The Effect of Fish-Extracted Collagen on the Growth of Malignant Cells

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Abstract

Collagen, the protein that makes up the majority of extracellular matrix structure, is essential in cell communication and proliferation. Research has found that collagen is also responsible for promoting many types of cancer growth because it both provides the infrastructure needed for the cells to expand, and it lessens the need for cell-cell interactions, therefore promoting cell motility. Clinically, collagen is used to treat severe burn victims as it assists in regenerating skin—this therapy typically involves bovine collagen or synthetic collagen. While these forms of collagen are readily available to clinicians, they are expensive, and it would be extremely useful to identify lower-cost alternatives. Recently, it has been discovered that tilapia, a fish popular for dietary consumption, and is now farmed all over the world, possesses high concentrations of collagen in its skin that can be easily extracted. Our hypothesis is that tilapia collagen could serve as a substitute to collagen derived from other sources in cell culture. By examining how malignant cells respond to tilapia collagen, we can determine if adding collagen to the media and/or as a coating on the surface of the tissue culture flasks in which these cells are grown on, creates a more "authentic" environment for the cells to grow in, better mimicking what cells would encounter *in vivo*. Firstly, we employ a cell-proliferation assay, comparing the impact that tilapia-derived collagen has on cell proliferation rate, with the findings providing insight into both cell growth and wound healing. We also utilized colony-forming assays to determine whether cancer cells respond differently to cancer drugs in the presence of collagen. This would give insight into the feasibility of using tilapia collagen in further cancer research without significant changes to traditional cell culture.

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Acknowledgements

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Statement of Purpose

The purpose of this study was to determine if tilapia-derived collagen has a promoting effect on the growth of breast cancer cells *in vitro*, with the goal of determining the feasibility of use as a substitute to more expensive cell culture reagents. In addition, this new potential cell culture reagent needed assessment to determine if and how tilapia collagen affects the behavior of this malignant cell culture model system. To those ends, in this study we first tested and compared the growth of these cells in different concentrations of collagen and ran a colony-forming assay. We also tested to see if the presence of collagen influences cell growth and/or drug resistance within MCF7 breast cancer cells via MTS colorimetric cell proliferation assay.

Introduction

The basis of this project was to observe how added tilapia collagen in cell cultures promotes cell proliferation and growth in malignant cancer cells. Previous research has shown that type 1 collagen, found in high concentrations in cytoplasm, promotes cell regrowth and proliferation (3). Initially used for burn victims, doctors would apply collagen-rich bandages, from bovine, equine, or porcine sources, or skin grafts to promote skin regrowth (1). In countries with less access to these resources, intact tilapia skin was used as an alternative because it is rich in collagen, inexpensive, and abundant in those countries (1).

The initial idea to use tilapia skin occurred in 2017 in Brazil, when it was discovered that tilapia skin could, indeed, be used as bandages for burn victims (1). The lack of accessibility to both human skin and the typical animal skin that is used to treat burns led to this discovery. Typically, doctors would use bandages and creams to dress wounds, but this method was difficult and inefficient as the bandages would dry out and had to be changed every day. An idea to use tilapia skin was centered around the fact that it can hold moisture better than gauze can, and has a high collagen content that is thought to promote keratinocyte and skin fibroblast cell regrowth.

Collagen bandages offer more therapeutic benefits than typical wound dressings because they not only prevent infection, but also promote regrowth and provide more substantial protection of the wound from the environment than traditional bandages and dressings (2). Collagen creates a barrier that is impermeable to bacteria, keeping out further complications like bacterial infection. One study involving clinical assessment of open wounds such as ulcers, postoperative wounds, and infection sites, suggests that using collagen bandages in replacement of typical wound dressings such as silver sulfadiazine or nadifloxacin, reduces loss of fluids and

could potentially help the patient avoid skin grafting (2). While the study showed that both groups with or without collagen had good healing results, the group treated with collagen had earlier cell motility, and overall a less painful recovery. This reinforces the idea that collagen is beneficial in wound treatment and healing, and supports the idea that further research into collagen and its properties on cell growth of all kinds is merited.

When researchers tested tilapia skin to determine collagen content, they were shocked at the amount of both collagen 1 and collagen 3 they found present in tilapia skin. The presence of this collagen is significant because these types of collagen have been previously shown to promote healing through cell regeneration (3). Other researchers have explored the idea of using tilapia in medicine (4,5,6). Not only can tilapia skin be used as a bandage, but collagen contained within tilapia skin has shown to have many other applications, including uses in the food industry, cosmetics, and as pharmaceutical drug coatings (6).

