Identify the effect of culture media in the viability of cultured gonad cells for fresh water snail *bellamya bengalensis in vitro*

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Abstract

The present study carried out during period from April 2013 –October 2013 at laboratories of college of science Qadisiyah University, to identify the effect of culture media on the viability *in vitro* cultured gonad cells of fresh water snail *bellamya bengalensis* and investigate the optimal culture media that suitable for gonad cells cultured *in vitro*. The fresh water snails *bellamya bengalensis* were collected from shamyiah River –Dewanyah province , the snail were dissection out to obtain the gonad cells, the gonad cells were cultured in (RPMI-1640,S-MEM media), the gonad cells were cultured in RPMI-1640 medium revealed higher viability rate compare with S-MEM medium and control ,according to our results , RPMI-1640 medium was suitable and had positive effects on the viability of gonad cells for *bellamya bengalensis in vitro* cultured**.**

*Key words***:**RPMI-1640 medium, S-MEM medium

Introduction

The most intensively studied group of fresh water invertebrates achieved to enhance and improve the cell cultures conditions (Rinkevich, 1999), and these aims was not realized in the last years completely, because of the fresh water invertebrates has low cell differentiation *in vivo*, therefore the optimal source of high level proliferation for these cells can be obtained by *in vitro* maturation tissue cultures. A lot of papers and studies tried to maintained high rate proliferation and viability bivalve cells, especially from mussels and oysters reported (Takeuchi *et al*., 1999). In the other hand there was many studies indicated that no mortal cell line from fresh water molluscs has been demonstrated (Dixon and Wilson, 2000).as well as there was a lot of reasons indicated as obstacles or difficulties that prevent high rate proliferation *in vitro* such as the failure of cell cultures conditions and

specific growth factors which promote cell proliferation in fresh water invertebrates *in vitro* (Dixon *et al*., 2002).

In that time there was many tries to maintain the cell viability for short time by use cell culture procedure for mussel mantle cells to maintained its (Cornet, 2000).Also here was few techniques have been used to obtain high cell proliferation or viability rate by established cell lines from fresh water invertebrates, where a large number of organs and cells from fresh water snails have been cultured *in vitro*, including epithelial cells from embryos, gills, mantles and digestive glands (Auzoux *et al*., 2003). Only short –term (approximately two week) cell culture have been proved, in these techniques, the cell viability maintained as much as 12 days (Tamse *et al*., 1995).While there was papers reported that the contamination by microorganisms species represented the main reason for failure of maintained the cell viability for long-term culturing *in vitro* for most common fresh water invertebrates (Auffret and Oubella, 2007).

The various knowledge about *in vitro* maintenance of fresh water invertebrates cells viability, providing convincing evidence for the potential *in vitro* cell cultures in various scientific field .A lot of procedures *in vitro* cell culture conditions are reported in order to enlarge scope knowledge of fresh water invertebrates cell cultures, but there are still in primary stages ,because of a lot of obstacles and difficulties came from primary fresh water invertebrates cell cultures, such as maintain aseptic conditions ,evade the contamination ,cellular isolation(Auffret and Oubella, 2007).

In this paper we tried to study the *in vitro* proliferation of gonads cells for *bellamya bengalegensis* by using tow culture media(RPMI-1640,S-MEM) while the most important aims of this study :

1-study the viability of gonads cell for *bellamya bengalegensis in vitro* using different culture media.

2-identify the optimal culture media for culturing the gonads cell by comparing the viability ratio between two culture media *in vitro*.

Martial and methods

Sample collections

Fresh water snails of the species *Bellamya bengalensis* (average size of 2.5 cm) were collected from Shamyiah river in Dewanyah province in April 2013 by simple hand picking method, freshly collected specimens were carried out to the laboratory of college science – Qadisyah university and placed in aquarium and half of fresh water in the aquarium was renewed every two days, the animals was fed with lettice and introduced to analysis, then the specimens dissected out, and the gonads of *B.bengalesis* have been taken out carefully and gently to prepare them to cell culture studies(Kamble&Gaikwad,2012).

