Effect of Cell Concentrations and Culture Media on Hamster Splenocyte Viability During *in vitro* Cultivation

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ABSTRACT

Syrian golden hamster has been widely used as experimental model in several diseases, although their immune background still not fully understood due to the limited of antibodies *in vitro* cultivation of their immune cells. Therefore, knowledge of mouse immunity has often been substituted to explain the results of hamster experiments. However, as these immune systems have also been reported to be different., this methodology may be problematic. In order to study the hamster immunity, *in vitro* culture of hamster immune cells is required. In this study, we therefore proposed to optimize the conditions necessary to study the hamster splenic cells, which contains major source of immune cells. Normal hamster splenocytes at $1x10^5$, $1x10^6$, and $1x10^7$ cells/mL were used to optimize culture conditions to obtain highest possible cell viability. Various media and supplements such as RPMI, DMEM, IMDM and 10-20% FBS were titrated. The cultures were performed over 1-4 days as has been done in most immunological studies. Our results demonstrated that after 2 days of culture, $1x10^7$ cells/mL gave the maximum viabilities of 78.77% while $1x10^5$ and $1x10^6$ cells/mL concentration yielded 62.32% and 63.49% viability. After 4 days, the same $1x10^7$ cells/mL also gave the maximum viabilities at 69.22%. A 15% FBS provided maximum viability of the $1x10^7$ cells/mL concentration. RPMI medium provided an optimal viability of 70% after 4 days for the hamster splenic cells. RPMI and DMEM were subsequently deemed to be suitable for hamster splenocytes. This novel results for optimization of culture conditions will therefore be useful for study of hamster immune cells in other immunological techniques.

Keywords: Hamster, Splenocyte, Immune cells

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Introduction

Hamster has been widely used as an experimental animal model in several diseases such as bacterial [1], parasitic [2-3] and viral infections [4], metabolic disorder [5] and some cancers [6-7]. The common species of hamsters used are Syrian hamster or *Mesocricetus auratus* [1-7] and Chinese hamster or *Cricetulus griseus* [8-9]. The reasons why Syrian golden hamster is selected for commonly using as animal model are easy to handle [10] and be susceptible to many pathogens such as bacteria [1] and virus [4]. Moreover, it has a rapid development and easily to be induced to cause tumors by several oncogenic agents [6-7, 10-11]. However, the immune background knowledge of hamster remains limited due to monoclonal antibodies, reagents and tools that specific to hamster. A lack of hamster-specific antibodies leads to immune response data in hamsters against microbial infection is still limited. Authors who used hamsters as experimental models usually explained their immune responses based on mouse model. Most of the molecules in the immune system between mouse and hamster are similar except some major parts of T cells [1]. There are several studies on T cell activation and molecules including T cell receptors [12-14], CD3 [12, 15], CD4 [15-17], CD8 [18-19], CD28 [20-22], CD40/CD40L [23-24], LFA-1 [18, 25-26], NKG2D [19, 27-28], and CD2 [20, 29]. These molecules play roles in T cell activation in other species but there are very rare studies in hamster. There was a study reported that hamster and mouse are different in transcriptome analysis between them [30]. Moreover, a recent study revealed differential hamster and mouse immune responses of $CD4^+$ T cell subsets after leptospiral infection [1]. Thus, substitution of mouse immunological knowledge for that of hamsters may be misleading. It is therefore essential to examine normal hamster immune cells before being exposed to microorganisms, hormones, or induction of cancers so that a reference point can be established for alterations to immunological status.

To study hamster immune cells, *in vitro* cultivation is essential, hamster mononuclear cells previously could not be cultured over 48 hours in normal mouse's culture condition as most of the cells died within 2 days (Nakornpakdee, unpublised observation). Accordingly, optimization of culture conditions for hamster immune cells is necessary. As spleen plays major role in immunity, hamster splenocytes were therefore used, isolated and then cultivated with various conditions. Spleen leukocytes are composed of B cells and subsets of T cells which are non-adherent cells, and also dendritic cells and macrophages which are adherent cells [31-35]. The conditions were optimized by using varied common media, cell concentrations and Fetal Bovine serum (FBS) concentrations. Cell viabilities was also investigated using dyes after 1, 2, 3, and 4 days of incubation.

