MRS Advances

Kinetics for De-Extracellular Matrix(ECM)

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ABSTRACT

A kinetic model for ECM decellularization with Trypsin and Deoxycholic Acid as reactants in a batch system has been developed. The decellularization mechanism has been analysed by solving a series of ordinary differential equations relating heterogeneous reaction kinetics at the solid/liquid interface to mass transfer of reactant and products. In order to remove the Deoxyribonucleic acid (DNA) of porcine, special emphasis has been given to the surface area for regulating the depth and amount of the penetration of the reagent, the numerical analysis thereof, and the corresponding experiment were carried out. The effect of chemical reaction order, rate constants and mass transfer coefficients on the overall decellularization rate has been analysed and discussed.

INTRODUCTION

ECM is made up of macromolecules secreted by cells, such as collagen, laminin, fibronectin and glycosaminoglycns. ECM not only provide structural support for cells but also plays a key role regulating cell's dynamic behaviour. Decellularization is a process isolating ECM to use as a scaffold and it is achieved by using chemical agents, biological agents and physical agents [1]. Typical decellularization process involves enzyme digestion with collagenase, lipase and 0.02% trypsin/0.05% ethylenediaminietetraacetic acid (EDTA), chemical treatment with alcohol, 4% sodium deoxycholate, 3% triton-X and 1% sodium dodecyl sulfate, and physical treatment including mechanical massaging and lyophilization [2]. Both 3 day- and 14 day- process of decellularization were tested to compare effectiveness by using similar chemical reagents, including 0.02% trypsin/0.05% EDTA, 3% triton-

X and PBS, but different time frame on washing steps with 14 days process being more extensive. Decellularization process underlies the effect of molecular reaction and mass transfer process. Rate process equations used on extractive metallurgy was modified for this study to find kinetic limitations involving chemical reactions and mass transfer which influence final rates of production [3]. Chemical reactions and mass transfer involved in decellularization process were divided into 5 steps. In theory, improvement on the rate and effectiveness of decellularization process can be accomplished by identifying rate-limiting steps.

In general, the reaction kinetic equation consists of reactants T, A and B and products P and D. The reaction steps can be briefly expressed by the following stoichiometric reaction, Eq. 1, where a, b, c and d are stoichiometric coefficients of the species of A, B, P and D, respectively.

$$<T> + a\{A\} + b(B) \rightarrow c\{P\} + d<D> \quad [1]$$

For the kinetics model in aqueous solution, the overall reaction process exhibits heterogeneous chemical reaction kinetics at the solid/liquid interface and mass transfer contributions from the reactants and products. The identification of the reaction mechanism is rather complicated when the time scales for the mass transfer reaction and the heterogeneous reaction are comparable. From the chemical equation, the reaction can be represented by the following 5 steps of differential equations when the decellularization is assumed to be an irreversible process. As seen from the figure 1, the diffusion of the reactant is denoted by step 1, the diffusion-in of reactant through channels from surface to cell is denoted by step 2, the chemical reaction between the cell and reactants is denoted by step 3, the diffusion-out of products through channels from cell to surface is denoted by step 4 and step 5 represents the mass transfer of the product cells [3].

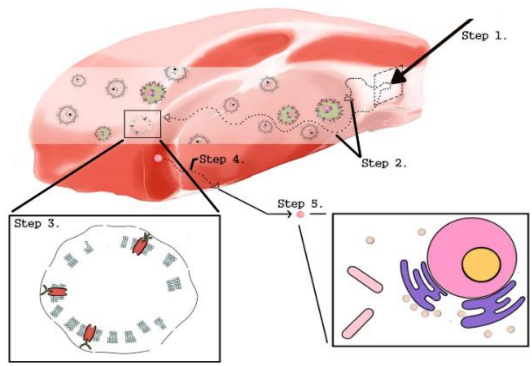

Figure 1. Schematic diagram of 5 steps of decellularization process of porcine tissue