The gonad cell culturing using culture media.

The Gonads explant from the *Bellamya bengalensis* were dissected out into small cellular fragments and placed in petri dish containing 2ml fresh water and the petri dishes were stirred during this period in order to make easier fragments disassociation, from the petri dish the suspension was taken out and put in a 15ml falcon tube. The suspension was centrifuged at 15°C, 150xg, for 4 minutes, the supernatant was discarded and the pellet of cells resuspended in 2 mL of fresh water . Using the same parameters the pellet of cells were resuspended in 2mL of cell culture media (Buchanan *et al*., 2001 ,Cristano,2009).

The cell suspension was transferred in a multi-wells and left at 15°C. Cells viability was checked every three days by using trypan blue dye (Sigma, 2007). The culture media was substituted tow times per week order to avoid possible contamination . This procedure was applied on both culture media in the same time using the same parameters(temperature ,speed of centrifuge device ,time) (Buchanan *et al*., 2001 ,Cristano,2009).

Results

The present study reported that there was significant differences(LSD($p \leq 0.05$):9.2) between the viability of gonads cell by using tow culture media PRMI-1640 and S-MEM medium as well as control.

There were significant differences ($p \leq 0.05$) in the viability of gonad cells at the first day between PRMI-1640 and S-MEM medium, when the viability reached to 94% in PRMI-1640 medium, while 85% in S-MEM medium compare with 3% in control.

Also the present study reported significant differences ($p \leq 0.05$) at the third day between these two cultures media, when the viability was 82% in PRMI-1640 medium, while 71% in S-MEM medium and 0% in control.

The significant differences($p \leq 0.05$) was continuous at the sixth ,ninth and twelfth days ,when the viability was(76%,54% and 33%) respectively in PRMI-1640 medium ,while the viability was (52%,22% and 0%) respectively in S-MEM medium(table1,figure1).

Table1:The viability of gonad cells using RPMI-164,S-MEM media

Culture Media	**1 day**	**3 day**	**6 day**	**9 day**	**12 day**
Control	3	0	0	0	0
RPMI - 1640	94	82	76	54	33
S - MEM	85	71	52	22	0

