Surface modification of implants in long bone

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Abbreviations: BIC, bone/implant contact; Coll, collagen type I; CS, chondroitin sulfate; ECM, extracellular matrix; FN, fibronectin; HA, hydroxyapatite; Hya, hyaluronic acid; TRAP, tartrate-resistant acid phosphatase

Coatings of orthopedic implants are investigated to improve the osteoinductive and osteoconductive properties of the implant surfaces and thus to enhance perimplant bone formation. By applying coatings that mimic the extracellular matrix a favorable environment for osteoblasts, osteoclasts and their progenitor cells is provided to promote early and strong fixation of implants. It is known that the early bone ongrowth increases primary implant fixation and reduces the risk of implant failure. This review presents an overview of coating titanium and hydroxyapatite implants with components of the extracellular matrix like collagen type I, chondroitin sulfate and RGD peptide in different small and large animal models. The influence of these components on cells, the inflammation process, new bone formation and bone/implant contact is summarized.

Introduction

Bone healing is a highly complex process which is conventionally divided into three overlapping steps: (1) inflammation, (2) repair and (3) bone remodeling. Each of them is characterized by a specific set of cellular and molecular events.1-3 Bleeding at the fracture site results in the development of a local hematoma. Inflammatory cells like granulocytes, macrophages, monocytes and lymphocytes infiltrate the hematoma and secrete cytokines and growth factors.1,2 Chemotactic effects induce further recruitment of inflammatory and mesenchymal cells, stimulation of angiogenesis and extracellular matrix synthesis.4,5 Over time the hematoma is reorganized in granulation tissue.6,7 Chondrocytes derived from mesenchymal progenitors and fibroblasts produce osteoid which is subsequently mineralized and form a soft callus between the fragments.6 In the next step, soft callus is gradually removed and replaced by mineralized bone matrix. The newly formed woven bone is called hard callus, is typically irregular and needs to be remodeled.7 This repair stage represents the most active period of osteogenesis with high levels of osteoblast activity. In the remodeling phase the woven bone is transformed into lamellar bone with the trabeculae being formed along the pressure trajectories. The most active cells during remodeling are osteoclasts which demineralize the matrix and degrade the organic components by proteinases.7,8 New bone is laid down in its shape, structure and mechanical strength by osteoblasts.7

In summary, the first 1 to 2 weeks, in which inflammation and revascularization occur, seem to be most critical for fracture healing.6,9 An early formation of granulation tissue could support the differentiation of mesenchymal cells into osteoblasts and thus provide a better requisite for bone remodeling. Coating of orthopedic implants aims at improved bone/implant contact (BIC), reduction of implant loosening and adverse reactions. Since the host response to surgical implants is mediated by regulatory interactions between the cells and the organic extracellular matrix,10-12 coating with components of the extracellular bone matrix (ECM) appears attractive to enhance bone healing around metallic and hydroxyapatite (HA) implants. Thereby, the ECM is not only a passive scaffold for cells. Several components of the ECM like collagen type I (Coll), chondroitin sulfate (CS) or RGD peptide containing proteins are able to bind cytokines and growth factors12,13 and can interact with bone cells via integrins or other specific cell surface receptors14 thus directly or indirectly influencing migration and cell adhesion as well as proliferation and differentiation of these cells.15,16

Osseointegration is influenced by the primary stability (mechanical stability) and secondary stability (biological stability after bone remodeling) of the implant in the bone. Thereby early bone formation and apposition is essential for secondary stability.17 In this review the promotion of early bone formation by components of the ECM is described.

