Medical Hypotheses 132 (2019) 109388

Contents lists available at ScienceDirect

Medical Hypotheses

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

Is selective protein adsorption on biomaterials a viable option to promote periodontal regeneration?

Ludovica Parisi[a,b,*], Andrea Toffoli[a,b], Beatrice Mozzoni[a,b], Federico Rivara[a], Benedetta Ghezzi[a,b], Miriam Cutrera[a], Simone Lumetti[a,b], Guido M. Macaluso[a,b,c]

[a] Centro Universitario di Odontoiatria, Università di Parma, Parma, IT, Italy
[b] Dipartimento di Medicina e Chirurgia, Università di Parma, Parma, IT, Italy
[c] Istituto dei Materiali per l'Elettronica ed il Magnetismo, Consiglio Nazionale delle Ricerche, Parma, IT, Italy

A B S T R A C T

Periodontitis is an inflammatory condition that can induce significant destruction of the periodontium, the set of specialized tissues that provide nourishment and support to the teeth. According to the guided tissue regeneration principles, the periodontium can be regenerated if the spatiotemporal control of wound healing is obtained, namely the tune control of cell response.

After material implantation, protein adsorption at the interface is the first occurring biological event, which influences subsequent cell response. With the regard of this, we hypothesize that the control of selective adsorption of biological cues from the surrounding milieu may be a key-point to control selective cell colonization of scaffolds for periodontal tissue regeneration.

Background

The periodontium is the set of specialized tissues that surround, support and nourish the tooth. It consists of hard and soft tissue components. Some structures serve the main purpose of anchoring teeth and also proper force dissipation during chewing: the alveolar bone, within which teeth alveoli are hosted, and the cementum and periodontal ligament (PDL), assuring the functional connection between tooth and alveolar bone. The anatomy of periodontium is completed by connective tissue and keratinized gingival epithelium that cover and isolate the other components from the oral cavity, thus preventing contamination and infection arising from the oral cavity [1,2].

However, in spite of this refined anatomy, periodontium is often hit by destructive diseases, namely inflammatory conditions of bacterial origin, recognized as one of the most common diseases of the mankind [3]. Periodontal diseases, comprising gingivitis and periodontitis, affect around 743 million people worldwide and according to the Global Burden of Disease Study (GBD, 1990-2010) are the sixth most prevalent pathological condition in the world, which affect not only the quality of life of the patients, but also their overall health and systemic well-being [4,5]. Without an adequate therapy, periodontitis leads to progressive destruction of the periodontium. As a consequence, individuals affected by periodontitis are at risk of multiple tooth loss, edentulism and masticatory disfunction [6].

Periodontal regeneration and its challenges

The ultimate goal of periodontal treatment is the regeneration, which includes the functional restoration of the tissue. However, the complex anatomy of the periodontium, the avascular nature of root surface and the demanding environment of the oral cavity, constantly exposed to bacteria, make of periodontium one of the most challenging tissues of the human body to regenerate.

With particular regard to the complex anatomy and physiology of periodontium, in the early 1970s clinicians had already conceptualized that the fast proliferation of gingival cells into the periodontal defects impeded bone regeneration and periodontal reattachment [7]. In the subsequent decade, the studies by Nyman and coworkers addressed the necessity of a selective periodontal defect repopulation to promote regeneration [8–11], leading to the formulation of the guided tissue regeneration (GTR) principles. GTR consists in the use of a barrier membrane to exclude the fast proliferative gingival cells, indeed 10 times faster than periodontal cells [12], impeding their ingrowth in the surgical wound over the root surface. This exclusion leaves space and time to the ingrowth of bone and PDL cells.

The clinical efficacy of GTR has been extensively validated in the last 30 years in several studies [13]. However, true periodontal regeneration, consisting of concomitant new cementum deposition, new periodontal fibers attachment with insertion into the cementum and new bone formation, is rarely achieved. The response of hard tissue

* Corresponding author at: Centro Universitario di Odontoiatria, Università di Parma, Parma, IT, Italy.
E-mail address: ludovica.parisi@unipr.it (L. Parisi).

https://doi.org/10.1016/j.mehy.2019.109388
Received 25 July 2019; Received in revised form 19 August 2019; Accepted 29 August 2019

components (bone, PDL and cementum cells) remains indeed unpredictable and depends on the characteristics of the defect. More specifically, cells often seem to repopulate the defect at random locations and moments, thus impeding sequential processes, but promoting overlapping phases. Therefore, an ideal implant material for periodontal regeneration should be endorsed with bioactivity, namely the capacity to establish a dynamic dialogue with the surrounding biological milieu [14,15], consistently allowing a timely regenerative response of the tissues.

