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Fibrils of different collagen types containing immobilised proteoglycans (PGs) as coatings: Characterisation and influence on osteoblast behaviour

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Abstract

Collagen, the main organic component of bone, is used as a coating on titanium implants and as a scaffold material in bone tissue engineering. Surface modifications of titanium which promote osteoblast adhesion, proliferation and synthesis of collagen by osteoblasts are desirable. One biomimetic approach is the coating of titanium with collagen in fibrillar form. Other organic components of bone may be bound to fibrils and exert additional effects. In this study, the collagen types I–III were compared regarding their ability to bind the proteoglycans decorin and biglycan, which are found in bone. More collagen was bound to collagen II fibrils than to those of types I and III. Therefore, titanium surfaces were coated with fibrils of collagen type II containing biglycan or decorin or neither to investigate the effect of the proteoglycans on human primary osteoblast behaviour. In addition, the growth factor TGF-β1 was adsorbed onto surfaces coated with fibrils of collagen type II containing biglycan or decorin or neither to investigate the influence of decorin and biglycan on the effect of TGF-β1 on osteoblasts. Fibril-bound biglycan and decorin influence primary osteoblast behaviour by themselves. The presence of substrate-bound biglycan or decorin influences the effect of TGF-β1. These results may be important when designing collagen-based coatings or scaffolds for tissue engineering, including those loaded with growth factors.

Keywords: Collagen; Decorin; Biglycan; TGF-β1; Titanium; Osteoblast

1. Introduction

Collagen, the main organic component of bone, is used as a coating on titanium implants and as a scaffold material in bone tissue engineering (Geissler et al., 2000; Roehlecke et al., 2001; Pieper et al., 1999; van Susante et al., 2001; Wollenweber et al., 2006). Proteoglycans (PGs) found in bone such as decorin and biglycan, consisting of a protein core connected to glycosaminoglycan (GAG) chains of chondroitin sulphate (CS), may bind to collagen fibrils in vitro (Schonherr et al., 1995; Pogany et al., 1994; Bierbaum et al., 2006). PGs can affect osteoblast behaviour and also bind and influence the effect of growth factors such as TGF-β1, which stimulates extra-cellular matrix production (Mundy, 1996; Hildebrand et al., 1994; Hausser et al., 1994; Takeuchi et al., 1994). This work had two principal aims. First, fibrils of the collagen types I–III were characterised with regard to the amount of decorin and biglycan bound. Secondly, the collagen type which bound the most decorin and biglycan was used to form artificial extracellular matrices for titanium surfaces in the form of coatings (a schematic view of the coatings is shown in Fig. 1) which are formed by adsorption of fibrils. The reaction of primary osteoblasts to titanium surfaces coated with fibrils containing decorin and biglycan was studied, as were the effect of TGF-β1 and the influence of decorin and biglycan on TGF-β1's effect.

2. Materials and methods

All chemicals were obtained from Sigma–Aldrich Chemie GmbH, Germany. Pepsin-treated collagen types I–III were derived from bovine skin, bovine tracheal cartilage, and human placenta, respectively. Decorin (molecular weight (MW) approximately 100 kDa, of which ca. 40 kDa is core protein and ca.

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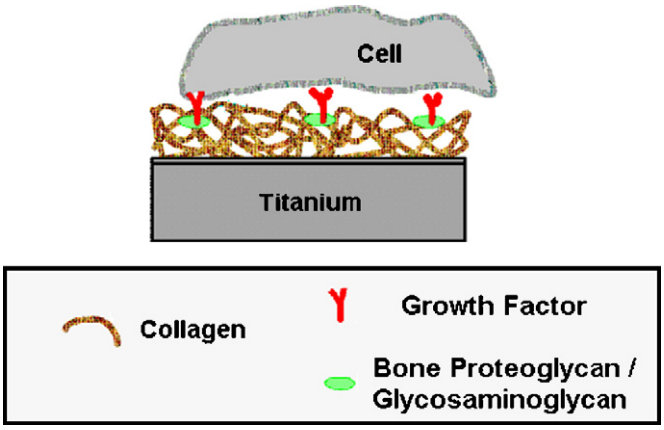

Fig. 1. Schematic view of artificial extracellular matrices in the form of coatings. Collagen fibrils containing proteoglycans (PGs) or glycosaminoglycans (GAGs) adsorb to titanium surfaces. In a second step, growth factors may adsorb prior to cell seeding.

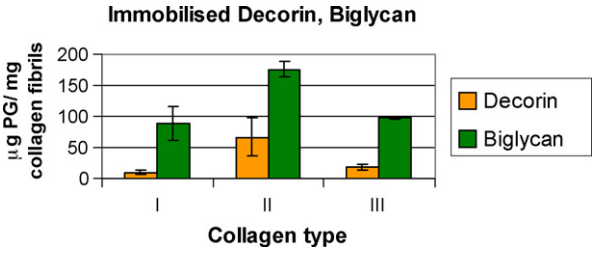

Fig. 2. Quantification of mass of decorin and biglycan immobilised per mg collagen fibrils of collagen types I–III formed. All experiments were performed in triplicate—error bars show standard deviation.

