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Dr. Dmitry Bulgin

applies cell–based technologies (*autologous bone marrow–derived and adipose tissue–derived mesenchymal stem cells*) and other proprietary methods in the field of:

-Reconstructive Dentistry

-Aesthetic & Reconstructive Surgery

-Traumatology & Orthopeadics

-Vascular (angiology) Medicine

-Wound Care

-Sports Medicine.



The main research interest:

The development of methods to accelerate the healing processes of tissues.

The tools and technologies for tissue and metabolic engineering to enable regenerative medicine.

Biological approaches for maintaining the human's performance and capabilities in the face of harsh accident conditions.

The research results and clinical outcomes have been presented at conference (as a speaker):	eren	ces	5	8			
(as a speaker).		2 - 2 8 - 2					
The First Symposium of Yangming and Nagasaki Universities, Nagasaki, Japan, November 29,	2004.						:
The International Symposium «Young Scientists Organizing Nagasaki Symposium of Internation	al Co	nso!	rtiu	m		•	•
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2012 eCM XIII Conference: Bone Fixation, Repair & Regeneration (Focus CMF, Spine, Trauma, Switzerland, June 24 – 26, 2012.	Vet),	Da	vos	',	•		•
5 th Vienna Biomaterial symposium, Vienna University of Technology, Vienna, Austria, November 2012	er 19	- 2	1,	3			•
UAE International Dental Conference & Arab Dental Exhibition AEEDC, Dubai, UAE, Februar	•y 5 -7	', 2(013				
1 st International Congress of Plastic Surgery - Fellows in Science, Ljubljana, Slovenia, September	er 18	- 2	1, ·				
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Bulgin Dmitry, Hodzic Enes. Autologous Bone Marrow-Derived Mononuclear Cells Combined With β -Tricalcium Phosphate (β -TCP) for Maxillary Bone Augmentation in Implantation Procedures. *Journal of Craniofacial Surgery*: November 2012 - Volume 23- Issue 6 - pp. 1728-1732.

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Bulgin Dmitry, Irha Ernst, Hodzic Enes, Nemec Boris. Autologous bone marrow derived mononuclear cells combined with β -tricalcium phosphate and absorbable atelocollagen for a treatment of aneurysmal bone cyst of the humerus in child. *Journal of Biomaterials Applicatios*: September 2013 - Volume 28 - Issue 3- pp. 343-353 [Epub ahead of print 2012 Jun 12].

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Prospective Technologies in Dental Tissues Regeneration

Interest in applications for dental tissues regeneration continues to increas as clinically relevant methods alternative to traditional treatments.

Dental tissues engineering is an opportunity that dentistry cannot afford to miss.

Recent progress in the studies of molecular basis of tooth development, adult stem cell biology, and regeneration will provide fundamental knowledge for that.

The impact of dental tissues engineering extends beyond clinical practice.

Dental tissues engineering could not have advanced to the current stage without the incorporation of interdisciplinary skill sets of stem cell biology, bioengineering, polymer chemistry, mechanical engineering, robotics, etc.

Thus, dental tissues engineering and regenerative dental medicine are integral components of regenerative medicine.

Citation: Bulgin D (2013) Prospective Technologies in Dental Tissues Regeneration. Oral Hyg Health 1: e102. doi:10.4172/2332-0702.1000e102



Replacing missing bone or adding mass to existing bone is often essential to the success of a dental implant.







An implant needs a critical mass of bone surrounding it in order to bind to it and deliver sufficient strength and stability.





If in the location where the implants are intended there is low mass of bone (width or height) a bone graft must be applied in order to maintain this critical bone mass.





A large variety of graft materials have been used for maxillary and mandibular atrophy to fill bone defects:

- AUTOGENOUS
- HOMOGENOUS (allograft)
- HETEROGENEOUS (xenograft)
- **SYNTHETIC** (*predominantly osteo-conductive*) substitutes.



