The effect of Cytochalasin B on cell adhesion in developing Sea Urchin embryos

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Introduction

Tissue morphology and development are topics that must be comprehended and studied if a more complete and accurate understanding of the biological mechanisms that constitute the fundamental processes that enable organisms to live and survive on a day to day basis is to be obtained. Each of the different tissues that constitute a given organism are a collection of cells that have been interconnected in such a way so that they perform a particular function (Wilt, et al., 2004). They function to work in tandem with one another in order to contribute towards maintaining the health and normal progression of the organism. However, the means by which cells associate with one another in order to form such tissues lie in various cellular behaviors and interactions such as adhesion, alterations in physical shape, and inhibition or stimulation of cell motility. (Wilt, et al., 2004). Cell adhesion is perhaps one of the most important factors that drive cells to associate with one another and hence promote the creation of tissues. Cellular adhesion refers to the action whereby cells physically bind to each other, each other’s plasma membrane, or the matrix (Cooper, et al., 2004). Cells do not randomly associate with one another, nor do they spontaneously form tissues from such associations. Rather, cell adhesion is a highly regulated processes, most often mediated by molecules referred to as cell adhesion molecules (CAMs) (Wilt, et al., 2004). Such adhesion molecules include transmembrane proteins called integrins, calcium dependent cadherins, fibronectins and...
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immunoglobulins among others (Wilt, et al., 2004). Cell adhesion typically takes places as a result of homophilic binding between CAMs. Homophilic binding refers to the processes whereby CAMs on different cells usually engage in binding to the same type of surface molecules. For instance, a cell with cadherin molecules would only preferentially bind to another cell that also had cadherin molecules expressed on its surface.

Cell adhesion can also be manifested due to the fact that several types of CAMs are bound to cytoplasmic plaque proteins (Gumbiner, 1996). Cytoplasmic plaque proteins function to bridge the cell adhesion molecules found on the cell surface to certain adhesion proteins that are found amongst cytoskeletal networks (Gumbiner, 1996). In this way, CAMs can associate with cytoskeletal movement and even induce or control it. For instance, it is known that integrin adhesion receptors are able to bind to the actin cytoskeleton by physical binding to actin microfilaments in conjunction with α-actinin, talin and vinculin. Cadherins too can be linked to the actin cytoskeleton by binding to α-catenin, which in turn is bound directly to the actin filaments (Wilt et al., 2004). Likewise, adherens junctions are also known to link cell binding sites to the actin microfilaments located within the cell. If these actin filaments are broken down via depolymerization, it should follow that the binding sites would be disrupted, hence causing cell adhesion to decrease. Considering additional such scenarios, it can be inferred that actin too plays a role in cell adhesion regulation. If actin filaments undergo depolymerization, then their associations with CAMs would be markedly reduced if not negated entirely. Such an event could lead to interferences in CAM homophilic binding, thus preventing or inhibiting cell adhesion.

In this laboratory, fertilized sea urchin embryos were used to investigate the effects of actin de-polymerization on cell adhesion. Sea urchin embryos were used in this laboratory because they were easily obtained and gametes could be obtained from both males and females with minimal effort. Furthermore, sea urchins are a prime organism to study the effect of actin de-polymerization in because the embryo can be
observed at multiple stages in development within a relatively short amount of time. The embryo undergoes its two cell, four cell, eight cell and sixteen cell stages within a matter of hours. Thus, cell adhesion can be tested at each of these stages without significant complications. Additionally, sea urchin embryos were desirable to study because they are large, and hence easily observable. Individual eggs can be distinguished with the naked eye if careful observation is practiced.

By studying actin de-polymerization and its effects on cell adhesion, mechanisms by which morphogenesis ensues can be better understood. Furthermore, determining what conditions stimulate cell dissociation provided insight with respect to such issues as maintaining tissue health, integrity and functionality. As equally as important, observing the role that the actin cytoskeleton plays in cell adhesion could provide important insight into those cases where cell adhesion plays a major role in diseases. For example, it was found that cell adhesion was prevalent in many forms of cardiovascular disease, in inflammatory bowel disorders such as ChronÕs disease, in hypersensitivity reactions and many other instances (Berg, et al., 2007). Perhaps by inducing actin de-polymerization in such cell aggregations, cell adhesion would decrease to the point where symptoms or causes of the disease would significantly dissipate.

