RESEARCH PROJECT –

“ 3D- PRINTABLE BIOPOLYMERS FOR SOCKET PRESERVATION TECHNIQUE: SOFT TISSUES RESPONSE ”

Introduction

It has been projected that 8.6 million individuals in the US will suffer from edentulism in the year 2050 [1] and the effect will be much greater in developing countries. Persons in the age group 35 to 45 years exhibit, in accordance with WHO guidelines [2], the maximum partial edentulousness prevalence and, because of lack of dental treatment, the condition can rapidly evolve to total edentulousness in older people. The phenomenon of the tooth loss, that can be connected to traumas, periodontal disease, traumatic extractions up to cancer of the mouth, may lead into moderate to severe bone deficiencies.
A bone defect is defined as an anatomical condition which doesn’t allow the conventional placement of implants [3]. In order to restore the lost anatomy and function, alveolar bone augmentation is often required. Many progresses were made in the last decades, but still several challenges exist concerning hard tissue augmentation procedures.
Bone substitutes and scaffolds are the main key materials for bone augmentation techniques.

Scaffolds include:

- non resorbable membranes
- resorbable membranes
- titanium meshes

NON RESORBABLE MEMBRANES. It is considered the first generation of barrier membranes and have been extensively studied and clinically tested for several years. They are produced primarily in polytetrafluoroethylene and titanium grade 5 alloy (Ti-6Al-4V), materials that present excellent biocompatibility as well as structural integrity for the whole period of their implantation. These membranes are especially used for large segmental bone defects since an adequate mechanical stability.
Two main drawbacks must be considered: the need to be removed with a second surgical procedure after that an adequate bone volume has been restored, representing a potential risk for the newly regenerated bone tissue, and the exposure of the membrane to the oral environment that increases the risk of secondary infections [4,5].

RESORBABLE MEMBRANES. They have been developed primarily to avoid a second surgery to remove the membrane after bone regeneration, also, in case of exposure during the healing phase can be fast resorbed, avoiding the risk of infection. The material used to produce them can be classified in:

- Natural materials (collagen and chitosan), present excellent biocompatibility and enhance wound healing and bone formation.
- Synthetic materials, mainly poly(L-lactide) or poly(L-lactide-co-glycolide), which are the most studied and clinically used bioresorbable polymers approved from FDA.

TITANIUM MESHES. They are the only devices made up of metal used as a barrier membrane for guided bone regeneration. Due to their stiffness, titanium meshes need to be shaped and adapted to the bone defect profile during the surgery. This sensitive, time consuming and

laborious step increase the surgery's duration and the final outcome of the procedure will be deeply influenced by the operator's ability.

CUSTOMIZED TITANIUM MESHES. In the last five years titanium alloys have been employed also by means of additive manufacturing processes, in order to fabricate customized devices for bone regeneration [6].
Meshes obtained with 3D printing have several surgical advantages:

- more precise and no need of adaptation during surgery
- rigid and the space making effect is more guaranteed
- margins and corners round

However, there are still some important limitations:

- no possibility to interact directly with the design of the mesh
- necessity of removal after several months
- unclear effects of post-production treatment on the surface of the mesh [6,7]
- high costs (average of 200€ / mesh)

BONE SUBSTITUTE. They can be divided into:

- autografts: harvested from the same individual
- omologous graft: human derived
- eterologous graft: animal derived demineralized de-hydrated bone
- alloplastic grafts: synthetic (ceramic, polymeric, metallic)

All the bone substitutes available on the market have osteoconductivity property (the direct action of the material as a molecular signal on the native stem cells of the bone), but not osteoinductivity (the capacity of the biomaterial to create a network for the blood cells of the host in order to provide a 3D architecture into the bone defect). Osteoinductivity is confined only to autografts bone substitute [8,9]. Therefore, the golden standard for bone regeneration remains the autogenous bone, despite its invasively and high morbidity. The most common bone substitutes used in clinical practice are eterologous grafts. The constant availability as well as a reasonable price make the first choice in dentistry and implantology.. Finally, synthetic bone substitutes are very common in dentistry, except the use of beta tricalcium phosphate alone or mixed with hydroxyapatite.

