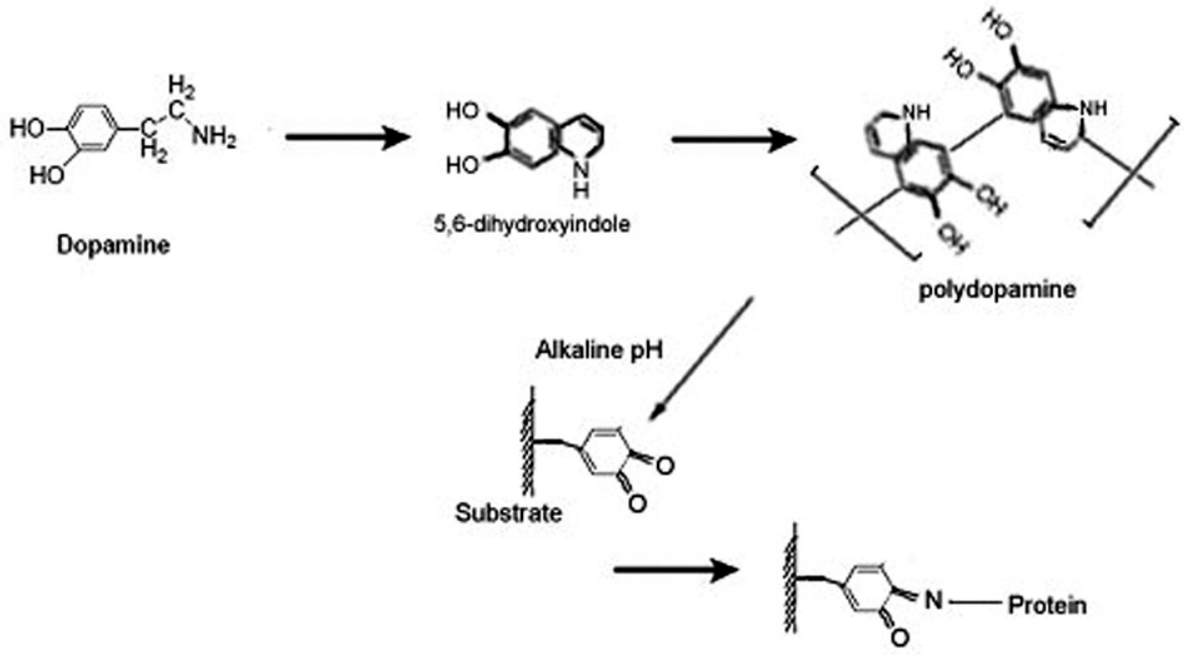

Figure 7. Schematic diagram showing polymerization of dopamine under alkaline pH and the equilibrium shift towards the quinone functional groups for reactivity with proteins.

3.2.5. Comparison of the various immobilization techniques

Several methods of immobilizing angiogenic growth factors onto substrates have been studied and reported [61-66]. A summary of a short study investigating the efficacy of immobilization of VEGF via various modes of functionalization on Ti-6Al-4V including physical adsorption, cross-linking and covalent binding (adapted for orthopaedic applications) is presented here to evaluate the effectiveness of each technique. As physical entrapment is not suitable in this case of improving the bone-implant interface via the surface of the implant material, therefore this system is not investigated. Table 1 summarizes the parameters of the binding efficiency, cytotoxicity, release profile and number of steps required for the fabrication of the substrates.

Although physical adsorption had the highest rate of binding however there was also uncontrolled release of the factors from the substrate which may be undesirable [67-69]. A measurement of the percentage of factors released into the solution over a 30 day period showed that more than 30% of the factors were released. A number of studies have examined simple coating or loading of factors onto implants [67-73] in order to provide local and sustained delivery after implantation. However with this strategy some studies showed an uncontrolled initial burst in the release kinetics of factors from such implants [67-69]. High levels of factors in the local microenvironments of these implants may be detrimental to healing and may promote tumorogenesis [74]. To avoid the deleterious effects, secure immobilization strategy would be preferred [61, 64-66]. Immobilization of growth factors on implants have been shown to promote desirable cell substrate interactions and enhance cell functions [62,

