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Review Article

Osseo Regeneration of the Cranio-Maxillo-Facial and Oro-Dento-Alveolar Complexes Using a Novel Self-Assembling Peptide Nanofibrous Hydrogel Scaffold and Its Composites

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Abstract

Introduction: Craniofacial reconstructive surgery allows the repair and restoration of defected tissues and/or organs, yet, with limitations. Regenerative engineering of complex tissues requires a precisely formulated combination of cells, spatio-temporally released bioactive growth factors, and a specialized scaffold system to provide support. Hence, auto-grafts (current gold standard treatment choice) are associated with numerous disadvantages. Thus, development of new alternative biomaterials for bone regeneration continues to be a hot research topicin Dentistry. Therapeutic transplant of human mesenchymal stem cells into bone defects is an appealing strategy for bone tissue engineering nonetheless, a main challenge for its clinical application relates to the need of adequate scaffolds for cell delivery and support. PuraMatrix™ is a novel and commercially-available self-assembling peptide nanofibrous scaffold with appealing properties for osseo-regeneration. Despite promising results in other medical disciplines, its potential in Dentistry is largely under-investigated. This review provides an up-to-date overview of the use of PuraMatrix™ for cranio-maxillo-facial and oro-dento-alveolar complexes bone regeneration and repair.

Materials and Methods: A structured literature search was performed on PUBMED using the search terms "PuraMatrix" and "Bone". A total of 29 articles were found, *though only studies related to* Bone Regeneration *are included.*

Results: 7 articles dealing with potential use and application of PuraMatrix™ for bone tissue engineering were identified. Briefly, PuraMatrix™ supports the survival, proliferation and migration of osteoblast-like stem cells without interference with their osteogenic differentiation. Stem cells seeded in PuraMatrix™ and supplemented with osteogenic induction media showed early extra-cellular matrix formation and mineralization in addition to increased osteogenicity. Furthermore, the functionalization of RADA16 with bone bioactive motifs enhanced cell attachment, proliferation and osteogenic differentiation *in vitro*. In animal studies, combination of the nano-material with other porous biomaterials (DBM) and growth factors (BMP-2, PRP) further enhanced the capacity of PuraMatrix™ for *in vivo* osteoblast growth and osteogenesis.

Conclusions: Although the number of studies identified in this review is limited; available/accruing evidence suggests PuraMatrix™ is a promising carrier/scaffold for stem cell based craniofacial bone tissue engineering. Additional studies are deemed necessary to assess safety, efficacy, impact potential and cost-effectiveness of this new technology on future regenerative Cranio-Maxillo-Facial fields.

Keywords: *Hydrogel; Nano fiber; PuraMatrix; Bone; Stem Cells; Tissue Engineering*

Abbreviation: ECM: Extracellular matrix; PM: PuraMatrix™; RCTs: Randomized clinical trials; BMMSC: Bone marrow mesenchymal stem cells; BIC: bone-to-implant contact; rh-BMP-2: Recombinant human bone morphogenetic protein 2; PRP: Platelet-rich plasma; PLGA: Polyglycolic-co-lactic acid; DBM: Demineralized allograft cancellous bone matrix

Introduction

Healing of bone fractures and regeneration of critical-sized defects remains a challenge in contemporary Oral and Maxillo-Facial surgery. Use of auto-grafts is the "gold standard" choice in these cases; however several disadvantages associated with their use (i.e. limited donor supply, increased morbidity, lengthening of the surgical intervention, to list a few.) seriously limit their application [1-3]. Alternative allogeneic and xenogeneic "graft substitutes" offer solution to some of the a fore mentioned inconveniences, none the less increased risk of (a) anti genicity and (b) foreign body reactions reduce their biological performance, while (c) high-cost manufacture restricts their clinical application [1,4]. Despite various alternative biomaterials have been tested (i.e. beta-tricalcium phosphate, hydroxyapatite and polymers) a simple, inexpensive and universal scaffold for tissue regeneration is still not available [1-3]. Thus, the development of new alternative biomaterials for bone regeneration continues to be a hot research, development and innovation topic, especially in the field of Dentistry. On the other hand, self-assembly is a well-known phenomenon within nature, consisting of the spontaneous organization of molecules - under thermodynamic equilibrium conditions-; into highly organized and stable macromolecular entities with specific functions [5]. In connective tissues, self-assembling of collagen and other proteins (such as laminin and fibronectin) result in extracellular matrix (ECM) formation. Because of this and their capacity to form hydrogels, these proteins (especially collagen I) have been extensively used for the development of scaffolds for regeneration [5]. In the last decades, advances in cell biology and tissue-engineering allowed identification of key proteins involved in ECM self-assembling. The discovery enabled scientists to create "new generation peptides" for manufacturing simpler and customizable scaffolds for regeneration [5].