Among the different 3D printing techniques, the fused deposition modelling (FDM), which consists of extrusion of a filament in a series of layers on a plate to create a three-dimensional object, offers the potential to design and fabricate, at reasonably low cost, highly reproducible, bioresorbable 3D scaffolds with a fully interconnected pore network. Typical materials used with FDM with biodegradable and bioresorbable properties are poly(lactic acid) (PLA), poly(ε-caprolactone) (PCL) [10].
In the last few years, polylactic acid (PLA) has become one of the leading biomaterials FDA-approved in biomedical field, thanks to its interesting properties such as being a thermoplastic, bioresorbable polymer with good mechanical behaviour. On the biological point of view the resorption pattern is one of the most important aspects because for an optimal bone growth a device has to last for a minimum time of 4 to 6 months. Young's modulus of PLA is around 3 Gpa, and tensile strength ranging from 50 to 70 Mpa. Due to its low elongation at break and a Tg close to 60 °C, PLA is considered a very brittle material so, this limits its use in applications requiring high plastic deformations at higher stress levels

[11]. So, PLA can be combined with other materials to reduce its fragility as hydroxyapatite (HA), tricalcium phosphate (TCP), brushite and monetite [12].
Hydroxyapatite is the main component and crystal structure similar to biological hydroxyapatite of human hard tissues and can be used as an additive to modify common 3D promised biological raw materials, increasing the mechanical properties and osteogenic activity of the material. However, the resorption rate of HA is extremely slow as compared with TCP, that is well known material by their synergy with environmental tissues and ability to induce osteoconductivity. Therefore, due to high mechanical properties and exceptional bone remodelling capacity, β-TCP can be useful to be used along with PLA polymer to create tissue scaffolds.
Dicalcium phosphate dihydrate (brushite) cement is also a biocompatible material that can be resorbed under physiological conditions. In vivo studies investigating the biological reaction to and degradation of brushite cements have reported complete or extensive resorption, in addition to fragmentation or long-term stability of the cement [13]. Crystallographic and spectroscopic analyses of retrieved brushite cement implants have shown however that a marked reduction in the rate of resorption occurs following the formation of hydroxyapatite in the cement, the presence of which is thought to be caused by hydrolysis of the brushite [14].
Although dicalcium phosphate anhydrous (monetite) has similar chemical composition of the brushite, its behavior in vivo is quite different, mainly due to differences in water solubility at physiological pH. Monetite does not reprecipitate into HA in vivo, and recent animal studies have demonstrated its good osteoconductive and osteoinductive properties, as well as being largely resorbable in vivo [15].
Polycaprolactone (PCL) is another aliphatic polymer, FDA-approved, belonging to the polyesters' family. PCL is one of the most preferred polymers in biomedical field. Differently to PLA, PCL has low tensile strength (about 23 MPa), but a high elongation at break (4700%), which gives it a highly elastic behaviour and Young's modulus from 0.2 to 0.4 Gpa, very smaller than that of the PLA [16]. It has been found that PCL has a high biocompatibility, even if a little lower than polylactides but, despite this, it is still widely used in biomedical field due to its higher stability [17]. The complete degradation of the polymer takes 2-3 years, that is a relatively long time, but it is possible to reduce it combining PCL with other elements such as hydroxyapatite (HA) or tricalcium phosphates [18].

The scope of this research was to introduce into the medical/dental community a novel concept for bone augmentations, with the following characteristics: printing a fully resorbable device, avoiding the second surgery and with an affordable price, by testing the materials in socket preservation procedures.

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Materials and methods

The clinical study has been conducted in the University Department Trisakti Indonesia and the protocol has been evaluated and cleared by the internal Ethical Committee with the following number….. All the proposed treatments were conducted according the Declaration of Helisinki for human rights.

a- Sample size calculation

For the calculation of the sample size the following formula was used

$$n = \left[\left(\frac{Z\alpha}{2} + Z\beta\right)^2 * \frac{\sigma^2 + \sigma^2 + \sigma^2}{(\Delta)^2}\right] + (m-1) * k$$

Where :
n is the total size for all groups
Z α/2 is the critical value for the level of significance (5%) = 1.96
Zβ is the critical value related to the power (80%) =0.84
σ is the standard deviation in every group = 0.5
Δ is the clinically minimal difference between the groups= 0
m is the number of planned treatments =3
k is the correction of drop outs=1

39 patients were requested to start the study.