The original extraction process was developed to extract gelatin—a denatured form of collagen—from salmon (4). A more efficient method was developed for tilapia collagen extraction (7), which creates a product that is closer to the product we ultimately decided to use in our experiments. Due to constraints in our ability to extract tilapia directly and efficiently, we elected to purchase 100% tilapia extracted collagen for use in our experiments.

One interesting advantage tilapia collagen has over bovine collagen is that the latter poses an issue to certain vegetarians and Hindus, given that consumption of cows and items derived from cows is against their beliefs. By replacing this bovine collagen with tilapia collagen, a large variety of products are now available for these groups of people that would otherwise not be. Thus, this new source of collagen will impact both the medical field as well as the food industry in a manner that is generally acceptable to a majority of people around the world. Once tilapia

collagen was established to be biologically safe (7), these prospective ideas became more plausible. Coupled with the ability to extract collagen (4,5) the potential uses of this protein source are many.

When testing gelatin created from fish collagen, researchers found that collagen from fish sources can enhance cell adhesion, cell growth, and wound healing in HaCaT cells (a keratinocyte cell line from adult human skin) that had been exposed to H_2O_2-induced cellular damage (5). Cell adhesion is necessary in all cells, but especially the most aggressively malignant cell cultures because it serves as a growth signal to cells, promoting proliferation, migration, and differentiation. Extremely malignant cell types, on the other hand, either secrete their own growth-promoting cell signals (autocrine stimulation), or the signal receptor pathways have mutated to the point that they no longer need external signaling in order to grow and proliferate. By increasing the ability of relatively normal, non-malignant HaCaT cells to do this, it created a more realistic model system in terms of similarity to actual skin tissue that could give researchers a more specific insight into how these cells 'think' and function in terms of wound healing.

Collagen is a normally insoluble fibrous protein found in the extracellular matrix (ECM) of cells and makes up 25% of the total protein mass found in vertebrates (4). It promotes cell growth because it provides structural support and helps maintain tissue homeostasis by creating the scaffolding that allows the cell to have the appropriate space to carry out necessary functions (3). Previous research has also found that collagen contains a high amount of arginine-glycine-aspartic acid amino acids, which research has shown also promotes cell adhesion and proliferation (6). There are currently 27 identified types of collagen, but most research focuses on type 1 because it is the most abundant in the human body.

The ability of collagen to promote cell growth is transferrable to cancer cells. Studies utilizing a model system for lung metastasis show that tumor growth was negatively affected when collagen 1 was depleted from the cellular environment (8). Moreover, as cancer cells spread, collagen fibers encountered by the cells would strengthen and remodel to better fit the metabolic needs of the cancer cells. So, from the idea that depleting collagen levels would decrease cell growth, comes the idea that increasing collagen levels would promote cell growth (3). It was once believed that collagen acted as a barrier, prohibiting cancer from spreading as rapidly, but as it turns out, collagen can also promote cancer cell growth by changing the microenvironment (3).

Collagen protein fibers typically comprise up to 90% of the extracellular matrix (ECM), which is where cancer tumor cells initially invade as they progress through the body—in fact, the definition of "malignancy" is the point when cancer cells move beyond the tissue of origin into other tissues, and the ECM (9). This is important because the ECM is the location where signaling that is involved in proliferation, migration, invasion and other important functions of the cells takes place (10). Because the ECM is so rich in collagen, when cancer cells invade, the contents of the ECM promote the spread of cancer by essentially building "pathways" for the cancer to follow and promoting cell adhesion as well. In addition, collagen is involved in sustaining a phenomenon termed "epithelial-mesenchymal transition (EMT)" which is the controlling of different proteins to promote or obstruct cell-cell contacts (10). When collagen is present in normal amounts and cancer encounters the ECM, EMT promotes the downregulation of epithelial proteins and an upregulation of mesenchymal proteins. This in effect causes the loss of cell-cell contact and therefore leads to more cellular motility, and ability to invade additional

tissues. Essentially, collagen helps increase the ability of the cancer cells to move around and therefore spread (10).

Creating a better in vitro cell culture model system

Two-dimensional ("2D") models of cells are traditional for research; some research has indicated that a three-dimensional cell culture model, an artificially created environment in which cells are permitted to grow or interact with their surroundings in all three dimensions, more closely mimics conditions inside the body. By growing the cells in a three-dimensional pattern, cells can more readily transfer nutrients, communicate, and allow ECM interactions that cannot normally take place in 2D models (14). However, using 3D models in research has its own set of disadvantages: they are expensive to create and hard to observe. To keep expenses low, researchers often fall back on the less accurate 2D models for cell culture, at the risk of cells not being able to communicate and proliferate as readily, and not effectively mimicking *in vivo* physiological conditions.

