Journal of Non-Crystalline Solids xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Journal of Non-Crystalline Solids

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

Collagen/fibrin microbeads as a delivery system for Ag-doped bioactive glass and DPSCs for potential applications in dentistry

Xanthippi Chatzistavrou [a,*], Rameshwar R. Rao [b], David J. Caldwell [b], Alexis W. Peterson [b], Blake McAlpin [a], Yuan-Yuan Wang [a], Li Zheng [a], J. Christopher Fenno [c], Jan P. Stegemann [b], Petros Papagerakis [a,*]

[a] Orthodontics and Pediatric Dentistry, School of Dentistry, University of Michigan, Ann Arbor, MI, USA
[b] Department of Biomedical Engineering, University of Michigan, Ann Arbor, MI, USA
[c] Biologic & Materials Sciences, School of Dentistry, University of Michigan, Ann Arbor, MI, USA

ARTICLE INFO

Article history:
Received 30 October 2014
Received in revised form 9 March 2015
Accepted 24 March 2015
Available online xxxx

Keywords:
Collagen/fibrin microbeads;
Silver doped bioactive glass;
Dental Pulp Stem Cells;
Antibacterial properties;
Cell viability;
Cell differentiation

ABSTRACT

Silver doped bioactive glass (Ag-BG) and Dental Pulp Stem Cells (DPSCs) have shown promising properties for dental tissue regeneration. This study presents the fabrication and characterization of composite collagen/fibrin microbeads that aimed to incorporate and successfully deliver both Ag-BG and DPSC. Cell viability, cell differentiation in vitro and in vivo, and antibacterial properties of the systems against *Escherichia coli* (*E. coli*), *Streptococcus mutans* (*S. mutans*) and *Enterococcus faecalis* (*E. faecalis*) were evaluated. Cell viability and proliferation of DPSCs within the delivery system were found unaltered in both combinations (Beads/DPSCs and Beads/DPSCs/Ag-BG). Our results show that Ag-BG and DPSCs were successfully incorporated and proliferated within the collagen/fibrin microbeads. DPSCs continued to proliferate up to 6-weeks in cell culture but cell differentiation into odontoblast-like cells was observed only in vivo. The beads containing Ag-BG strongly inhibit bacterial growth in the experimental groups (beads alone, Beads/Ag-BG and Beads/DPSCs/Ag-BG). Our data suggests that collagen/fibrin microbead paste could have clinical applicability in the regenerative dentistry field.

1. Introduction

Cell-based therapy is a promising approach in tissue regeneration. However, a purely cellular treatment cannot be always applied. This leads to an increased interest in using a combination of cells, matrix and other bioactive agents [1,2]. Aguado et al. have shown that direct injection of naked cells decreases cell viability, while hydrogel carriers can prevent this result [3]. A defined extracellular matrix can be used as a delivery vehicle, providing space-filling properties. Appropriate biomaterials incorporating stem and progenitor cells have been already used extensively in order to both maintain their self-renewal [4] and direct their function [5,6]. The composition of the matrix that surrounds the cells is important, as it could regulate cell–matrix interactions, and could potentially be used to control cell differentiation. As such proteins and naturally derived polysaccharides find applications in these approaches due to their biological relevance and biocompatibility.

Cellular microenvironments as hydrogels have extensively been applied in tissue engineering [7]. The development of "microbeads" that can mimic the function of a 3D tissue has been suggested as a way to culture and deliver cells in a minimally invasive manner for tissue regeneration [8]. Microbeads are comprised of biological macromolecules that surround the encapsulated cells [9,10], while a cell-friendly process is required in which cell viability can be maintained, simultaneously with the creation of robust and functional structures. Moreover these structures can promote specific cell phenotypes and subsequent self-assembly of microbeads into larger tissue structures [11–13]. Cell function can be guided and determined by many different factors such as the composition of the microbead, the cell density, the mechanical properties of the matrix, and the incorporation of other entities as growth factors and/or inorganic bioactive particles. Consequently, cell compatibility could be enhanced and cell adhesion, proliferation and/or differentiation could be promoted, by using naturally derived materials such as the microbead matrix and tailoring their properties (density, stiffness, cohesiveness) in order to control their macro-size characteristics [14,15].

Moreover, hydrogel microbeads have been already fabricated incorporating cells within spherical units of defined extracellular matrix, including collagen, fibrin, agarose, and chitosan [14,16]. In some cases, cells have been successfully embedded directly inside the microbeads, including microbeads made of alginate and agarose [17,18]. However, this surrounding insoluble matrix cannot provide biological cues to incorporated mammalian cells, as these cells do not have receptors for attachment to these polysaccharide materials. Because of this, other materials like fibrin, collagen, as well as blends of materials, have been used in order to enhance cell–matrix interactions with microbeads [14,19–21].

