The Utilization of MG-63 Osteosarcoma Cells as a Biocompatibility Model for Bone Regeneration in Manufactured Scaffolds

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GH 401: Honors Thesis

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Abstract

When a bone is broken beyond traditional repairs, medical advancements have created a new pathway for bone transplants that involves manufactured scaffolds. These scaffolds can be 3D-printed to any size, and donated bone cells can be placed onto scaffolding, and regeneration of the bone will occur. This is due to the scaffold mimicking tissue structures that are naturally found in the body acting as an anchor for the bone cells. The choice of the scaffold material is important to consider in order to help the tissue heal properly while also being able to withstand damages that come with daily life. Graphene-based nanocomposites allow for flexibility in the bone; however, it is necessary to run a biocompatibility evaluation to enhance the scaffolding material and test structure properties to ensure success in treatment later. The osteoblast-like MG-63 human osteosarcoma cell line is utilized in bioengineering to test the biocompatibility of a variety of materials due to its cost and ability to behave like a healthy osteoblast. Our lab evaluated the induction of osteoblast-like activity in this cell line when introduced to different supplements. The experiments conducted reproduced similar results to past experiments, indicating that the MG-63 cell line induces the osteogenic marker alkaline phosphatase upon treatment with TGFβ and/or vitamin D supplements.

Keywords: MG-63 Cells, Alkaline Phosphatase, Calcitriol, TGFβ

Utilizing MG-63 Osteosarcoma Cells as a Biocompatibility Model for Bone Regeneration in Manufactured Scaffolds

Fracture Healing Techniques

Within modern day medicine, multiple breakthroughs have been made regarding bone healing and regeneration. For most patients with fractures, an individual can go into a physician's office, get high-definition x-ray imaging and diagnostic tests run, and leave with a cast or brace, and in some instances, a procedure to properly set the bone. What is often overlooked in healing fractures is that in certain instances, a fracture cannot be mended by normal means of treatment, or an infection causes enough damage that a bone can no longer produce healthy cells that can help in the regeneration process. When these fractures and infections occur, newer technologies are required to help the bone heal because the body no longer has the ability to heal itself properly.

Because of this disparity for major fractures and infections, research is being redirected towards finding materials that can act as a scaffold to help regeneration of current living cells or reintroduce healthy osteocytes to the patient to encourage proliferation and lead to healthy tissue growth. Bone scaffolding is an innovative technology that acts as a transplant that can be 3D printed to be any shape needed, and cells can be placed onto the scaffolding where they can grow and take shape of the human bone (Tariverdian et. al, 2019). Scaffolding for bone regeneration is constantly evolving to help patients with a variety of different ailments, and this scaffolding can be specific to the bone that has experienced a fracture. Initial research that was conducted to help find suitable scaffolding involved the utilization of human cadavers as a means for treatment. The benefits of using cadaver donated tissue is that human donated tissue has antibodies on the surface of the tissue that help prevent immediate immune rejection are already present in the

sample, but multiple drawbacks prevent cadavers from being a perfect transplant option. The primary setback for utilizing cadaver donated tissue is bloodborne pathogens and other pathogens that are present in the cadaver could be transmitted to the patient receiving care. This is due to the cadaver harvested sample being contaminated from pathogens when the person was still living being placed in a healthy host, and the pathogens from the contaminated tissue samples spreads to other parts of the body and infect the new host as well, (Talamonti et. al. 2019). In order to avoid potential infections due to grafting from cadavers, newer technologies have allowed scientists to 3D print scaffolding materials to be able to seed cells onto grafts and implant them into patients who are seeking bone treatment. This allows scaffolds to be manufactured with stronger materials such as metals, which allow for corrosion resistance, fracture toughness, and biocompatibility, (Chang et. al, 2014). Multiple labs are currently testing different printed scaffolds made of a variety of materials such as plastic, collagen, metal alloys, and others. While these 3D printing models are becoming more popular to test and research, problems arise when the biocompatibility of these scaffolds are being tested.

Utilizing MG-63 Osteosarcoma Cells

In order to be confident in utilizing bone scaffolding as a means of transplanting, it is important that the materials being studied are compatible with human tissue before being implanted. This is to ensure that cells that need to be placed onto the scaffolds are able to adhere and proliferate at a rate necessary to help the regeneration process. While it may be understandable to utilize cells that will be directly implanted into an individual as the best guide to test biocompatibility, multiple drawbacks, such as cost, preservation, and needed maintenance of the cell, make this an unachievable option for a majority of research labs. Because of this, the lab that I work in has resorted to utilizing different, cheaper cells that have the ability to behave

as an osteoblast, without paying for human bone marrow cells. In order for a cell to be utilized for this experiment, osteoblastic properties, such as synthesizing bone matrices and bone mineralization, need to occur in the cellular model to be able to mimic the regular biological functions and properties of a healthy bone cell. To be able to meet these needs, special care needs to be taken in selecting the model cells to ensure that adhesion will occur for the following procedures, as well as ensure that the cells do behave like an osteoblast and not an osteosarcoma.