RAD16-I (commercially available as PuraMatrix™, BD Bioscience, Bedford, MA, USA) is one of such newly-designed peptides for tissue-engineering. The 16-amino-acid peptide (Figure 1) derives from and alternating sequence of hydrophilic and hydrophobic amino-acids [Arginine (R), Alanine (A) and aspartic acid (D)] which - under physiologically salt and pH conditions -organize in B-Sheet configuration forming insoluble membranes [5]. Compared to other 3-dimensional cell culture systems, PuraMatrix™ excels at mimicking the natural ECM as both its nano scale network and biomechanical properties are similar to those of collagen I [5]. The material is also "inert" or "non-intrusive" as absence of cytokines and growth factors allows cells to migrate, proliferate and interact in a permissive/natural environment composed exclusively of synthetic proteins and 99.5% water [5-7]. This not only makes PuraMatrix™ an ideal nano scaffold for in vitro cell study, then again opens the door for further functionalization of the matrix in order to fulfill individual culture requirements or functions; i.e. cell differentiation [5]. The aforementioned is of vital importance in the race for developing next generation biomaterials as XXI century scaffolds should have both, instructive and inductive capacity over cells [5,8]. Furthermore, the development of new strategies for bone regeneration requires not only new biomaterials, yet also an entire bioengineering platform which assists regeneration [5]. This platform should be (a) biocompatible and safe, (b) guarantee biomineralization;(c) allow functional osteoblast migration, proliferation and differentiation; (d) permit the establishment of an adequate vascularization network and (e) maintain the biophysical and biomechanical properties of the bone [5].

In this context, PuraMatrix™ is a naturally-appealing strategy for cranio-facial bone regeneration as it offers several advantages over conventional scaffolds (Figure 2): (a) it is a liquid/solution that may be easily and directly injected into bone defects [5], (b) as far as it is known, does not produce immune responses or reactions [5], (c) self-polymerizes rapidly under physiological conditions forming solid

3-dimensional nano fiber hydrogels which adopt the geometry of the defect [5], (d) allows cell and growth-factor encapsulation and delivery into target tissues [5,6], (e) supports cell adhesion, survival, migration and proliferation [5,6], (f) has a porous 3-dimensional structure which allows free cell-to-cell contact (unlike other encapsulation materials were cells are surrounded by the matrix and must digest it in order to interact and migrate) [6] and (g) may be easily functionalized in order to meet individual needs of the target site [5]. An example of this would be the possibility to modify PuraMatrix™ in order to include bioactive motifs for biomineralization to promote calcium hydroxyapatite crystal nucleation and development [5].

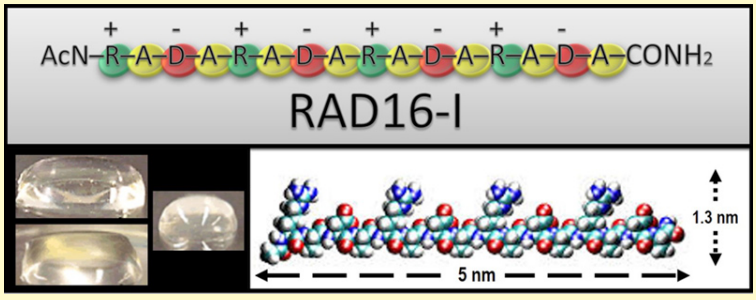

Figure 1: *The RAD16-I (PuraMatrix™) molecular amino-acid sequence.*

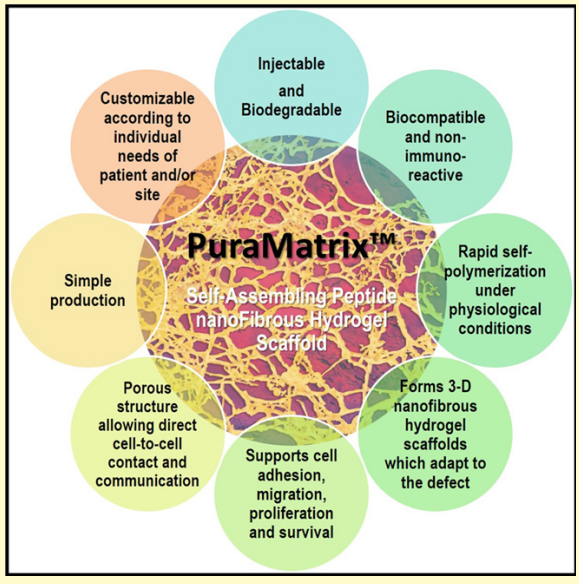

Figure 2: *Electron micrograph of PuraMatrix™ peptide hydrogel and main characteristics as a bio-scaffold.*