Methodology

1. Animals

Female Syrian golden hamsters (4–6-week-old) were maintained in the animal unit at the Faculty of Medicine, Khon Kaen University (Khon Kaen, Thailand). All experiments were considered by Animal Ethic Committee of KKU and performed in accordance with institutional guidelines (IACUC-KKU-54/62).

2. Hamster splenocyte isolation

Normal Syrian golden hamsters were sacrificed, and their spleens were removed. Their spleens were homogenized as cell suspension in 10% FBS in Roswell Park Memorial Institute (RPMI) media containing penicillin (100 U/mL), streptomycin (100 μg/mL). The media were adjusted to pH 7.2-7.4. All reagents were purchased from Gibco, Thermo Fisher Scientific, MA, USA. After washing, 5 ml of media per spleen was added to resuspend the cell pellet and centrifuged at 500 xg for 5 minutes at 4 °C. To lyse red blood cells, 3 mL of Ammonium Chloride Potassium (ACK) lysing buffer was added and leave for 5 minutes in ice box. The desired medium volume was added to resuspend cells to a final known volume. Lastly, stained the cells with trypan blue (Gibco, Thermo Fisher Scientific, MA, USA) and counted live cells using a hemacytometer under the light microscope.

3. FBS concentration and cell number optimization

The $1x10^5$, $1x10^6$ and $1x10^7$ hamster splenocytes/mL were seeded in 24-well plates, cultured with RPMI containing penicillin (100 U/mL), streptomycin (100 μg/mL) and supplemented with either 10%, 15% or 20% inactivated FBS. All conditions were then incubated at 37 °C and 5 % CO_2. The media was not changed until day 4.

4. Media optimization

Optimal hamster spleen cells, $1x10^7$ cells/mL were seeded in 24-well plates, cultured with either RPMI, Dulbecco's Modified Eagle Medium (DMEM) or Iscove's Modified Dulbecco's Medium (IMDM) containing penicillin (100 U/mL), streptomycin (100 μg/mL) and supplemented with optimal FBS concentration (15% FBS). The media were adjusted to pH 7.2-7.4. All media were purchased from Gibco, Thermo Fisher Scientific, MA, USA. All conditions were then incubated at 37 °C and 5 % CO_2. The media was not changed until day 4.

5. Cell viability

Cells viabilities were determined by gathering cells after 1, 2, 3 and 4 days after cultivation using trypan blue staining. The 0.4% trypan blue was used to check cell viability [36]. The percentage of viable cells were calculated by dividing live cell count by total cell count which obtained from live cell count and dead cell count.

6. Statistical analysis

Data were shown as mean ± Standard Error of mean (SEMs). As the data was found not to be normal distribution. The Kruskal Wallis test was then used to analyze the data in each parameter. The comparison data with *P* values <0.05 were considered as statistically significant differences.

Results

1. Effect of cells and FBS on cell viability

1.1 Higher cell numbers increase cell viability when cultured for 2 days

The average cell viability of fresh splenocytes was 80.48% on the day of isolation. The viability of hamster splenic cells was varied depending on cell numbers and FBS concentrations **(Fig 1)**. Our results demonstrated that at $1x10^5$ cells/mL and 10% FBS supplementation in media, average cell viabilities were 70.84% and 62.32% after being cultured for 1 and 2 days while the corresponding viabilities was 66.75% and 56.27 when 15% FBS was used and 61.53% and 61.48% in 20% FBS. **(Fig 1)**. This indicates that low cell numbers gave low cell viabilities despite an increase percent of FBS. At $1x10^6$ cells/mL and 10% FBS, the average cell viabilities on day 1 and day 2 were 72.21% and 58.96%, respectively. The viabilities were 69.78% and 63.49% in media supplemented with 15% FBS and 68.19% and 62.52% in 20% FBS. For $1x10^7$ cells/mL, the average cell viabilities were 74.90% and 73.66% after incubation 1 and 2 days when 10% FBS was used. The cell viabilities of 15% FBS were 77.29% and 78.77% and 20% FBS viabilities were 75.35% and 69.85% after incubation 1 and 2 days, respectively. These viabilities decreased by about 20-50% after incubation for 3 and 4 days in every FBS concentration in both $1x10^5$ and $1x10^6$ cells/mL. In contrast, only $1x10^7$ cells/mL condition exhibited greater than 60% viability after being cultured for 3 and 4 days. There were no significant differences between any cell concentrations.

