The Significance of the Patient Innate Immune Oxidative Burst for Drug Delivery Vectors and Biomaterials (Implanted Medical Devices)

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Introduction

An enormous variety of biomaterials are used within the field of tissue engineering and regenerative medicine (TERM) and then further as vectors in degradable controlled drug delivery systems (DDS). Their interaction with the immune defence mechanism of the patient is important for the performance of the device. After implantation leukocytes will infiltrate the graft, initiated by acute inflammation and reactive oxygen species (ROS) are formed. When a graft induces a high degree of cell activation and ROS formation is triggered, this may lead to an accelerated degradation rate of the material. With biocompatibility testing only, it is still hard to predict the exact immune reaction when implanted, since a reaction is patient specific. Reactive oxygen species (ROS) are very important in regulating inflammatory responses, but are often overlooked during biocompatibility testing despite their importance.

The aim of this research was to find a sensitive and fast assay to predict the performance of different biomaterials that are used in TERM and DDS. An advantage of such an assay would be that it could predict the performance of the biomaterial patient specifically, with a potential for the healthcare provider to use it as a standard pre-screen before an (surgical) intervention.

The biomaterials that were tested were produced commercially or made by the department, and consisted of hydroxyapatite-silk fibroin-hyaluronic acid (HA-SF-HA) powder (made by the department), hydroxyapatite (HA) powder (made by department), Hyaff-11 PLA mesh (F.A.B. S.r.l – Italy), Permacol (TSL) and Hyaff-11p75HE PCL-PLA mesh (F.A.B. S.r.l – Italy).

Methods

To measure the materials' reaction a chemiluminescence kit was used (Knight Scientific, UK), which contained the following reagents: Adjuvant K, Blood dilution buffer, Formylmethionyl-leucyl-phenylalanine (fMLP) (10 µmol/L), Pholasin (10 mg/L), Phorbol 12-myristate 13-acetate (PMA) (10 µmol/L). This chemiluminescence kit was used to test the biocompatibility of the materials used in the experiment, by measuring the production of ROS as a result the material. During the experiments two components were used as stimuli; one was fMLP that is a peptide that can bind to a specific G-protein linked membrane receptor and the other PMA bypasses the membrane receptor but activates the same NADH oxidase as fMLP. When there is a high reaction with fMLP and almost no PMA reaction it means that the reaction is due to phagocytosis, and the other way around means that a normal pathway is activated for apoptosis. The reaction before injection of fMLP gives information about the cell activation, when there is a high signal before fMLP of one of the materials it means that there is ROS production and saturation is more likely. When saturation of the biomaterial occurs, there will be (almost) no signal after fMLP and PMA.

For all of the experiments with different biomaterial sets the same settings were used, the binning was set to 4x4, an exposure time of 10 seconds and a sensitivity of 10. With a binning of 4x4 the signal to noise ratio is decreased but the resolution reduced. Prior to addition of the fMLP the well plate was incubated in the machine for 30 minutes and after another 20 minutes of measuring fMLP reaction PMA was added. Throughout which chemiluminescence was measured continuously using FDSS/µCELL (Hamamatsu, Japan).

To test different materials' reaction on freshly derived human whole blood of a finger puncture a kinetic plate reader, FDSS/μCELL was utilised. The device consists of a dispenser head with disposable tips, stages for the assay plate and for two different compound plates, an excitation light source, removable emission filters, camera lens and camera itself. Within the device it is possible to measure the kinetics of luminescence or fluorescence, by dispensing the compound into the assay plate and at the same do the detection in our assay for upto 96 wells simultaneously. Black plates with a chimney well (Greiner bio-one) were used to avoid cross talk.

Conclusions

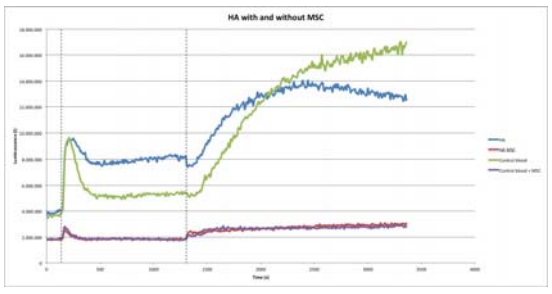
HA-SF-HA powder showed consistently and greatly elevated levels of ROS compared to the remaining biomaterials. Not only in the donor graphs, but also in the combined graph for n=4/material/donor, which showed a greatly elevated signal before fMLP addition. Also the PMA addition showed that the biomaterial was already saturated by cell activation. From this a careful conclusion can be made, The ROS release profile of this material was for three donors even three times as big as the no material control, in one of them even more than three times .

