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Bioactive Glass Coatings for Adherence to Bone Tissue

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Abstract

The purpose of the experiments for this research project was to determine whether certain compositions of borate-based glasses are bioactive and can be used to bond titanium pins to bone. Two different major experiments were done. The first experiment was done to determine the bioactivity of the glass. The second experiment was done to simultaneously determine the bioactivity of the glass and determine whether or not the glass would bind to titanium and bone.

The majority of the research consisted of working with different compositions of borate-based glass, making glass pins, and coating titanium pins with the glass. The making and coating of pins was done in the Materials Research Center (MRC) at UMR. Various glasses were used and various synthesis techniques for the glasses were used.

The smaller but most important part of the research was done in the UMR animal lab. This part of the research involved performing surgery on rats to test the viability of the glass disks *in vivo*.

Introduction

Previous research has been done with bioactive glass. The research is mainly in the development phase and bioactive glass is not being currently used for commercial applications. The main differences between the established bioactive glass and the particular glass tested in this experiment is the chemical composition. Some established bioactive glasses have different amounts of borate with other chemicals.

The exact composition of the glass was not the focus of my research project. The synthesis procedure for the glass pins and the glass coating on the titanium pins was a trial-and-error procedure, and was mostly done by graduate students working in the MRC. The major focus of this paper will be the implanting experiments and the theory.

Theory

The potential of this research is that a type of glass could be developed that would improve the healing process for people who break bones and need to have titanium pins inserted into the bone. An ideal glass is one that can simultaneously bind to titanium and bone. The titanium pins can be coated with the glass and then inserted into the bone.

Once the pins are inserted, the glass should form a strong bond with the surrounding bone. The titanium pins themselves don't bond to bone as well as some other substances. One such substance that has been shown to improve bonding strength with bone is borate glass. This strong bond will speed the healing process by providing an indirect bone to pin bond.

Several other borate-based glasses have been shown to be bioactive – i.e. the glass provides a porous surface upon which biological tissues such as bone can permeate and proliferate. Prior to inserting the glass into a biological system such as the body of a rat, the glass needs to be soaked in bone marrow tissue.

Soaking the glass in bone marrow tissue requires a separate group of experiments to harvest the marrow stem cells and infuse the glass plates with them. The ideal conditions for inoculating the glass plates with cells can also be determined by practicing with some other types of bone cells instead of just marrow cells. The experiments for determining the conditions for inoculating the glass plates are discussed in the section titled "related experiments".

For the main experiments the marrow cells are taken from a live rat and cultured to keep them alive. The bone marrow tissue is taken from the femur of sacrificed rats, and it contains bone stem cells. Bone stem cells are generic cells that can develop into bone cells given the right biological environment. The porosity of the glass was an important aspect of the composition. The successful growth of bone on the glass required pores of a particular size.

Experiment 1

The first experiment's main purpose was to determine the right porosity and composition of glass to maximize the bioactivity. Two different types of borate glass were used, one called H12 and another called 45S5. Two different types of commercial bioactive glasses were also used to compare to the H12 and 45S5.

This experiment was repeated several times to test different compositions and porosities of the experimental glasses. Small disks were made for each type of glass, each about 3 mm thick and 2 cm in diameter.

Surgery was performed on the rats and the disks (which had been soaked in the bone marrow solution for 2 days) were inserted into the rats. The rats were shaved and put under anesthesia and four shallow incisions were made in the skin on their backs near the spine. The incisions were only skin-deep, because the disks needed to be inserted just underneath the skin.

The skin was separated from the fascia underneath by probing a small area on each side of the cuts with a blunt tool. Once a pocket was made, the disks were inserted underneath the skin in each of the four cuts. The wounds were then closed with staples and the disks were allowed to stay in the rats for 6 weeks. The rats recover fairly quickly from the wounds to the skin. They can walk around without much problem almost immediately after waking up from the anesthesia.

After six weeks the rats were sacrificed and the disks were extracted and analyzed. Eight rats were used for the initial experiment. The H12 disks showed about 50% of the amount of bone growth as the commercial disks. The 45S5 showed more bone growth than the H12, averaging about 75% of the growth on the commercial disks. The disks are analyzed for growth by placing them in a clear thermoset polymer solution and grinding them down into a thin section with a representative slice.

Experiment 2

The second experiment was to simultaneously affirm the bioactivity of the glass while testing the bonding capability of the glass to bone and titanium. The samples made were 16 small cylindrical plugs of either pure titanium, glass coated titanium, or pure glass plugs.

The pure titanium pins were used as a control to compare with the glass coated titanium pins. The pure glass pins were used to further assess the bioactivity of the glass. Eight rats were used again for this surgery.

The rats were put under anesthesia using isoflurane gas. The shin bone was used to test the glass for two reasons: it is easily accessible to surgery because there is very little muscle covering on the shin, so very little tissue has to be cut through to access the bone.