1.2 The 15% FBS gives maximum cell viability

Our results demonstrated that media supplemented with 10% and 20% FBS gave different viabilities. The 15% FBS showed the highest viabilities (77.29%) on day 1 after incubation. Supplementation with 15% FBS in media also gave higher than those of 10% and 20% on day 2, 3 and 4 in almost cell concentration. At $1x10^5$ cells/mL, media supplemented with 10%FBS gave the highest viability on early days **(Fig1a)**. In contrast, 15% FBS in media had the highest cell viability at $1x10^6$ and $1x10^7$ cells/mL after cultivation for 4 days **(Fig 1b and 1c)**. Although there were no significant differences among all these FBS concentrations, 15%FBS in media tend to have higher cell viabilities than 10% FBS and 20% FBS. Therefore, 15% FBS and $1x10^7$ cells/mL were selected for further medium optimization.

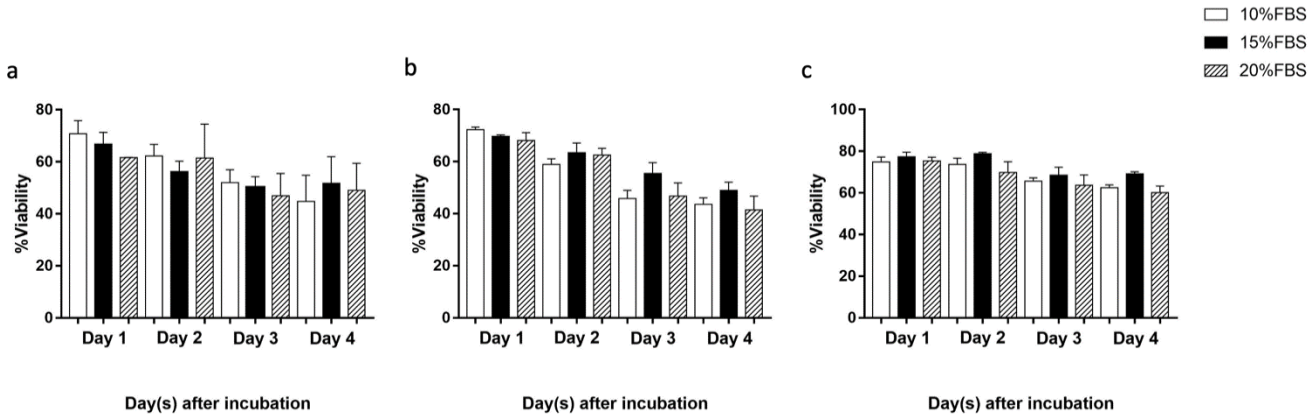

Figure 1 Viabilities of hamster splenocytes after cultivation with various cell numbers and FBS concentrations.

Hamster splenocytes at $1x10^5$ (a), $1x10^6$ (b) or $1x10^7$ (c) cells/mL were cultured in 24-well plates with RPMI media supplemented with 10% (white bar), 15% (black bar) or 20% (pattern bar) inactivated FBS. All conditions were then incubated at 37 ℃ and 5 % CO_2. Cells were sampled to stain with trypan blue, investigated under microscope and calculated for percentage of viable cells on days 1, 2, 3 and 4 after incubation. The data are shown as mean ± SEM. Statistically significant differences were evaluated using Kruskal Wallis test. The asterisks (*) and (**) indicate statistical significance at $p < 0.05$ and $p < 0.01$.

2. The RPMI is the most suitable medium

We further explored for the effect of different media on cell viability. Three different media including RPMI, DMEM and IMDM were selected for further optimization of cell viability using optimal 15% FBS with $1x10^7$ cells/mL cultivation. We found that the viabilities of cell cultured with RPMI media showed significantly higher than DMEM and IMDM **(Fig 2a).** The average viability of RPMI was about 70% for 4 days of cultivation **(Fig 2a)** while the average cell viability of fresh splenic cells was 75.92%. RPMI seemed to be appropriate media for hamster splenocytes.