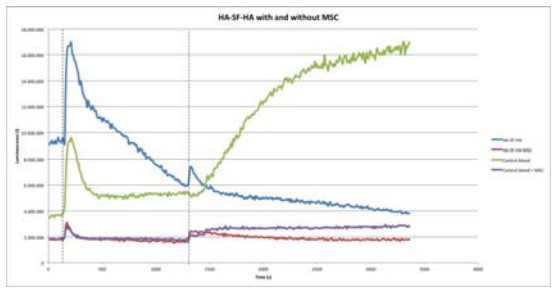
Another interesting feature that was studied was the immunoprotective feature of MSCs. In these experiments the biomaterials were first incubated with the MSCs. By carrying out various experiments with and without MSCs, it was seen that the blood with MSCs control showed a more reduced reaction compared to the blood control. From the results from these experiments it was directly seen that the biomaterials with blood and MSCs showed reduced ROS. From HA-SF-HA as cause of a severe reaction to HA-SF-HA in combination with MSCs the difference was a luminescence intensity of 14 million, which is almost 6 times lower. Even in the HA-SF-HA in combination with MSCs an appropriate PMA reaction is seen, without the MSCs the biomaterial was already saturated by cell activation and there was no PMA reaction.

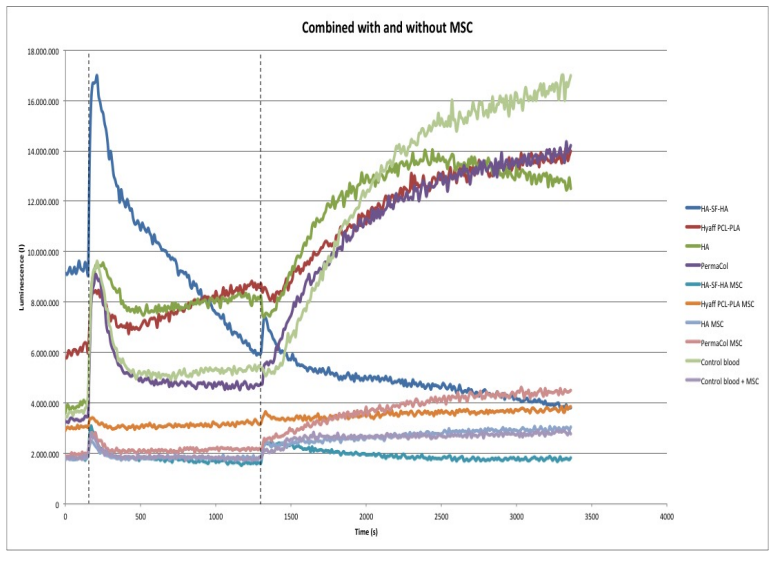
The aim of the research was to develop a sensitive and fast assay to predict the performance of biomaterials that are used in TERM and drug delivery systems. Previous research was done using a microplate luminometer, where everything had to be added one well at a time. The new fully automated parallel kinetic plate reader that was used in these experiments utilised a much faster measurement and simultaneous dispensing of the components. In these experiments the reproducibility was successfully tested, by redoing the experiments in the same conditions and by using different blood donors all in quadruple. From the results it can be stated that predicting the performance of biomaterials using this automatic kinetic plate reader and by using the discussed conditions seems to be a powerful analytical tool. The future perspective of the assay therefore lies in the use as a standard pre-screening before an (surgical) intervention, in which a biomaterial is implanted. This will provide a faster and not interrupted integration with the host.

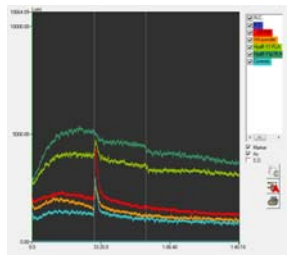
Biomaterials are tested for biocompatibility and degradation, but causing reactive oxygen species (ROS) to be formed is often overlooked; but this may be important for the efficacy of the biomaterial. ROS are short-lived but very active cytotoxic chemical species; they consist of both molecules that contain unstable oxygen or radicals. There are different ways for ROS to be formed in the human body, among them the respiratory burst of inflammatory cells such as PMNs and monocytes to kill microbes. There are various cell types that can produce ROS but phagocytes are the main type, like PMNs, monocytes, macrophages and dendritic cells. Besides the involvement of ROS in killing microbes it also has a function in for instance cell signalling and removing cells that are in apoptosis or necrosis. ROS consist of very unstable species such as hydroxyl radicals (•OH) and super oxide anion (O_2•−). and there are some ROS that are long-lived like hydrogen peroxide (H2O2). The adverse effects are for instance an acceleration in degradation time or loss of mechanical properties. The respiratory burst is a process that involves NADH oxidase, which generates super oxides from molecular oxygen. When PMNs are in contact with for instance growth factors, cytokines or fragments of bacteria this generation of O_2•−is initiated. O_2•−at their turn can be converted into H2O2, by superoxide dismutase (SOD). The materials' reaction and potential formation of ROS is very important to prevent unwanted and proinflammatory reactions