EXPERIMENTAL PROCEDURE

Frozen porcine adipose tissue was purchased from the meat market and was kept in -20°C. The tissue was thawed at 4°C overnight before using and was manually grinded to facilitate cell lysis. To cleave protein interactions, the tissue was treated with 0.05% Trypsin/0.02% EDTA solution (Concentration of Reactants: C_{Ab}, C_{As}, C_{Ax}). Then, 3% Triton-X solution and 4% Deoxycholic acid solution was used to disrupt

lipids and lyse cells. The tissue was subjected to a 4% Ethanol and 0.1% Peracetic acid solution followed by phosphate-buffer saline (pH 7.4) and water washes. After treating with Isopropanol, which denatures cell proteins and DNA, the resulting material was rinsed in distilled deionized water [4],[5]. After decellularization process, the structure of the ECM was examined using scanning electron microscopy (SEM). Hexamethyldisilazane (HMDS) was used as a drying agent and was coated with Palladium for SEM. The SEM analysis of decellularized ECM show the structure comprised of collagen-fiber network [6]. The ordinary differential equations (ODE), Eqs. 2-6, and relating equations are almost impossible to solve with an analytical method when the surface area and diffusion limiting steps are changing. With the advent of computer software, a numerical solution can be obtained by the Runge-Kutta 4th order method using MATLAB. In this system it was assumed β=0.5.

$$\frac{dC_{As}}{dt} = nk_{m1}(C_{Ab} - C_{As}) - nk_{m2}(C_{As} - C_{Ax}) \quad [2]$$

$$\frac{dC_{Ax}}{dt} = nk_{m2}(C_{As} - C_{Ax}) - n\beta k_1 C_{Ax}^{l} \quad [3]$$

$$\frac{dC_{Px}}{dt} = nk_1 C_{Ax}^{l} - n\frac{dC_{Ps}}{dt} \quad [4]$$

$$\frac{dC_{Ps}}{dt} = nk_{m3}(C_{Px} - C_{Ps}) - nk_{m4}(C_{Ps} - C_{Pb}) \quad [5]$$

$$\frac{dC_{Pb}}{dt} = k_{m4}(C_{Ps} - C_{Pb}) \quad [6]$$

Nomenclature (reactants: Deoxycholic Acid/Trypsin, products: Intracellular contents)

C_{Ab} molar concentration(mol/L) of reactants at bulk Unit.
C_{As} molar concentration(mol/L) of reactants at tissue surface
C_{Ax} molar concentration(mol/L) of reactants at cell
C_{Pb} molar concentration(mol/L) of products at bulk
C_{Ps} molar concentration(mol/L) of products at tissue surface
C_{Px} molar concentration(mol/L) of products at cell
k_1 overall chemical-reaction coefficient(See in Table 1) of forward direction
k_{m1} overall mass transfer coefficient of reactant from bulk to tissue surface
k_{m2} overall mass transfer coefficient of reactant from tissue surface to cell
k_{m3} overall mass transfer coefficient of product from cell to tissue surface
k_{m4} overall mass transfer coefficient of product from tissue surface to bulk
β fraction(no unit) that reactants spatially occupied
l reaction orders(no unit) with respect to individual species
n fractional volume(m^2)of the interphase

DISCUSSION

Theoretical Consideration and Discussion

To solve an initial-value-problem of ODE, $C_{Ab}{}^{o}$=0.1 and $C_{As}{}^{o} = C_{Ax}{}^{o} = C_{Px}{}^{o} = C_{Ps}{}^{o}$ $C_{Pb}{}^{o}$ =0 mol/dm^3 were assumed as the initial values. C_{As}, C_{Ax} C_{Px} and C_{Ps} were calculated as a function of time for chemical reaction order, l= 0.5. To simulate this model, four different conditions are tested (values and units are given in Table 1). The choice of these values was made in light of practical implication. The coefficients assigned in the kinetics model is the specific value used on diffusion of the ions in the

solution. The solid/liquid interface area and diffusion rate are simulated to be 10 times or more different depending on the presence or absence of the open channel.