	Binding Efficiency (50ng loading)	Cytotoxicity	Factor release overtime	Active form	Number of steps required for fabrication
Physical adsorption (via simple coating)	86%	0.677	"/> 30% after 1 month	Soluble	Single step
Cross-linking (via glutaraldehyde cross-linking)	56%	0.449	Nil	Immobilized	Three steps
Covalent Binding (via polydopamine conjugation)	52%	0.841	Nil	Immobilized	Two steps

Table 1. please add caption

63]. Furthermore it has been demonstrated that immobilized factors is more effective in promoting proliferation of cells compared to soluble factors [65]. Both immobilized and soluble factors bind to receptors on cells, however they have differing effects due to the fact that soluble factors are internalized and subsequently degraded, while immobilization inhibits internalization and prevents down regulation [64, 75], thereby enabling the factors to stimulate proliferation for an extended period of time. A comparison of cross-linking and covalent binding shows that they come quite close in terms of binding efficiency and there is no release of growth factors into the solution which is the preferred methodology.

From the cytotoxicity indications (Table 1) follows that there is a lower cell viability with glutaraldehyde cross-linking compared to the other groups. This may be due to the fact that glutaraldehyde is known to be toxic and is able to kill cells quickly by cross-linking with their proteins. There have also been reports of its toxicity implicated in poor cell growth, attachment and apoptosis [58-60] by other groups. Although glutaraldehyde cross-linking effectively anchors a high density of factors onto the titanium substrate surface and the molecules are also more firmly attached than those which are physically adsorbed however the associated toxicity has made it unsuitable for clinical applications. The use of covalent immobilization with polydopamine looks promising. Polydopamine has been found to be able to form thin adherent films onto a wide variety of metallic substrates via covalent bonds and various strong intermolecular interactions including metal chelation, hydrogen bonding and π-π interactions [76] which cannot be disrupted by normal mechanical forces. The use of this bioreactive layer for covalent bioconjugation with bioactive factors for orthopaedic applications holds great potential. Although it will not be possible to have a universal means of immobilization, however it is vital to develop a viable methodology which can provide for secure immobilization with good interactions for orthopaedic implants. The choice of the technique would depend on the nature of the bioactive factors, substrates and their application. The development of surface modification procedures that do not affect the integrity of the substrate and bioactivity of the growth factors are crucial in producing the desired surface functionalization effect. This would provide us with a secure and efficient method of attaching bioactive

molecules to titanium implant material surface conferring enhancement of cell-implant interactions beneficial for orthopaedic applications.

4. Conclusions

There is an ever growing need for orthopaedic advancement with the high prevalence and impact of musculoskeletal diseases. 50% of the world's population over 65 suffer from joint diseases and more than 25% of population over 65 require health care for joint related diseases. The instances for failed joint replacements associated with osteolysis and bone defects is increasing. There is an urgency to increase the success of bone implant fixation and the longevity of implant. Fixation of orthopaedic implants has been one of the most challenging and difficult problem faced by orthopaedic surgeons and patients. Fixation can often be achieved via direct biological fixation by allowing tissues to grow into the surfaces of the implants or with the use of bone cement acting as a grouting material. Whether cemented or cementless fixation are employed, the problems of micromotion and the generation of wear particles may eventually necessitate further surgery. Revision surgery poses increased risks like deep venous thrombosis, infection and dislocation, in addition to being an economic burden to the patient. Therefore the enhancement of implant integration would bring enormous benefits. Titanium alloy is one of the most frequently used material in orthopaedic implants. However despite the good inherent bioactivity and biocompatibility exhibited by titanium alloys, osseointegration with host tissue is still not definite, the lack of bioactivity may cause implant failure at times. Fixation of orthopaedic implants has been one of the most challenging and difficult problem faced by orthopaedic surgeons and patients. With the ever growing number of patients requiring orthopaedic reconstructions the development and evolvement of titanium alloys with structural and biological potential to manage bone healing impairment and defects would be desirable.

Author details

Wilson Wang* and Chye Khoon Poh

Department of Orthopaedic Surgery, National University of Singapore, Kent Ridge, Singapore

References

[1] Lausmaa J, Linder L. Surface spectroscopic characterization of titanium implants after separation from plastic-embedded tissue. Biomaterials. 1988;9:277-80.