Actin de-polymerization was induced by using a known de-polymerizing agent, cytochalasin B. Cyotchalasin is also known to actually inhibit the elongation of actin filaments by binding to the positive (ÒplusÓ) ends of the filament (Cooper, et al., 2004). Cells often undergo changes in shape and motility as a result of actin polymerization inhibition and de-polymerization, signifying the importance of these filaments in such processes (Cooper, et al., 2004). Previous studies have shown that a certain concentration of cytochalasin in solution must be reached, above which effects to cell adhesion and shape are evident. Below such a concentration, associations between cells may not be disrupted in the least, making analysis of the actual effects of
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cytochalasin impossible (Schnittler, et al., 2001).

The experiment in which the effects of cytochalasin B on cell adhesion were studied, was carried out by administering varying concentrations of cytochalasin B to sea urchin embryos in different stages of development. Developing embryos were placed in the varying concentrations of cytochalasin at 2, 4 and 8 cell stages by a transfer pipette. They were placed in 3ml of salt water containing differing concentrations of cytochalasin B and were floated in an aquarium cooled to 13 C for a period of 24 hours, so as to allow development to proceed up to the 24 hour mark. Another batch of embryos served as the control group, having no exposure to cytochalasin B. The degree of cell adhesion was evaluated by calculating the percentages by which cell circumferences of contiguous cells were shared with one another. The flatter and more linear the contours of the cells appeared to be when touching each other, the more compact the associations between the cells were, and cell adhesion was considered to be high. When percentages of shared cell circumferences of adjoining cells in an embryo were low, cell adhesion was considered to be low because less of the cell circumference was coming in contact with the circumference of other cells. In this laboratory, it was hypothesized that cell adhesion will decrease with increasing concentrations of cytochalasin B.

Materials and Methods

Making up various Cytochalasin B concentrations

Working cytochalasin B concentrations of 1ug/ml, 5ug/ml, 10ug/ml, and 20ug/ml were made up as stock solutions in 12ml of salt water. A stock solution of 1mg of cytochalasin B dissolved in 1ml dimethyl sulphoxide (DMSO) was used, generating a 1mg/ml solution. The 1ml of DMSO was added by pulling it up through a hyperdermic needle and injecting it into the top of the cytochalasin bottle that contained 1mg of dry cytochalasin B. It was determined that in 12ml of saline water, 12ul of the cytochalasin B solution would be necessary because 1mg/ml is equivalent to 1ug/ul. Thus, for a 1ug/ml solution to use in experiments, 12 ul would be needed (1ug = 1ul).
Similarly, for a concentration of 5ug/ml, it was observed that such a value is 5 fold of 1ug/ml and so the volume used to make up a 1ug/ml solution in 12ml of solvent was simply multiplied by 5 to yield 60ul of cytochalasin B in 12ml of salt water. For 10ug/ml, 120ul (double 5ug/ml) was needed, and for 20ug/ml, 240ul of cytochalasin was used. 3ml portions of each cytochalasin stock solution were then dispensed into sterile, empty 15ml Falcon tubes that were accurately labeled with the concentration of cytochalasin in solution, as well as the stage of development the embryo was in when placed in the solution.

*Obtaining sea urchin gametes*

Eggs and sperm were obtained from adult *Lytechinus pictus* specimens. Specimens were housed in a salt-water aquarium kept at a steady temperature of 13 C. To induce the shedding of gametes, two specimens were picked from the aquarium at random. Each was carefully flipped upside down on a piece of parafilm paper. 3ml of 0.5M KCl was drawn up into a hyperdermic needle which was then injected into the mesodermal space known as the coelom of the sea urchins. The specimens were then wrapped in approximately 1ft of paper towel and continuously shaken for 30 seconds in a vigorous manner. The specimens were then replaced on the parafilm in the inverted position as previously, and were untouched. After several minutes had elapsed, each specimen was lifted from the parafilm to observe whether or not eggs or sperm had been shed. A distinct orange liquid was observed in the discharge of one specimen, indicating it was a female releasing eggs. The second specimen was found to be a male as it yielded a viscous opaque-white liquid that was determined to be sperm. Both the white and orange liquids were observed under a compound light microscope and 10X magnification to verify that both eggs and sperm had been obtained. A transfer pipette was used to collect eggs into a 50mL Falcon tube containing the salt water from the aquarium in which the *L. pictus* specimens were obtained. Sperm was collected into a 2ml ependorff tube, using a transfer pipette. This tube was kept on ice to maintain sperm viability.
Fertilization of sea urchin eggs

