bacteria leakage experiment

Protocol

1. Using syringe to take 50ml bacteria culture of Bacillus subtilis and inject it into one opening of the dialyzer for bacteria circulation (Connected with inner cavity of fiber tube). Then, using another empty syringe to block another opening and taking the air from the inner cavity. The bacteria culture can flow into the new syringe by this way. When the fluid is flowed into the new syringe, bacteria culture has been already filled into the inner cavity of the fiber tube. At the same time, keeping the both sides of syringe still blocking the openings..
2. Pouring 200ml sterilized water into the opening which connected with the simulated blood circulation. Shaking the dialyzer to make sure that the sterilized water completely contacts with the outer wall of the fiber tube.
3. Sealing two openings which connected with the simulated blood circulation by the foil
4. Stilling the device for 24h.
5. After 24h, shaking the device appropriately, then sampling liquid from the opening for bacteria circulation and the opening for blood circulation respectively. The liquid is cultured in LB solid medium with no antibiotic selection for observation.

All the operation must in the aseptic environment and all the appliance should be sterilization.

protein leakage experiment

Protocol

1. Adding 500ml aseptic M9 culture medium and inoculating the Bacillus subtilis (Bacillus subtilis was grown in LB medium overnight, bacteria was centrifugated and added into M9 medium) into the conical flask which stores the bacteria for bacteria circulation. At the same time, adding the 500ml aseptic culture medium into the conical flask storing the “blood”. Sampling from two flasks, and heating the sample for 10min. Then doing BCA protein assay to measure its protein concentration. After assembling the tube system, the device would work for 4h. All the operations must be done in the aseptic environment and all the appliances should be sterilized. Due to the inner tube of the device is sealed, the fluid can circulate in the normal environment without sterilization. Setting the flow of the simulated blood vessels at 134ml/min, the flow of the simulated blood circulation and bacteria circulation at 60ml/min.
2. After working 4h ,sampling from two flasks respectively and heating the sample for 10min. Then doing BCA protein assay to measure the protein concentration.
3. Comparing the change of protein concentration.

uric acid permeating experiment

protocol

1. 500ml LB medium with uric acid of 420μg/L is added into the blood storage conical flask and 500ml blank LB medium is added into the bacteria storage conical flask. Connect tubes, dialyzer and conical flasks, set parameters. Operations mentioned above are conducted in super clean bench and apparatus is sterilized;
2. Set the flow rate of the peristaltic pump in human body blood vessel simulation part to the same flow rate as the simulated vein. Set the flow rate the peristaltic pumps in the simulated blood extracorporeal circulation part and the bacterial circulation part to the

flow rate the experiment planed. Put the two conical flasks into magnetic heating stirrer and set the temperature to 37℃ ;

3. Take a sample from each of the two conical flasks. Run the whole device, and start timing when the medium replenishes the dialyzer, collect samples every 7 minutes(femoral vein situation) or 10 minutes(cephalic vein situation) from the two conical flasks;