b- Patients enrolment

Patients with no exclusion of sex and race > 18 years old who have at least one tooth to be extracted. The subjects had to be medically healthy, with no assumption of bifosphonates and light – medium smokers (maximum 10 cigarettes/day). Pregnancy and lactation were considered as exclusion criteria.
After the enrolment all the subjects were randomly allocated to 3 different groups by using the software www.randomizer.org

- TEST 1 (described in the following paragraphs)
- TEST 2 (described in the following paragraphs)
- CONTROL (described in the following paragraphs)

c- Surgical treatment

All the extractions were performed without flap elevation , a careful cleaning of the socket was done, without grafting any additional bone substitute. A periapical X-ray was taken immediately after the extraction. Only after the extraction the operator was allowed to open the sealed envelope containing the result of the randomization, according to the following :

- TEST 1 : a 3D printed disk of poli-D-lactic acid with 10% of hydroxyapatite had to be trimmed inside the gingival margin and ensured with a crossed mattress suture. A picture was taken. (Fig.1)

- TEST 2 : a 3D printed disk of poli- **ε** caprolactone with 20% of β- tricalcium phosphate had to be trimmed inside the gingival margin and ensured with a crossed mattress suture. A picture was taken.
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- CONTROL : extraction open to heal. A picture was taken.

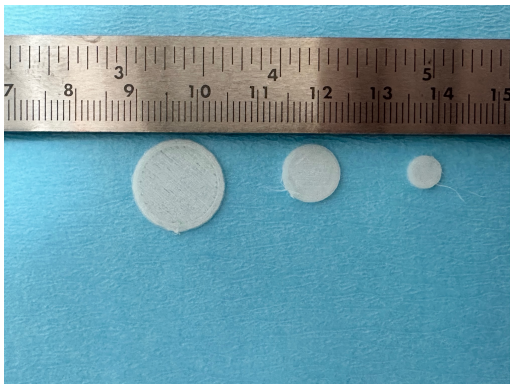


Fig. 1: example of 3D printed disks with different diamters.

d- Follow up

Follow up schedule was as following:

- 10 days : suture removal and picture
- 30 days : picture
- 45 days : X-ray and picture

Data extraction and evaluation

Clinical pictures were taken at every step of the research in order to evaluate the progression of the healing of the soft tissues.
A blind examiner received all the clinical photos and by the use of the open source software Image J (https://imagej.net) the distance between the buccal and the lingual gum margin will be measured in pixels and then converted in millimetres, after the equalization generated by means of the same software. (Figure 2)

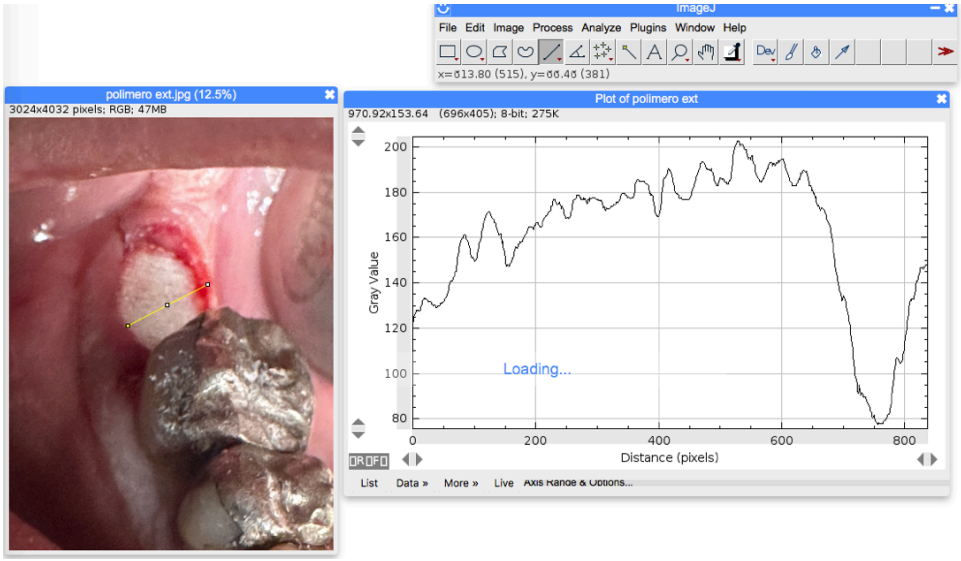


Fig.2 : calculation of the area of the sockets with Image J.