Indeed, studies suggest that PuraMatrix™ is a promising scaffold for stem cell-based engineering of mineralized dental tissues [19]. Studies regarding its application on bone tissue engineering report Mesenchymal Stem Cells (MSCs) and osteoblasts seeded in the scaffold survive, proliferate and successfully differentiate into osteogenic linage [9,10]. Combination with other porous biomaterials and peptides (such as poly-HIPE polymer and P24 peptide) further enhanced PuraMatrix™' s capacity for *in vivo* osteoblast growth and

osteogenesis, suggesting potential use/application for bone tissue engineering is genuine [5,10,11]. Hence, the purpose of this article is to provide an up-to-date overview on the application of PuraMatrix™ during craniofacial bone tissue engineering.

Materials and Methods

A structured literature search (Figure 3) was performed on PUBMED (May - July 2015) using MESH search terms "PuraMatrix" and "Bone" according to the following search strategy: "PuraMatrix [all fields] AND "Bone" [all fields]". Results were limited by: seniority (5 years since publication), language (English) and full text availability. A total of 29 articles were found. Focus was centered on the use/application of self-assembling peptide hydrogel PuraMatrix™ in Bone Regeneration; reason why a total of 15articles (related to regeneration of other tissues) were excluded from this review. Four out of remaining 14 studies were also excluded because of no "full text" availability. Data from remaining articles was abstracted and compiled in tables for latter appraised by authors. Decision to exclude one more article was taken during this stage after authors realized the article used RADA-P24 (JI'ER Biotechnology Co. LTd., Shanghai, China) instead of PuraMatrix™ (BD Bioscience, Bedford, MA). It is noteworthy additional manual search on PuraMatrix™'s official webpage (www.puramatrix.com) was performed to fetch for published or unpublished data. Despite finding 9 articles, none was added to this review as 5 publications were repeated and 4 were not available in Full-text format. In the same context, additional manual search on clinicaltrials.gov (http://clinicaltrials.gov) was performed in order to fetch finished or ongoing human randomized controlled clinical trials (RCTs); however no results were found. Results and methodology flow diagram are reported according to the "PRISMA STATEMENT" (http://www.prisma-statement.org/statement.htm)

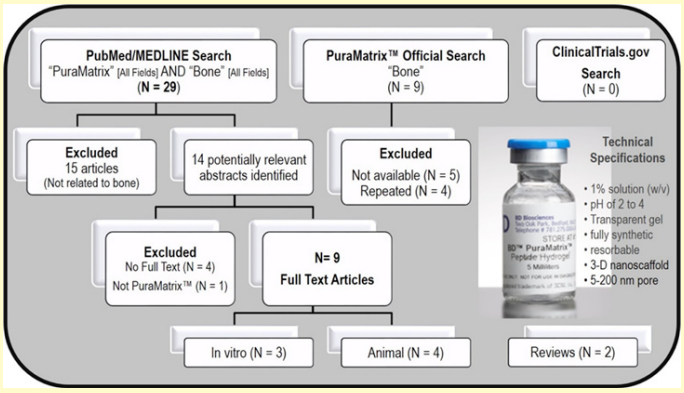

Figure 3: *Flow-chart of performed systematic review methodology and reported findings/studies.*

Results and Discussion

In total, 7 studies dealing with potential use and application of PuraMatrix™ in bone tissue engineering were found (3 in vitro studies and 4 animal trials - for more details please refer to Table 1 and Table 2, respectively). It is noteworthy that no RCTs were found; signifying that this particular topic and biomaterial is a novel field of research and development within bone regeneration and tissue engineering fields.

In vitro Studies

PuraMatrix™ supports *in vitro* survival, proliferation and migration of osteoblast-like cells

Undifferentiated BMMSC seeded within PM™ retained fibroblast morphology as they migrated within the scaffold [13]. Once inside, cells remained viable and developed multiple "processes" extending into the matrix and other cells [12]. Aforementioned protrusions are typical in cells cultured on 3-dimensional culture systems and are thought to participate in communication through direct cell-to-cell contact. Iwai et al. (who also reported the appearance of these protrusions) described the phenomenon to occur as early as 3

days into PuraMatrix™ culture [14]. Regarding cell proliferation, Chen et al. reported only undifferentiated BMMSC proliferated within the matrix [12]. This is within the expected as differentiation to osteoblasts (terminal-line cells) reduce mitogenesis and cell division. Finally, PuraMatrix™ did not interfere with BMMSC proliferation as no differences regarding this parameter were noticed when comparing cells seeded onto 2D-culture system and the nanofibrous scaffold [12].