Table 1. The mass transfer and heterogeneous reaction coefficients values and units

	k_1	k_{m1} & k_{m4}	k_{m2}	k_{m3}
Unit	$mol^{0.5}dm^{-3.5}s^{-1}$	$dm^{-2}s^{-1}$	$dm^{-2}s^{-1}$	$dm^{-2}s^{-1}$
a	2.5×10^{-7}	5.0×10^{-6}	1.0×10^{-6}	1.0×10^{-6}
b	2.5×10^{-7}	5.0×10^{-6}	2.5×10^{-6}	2.5×10^{-6}
c	5.0×10^{-7}	5.0×10^{-6}	5.0×10^{-6}	2.5×10^{-6}
d	5.0×10^{-7}	5.0×10^{-6}	5.0×10^{-6}	5.0×10^{-6}

With the chemical reaction on the membrane causing burst of intracellular matrix being an exception, limiting steps due to chemical agents effecting on chemical reaction control (a, b) or mixed control (c, d) can be improved for better effectiveness on the entire de-ECM process. The volume of the ECM system, the units of mass transfer and heterogeneous reaction coefficients for simulation were summarized in Table 1. The enzyme reaction was chosen to illustrate one of the advantages of this model. This enzyme process is usually known to be controlled by chemical reaction limiting at the interface of solid and liquid. The system parameters are: k_1: 2.5×10^{-7}; k_{m1}: 5.0×10^{-6}; k_{m2}: 1.0×10^{-6}; k_{m3}: 1.0×10^{-6}; k_{m4}: 5.0×10^{-6} (Units in Table 1). Fig. 2 (a) shows the simulation results for a system controlled by a chemical reaction controlled mechanism. The product concentration at cell, C_{Px}, increases with progress of time and reached 0.12 mol/dm^3. The system parameters are: k_1: 2.5×10^{-7}; k_{m1}: 5.0×10^{-6}; k_{m2}: 2.5×10^{-6}; k_{m3}: 2.5×10^{-6}; k_{m4}: 5.0×10^{-6}. Fig. 2 (b) shows the simulation results for a system controlled by a chemical controlled mechanism when the diffusion coefficient in & off in cell was increased by 2.5 times. The product concentration at cell, C_{Px}, increases with progress of time and reached 0.15 mol/dm^3.

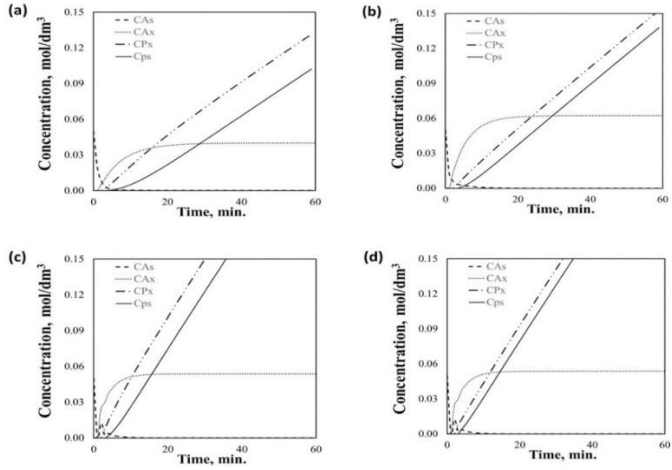

Figure 2. Simulation results and conditions used on (a~d) in Table 1. (a) and (b) for chemical reaction limiting ;(c) and (d) mixed contribution

The penetration of reactants and products thorough the small channel in porcine will be the main parameter to control the final product concentration in the bulk solution. Using the same system given in Table 1, but k_1: 5.0×10^{-7}; k_{m1}:5.0×10^{-6};