Collagen is a very fibrous substance that acts as scaffolding for the cells that it encounters. This extra support allows the cells to grow on different microscopic planes within the plate, rather than flat on the surface. Adding collagen—and in this case tilapia-extracted collagen—to promote the growth of cells in culture could potentially serve as an effective "compromise," boosting the proliferation rate and making a lower-cost, 2D model cell culture system more analogous to conditions found in actual cancerous tumors.

To understand how cancer works, Weinberg contends that we must first understand a minimum of six biological processes he terms "hallmarks of cancer(9)." These hallmarks appear to be biological commonalities across all cancer cells, or barriers that cells must overcome in

order to develop into full-blown metastatic cancer and help us understand and develop therapeutic and preventive clinical strategies against them. These hallmarks include: sustaining proliferative signaling; evading growth suppressing signals; activating invasion and metastasis; enabling DNA replicative immortality (normal cells seem to reach a limit in terms of number of cell divisions, termed the "Hayflick Limit"); inducing growth of new blood vessels (termed "angiogenesis"); and resisting cell death (15). Most traditional chemotherapeutic agents to date are used to exploit how cancer activates invasion and metastasis and sustains proliferation signals. It is important however, to keep in mind the other four hallmarks for therapy, as they might provide an explanation for why the cancer cells act in specific ways.

Tilapia-extracted collagen to create a better model system

The discovery of collagen-rich tilapia skin has led to many advancements in ideas and products throughout the world. What started as a basic idea to satisfy a lack of resources, has exploded into hundreds of journals and research articles outlining other ways this collagen can be useful to us. By extending this idea further into the realm of cancer research, if successful, could be advantageous. Creating a better model system at a cheaper cost that more closely replicates the physiological conditions of cancer cells in the body, will open the door for future research to be done and create endless research and clinical possibilities, particularly at smaller universities where financial constraints are significant.

Cancer Chemotherapy

Chemotherapy is one of the most common methods used to treat, and hopefully cure, cancer in a patient. It works by using chemical compounds to target specific metabolic vulnerabilities in cancer cells. This would include DNA replication and metabolic targets that

impact different phases of the cell division cycle and kills the cells. Alternatively, some chemotherapeutic agents are used with the goal of shutting off certain types of intracellular signals that induce cells to grow and divide, or to block certain normal processes in cells and tissues which have been "hijacked" by cancer cells—such as triggering new growth of blood vessels.

Because cancer cells are dividing at a rapid rate and are highly active metabolically, cancer cells tend to be more vulnerable than normal cells—this difference is implied in what is known as a "therapeutic index" (11), where chemotherapeutic drugs can successfully affect cancerous cells at doses below that which causes toxicity and other significant adverse side effects in patients (12). Chemotherapy drugs include DNA alkylating agents, antimetabolites, anti-tumor antibiotics, topoisomerase inhibitors, and receptor inhibitors (12). The negative side of using these drugs in therapy is that they can frequently affect healthy cells, especially cell populations which are rapidly dividing, such as bone marrow (which makes red and white blood cells), hair, and the digestive tract. And so, patients typically see some fairly severe side effects.

On the other hand, using chemotherapy drugs or other anti-cancer drugs are useful in cancer research, because the effects observed in cancer cells under research conditions can be helpful to predict outcomes in the patient. Perhaps just as importantly, even less than highly effective drugs used in the research lab can provide important information about the biology of cancer cells, as well as provide insight into potential specific targets for which a more effective compound might be developed (13).

To that end, it is vitally important that when creating a new model system for growing cells—be them cancer cells or other types of cells— intended for therapeutic use, to note how cells react in various environments and how closely this relates to the *in vivo* conditions within

actual patients. Creating "biologically authentic" environments *in vitro*, including a collagen-rich environment that we are interested in, can tell a researcher a lot about what could happen to cells *in vivo* if we either change the environment inside a patient, or subject the patient to chemotherapy. If the environment created does not accurately replicate the *in vivo* biological system, then the data that is collected will likely not be as predictive as it could be.

