Carbohydrate Polymers 98 (2013) 665–676

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Review

Chitosan stabilizes platelet growth factors and modulates stem cell differentiation toward tissue regeneration

Alberto Busilacchi [1], Antonio Gigante [1], Monica Mattioli-Belmonte [1], Sandra Manzotti [1], Riccardo A.A. Muzzarelli *

Department of Clinical and Molecular Sciences, Università Politecnica delle Marche, Via Tronto 10-A, IT-60126 Ancona, Italy

ARTICLE INFO

Article history:
Received 15 May 2013
Received in revised form 17 June 2013
Accepted 19 June 2013
Available online xxx

Keywords:
Chitosan
Platelets
Growth factors
Stem cells
Wound healing
Tissue regeneration

ABSTRACT

The idea of using chitosan as a functional delivery aid to support simultaneously PRP, stem cells and growth factors (GF) is associated with the intention to use morphogenic biomaterials to modulate the natural healing sequence in bone and other tissues. For example, chitosan–chondroitin sulfate loaded with platelet lysate was included in a poly(D,L-lactate) foam that was then seeded with human adipose-derived stem cells and cultured *in vitro* under osteogenic stimulus: the platelet lysate provided to the bone tissue the most suitable assortment of GF which induces the osteogenic differentiation of the mesenchymal stem cells. PDGF, FGF, IGF and TGF-β were protagonists in the repair of callus fractures. The release of GF from the composites of chitosan–PRP and either nano-hydroxyapatite or tricalcium phosphate was highly beneficial for enhancing MSC proliferation and differentiation, thus qualifying chitosan as an excellent vehicle. A number of biochemical characteristics of chitosan exert synergism with stem cells in the regeneration of soft tissues.

Contents

Abbreviations: ADSC, adipose derived stem cell; AFM, atomic force microscopy; ALP, alkaline phosphatase; BMP, bone morphogenetic protein; BMSC, bone marrow stem cells; $Ca_3P_2O_8$, tricalcium orthophosphate; DMEM, Dulbecco modified Eagle's medium; ECM, extracellular matrix; ESC, embryonic stem cells; FGF, fibroblast growth factor; GAG, glycosaminoglycans; GF, growth factors; GFP, green fluorescent protein; H&E, hematoxylin and eosin; HPC, hematopoietic progenitor cells; HSC, hematopoietic stem cells; IGF, insulin-like growth factor; NMI-chitosan, N-methylimidazole chitosan; MSC, mesenchymal stem cell; PDGF, platelet derived growth factor; PDLSC, periodontal ligament stem cells; PRP, platelet-rich plasma; RGD, Arg-Gly-Asp tripeptide; SA-chitosan, salicylate-modified chitosan; SOD, superoxide dismutase; TGF-β, transforming growth factor-β; VEGF, vascular endothelial growth factor; TXA_2, thromboxane A_2.

* Corresponding author. Tel.: +39 071 36206/220 6068; fax: +39 071 36206/220 6066; mobile: +39 3480379279.
E-mail address: muzzarelli.raa@gmail.com (R.A.A. Muzzarelli).
URL: http://www.chitin.it (R.A.A. Muzzarelli).
[1] Tel.: +39 071 220 6068; fax: +39 071 220 6066.

0144-8617/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2013.06.044

1. Introduction

The present article reviews the associations of chitosans (a family of cationic partly acetylated and optionally modified (1-4)-2-amino-2-deoxy-β-D-glucans) with growth factors, either from platelets or stem cells, mainly intended for the regeneration of bone and soft tissues.

Chitosans and platelets were described for the first time by C. Rouget (1859) and G. Bizzozero (1881), respectively (Coller, 1984; Gravela, 1989; Muzzarelli, 1977; Muzzarelli, Boudrant, et al., 2012; Paweletz, 2001; Ribatti & Crivellato, 2007). Nearly one century had to elapse before platelets were studied for the development of wound dressing endowed with exceptionally high hemostatic activity, manufactured from chitosans from marine diatoms and crustaceans.

In particular the diatom chitin/chitosan fibers were found to have superior hemostatic activity compared to the other chitosans (Fischer, Bode, Demcheva, & Vournakis, 2007). Examples include Syvek-Patch® whose chitin fibers are obtained from aseptic culture of the diatom *Thalassiosira fluviatilis*, and hydrogels consisting of either partially or fully deacetylated chitosans. Chitin/chitosan fibers tightly bind major plasma proteins and a specific sub-set of platelet surface proteins, resulting in the acceleration of fibrin gel formation when platelet integrins contact plasma protein-saturated chitin/chitosan fibers (Fischer et al., 2005). The essential reasons for the exceptional performances reside in the chemical nature and tertiary/quaternary structure of the chitin/chitosan whose surface is recognized by the platelets: *via* the staining of the activation marker P-selectin, it was possible to reveal the sudden activation of the platelets whose pseudopodia make a robust contact immediately upon activation.

In a time scale of nano- to micro-seconds, the consequences are the start of the coagulation cascade, the red blood cell agglutination, thrombin generation and fibrin mesh synthesis in the microenvironment of the polysaccharide. The mechanical constriction is then augmented by the vasoconstriction generated by the platelets through the clot retraction.