Collagen Type I on Ti and HA Implants

Collagen type I is the major structural protein in bone. Coating with Coll enhanced in vitro adhesion, migration and differentiation of osteoblasts on Ti disks.18,19 Furthermore, the osteoconductive properties of Coll in cancellous and cortical bone are well documented.20-22
Coating of Ti pins with Coll showed that the cellular reaction on the implants appeared more intense around the Coll-coated implants in the early stages of bone healing in a rat tibia model compared with uncoated pins. At four days after implantation reparative granulation tissue was seen around the Coll-coated implants with 70% of the surface being surrounded by loose granulation tissue. Cathepsin D, osteopontin and osteonectin-positive cells were detected earlier and in higher number around Ti/Coll pins compared with Ti pins (Fig. 1). Cathepsin D is an important marker for cells of the monocyte/macrophage lineage and is known to play a role in bone remodeling under both physiological and pathological conditions. Osteopontin and osteonectin are non-collagenous proteins of the ECM. They are produced by osteoblasts and osteocytes and are involved in fracture healing. The early appearance of these proteins around Coll-coated Ti pins indicated an earlier onset of the bone remodeling process compared with uncoated Ti pins. After 28 days both coated and uncoated implants were surrounded to a great extent by newly formed lamellar bone with small parts of dense fibrous tissue connecting the distinct bone lamellae. The bone was in close contact with the implants without intervening fibrous tissue (Fig. 2B and C). Both the direct BIC and the amount of newly formed bone were greater in Coll-coated implants compared with uncoated implants without reaching statistical significance. In a sheep tibia model, coating of Ti implants with Coll under loaded conditions (external fixator pins) was investigated (Fig. 3). The extraction torque of the external fixation pins was not altered by the Coll-coating after 6 weeks of implantation. However, the apparent new bone formation and significantly increased activity of osteoblasts around the external fixator pins...
suggested increased bone remodeling around the Coll-coated implants. All data indicated that the faster remodeling by Coll-coating affects primarily the earlier stages of bone healing without altering mechanical stability.

The favorable in vivo results of coating Ti implants with collagen type I were confirmed by other groups. In the dog mandible a significantly increased peri-implant bone formation was found around Coll-coated screws implants after 3 mo as compared with uncoated screws. In the femur condyle of goats the amount of new bone formation was significantly higher in the cavities of cylindric Ti implants after 5 and 12 weeks. In a rabbit femur condyle model Coll-coating of Ti implants enhanced the osseointegration rate after 4 weeks as evaluated by histomorphometry, push-out test and bone microhardness measurements. Coll-coating of commercially available dental implants investigated in the dog mandible resulted in significantly higher BIC after 3 and 8 weeks. In addition, analysis of cells near the bone implant phase showed cellular and molecular profiles of osteoblasts in a more advanced stage of differentiation.

In contrast, Svehla et al. did not observe improved osseointegration of porous Ti cylinders coated with collagen gel implanted into the diaphysis of the sheep tibia. Different animal models, time frames and coating techniques make it difficult to directly compare the different studies.

However, Coll is effective in promoting cellular adhesion and spreading through RGD sequences recognized by integrins. The RGD sequences are able to interact directly with bone cells like osteoblasts, osteoclasts and their precursor cells. Salaszyk et al. described strong adhesive interactions of human mesenchymal cells on Coll-coated surfaces in vitro. Furthermore the adhesion to Coll promotes the differentiation of these cells into the osteogenic line. In addition Coll serves as substrate for collagenases and matrix metalloproteinases. It is therefore able to enhance bone resorption and bone formation resulting in increased bone remodeling.

Hydroxyapatite as bone substitute material is known to be a bioactive and biocompatible material with excellent osteoconductive properties. Combination of HA with Coll was found to be particularly suitable for bone replacement with good bone cell attachment, biodegradation by macrophages and induction of basic multicellular units in the surrounding tissue. In a rat tibia model the tissue reaction to HA combined with Coll (HA/Coll) was evaluated. The number of cathepsin D-positive cells was significantly increased around HA/Coll implants at day 6 (p < 0.01), 14 and 28 (p < 0.05). These results suggest that a certain amount of phagocytotic cells, which is required for a successful tissue turnover in the interface region, appeared earlier and prevailed for a longer period around HA/Coll implants. These findings reflect a higher bone remodeling activity around the Coll-modified implants compared with the unmodified HA. Furthermore, the low number of multinucleated cells and markedly decreased macrophage numbers after day 6 indicate that only a short inflammatory reaction took place reflecting the early stages of physiological bone healing. As a result of these early events clusters of newly formed woven bone could be detected around HA/Coll implants at day 6, but not around the implants of pure HA. Histomorphometric analysis showed an averaged BIC of 50.8% around HA/Coll implants and 28.5% around pure HA implants, representing a statistically significant difference (p < 0.05).