The cell that my lab selected to be used for the testing of our bone scaffolding material was the MG-63 osteosarcoma cell line. We selected this cell line because there are supplements that can be added to the growth media that can induce the cell to manufacture proteins that aid in the formation of bone matrices and mineralization. These proteins then act as protein biological markers that prove that osteoblast-like behavior is occurring in the cells. This is beneficial because MG-63 cells naturally produce other proteins that aid in bone matrices formation, such as MMP-9, so testing for osteoblast like behavior can be hard to identify, (Pautke et. al, 2004). By having other proteins behave as biomarkers for osteoblast-like behaviors, we can ensure that the data collected is accurate and osteoblastic properties are present.

One of the primary biomarkers present in a majority of osteoblast cells is alkaline phosphatase (ALP). This protein is highly beneficial in human bone cells because it helps with the calcification of young cells and allows for healthy bone regeneration. If successful, the induction of alkaline phosphatase production would indicate that the MG-63 human osteosarcoma cell line would be a perfect biocompatibility model to test the viability of the graphene-based nanocomposite scaffolds. While experiments have been done to induce alkaline phosphatase presence in MG-63 cells, our goal is to be able to choose a method that is cost-efficient while also getting the best results for induction of biomarkers that indicate osteoblast-

like behavior. Therefore, my goal for this research is to test different supplements that can induce production of alkaline phosphatase to identify which protein/vitamin gives the best results of osteoblast-like induction. This will confirm if MG-63 cells can be utilized as a biocompatibility model for our scaffolds. In order to identify if these cells can be used, three questions need to be answered to determine the best protein and/or vitamin to use to induce osteoblast-like behaviors.

1. What protein can be used as a biomarker for these cells?

To test the viability of the MG-63 osteosarcoma cell line, it is important to identify different biomarkers that are not only present in healthy osteoblasts, but also are absent in cancerous cells. This will help identify if the supplements that are used for the experiment are altering the cellular components of the osteosarcoma cells and ensure that the supplements are not being wasted. By identifying what proteins we want to utilize as a biomarker, we can then select the proper equipment and supplements needed to be able to identify osteoblast-like behavior that is induced within the cells.

2. What supplements can induce osteoblastic behavior?

In order to prevent wasting supplement samples and avoid potential cell death, it is important to identify previous experiments that have tested the inducibility of osteoblast-like behavior in MG-63 cells. This will ensure that we can compare the results of our experiment with that of another lab to identify if things go wrong. Not only that, but by utilizing samples that have already been tested, we can ensure that the results we get should be positive, and so money is not being wasted on different supplements that do not work. This will allow us to bypass trial and errors, as well as allow us to achieve the results we are looking for quickly to be able to move on to testing our cells and their inducibility.

3. How can the induction of biomarkers in MG-63 cells be measured in a laboratory?

To be able to identify that the induction of protein biomarkers has taken place in the MG-63 osteosarcoma cells, different experimental methods can be used to show the concentration of the biomarker present. Multiple methods for protein probing exist that can give both qualitative and quantitative results. By selecting a combination of different methods to test protein concentration levels, we can guarantee that the results that we obtain are not skewed by just one experiment. This will allow us to be able to see and quantify the results we achieved, as well as show potential failures in the experiments that need to be fixed to obtain proper results.

Overall, the purpose of this paper is to determine if the MG-63 human osteosarcoma cell line can act as a biocompatibility model for testing bone scaffolding materials. The hope of this research is to be able to utilize these cells in further bone scaffolding material testing. This will allow for future experiments to focus on forming scaffolds from different materials to aid in bone regeneration.

Methods

Cell Culturing

Even though the MG-63 human osteosarcoma cell line is able to undergo a lot of different homeostatic strains, best results with laboratory testing require that the cells be handled in a clean, warm, and sterile environment for optimal cell growth. In order to achieve this, subcultures of cells are made within a biosafety cabinet that has been sterilized with 70% ethanol and UV radiation. Subcultures are plated in tissue culturing flasks with media consisting of minimum essential media (MEM), fetal bovine serum (FBS), and pen-strep antibiotics, and then placed in an incubator set to 37°. Once subculturing of the cells grows to 80% confluency of the tissue culture flask, MG-63 osteosarcoma cells are dissociated via trypsin and plated in a new flask with fresh media. All growth media ingredients as well as trypsin and culture flasks are

purchased from ThermoFisher Scientific and are kept in a cold environment when not being utilized.