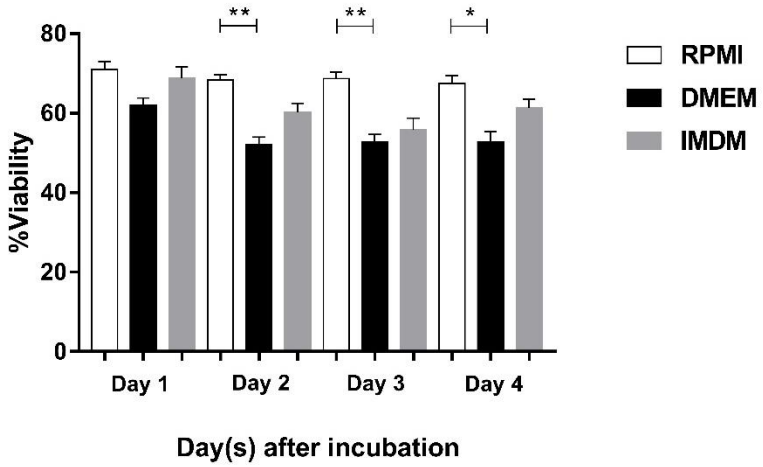

Figure 2 Viabilities of hamster splenocytes after cultivation with various media.

Hamster splenocytes at1x10^7 cells/mL were cultured in 24-well plates with either RPMI (white bar), DMEM (black bar) or IMDM (grey bar) media containing 15% inactivated FBS. All conditions were then incubated at 37 °C and 5% CO_2. Cells were sampled to stain with trypan blue, investigated under microscope and calculated for percentage of viable cells on days 1, 2, 3 and 4 after incubation. The data are shown as mean ± SEM. Statistically significant differences were evaluated using Kruskal Wallis test. The asterisks (*) and (**) indicate statistical significance at $p < 0.05$ and $p < 0.01$.

Discussion and Conclusions

Hamsters have been used increasingly in laboratory because of their susceptibilities, easily breeding, rapid development and short life cycle. However, limitations exist knowledge of their immunological background. This may have been due to the limitation of monoclonal antibody available for immune cell studies. Previous researchers have often explained hamster phenomenon based on mouse modeling although many reports had demonstrated that mouse and hamster were different in several aspects. Lymphocytes are main key important of the immunity. T and B cells are important for specific adaptive immunity against various infections [37-38]. In this study we therefore focus on hamster splenocytes. *In vitro* cultivation of splenocytes is the main investigation on mouse and hamster systemic immune cells. Our observation found that using mouse cell culture conditions, hamster splenocytes could not survive longer than 48 hours. When compared with mouse, the percentage PI of cells within spleen slices was around 20% [39]. This makes the difficulty on mononuclear cells study especially on T and B lymphocytes. Our result shown here is the first investigation to explore the optimal viability of these cells. We found for the first time that using higher number of cells made more cell survival when culture for more than 2-4 days. Keeping cells at an optimal density for continued growth are very important and will stimulate them further proliferation. Higher number of hamsters splenocytes needed for their survival might be due to the low growth rate of such cells compared to mouse cells. Maintaining of their log phase growth will maximize the number

of healthy cells. Although there was no statistically significant in viability among different number of cell culture, at $1x10^7$ cells/mL (in 24 wells) was found to be the best and we therefore suggest to use this cell number in *in vitro* culture for 3-4 days.

Trypan blue (TB) is an exclusion dye that usually used for cell viability detection. This method is simple, rapid technique and commonly used in laboratory. The dye stains cells based on membrane permeability. Intact membrane of live cells will exclude the dye whereas compromised membrane of dead cells will not. Up on entering the dead cell, trypan blue binds to intracellular protein, thereby cells appear blue color [36]. However, trypan blue staining had been previously reported in Jurkat cell line [40-41] and murine hybridoma cells [42] to change cell morphology rendering of cell rupture in dead cells or dying cells and disappear after staining. Thereby, under estimation of actual dead cells will lead to over-estimation of cell viability by trypan blue [40-42]. The use of PI may reflect the real cell viability. As our study used trypan blue as viability test, the number reported might be higher than real situation.

