IMPACT OF MODIFIED HYALURONAN ON BINDING OF BIOLOGICAL MEDIATORS

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Introduction

Titanium and its alloys exhibit sound biocompatibility and are widely used as implant materials. However there is still the need for improving the integration of implants into the surrounding tissue. The cells between the interface of the implant and the tissue are mainly held responsible for this integration. Besides direct interactions with the extracellular matrix (ECM) they are mainly influenced by growth factors and cytokines (biological mediators). In tissues the ECM serves as a scaffold for cells and also as a reservoir of growth factors. Through targeted use of ECM components we aim on creating implant coatings with a defined microenvironment to selectively influence in vivo mediators and thereby improving cell adhesion, proliferation and differentiation (FIGURE 1) [1,2,3,4]. The artificial extracellular matrices are mainly based on collagen-I and modified hyaluronic acid derivatives (HAD).

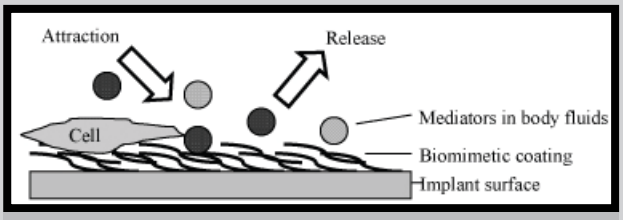

FIG. 1. Biomimetic implant coatings as attachment sites for cells and as reservoir for growth factors.

While the binding of GAGs and proteins mainly depends on electrostatic interactions of the negatively charged sulfate groups with the positively charged amino acids we have focused our investigation on HADs with different degrees of sulfate modification.

Through binding mediators can be 1) accumulated close to the implant, 2) activated by conformational change and/or 3) protected from degradation. We have emphasized on growth factors and interleukins involved in healing and remodeling of bone (i.e. BMP-2) [5,6,7,8]. We will present the newest developments in characterizing the differently modified model substances on binding and activity of selected mediators and their impact on osteoblastic cells.

Materials and methods

In vitro fibrillogenesis: Collagen was prepared as described in [1]. For copolymerization different HADs were added before starting fibrillogenesis. Fibril formation was monitored at 313 nm. The amount of collagen integrated as well as the integration of HADs into fibrils was determined as described in [1].

Atomic force microscopy (AFM): The morphology of the fibrils was examined by AFM with collagen fibril suspensions on Glimmer using a Bioscope instrument (Digital Instruments/Vecco) as described in [9].

ELISA: HADs were chemically coupled to polystyrol plates. Mediators were incubated in different concentrations for 24h at 4°C. Supernatants were used for Sandwich-ELISA analysis, while bound growth factors were analyzed by Direct-ELISA.

Surface Plasmon Resonance (SPR): Growth factors were amine coupled to a CM-5 chip and varying concentrations of different HADs were injected over the surface.

Cell experiments (in preparation): Osteoblastic cell lines and hMSCs will be seeded on collagen/HAD coated polystyrol plates with and without biological mediators and the metabolic activity will be determined by MTS-formazan assay after 24h. We will further investigate cell attachment, collagen synthesis, Ca phosphate accumulation and proliferation.

Results and discussion

The integration of collagen monomers during in vitro- fibrillogenesis is not significantly changed by the amount of HADs added and the derivatives are integrated according to the amount of HAD used.

Therefore it is feasible to regulate the amount of HAD in collagen fibrils by adjusting the ratio without disturbing collagen fibrillogenesis. However, with increasing HAD/collagen ratio the kinetics of fibril formation is altered and fibril diameters is changed as determined by turbodimetric measurements at 313 nm and atomic force microscopy. This is in compliance with published data on chondroitin sulfate derivatives [9]. Nevertheless fibrils exhibited a natural banding pattern and seemed to be equally stable even at higher HAD to collagen ratios. Interaction studies with HAD and different biological mediators revealed differences in binding depending on which mediator and sugar derivative used: As expected the degree of sulfate modification plays an important role in mediator binding of HADs (FIGURE 2).

These findings were confirmed by a biophysical- (SPR) as well as an immuno-biochemical assay (ELISA).

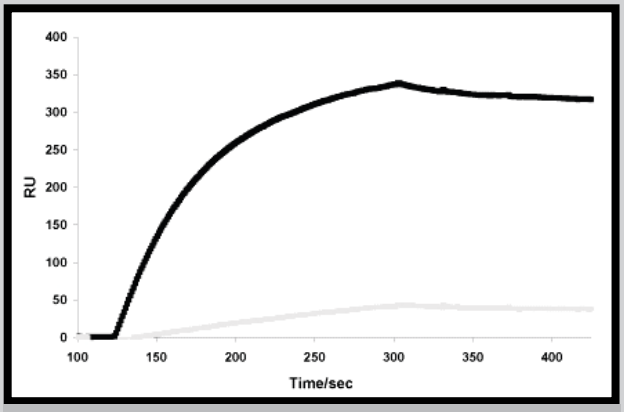

FIG. 2. SPR- interaction study of immobilized recombinant human growth factor with 50µg/ml soluble sulfated (black) or unmodified (grey) HAD. RU: Resonance Units.

Conclusion/Summary

By varying the HAD type and composition with copolymerizing fibrillar collagens our approach seems feasible to create customized artificial extracellular matrices. These matrices can be used to establish a defined environment at the implant surface selectively attracting and storing biological mediators relevant for bone healing and remodeling. Coating titanium implants with these matrices seems promising to improve healing of bone prosthesis and therefore to enhance stability of the regenerated tissue.

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THE ROLE OF DECELLULARIZATION IN BIOMATERIALS MANUFACTURING FROM XENOGENEIC TISSUES

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Abstract

Biological heart valves have represented an important area of the tissue-derived biomaterials. Decellularization processes are considered to be useful for manufacturing of biodegradable scaffolds which make it possible to create living and functioning tissues. These processes result in elimination of most disadvantages of GA-stabilized tissues. Acellular tissues may be obtained using various chemical, enzymatic and mechanical methods. Decellularization processes give the possibility of creating biomaterials for cell seeding which are not immunogenic, cytotoxic and calcifying.

[Engineering of Biomaterials, 77-80, (2008), 19-20]

Introduction

Biological heart valves manufacturing has represented an important area of the tissue-derived biomaterials use over the past 40 years. During this time period, various allogeneic and xenogeneic tissues have been investigated and applied for this purpose [1]. Although many allograft features such as relative resistance to infection and also hemodynamic properties are very important for biomaterials fabrication [2-4], these tissues have limited supply.

On the other hand, fabrication of xenogafts is not limited. The immunological barrier has been broken due to various stabilization processes [5].

Glutaraldehyde (GA) is a chemical cross-linking reagent routinely used in stabilization processes. Apart from stabilization of extracellular matrix, GA-treatment leads to reduction of immunological response [5]. However, GA in tissue-derived biomaterials is responsible for their premature calcification as well as thrombosis [6] and cytotoxicity [7]. Although GA-modified biomaterials are biologically inert, cellular debris is not completely removed from them.

Decellularization processes are considered to be useful for manufacturing of biodegradable scaffolds which make it possible to create living and functioning tissues [8]. It is widely accepted that decellularization processes result in elimination of most disadvantages of GA-stabilized tissues. However, till now little is known about the safety of decellularized xenogeneic tissues.