Colloids and Surfaces B: Biointerfaces 156 (2017) 221–226

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Scaffold requirements for periodontal regeneration with enamel matrix derivative proteins

Alessandra Apicella[a,1], Peggy Heunemann[b,2], Laurent Dejace[a], Matteo Marascio[a], Christopher J.G. Plummer[a,*], Peter Fischer[b,*]

[a] Laboratoire des Technologie des Composites et Polymères (LTC), Ecole Polytechnique Fédérale de Lausanne (EPFL), 1015 Lausanne, Switzerland
[b] Institute of Food, Nutrition and Health, ETH Zurich, 8092 Zurich, Switzerland

ARTICLE INFO

Article history:
Received 27 November 2016
Received in revised form 4 May 2017
Accepted 6 May 2017
Available online 9 May 2017

Keywords:
EMD
Bone grafts
Protein precipitation
pH
Protein aggregation

ABSTRACT

Periodontitis affects the attachment of natural teeth, and infection or inflammation associated with periodontitis may affect peri-implant tissues. Enamel matrix derivative (EMD) proteins provide stimulation for self-regeneration of the damaged tissue when applied to wide intrabony defects as part of a mixture with bone graft material. As a first step of the process enhancing cell proliferation and ligament formation, we demonstrated that EMD protein precipitation depends strongly on the physical and chemical characteristics of the bone grafts used in the mixture. To guarantee optimum protein-stimulated self-regulation, the pH of the initial EMD formulation must therefore be adjusted between 3.9 and 4.2 in order to compensate the change in pH induced by the bone graft. Moreover, the interaction between the two components resulted in precipitates of different shape and size differently covering the grafts. This outcome might potentially have clinical implications on cell attachment and periodontal ligament extension, which deserve further in vitro and in vivo tests.

1. Introduction

Periodontitis is an inflammatory condition of the periodontium, the tissue that surrounds and supports teeth. Between 2009 and 2012 it affected around 46% of the adult population in the US, causing mild, moderate and severe loss in tooth attachment [10]. Several methods have been developed for the restoration of periodontal tissue. Periodontal debridement remains the standard in the treatment of this inflammatory process for initial therapy in non-surgical and surgical treatment [8,41]. Together with new methods and technologies it represents a viable treatment on the long term for maintaining attachment levels [34]. In case of severe periodontitis generating highly deep pockets, surgery is preferred. However, it has been demonstrated that the combination of surgical procedures with periodontal debridement results in greater probing depth reduction [1]. Despite its success, regeneration induced by surgical procedures is not fully predictable yet. For this reason, scientists and medical doctors actively look for new methods and materials to combine with surgical procedures promoting regeneration. As a result, the focus of the clinical research shifted from a purely surgical to biologically oriented treatment of periodontal disease and bone defects [42].

In this landscape, enamel matrix derivative (EMD) proteins have been tested in non-surgical and surgical procedures for periodontal tissues regeneration. Porcine EMD is a purified acid extract obtained from 6-month-old pig tooth germs. Although, there was no significant benefit of EMD adjunctive to non-surgical procedures [13,29], it has been found to show unique healing and regenerative properties [22] combined with surgery. Thus, patients treated with EMD have experienced significant regeneration of the damaged tissue in as little as a few months. The regeneration process begins with application of the EMD to the defect site, where physiological conditions cause the proteins to precipitate. The precipitated EMD has been demonstrated to favor cell proliferation and extension of ligaments from the periodontium, filling in the defect [14,7,33]. In order to provide the product with the necessary rheological properties for the application, a combination of the protein matrix with a polymer acting as a carrier has been also developed [7,11,15,31]. However, wide bone defects at the tooth attachment site often require application of bone grafts [24]. Indeed, any product intended for the regeneration of the tissue should not only mediate regeneration, but also create an environment capable of

* Corresponding author.
E-mail addresses: christopher.plummer@epfl.ch (C.J.G. Plummer), peter.fischer@hest.ethz.ch (P. Fischer).
[1] Present address: Swiss Integrative Center for Human Health (SICHH), Passage du Cardinal 13B, 1700 Fribourg, Switzerland.
[2] Present address: Novartis Pharma Stein AG, Schaffhauserstr. 101, Postfach 4332 Stein, Switzerland.

http://dx.doi.org/10.1016/j.colsurfb.2017.05.013
0927-7765/

supporting structures [27,43]. One of the limitations of EMD is its semifluid formulation, which prevents the space maintenance typical of grafts materials. Combinations of EMD with bone graft materials may represent a solution [16,21,25,27].

Within the last few years many authors focused their works on the therapeutic effect of EMD combined with different kinds of grafts. Bovine grafts [17–19,5,35,38,40], alloplastic synthetic bone graft (BG) [39], and demineralized or mineralized freeze-dried bone allografts [32,6] have been tested and they have all shown good osteoconduction, providing suitable surfaces for cell attachment and proliferation. The combination of EMD with different bone graft materials has been also investigated, and a review by Miron summarizes the most important results from in vitro, in vivo and clinical studies [26]. A main conclusion of this review is that combining EMD with bone grafts does not appear to result in quantitatively or quantitatively improved regeneration, nor does it reduce process times with respect to separate application of the individual components. On the other hand, Matarasso et al. [23] conclude that combining EMD with bone grafts may result in additional clinical improvements as higher clinical attachment and probing depth reduction. However, there remains a lack of fundamental insight into how materials properties and interactions might be tailored in order to create useful synergy between the EMD and the bone graft.