Cell Attachment and Proliferation on Nanocomposite Material

MG-63 osteosarcoma cells were seeded on a bone scaffold matrix consisting of graphene embedded thermal plastic and allowed to proliferate for nine days. Scaffolds consisting of MG-63 osteosarcoma cells were then incubated with DAPI stain to identify areas of growth with both high and low density to identify if the cells would adhere to the initial scaffold material.

Treatment of MG-63 Cells with TGFβ and Calcitriol

MG-63 cells were harvested from the subculturing plate using trypsin and were seeded onto a 24 well plate with a density of $0.2x10^5$ cells/mL. After allowing the cells to recover from transfer for 24 hours, media in the wells is aspirated and the wells were treated with new media samples. Four wells were treated with regular growth media (MEM, FBS, pen-strep), four were treated with growth media that contains 5 μL of 100 μg/mL hTGFβ1, four were treated with growth media that contains 4.2 uL of 1.25 x 10-4 M (50 ug/mL) D3 (calcitriol), and the last four were treated with a combination of growth media, hTGFβ1, and calcitriol. After 2 days of growth in treated media, then half of the cells were harvested for western blotting using SDS lysis buffer, and the other half was treated with an alkaline phosphatase activity kit.

Alkaline Phosphatase Activity Assay

Half of the MG-63 cells that remain in the 24 well plate are fixed and stained utilizing the REPROCELL Stemgent Alkaline Phosphatase Staining Kit II and are imaged utilizing regular microscopy. Yellow color indicates that there is no presence of alkaline phosphatase, while red color indicates the presence of alkaline phosphatase. Staining occurs in a non-sterile environment and cells are stored in a dark environment for five minutes while the stain sits on the cells.

Western Analysis

The wells that were lysed with 1x sample loading buffer are then tested utilizing a protein concentration quantification via the Bio-Rad protein assay. After the assay, appropriate amounts (15-40 µg) of protein are loaded into each well of the SDS PAGE gel, resolved using PAGE protocol, and are transferred to a PDFV membrane. After the transfer, membranes are blocked using 5% PBST+M for one hour. Membranes are then washed with 1% PBST+M and treated with β-tubulin antibodies and TRA-2-54-2J ALP antibodies for 12-24 hours (overnight). Membranes then are washed with 1% PBST+M and blocked with anti-mouse antibodies for one hour. Last, membranes are washed with PBST and PBS before being probed with a chemiluminescent kit for 5 minutes and then imaged in the Indiana State University biology department's dark room. This imaging allows for a high sensitivity which will show even the smallest detection of alkaline phosphatase.

Results

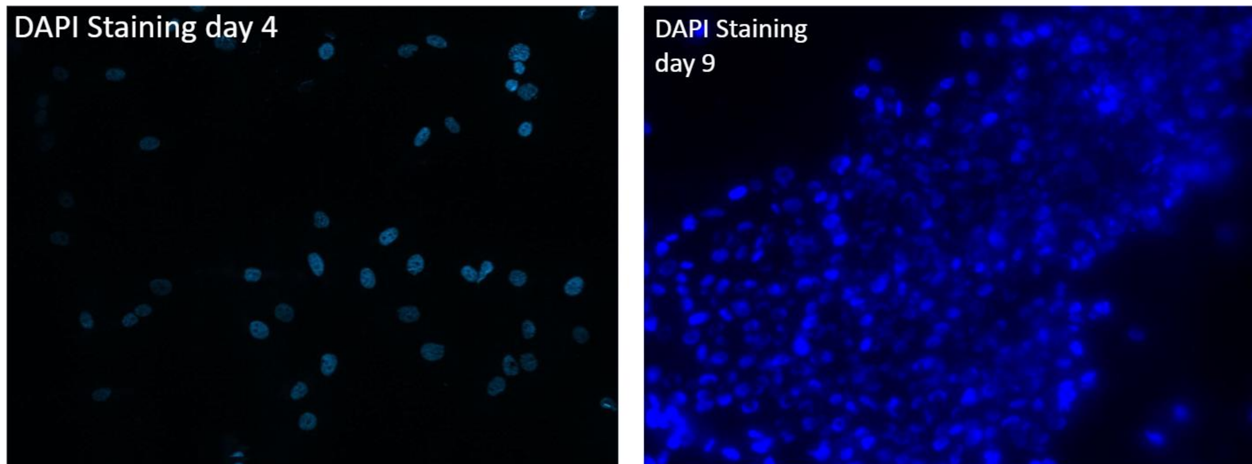
Cell Attachment

The results of our initial attachment to the nanocomposites indicated that even untreated MG-63 osteosarcoma cells have the ability to adhere and proliferate on thermoplastic scaffolding. The DAPI stain, which illuminates blue in the presence of healthy cell nuclei, shows that over the course of culturing, cells continued to proliferate and multiply on the scaffold material. This indicated to the lab that we were able to continue utilizing MG-63 human osteosarcoma cells, as well as be able to expand upon the nanocomposite material, and alter different components, such as porosity, polymer type, and graphene content of the scaffolding. In addition, the results of the DAPI stain show that without prior treatment, cell proliferation