Farnesol as a Cancer Drug

The anti-cancer drug that we used in this study was farnesol. This "orphan drug" (as categorized by the FDA), that has remained mostly undeveloped commercially due to lack of profitability, is found mostly in nature within the essential oils of plants. Farnesol is a relatively new anti-cancer drug, and therefore its mechanism of attack is not understood completely. It has many uses, including anti-inflammatory effects and alleviating allergic asthma and edema, though the most important property of farnesol for our study is its anti-cancer effects. Farnesol has an antiproliferative effect on cells due to its ability to induce apoptosis, and/or upregulate regulatory proteins which delay or halt the cell cycle altogether (17). We used various concentrations of this drug in order to observe the potential of some cells living after applying the drug.

In one study, scientists observed the effect of farnesol and other anti-cancer drugs on pancreatic cancer cells to determine when the drug plays a role on the cell cycle. They determined that farnesol, and the other drugs, have a G_0/G_1 phase cell cycle arrest on cells that correlated with an increase in cyclin kinase inhibitor proteins (18). This is important to note because if the drug effects the cells in the early phases of proliferation, like the G_0 or G_1 phase, it could have a stronger effect on cells and kill more of the living cells. These phases are crucial because during this time, the cells are preparing for DNA replication and are vulnerable.

Methods and Procedures

Colony-Forming Assay

This procedure is based on a protocol developed by Shoemaker, et al (19). For this set of experiments, we used Premium Marine Collagen Peptides by Zammex to pre-treat tissue culture plants onto which we would apply a low density (~1-5 cells/cm^2). The only ingredient in the collagen was from tilapia non-GMO Type I and III collagen. This simplified the procedure for extracting the collagen from tilapia ourselves and allowed us to work solely with the cells. We used this collagen to create different concentrations of collagen mixed with water.

The three concentrations we decided to use were 0.5 mg/ml, 1.0 mg/ml, and 2.0 mg/ml. We created these concentrations by measuring out a designated amount of collagen, adding it to a 50mL flask and then adding the appropriate amount of water. To make sure the collagen was evenly distributed and dissolved, we left them on a laboratory rocker over night as a constant speed and temperature.

Next, we applied 1mL of the 0.5 mg/ml mixture to a sterile culture dish. We repeated this step twice for each concentration for a total of 6 plates. Next, we left them in the sterile hood over night for the water to evaporate, leaving only the collagen. We also plated two culture dishes with just 1mL of distilled water as a control.

After the dishes were dried, approximately 500,000 MCF-7 cells were plated in a volume of 3 ml of standard Dulbecco's Modified Eagle Medium (DMEM). Cultures were incubated in humidified 37°C incubator with an atmosphere of 5% CO2 in air, and control plates were monitored for growth using an inverted microscope. At the time of maximum colony formation, (7 to 21 days in culture), final colony counts were obtained with a Bausch and Lomb Omnicon (FAS II) image analysis system. We performed our counting procedure on day 9 of the cells

being incubated, based on a plateau of growth observed. Objects presenting a circular profile minimum approximate diameter of 60 um were scored as colonies by the system. For quality control purposes, control plates were also counted on the day of plating (Day 0) or the following day (Day 1).

The colony forming assay tests the ability of cells to grow into colonies. It essentially gives us information about the viability of a cell and its potential for continued growth and reproduction. We chose this assay to see if there were more viable cells and better cell replication present on plates with or without collagen present.

MTS Assay

The next assay that we performed was the MTS Assay. We started by creating our collagen mixtures again of 0.5 mg/ml, 1.0 mg/ml, and 2.0 mg/ml, and allow them to mix at room temperature overnight. Then we plated a 96-well plate with 24 wells of control, which just consisted of purified water, and 24 wells of each concentration of collagen. We allowed these to dry in aseptic conditions overnight, and then plated our cells the next day. After 24 hours, we exchanged the media in each well and added fresh DMEM to the cells.

After allowing an additional 24 hours of growth, we then added DMEM with our cancer drug (farnesol) in various concentrations (0, 20, 40, and 80 μM) to the cells. We chose to use farnesol because of our accessibility to it and its known effect on cancer cells. After exposing the cancer drug to the cells for 24 hours, we examined cell viability using an MTS assay to determine how many cells were still living. Following the manufacturer's recommended protocol, briefly, we added 20 μM/well of the MTS reagent and incubated the plates for 3 hours at 37^{o}C. After the incubation period, we measured the absorbance of the collagen treated verses untreated cells using a plate reader at OD= 490 nm.

