# Bioglass as space filling bone substitute

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## 1 Introduction

Today, bone transplants and substitutions are a frequently used surgical technique, bones are transplanted at least 10 times more often than any other transplantable organ [1]. Bone transplants or grafts have come a long way since they have been used only for structural replacement of defect bone. A successful bone graft should not only support normal forces, but also incorporate itself into the operation site, revascularize as new bone forms and desirably also accelerate the normal healing process.

An ideal bone substitute has to provide three attributes to successfully replace a piece of bone in a living organism. First, it should provide an osteoconductive matrix. Second, there have to be osteoinductive properties or factors present and third, osteogenic cells have to be present in the graft or must easily be able to grow into the graft. The easiest way to satisfy all three conditions is to use an autogenous bone graft, i.e. from within the same body/patient. However, there are several potential complications involved with autogenous grafting, such as donor-site morbidity, limited availability for harvest, and increased operative blood loss. It has therefore become necessary to find suitable alternatives, particularly when a large graft is required [1].

The motivation to incorporate the favorable properties of different materials into an effective bone graft compound has led to the manipulation and development of various new synthetic bone graft products. The most interesting and potentially useful substitutes are composite grafts, such as an osteoconductive material inlayed with mesenchymal cells synthetically produced from cell culture.

### 1.1 Bioactive glass

Bioactive glasses have been developed in the late 1960s by Larry Hench et al. [2, 3]. The high biocompatibility of this class of glasses has led to extensive use as implant materials in the human body to repair and replace diseased or damaged bone, albeit the structural parameters of these surface reactive ceramics make them not suitable for load-bearing applications [4], but more suited for replacement of non-structural parts of the human body.

The original bioglass composition is also known as 45S5 glass and is composed of $SiO_2$, CaO, $Na_2O$ and $P_2O_5$.[4] In its simplest form, the body thinks that the calcium loaded glass powder is in fact bone material and stimulates the re-growth of new bone material between the fractures, where the bioglass has been used as a filling material. Exemplary use of bioactive glass material includes packing into the cavity left by an extracted tooth as well as tiny cast glass implants being used in the middle ear [5].

There can be found many applications of Bioglass in general, but most of them are restricted to where the bone substitute is used in reconstructive surgery like bone substitution during operations or reconstructions in the facial region. Bioactive glasses can be used as synthetic bone graft materials for orthopaedic, cranio- and maxilofacial and periodontal repair, have been used for the embedding of cochlear implants into the skull, for keratoprosthesis, for anchoring dental implants and for bone tissue engineering scaffolds (see [6] and [7] for two exemplary works).

## 2 Research question

In the course of the MAS ETH in Medical Physics I had the chance to visit a lecture series in 'Medizinischer Akustik'. During this lecture we learned a lot about cochlear implants. These kind of im-

plants are a surgically implanted hearing aid which does not amplify the sound, but directly stimulates the still functioning auditory nerves of the wearer. The whole system is composed of different parts, some outside the implantee and some inside which can be generalized into five parts (adapted from [8]): A **microphone**, which picks up the sound from the environment, a **speech processor** which filters the incoming sound and sends the processed signals to the **transmitter**, where a coil held in position by a magnet placed behind the ear transmits the processed sound to the **receiver and stimulator** by electromagnetic induction. The receiver/stimulator is secured in a bone cavity beneath the skin and converts the signals into electric impulses and sends them to the **electrodes** which are wound through the cochlea (in an array of up to 22 electrodes) which then send the impulses to the auditory nerve system inside the skull.

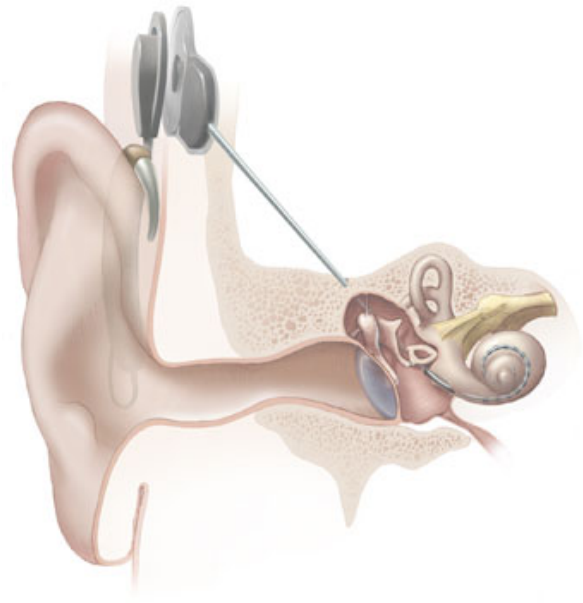

Fig. 1: Illustration of cochlear implant from the National Institute on Deafness and Other Communication Disorders at the National Institutes of Health (from [8]).