Eggs were fertilized by pipetting 3ml of sperm located in the ependorff tube with a transfer pipette into a 50ml Falcon tube containing 20ml of aquarium salt water, into which *L. pictus* eggs were previously pipetted. The addition of the sperm led to the fertilization of the vast majority of all eggs that were later observed on a glass slide under a compound light microscope at a 10X magnification, when obtained from the tube to which the sperm was added. The tube in which fertilization was induced was floated in the 13 C aquarium due to the fact that embryos from *L. pictus* will only develop if kept in a temperature range of 12 – 14 C.

Monitoring developmental stages of *L. pictus* embryos

The developmental progress of the developing *L. pictus* embryos once eggs were fertilized was accomplished via periodic check ups. Such check-ups included pipetting a 1ml sample of the embryos in the 50ml Falcon tube onto a clean glass slide. Observations of development were made under a compound light microscope at 10X magnification. When it was observed that the embryos were at one of the three desired developmental stages (2-cell, 4-cell, 8-cell), a photograph of the embryo was captured using a Canon A20 digital camera by pointing the camera lense directly through the eye piece of the microscope. 2 ml of the embryos were pipetted from the stock Falcon tube into the 15ml Falcon tubes labeled with the appropriate cell stage and cytochalasin concentration. For instance, if embryos were observed at the 4-cell stage, 2 ml of the embryos were pipetted using a sterile transfer pipette, into a 15ml Falcon tube labeled as containing a 3ml solution of 1ug/ml cytochalasin at the 4- cell stage. 2mL samples were then also immediately pipetted into 15ml Falcon tubes that were labeled as containing 5ug/ml cytochalasin at the 4-cell stage, 10ug/ml cytochalasin at the 4-cell stage and 20ug/ml cytochalasin at the 4-cell stage. This process was repeated for both the 2-cell and 8 cell stages. After embryos were dispensed into the appropriate Falcon tubes, all tubes were left to float in the 13 C aquarium for a 24 hour period to allow for
cytochalasin to inhibit cell adhesion in the embryos.

**Analysis of cell adhesion in 24 hour *L. pictus* embryos**

After 24 hours had elapsed from the point of administering the *L. pictus* embryos in varying concentrations of cytochalasin, all tubes were removed from the 13 C aquarium. A 1ml sample was pipetted from each tube, making sure the transfer pipette touched near the bottom of the tube near where the eggs would have sunk due to the fact that they are heavier than water. Each 1ml sample from each tube was dispensed on a separate clean glass slide and then placed under a compound light microscope at 10X magnification. Embryos were observed for signs of cell adhesion inhibition. Photographs were taken of embryos from each tube of a differing cytochalasin concentration and cell stage with the Canon A20 digital camera as previously mentioned. All captured photographs were uploaded to a Dell Inspiron PC with Windows Vista via a USB cable that attached the Canon A20 camera to the computer. Photographs were labeled according to the stages of development and concentration of cytochalasin the embryos in the given photo were exposed to. For instance, an embryo placed in 5ug/ml cytochalasin solution at the 4-cell stage was labeled as Ò4-cell_5cytochalasin.Ó Once all photographs were labeled, they were assembled on a Microsoft Word document according to whether they were control or experimental specimens, and what stage in development they belonged to (2 cell, 4 cell, 8 cell).

Analysis of cell adhesion took place based on what percentage of cell circumferences of cells in a given embryo were shared amongst contiguous cells. The ocular units on the microscope used to measure cell diameter had to be converted to micrometers. It was found by using a clear plastic ruler that 8 ocular units at a 10X magnification corresponded to 1mm on the ruler. Hence, 1mm/8 ocular units X (1000 micrometers/1mm) = 125 micrometers; 1 ocular unit was equivalent to 125 um. Each ocular unit had 10 subdivision marks, meaning each division mark was 12.5 micrometers. Knowing these conversion factors, the length of membrane or cell circumference that
each cell shared with a contiguous cell could be measured by lining up the
ocular ruler to the region at which the circumference was being shared. Such
measurements were usually taken in units of division marks, and then converted into
micrometers, with the knowledge that one division mark was 12.5 micrometers. This
value in micrometers was divided by the total circumference of a typical cell, which was
constant (393 um). Such a calculation would yield a percentage of shared circumference.
Since it is known that compaction corresponds to high cell adhesion, relatively low
percentages of shared circumferences indicated that cell adhesion rates were low. In
contrast, high percentages in circumference shared by contiguous cells indicated high
rates of cell adhesion. In this manner, the hypothesis and related questions could be
evaluated.