The hypothesis

Proteins are the first molecules to interact with the material surface after its positioning, thus rendering impossible the direct cell-material interaction and determining the response of cells in term of adhesion, growth and migration. Therefore, according to the previous discussion, we believe that controlling the selective adsorption of proteins from blood plasma, is a viable approach to design materials tailored for periodontal regeneration.

Specifically, our hypothesis assumes that the selective adsorption of blood plasma proteins can control the spatiotemporal adhesion of progenitor cells, pushing the subsequent cascade of events to periodontal regeneration instead of periodontal repair.

Evidence supporting the hypothesis

The dynamics of protein when adsorbed on surfaces depend both on the protein and the biomaterial properties and aim to minimize the system energy [16]. In simple systems, namely when pure protein solutions are applied, while proteins approach to a surface, they interact with the substrate through the surface domains they exposed in native conditions. Subsequently, driven by the need of more favorable conformational states, rearrangements of their original structure take place, resulting in loss of the original secondary structure and in gain of energy [17]. Protein adsorption onto surfaces is clearly complicated when complex mixtures, i.e. blood or saliva, are considered. In those cases, different proteins mutually compete for the adsorption on exposed surfaces, each following the above-mentioned mechanisms, and biomaterial surface will be eventually enriched of proteins presenting the highest affinity to it, a process known as the Vroman effect [18]. As a result of those dynamics, the final composition of the adsorbed layer, which will also determine subsequent cell response, is randomized by the type, the amount and the relative affinity of proteins recruited from the surrounding milieu [8,19].

The hypothesis we propose concerns to the possibility to control the adhesion and the response of cells in a spatiotemporal dimension by selectively modulating protein adsorption at the interface of biomaterials.

Plenty of options are available to induce selective protein adsorption on biomaterials.

Since some proteins are preferentially adsorbed by specific chemical groups, pioneering studies in the early 2000's aimed to control protein adsorption by enriching surfaces with functional groups. Tegoulia et al. showed for example that the enrichment of a gold monolayer with thiol (-SH) groups ameliorated fibrinogen adsorption and neutrophil adhesion, without any significative effect on microorganism biofilm formation [20]. Still *in vitro*, Martins et al. observed that the combination of an ideal percentage of hydroxyl-terminated alkanethiols was capable of ameliorating albumin over fibrinogen adsorption, if compared to the same surface enriched with methyl-terminated alkanethiols. No results were reported for cell response as a consequence of this competitive adsorption [21]. By using a similar approach, on the *in vivo* level, Tang et al. demonstrated that due to a selective adsorption of complement proteins, a stronger inflammatory response was triggered on a gold surface if enriched with mercaptoglycerol or mercaptoethanol functional groups, than if enriched with L-cysteine or with glutathione [22].

Some years later, Oliveira et al. paved the way to the possibility of selectively binding proteins by means of surface receptors. In particular, the authors immobilized at the interface of polycaprolactone (PCL) fibers monoclonal antibodies (mAbs) recognizing different growth factors (GFs). Different mAbs were immobilized in different patterns and designs, endorsing the surface with the capacity to selectively adsorb the specific growth factor from a complex biological fluid on different areas of the same surface. Furthermore, cell culture assays confirmed the bioactivity of the bound molecules and the benefit to develop platforms to implement personalized therapies to specific medical conditions [23].

The use of mAbs, however, bears a high risk to elicit an adverse response of the host immune system. With the regard of this, aptamers represent a viable alternative that has been recently proposed to the use of mAbs. Aptamers are short oligonucleotides that in the presence of a target molecule are capable to adopt a specific three-dimensional (3D) conformation in order to selectively bind it with high affinity. Differently from the conventional view of nucleic acids as carriers of genetic information, aptamers are thus more like globular molecules, whose functionality is based on their complex 3D structure [24]. Aptamers selected for recognizing cell surface receptors have been exploited to confer biomaterials the capacity to sustain cell response [25–27]. However, only in 2016, aptamers were used as selective binding molecules to confer biomaterials the capacity to retain specific cues from a complex mixture, thus triggering specific cell responses [28]. A hyaluronic acid-based hydrogel with aptamers selected to recognize fibronectin showed the capacity to sustain cell adhesion and migration by selectively retaining fibronectin from the culturing serum. Similarly, fibronectin-recognizing aptamers dramatically increased the number of adhering cells to chitosan in a way proportional to the quantity of aptamers used [29,30].

On a similar level, Zhang et al. has recently published the possibility to mediate cell behaviors by a spatial and specific regulation of protein adsorption at implant surfaces. In this case, materials were designed with a non-uniform spatial distribution of charges on their surface. In such instance, cell analysis showed that an increase in charge density promoted cell adhesion and philopodia formation, if compared to non-uniform distribution [31].