We chose to perform an MTS assay to see if collagen promoted drug resistance and/ or increased growth following the cancer drug treatment. Adding farnesol is analogous to a wounding event, therefore observing the number of living cells following the treatment can tell us a lot about how the presence of collagen influenced the results.

Statistical Analysis

Samples were tabulated and subjected to analysis for statistical significance using a two-tailed unpaired Student's *t*-Test (21). Samples with a *p*-value less than, or equal to 0.05 were considered statistically significant. Those that were considered statistically significant were marked with an asterisk on the graph.

Results

The colony forming assay produced results shown in Figure 1, and Table 1 below. Each blue dot on the plates shown at the top of Figure 1 is a viable colony and the values listed in the table below come from averaging all the data across trays with the same plated collagen concentration. Any colonies smaller than approximately 0.25 mm were omitted. We deidentified and labeled the trays alphabetically so that there was no biased when counting the viable cells. We then later determined the concentration associated with each tray to record the data.

Table 1: Raw data from the Colony Forming Assay

Tilapia Collagen				
	Untreated Control	0.5 mg/ml	1.0 mg/ml	2.0 mg/ml
Replicate 1	61	123	166	75
Replicate 2	55	111	186	40
Replicate 3	67	127	150	25
Replicate 4	49	91	126	36
Replicate 5	40	130	200	60
Replicate 6	70	140	165	90
Replicate 7	35	76	92	20
Replicate 8	42	89	90	31
Average	**52.38**	**110.88**	**146.88**	**47.13**

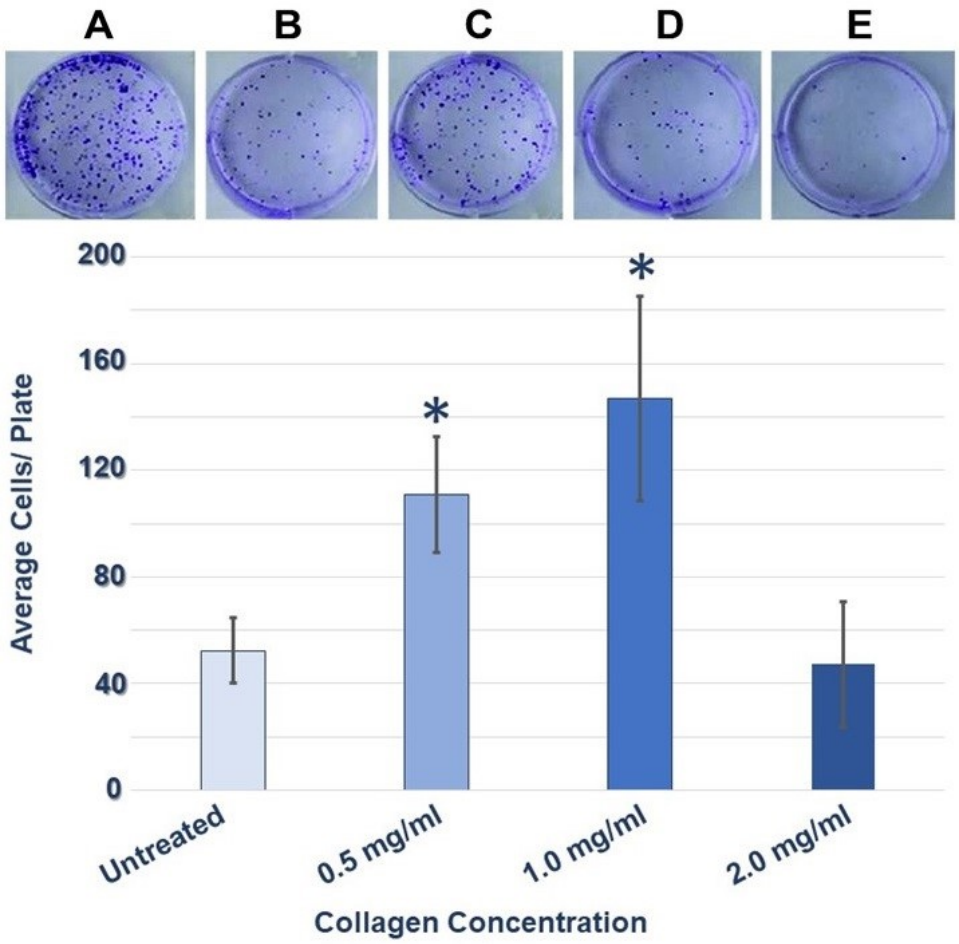

Figure 1. Effect of tilapia collagen on MCF-7 cell proliferation and migration. Approximately 5 x 10^5 MCF-7 breast cancer cells were plated on 35mm tissue culture plates pretreated with 0-2mg/ml tilapia collagen solution in ddH_2O. After 9 days of incubation, colonies were counted, samples were deidentified with letters, as shown at the top of the figure, and counted. Each plate contained a different concentration of collagen. The graph represents the average number of colonies on each plate (n=8) ± Standard deviation. * $p \leq 0.05$ vs. untreated.

