RGD peptides inhibit osseointegration of hydroxyapatite implants

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Introduction

The RGD peptide, a sequence found within many extracellular matrix proteins including fibronectin (FN) and vitronectin (VN), has been widely used to functionalize biomaterials in order to promote cell adhesion. However, our laboratory has shown that hydroxyapatite (HA) is very efficient at adsorbing FN and VN from blood/serum and therefore we questioned whether RGD peptides would improve osseointegration of HA implants. In fact, our prior in vitro studies showed that significantly more mesenchymal stem cells (MSCs) adhered to HA disks coated with fetal bovine serum (FBS) as compared with disks sequentially coated with high concentrations of RGD plus FBS, indicating that RGD can inhibit cell adhesion under some circumstances. Notably, it is well-established that RGD peptides induce significantly less integrin activation than intact matrix proteins such as FN.

We previously used FBS as an in vitro mimic for the coating of an HA implant with blood that would occur in vivo. The goal of the current study was to examine whether RGD influences the adhesion of MSCs to HA disks that were implanted for 30 minutes in the bone microenvironment to allow endogenous protein adsorption. We also monitored bone formation on RGD-coated HA disks that were implanted for 5 days in rat tibiae. Collectively, our results suggest that RGD is detrimental to the osseointegration of HA biomaterials.

Methods

Preparation of HA disk: HA powder (Fisher) was pressed into disks under 1000 psi. The disks were sintered at 1000°C for three hours and allowed to cool.

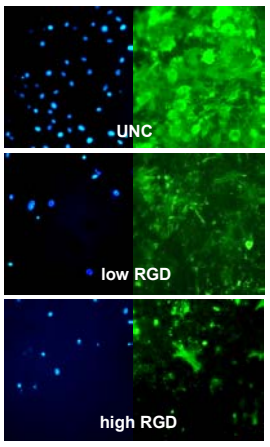
Cell Adhesion: HA disks with various concentrations of RGD peptides were implanted into rat tibial osteotomies for 30 minutes. Disks were then retrieved, and washed extensively to remove loosely-bound proteins. Human MSCs were subsequently seeded onto the disks and allowed to attach in serum-free medium for 1 hr. Disks were washed again, and cells were visualized by staining with phalloidin-Alexa 488 and DAPI. Cell adhesion was quantified by counting cells from 3 different fields, with 5 implanted disks assessed per experimental variable.

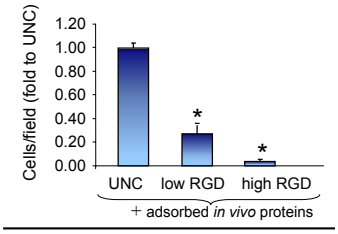
Bone formation: Uncoated (incubated in saline) or RGD-coated HA disks were implanted into rat tibiae for 5 days. The tibiae, with implants in place, were subsequently retrieved and embedded in plastic. Tibial sections were then stained with Goldner's Trichrome and bone formation was quantified using Bioquant software.

Results/Discussion:

Uncoated and RGD-coated HA disks were implanted into rat tibial osteotomies for 30 minutes to allow the adsorption of endogenous proteins. The disks were then retrieved, washed extensively, and MSCs were seeded onto the disks and monitored for cell adhesion. As shown in Figure 1, significantly less MSCs adhered to retrieved RGD-coated disks, as compared with uncoated disks, indicating that RGD interfered with some aspect of MSC binding to HA. We initially hypothesized that RGD might attenuate protein adsorption, however Western blots of desorbed endogenous proteins revealed that equivalent amounts of FN and VN were deposited on uncoated and RGD-coated disks (not shown). To determine whether the inhibitory effects of RGD on initial cell adhesion influenced implant integration, we implanted uncoated and RGD-coated disks into tibiae for 5 days. Staining of mineralized bone revealed that significantly less bone was formed on the RGD-coated disks (Figure 2).

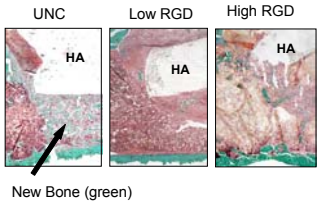
Fig. 1: Attachment of MSCs to HA disks exposed to a tibial microenvironment

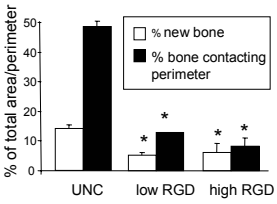




HA disks were either left uncoated (UNC) or coated overnight with 1µg/mL RGD (low RGD), or 1000 µg/mL RGD (high RGD). The disks were implanted into rat tibiae for 30 minutes, retrieved and washed. MSCs were then seeded onto the disks and allowed to adhere for 1 hr. Adherent cells were visualized by co-staining with phalloidin and DAPI, and cells were quantified by counting multiple fields from 5 implants per treatment group. Values represent means + SEMs (* $p < 0.05$ relative to UNC)

Fig. 2. New bone synthesis on HA tibial implants





HA disks were either left uncoated (UNC) or coated with 1 µg/mL RGD (low), or 1000 µg/mL RGD (high). The disks were then implanted into rat tibiae for 5 days. The tibiae, with implants in place, were subsequently excised, plastic embedded, sectioned, and stained with Goldner's Trichrome. Bioquant imaging software was used to quantify both the total amount of newly-synthesized bone, and the amount of bone directly contacting the perimeter of the implant. Data shown represent the means and SEMS for 5 retrieved implants per treatment, with at least 3 sections per implant counted (* $p<0.05$ to UNC).

Conclusions:

Although RGD peptides are widely used to functionalize biomaterials, our laboratory has found that RGD inhibits the osseointegration of HA implants. While the mechanism for this phenomenon is not currently understood, we hypothesize that integrins on the surface of MSCs bind the RGD peptides rather than the native proadhesive proteins, and are therefore unable to fully activate the integrin-dependent signaling cascades necessary for cell attachment, survival, and/or osteoblastic differentiation.