Cell adhesion rates were graphed according to percentages of shared/contiguous
circumferences of cells in a particular developing embryo, exposed to a particular
concentration of cytochalasin B. A chart was formulated such as this:

<table>
<thead>
<tr>
<th>Cell Stage</th>
<th>[Cytochalasin B]</th>
<th>Circumference shared by adjoining cells</th>
<th>Total cell circumference</th>
<th>% circumference shared</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>1ug/ml</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1ug/ml</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>1ug/ml</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

whereby division marks converted into micrometers from the ocular scale were
recorded and divided by the total circumference of a cell (393 micrometers) to generate
a percentage of circumference that is shared with other cells. See pages
11/08/08(281)MJO – 11/08/07(292)MJO for accurate calculations. From such data, line
graphs were generated that illustrated the patterns that occurred with respect to cell
adhesion in the developing *L. pictus* embryos. If percentages of shared cell
circumferences decreased in value, it was concluded that cell adhesion had
decreased. In the cases of the 4-cell and 8-cell embryos, averages of shared cell
circumferences were taken. In the 4-cell embryo, four cells were touching
each other. Thus, there were four values that were found with respect to the percentages that each cell shared its circumference with another contiguous cell. The values for each two cells in an association with one another were added up and then divided by the total number of associations that took place between the different cells in the embryo. In the 4-cell stage embryo, the value was divided by four, and in the 8 cell embryo, the value was divided by 8. One embryo from each respective cell stage and distinct cytochalasin concentration was analyzed for the generation of data in this laboratory, and this entire experiment consisted of a single trial.

Controls in this experiment were those embryos that were not exposed to any concentration of cytochalasin B whatsoever. Such embryos served to indicate to the researchers what a normally developing *L. pictus* embryo should look and behave like. The controls also showed to serve how cell adhesion is manifested in developing urchin embryos that are not exposed to any factor that could alter its development patterns. The shared circumference percentages would serve as a template on which to base any differing or surprising data that could be obtained from the embryos that were exposed to varying cytochalasin B concentrations.

**Materials Required:**

*Lytechinus pictus* (from Marinus Scientific, Long Beach, CA)
Transfer Pipettes
Micropipettes
Micropipette tips
Paper towels
Beakers (50mL, 100mL)
Salt water
0.5M KCl (Potassium Chloride solution)
Hyperdermic needle (3mL)
Dimethyl Sulphoxide Buffer (courtesy of Dr. R. L. Morris).
Cytochalasin B-1mg/ml (from Sigma Chemical Company)
Tweezers
Forceps
Glass slides
Cover slips
Valap (Vaseline/paraffin wax mixture, see pg É.MJO)
Compound light microscope
Test tubes
Petri dishes
Graduated cylinders
Cytochalasin B did appear to have an effect on cellular adhesion in developing *Lytechinus pictus* embryos. Percentages of shared circumferences of contiguous cells were generally lower amongst embryos in the experimental groups as opposed to the control groups, although cell adhesion did not change much in response to increasing concentrations of cytochalasin B in some cases. In several instances, cellular adhesion rates actually increased rather than decreased as expected, in the presence of higher concentrations of the reagent. Analysis of embryos in the 2-cell stage of development was made difficult due to the fact that almost all embryos in this stage failed to divide. Many cells died or become misfigured, and several others appeared to be apoptotic in nature. In fact, only embryos in the Falcon tube containing a concentration of 5µg/ml of cytochalasin B seemed to remain viable enough for analysis.
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**Fig 1.** Cell adhesion is lower in the embryo exposed to a solution containing 5ug/ml of cytochalasin B in comparison to the control sample. All other embryos in the 2 cell stage failed to survive, illustrated by the lack of data in this graph.