The next analysis that we performed was the MTS assay. After calculating the viability of the cells following the addition of the cancer drug, we came up with the following graph to exhibit the data.

Table 2. Raw data from MTS assay

MTS Assay												
Plate 1	**Untreated**			**0.5 mg/ml**			**1mg/ml**			**2mg/ml**		
	1	2	3	4	5	6	7	8	9	10	11	12
Control	1.083	1.264	1.257	1.387	1.463	1.414	1.545	1.682	1.536	1.335	1.149	1.139
	1.212	1.359	1.324	1.295	1.343	1.274	1.584	1.599	1.586	1.443	1.269	1.161
5ul	0.582	0.629	0.627	0.629	0.628	0.621	0.682	0.701	0.730	0.705	0.630	0.701
	0.668	0.655	0.637	0.662	0.645	0.646	0.712	0.737	0.677	0.683	0.699	0.661
10ul	0.394	0.396	0.408	0.366	0.396	0.403	0.496	0.505	0.536	0.434	0.397	0.409
	0.389	0.394	0.385	0.386	0.384	0.375	0.472	0.475	0.481	0.424	0.397	0.409
20ul	0.261	0.281	0.261	0.321	0.333	0.387	0.364	0.373	0.417	0.321	0.289	0.322
	0.297	0.286	0.284	0.307	0.340	0.315	0.398	0.371	0.388	0.338	0.309	0.300

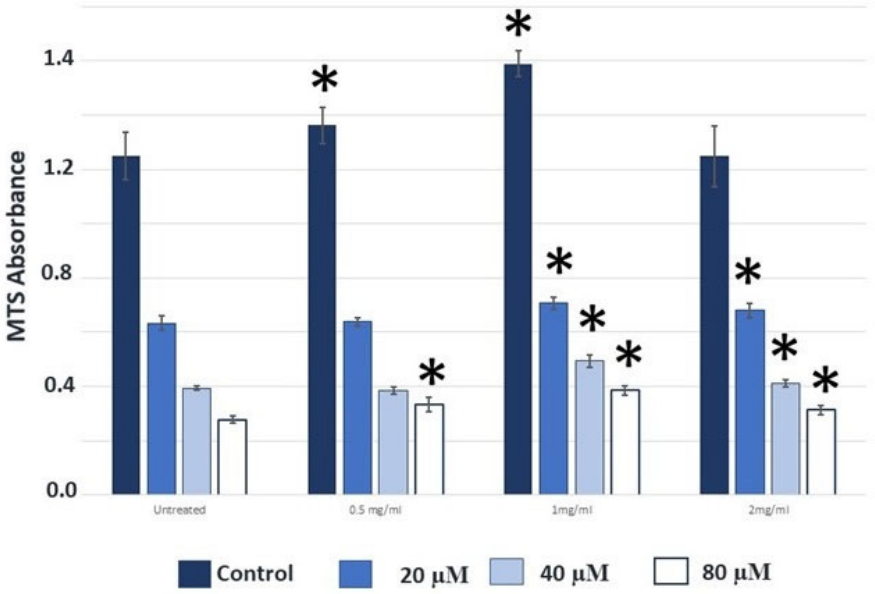

Figure 2 – Effect of tilapia collagen on chemosensitivity of MCF-7 breast cancer cells. Cells were plated into 96-well plates at a density of 5,000 cells per well in wells pre-coated with 0, 0.5, 1.0, or 2.0 mg/ml tilapia collagen solution. Cells were allowed to grow for 24 hours prior to exposure to farnesol (0-80 µM) for 24 hours. At the end of exposure period, cells were assayed with MTS reagent. Graph represents average ± standard deviation ($n \geq 6$). * $p \leq 0.05$ vs. wells absent of tilapia collagen.