A fundamental requirement is the EMD precipitation as it triggers cells proliferation and periodontal ligament extension at the site of the defect. As discussed in detail in our previous work, the influence of environmental factors such as pH and temperature on EMD precipitation, and the folding and unfolding of its constituent proteins is thought to be crucial for the effectiveness of EMD-based products, precipitation of the EMD without excessive unfolding being important for its therapeutic activity [3]. It follows that the conditions under which EMD is mixed with bone graft materials should be chosen so as to promote EMD precipitation in its folded state, as well as homogenous distribution of the EMD precipitates at the bone graft surface and within its volume. Indeed, the quality of the interaction between the grafts and the EMD could play a key role in enhancing cells attachment and periodontal ligament extension having clinical returns. To our knowledge, there is not any study focusing on this kind of analysis so far.

In the present work we have investigated the behavior of EMD in combination with natural bone graft material and a calcium phosphate bone graft substitute. Emphasis has been laid to the evolution of the pH after mixing, aggregate and precipitate sizes, conformational changes, and the influence of porosity on the distribution of the EMD at the surface and in the volume of the bone graft granules. Our main goal was to highlight some of the materials properties playing a fundamental role in the interaction between EMD and grafts, which could be of potential clinical interest. Selected specimens were also modified by addition of arginine, which we have shown previously to stabilize EMD with respect to unfolding, without impeding precipitation at physiological pH [3].

2. Experimental

2.1. Materials

EMD solution (31 mg/mL, pH 4.8) was provided by Biora-Straumann (Malmo, Sweden). It is the active component of EMDOGAIN® gel commercially distributed by Straumann AG. Specimens were prepared at different initial pH (3.4, 3.7, 3.9, and 4.2) by addition of acetic acid (200, 26, 24, and 8 μL, respectively) to 2 mL of the original EMD solution. The final concentration of each specimen was maintained at 31 mg/mL for a final volume of 100 mL.

0.5 M of arginine was added to 50 mL of each specimen as described elsewhere [3]. The bone graft substrates selected for investigation were spongious natural bone granules with a diameter between 0.25 and 1 mm (SNB – Geistlich Bio-Oss®) and hydroxyapatite-based granules (HB – RevisiOs BV OsOpia®) with diameters of between 0.25 and 1 mm. These were added to the EMD and EMD-arginine solutions at a concentration of 1 g/mL. The evolution of the pH of the EMD and EMD-arginine solutions and the extent of precipitation were monitored before and after addition of the bone graft.

2.2. Measurement of the pH

5 mL of Milli-Q water were added to freshly mixed EMD- and EMD-arginine-bone graft specimens in order to increase the volume sufficiently for measurement of the subsequent changes in pH (Mettler Toledo MP120pH). The total measurement time was approximately 24 h, measurements initially being taken at intervals of a few minutes, and then at progressively greater intervals up to about 15 h (t = 0, 15, 30, 60, 180, 360, 600, 900 min). All measurements were performed at least threefold at room temperature and mean values are reported.

2.3. Fluorescence spectroscopy

Fluorescence spectroscopy was used to quantify the extent of EMD aggregation subsequent to mixing with the bone graft materials. A kit provided by Enzo Life Science for measuring protein aggregation was used to prepare the specimens. The procedure involved mixing ProteoStat® Protein Aggregation Assay with a specific dye for aggregation detection and Milli-Q water in the proportions: 20 μL aggregation assay/10 μL dye/170 μL Milli-Q water. 2 μL of the resulting solutions were added to 100 μL of the EMD or EMD-arginine specimens on a plastic plate for fluorescence detection. The same procedure was used to prepare specimens containing 100 mg of SNB and HB granules. The fluorescence measurements were made at 10 min intervals at room temperature over a total time of 50 min. The time required for specimen preparation and to set up the measurement prevented data acquisition at time zero, so that the first measurement corresponds to 10 min. Measurements were performed threefold and mean values are reported.

2.4. Particle size detection

A Malvern Zetasizer was used to determine the size of the aggregates suspended in solution, and the precipitate sizes immediately after precipitation in the presence of the bone graft materials (pH 3.9 and 4.2). The measurements were performed at room temperature and in triplicate for each type of specimen.

2.5. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM)

The morphology of the EMD precipitates at the surface of the bone grafts was investigated by SEM (Philips XLF30-FEG) in secondary electron imaging mode. Drops of the specimens were placed on SEM specimen holders, dried, and gold coated. An accelerating voltage of about 3 kV was used throughout in order to avoid excessive charging and loss of definition in the surface structure.

The morphology of solvated EMD aggregates was investigated using a Tecnai Spirit BioTWIN TEM (FEI) operated at 80 kV in bright field mode. After removal from storage, selected specimens were subjected to negative staining using a two-droplet sequential method, as described in the literature [30].