To use different media including RPMI, DMEM and IMDM supplemented with optimal FBS concentration and optimal cell number, our results revealed that RPMI had significantly higher cell viabilities than DMEM and IMDM media**.** According to data from manufacturer and publications, RPMI media has higher concentration of phosphate, lower concentration of calcium and medium concentration of glucose when compared to IMDM and DMEM media **[**43-46**].** Several publications discussed that the different composition of each media affects the cell proliferation and differentiation **[**44-45, 47**].** Our result in this study indicated that RPMI is still the best medium among tested for cultivation of hamster splenocytes**.** This might be due to the high nutrition or enriched components when compared to DMEM and IMDM**.** Both DMEM and IMDM are widely used basal medium for supporting the growth of many different mammalian cells. The DMEM contains low concentration of glucose (1 g/L) while IMDM is suited for rapidly proliferating, high-density cell cultures, including Jurkat, COS-7, and macrophage cells. In addition, we found in this report that 15% FBS and high percent of hamster mononuclear cells were needed to maintain high cell viability. All this information might indicate the high nutritional requirement of hamster mononuclear cells than those of mice.

In conclusion, although we showed in our study that RPMI with high cell number and 15**%** FBS were the best conditions to maintain hamster mononuclear cell viability for 3-4 days, the percent viability is still lower than those found in mouse**.** Other conditions and several supplements should be investigated to maintain hamster immune cells before those cells could be further investigated to obtain the immunological knowledge background of the hamster**.**

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References

1. Nakornpakdee Y, Sermswan RW, Maneewatchararangsri S, Wongratanacheewin S. Hamster IFN-gamma+CD4+ and IL-4+CD4+ T cell responses against leptospires are significantly higher than those of mice. Asian Pacific journal of allergy and immunology. 2018; 36(4): 265-272.
2. Melby PC, Chandrasekar B, Zhao W, Coe JE. The hamster as a model of human visceral leishmaniasis: progressive disease and impaired generation of nitric oxide in the face of a prominent Th1-like cytokine response. Journal of immunology. 2001; 166(3): 1912-1920.
3. Borji H, Khoshnegah J, Razmi G, Amini H, Shariatzadeh M. A survey on intestinal parasites of golden hamster (Mesocricetus auratus) in the northeast of Iran. Journal of parasitic diseases : official organ of the Indian Society for Parasitology. 2014; 38(3): 265-268.
4. Miller LJ, Nasar F, Schellhase CW, Norris SL, Kimmel AE, Valdez SM, et al. Zika Virus Infection in Syrian Golden Hamsters and Strain 13 Guinea Pigs. The American journal of tropical medicine and hygiene. 2018; 98(3): 864-867.
5. Li Z, Xiong C, Mo S, Tian H, Yu M, Mao T, et al. Comprehensive Transcriptome Analyses of the Fructose-Fed Syrian Golden Hamster Liver Provides Novel Insights into Lipid Metabolism. PloS one. 2016; 11(9): e0162402.
6. Pour P, Kruger FW, Althoff J, Cardesa A, Mohr U. Cancer of the pancreas induced in the Syrian golden hamster. The American journal of pathology. 1974; 76(2): 349-358.
7. Pour P, Althoff J, Kruger FW, Mohr U. A potent pancreatic carcinogen in Syrian hamsters: N-nitrosobis(2-oxopropyl)amine. Journal of the National Cancer Institute. 1977; 58(5): 1449-1453.
8. Luo D, Ni B, Zhao G, Jia Z, Zhou L, Pacal M, et al. Protection from infection with severe acute respiratory syndrome coronavirus in a Chinese hamster model by equine neutralizing F(ab')2. Viral immunology. 2007; 20(3): 495-502.
9. Ike K, Murakami T, Komatsu T, Uchida Y, Imai S. Susceptibility of Chinese hamsters (Cricetulus griseus) to the infection of Babesia microti. The Journal of veterinary medical science. 2005; 67(3): 333-336.
10. Xiangdong L, Yuanwu L, Hua Z, Liming R, Qiuyan L, Ning L. Animal models for the atherosclerosis research: a review. Protein & cell. 2011; 2(3): 189-201.