L.S.D (($p \leq 0.05$):9.2

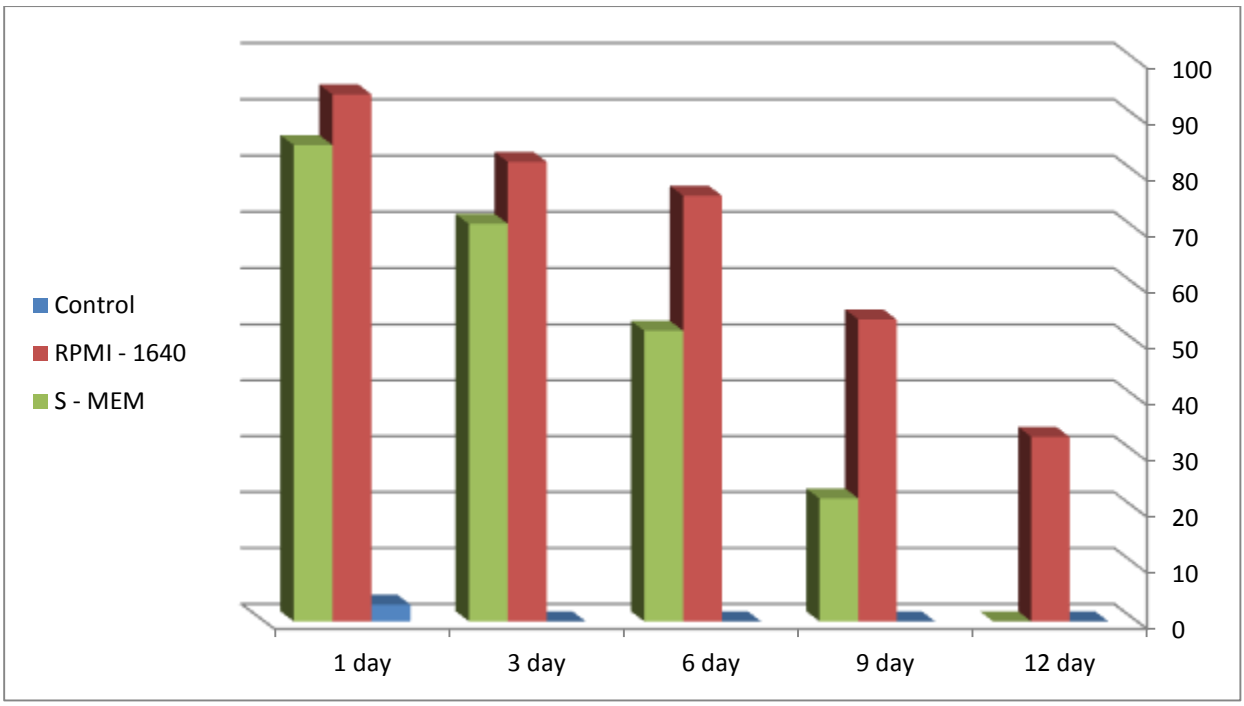

Figure 1: showed the comparison between the viability in RPMI-1640, S-MEM media.

Discussion

Because it was described that the medium can influence on cell growth and survival, the present study identified which media had the most effect on cell viability and cell differentiation. The tow culture media S-MEM and RPMI 1640 are used in this study, were generally available for supporting the growth of different types of cells. However, there are some differences in their composition, and this difference could potentially effect on cells viability (Liu *et al*., 2009). RPMI 1640, was first use to maturation the lymphocytes *in vitro*, is simple in ingredients which rich in amino acids ,organic compounds and glucose ,while the S-MEM medium , modified from Basal Medium Eagle, is rich in nutrition and have different characteristics(Kim *et al*. ,2005). The present study demonstrated that gonads cells cultured in RPMI-1640 maintained higher cell viability until twelfth day, compared with the viability of gonads cell that cultured in S-MEM medium that maintained the viability of cells till the ninth day.

These results might specific related with concentrations of glucose ,calcium and phosphate in both culture ,the Glucose is a central source of energy for all cells in different organisms ,the most common media contain varying concentrations of glucose ranging from 1 to 4.5 g/L (Li *et al*. 2007). S-MEM (low glucose) contains a lower concentration of glucose (1 g/L) than RPMI 1640 (2 g/L). In addition, S-MEM haven't calcium in their components and a lower concentration in phosphate (0.122g/L) than RPMI 1640 which contains(1.1 g/l) of calcium and (1.8g/L), in this point , there was a couple of studies about the effects of calcium and glucose on cell culture ,and these studies reported that glucose and phosphate have positive role to maintain actively cells viability and proliferation *in vitro* (Li *et al*., 2007;Liu *et al*., 2009).

Based on viability assay, the present study found no statistical differences between S-MEM and RPMI 1640 groups in terms of cells viability at the first six days. The results are agree with the findings of Maeno's (2005) and Lopez-Cazuax's(2006) studies , in these approach they found that rich calcium induced cell proliferation .as well as , some reports also demonstrated that lower calcium concentration didn't effect on cell viability (Youshimura *et al*., 1996). In addition, glucose may also had positive effect on cell proliferation and cell viability ,when previous studies have demonstrated that high glucose induced cellular senescence or apoptosis, while reduction of glucose enhanced proliferation and viability (Stolzing *et al*., 2006; Kim *et al*., 2005),these findings disagree with the present study that reported the high concentration of glucose in RPMI 1640 compare with low concentration in S-MEM, might had influence to maintain the viability of gonads cell over two weeks.

The absent of calcium and reduced of glucose in S-MEM could be responsible for the reduced its efficiency of medium to maintain cells viability ,This thought compatible with Eklou-Kalonji *et al*(1998) they confirmed the extracellular Ca^{2+} could be of great importance in the regulation of osteoblastic proliferation and differentiation.