Taking together, different evidence from the literature pinpoint the possibility to control the selective adsorption of proteins from complex mixtures and to exploit this selective adsorption for tailoring cell response in terms of adhesion. Translating this concept to the GTR principles we previously discussed, we can thus speculate that tailoring the biomaterial interface by inducing selective adsorption of proteins from complex mixtures, i.e. blood, could be a viable method to control the spatial response of cells. However, we still miss evidence if it could be possible to control the adhesion also on a temporal dimension.

Evidence against the hypothesis

In spite of the numerous *in vitro* evidence that support the possibility to control cell response by tailoring the adsorption of proteins at the interface, we still lack sufficient *in vivo* evidence to justify the applicability of this methodology in periodontal regeneration procedures. However, there are plenty of *in vivo* evidences showing that the selective modulation of cell colonization and response through the use of multi-phasic scaffolds is important to enhance periodontal regeneration. Biphasic scaffolds, for instance, have been repeatedly developed to facilitate the separate regeneration of alveolar bone and periodontal ligament. On the other hand, the use of triphasic scaffolds, which further supplies the presence of a compartment supporting cementum regeneration has also been proposed. However, it still remains a vastly unexplored area [13,32–36].

With the regard of this little discussion, we can thus reinforce the hypothesis that a selective control of cell response is important to support the regeneration of periodontium. Furthermore, we can also

speculate that methods to control selective cell response should be considered as potentially beneficial for promoting periodontal regeneration.

Conclusions

Due to its complex and unique anatomy, which includes hard and soft components at the interface of the oral cavity, periodontium is one of the most challenging tissues to regenerate. Since protein adsorption on biomaterials plays a key role in the modulation of subsequent host reactions and consequent tissue regeneration, we hypothesized that its control is the key point to obtain advanced multi-phasic biomaterials capable of consistently eliciting periodontal regeneration.