Progressive soft tissues closure was considered as primary outcome, bone filling of the extraction socket as secondary one and both analysed and stratified according to site, the age and the gender.

Statistical analysis

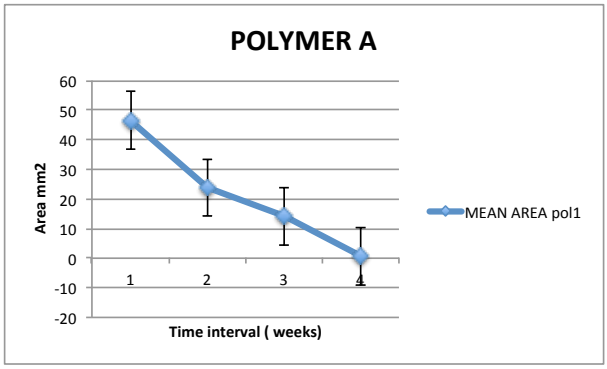
The following hypothesis were considered: there is no difference between Control and Test Groups (Null hypothesis) and there is a difference between the two groups (Hypothesis 1). T-student test was performed with a significance level of 0.05.

Results

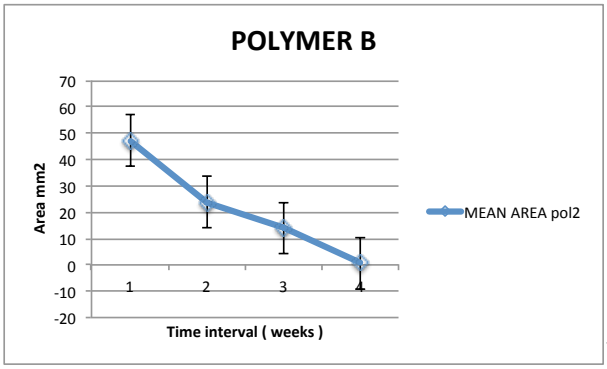
39 subjects , 13 per group, (20 males and 19 females, age 20-70) were enrolled in the study and no one of them reported major complications (infection, swelling, pain) throughout the duration of the follow up. The total number of extracted teeth was 39, 10 molars (upper and lower), 20 premolars (upper and lower), 9 front teeth (upper and lower incisors). The mean of the initial exposed area was 46.5± 8,25 mm^2 ; 47.1 ±8,67 mm^2 and 45.6± 7.25 mm^2 on Group A ,Group B and Control Group respectively. At the end of the observation (4 weeks) the mean of the exposed area was 0.6±0,84 mm^2 ; 0.6±0,7 mm^2 and 1.2±0,9 mm^2 for Group A , B and Control Group respectively.

Student t-test was performed for the stratification of the results anterior and posterior teeth and the polymer type with the primary outcome.
The first analysis compared the outcomes between the 3 groups and t value was 2.33 (above the critical t value), therefore the null hypothesis can not be accepted. The second stratification was done for posterior sites between the 3 groups and t value was 1,16 (below the critical t value), therefore the null hypothesis can be accepted. Similar results were obtained for the other sites.

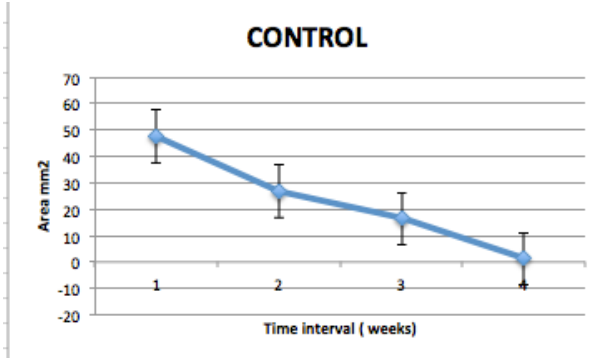
The observation period was 4 weeks and the total resorption of the disk and the closure of the sockets was observed.



a



b



c

Figure 4 a,b,c: polymer A is poli-D-lactic acid with 10% of hydroxyapatite; polymer B is poli- **ε** caprolactone with 20% of β- tricalcium phosphate; c is the control group.

Benefits (rewards) for the participation to the study

All the participants were treated free of charge for the extraction (regardless site and complexity) and received 10% discount on the implant-prosthetic treatment.

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