Looking at Figure 1, a decrease in the cell circumference shared by cells in the same embryo was in fact noted amongst embryos exposed to a 5ug/ml concentration of cytochalasin B, when compared to the control embryo. However, only one embryo in the control group was observed in order to obtain data for the control population at an exposure level of 5ug/ml of the inhibiting reagent. As a result, data in this category is not completely reliable. Cell adhesion decreased in the presence of cytochalasin, but the effect of cytochalasin B as an inhibitor of cell adhesion was not manifested in this instance, as there was a dearth of data.

In the embryos at the 4-cell stage of development, survival rate of the embryos was far more successful than that experienced amongst the embryos that were placed in cytochalasin B solutions when at the 2-cell stage of development. Control embryos appeared to be healthy and robust, with an average circumference of 393 um. Similarly, it would appear as though control embryos grew at a steady rate as the cells in each embryo appeared to be roughly the same shape and same levels of cell adhesion to one another amongst all cells.
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Fig 2. Cell adhesion rates as cytochalasin levels are varied. Cell adhesion is significantly less prominent in embryos exposed to cytochalasin B than in those that are not exposed to the reagent as is evidenced by the high percentage of shared circumference of contiguous cells that is shared in the control embryos.

The percentage of cell circumference that was shared with contiguous cells in the control embryo was calculated to be a much higher value than was found amongst any of the embryo cell stages immersed in cytochalasin that were used in the successful completion of this laboratory. When 4-cell stage embryos were exposed to cytochalasin, the percentages of shared cell circumference drastically decreased compared to the control embryo. According to Figure 2, when embryos were exposed to a 1ug/ml concentration of cytochalasin B, the percentage of cells that shared a same circumference dipped down gradually, before rising rapidly when the embryos were exposed to 10ug/ml of the reagent. Such dramatic shifts in the percentages of shared circumferences of contiguous cells indicated violent fluctuations in the level of cell adhesion that was occurring within these embryos as well. Cell adhesion seemed to decrease when cytochalasin B concentration was initially increased from 1ug/ml to 5ug/ml. However, compaction was observed as cytochalasin concentrations continued to rise, and then ended with another decrease in adhesion as concentrations of the inhibiting reagent reached 20ug/ml. According to Figure 2, the presence of cytochalasin B did not have a clear-cut effect on cell adhesion as the percentages of shared circumferences of contiguous cells, was unstable.
Cell shape varied significantly depending on what concentration of cytochalasin B the embryos were exposed to. As cytochalasin concentrations increased, a qualitative observation was made that cell shape varied to such an extent that it influenced the ability to maintain cell shape or control formation of cell adhesion.

![Figure 3](http://icuc.wheatoncollege.edu/bio254/2007/mophir/index.htm)

**Fig 3.** Cell adhesion effects in *L. pictus* embryo (at the 4-cell stage) exposed to 1ug/ml cytochalasin B solution. Prominent sharing of cell circumference as cells are delineated by elongated, broad lines that appear very linear and distinct in nature. Such organization and order is evidence of compaction that is occurring as a result of efficient cell adhesion amongst the cells of the embryo.

![Figure 4](http://icuc.wheatoncollege.edu/bio254/2007/mophir/index.htm)

**Fig 4.** *L. pictus* embryo at the 4 cell stage in the presence of 5ug/ml of cytochalasin B solution. Cell adhesion has markedly decreased as the linear bands that separated one cell from another are now gone. Cells in the embryo are oval in shape and the amount of circumference each cell shares with its neighboring cells is much less than amongst cells in Figure 3.

![Figure 5](http://icuc.wheatoncollege.edu/bio254/2007/mophir/index.htm)

**Fig 5.** *L. pictus* embryo at the 4-cell stage in the presence of 10ug/ml of cytochalasin B solution. Cell adhesion has again become more prominent as cell membranes have become more elongated, and cells themselves have taken on a fuller, rounder shape, increasing the amount of circumference that is common to contiguous cells.

Cell shape amongst embryos in each concentration of cytochalasin B solution was somewhat different. As cytochalasin concentrations increased, cells deviated more and more from the typical shape of an egg cell. These abnormalities could have played a role in generating the data that was obtained with respect to the degree of cell adhesion in
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embryos at different cytochalasin B concentration levels. Cells that are more elongated or or otherwise not conforming to the typical round shape of a sea urchin embryo can induce cells to share more of their circumferences with each other, and hence could lead to higher rates of adhesion than is in reality taking place between the cells.