On one side, evidence of the literature sustain our hypotheses at an *in vitro* level, showing that the selective control of cell response through different methods is a suitable method for controlling cell response, particularly in terms of adhesion. On the other hand, we still miss strong evidence on the *in vivo* counterpart. We can thus conclude that the selective control of protein adsorption at material interface may be a suitable method to tailor the bioactivity of materials for periodontal regeneration. In particular, materials could be envisaged as able to capture specific cues and mediators from patient's own blood and to concentrate them where they are needed and, in the quantity, they are needed on material itself. However, on the opposite, evidence to support the possibility of controlling the temporal sequence of events when dealing with complex structures like the periodontium and *in vivo* data confirmation are required.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] Nanci A, Bosshardt DD. Structure of periodontal tissues in health and disease. Periodontol 2006;2000(40):11–28.
[2] Chapple ILC, Mealey BL, Van Dyke TE, et al. Periodontal health and gingival diseases and conditions on an intact and a reduced periodontium: consensus report of workgroup 1 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. J Clin Periodontol 2018;45:S68–77.
[3] Haffajee AD, Socransky SS. Microbial etiological agents of destructive periodontal diseases. Periodontol 1994;2000(5):78–111.
[4] Tonetti MS, Jepsen S, Jin LJ, Otomo-Corgel J. Impact of the global burden of periodontal diseases on health, nutrition and wellbeing of mankind: a call for global action. J Clin Periodontol 2017;44:456–62.
[5] Winning L, Patterson CC, Cullen KM, et al. The association between subgingival periodontal pathogens and systemic inflammation. J Clin Periodontol 2015;42:799–806.
[6] Papapanou PN, Sanz M, Buduneli N, et al. Periodontitis: consensus report of workgroup 2 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. J Clin Periodontol 2018;45:S162–70.
[7] Giannobile WV. Treatment of periodontitis: destroyed periodontal tissues can be regenerated under certain conditions. J Periodontol 2014;85:1151–4.
[8] Gottlow J, Nyman S, Karring T, Lindhe J. New attachment formation as the result of controlled tissue regeneration. J Clin Periodontol 1984;11:494–503.
[9] Gottlow J, Nyman S, Lindhe J, Karring T, Wennstrom J. New attachment formation in the human periodontium by guided tissue regeneration – case-reports. J Clin Periodontol 1986;13:604–16.
[10] Nyman S, Lindhe J, Karring T, Rylander H. New attachment following surgical-treatment of human periodontal-disease. J Clin Periodontol 1982;9:290–6.
[11] Nyman S, Gottlow J, Karring T, Lindhe J. The regenerative potential of the periodontal-ligament – an experimental-study in the monkey. J Clin Periodontol 1982;9:257–65.
[12] Caton J, Nyman S. Histometric evaluation of periodontal surgery. III. The effect of bone resection on the connective tissue attachment level. J Periodontol 1981;52:405–9.
[13] Vaquette C, Pilipchuk SP, Bartold PM, Hutmacher DW, Giannobile WV, Ivanovski S. Tissue engineered constructs for periodontal regeneration: current status and future perspectives. Adv Healthc Mater 2018;7:e1800457.
[14] Williams DF. On the mechanisms of biocompatibility. Biomaterials 2008;29:2941–53.
[15] Parisi L, Toffoli A, Ghiacci G, Macaluso G. Tailoring the interface of biomaterials to design effective scaffolds. J Funct Biomater 2018;9:E50.
[16] Chen LN, Yan C, Zheng ZJ. Functional polymer surfaces for controlling cell behaviors. Mater Today 2018;21:38–59.
[17] Bryan PN, Orban J. Proteins that switch folds. Curr Opin Struct Biol 2010;20:482–8.
[18] Hirsh SL, McKenzie DR, Nosworthy NJ, Denman JA, Sezerman OU, Bilek MMM. The Vroman effect: competitive protein exchange with dynamic multilayer protein aggregates. Colloids Surf B-Biointerfaces 2013;103:395–404.
[19] Krishnan A, Siedlecki CA, Vogler EA. Mixology of protein solutions and the Vroman effect. Langmuir 2004;20:5071–8.
[20] Tegoulia V, Rao W, Kalambur A, Rabolt F, Cooper S. Surface properties, fibrinogen adsorption and cellular interactions of a novel phosphorylcholine-containing self-assembled monololayer of gold. Langmuir 2001:4396–404.
[21] Martins MCL, Ratner BD, Barbosa MA. Protein adsorption on mixtures of hydroxyl- and methylterminated alkanethiols self-assembled monolavers. J Biomed Mater Res Part A 2003;67A:158–71.
[22] Tang LP, Hu WJ. Molecular determinants of biocompatibility. Expert Rev Med Devices 2005;2:493–500.
[23] Oliveira C, Costa-Pinto AR, Reis RL, Martins A, Neves NM. Biofunctional nanofibrous substrate comprising immobilized antibodies and selective binding of autologous growth factors. Biomacromolecules 2014;15:2196–205.
[24] Song KM, Lee S, Ban C. Aptamers and their biological applications. Sensors (Basel) 2012;12:612–31.
[25] Guo J, Gao X, Su L, et al. Aptamer-functionalized PEG-PLGA nanoparticles for enhanced anti-glioma drug delivery. Biomaterials 2011;32:8010–20.
[26] Hoffmann J, Paul A, Harwardt M, et al. Immobilized DNA aptamers used as potent attractors for porcine endothelial precursor cells. J Biomed Mater Res A 2008;84:614–21.
[27] Chen N, Zhang Z, Soontornworajit B, Zhou J, Wang Y. Cell adhesion on an artificial extracellular matrix using aptamer-functionalized PEG hydrogels. Biomaterials 2012;33:1353–62.
[28] Galli C, Parisi L, Piergianni M, et al. Improved scaffold biocompatibility through anti-Fibronectin aptamer functionalization. Acta Biomater 2016;42:147–56.
[29] Parisi L, Galli C, Bianchera A, et al. Anti-fibronectin aptamers improve the colonization of chitosan films modified with D-(+) Raffinose by murine osteoblastic cells. J Mater Sci-Mater Med 2017;28.
[30] Parisi L, Toffoli A, Bianchi M, et al. Aptamer-doped chitosan modulates cell morphology by integral-mediated pathway. Materials 2019;12.
[31] Zhang KJ, Xing J, Chen JQ, et al. A spatially varying charge model for regulating site-selective protein adsorption and cell behaviors. Biomater Sci 2019;7:876–88.
[32] Reis ECC, Borges APB, Araujo MVF, Mendes VC, Guan LM, Davies JE. Periodontal regeneration using a bilayered PLGA/calcium phosphate construct. Biomaterials 2011;32:9244–53.
[33] Requicha JF, Viegas CA, Hede S, Leonor IB, Reis RL, Gomes ME. Design and characterization of a biodegradable double-layer scaffold aimed at periodontal tissue-engineering applications. J Tissue Eng Regener Med 2016;10:392–403.
[34] Park CH, Rios HF, Jin QM, et al. Biomimetic hybrid scaffolds for engineering human tooth-ligament interfaces. Biomaterials 2010;31:5945–52.
[35] Costa PF, Vaquette C, Zhang QY, Reis RL, Ivanovski S, Hutmacher DW. Advanced tissue engineering scaffold design for regeneration of the complex hierarchical periodontal structure. J Clin Periodontol 2014;41:283–94.
[36] Vaquette C, Fan W, Xiao Y, Hamlet S, Hutmacher DW, Ivanovski S. A biphasic scaffold design combined with cell sheet technology for simultaneous regeneration of alveolar bone/periodontal ligament complex. Biomaterials 2012;33:5560–73.