Property *(MESH Definition)*	Cell Line	Scaffolds	Follow-up	Results	Ref.
Attachment *(Adherence of cells to surfaces or to other cells)*	MC3T3E1	RADA16 / ALK RADA16 / DGR RADA16 / PRG Control (RADA16, ALK, DGR, PRG)	14 d	Cell attachment significantly higher in DGR- and PRG- RADA16 scaffolds vs. RADA16 controls.	[8]
Survival *(Continuance of life or existence especially under adverse conditions)*	human BMMSC	PM™/HELISTAT BMMSC/PM™/HELISTAT	N/A	Seeded cells remained viable within scaffolds (MMT assay). BMMSC appeared completely surrounded and encapsulated in PM™.	[16]
	human BMMSC	0.25% PM™ 2D culture	21 d	Cells retained normal fibroblast-like morphology within PM™ until osteogenic induction was made.	[12]
	human BMMSC	PM™/DBM DBM		Cells retained normal fibroblast-like morphology.	[13]
Proliferation *(Processes involved in increasing cell number including cell division).*	human BMMSC	0.25% PM™ 2D culture	21 d	Obvious proliferation was seen only on undifferentiated BMMSC. No differences in cell proliferation rates in PM™ and control groups were noticed.	[12]
	MC3T3E1	RADA16 / ALK RADA16 / DGR RADA16 / PRG Control (RADA16, ALK, DGR, PRG)	14 d	Significant increase in cell proliferation in RADA16/DGR and RADA16/PRG vs. RADA16 control.	[8]
Migration	human BMMSC	0.25% PM™ 2D culture	21 d	Cells migrated successfully into PM™.	[12]
	HMS0014	1% PM™ 2D culture	14 d	Cells migrated freely within PM™. Appearance of cell processes extending into the matrix was noticed since 3 days of culture.	[14]
	MC3T3E1	RADA16 / ALK RADA16 / DGR RADA16 / PRG Control (RADA16, ALK, DGR, PRG)	14 d	Cells migrated freely within PM™. Appearance of cell processes extending into the matrix was noticed.	[8]
	human BMMSC	PM™/DBM DBM	14 d	Cells migrated into PM™	[13]

Differentiation	human BMMSC	0.25% PM™ 2D culture	21 d	Once differentiated, cells changed from fibroblast- to osteoblast-like shape and formed small cell clusters. Early ECM production and mineral nodule formation was reported in PM™ (7 day vs. 14 days in control). Mineralization sites within PM™ located in and around osteoblast clusters Non-induced PM™ cultures also showed some mineral deposition (positive Von Kossa staining) No intergroup differences in BMP-2, RunX and OP gene-expression were noticed.	[12]
	human BMMSC	PM™/DBM DBM	14 d	Early ECM production and mineral nodule formation was reported in PM™ vs. control	[13]
	HMS0014	1% PM™ 2D culture	14 d	PM™ group exhibited active osteogenic differentiation. Increased ALP activity (P<0.01), Calcium volume (P<0.05) and Osteocalcin volume (P<0.01) were noticed on experimental group. Calcium and Osteocalcin volume was 2x and 4x times higher than control.	[14]
	MC3T3E1	RADA16 / ALK RADA16 / DGR RADA16 / PRG Control (RADA16, ALK, DGR, PRG)	14 d	RADA16/DGR and RADA16/PRG showed significantly higher ALP activity and staining than RADA16 control. Osteocalcine volume in all modified RADA16 scaffolds was higher than RADA 16 control. RADA16/PRG showed the highest osteocalcine concentration among modified scaffolds (P<0.05)	[8]

Table 1: *In vitro findings on prospective use/application of PuraMatrix™ for Bone Tissue Engineering.*

PuraMatrix™ supports *in vitro* osteogenic differentiation of osteoblast-like cells

