Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biomaterials 26 (2005) 7046–7056
Biomaterials
www.elsevier.com/locate/biomaterials

The effect of the addition of a polyglutamate motif to RGD on peptide tethering to hydroxyapatite and the promotion of mesenchymal stem cell adhesion

A.A. Sawyer[a], D.M. Weeks[b], S.S. Kelpke[c], M.S. McCracken[d], S.L. Bellis[a,b,*]

[a]*Department of Biomedical Engineering, University of Alabama at Birmingham, Birmingham, AL 35294, USA*
[b]*Department of Physiology and Biophysics, University of Alabama at Birmingham, 1918 University Blvd., McCallum Building Room 982, Birmingham, AL 35294, USA*
[c]*Department of Surgery-Transplantation, University of Alabama at Birmingham, Birmingham, AL 35294, USA*
[d]*Department of Prosthodontics and Biomaterials, University of Alabama at Birmingham, Birmingham, AL 35294, USA*

Received 4 January 2005; accepted 5 May 2005
Available online 16 June 2005

Abstract

Mimicking endogenous bone-binding proteins, RGD peptides have been synthesized with polyacidic amino acid domains in order to ionically tether the peptides to bone-like synthetic biomaterials, including hydroxyapatite (HA). However, a direct comparison of unmodified RGD with polyacidic-conjugated RGD has not been performed, and thus a benefit for the acidic domain has not been established. We evaluated the peptide/HA bond of RGD peptides with and without an attached polyglutamate sequence (E_7), as well as examined mesenchymal stem cell (MSC) adhesion and morphology as they were affected by the conjugated peptide. We found that significantly more E_7RGD was bound to HA than RGD at all coating concentrations tested, and moreover, more E_7RGD was retained on the HA surface even after extended washing in serum-free media. Consistent with in vitro results, higher levels of E_7RGD than RGD remained on HA that had been implanted in vivo for 24 h, indicating that the polyacidic domain improved peptide-binding efficiency. At several peptide concentrations, E_7RGD increased cell adhesion compared to RGD surfaces, establishing a biological benefit for the E_7 modification. In addition, HA pre-coated sequentially with low-density E_7RGD (1–10 μg/ml) and serum (FBS) stimulated cell adhesion and spreading, compared to either coating alone, suggesting that an ionic linkage allows for the potential adsorption of serum proteins to unoccupied sites, which may be important for bone formation in vivo. Collectively, these results suggest that tethering peptides to HA via a polyglutamate domain is an effective method for improving the peptide/HA bond, as well as for enhancing MSC adhesion.

Keywords: Hydroxyapatite; Mesenchymal stem cell; RGD peptide; Cell adhesion; Cell spreading; Bone

1. Introduction

A principal goal of tissue engineering is to repair or replace damaged tissues or organs by implanting engineered biological substitutes, or by delivering factors to the injured site that induce in vivo regeneration. The former approach often involves extracting stem cells from a patient, and growing the cells ex vivo on some type of biomaterial scaffold that is permissive for, or promotes, cell differentiation along a tissue-specific lineage. Not surprisingly, there is currently much interest in modifying scaffolds with biological entities to mimic the host environment in a way that supports tissue development. Since the finding that extracellular matrix (ECM) proteins contain signaling domains which can be synthesized as bioactive peptide fragments, many biomaterials have been modified with short peptides

*Corresponding author. Department of Physiology and Biophysics, UAB, 1918 University Blvd, McCallum Building Room 982, Birmingham, AL, 35294-0005, USA. Tel.: 205 934 3441; fax: 205 975 9028.
E-mail address: bellis@physiology.uab.edu (S.L. Bellis).

0142-9612/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2005.05.006

(reviewed in [1–3]) in an effort to enhance cell-material interactions, and mimic the role of ECM proteins. The best studied of these peptides is the RGD sequence, a motif that binds to integrin cell adhesion receptors and thereby promotes cell attachment to the material surface. Several in vivo studies have shown that osseointegration of implant biomaterials, including titanium [4–6], polymers [7,8], and hydrogels [9], is significantly improved when RGD peptides are coupled to the biomaterial surface prior to implantation.