Bone-graft materials usually have one or more components:

OSTEOCONDUCTIVE MATRIX, which acts as scaffold to new bone growth



OSTEOINDUCTIVE PROTEINS, which support mitogenesis of undifferentiated cells

OSTEOGENIC CELLS, which are capable of forming bone in the appropriate environment





Many clinicians consider harvested autologous bone (i.e. taken from the same individual) as the **"gold standard"** material for the reconstruction of osseous defects.



autological bone fragment from posterior iliac crest

Autologous bone grafts by their very nature are able to deliver a physiologically optimized combination of osteogenic cells and growth factors in a mineralized scaffold. However, limited availability of donor sites, requirement for an additional surgery to obtain the graft material, and extra chair time are the limitations of this technique.

A large amount of harvested bone raises the risk of postoperative functional or cosmetic morbidities at the donor site.











The autologous application of human **bone marrow cells** which are not expanded ex vivo has medico-legal advantages in clinical applications.

Defect repair and bone ingrowth, maturation, and modeling are cell-mediated processes.



Most clinical trials report successful bone regeneration after the application of mixed cell populations from bone marrow.



Bone marrow derived mononuclear cells (BMMNCs) secrete many kinds of growth factors and represent a potential key component in autologous graft for bone regeneration.

Growth factors that encourage the formation of new bone have been identified and applied to heal bone defects around medical and dental implants and without implant placement.

To reduce bone harvesting, various synthetic bone void fillers with improved biocompatibility have been developed.



The extracellular matrix of bone has been described as a composite material composed of collagen type I fibrils mineralized with nanocrystals of hydroxyapatite.

Bernhardt A, Lode A, Boxberger S, Pompe W, Gelinsky M. J Mater Sci Mater Med 2008;19:269.

Synthetic bone replacement materials are osteoconductive scaffolds that encourage the growth of new bone by apposition from adjacent bone surfaces or from bone-forming cells.

After sufficient bone has grown throughout the defect site, the scaffold no longer plays a role in the reparative process and, ideally, is resorbed. These materials are made from calcium salts:

- CALCIUM SULFATE
- CALCIUM CARBONATE
- CALCIUM PHOSPHATE
- BIOACTIVE GLASSES CONTAINING CALCIUM OXIDE

Highly biocompatible materials with retention of the natural porous trabeculation, architecture, and natural mineral content
closest components of human cancellous bone.



The class of calcium phosphate bone replacements is composed:

- porous ß-tricalcium phosphate (ß-TCP)
- dense hydroxyapatite (HA)

porous HA that has retained the structure of
 Coral (the exoskeleton of which has been chemically converted, in full or in part, from calcium carbonate into HA)

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- biphasic mixture of TCP and HA

Recently highly pure porous β -tricalcium phosphate (β -TCP) represents a very interesting practical tool for bone regeneration β -TCP is biocompatible porous material, new bone formation takes place along the surface in their micropores.

The open and interconnected porosity of β -TCP allows body fluids to circulate throughout its entire extent.

The range of pore sizes encourages tissue ingrowth.

β-TCP shows resorbable characters during bone regeneration, and can be completely substituted for the bone tissue after stimulation of bone formation.



Scanning electron micrograph of highly pure porous β -TCP:

(a) Macrostructure has a macrodiameter of 0.5 - 1.5 mm, (b) micropores of $100 - 400 \mu$ m, various size of micropores are seen. Hydroxyapatite can be produced with either a dense or macroporous morphology, and is typically sintered at temperatures above 1000 °C in granular or block forms.



The high heat of sintering produces a material that cannot be reshaped to fit into a bone defect (i.e. if in block form) and is **nonresorbable.**

Hydroxyapatite is a brittle material with low fracture resistance.

Hydroxyapatite is biocompatible, osteoconductive, and bioactive (i.e. it develops a direct, adherent bond with bone).







Biphasic calcium phosphate products which contain **hydroxyapatite** and β -**TCP** in various ratios are aimed at the provision of a bone grafting material which is able to degrade within a physiologically optimized time frame, while providing some measure of mechanical stability until sufficient bone in-growth has occurred.