[2] Niinomi M. Mechanical biocompatibilities of titanium alloys for biomedical applications. J Mech Behav Biomed Mater. 2008;1:30-42.

[3] Puleo DA, Nanci A. Understanding and controlling the bone-implant interface. Biomaterials. 1999;20:2311-21.

[4] Branemark R, Branemark PI, Rydevik B, Myers RR. Osseointegration in skeletal reconstruction and rehabilitation: A review. J Rehabil Res Dev. 2001;38:175-81.

[5] Mano T, Ueyama Y, Ishikawa K, Matsumura T, Suzuki K. Initial tissue response to a titanium implant coated with apatite at room temperature using a blast coating method. Biomaterials. 2002;23:1931-6.

[6] Rossi S, Tirri T, Paldan H, Kuntsi-Vaattovaara H, Tulamo R, Narhi T. Peri-implant tissue response to TiO2 surface modified implants. Clin Oral Implants Res. 2008;19:348-55.

[7] Migirov L, Kronenberg J, Volkov A. Local tissue response to cochlear implant device housings. Otol Neurotol. 2011;32:55-7.

[8] Suska F, Emanuelsson L, Johansson A, Tengvall P, Thomsen P. Fibrous capsule formation around titanium and copper. J Biomed Mater Res A. 2008;85:888-96.

[9] Kyriakides TR, Hartzel T, Huynh G, Bornstein P. Regulation of angiogenesis and matrix remodeling by localized, matrix-mediated antisense gene delivery. Mol Ther. 2001;3:842-9.

[10] Nebe JB, Muller L, Luthen F, Ewald A, Bergemann C, Conforto E, et al. Osteoblast response to biomimetically altered titanium surfaces. Acta Biomater. 2008;4:1985-95.

[11] Akbar M, Fraser AR, Graham GJ, Brewer JM, Grant MH. Acute inflammatory response to cobalt chromium orthopaedic wear debris in a rodent air-pouch model. J R Soc Interface. 2012;9:2109-19.

[12] Perona PG, Lawrence J, Paprosky WG, Patwardhan AG, Sartori M. Acetabular micromotion as a measure of initial implant stability in primary hip arthroplasty. An in vitro comparison of different methods of initial acetabular component fixation. J Arthroplasty. 1992;7:537-47.

[13] Kaufman AM, Alabre CI, Rubash HE, Shanbhag AS. Human macrophage response to UHMWPE, TiAlV, CoCr, and alumina particles: analysis of multiple cytokines using protein arrays. J Biomed Mater Res A. 2008;84:464-74.

[14] Pap T, Claus A, Ohtsu S, Hummel KM, Schwartz P, Drynda S, et al. Osteoclast-independent bone resorption by fibroblast-like cells. Arthritis Res Ther. 2003;5:R163-73.

[15] Castellani C, Lindtner RA, Hausbrandt P, Tschegg E, Stanzl-Tschegg SE, Zanoni G, et al. Bone-implant interface strength and osseointegration: Biodegradable magnesium alloy versus standard titanium control. Acta Biomater. 2011;7:432-40.

[16] Coelho PG, Granjeiro JM, Romanos GE, Suzuki M, Silva NR, Cardaropoli G, et al. Basic research methods and current trends of dental implant surfaces. J Biomed Mater Res B Appl Biomater. 2009;88:579-96.

[17] Morra M. Biomolecular modification of implant surfaces. Expert Rev Med Devices. 2007;4:361-72.

[18] Rey C. Calcium phosphate biomaterials and bone mineral. Differences in composition, structures and properties. Biomaterials. 1990;11:13-5.

[19] de Groot K, Wolke JG, Jansen JA. Calcium phosphate coatings for medical implants. Proc Inst Mech Eng H. 1998;212:137-47.

[20] Barrere F, van der Valk CM, Meijer G, Dalmeijer RA, de Groot K, Layrolle P. Osteointegration of biomimetic apatite coating applied onto dense and porous metal implants in femurs of goats. J Biomed Mater Res B Appl Biomater. 2003;67:655-65.

[21] Geesink RG. Osteoconductive coatings for total joint arthroplasty. Clin Orthop Relat Res. 2002:53-65.