Since the implant is generally worn for a very long time a secure placement and embedding into the skull is crucial for the acceptance of the implant and for the well-being of the patient. It has been shown that bioglass-coated metallic surfaces have a good integration into the bone and that osteoblast-like cells proliferate on a bioactive glass surface [9], so the coating of the receiver/stimulator with bioglass would make it a suitable implant. Albeit it has also been shown that there are biologically more well suited materials (e.g. hydroxyapatite) for the coating of metallic surfaces [10], I suppose that an implant coated with bioglass would greatly facilitate the implantation process of the receiver of a cochlear implant in the skull behind the ear. Since the resulting cavity could be filled with bioglass powder this would also facilitate the secure ingrowth and placement of this implant in the operation site (see Fig. 2 for an overview). It has been said before that implants, which are made fully from bioglass are not suited to replace bone at load bearing sites in the body. They are although well suited to fill non nicely formed cavities since bioglass can be applied in the form of a fine powder to fill the cavities in the bone surrounding the implant [11]. The combination of this space filling properties and the ability to coat the surfaces of implants with bioglass make the chosen material well suited to kill two birds with one stone.

In this particular problem the mechanical strength of the bone substitute is a secondary question, so I would like to focus more on the examination of the material in terms of biocompatibility. It is known that bioglass coatings help to integrate metallic implants in the human body. I would like to investigate this osseo-integrating properties of a bioglass coating and bioglass filling of the resulting cavity and study how fast the bone-building cells are able to grow into and through differently thick and differently dense packed bioglass powder.

Two of the questions I would like to answer are:

- Generally: Is bioglass powder a good surface for osteoblasts to grow on and a good volume to grow into?
- Does the packing density of the powder influence the ingrowth of the cells? This question is important to answer, since since the necessary powder volume needed to fill the cavity might not be easy to asses in the operating theatre by the surgeon during the planning of the operation, so he is prone to use more than needed and to pack more than enough

powder in-between the receiver/stimulator and bone than needed and thus potentially overfilling the cavity, resulting in a higher packing density.

There are some more questions I would like to answer (How well are the 'phases' bone, powder, implant coating and implant integrated after the healing process, are there certain combinations of powders, which are more suited as a filling material, etc.), but this is beyond the scope of this homework.

# 3 Description of experiments

## 3.1 Influence of packing density

Kontonasaki et al. [12] have shown that when pellets of bioglass powder are immersed in simulated body fluid (SBF, described in [13]) as soon as 24 hours after immersion an initially already present amorphous layer of Hydroxy carbonate apatite (HCAp) is converted to a crystalline phase of small spherical cells of apatite, smaller than 10 $\mu$m in size. After 48 hours of immersion the surface of the specimen is fully covered by a layer of apatite with aggregates of approximately 10–20 $\mu$m size. Other authors reported different timeframes [12, p. 1170] for bioglass in bulky form, but all references cite, that after 24 hours the amorphous phase starts to crystallize into HCAp, which promotes bone growth. Kontonasaki et al. showed the formation of HCAp on only one configuration of pellets (13 mm in diameter and 0.8 mm in thickness), for only one packing density (a quantity of 0.22 mg pressed in a vacuum press with one defined force). I would like to study the influence of differently dense packed bioglass powders, to simulate the different packing of the powder between the cavity in the skull and the covered implant.