While the ß-TCP exhibits quick osseous organization and is replaced by newly formed bone within a short period, the HA content ensures that the volume remains unchanged.

Collagen as a material for scaffold-based therapy



Scanning electron micrograph of porous atelocollagen sponge :

- (a) macrostructure
- (b) micropores of $50-500 \mu m$, various size of micropores are seen.

The atelocollagen is a cross-linked collagen material with abundant micropores

It has large pores that permit cellular entry and is degraded in vivo.

These characteristics suggest that this material may be a good candidate for use as scaffolding for implantation of cells.



Synthetic materials require the addition of bioactive factors or cells to promote tissue regeneration.



Recently, the transplantation of autogenous BMMNCs combined with synthetic grafting materials has been inaugurated and introduced as a clinical procedure in oral and maxillofacial surgery.



To date there has been no graft material which can be regarded as completely satisfactory.

My experience with freshly isolated autologous bone marrow derived mononuclear cells combined with synthetic biphasic calcium phosphate ceramic and absorbable atelocollagen for augmentation of the extremely atrophic maxilla and mandible is presented.

The techniques are based on stimulation of natural events continuously present in living bone, that is, the process of bone remodeling and offering both osteoinduction and osteoconductive features.

Cases breakdown Preoperative radiological evaluation



A 57-year-old man.

Panoramic X-ray image before augmentation procedures (horizontal and vertical augmentation, left maxillary sinus lifting).

A A Pa pro lef

A 46-year-old woman.

Panoramic X-ray image before augmentation procedures (horizontal and vertical augmentation, left and right maxillary sinus lifting).



A 48-year-old man.

Panoramic X-ray image before augmentation procedures (horizontal and vertical augmentation).

Materials and methods: Patients

Maxillary bone augmentation with the aim of embedding implants was performed under deep sedation in three healthy patients with advanced atrophy of the alveolar bone of maxilla.

TABLE 1. Case Breakdown							
Case Age G		Gender	Implant Placement Site in Maxillary Bone	Maxillary Bone Augmentation Method	Number of Implants in Maxillary Bone		
1	57	Male	Left upper 1, 3, 6 Right upper 3, 6	Horizontal and vertical augmentation	5		
				Left maxillary sinus lifting			
2	46	Female	Left upper 2, 3, 5, 6 Right upper 2, 3, 6	Horizontal and vertical augmentation	8		
				Left and right maxillary sinus lifting			
3	48	Male	Left upper 2, 4, 6 Right upper 6	Horizontal and vertical augmentation	4		

Bone marrow derived mononuclear cells preparation:



- (a) the bone marrow harvesting from posterior iliac crest
- (b) collection of bone marrow in plastic bag
- (c) the bone marrow processing by using Cell Separation System SEPAX S-100
- (d) qualitative assessment of BMMNCs population by haematoxylin and eosin cytological staining (magnification x 400).

Freshly isolated autologous BMMNCs in combination with synthetic biphasic calcium phosphate ceramic



At the time of surgery, BMMNCs and **synthetic biphasic calcium phosphate ceramic** granules were mixed, and excess fluid volume was removed using filtration and lowgrade vacuum.

A fully synthetic, two-phase calcium phosphate ceramic consisting of



Micro- and macroporosity (magnification 25x)

nano structure (magnification 1000x)

- 60% hydroxyapatite (HA)
- 40 % ß-tricalcium phosphate (ß-TCP)

Surgical grafting and dental implants procedures

Maxillary bone augmentation with simultaneous implants placement.



- A, Maxillary bone augmentation site preparation.
- B, Implants placement.
- C, Sinus filled by synthetic biphasic calcium phosphate ceramic combined with BMMNCs.
- **D**, Surgical sites are closing with silk non-absorbable sutures.