[22] Wennerberg A, Albrektsson T. Structural influence from calcium phosphate coatings and its possible effect on enhanced bone integration. Acta Odontol Scand. 2009:1-8.

[23] van den Beucken JJ, Vos MR, Thune PC, Hayakawa T, Fukushima T, Okahata Y, et al. Fabrication, characterization, and biological assessment of multilayered DNA-coatings for biomaterial purposes. Biomaterials. 2006;27:691-701.

[24] Bierbaum S, Hempel U, Geissler U, Hanke T, Scharnweber D, Wenzel KW, et al. Modification of Ti6AL4V surfaces using collagen I, III, and fibronectin. II. Influence on osteoblast responses. J Biomed Mater Res A. 2003;67:431-8.

[25] Liu Y, de Groot K, Hunziker EB. BMP-2 liberated from biomimetic implant coatings induces and sustains direct ossification in an ectopic rat model. Bone. 2005;36:745-57.

[26] Hall J, Sorensen RG, Wozney JM, Wikesjo UM. Bone formation at rhBMP-2-coated titanium implants in the rat ectopic model. J Clin Periodontol. 2007;34:444-51.

[27] Schmidmaier G, Wildemann B, Cromme F, Kandziora F, Haas NP, Raschke M. Bone morphogenetic protein-2 coating of titanium implants increases biomechanical strength and accelerates bone remodeling in fracture treatment: a biomechanical and histological study in rats. Bone. 2002;30:816-22.

[28] Sumner DR, Turner TM, Urban RM, Turek T, Seeherman H, Wozney JM. Locally delivered rhBMP-2 enhances bone ingrowth and gap healing in a canine model. J Orthop Res. 2004;22:58-65.

[29] Saito A SY, Ogata S, Ohtsuki C, Tanihara M. Accelerated bone repair with the use of a synthetic BMP-2-derived peptide and bone-marrow stromal cells. J Biomed Mater Res A. 2005 Jan;72:77-82.

[30] Choi J-Y, Jung U-W, Kim C-S, Eom T-K, Kang E-J, Cho K-S, et al. The effects of newly formed synthetic peptide on bone regeneration in rat calvarial defects. J Periodontal Implant Sci. 2010;40:11-8.

[31] LeBaron RG, Athanasiou KA. Extracellular matrix cell adhesion peptides: functional applications in orthopedic materials. Tissue Eng. 2000;6:85-103.

[32] Ranieri JP, Bellamkonda R, Bekos EJ, Vargo TG, Gardella JA, Jr., Aebischer P. Neuronal cell attachment to fluorinated ethylene propylene films with covalently immobilized laminin oligopeptides YIGSR and IKVAV. II. J Biomed Mater Res. 1995;29:779-85.

[33] Schliephake H, Scharnweber D, Dard M, Rossler S, Sewing A, Meyer J, et al. Effect of RGD peptide coating of titanium implants on periimplant bone formation in the alveolar crest. An experimental pilot study in dogs. Clin Oral Implants Res. 2002;13:312-9.

[34] Alam MI, Asahina I, Ohmamiuda K, Takahashi K, Yokota S, Enomoto S. Evaluation of ceramics composed of different hydroxyapatite to tricalcium phosphate ratios as carriers for rhBMP-2. Biomaterials. 2001;22:1643-51.

[35] Lin M, Overgaard S, Glerup H, Soballe K, Bunger C. Transforming growth factor-beta1 adsorbed to tricalciumphosphate coated implants increases peri-implant bone remodeling. Biomaterials. 2001;22:189-93.

[36] Siebers MC, Walboomers XF, Leewenburgh SC, Wolke JC, Boerman OC, Jansen JA. Transforming growth factor-beta1 release from a porous electrostatic spray deposition-derived calcium phosphate coating. Tissue Eng. 2006;12:2449-56.

[37] Reiner T, Gotman I. Biomimetic calcium phosphate coating on Ti wires versus flat substrates: structure and mechanism of formation. J Mater Sci Mater Med. 2010;21:515-23.

[38] Hirano Y, Mooney DJ. Peptide and protein presenting materials for tissue engineering. Adv Mater. 2004;16:17-25.

[39] Novaes AB, Jr., de Souza SL, de Barros RR, Pereira KK, Iezzi G, Piattelli A. Influence of implant surfaces on osseointegration. Braz Dent J. 2010;21:471-81.