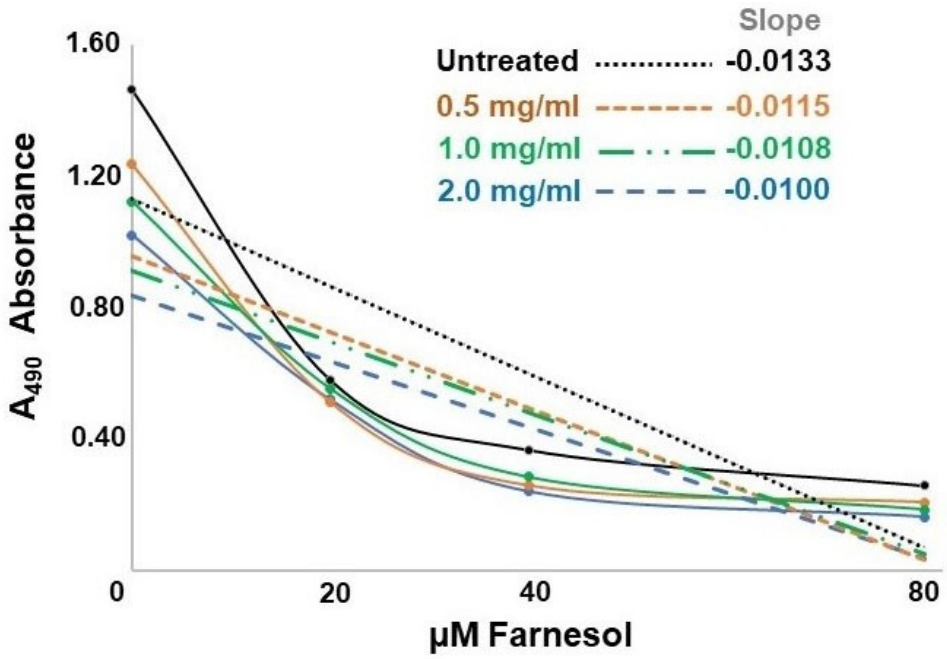

Figure 3 – Linear slope analysis of tilapia collagen on chemosensitivity of MCF-7 breast cancer cells. Results as described previously, in figure two were plotted in line form, and then a linear trendline was generated to calculate average slope versus dose.

Analysis/Conclusion

The results we collected from the colony-forming assay indicate that there is a pro-growth and migration effect from addition of tilapia collagen for MCF7 cells, but also that too much collagen prohibits cell growth. There is a statistical significance between the untreated plate and the plate treated with 0.5 mg/ml of collagen. This indicates that having the collagen present likely created more favorable conditions for cells to grow and adhere to the plate. When we increase the collagen concentration to 1.0 mg/ml, there is another slight increase in the number of cells present, but it is not statistically significant relative to 0.5 mg/ml. On the other hand, when we increased the concentration to 2.0 mg/ml, there appears to be an inhibiting effect, given that there were fewer cell colonies present than the untreated plate. It is possible that the cells were overcrowded by the amount of collagen on the plate and therefore couldn't spread and grow as rapidly and efficiently as the cells on the other plates. Another possibility is that excess collagen blocked other signals that the cells need to grow.

The data from the MTS assay presents a similar finding. When analyzing samples that did not receive any farnesol, the results support what we noticed with the colony-forming assay; Cells grew more abundantly in 1 mg/ml collagen plates, and the 2 mg/ml collagen caused an inhibiting effect. As we look at cells grown in the 1 mg/ml plates, they did statistically significantly better at surviving/growing than the untreated cells following the farnesol treatment. Furthermore, when we compare the number of cells present following the 10 μM farnesol treatment for cells grown in 0.5 mg/ml and 1 mg/ml we also observe a large increase in the cells plated with 1 mg/ml collagen. This clearly supports our hypothesis that collagen influences the growth following a 'wounding event' such as our anti-cancer treatment. The cells grown in the

optimal concentration of collagen exhibited better wound healing and growth factors than other plates.

On the other hand, in spite of the improved growth characteristics of collagen, and some statistically different responses to the anti-cancer drug farnesol (Figure 2.), when we subjected the dose-response curves to a linear slope analysis (Figure 3.), we find that the average curve slope versus drug dose does change, but not to the point where we would consider the difference in slope to be of significant concern in future experiments. Any time that dependent variables are changed in an experiment, there is always the risk than an untoward effect will occur. This is especially of concern regarding *in vitro* drug efficacy studies prior to entering an *in vivo* or clinical model system. Such effect may be "pharmacokinetic," such as theoretically our treatment may act as an absorbent, preventing some of the drug from encountering the cell. A more insidious potential effect is that the tilapia collagen affects the internal biochemistry of the cell, resulting in the synthesis or expression of molecules which confer resistance. By conducting this "slope-index" analysis, we conclude that adding tilapia collagen may better mimic the growth characteristics of malignant disease while at the same time not significantly impacting the cellular response to a drug that the cancer cells are known from previous studies to be sensitive to.