During osteogenic induction, BMMSC cells underwent dramatic morphology changes shifting from fiboblast-like-toosteoblast form [12]. Cell distribution was also altered as osteoblasts grouped into small clusters whereas undifferentiated BMMSC remained isolated within the matrix [12]. ECM production and mineralization (measured by ECM secretion and mineral nodule formation) was noticed earlier in induced PM™ cultures (day 7) vs. 2D-controls (day 14) [12,14]. Interestingly, non-induced PM™ cultures also showed some mineral deposition (measured by positive Von Kossa staining) [12]. Slight osteogenic differentiation of these particular cultures despite absence of osteoinductive media or any other extrinsic osteogenic growth factor suggest PM™'s architecture may positively influence BMMSC differentiation none the less, more studies are needed in order to test this hypothesis. Calcium X-ray density analyses of induced PM™ cultures revealed mineralization nodules occurred at specific localizations within the nano-scaffold. Further analysis using X-ray intensity maps evidenced most calcium deposits appeared in and/ or around osteoblast clusters [12]. Complementary analyses of oteogenic marker gene-expression during culture induction showed no differences between PM™ and 2D cultures regarding the expression of BMP-2, Runx2 and OP [12]. A second study by Iwai et al. studied HMS0014 cells cultured on PM™ [14]. In his study, cells were seeded into 1% PM™ and then cultured for 14 days under: (a) normal or (b) osteogenic induction conditions. Results from

this study are similar to those reported for BMMSC, indicating survival and active osteogenic differentiation occurred under osteogenic induction. HMS0014 on induced-PM™ showed increased ALP activity ($P<0.01$) and nearly 2x- and 4x times higher Calcium ($P<0.05$) and Osteocalcin ($P<0.01$) volumes than 2D induced controls [14].

Application and Model ***(Bone Defect dimensions)***	**Species**	**Groups**	**Follow-up**	**Results**	**Ref.**
Implant Osseo-integration & Implants placed on previously grafted alveolar sockets *(10mm height x 10mm diameter)*	Dog (N=?)	PM PM / dog BMMSC PM / dog BMMSC / PRP Control (defect only)	8, 16 w	Bone formation Control and PM groups exhibited cavities invaded by fibrous tissue. PM/dog BMMSC showed partial bone formation. PM/dog BMMSC/PRP showed mature bone formation. PM/dog BMMSC and PM/dog BMMSC/PRP exhibited greater new-bone volume than PM and controls (significance not reported). BIC in PM group was 40.77% + 12.85%. PM/dog BMMSC showed 50.35% + 8.02% and PM/dog BMMSC/PRP exhibited 55.64% + 4.97%. BIC in Control groups was 30.57% + 2.50%. PM/dog BMMSC BIC was significantly greater than control ($P<0.05$) PM/dog BMMSC/PRP BIC was significantly greater than control and PM ($P<0.01$) No significant differences were noticed between (a) PM and control and (b) PM/dog BMMSC and PM/dog BMMSC/PRP	[17]
Periodontal Regeneration & King and Hughes' Periodontal wound model (18). *(2mm height x 2mm depth x 3-4mm width)*	Rat (N=24)	Human BMMSC / PM / HELISTAT (Collagen sponge, Integra Life Sciences corp.) PM / HELISTAT Control (defect only)	1, 4 w	All animals survived. No adverse reactions were noticed. Cell viability in vivo Human DNA was detected only were hBMMSC were implanted. Cells were located mainly in the periodontal defect. hBMMSC numbers decreased significantly over time. No cells were detected at 4 w (PCR method). Bone Formation At 1w, control group defects were filled with loose-connective tissue. No new bone formation was found in any of the other samples. At 4w, newly formed bone was found in all samples. Partial regeneration of damaged periodontal ligament and cement was noticed. No significant differences between treated groups were found regarding (a) the amount of newly formed bone and (b) the healing of periodontal ligament and cementum. Damaged dentin did not heal. Bone volume density was significantly higher in hBMMSC vs. non-hBMMSC groups only at 1w. Osteoclast numbers were significantly reduced in hBMMSC treated group vs. control at 1 and 4w. No osteoclasts were found in healthy controls (no defect).	[16]

Bone Regeneration & Calvarial defect under titanium dome. *(6mm diameter x 1mm depth)*	Rabbit (N=5)	PM rhBMP-2 / saline PM / rhBMP-2 Control (Defect only)	8 w	All animals survived. Newly formed bone was seen in all groups. Maximum height of newly formed bone in PM/rhBMP-2 was significantly higher than control (P<0.01) and rhBMP-2 or PM alone (P<0.05). Area of newly formed bone in PM/rhBMP-2was significantly higher than control (P<0.05) Average regenerated tissue in control was 35.8 + 10.35%. PM and rhBMP-2 exhibited 48.94 + 11.33% and 47.94 + 5.65% respectively. PM/rhBMP-2 showed 58.06 + 14.84% and was significantly higher than control (P<0.05).	[15]
Bone Regeneration & Femoral defect (20mm depth x unknown)	Goat (N=12)	DBM / goat BMMSC PM / DBM / goat BMMSC	8, 16 w	All animals survived. PM formed a 3-dimensional interlacing network in the inner wall of DBM. Bone Formation At 8 w, all animals experienced some new bone formation however, no union occurred. PM treated group showed massive osteoid formation, whereas control exhibited cartilage-like tissues with less osteoid. At 16 w, bone union occurred in all samples. PM-enriched group was completely reconstructed while control was partially regenerated. PM treated group exhibited extensive lamellar bone while control had abundance of woven bone. Volume of new bone was significantly higher in the PM group vs. control (P<0.05). Mean Bone Density was significantly higher in PM/DBM/goat BMMSCS group vs. control at 16 w(P<0.05), however no difference was found at 8 w.	[13]