In order to achieve successful cell–material interactions, pro-adhesive peptides like RGD must be anchored strongly to the biomaterial surface. Without an efficient linkage to the underlying substrate, cells can remove mobile integrin ligands by internalization [10,11], disruption of focal adhesion formation can occur if the ligands are unable to withstand cell contractile forces [12–14], and weakly bound peptides could release into solution and actually bind cell surface integrins, blocking cell adhesion to the biomaterial [15]. The most common method of peptide immobilization on an inert biomaterial is through formation of a covalent bond between the material surface and the bioactive molecule. This is generally accomplished via functional moieties such as hydroxyl-, amino-, or carboxyl groups present on the biomaterial surface and the synthetic peptide (reviewed in [1–3]). Many metals, polymers, and silica substrates can be readily modified with peptides; however, methods for functionalizing calcium phosphate-type biomaterials, such as hydroxyapatite (HA), are limited. HA is the principal mineral constituent of the natural bone matrix, and, accordingly, synthetic versions of this material are being widely used in orthopaedic and dental applications. Numerous studies have shown that HA promotes more rapid and extensive bone formation than most other biomaterials (reviewed in [16]), and thus there is a compelling rationale for exploring the possibility that biomimetic peptides can improve the performance of this material.

In vitro studies from our laboratory have shown that RGD, when passively adsorbed to HA surfaces, can increase the adhesion of mesenchymal stem cells (MSCs), a multipotent cell type that has the ability to differentiate along the osteoblast lineage [17]. However, it is not known whether non-specifically adsorbed peptides will be able to withstand exposure to the in vivo environment for a period of time long enough to promote cell adhesion on implanted-HA biomaterials, or whether non-specific adsorption orients the RGD in a conformation that is optimal for MSC recognition.

It is known that highly acidic amino acid domains, present in many endogenous bone-binding proteins such as statherin [18], osteonectin [19,20], bone sialoprotein [21,22], osteopontin [23], and dentin phosphoryn [24], promote tight attachment to biominerals by interacting with inorganic ions and mineral surfaces. Recently, acidic amino acid sequences isolated from, or mimicking, the bone-binding regions of these proteins have been used to ionically anchor bioactive peptides in a favorable orientation on inorganic surfaces [25–28]. These sequences include natural HA-binding domains [26,27], as well as phosphonates [29], polyaspartates [30], and polyglutamates [25,28]. Even though these interactions are ionic rather than covalent, it is thought that a synthetic peptide that incorporates a polyacidic region and a cell attachment motif may stimulate cell adhesion on HA because the adhesive peptide is reliably tethered to the biomaterial surface and optimally oriented toward cell receptors [1,25,26,28].

As with non-specifically adsorbed RGD [17], HA surfaces coated with RGD peptides conjugated to a heptaglutamate domain (E_7) stimulated better adhesion and differentiation of osteoblast-like cells than uncoated HA surfaces [25,28]. However, a direct comparison of non-specifically adsorbed RGD with RGD containing an HA-binding domain has not been reported, and therefore it is not known whether an ionic linkage truly improves peptide tethering to HA, or more importantly, whether anchoring domains contribute to a biological benefit. Thus, the objectives of our study were to examine, both in vitro and in vivo, the peptide/HA bond of RGD peptides with and without an attached E_7 sequence, as well as to evaluate the effects of such a modification on cell adhesion and morphology. We find that RGD peptides conjugated with an E_7 domain bind more efficiently to HA compared to unconjugated RGD peptides and that the polyacidic region does not interfere with cell recognition of the RGD domain.

2. Materials and methods

2.1. Preparation of peptide and serum-coated disks

HA powder (HA, Fisher Scientific, Pittsburgh, PA) was pressed into disks using a 5/8″ or 1/4″ hardened steel-pressing die under 3000 1b or 2000 lb of pressure, respectively. Pressed disks were sintered at 1000 °C for 3 h and allowed to cool in the furnace at decreasing intervals. The smaller disks were used for all experiments, except cell attachment and morphology, which utilized the larger HA disks.

The peptide GPenGRGDSPCA (RGD, 948.1 g/mol), which has reported selectivity for the VN receptor [31], was synthesized by American Peptide Co., Inc. (Sunnyvale, CA). Synthesized modifications of the above peptide sequence included GPenGRGDSPCA–FITC (RGD–FITC, 1465.6 g/mol), EEEEEEEGPenGRGDSPCA (E_7RGD, 1851.9 g/mol), and EEEEEEEGPenGRGDSPCA–FITC (E_7RGD–FITC, 2369.4 g/mol). For cell blocking experiments, the peptide EEEEEEE (E_7, 921.8 g/mol) was also synthesized. The lyophilized peptides were reconstituted in deionized water