continued to occur on the scaffolding material, as the density of viable cells increased from day four to day nine.

Figure 1.

MG-63 cells attach and proliferate on nanocomposite materials.



This difference in density shows that the graphene-based nanocomposite is nontoxic to the untreated MG-63 osteosarcoma cells, and that the scaffold material can be utilized for later testing when alterations to the matrix occur.

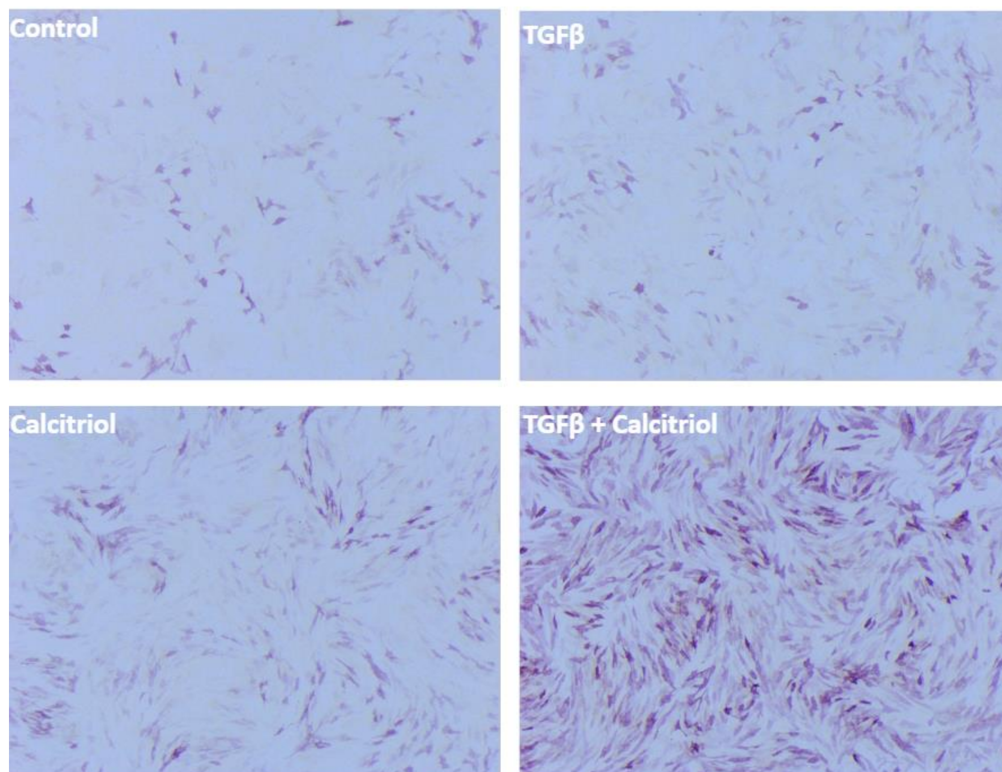
Alkaline Phosphatase Assay

In light of our cells being seeded on scaffolding material, it was important to test that osteoblast-like behavior is not affected by said material. In order to ensure that no changes occurred in the cell that would cause problems with proliferation and cell behavior later. To continue with sample testing, the osteoblast-like behavior of MG-63 cells needed to be induced, and alkaline phosphatase needed to be probed. To obtain our results, we stained a control well containing MG-63 cells with regular growth media, a culture well of MG-63 cells treated with TGFβ, a well of MG-63 cells treated with calcitriol, and a well of MG-63 cells treated with both TGFβ and calcitriol. This 24 well plate was then placed into an incubator and let sit for 2-3 days, only to be removed when alkaline phosphatase should be present in the cells, and the protein can

be probed using the activity assay kit. This kit allows for alkaline phosphatase concentrations to be visualized, with cells containing alkaline phosphatase being stained pink, and cells not containing the protein will remain a yellow/translucent color. In addition, this stain also allows us to see differences in concentration levels, as a darker stain indicates more protein present in the sample material.

Figure 2.

MG-63 Cell Alkaline Phosphatase Activity.