Results

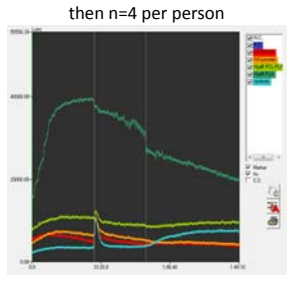




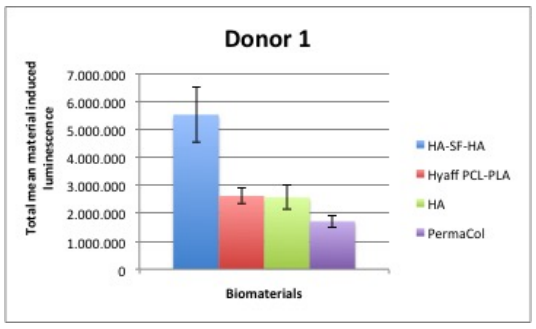


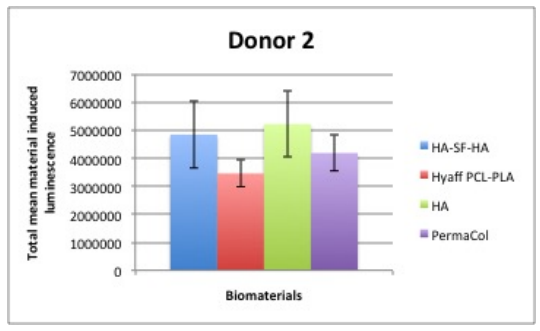


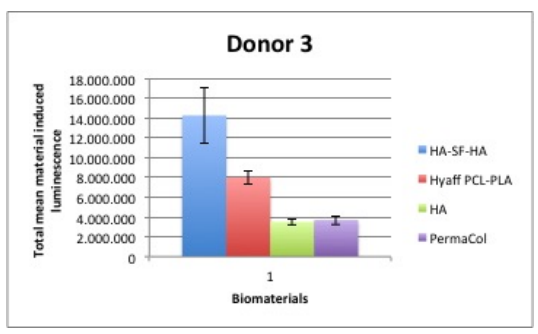
Materials with blood (4 donors then n=4 per person

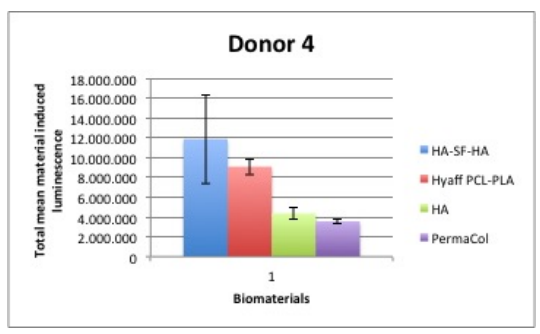


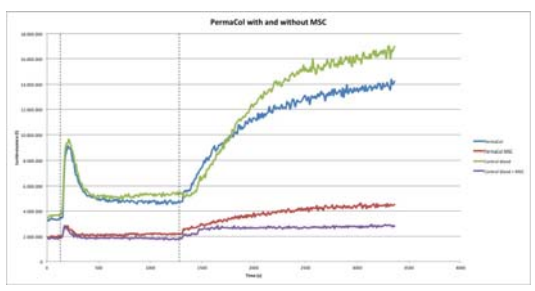
3 donors, then n=4

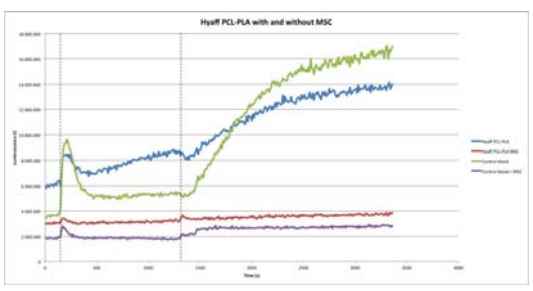












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