k_{m2} : 5.0×10^{-6}; k_{m3} : 2.5×10^{-6}; k_{m4}: 5.0×10^{-6} (Units in Table 1) and the same parameters, the system is assumed to be controlled by mixed control of enzyme reaction and products diffusion out from the cell to the surface. When the overall reaction is controlled by mixed contributions, it is noted that mass transfer of product from the cell to surface is more important as seen in Fig. 2 (c & d). In such cases, optimization of the decellularization process is difficult due to the effects of chemical reaction order on C_{Px} and C_{Pb}. From this simulation, the product diffusion out from the cell to the surface should be increased. In a mixed controlled reaction, neither chemical reaction nor mass transfer can be ignored since both play an important role in determining the overall reaction. Even if countless chemical reaction took place to cause cell lysis, the step involving diffusion-out through channels from cell to surface turned out to be the most critical step and DNA assay was performed in order to confirm. DNA assay was done with Invitrogen Qunanti-iT Picogreen dsDNA kit with washing steps for 24 hours or more in conjunction with controlled chemical reaction and diffusion rate efficiently removed intracellular content of cells while retaining the native composition and structure of ECM as the result shows on Fig. 3. Creating channels were the key to enhance the flow rate to diffuse out any cellular debris, chemical reagent, or any solutions used during washing steps.

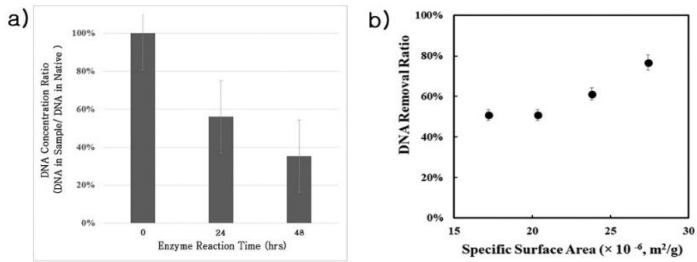

Figure 3. (a) DNA concentration ratio over enzyme reaction time showing decrease in concentration as time progresses; (b) Effect of specific surface area on the removal efficiency of DNA

Decellularized ECM was observed under SEM to prove the kinetic theory shown above. Decellularization process involves several steps involving reactant reaching the plasma membrane and product reaching out of the tissue [7]. Among these steps, mass transfer of product from the cell to surface was most important and decellularization included massive perforations on the surface of the tissue. Fig. 4 shows the SEM result of the decellularization process that was followed by the kinetic theory proven to be more effective than the one without placing holes on the surface [8],[9]

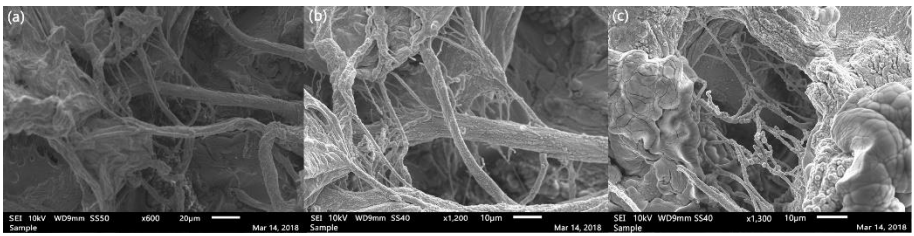

Figure 4. SEM images of decellularized ECM (a) 600X (b) 1200X, (c) 1300X. Representative images confirms the intact extracellular matrix after the decellularization process.

CONCLUSIONS

A series of experiments were performed to extract cell's intracellular contents from porcine adipose tissue in aqueous solution with Trypsin and deoxycholic Acid as reactants. Although reactant and product mass transfer in the bulk had some effect on the overall decellularization process of porcine, the decellularization process reaction was found to be mixed control; chemical reaction at the interface and product mass transfer control between cell to the surface of tissue. The kinetic theory of decellularization involve 5 steps, involving reactant reaching tissue, getting into cells, product getting out of cells and eventually getting out of the tissue. The theory shows the mass transfer of product from the cell to surface, which is step 4, is the most important step determining effectiveness of decellularization process. In order to facilitate the process on step 4, method involving mechanically placing holes on the surface of tissue was selected and was proven to support the statement that mass transfer of product from the cell to surface is indeed the most important step during the whole decellularization process.

ACKNOWLEDGMENTS

This work was supported by the Korea Institute of Machinery and Materials (No. CRC-15-03-KIMM).

DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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