60 kDa GAG chain) and biglycan (MW 200–350 kDa, of which ca. 45 kDa is core protein and 155–295 kDa GAG chains) were both derived from bovine articular cartilage. Fibrils of collagen types I–III containing decorin and biglycan were formed at 37 °C in a 30 mM phosphate buffer at pH 7.4 at different PG:collagen ratios according to the method of Bierbaum (Bierbaum et al., 2006). PG content of fibrils was determined colormetrically according to the method of Chou (Chou et al., 2006). Fibril mass was determined using the Lowry protein assay (Lowry et al., 1951). All experiments were performed in triplicate. Fibrils were separated by centrifugation at 10,000 × *g* for 15 min and resuspended in buffer to form a suspension with a "concentration" of 1 mg fibrils/ml buffer. Titanium surfaces were coated with fibrils by adsorption from this suspension for 15 min, rinsed three times and air-dried. In a second step, TGF-β1 with a concentration of 10 ng/100 μl phosphate-buffered saline (PBS) was allowed to adsorb to fibril-coated surfaces overnight. Adsorption of both fibrils and TGF-β1 proceeded according to the methods of Fischer (Fischer et al., 2003). Subsequently, surfaces were seeded with primary osteoblasts from human knee (HKO). HKO were obtained from PromoCell GmbH, Germany and cultured in Osteoblast growth medium (PromoCell GmbH): the donor was a 76-year-old woman. Adhesion, proliferation and collagen synthesis were determined as described previously (Geissler et al., 2000). All experiments were performed three times.

3. Results

Collagen type II fibrils bound more PGs than fibrils of types I and III, and considerably more biglycan than decorin was bound by all collagen types (Fig. 2). Biglycan promoted the formation of focal adhesions (Fig. 3). Decorin and especially biglycan promoted HKO proliferation (Fig. 4) but did not appear to influence collagen synthesis (Fig. 5). TGF-β1 adsorbed onto collagen fibril coatings reduced the proliferation (Fig. 4) and enhanced the collagen synthesis of HKOs (Fig. 5). However experiments with TGF-β1 applied together with collagen fibril coatings containing biglycan and decorin yielded different results. The decrease in proliferation was lower and the increase in collagen synthesis was higher when decorin was present instead of biglycan.

4. Discussion

Collagen II bound more decorin and biglycan than collagen I and III and all three collagen types bound more biglycan than decorin (Fig. 2). Both PGs are believed to interact with collagen via their protein cores (Schonherr et al., 1995) and GAG chains (Pogany et al., 1994). It may be that the longer GAG chains of biglycan promote GAG-mediated binding as reported previously (Obrink et al., 1975; Obrink, 1973; Obrink and Sundelof, 1973). It has been reported in the literature that collagen II has a higher affinity than collagen I for biglycan (Douglas et al., 2006; Bidanset et al., 1992; Vynios et al., 2001) and CS (Negroiu et al., 1992).

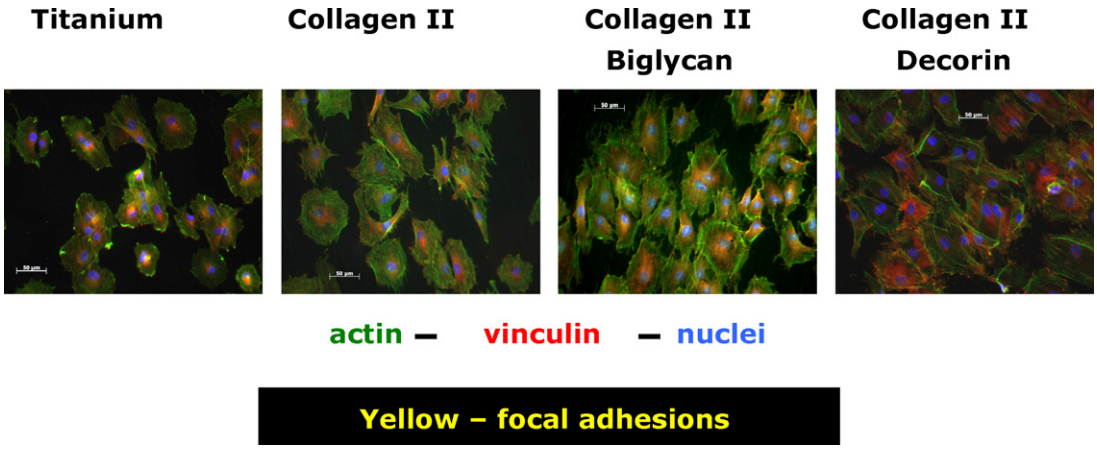

Fig. 3. Formation of focal adhesions by rat osteoblasts. Actin, vinculin and nuclei appear green, red and blue, respectively. A yellow colour indicates the formation of focal adhesions. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)