* Corresponding authors.
E-mail addresses: xchatzis@umich.edu (X. Chatzistavrou), petrosp@umich.edu (P. Papagerakis).

http://dx.doi.org/10.1016/j.jnoncrysol.2015.03.024
0022-3093/

Fibrin is a clotting protein derived from the blood plasma protein fibrinogen and is significant in the wound healing process [22,23]. Fibrin has been used in different applications in the field of tissue engineering and regenerative medicine, where specific physical and biological properties are required [24]. The application of fibrin can be grouped into three main areas: fibrin as cell matrix material alone, fibrin as a cell matrix material combined with a polymeric scaffold, and as a delivery system for growth factors or other therapeutic agents. The fibrin system has been used as scaffold component and cell delivery vehicle in vivo because of fibrin's ability to support cell adhesion, growth and differentiated function [25]. In particular, fibrin is already used in the tissue engineering of skin, vascular, bone and cartilage. However, fibrin could not be used alone on fabricating robust cellular scaffolds due to low mechanical properties [26]. Additionally, collagen is of particular interest, as it is a naturally derived material, biodegradable and biocompatible [27,28], and has been investigated for numerous applications as well, such as in vascular [29], skin [30], ligament [31], and bone [32] tissue engineering. Applications of collagen in bone tissue regeneration showed that it acts as a bioactive component supporting cell attachment, proliferation and initiating osteogenic differentiation [14].

Apart from cell delivery, microbeads have been also used for delivering inorganic bioactive materials. This approach aims to enhance their bioactive characteristics and to increase their capability of inducing cell differentiation and consequently tissue regeneration [33]. A bioactive and antibacterial sol–gel derived bioactive glass in the system SiO_2 58.6–CaO 24.9–P_2O_5 7.2–Al_2O_3 4.2–Na_2O 1.5–K_2O 1.5–Ag_2O 2.1 wt.% has been already studied for applications in tissue engineering [34]. The developed Ag-doped bioactive glass (Ag-BG) possesses strong and long lasting antibacterial properties combined with the bioactive behavior [35]. These characteristics make Ag-BG a very promising bioactive glass in tissue engineering. Natural extracellular matrix (ECM) has been used as an advanced vehicle for the delivery of Ag-BG. The new ECM/Ag-BG composites showed enhanced antibacterial and bioactive properties [35]. However the additional incorporation of cells for simultaneous cell delivery has not been studied. Additionally, the capability of these systems to guide cell behavior is unclear.

We hypothesized that cells combined with bioactive and antibacterial glass incorporated into biodegradable microbeads could be directly injected into a defect area in a minimally invasive procedure with little stress for the patient. Based on this, the aim of this work was to develop a new system for delivering cells combined with an antibacterial and bioactive agent for applications in tissue regeneration of defect areas. The emulsification method was applied to fabricate early microbead formulations made of collagen/fibrin, containing embedded human Dental Pulp Stem Cells (DPSCs) and Ag-BG. The resulting systems were characterized in terms of cell viability, proliferation, morphology, antibacterial activity and cell differentiation.

2. Methods

2.1. Fabrication

Collagen/fibrin beads were fabricated as previously described [36]. Bovine type I collagen (MP Biomedicals, Solon, OH) was dissolved at a concentration of 4.0 mg/ml in 0.02 N acetic acid. Bovine fibrinogen (Sigma Aldrich, St. Louis, MO) was dissolved in serum-free Dulbecco's modified Eagle's medium (DMEM, Thermo Scientific, Logan, UT) at a concentration of 4.0 mg/ml clottable protein. Fibrin and collagen were combined to form a total protein concentration of 2.5 mg/ml (mass ratio 50/50) and added to a mixture of 2% bovine thrombin (1 UT/ml, Sigma), 5% 0.1 N NaOH, 10% 5×-concentrated DMEM, 10% FBS, and 1.0 mM glyoxal (Sigma). Ag-BG particles were placed in a sonication water bath for 1 h prior to incorporation into microbeads to disrupt particle aggregates [37]. Dental Pulp Stem Cells (DPSCs) were incorporated into microbeads (Beads/DPSCs) at a cell concentration of (1×10^6 cells/ml), while microbeads with DPSCs and Ag-doped bioactive glass (Ag-BG 2.5 mg/ml) were also fabricated (Beads/Ag-BG/DPSCs) by adding both cells and Ag-doped bioglass directly to the gel mixture prior to emulsification. The cell–microbead mixture was then pipetted into a bath of 100 cSt polydimethylsiloxane (PDMS, Xiameter, Dow Corning), that was cooled to 4 °C and stirred at 600 rpm for 5 min with a double-bladed impeller. Concurrent gelation of the fibrin and collagen components was then induced by raising the temperature of the bath to 37 °C. Microbeads were removed from the oil phase by centrifuging the mixture at 200 ×*g* for 5 min and then washing the mixture with phosphate buffered saline (PBS, Life Technologies) supplemented with Pluronic L101 (BASF, Florham Park, NJ). Cell viability, proliferation, and cell morphology with and without the presence of Ag-BG were observed by live/dead staining (Life Technologies) and actin/nuclei staining (Life Technologies). The morphology of the beads was observed with a reflectance confocal microscope.