These findings support the hypothesis from other authors that the addition of collagen to HA implants can induce ossification processes in the early stage and thus provide better conditions for bone remodeling in the interface region.

**Chondroitin Sulfate in Ti and HA Implants**

Chondroitin sulfate (CS) is an important glycosaminoglycan found in cartilage as well as in cancellous and cortical bone. It is part of proteoglycans like decorin and aggregan and consists of a...
repeating disaccharide unit of D-glucuronic acid linked to N-acetylgalactosamine. The galactosamine residues are sulfated in positions 4 or 6. These sulfate groups as well as the carboxyl groups were assumed to interact with mineral structures such as hydroxyapatite in bone. Besides Coll, CS appears to be promising as a further addition to biomaterials to enhance bone healing by creating an artificial matrix for osteoblasts. It can mediate the binding of bone-like cells like osteoblasts and osteoclasts to the matrix and capture soluble molecules such as growth factors into the matrix and at cell surfaces.

In vitro studies have shown that the coating of Ti surfaces or textile scaffolds with Coll/CS enhanced mesenchymal cell adhesion, spreading and differentiation.

In a standardized rat tibia model Rammelt et al. investigated the effects of Coll/CS-coating on bone remodeling and bone healing. Four days after implantation of the coated Ti pins a reparative granulation tissue was seen around the Coll/CS-coated implants with a high amount of infiltration of mononuclear macrophages displaying immunoreactivity against cathepsin D. At the same time a primitive fibrin network of mainly granulocytes, fibroblasts and few macrophages was observed around uncoated Ti. The number of TRAP-positive osteoclasts at the newly deposited osteoid and woven bone at the implant surface was significantly higher around Coll/CS implants compared with Coll-coated implants or pure Ti (p < 0.05). Furthermore, the number of osteopontin-positive cells was significantly increased around the Coll/CS implants at this time point (p < 0.05) indicating an early bone remodeling. After 28 d BIC was significantly higher (p < 0.05) around Coll/CS-coated Ti pins (89.5%) compared with pure Ti (63.9%) and Coll alone (76.1%).

Further investigations focused on large animal models. In a sheep experiment under loaded conditions the number of osteopontin-positive osteoblasts was significantly increased in the interface around Coll/CS-coated Ti external fixation pins. The extraction torque was slightly higher than that of the uncoated external fixation pins (Fig. 3). These data support findings from other groups that have shown that proteoglycans were able to improve cell binding and accelerate differentiation of bone like cells in vivo. Around unloaded Ti screws in the tibia head these effects were not observed, which may be explained by the increased bone remodeling under loaded conditions according to Wolff’s law.

Several other animal studies confirmed the advantages of Coll/CS-coating. Stadlinger et al. showed a significantly higher BIC on Coll/CS-coated cylindric Ti implants (40%) compared with Coll-coating (30%) in the mandible of minipigs 6 mo after implantation. The same group studied bone formation using threaded dental Ti implants with different CS content in minipigs. One month after implantation more mature stages of bone formation were reached around the Coll/CS-coated implants in comparison to control independent of the CS amount. The significantly higher BIC after one month was equalized after 2 mo indicating that Coll/CS-coating enhances early bone formation.

The addition of CS to HA/Coll appears attractive in order to further increase the osteoconductive properties of these bone substitute composites. The influence of cylindric CS-modified
HA/Coll implants on the host response was examined in the rat tibia. The HA/Coll implant was not designed to be absorbed by the host tissue. Rather, the tissue reaction of the implants could be observed in the interface around the implant. At the early stages of tissue reaction (days 4 and 7) a significantly higher number of cathepsin D-positive and TRAP-positive cells were seen around HA/Coll/CS implants as compared with HA/Coll implants indicating a more rapid tissue reaction and appearance of phagocytic cells around HA/Coll/CS implants. From the seventh day on, islands of woven bone were observed at the interface around the CS-coated HA/Coll implants, but not around the HA/Coll implants. On day 28 after implantation all implants were surrounded by newly formed lamellar bone with increased amount of BIC and higher amount of newly formed bone around the Coll/CS-coated implants.