[40] Mavrogenis AF, Dimitriou R, Parvizi J, Babis GC. Biology of implant osseointegration. J Musculoskelet Neuronal Interact. 2009;9:61-71.

[41] Freed LE, Guilak F, Guo XE, Gray ML, Tranquillo R, Holmes JW, et al. Advanced tools for tissue engineering: scaffolds, bioreactors, and signaling. Tissue Eng. 2006;12:3285-305.

[42] Laschke MW, Harder Y, Amon M, Martin I, Farhadi J, Ring A, et al. Angiogenesis in tissue engineering: breathing life into constructed tissue substitutes. Tissue Eng. 2006;12:2093-104.

[43] Boontheekul T, Mooney DJ. Protein-based signaling systems in tissue engineering. Curr Opin Biotechnol. 2003;14:559-65.

[44] Ferrara N, Davis-Smyth T. The biology of vascular endothelial growth factor. Endocr Rev. 1997;18:4-25.

[45] Deckers MM, Karperien M, van der Bent C, Yamashita T, Papapoulos SE, Lowik CW. Expression of vascular endothelial growth factors and their receptors during osteoblast differentiation. Endocrinology. 2000;141:1667-74.

[46] Kaku M, Kohno S, Kawata T, Fujita I, Tokimasa C, Tsutsui K, et al. Effects of vascular endothelial growth factor on osteoclast induction during tooth movement in mice. J Dent Res. 2001;80:1880-3.

[47] Engsig MT, Chen QJ, Vu TH, Pedersen AC, Therkidsen B, Lund LR, et al. Matrix metalloproteinase 9 and vascular endothelial growth factor are essential for osteoclast recruitment into developing long bones. J Cell Biol. 2000;151:879-89.

[48] Nakagawa M, Kaneda T, Arakawa T, Morita S, Sato T, Yomada T, et al. Vascular endothelial growth factor (VEGF) directly enhances osteoclastic bone resorption and survival of mature osteoclasts. FEBS Lett. 2000;473:161-4.

[49] Mayr-wohlfart U, Waltenberger J, Hausser H, Kessler S, Gunther KP, Dehio C, et al. Vascular endothelial growth factor stimulates chemotactic migration of primary human osteoblasts. Bone. 2002;30:472-7.

[50] Midy V, Plouet J. Vasculotropin/vascular endothelial growth factor induces differentiation in cultured osteoblasts. Biochem Biophys Res Commun. 1994;199:380-6.

[51] Gerber HP, McMurtrey A, Kowalski J, Yan M, Keyt BA, Dixit V, et al. Vascular endothelial growth factor regulates endothelial cell survival through the phosphatidylinositol 3'-kinase/Akt signal transduction pathway. Requirement for Flk-1/KDR activation. J Biol Chem. 1998;273:30336-43.

[52] Roberts WG, Palade GE. Increased microvascular permeability and endothelial fenestration induced by vascular endothelial growth factor. J Cell Sci. 1995;108 (Pt 6): 2369-79.

[53] Soker S, Machado M, Atala A. Systems for therapeutic angiogenesis in tissue engineering. World J Urol. 2000;18:10-8.

[54] Alon T, Hemo I, Itin A, Pe'er J, Stone J, Keshet E. Vascular endothelial growth factor acts as a survival factor for newly formed retinal vessels and has implications for retinopathy of prematurity. Nat Med. 1995;1:1024-8.

[55] Villars F, Guillotin B, Amedee T, Dutoya S, Bordenave L, Bareille R, et al. Effect of HUVEC on human osteoprogenitor cell differentiation needs heterotypic gap junction communication. Am J Physiol Cell Physiol. 2002;282:C775-85.

[56] Emerson RH, Jr., Head WC, Emerson CB, Rosenfeldt W, Higgins LL. A comparison of cemented and cementless titanium femoral components used for primary total hip

arthroplasty: a radiographic and survivorship study. The Journal of arthroplasty. 2002;17:584-91.

[57] Ito Y. Covalently immobilized biosignal molecule materials for tissue engineering. The Royal Society of Chemistry 2008. 2007;4:46-56.