In comparison, figure 6 represents a control embryo at the four cell stage that was not exposed to cytochalasin B at all.

![Image](image_url)

**Fig 6.** Shared circumference is very evident in this photograph due to linear, long lines that indicate where circumferences of two contiguous cells is being shared and is of the same length. The more linear the region at which cells are contiguous with one another is, the higher the degree of compaction.

Without cytochalasin B, cell shape is more defined and shared circumference of contiguous cells in a given embryo is outstanding and more readily observable than the shared circumference evidenced in embryos exposed to cytochalasin B.

Figure 7 depicts a pattern in cell adhesion behavior reminiscent of Figure 2, however instead of cell adhesion decreasing from 10ug/ml to 20ug/ml as in Figure 2, it is increasing here. The percentage of cell circumference that is shared by contiguous cells in a developing embryo increases, indicating an increase in cell adhesion. This increase is similar to the followed by a rapid increase from 5ug/ml to 10ug/ml that is exhibited in Figure 2. that is of the same magnitude (same slope) as that was found in Figure 2. Such a pattern may indicate unique cell adhesion properties in *L. pictus* embryonic cells during development. The percentage of shared circumference amongst contiguous cells in the control group was found to be lower than the percentage of shared circumference found in cells of embryos that were exposed to 20ug/ml of cytochalasin B.

Nevertheless, it was found that the cell adhesion rate in the control group is still higher than those in the experimental group on average.
Fig 7. Cell adhesion decreases as cytochalasin concentrations increase. A sharp increase in shared circumferences between cells in embryos when exposed to a 20ug/ml cytochalasin B concentration.

It was observed that cell adhesion is indeed significantly lower amongst developing embryos that were exposed to varying concentrations of cytochalasin B than in those embryos that were not exposed to inhibiting drug at all. It was also observed that cell adhesion displays a varying pattern as evidenced in both Figures 2 and 7, such that cell adhesion increases or decreases when embryos of *L. pictus* are exposed to cytochalasin concentrations of as high as 20ug/ml, depending on what cell stage is present, whereas cell adhesion was much lower when embryos were exposed to 10ug/ml.

A unique aspect of cell associated adhesion may have been identified as a result of administering such a wide range of concentrations of the reagent that was supposedly to inhibit cell adhesion from occurring at sufficiently high concentrations.

**Discussion and Conclusions.**

In this laboratory, the hypothesis that cell adhesion would decrease with increasing concentrations of cytochalasin B was not fully supported. While there is reason to suspect that cytochalasin B did play an active role in contributing to the significantly lower percentages of shared circumference in contiguous cells in experimental embryos that were exposed to cytochalasin as opposed to the control group, some observations were made that completely invalidate the hypothesis. For instance, it was found that cell adhesion actually increases when embryos at the 4-cell...
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stage are exposed to a cytochalasin B concentration of 20ug/ml. At this concentration, cell adhesion would rationally have been thought to be non-existent and cells would be dissociated from one another. Nevertheless, cell adhesion appears to be prominent and in full force as the percentages of cell circumferences shared in embryos that were incubated in the presence of 20ug/ml of the reagent were higher than those percentages found for embryos in 10ug/ml. Furthermore, a distinct conclusion cannot be made in this laboratory due to the fact that only 1 embryo for each cell stage was used in measurements of shared circumferences of contiguous cells for both control and experimental batches. These embryos may have been injured, sick, mutants or in other ways defective, thus skewing the data in one way or another.

A qualitative conclusion that was determined in this laboratory was that the presence of cytochalasin B in solution induced the deformation of cells in developing embryos. In general, cells in embryos that were developing in the presence of varying amounts of cytochalasin B appeared to be incorrectly formed, disfigured and in many instances did not resemble cells at all. In comparison, embryos incubated without the presence of cytochalasin did not manifest such striking characteristics. This would lead one to conclude that cytochalasin may play some role in induced cell disfigurement, and indeed it does. Several studies have indicated that growing any type of cell in the presence of cytochalasin B has tended to lead to weird cell shape and incorrect formation (Chamorro, et al., 1986).