A number of manufacturers are today producing chitosan-based hemostatic bandages approved by FDA and certified by CE and NATO (see Table 2 in Muzzarelli, 2009), currently used in emergency and under extreme conditions in consideration of their exceptional performances (Devlin, Kircher, Kozen, Littlejohn, & Johnson, 2011; Klokkevold, Fukayama, Sung, & Bertolami, 1999; Pozza & Millner, 2011; Pusateri et al., 2003; Sugamori, Iwase, Maeda, Inoue, & Kurosawa, 2000; Weiner, Fischer, & Waxman, 2003; Whang, Kirsch, Zhu, Yang, & Hudson, 2005). This applied research area developed at a very high pace, while the basic knowledge on chitosan combined with platelet-rich plasma or single GF for tissue regeneration progressed slowly.

Many GF have well established roles in embryonic development and skeletal homeostasis. Angiogenic factors are important in consideration of the transport of oxygen, nutrients and circulating cells: thus, angiogenesis is regulated by soluble compounds such as VEGF, PDGF, FGF and IGF. Among osteogenic factors, the members of the highly conserved TGF-β superfamily play an important role in embryonic development, tissue morphogenesis, cell proliferation and cell differentiation; TGF-β1 and TGF-β2 inhibit bone resorption and osteoclast activity, and trigger rapid maturation of collagen in early wounds. Because fracture healing is characterized by inflammation, renewal and remodeling phases, the control of inflammation involves the manipulation of pro-inflammatory cytokines and growth factors temporally and spatially released following bone injury. It was shown that inflammatory compounds including TNF-α, interleukins, interferon-γ and prostaglandins stimulate the migration and differentiation of osteoblasts and osteoclasts. Several authors discussed the role of various factors in bone regeneration, and examined the requirements for protein, cell and gene therapy for bone regeneration, with emphasis on the materials and methods for controlled delivery (Panuganti, Schlinker, Lindholm, Papoutsakis, & Miller, 2013; Takayama & Eto, 2012; Vo, Kasper, & Mikos, 2012).

Pessimistic forecasts on the application of GF in the management of chronic ulcers were based on the rationale that GF are inactivated by high levels of MMP in the ulcers, have scarce bioavailability and high manufacturing cost (Sweeney, Miraftab, & Collyer, 2012). Therefore, their use would be justified only in the presence of a functional delivery aid or scaffold capable to offset said drawbacks, or even enhance the activity of the GF: the covalent immobilization of BMP-2 onto chitosan nanofibers imparts higher bioactivity, and greater cell proliferation, when compared with plain absorption (Zustiak, Wei, & Leach, 2013). Moreover, chitosan is an inhibitor of MMP (Kim, Ahn, Kong, & Kim, 2012).

Besides the inhibitory activity against MMP, characteristic properties of chitosan appreciated for tissue engineering in regenerative medicine are the following: atoxicity; biodegradability; availability in various physical forms; chemical and enzymatic versatility; mucoadhesivity; suitability for controlled release of cytokines, extracellular matrix components and antibiotics and for retention of the normal cell morphology; entrapment of GF to accelerate the healing; stimulation of integrin-mediated cell motility and increased *in vitro* angiogenesis; integrin-dependent regulation of the pro-angiogenic transcription factor Ets1; promotion of the attachment, proliferation and viability of seeded stem cells.

Basic information on chitins and chitosans is amply available in the bibliographies of most recent review articles (Muzzarelli, Boudrant, et al., 2012; Muzzarelli, Greco, Busilacchi, Sollazzo, & Gigante, 2012; Ravi Kumar, Muzzarelli, Muzzarelli, Sashiwa, & Domb, 2004). In particular, the following characteristics that qualify chitosans in wound healing can be recalled: chemoattraction and activation of macrophages and neutrophils to initiate the healing process; promotion of granulation tissue and re-epithelialization; limitation of scar formation and retraction; analgesic and hemostatic activities; activation of immunocytes; release of glucosamine and N-acetylglucosamine monomers and oligomers that stimulate cellular activities besides being used as building blocks in the synthesis of the ECM; intrinsic antimicrobial activity.

Platelet-rich plasma has been proposed for therapeutic purposes and for tissue engineering as well (Borzini & Mazzucco, 2005; Crovetti, Martinelli, & Issi, 2004; Ehrenfest, Rasmusson, & Albrektsson, 2009; Gigante et al., 2010, 2012; Marx, 2004; Mazzucco, Balbo, Cattana, Guaschino, & Borzini, 2009; Okamoto et al. (2003); Rodriguez-Merchan, 2012; Whitaker, Quirk, Howdle, & Shakesheff, 2001). PRP is obtained easily on the day of surgery from autologous whole blood (Kutlu, Aydin, Akman, Gumusderelioglu, & Nohutcu, 2013; Weibrich, Kleis, & Hafner, 2002), thus the autologous origin excludes the risk of transmission of diseases (Soyer, Cakmak, Aslan, Senyucel, & Kisa, 2013).

GF tend to be released quickly from PRP, and as a consequence, they soon lose their activity, and their clinical efficacy. Although centrifugation, freeze-thawing and thermal cycles are simple operations (Leytin, Mody, Semple, Garvey, & Freedman, 2000; Picker, 2011; Zaky, Ottonello, Strada, Cancedda, & Mastrogiacomo, 2008) the handling requires precautions, because the platelet density values in PRP may change according to the preparation technique, ranging from 2- to 7-fold above physiological levels (Santo, Duarte, et al., 2012; Santo, Gomes, et al., 2012). Therefore, a major concern comes from the fact that hyper-concentration of platelets induces further platelet activation and leads to decrease or loss of platelet functionality (Gollehon, King, & Craig, 1998). For these reasons, scaffolds suitable for the stabilization of platelets and GF are sought.