After utilizing the REPROCELL Stemgent alkaline phosphatase staining kit II, the present concentration of alkaline phosphatase was evident in the treated cells. For the first image panel, we can identify that there is a lack of alkaline phosphatase that is normally found in MG-63 osteosarcoma cells. A similar result can be seen when cells are treated with just TGFβ which indicates that alkaline phosphatase is not inducted with just this supplement. When the cells

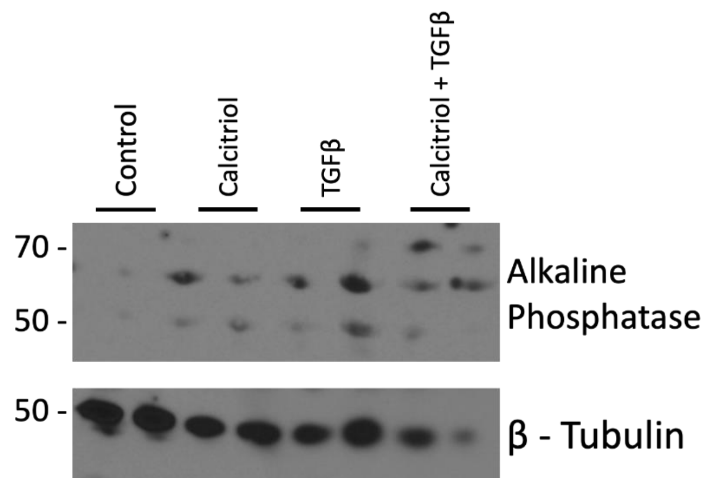
treated with calcitriol are stained, more cells appear pink, indicating that induction of alkaline phosphatase has occurred, but the concentration of the protein is minimal. After treatment with both TGFβ and calcitriol, dark pink and almost red stain can be identified, indicating that the mixture of the two supplements induces the highest rate of alkaline phosphatase production.

Western Analysis

PAGE and western analysis of whole cell lysates were utilized to identify the levels of alkaline phosphatase between the control and supplemented cells. Protein concentrations from the whole cell lysates were determined to help normalize the gel loading, an anti-beta-tubulin was utilized as a loading control. To probe for alkaline phosphatase, we utilized TRA-2-54-2J ALP antibodies due to its high sensitivity to the protein. After incubation, transfer membranes that were probed with the antibodies, and then imaged using chemiluminescence, due to its high sensitivity to anything that is illuminated. This allows for all isoforms of alkaline phosphatase to be imaged, as well as allows for identification of poor lab techniques that would indicate that the western analysis needs to be rerun.

Figure 3.

Western analysis of alkaline phosphatase.



Results of the western show several isoforms of alkaline phosphatase present in all of the cells regardless of supplemental treatment, with concentration levels of alkaline phosphatase present being easily identified by the darkness of the band. Beta tubulin was also probed because all cells in the human body form this protein.

Discussion

The following subsections act as a way of breaking down the process and importance behind each experiment conducted, as well as further explaining the results of the experiment and what it means for further research. While we haven't tested the biocompatibility of these cells on the scaffolds yet, our current preliminary findings allow us to have a baseline for when we start growing and seeding our cells on the graphene scaffold. Not only that, but it is important to note that while the majority of this project was done in a laboratory setting, there were some elements of literature review necessary to start this project. The literature review that was done before the project started will be discussed below as well to show insight into choices that were made to help with the formation of this project.

Protein Identification

Before any experimental work could be conducted on the MG-63 osteosarcoma cells to identify if they were going to be a candidate cell type for a biocompatibility model, literature review had to be conducted in order to understand what proteins could be identified that osteoblast-like induction occurred. From this search, it was discovered that alkaline phosphatase is the ideal protein marker to identify if the osteosarcoma cells are able to be viable bone models for the experiment for a multitude of reasons. First, alkaline phosphatase is known as one of the first genes that are expressed in the calcification process of bone, (Golub et. al, 2007). This

protein is a massive biomarker in any developing bone cell because as the gene for alkaline phosphatase is turned on, the production of the protein begins. This protein helps ensure that there is a high concentration of phosphate present on the surface of the osteoblast when the bone is being formed. This increase in phosphate concentration helps calcium ions bond to the cell, and thus allow the cell to calcify and harden into a bone cell.