Table 2: *In vivo studies evaluating prospective use/application of PuraMatrix™ for Bone Tissue Regeneration.*

Functionalized PuraMatrix™ increases biocompatibility and osteogenicity

As it was previously mentioned, PM™ may be further customized in order to fulfill specific functions. In Horii et al.'s study, RAD16-Iwas functionalized adding one of the following three bioactive motifs: (a) Osteogenic growth factor peptide (ALK), (b) Osteopontin cell adhesion motif (DGR) or (c) specially designed 2-unit binding sequence (PRG). Biocompatibility and osteogenicity of these "second generation" scaffolds was tested *in vitro* using MC3T3-E1 cells [8]. After 2 weeks of continuous culture, MC3T3-E1's attachment and proliferation was significantly improved in DGR- and PRG-modified scaffolds [8]. Cell attachment increase was within the expected as both motifs (DGR and PRG) contain well-documented RGD cell attachment sequences. Interestingly, it was also reported PRG concentrations as low as 1% may be sufficient for effectively increase cell adhesion and migration of MC3T3-E1 cells on RAD16-I, proving even minor modifications to PM™'s sequence may dramatically improve its *in vitro/in vivo* performance. Regarding osteogenicity, Horii et al. Reported ALK- and PRG-modified RAD16-I scaffolds presented significantly higher osteogenic potential (measured by ALP activity and staining) than non-functionalized controls [8]. Osteocalcin volume concentration (a late osteogenic biomarker) was also significantly increased in all modified scaffolds, among which PRG-modified RADA16 showed the highest concentration [8].

Collectively, the aforementioned analysis suggests that *the functionalization of PuraMatrix™ seems to be a simple, effective and inexpensive strategy (or at least less than polymer or natural collagen functionalization) to create customized/improved "second generation scaffolds"withosteoconductive and osteoinductive properties suitable for bone tissue engineering.*

Pre-clinical Animal (*in vivo*) Studies

Pre-clinical models found on this review (Table 2) evaluated the prospective use of PuraMatrix™ for bone tissue engineering using four different animal species (dog, rat, rabbit and goat) and two models: (a) treatment of critical-sized bone defects (Calvarial [15], Femoral [13] and Periodontal [16]) and (b) implant osseointegration [17]. Outcomes as well as defect type, location and size were not uniform; hence results are presented in narrative format. Since the scaffold is composed solely by peptides and water, osteogenicity was achieved using the following strategies: (a) combination with Bone Marrow Mesenchymal stem cells [13,16,17], (c) combination with growth factors (rhBMP-2 [15] and PRP [17]) and/or (d) mixing with other biomaterials (collagen membrane HELISTAT [16] or DBM [13]). Main source of mesenchymal stem cells were same species animals, with exception of Tcacencu's study [16] in which human BMMSCs were used with no adverse effects.

PuraMatrix™ in the treatment of critically-sized bone defects

PuraMatrix™ in large bone defects:

Treatment of trans-segmental femoral defects using P™-modified DBM grafts was addressed by Li., et al. [13]. This particular model has been extensively used in the literature as a challenging environment for testing bone grafts in outsized defects; hence it was also used as a way to test PM™'s potential in large bone defects. Rationale behind PM™ and DBM combination came from the evidence reporting that the loss of chemical and physical reconstituents during DBM production often led to gross pore size (200-900um) and low in vitro adhesiveness of cells and growth-factors. Thus, addition of PM™ was thought as a way to increase osteo progenitor cell adhesion and migration within DBM grafts. This is plausible as PM™ forms 3-dimensional nano scale networks (10nm fibers and 2-300 nm pores) within several micro scale scaffolds [10,13]. In Li's study, critically-sized defects on the mid-diaphyses of goat femurs were treated using (a) BMMSC/DBM or (b) PM™/BMMSC/DBM construct [13]. Quantitative assessment of new bone formation was made at 8 and 16 weeks post-surgery by means of histologic- and radiological analysis. It must be noticed authors reported uneventful healing in all animals and no adverse reactions to the biomaterials. Radiographic evaluation 8 weeks post-surgery revealed that both groups experienced partial bone union however, PM™-treated femurs exhibited evident and abundant cortical bone formation (unlike controls in which cortical bone was poorly seen) [13]. Histological analyses of the same samples revealed that massive osteoid formation had occurred in the PM™ group, whereas extensive cartilage-like tissues and minor osteoid was found in control lesions. Intergroup differences in new bone volume and density were non-significant at this stage. At 16 weeks, radiographic bone union was complete in both groups. Cortical bone on PM™ group appeared completely reconstructed whereas non-PM™ femurs still showed partial regeneration in one of their sides. Histological analyses revealed extensive lamellar bone in PM™-treated group whereas woven bone was still predominant on controls. Quantification of bone formation using radiological scores revealed de novo/new bone volume and density was significantly higher in the PM™ group [13].