11. Martinez BD, Barato Gomez PA, Iregui Castro CA, Rosas Perez JE. DMBA-Induced Oral Carcinoma in Syrian Hamster: Increased Carcinogenic Effect by Dexamethasone Coexposition. BioMed research international. 2020; 20201470868.
12. Pan Q, Brodeur JF, Drbal K, Dave VP. Different role for mouse and human CD3delta/epsilon heterodimer in preT cell receptor (preTCR) function: human CD3delta/epsilon heterodimer restores the defective preTCR function in CD3gamma- and CD3gammadelta-deficient mice. Molecular immunology. 2006; 43(11): 1741-1750.
13. Niu K, Xu J, Cao Y, Hou Y, Shan M, Wang Y, et al. BAP31 is involved in T cell activation through TCR signal pathways. Scientific reports. 2017; 744809.
14. Montaudouin C, Boucontet L, Mailhe-Lembezat MP, Mariotti-Ferrandiz ME, Louise A, Six A, et al. Endogenous TCR recombination in TCR Tg single RAG-deficient mice uncovered by robust in vivo T cell activation and selection. PloS one. 2010; 5(4): e10238.
15. Bandaru P, Rajkumar H, Nappanveettil G. Altered or Impaired Immune Response to Hepatitis B Vaccine in WNIN/GR-Ob Rat: An Obese Rat Model with Impaired Glucose Tolerance. ISRN endocrinology. 2011; 2011980105.
16. Hegazy AN, West NR, Stubbington MJT, Wendt E, Suijker KIM, Datsi A, et al. Circulating and Tissue-Resident CD4(+) T Cells With Reactivity to Intestinal Microbiota Are Abundant in Healthy Individuals and Function Is Altered During Inflammation. Gastroenterology. 2017; 153(5): 1320-1337 e1316.
17. Reed J, Wetzel SA. CD4(+) T Cell Differentiation and Activation. Methods in molecular biology. 2018; 1803335-351.
18. Lotscher J, Marti ILAA, Kirchhammer N, Cribioli E, Giordano Attianese GMP, Trefny MP, et al. Magnesium sensing via LFA-1 regulates CD8(+) T cell effector function. Cell. 2022; 185(4): 585-602 e529.
19. Chen T, Zhang T, Liu C, Wang C, Ding S, Shao Z, et al. NK cells suppress CD8(+) T cell immunity via NKG2D in severe aplastic anemia. Cellular immunology. 2019; 3356-14.
20. de Jong R, Stokkers P, Lamme E, Kool JM, Borst F, Brouwer M, et al. Regulation of T-cell differentiation by CD2 and CD28 accessory molecules. Immunology. 1991; 74(2): 175-182.
21. Hui E, Cheung J, Zhu J, Su X, Taylor MJ, Wallweber HA, et al. T cell costimulatory receptor CD28 is a primary target for PD-1-mediated inhibition. Science. 2017; 355(6332): 1428-1433.
22. Esensten JH, Helou YA, Chopra G, Weiss A, Bluestone JA. CD28 Costimulation: From Mechanism to Therapy. Immunity. 2016; 44(5): 973-988.
23. Lee HS, Jeong GS. Chrysophanol Mitigates T Cell Activation by Regulating the Expression of CD40 Ligand in Activated T Cells. International journal of molecular sciences. 2020; 21(17).