Not only does alkaline phosphatase increase the phosphate present on the cell's membrane, but alkaline phosphatase also aids in reducing the concentration of pyrophosphate, which acts as an inhibitor for bone mineralization, (Vimalraj, 2020). Alkaline phosphatase in the body also plays a role in the formation of hydroxyapatite, which is a necessary chemical compound that is utilized in the body to form extracellular matrixes in osteoblasts and osteocytes. This protein aids in the formation of hydroxyapatite by maintaining an adequate level of inorganic phosphorus, which is one of the two main components for the reaction that forms the compound, (Penido et. al, 2012). It is also important to note that this protein occurs in other bone and tissue samples, but the use of probing for this in MG-63 cells is that the osteosarcoma cell line has to be supplemented with vitamin D in order to produce alkaline phosphatase, while other cells that produce this protein do not need induction, (Clover et. al, 1994). By treating the cells with Calcitriol (a vitamin D supplement), not only is alkaline phosphatase production significantly increased, but cells that are treated with calcitriol have an increase in the potential for cell survival, which would help cells that are being seeded on a nanocomposite scaffold that is not normally utilized for tissue culturing, (Gocek et. al, 2009).

Supplements For Cells

As stated previously, MG-63 osteosarcoma cells do not produce a high concentration of alkaline phosphatase when left to grow alone with no supplemental treatment. However, it is

known that there are different supplements that can be added into the growth media for the cells that can help induce alkaline phosphatase production. Our lab selected calcitriol, which is known as 1,25-dihydroxyvitamin D3, due to other laboratories identifying that this compound helps rapidly increase the production of alkaline phosphatase in this cell line, (Clover et. al, 1994). In addition, TGFβ, also known as transforming growth factor-β, is an important polypeptide chain found in every cell of the human body that helps regulate homeostasis. This polypeptide chain was selected to help the induction of alkaline phosphatase, as other research labs have proven that combining TGFβ and calcitriol significantly increases the production of alkaline phosphatase within cells, (Bonewald, et. al, 1992). By combining the two supplements, the production of alkaline phosphatase should be easy to identify with western analysis and the alkaline phosphatase activity assay. It’s important to note that one of the major drawbacks when it comes to utilizing TGFβ is that it is quite expensive (50μg of TGFβ costs $496), so by having another supplement to combine with the protein allows for a cheaper alternative to using just TGFβ.

Alkaline Phosphatase Activity Assay

There are a multitude of different methods that can be used in order to identify protein concentrations within a given cell line or cell type. For this project, we wanted to both quantitatively as well as qualitatively identify if the cells produced alkaline phosphatase. In order to do this, we utilized an activity assay that changes color when alkaline phosphatase was present, as well as western analysis which shows the size of the protein being probed. This not only allowed us to see color changes within cells containing alkaline phosphatase, but also allowed us to identify any isoforms that might exist of alkaline phosphatase.

With the alkaline phosphatase activity assay, a pink color indicates that alkaline phosphatase is present, while no color or a yellow color indicates that no alkaline phosphatase is present. After analyzing our samples, our control showed that some of the MG-63 osteosarcoma cells naturally produce alkaline phosphatase without supplemental treatment, but a majority of the cells do not present this protein naturally. This matches other scientific discoveries, as this cell type does not naturally produce high concentrations of alkaline phosphatase on its own. Instead, alkaline phosphatase needs to be induced within MG-63 cells, which is why we had other culture wells on the 24 well plate with other concentrations of different supplements. We then tested wells containing just MG-63 cells that were treated with TGFβ to see if there was any major induction that would happen between just adding the one supplement versus adding a combination of supplements to the growth media. As shown by the results, TGFβ alone does not induce high enough activity for alkaline phosphatase. In fact, some wells showed that there was no difference between TGFβ, and the control based on color alone, which indicates that TGFβ cannot solely induce the presence of alkaline phosphatase in these cancer cells.

Changes in the alkaline phosphatase activity assay begin to be visible when calcitriol is added to the MG-63 growth media. When comparing the images of the calcitriol treated cells versus either control or TGFβ treated cells, there is a noticeable pink hue that is consistent throughout a majority of the cells with darker hues of pink and red becoming more visible as the stain is allowed to sit. This indicates that calcitriol is inducing the production of alkaline phosphatase which matches literature review and other scientific reports as well. The major change in activity can be visible when MG-63 osteosarcoma cells are treated with a supplement mixture of both TGFβ and calcitriol. In these images, there is not a single cell that's visible that has not been induced to begin producing alkaline phosphatase. Instead, there are multiple cells

that have a dark pinkish red pigment, indicating that these treated cells are producing high levels of alkaline phosphatase. This result agrees with other literature values and results, as accommodation of TGFβ and calcitriol showed that higher inductions of alkaline phosphatase occurred when compared to regular MG-63 osteosarcoma cells, (Bonewald et. al, 1994). The activity assay thus acts as our first positive result, and results show that the MG-63 cell line does have the ability to behave like an osteoblast.