Sinus lift and bone augmentation procedures



A, Alveolar crest incision at the beginning of the bone augmentation procedure.
B, The planned maxillary bone augmentation site with advanced atrophy.
C, Sinus lift procedure: window preparation and sinus membrane elevation.
D, The BMMNCs and *Ossceram nano*® mixture grafting. The graft material isolation from the soft tissues by membrane.

Approximately 7 months later, dental implants were inserted into the augmented maxilla.



A, Embedding of the implantation material into the augmented maxilla (7 months after augmentation procedure).

B, Surgical site reopening for revision of implant integration after 3 months from the date of implants placement.

C, Histological condition of graft tissue 7 months after augmentation procedure. Hematoxylin and eosin stain method. Original magnification x200.

Patient S. L., 46 y. o., female (Case 2)



Advanced Maxillary Atrophy before augmentation

in 10 months after augmentation reopening for revision of implant integration

Patient S. L., 46 y. o., female (Case 2)





Favorable bone bonding was seen in the embedded implantation material in all of the cases

A 57-year-old man. A 46-year-old woman. A 48-year-old man.



A, Before augmentation procedure.B, At one month after operation.C, At twelve months after operation.

Radiological evaluation of healing period

The panoramic radiographic imaging was taken at 1 and 12 months after operation to evaluate the extent of reconstruction at grafted sites by using x-ray image analysis Planmeca Romexis software (Planmeca Oy, Helsinki, Finland).

Case Number and Side (Right/Left)	The Original Bone Height at the Graft Site	In 1 Month	In 12 Months	Bone Augmentation Rate From Original Bone Height to 12 Months
Case 1 Rt	9.2	19.7	19.4	52.57
Case 1 Lt	6.2	18.4	17.0	63.52
Case 2 Rt	4.0	17.4	15.2	73.68
Case 2 Lt	3.0	16.5	14.4	79.16
Case 3 Rt	3.2	14.1	12.7	74.80
Case 3 Lt	4.3	14.2	13.1	67.17
Mean (SD)	4.98 (2.35)	16.71 (2.25)	15.3 (2.53)	68.48 (9.59)

The mean increasing height of the graft site was 16.7 mm at 1 month and 15.3 mm at 12 months; that is, the height of <u>the graft site showed approximately 8.4% reduction</u> from 1 to

12 months.

Isolation of synthetic biphasic calcium phosphate ceramic by absorbable atelocollagen membrane from the oral cavity.







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Isolation of synthetic biphasic calcium phosphate ceramic by absorbable atelocollagen membrane from the oral cavity



After a recovery period of approximately 12 months the upper structures were mounted.









Patient : E. F., 59 y. o., female

Diagnose: Advanced Maxillary and Mandibular Atrophy, bone resorbtion, Parodontosis, gum recession.



Window preparation and sinus membrane elevation









Isolation of synthetic biphasic calcium phosphate ceramic by absorbable atelocollagen membrane from the oral cavity



After a recovery period of approximately 12 months the upper structures were mounted.



CONCLUSIONS

Within the constraints of our clinical outcomes, freshly isolated autologous BMMNCs in combination with synthetic biphasic calcium **phosphate ceramic** and absorbable atelocollagen membrane (as a barrier membrane) led to significant improvements clinically as well as radiographically and, hence, can be successfully used in the treatment of extremely atrophied maxilla and mandible.

This method does not require cell culturing, and lacks any risk of immune reaction as the grafts are autologous in nature.

The adjunctive clinical benefit of the BMMNCs preparation can be explained on the basis of tissue engineering,

i.e., tissue engineering generally combines three key elements for regeneration:

- 1) scaffolds or matrices
- 2) signaling molecules or growth factors3) cells .

The advantages of this method for clinical use:

- **the application of the cells immediately** after the bone marrow is collected, consequently the surgery can be performed the same day;

- **the cells do not need to be expanded** *in vitro*, they preserve their osteogenic potential to form bone and promote the proper bone defect healing.

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The research results and clinical outcomes have been presented at conferences (as a speaker)		23	* .*	.*	•	•
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