Collectively, these findings suggest that the modification of DBM grafts with PuraMatrix™ may provide significant additional benefits on bone formation, maturation and remodelling. The aforementioned strategy appears as a simple and viable approach for enhanced and accelerated wound healing in large bone defect; and thus could be also applied in the treatment/regeneration of extensive maxillo-facial defects.

PuraMatrix™ in craniofacial defects:

Ikeno., et al. evaluated use/application of rhBMP-2-enriched PM™ in the treatment of small craniofacial bone defects [15]. In their study, critically-sized calvarial defects on rabbits were treated with either: (a) PM™, (b) rhBMP-2 (25ug/mL) or (c) a combination of both. Untreated bone defects were used as negative controls [15]. After 8 weeks, animals were euthanized and ground sections were obtained for histomorphometric analysis. It must be noticed all animals experienced uneventful healing and no adverse effects to the therapy were reported. Study results showed newly formed bone in all groups however; the amount, height and area were significantly higher in the PM™/rhBMP-2 combined group vs. control ($P<0.05$) [15]. Osteogenic potential of the biomaterial on its own was lower than that of PM™/rhBMP-2 combination yet similar to that of rhBMP-2-alone; nonetheless despite how promising this may sound

(since rhBMP-2 is highly osteoinductive); no significant differences with control were found [15]. An interesting thing about this study was the fact that used rhBMP-2 concentration was of at least 10 to 50 times lower than that of most experimental and clinical studies evaluating rhBMP-2 on bone regeneration [15]. On one hand, this could explain why the PM™- and rh-BMP-2-groups had similar results with no significant differences vs. control (maybe concentration was too low), however on the other and more interesting side, the fact that PM/rhBMP-2 group showed significant bone regeneration (despite low rhBMP-2 concentration) suggesting that PuraMatrix™ may be an effective delivery system for clinical application of rhBMP-2in craniofacial bone tissue engineering. According to authors, in vitro release kinetics for PM™/rhBMP-2 constructs were fast in the first two hours and then slower over time, until reaching plateau at 30 to 50 hours of application. Functional protein release was observed to occur for over a 2 to 3 week period [15]. The fore mentioned indicates that long term functional delivery of rhBMP-2 (in an appropriate time for bone regeneration to occur) is possible using PuraMatrix™. Additional benefits of this particular regeneration strategy include: (a) efficient reduction of the rhBMP-2effective dosesto-clinically accepted concentrations, (b) increased safety for in vivo application and (c) user-friendly/malleable application in complex defects such as alveolar clefts, periodontal intra-bony defects, peri-implant defects, etc [15].

Tcacencu., et al. evaluated the use of human BMMSCs on PM™-modified HELISTAT collagen membranes for the treatment of periodontal intra-bony defects [16]. Defects were prepared using King and Hughes's rat periodontal wound model [18]: extra-oral incisions at the lower mandible border were made. Both, fascia and masseter muscle were retracted to permit for direct access to the mandibular bone. Afterwards, all tissues overlying distal root of the first molars (bone, periodontal ligament, cementum and superficial dentine) were removed preserving tooth vitality. Defects were treated using the following strategies: (a) BMMSC/PM™/HELISTAT, (b) PM™/HELISTAT or (c) none (defect only) [16]; and flap was positioned using sutures. Quantitative assessment of new bone formation was made at 1 and 4 weeks post-surgery by means of histomorphometric analysis. Histological evaluation at 1 week post-surgery revealed all lesions remained unhealed. No new bone formation was found thus, defects were filled with loose connective tissue. At 4 weeks, all groups exhibited new bone formation and various degrees of periodontal ligament and cementum repair. No Dentin regeneration was observed. Intergroup differences on the wound healing rate were noticed. Quantitative assessment of bone tissue volume/density surrounding treated roots at 1 and 4 weeks revealed significant differences favouring both experimental groups over control. Intergroup comparison further indicated BMMSC/PM™/HELISTAT-treated lesions had significantly more bone than PM™/HELISTAT groups. It is worth mentioning here in that the absence of PM™- and HELISTAT-alone controls in Tcacencu's study makes it difficult to determine the real potential and efficiency of PuraMatrix™ in periodontal bone regeneration. The reported results indicate that the PuraMatrix™/HELISTAT combination may enhance bone tissue regeneration, nonetheless whether this is an effect of the nano scaffold or its combination with HELISTAT, remains unknown.