An incision was made in the skin of each of the shins of the rats. The incision leaves bare a small flat surface of bone where a small hole 2 mm in diameter was drilled out of the bone. One sample of the pins was inserted into each of the holes drilled in the bones. The rates generally recover quickly from this surgery. As with the subcutaneous implants, the rats can usually walk around easily immediately after waking up from the anesthesia. The wounds were stapled up and the pins were left in the tibia of the rats for 6 weeks. The rats were then sacrificed and the tibias of the rats were solidified in the polymer and ground down for analysis.

Related Experiments

For these experiments, porous glass disks are used to grow bone cells on. Bone cells migrate into the pores of the glass disks while the glass is soaking in the marrow solution. The marrow solution is made from the marrow cells taken from the femur of a live rat. Once the marrow cells are taken from the femur of a live rat, they are placed in a medium solution that contains plenty of nutrients for the cells to survive and replicate.

There are certain conditions that optimize the growth and inoculation of cells in the pores of the glass disks. The pore size of the disks is an important concern. If the pores are too big, there will be less total surface area for the cells to bind to the surface of the glass. When the pores are too small, the cells cannot migrate from the outer solution into the pores to attach to the surface of the disks. If the pores are the right size, the cells easily migrate into the pores and attach themselves to the large surface area of the disks. Marrow cells are difficult to attain because a live rat has to be sacrificed to get them.

To minimize the amount of rats sacrificed and still experiment with the pore size on the glass disks, different types of cells are used other than marrow cells. Other types of bone cells are approximately the same size as marrow cells and are much easier to obtain. In particular, some types of bone cancer cells can be grown in culture dishes in an incubator and used to experiment with the pore size of the glass.

Bone cancer cells tend to work well for this type of experimentation because they grow and replicate very quickly, so large colonies of them can be made in about a day. These cells are ironically called UMR cells. They tend to be very big and their colonies can be seen with the naked eye.

The following is an example of the experiments used to test for the correct pore size in the glass. A sample of the UMR cells are incubated in an oven until the cells form colonies and completely cover the bottom of the plate. Once the cells cover the plate,

they are "split". When the cells are incubated in the plates, they attach themselves to the surface of the plate. A chemical called trypsin is added to the cells. Trypsin causes the cells to detach from the bottom of the plate, and float in the fluid. A fraction of the total cells are taken off of the plate and put onto a new plate to form new colonies. In this way, large numbers of cells can be generated out of just a few cells.

Once a solution of the cells is obtained, the glass disks are immersed in the fluid with suspended cells. The suspended cells migrate into the pores on the disks and the cells are allowed to grow on the surface of the glass. In this way, many different disks with many different pore sizes can be tested to see which size pores are most conducive to cell growth.

Results and Discussion

Experiment 1 showed that the newly tested glasses did exhibit some bioactive behavior. The 45S5 pieces and the H12 pieces showed that after presoaking in bone marrow solution, and incubation under the skin of the rats, there was significant bone growth inside the pores on the large surface area of the glass. The pores not only provide a place for the bone stem cells to reside, but they also provide a large surface area to promote bone proliferation. The surface and chemical structure of the glass itself appears to aid in bone proliferation.

There were some design problems in the second experiment which prevented a proper analysis of the efficiency of the glass-titanium-bone interface. First, the porosity of the glass was somewhat difficult to control in the synthesis. If the pores were too big, the glass was unstable and in would easily crumble. If the pores were too small, the bone marrow solution wouldn't penetrate as easily and it reduces the efficiency of the bone growth.

The second problem occurred with the method of insertion into the rat's bone. The method involved using a small drill with only a 2 mm diameter bit. The hole was drilled in the shin of the rat, where there was little muscle cover (this reduces the amount of tissue destroyed on the rat, speeding its recovery). The bone at the shin position is pretty thin, and it is difficult to find the right spot to drill.

The entire length of the pins is only about 4 mm long. When the pins are inserted into the hole in the bone, it tends to come out after the wound is closed. This was the problem with three of the eight tested samples.

When the would was opened after six weeks to examine the adherence of the pins into the bone, the pin had actually come out of the hole and was resting along the shin just underneath the skin.

The samples that did stay in the bone adhered well and seemed to have made a good bond with the bone. Without having a good number of samples to analyze, however, it is difficult to say whether the glass coating on the pins provided and improved method of implanting titanium pins.

Conclusions

The first experiment confirmed that there is good reason to conduct further experiments on bioactive glasses. It showed that the new glasses tested, 45S5 and H12, exhibited bioactive behavior. In order for the glass to promote bone growth it is important to determine and comply with standards for the porosity and composition of the glass.

The design of the second experiment needs to be improved and optimized before any conclusions can be drawn about the viability of using bioactive glass to coat titanium pins before implanting them. The pins that were successfully implanted showed promise in bonding with the bone, however more experiments are necessary to confirm how well the implants work.