Ag-BG fabrication has been already presented in detail elsewhere [34]. Briefly, the fabrication protocol is based on the sol–gel process by mixing the solution stage of the 58S sol–gel bioactive glass (in the system SiO_2 58–CaO 33–P_2O_5 9 wt.%), with the respective solution stage of the sol–gel glass in the system SiO_2 60–CaO 6–P_2O_5 3–Al_2O_3 14–Na_2O 5–K_2O 5–Ag_2O 7 wt.%, as it has been presented in detail in our previous work [38,39]. After extended stirring, the final homogeneous solution follows a specific heat treatment: aging at 60 °C, drying at 180 °C and stabilization up to 700 °C. The final sol–gel derived Ag-doped bioactive glass (Ag-BG) is in the system SiO_2 58.6–CaO 24.9–P_2O_5 7.2–Al_2O_3 4.2–Na_2O 1.5–K_2O 1.5–Ag_2O 2.1 wt.% [34]. The novel Ag-BG is fabricated in powder form with particle size around ~25 μm.

The laboratory of Dr. Kaigler at the University of Michigan kindly provided DPSCs. DPSCs were isolated from pulp tissue that was gently separated from the crown and root, and subsequently digested in a solution of 3 mg/ml collagenase type I (Sigma, St. Louis, MO, USA) and 4 mg/ml dispase (Sigma, St. Louis, MO, USA) for 1 h at 37 °C. Single-cell suspensions were obtained by passing the cells through a 70-mm strainer (Falcon). Single-cell suspensions (0.5 to 1.0×10^3/well) were seeded onto 6-well plates (Costar) containing alpha modification of Eagle's medium (GIBCO/BRL) supplemented with 15% fetal bovine serum (FBS; Hyclone), 100 U/ml penicillin and 100 mg/ml streptomycin (Sigma, St. Louis, MO, USA), and incubated at 37 °C in 5% CO_2. The DPSCs were confirmed to be actually stem cells according to the methods described previously [40]. DPSCs were then cultured at 37 °C in T75 flasks.

2.2. DPSC odontogenic differentiation in vitro and in vivo

Previous studies of solid polymer scaffolds have shown that differentiation of DPSC may be stimulated by the presence of a tooth slice [41]. In our study, we used sterilized pulpless tooth slices incubated in culture medium to stimulate the odontogenic differentiation. Beads with Ag-BG and DPSCs, as well as DPSCs alone (as a control group) were seeded in the hole of the tooth slice. Regular and differentiation cell culture media were used for culture up to 6 weeks.

The same sample groups seeded in tooth slices were studied in vivo. An approved protocol from the University Committee on and Use and Care of Animals (UCUCA) for small animals was applied during this study. Beads were implanted into the dorsal surfaces of 10-week-old mice with severe combined immunodeficiency (CB-17 scid mice, NIH-bg-nu-xid; Harlan–Sprague–Dawley; Harlan Laboratories, Indianapolis, IN). The implants were recovered after 8 weeks, then fixed with 4% formalin, decalcified with buffered 10% EDTA (pH 8.0), and finally they were embedded in paraffin. Sections (5 mm) were deparaffinized and stained with hematoxylin–eosin.

2.3. Antibacterial activity

The bactericidal properties of the three groups: microbeads alone (Beads), microbeads with Ag-BG (Beads/Ag-BG) and microbeads incorporating Ag-BG and DPSCs (Beads/Ag-BG/DPSCs) were tested against

Please cite this article as: X. Chatzistavrou, et al., Collagen/fibrin microbeads as a delivery system for Ag-doped bioactive glass and DPSCs for potential applications in dentistry, J. Non-Cryst. Solids (2015), http://dx.doi.org/10.1016/j.jnoncrysol.2015.03.024