There are many other factors that could have played a major role in obtaining the results and data that were found in this experiment. Interferences with the receptor/ligand binding associations of CAMs could have lead to decreased or increased cell adhesion, depending on what type of interference was made. Observation of cell adhesion or lack thereof could have been prevented due to the fact that the cells in the embryo were all kept in relative proximity to one another due to the presence of the hyaline layer that traps cells inside the embryos and prevents them from moving too far apart from one another. Additionally, an enzyme or other ligand molecule could have interfered with proper binding and hence cells could not associate with one another properly. An explanation as to why cell adhesion increased in certain instances could be due to the fact that cadherins bind in the presence of calcium in solution (Cooper, et al., 2004). Calcium is indeed present in sea water. As a result, cadherins could have associated with one another, irregardless of whether cytochalasin B was in solution; holding cells together even if actin filaments were being de-polymerized or inhibited. The occurrence of deformed cells when embryos were incubated in the presence of cytochalasin B could be due to the fact that actin filaments play a major structural role in supporting the cytoskeleton of the cell (Cooper, et al., 2004). If these actin filaments are de-polymerized, the entire structure of the cell is compromised and is now susceptible to changing its shape and behavior. Furthermore, since adhesion still persisted in the presence of cytochalasin, it could be that the threshold for disrupting actin filament formation was not crossed.
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The fact that the percentages of shared cell circumferences amongst contiguous cells was consistently higher in control embryos rather than experimental ones showed that cytochalasin did play a role in inhibiting cell adhesion in certain instances. It can also be concluded that cytochalasin B did induce cell adhesion to decrease in some embryos, as several trends indicated that when concentrations of the reagent were high or low, that cell adhesion was markedly lower in experimental embryos than in control embryos.

Discrepancies could be explained by incorrectly making the concentration of the given cytochalasin solution, such that it was less or more concentrated than was labeled or intended. Additionally, the cytochalasin B that came in powder form may not have been fully dissolved in DMSO before use, and hence not all cytochalasin B compound was utilized in applying the reagent to various embryo environments. The embryos themselves only develop in a temperature range of 12-14 C. Perhaps the temperature in the aquarium in which the tubes were floating had increased overnight, thus inhibiting the embryos from developing normally, inducing them to die.

This experiment could have yielded higher quality results if it was refined in several ways. For instance, developing embryos could have been placed in their respective cytochalasin concentrations immediately at the point at which they arrived at a certain cell stage. For example, embryos could have been monitored under a microscope until the two cell stage was immediately complete. At this point, the embryo could have been put into the cytochalasin solution so as to get a more accurate representation of how cytochalasin inhibited cell adhesion and other cellular interactions. Another refinement to this experiment includes adding more cytochalasin concentration values so as to get a better understanding of at which concentrations cytochalasin affects cell adhesion processes, and at which concentrations it does not. Thirdly, it would be beneficial to keep the embryos in shallower water so as to allow them greater access to oxygen. If eggs are layered on top of each other, the ones on the bottom will suffocate and die. By using a shallower container such as a petri dish or bowl, the eggs can spread out along the greater surface area and have better access to oxygen, increasing survivability, and hence allowing for observation of development in the presence of cytochalasin. A final refinement that would be beneficial to this laboratory is the presence of 0.2um filtered sea water instead of using sea water from the aquarium. This filtered sea water would have a better chance of being sterile than simply taking water from an aquarium where different microorganisms and pathogens can invade the water and in turn, induce poor health and death to the developing embryos.

To expand on this experiment, it would be advisable to conduct a study whereby the concentrations of cytochalasin B remain the same, but the types of cells studied, be varied. Previous studies have dealt with mesodermal tissue aggregations and the effects of cytochalasin on cell adhesion in such tissues. Studies regarding cell adhesions in sea urchin embryos are not well known and the feasibility of such a study could be subject to debate. A study whereby
varying concentrations of cytochalasin B are applied to developing tissue cells or tissue in general at differing stages in development, would be ideal. Degrees of cell adhesion could be measured by observing how many cells lose affinity for one another and separate entirely from the tissue. Conversely, cell adhesion could be simply measured as has been outlined in this paper; observing the percentage of cell circumference that is shared by contiguous cells in a given area of the tissue.

References