24. Kennedy MK, Picha KS, Fanslow WC, Grabstein KH, Alderson MR, Clifford KN, et al. CD40/CD40 ligand interactions are required for T cell-dependent production of interleukin-12 by mouse macrophages. European journal of immunology. 1996; 26(2): 370-378.
25. Sharma A, Lawry SM, Klein BS, Wang X, Sherer NM, Zumwalde NA, et al. LFA-1 Ligation by High-Density ICAM-1 Is Sufficient To Activate IFN-gamma Release by Innate T Lymphocytes. Journal of immunology. 2018; 201(8): 2452-2461.
26. Wang Y, Shibuya K, Yamashita Y, Shirakawa J, Shibata K, Kai H, et al. LFA-1 decreases the antigen dose for T cell activation in vivo. International immunology. 2008; 20(9): 1119-1127.
27. Ng YY, Tay JCK, Li Z, Wang J, Zhu J, Wang S. T Cells Expressing NKG2D CAR with a DAP12 Signaling Domain Stimulate Lower Cytokine Production While Effective in Tumor Eradication. Molecular therapy : the journal of the American Society of Gene Therapy. 2021; 29(1): 75-85.
28. Peng P, Lou Y, Wang S, Wang J, Zhang Z, Du P, et al. Activated NK cells reprogram MDSCs via NKG2D-NKG2DL and IFN-gamma to modulate antitumor T-cell response after cryo-thermal therapy. Journal for immunotherapy of cancer. 2022; 10(12).
29. Bierer BE, Hahn WC. T cell adhesion, avidity regulation and signaling: a molecular analysis of CD2. Seminars in immunology. 1993; 5(4): 249-261.
30. Tchitchek N, Safronetz D, Rasmussen AL, Martens C, Virtaneva K, Porcella SF, et al. Sequencing, annotation and analysis of the Syrian hamster (Mesocricetus auratus) transcriptome. PloS one. 2014; 9(11): e112617.
31. Bronte V, Pittet MJ. The spleen in local and systemic regulation of immunity. Immunity. 2013; 39(5): 806-818.
32. Yi HJ, Lu GX. Adherent and non-adherent dendritic cells are equivalently qualified in GM-CSF, IL-4 and TNF-alpha culture system. Cellular immunology. 2012; 277(1-2): 44-48.
33. Malheiro V, Elbs-Glatz Y, Obarzanek-Fojt M, Maniura-Weber K, Bruinink A. Harvesting pre-polarized macrophages using thermo-responsive substrates. Scientific reports. 2017; 742495.
34. Gatla H, Uth N, Levinson Y, Navaei A, Sargent A, Ramaswamy S, et al. Enabling Allogeneic T Cell-Based Therapies: Scalable Stirred-Tank Bioreactor Mediated Manufacturing. Frontiers in medical technology. 2022; 4850565.
35. Amadori M, Soares-Filipe JF, Riva F, Vitali A, Ruggeri J, Lacetera N. Characterization of the blastogenic response to LPS of bovine peripheral blood mononuclear cells. PloS one. 2018; 13(10): e0204827.
36. Strober W. Trypan Blue Exclusion Test of Cell Viability. Current protocols in immunology. 2015; 111A3 B 1-A3 B 3.
37. Misumi I, Mitchell JE, Lund MM, Cullen JM, Lemon SM, Whitmire JK. T cells protect against hepatitis A virus infection and limit infection-induced liver injury. Journal of hepatology. 2021; 75(6): 1323-1334.

38. Buendia AJ, Ortega N, Caro MR, Del Rio L, Gallego MC, Sanchez J, et al. B cells are essential for moderating the inflammatory response and controlling bacterial multiplication in a mouse model of vaccination against Chlamydophila abortus infection. Infection and immunity. 2009; 77(11): 4868-4876.
39. Finetti F, Capitani N, Manganaro N, Tatangelo V, Libonati F, Panattoni G, et al. Optimization of Organotypic Cultures of Mouse Spleen for Staining and Functional Assays. Frontiers in immunology. 2020; 11471.
40. Chan LL, Kuksin D, Laverty DJ, Saldi S, Qiu J. Morphological observation and analysis using automated image cytometry for the comparison of trypan blue and fluorescence-based viability detection method. Cytotechnology. 2015; 67(3): 461-473.
41. Chan LL, Rice WL, Qiu J. Observation and quantification of the morphological effect of trypan blue rupturing dead or dying cells. PloS one. 2020; 15(1): e0227950.
42. Altman SA, Randers L, Rao G. Comparison of trypan blue dye exclusion and fluorometric assays for mammalian cell viability determinations. Biotechnology progress. 1993; 9(6): 671-674.
43. Zimmermann J, Radbruch A, Chang HD. A Ca(2+) concentration of 1.5 mM, as present in IMDM but not in RPMI, is critical for maximal response of Th cells to PMA/ionomycin. European journal of immunology. 2015; 45(4): 1270-1273.
44. Wu X, Lin M, Li Y, Zhao X, Yan F. Effects of DMEM and RPMI 1640 on the biological behavior of dog periosteum-derived cells. Cytotechnology. 2009; 59(2): 103-111.
45. Lopez-Cazaux S, Bluteau G, Magne D, Lieubeau B, Guicheux J, Alliot-Licht B. Culture medium modulates the behaviour of human dental pulp-derived cells: technical note. European cells & materials. 2006; 1135-42; discussion 42.
46. Meijer JJ, van Dijken JP. Effects of glucose supply on myeloma growth and metabolism in chemostat culture. Journal of cellular physiology. 1995; 162(2): 191-198.
47. Hou LT, Li TI, Liu CM, Liu BY, Liu CL, Mi HW. Modulation of osteogenic potential by recombinant human bone morphogenic protein-2 in human periodontal ligament cells: effect of serum, culture medium, and osteoinductive medium. Journal of periodontal research. 2007; 42(3): 244-252.