The influence of packing density would be studied with differently pressed pellets, with the difference that I would apply the force manually and apply much less force than Kontonasaki et al. (they spoke of a force of 7 t!). I would like to study the influence of packing density as observed in situ in the operation theatre, where the implant with the surface coating is embedded in the cavity which has been milled into the skull. The surrounding is filled with the bioglass powder before closing the wound. Since we would like to extract a meaningful parameter which can be used in vivo in the operating theatre, we would record the force for hand-pressed pellets with a push force gauge for the different pellets, while taking care that we always use the same amount of bioglass powder for producing the pellets

Fig. 2: Illustration of desired pellet configuration in situ. Black is the metal implant, in the experiment case just a dummy metal made from the same material as the real implant as in the in-vivo case. Dark grey is the bioglass coating on the surface of the implant, light grey is the hand-pressed outer pellet made from bioglass powder. The surrounding grey should depict the skull bone and the fleshy pink should depict the skin of the patient.

After we've generated a big enough sample population, we would prepare the bioglass samples generally in the same manner as Price et al. [9] and Kontonasaki et al. [12]. In addition we would use pellets where the core is a metal pellet scaled according to the dimensions of the implanted receiver/stimulator, so that the pellets still fit in a well plate for incubation. After preparing several samples with multiple packing densities of the surrounding bioglass powder we would then be able to prepare a cell viability assessment and study the influence of packing density of the pellets. Since we are aiming to study the influence of packing density in an environment as close to the desired application we would not polish the disks as mentioned in [12], but leave the surface of the pellets unaltered.

## 3.2 Growth rate and cell 'health'

Following the preparation of the pellets we would put them in a well plate and immerse the plates with SBF where we would also add osteoblast cells (e.g. MG63 osteoblast-like cells, originally isolated from a human osteosarcoma like suggested in [9]). While also keeping a control-group, the plates would be incubated at 37°C, 5% $CO_2$ during some days (or even weeks). Afterwards the amount of cells grown on and into the pellets could be extracted with standard cell viability and counting tests. The cells would need to be extracted from the differently packed pellets as follows: detach the osteoblasts that have grown into the pellet with trypsin (or another agent suited for the task), wash all cells out of the pellet (all cells, including healthy and non healthy ones), centrifuge the suspension to isolate the cells from the medium including the trypsin, re-suspend in medium and count them using a Neubauer chamber (as learned in the block course with Heike...).

After extraction of the cells from the pellets the viability can be assessed using a marker of cell health. My girlfriend (a pharmacist) suggested a dye exclusion method, e.g. with trypan blue to selectively mark dead cells. With this test the fraction of living to healthy cells can be calculated which then can be backtracked the force applied while pressing the pellets into the simulated cavity thus giving the surgeon a mean to control the applied pressing force during the operation.

# 4 Expected results

## 4.1 Growth rate and penetration depth

It has been shown before, that bioactive glasses are viable surfaces for cells, especially bone cells [9]. I expect no difference to this findings in the outcome of our experiments.

Osteogenic cells like to grow in and on differently packed pellets of bioglass in general, but I presume that we will find a small difference in the amount of cells grown into the pellets depending on the packaging density. If the pellets are very strongly packed, the cells do not grow well into the pellet. With the proposed method we cannot directly measure the penetration depth of the osteoblasts into the pellet, since we only have a measure of the total account of cells that have grown on the whole implant. But since we use the same amount of bioglass powder for every sample we should be able to correlate the total amount of found cells to the penetration depth of the cells into the pellet. The penetration into the pellet could be measured directly using electron microscopy, but this is above the scope of this homework.

## 4.2 Cell health

I presume that we observe no statistically significant difference in cell health between the different packaging density, since the osteogenic cells will be able to grow into the pellet and be 'happy' even when the pellet is densely packed. Since I try to minimize the influence factors, I would only study one type of bioglass powder, with a certain well known particle size for a first round of experiments. It has also been shown that the particle distribution of bioactive glass powder does not influence the outcome of healing, at least in terms of mechanical stability [11], so I suppose that this dependence can be left out for the moment.

# 5 Discussion

I postulate that my experiments predict to that the filling of the skull cavity from the implantation of a cochlear implant is a feasible way of achieving a good integration of the implant into the bone without imposing healing problems to the patient. Moreover, I suppose that the method is a fairly easy method for the surgeon, since he can put as much powder as needed into the cavity and press it with a special force gauge until the desired packaging density is reached. This facilitates the operation procedure, since the milling of the cavity does not need to be controlled extremely precise and the arising variations can be overcome with the use of bioglass powder. Nonetheless, these findings are all taken from bench experiments, to really prove this assertions, clinical trials need to be carried out.

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