The key finding from our experiments is that there is a balance, perhaps even "dose-dependent" phenomenon in the amount of collagen present between pro-growth and anti-growth effect in MCF7 cells. When there is too much collagen present, it causes a decrease in the proliferation of the cells. As we transition to look at how the cells respond to the cancer drug in different environments, we see clearly that each plate is affected by the cancer drug. However,

when 1 mg/ml of collagen was present, there were more viable cells following the cancer drug treatment.

These findings give us insight into creating a better, more clinically-relevant model system for studying cancer cells *in vitro*, which is tremendously more efficient and less expensive than *in vivo*, much less clinical alternatives. Moreover, if our early, *in vitro* models are more in alignment with the other systems, then any significant findings *in vitro* will be more likely to be successful *in vivo* and in clinical trials, avoiding unnecessary "false positive" situations. If an institution uses collagen on their plates, the cells will be more robust and easier to study. We find from this study that 1.0 mg/ml is the ideal concentration of collagen to be effective from the three concentrations we observed.

Another interesting thing to note is that when there is a lot of collagen present, the cells seem to do poorly, as noted in the colony forming assay. However, when we look at the MTS Assay, and how the cells in the 2.0 mg/ml plate reacted to the cancer drug in reference to the untreated plate reacting to the cancer drug, the plate with collagen had more surviving cells. This proves that the presence of any amount of collagen influences the cells' growth.

In relation to wound healing, this experiment proves more definitively that collagen has a huge impact on the growth of cells. While we were not able to perform a wound-healing assay, we can still draw from our data that cells perform better when collagen is present. Specifically, in reference to the MTS assay, we saw that there were more viable cells following the farnesol treatment when there was any amount of collagen present with the cells. Ultimately, the collagen either promoted drug resistance or the collagen helped with regrowth of surviving cells after the treatment. This is advantageous to doctors that specialize in burns because it reiterates the

important fact that collagen promotes cell proliferation and a patient can potentially see a faster recovery if collagen is directly involved in their healing process.

In total, I would say that collagen is very influential in the growth of cells as mentioned in the introduction of this article. Many scientists have concluded that it can promote growth of keratinocytes to benefit wound healing and we have shown that it also influences cancer cells in a similar way. This study showed that there is an optimal concentration of collagen that promotes the most cell growth which will be beneficial moving forward. Knowing that 1.0 mg/ml had the best response among the cells allows for further tests to be done while allowing that variable to be held constant. Moving forward, I think it is important to test how other cancer drugs effect the growth of cells when collagen is present.

While we can draw conclusions about what we think might happen to cancer cells within a person, we do not know for certain the various ways in which collagen effects a person. It could be important for future researchers to look at the effects of a collagen-rich diet versus heavy usage of collagen-rich products in relation to a person's cells. Growing cells on a collagen coated plate cannot be directly compared to the complex ways in which the human body uses collagen, but gaining a well-rounded understanding of how cancer cells 'think' and act in different environments is helpful when treating patients that have cancer. While this study might not have a direct link to what cancer cells experience *in vivo*, it does tell us a lot about the environment cancer cells prefer and how they respond to changing environments. Moving forward, I think it would be helpful to also test different types of cancer cells to see how they react in the various environments. Gathering as much information as possible on the growth dynamics of cancer cells could help in determining the best course of action used to treat them in the future.

Reflection

Overall, we were able to collect good data on the cells that we grew. We had a lot of difficulties in the beginning with our cells dying when we pulled them out of the liquid nitrogen tank. We also had a lot of issues with mold forming within our cells from the incubator. While we managed to overcome these difficulties, it did force us to change the scope of the project slightly. Unfortunately, we did not get to perform a wound-healing assay which would've helped us see if the collagen promoted the cells growing back together following a wounding event. We also were not able to get skin cells to use in the study. The project took a turn towards cancer cells because that is all we were able to grow, but we managed to gather some great data about how resilient the cells are and how collagen effects their growth.

Working on this project was a great experience for me as a student because it promoted critical thinking and gave me an opportunity to apply my classwork. I really enjoyed developing the research plan and then watching it come to fruition. Having to adapt to the various issues that we encountered taught me a lot about the struggles of research and gave me a deeper appreciation for this process.

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