Western Analysis

The reason that we selected to do an activity assay before doing any other form of testing was for both cost reasons as well as easy identification. When running western blot analysis, different antibodies and reagents can be expensive, so it is important to note if the protein being probed for is present in the cell. The activity assay acts as a cheaper alternative to identify if alkaline phosphatase is present in the cell, and results from the activity assay can assist in selecting an appropriate antibody. In addition, utilizing the activity assay first also allows us to identify if there is a problem happening with our western analysis procedure. If no bands of alkaline phosphatase form during the western blot analysis, but a color change happened with the activity assay, then we know there is something wrong with our blotting procedure and can change the procedure to obtain the best results. In addition, by using a color identifying assay, we are able to quickly identify the concentration levels that should be expected of each well, and thus can identify if something looks incorrect in the western analysis. This is also beneficial when it comes to roughly estimating the protein concentration per each well before sample loading the PAGE gel, and problems can be identified before reagents are wasted on a failed experiment. By knowing protein concentrations before western procedures even occur, we can

ensure that all samples have equal levels of protein present in the gel, and that the western analysis procedure runs smoothly and that the results are easy to interpret.

When running the western analysis, we utilized eight wells of the PAGE gel, so that we could have two of the same treated samples run simultaneously. This aided in identifying if an error occurred in the proliferation or probing process, as well as acted as a checkpoint to ensure that the data collected was not a fluke. Looking at the different alkaline phosphatase results of the western, the western results match that of the activity assay to an extent. The two control wells of the western blot contain very little, if any, alkaline phosphatase. These results are consistent with our activity assay, which shows little to no pink coloring when probing for alkaline phosphatase, indicating that very little protein is present.

One thing that is important to note is that the activity assay results did not match the western blot for the cells treated with only TGFβ. According to the western blot analysis, more alkaline phosphatase was produced in a well containing only TGFβ than either of the calcitriol wells, which does not appear to be the same as the activity assay performed before. This could be for a multitude of reasons, but the second well of TGFβ that was also analyzed by western blotting matches the activity assay, indicating that there could have been a problem with that one well. Errors that could have occurred are either with the staining procedure in the activity assay gave faulty results, the western was imaged improperly, or the wells that were utilized for the western blotting procedure were naturally producing more alkaline phosphatase. Even with the results that do not align, having the cells treated with TGFβ produce alkaline phosphatase does not prove that the cells can’t be utilized as a biocompatibility model, and instead, could be used for more biocompatibility models than originally believed.

The results for both the calcitriol only and the calcitriol and TGFβ wells for the western analysis show similar results as the activity assay, with a higher concentration of alkaline phosphatase being present in the calcitriol wells, and the combination of both supplements produces the most alkaline phosphatase. Not only did both the calcitriol and the calcitriol and TGFβ wells produce the darkest bands on the western blot, but they also showed the largest number of isoforms of alkaline phosphatase formed. These are visible with the lighter bands around the 65 kDa mark, indicating that alkaline phosphatase can have multiple different forms that are produced when induction of the MG-63 cells occurs.

In order to ensure that we were probing our westerns correctly and that we were not flooding our gels with too much protein, we also probed a second membrane for beta tubulin. The reason for this is that beta tubulin is present in every cell, and thus they act as a control to ensure that nothing is going wrong with the procedure. Beta tubulin also acts as loading control because protein loading should be the same across the SDS-PAGE gel, so by probing for beta-tubulin, the banding pattern that forms should be consistent across the gel. We utilized the same cell lysates for both beta tubulin as well as alkaline phosphatase, put probed utilizing anti-beta-tubulin on the second membrane instead of the antibody used to probe for alkaline phosphatase. The dark stains across all of the beta tubulin samples indicate that beta tubulin is present in high concentrations in all cells, which is to be expected and matches literature results. This indicated that our cells had been lysed properly, and that the results of our alkaline phosphatase western analysis are accurate and can therefore be used as an indicator for the presence of alkaline phosphatase in different cells.

After viewing the results of both the activity assay and the western analysis, the results that I have found have successfully reproduced past results. This indicated to the lab that we are

able to utilize these forms of testing when the cells are grown on in the scaffolding and indicates that the MG-63 cell line can be used as a biocompatibility model for future experiments. This not only gives us a baseline for when the scaffolding is used to grow the cells, but it also answers our major question of can the MG-63 osteosarcoma cell be utilized as a biocompatibility model. Both with the induction of alkaline phosphatase, as well as the inducibility of higher concentrations of alkaline phosphatase, it is clear that the MG-63 cell line can be utilized as a model for bone scaffolding. These cells can adhere and proliferate on the scaffolding, and they are able to produce alkaline phosphatase, which will help with identification in cellular activity when later testing is done on the scaffolding. While more testing will be needed to be able to identify if they will grow on the specific scaffolding we are testing, these preliminary findings ensure that we are able to reproduce results conducted by other labs and can thus move forward with the project.