[58] Gough JE, Scotchford CA, Downes S. Cytotoxicity of glutaraldehyde crosslinked collagen/poly(vinyl alcohol) films is by the mechanism of apoptosis. J Biomed Mater Res. 2002;61:121-30.

[59] Lin FH, Yao CH, Sun JS, Liu HC, Huang CW. Biological effects and cytotoxicity of the composite composed by tricalcium phosphate and glutaraldehyde cross-linked gelatin. Biomaterials. 1998;19:905-17.

[60] Huang-Lee LL, Cheung DT, Nimni ME. Biochemical changes and cytotoxicity associated with the degradation of polymeric glutaraldehyde derived crosslinks. J Biomed Mater Res. 1990;24:1185-201.

[61] Hall H, Hubbell JA. Matrix-bound sixth Ig-like domain of cell adhesion molecule L1 acts as an angiogenic factor by ligating alphavbeta3-integrin and activating VEGF-R2. Microvasc Res. 2004;68:169-78.

[62] Ito Y, Liu SQ, Imanishi Y. Enhancement of cell growth on growth factor-immobilized polymer film. Biomaterials. 1991;12:449-53.

[63] Karakecili AG, Satriano C, Gumusderelioglu M, Marletta G. Enhancement of fibroblastic proliferation on chitosan surfaces by immobilized epidermal growth factor. Acta Biomater. 2008;4:989-96.

[64] Sharon JL, Puleo DA. Immobilization of glycoproteins, such as VEGF, on biodegradable substrates. Acta Biomater. 2008;4:1016-23.

[65] Shen YH, Shoichet MS, Radisic M. Vascular endothelial growth factor immobilized in collagen scaffold promotes penetration and proliferation of endothelial cells. Acta Biomater. 2008;4:477-89.

[66] Yao C, Prevel P, Koch S, Schenck P, Noah EM, Pallua N, et al. Modification of collagen matrices for enhancing angiogenesis. Cells Tissues Organs. 2004;178:189-96.

[67] Patil SD, Papadmitrakopoulos F, Burgess DJ. Concurrent delivery of dexamethasone and VEGF for localized inflammation control and angiogenesis. J Control Release. 2007;117:68-79.

[68] Elcin AE, Elcin YM. Localized angiogenesis induced by human vascular endothelial growth factor-activated PLGA sponge. Tissue Eng. 2006;12:959-68.

[69] Kempen DH, Lu L, Heijink A, Hefferan TE, Creemers LB, Maran A, et al. Effect of local sequential VEGF and BMP-2 delivery on ectopic and orthotopic bone regeneration. Biomaterials. 2009;30:2816-25.

[70] Lode A, Wolf-Brandstetter C, Reinstorf A, Bernhardt A, Konig U, Pompe W, et al. Calcium phosphate bone cements, functionalized with VEGF: Release kinetics and biological activity. Journal of Biomedical Materials Research - Part A. 2007;81:474-83.

[71] Lode A, Reinstorf A, Bernhardt A, Wolf-Brandstetter C, Konig U, Gelinsky M. Heparin modification of calcium phosphate bone cements for VEGF functionalization. Journal of Biomedical Materials Research - Part A. 2008;86:749-59.

[72] Matsusaki M, Sakaguchi H, Serizawa T, Akashi M. Controlled release of vascular endothelial growth factor from alginate hydrogels nano-coated with polyelectrolyte multilayer films. J Biomater Sci Polym Ed. 2007;18:775-83.

[73] Petersen W, Pufe T, Starke C, Fuchs T, Kopf S, Raschke M, et al. Locally applied angiogenic factors--a new therapeutic tool for meniscal repair. Ann Anat. 2005;187:509-19.

[74] Ehrbar M, Djonov VG, Schnell C, Tschanz SA, Martiny-Baron G, Schenk U, et al. Cell-demanded liberation of VEGF121 from fibrin implants induces local and controlled blood vessel growth. Circ Res. 2004;94:1124-32.

[75] Place ES, Evans ND, Stevens MM. Complexity in biomaterials for tissue engineering. Nat Mater. 2009;8:457-70.

[76] Lee H, Dellatore SM, Miller WM, Messersmith PB. Mussel-inspired surface chemistry for multifunctional coatings. Science. 2007;318:426-30.