Although some previous studies regarded that maintain the cell culture activity increased in lower-calcium concentration (Matsumoto 1995; Youshimura *et al*. 1996) or was not

clear effect by the different concentrations of calcium which contained in the basal media (Eklou-Kalonji *et al*. 1998), while a lot of studies evidenced that high-Ca^{2+} medium improved higher viability in cell culture *in vitro* (Maeno *et al*. 2005). It was also proved that high calcium concentration and low phosphate concentration contained in different basal media enhance cell culture proliferation (Lopez-Cazuax *et al*. 2006). On the other hand, high glucose concentration influences on osteogenic differentiation and maintain cell viability (kim *et al*. 2005; Li *et al*. 2007). Thus, the present study could hypothesize that high glucose concentration may promote the gonads cell differentiation and have positive effect to maintain cell viability .

Also present study confirmed that high Ca^{2+} concentrations would improve proliferation and the optimal extracellular Ca^{2+} and Pi for proliferation and maintain cell viability for cell culturing *in vitro* was 1.1g/L ,1.8 g/L respectively, which are the concentrations supplied in RPMI-1640, as well as ,in previous studies it was indicated that low concentrations of glucose increased the percentage of proliferation for culturing cells *in vitro* and high glucose concentrations decreased the percentage of proliferation and differentiation for culturing cells *in vitro* by using different culture media (Kim *et al*. 2005), that is not in accord with our results which regarded that the high concentrations of glucose in RPMI-1640 medium enhance the viability of gonads cell compare with S-MEM that have less concentration of glucose.

In view of the above mentioned studies included our study, we can conclude reasonable reason to proof that the higher concentration of glucose ,calcium and phosphate in RPMI-1640 medium was the reason to maintain cell viability over tow weeks. We supposed that our results either agreed or disagreed with other studies because a lot of factors such as different of cells type, different culture media and experiment conditions ,this result supported by(Perka *et al*. 2000). In our study, the results reported that the high concentration for glucose and calcium in PRMI-1640 was the main reason for superiority of RPMI-1640 medium on S-MEM medium for maintain the viability of gonads cell, Thus, we think that the inclusion of RPMI-1640 of calcium at concentration (1.1 g/L) and phosphate(1.8g/L)performed several functions , it was help to retain the osmatic balance of

the cell and help regulate membrane potential by provision of calcium and phosphate ions, in the other hand the main source of energy is driven from carbohydrates generally in the form of sugars, the major sugars used are glucose, these points might help us to understand the positive effect of calcium ,phosphate and glucose and all of them on cells growth or cells viability of gonads.

تحديد تأثير الأوساط الزرعيه في حيويه الخلايا التكاثريه المزروعه لقوقع المياه العذبه بللاميا بنكالينسس خارج الجسم الحي

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أجريت هذه الدراسه في مختبرات كليه العلوم –جامعه الفادسيه خلال الفتره من نيسان ٢٠١٣ ولغايه تشرين الأول ٢٠١٣ لتمييز تأثير الأوساط الزرعيه في حيويه الخلايا التناسليه لقوقع المياه العذبه بللاميا بنكالينسس المزروعه خارج الجسم الحي, والتحري عن الوسط الزرعي المناسب للخلايا التناسليه المزروعه خارج الجسم الحي. تم جمع القوقع من نهر الشاميه –محافظه الديوانيه في شهر نيسان ٢٠١٣ ومن ثم تشريح القوقع للحصول على الخلايا التناسليه التي زرعت في الوسط الزرعي(RPMI-1640) و الوسط الزرعي (S-MEM).الخلايا التناسليه التي زرعت في الوسط الزرعي (RPMI-1640) أظهرت معدل عالي من الحيويه مقارنه بالوسط الزرعي(S-MEM) ومجموعه السيطره.وطبقا لهذه النتائج ,الوسط الزرعي (RPMI-1640) مناسب وله تأثير أيجابي على حيويه الخلايا التناسليه المزروعه لقوقع المياه العذبه بلاميا بنكالينسس خارج الجسم الحي.

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