Conclusion

In order to ensure that we could use the MG-63 human osteosarcoma cell line as a biocompatibility model, the induction of osteoblast like behavior in the cells had to occur. To do this, research was done to find that the best protein to use as a biomarker for identifying the induction of this behavior is alkaline phosphatase. Alkaline phosphatase was selected due to its easy inducibility, easy protein probing, and the supplements that can induce the production of the protein are affordable. After this selection, the two supplements, calcitriol and TGFβ, were selected to induce osteoblast-like behavior due to past findings showing positive results when utilizing these supplements, as well as the supplements are easily attainable.

The last part of this project was to test and see if we could induce the production of alkaline phosphatase and our MG-63 cell samples. The results that have been obtained duplicate

past results that exist, indicating that our experiments were successful. With our initial test of MG-63 cells growing on the graphene-based polymer scaffolds, we expected to see cell attachment and proliferation occur, with proliferation starting off slow and then multiplying exponentially. This is easily seen in our imaging, we're on day 4 very little DAPI stained cells are fluorescing, but by day nine there are many fluorescent cells present on the scaffolding material. This indicated to us that not only did our cells attach to the scaffolding, but they were also able to continuously grow on the scaffold material. This means that we can then move on with further testing as natural MG-63 cells do attach to our graphene based polymer, indicating that the cell passed the first stage of the biocompatibility test.

With the alkaline phosphatase activity assay, we expected to see little to no pink staining in our control wells, as well as our TGFβ treated wells, and having wells treated with calcitriol express darker pink staining. Our results of the alkaline phosphatase activity assay were to be expected with low concentrations of alkaline phosphatase present in our control samples and our TGFβ sample, and an increase in protein production when treated with calcitriol. Because these results matched our literature reviewed sources as well as past analyses, we can conclude that our results for are alkaline phosphatase activity assay as accurate. This allows us to conclude that calcitriol does need to be present in order to induce alkaline phosphatase; however, by having TGFβ present, the concentration of alkaline phosphatase jumps, as the stain is immediately darker than any other well. An explanation for this may arise in the understanding that TGFβ is a cytokine, a specific signaling protein that interacts with receptors on the cell surface of target cells to start a signal cascade within the cell. This cascade could affect the rate at which alkaline phosphatase is produced and thus could lead to a darker stain.

Questions began to arise when analyzing the western analysis that was conducted in the lab. The expected result of this experiment was to see little to no banding for our wells containing samples from our control cells and TGFβ treated cells, and darker bands for our calcitriol and calcitriol/ TGFβ wells. While we saw the expected banding, the dark banding pattern seen on the alkaline phosphatase blot under the TGFβ column is more prominent than expected. With the results of the protein activity assay, we had expected to see lighter banding with TGFβ but instead a darker band formed, indicating that there is a higher concentration of alkaline phosphatase present than initially assumed. Differences could have arisen between the alkaline phosphatase activity assay and the western analysis due to being taken from different cell samples; however, all cells were kept in the same controlled environment and the only alteration that occurred between samples was the supplements added to the media. The presence of isoforms could indicate why other bands appear lighter because all of the protein was not concentrated in one spot on the transfer membrane. The dark TGFβ could be due to the selected antibody we used to probe our membrane, as well as problems could have occurred with the imaging machine for the blot as well.

Future Direction

If given the opportunity to rerun this procedure, deeper analysis needs to be done on TGFβ treated samples and how much alkaline phosphatase this sample can produce, as well as test different antibodies that probed for western analysis to be able to make clear banding patterns without isoforms being present. This will allow for clear and concise data that can easily conclude that the MG 63 osteosarcoma cell line can be used as a biocompatibility model for graphene-based polymer scaffolds. Regardless, we did see each band where they were expected to be, indicating that our MG-63 cells are induced to behave like osteoblasts.

As shown both with the alkaline phosphatase activity assay, as well as the western analysis, the MG 63 human osteosarcoma cell line can be induced to behave like an osteoblast. Not only does this allow for further testing in our lab to understand if our graphene-based nanocomposite scaffolds can work as a scaffold material for bone transplants, put multiple other labs can use our findings as well. Other labs that are doing similar bone scaffolding research now have the opportunity to utilize a new cell line that can be more cost effective for preliminary results, as well as other labs that are using MG-63 cells now know of another function for these cells. Lastly, the hope of this research is to one day be utilized by medical professionals as a means of treatment for anyone suffering from a condition where their body cannot naturally heal anymore.

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