PuraMatrix™ in implant osseo-integration

Potential effects of PuraMatrix™ on implant osseointegration were evaluated by Kogho., et al. [18]. In this study, post-extraction sockets in dogs were allowed to heal for 4 weeks before implants(Nobel BioCare) were placed using the following grafts: (a) PM™, (b) PM™/BMMSC, (c) PM™/BMMSC/PRP or (d) none (defect only). Eight weeks later, animals were euthanized and bone biopsies were taken in order to assess bone-to-implant contact (BIC) [18]. Macroscopic findings during biopsy revealed partial bone formation occurred in control, PM™ and PM™/BMMSC groups. Histological findings confirmed sockets were filled with either (a) fibrous tissues (control and PM™ -alone groups) or (b) partial/immature bone (PM™/BMMSC group) [18]. In contrast, complete macroscopic bone formation was seen on the PM™/BMMSCS/PRP group. Further histologic analysis of samples revealed extensive bone formation and maturation within this particular group [18]. Considering this, minimal implant thread exposure also reported for this group should not come as a surprise. Regarding Bone-to-implant contact (BIC), use of PM™-alone provided a slight non-significant increase in the BIC vs. control (40.77% + 12.85% vs. 30.57% + 2.50%respectively). When BMMSC were added to the scaffold, further and significant increase of BIC was noticed (50.35% + 8.02%) nonetheless it was only significant when comparing with control and not PM™-alone group. The PM™/BMMSC/PRP group exhibited the highest BIC value within the study (55.64% + 4.97%). Intergroup comparisons revealed the BIC in this group was significantly higher than those of control and PM™-alone groups [18].

Findings suggest that *PuraMatrix™ may be useful for peri-implant bone regeneration and implant osseointegration when combined with BMMSC and (even more) BMMSC with growth-factor supplements* (i.e. PRP). Clinical applicability as well as efficacy of this approach remain to be elucidated, as the model used in this study offers favorable conditions for bone regeneration/osseointegration, thus offering little to no challenge for adequately testing the true regenerative potential extent of PuraMatrix™ as the "ideal" bio scaffold.

Conclusions

Injectable biomaterials and hydrogels, particularly those delivered in aqueous formats, are considered ideal delivery/carrier vehicles for cells, drugs and bioactive growth factors. This is particularly true due to allowing to fill complex 3-D defects with minimal-invasiveness [19]. PuraMatrix™ is a synthetic, injectable and self-assembling peptide nanofibrous hydrogel scaffold recently introduced in the bone tissue engineering field. The nano-fiber scaffold supports osteoblast-like stem cell viability, proliferation and migration without interfering with cell osteogenic differentiation. Demonstrated in vitro functionalization of the RADA16 sequence with bone bioactive motifs (a simple, easy and inexpensive way to improve the biomaterial properties) as well as in vivo combination with other porous biomaterials and growth factors may further enhance osteogenesis while allowing the potential development of "second generation" nano-fiber scaffolds; specially tailored for osseo regeneration. Ability to form scaffolds or networks capable of both replacing tissue function early after delivery and supporting tissue regeneration over a time period of weeks to months, is highly attractive. Yet, the number of studies available to date is limited. Nonetheless, accruing evidence [20] suggests that PuraMatrix™ may be a promising hydrogel scaffold for cell-/protein-based cranio-facial bone tissue engineering. Future studies will explore the application of PuraMatrix™ (and its composites) and assess its safety, efficacy, impact and cost-effectiveness as a potential bioengineering strategy for clinical craniofacial regeneration, and beyond.

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Conflict of Interest

Authors of this article declare having no conflict of interest. However, it is worth mentioning that the corresponding author has an ongoing partnership (via which PuraMatrix™ is obtained) with 3-D Matrix Medical Technology, Inc. - Europe SAS, Caluire, France, a subsidiary of 3-D Matrix, Ltd., Tokyo, Japan, where a family of peptide biomaterials is developed and manufactured for use in clinical applications and life science research.

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