These results could be further supported in a large animal model which is more clinically relevant than a small animal model. A critical midshaft defect of 3 cm was created in the tibia and filled with HA/Coll or HA/Coll/CS cement cylinders (Fig. 5A). Radiographic investigations of the HA/Coll implants showed no callus reaction until the 12th week whereas an initial callus reaction was seen around the HA/Coll/CS implants. After 3 mo newly formed woven bone was seen directly at the HA implant coated with Coll/CS (Fig. 5C and D). Direct bone contact at later stages of bone healing and the total amount of newly formed bone were increased around HA/Coll/CS compared with HA/Coll implants (Fig. 5B).

Taken together the results of the small and large animal model indicate additional effects through the addition of CS to Coll than Coll-coating alone. The negative surface charge of CS could be responsible for the observed effects. The negative charged sulfate or carboxyl groups can interact with positively charged amino acid sequences of growth factors thus modulating their activity. This might enhance the immobilization of the growth factors and other cytokines on the implant surface and thus stimulate cell activity around the implants.

In conclusion, CS can mediate the attachment of growth factors and cytokines to the implant surface by direct interaction with them, which should be the subject of further investigations.

**RGD on Ti and HA Implants**

The RGD peptide sequence (Arg-Gly-Asp) is an ubiquitous adhesive motif. In addition, it is responsible for the interaction of cellular integrin receptors with proteins of the ECM. Numerous in vitro studies have shown that RGD and related peptides are able to enhance adhesion, migration and osteoblast gene expression. The RGD sequence is present in several molecules of the ECM like collagen, fibronectin, osteopontin and osteonectin and is thought to play a role in bone formation.

![Figure 5.](image-url)
Thereby the no significant differences in bone formation were found with HA/Coll implants did not increase bone ingrowth in a sheep model. In contrast, Hya-coated HA implants did not increase bone ingrowth. The influence of Hya on osseointegration was more evident in trabecular than in cortical bone. In contrast, Hya-coated HA implants did not increase bone ingrowth in a sheep model.

Fibronectin, a major component of cell adhesive proteins, is known to play a role in facilitating cell attachment, spreading and differentiation. Differentiation of osteoblasts was enhanced on Ti surfaces coated with a recombinant fragment of FN containing the central cell binding domain. In a rat cortical bone model BIC, mechanical fixation, and functional osseointegration were significantly improved compared with uncoated Ti implants and implants coated with FN from human plasma. However, plasma FN coating also showed significantly higher mineralization compared with uncoated implants. In a cylindrical bone defect in the femur of mice acrylic rods coated with Ti and Ti/FN were investigated. The coating with FN from mouse plasma induced earlier osseointegration. BIC was significantly higher at day 5 after implantation around the Ti/FN rods.

Attachment and proliferation of osteoblasts was significantly increased on HA disks pretreated with FN together with fetal calf serum.

**Summary and Outlook**

Coating of titanium or hydroxyapatite implants with organic components of the extracellular matrix offers great potential to improve new bone formation and enhance bone/implant contact which in turn will improve healing time and implant stability. Nevertheless the sometimes conflicting results necessitate further investigations to individually tailor the ideal implant modifications. The increasing knowledge about the role of the ECM for recruitment, proliferation, and differentiation of cells and regeneration of tissue will eventually deal to the creating of an artificial ECM on the implant that could allow a defined adjustment of the required properties to support the healing process.

A potential strategy could be the sulfation of glycosaminoglycans to specifically address the functionalities of these ECM components. To further elucidate the basic mechanisms of the cell-matrix interactions during the early stages of bone healing in order to specifically address them with novel implant modifications, our group has used microdialysis on a bone defect model to detect the time-dependent course of the key mediators and cytokines during that process.
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