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Research Article

Research on the Isolation and Biological Reaction of Nano-Wear Debris from Artificial Joint

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Abstract: When artificial joint is abrased, it produced nano-wear debris, which can not only lead to aseptic loosening, decreasing the artificial joint service life, but also brings about biotoxicity, amyotrophy and neurotrophy around artificial joint. This study introduces a process of extracting nano-wear debris from human body repaired synovial fluid and experiment synovia fluid and analyses its advantages and disadvantages, furthermore, it makes a systematic summary of the research on biological response of nano-wear debris up to date.

Keywords: Biological response, nanometer, wear debris

INTRODUCTION

Prosthesis cinch is the common complication after prosthetic replacement of joint, having a direct influence on the service life of prosthesis, which constitutes the main cause of replacement after operation (Yang and Zhang, 2009). It has been thirty years since the application of replacement of total hip; however, there hasn't been an intact research system until now (Hakon, 2011). After implanted into the human body, artificial joint would encounter a complex operation environment (Liu, 2008). As for hip joint, it not only suffers from the erosion of body fluid, but also suffers from body mass loading of 1,000,000~3,000,000 cycles and impact annually. Thus the bio-tribology of joint during operation is the main cause affecting the quality and life of replaced joint, wherein the aseptic loosening of joint caused by artificial joint wear debris mainly contributes to failure of artificial join (Lv and Sun, 2009). High Molecular Weight Polyethylene (HMWPE) may induce immune reaction *in vivo*, leading to osteolysis around prosthesis, eventually causing prosthesis cinch and replacement failure (De and Yao, 2010). Wear debris of artificial joint caused the toxicity or immune reaction, initiating bone resorption and aseptic loosening, thus leads to aseptic loosening of artificial joint, the so-called 'wear debris illness'. Because of this, about ten percent of replaced artificial joints fail, resulting in a tough problem which affects the life of replaced joint throughout the world (Cai and Jifang, 2001). It is discovered that replaced artificial joint will create numerousnano-wear debris during long-time operation (Saldana and Vilaboa, 2010). Compared with those micro-artificial joint wear debris, these nano-wear debris tend to be swallowed by larger macrophages and tend to be swallowed by smaller skeletogenous cells as well, creating more osteolysis factor *in vivo*, thus resulting in aseptic loosening of artificial joint, reducing the service life of artificial joint (Eileen and John, 2005). In addition, recent research (Tipper *et al*., 2001) showed that nano-wear debris of artificial joint can enter human systemic circulatory system and be brought to tissue around joint, causing the atrophy and pathological changes of human muscle and nerve tissue, deteriorating human health. Thus, a lot of researchers have made deep researches on the abstraction and biological response of wear debris, this thesis is a review of abstraction of nano-wear debris from human body repaired synovial fluid ,and gives a relatively systematic summary of today's biological response of nano-wear debris.

ABSTRACTION OF NANO-WEAR DEBRIS

Generation of wear particles: No matter what kind of material or fixing way is used in prothesis, jog between material and bone interface will absolutely generate wear particles, such as bone cement particles, polythene particles, metal particles(e.g., Titanium-alloy Ti-Al-V, cobalt-chromium alloy Co-Cr-Moand stainless steel), ceramic particles (Toru *et al*., 2009) etc. Aseptic loosening of artificial joint has a close relationship with the composition, quantity, shape and wear debris

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physico-chemical property of wear debris particles (Jia *et al*., 2007).

Prosthesis friction generates different wear debris and these wear debris will stimulate cells, causing a serious of biological response. Wear debris particles of different materials implanted have different physical and chemical properties, which ask for different separation methods. In terms with different frictional couples and different materials used, different materials implanted into prosthesis will generate wear debris with different biochemistry properties, shape and topography. Thus, abstraction and separation of artificial joint wear debris should adopt different methods. In summary, there are acid condition separation method, acid separation method, alkali separation method and enzyme separation method, alky condition separation method and enzymolysis condition separation method.

Abstraction of nano-wear debris particles from repaired prosthesis: Wear debris are generated during long-time wear of artificial joint. Thus, the wear debris of implanted prosthesis joint in vivo exists in synovial fluid. Firstly sample of joint synovial fluid of patient with repaired prosthesis joint is acquired, which is conserved in brown glass bottle in formalin (Houshan, 2001) (37% formaldehyde by weight, 40% formaldehyde by volume), which contains 6-13% formaldehyde and water diluted.

Acid digestion: Specific steps of abstraction of nano-wear debris of artificial joint by acid digestion are as follows (Visentin *et al*., 2004):

- **Depuration of chemicals:** distill water and filter and purify dimethyl-carbinol using polycarbonate; depurate other chemicals, such as nitric acid (Ise *et al*., 2007) and caustic potash (KOH) using polytef
- **Delipidation of samples:** extract cryodesiccated tissue sample (Holsapple *et al*., 2005) twice using mixed liquor of chloroform and methanol, then pour out the solvent, heat the tissue. sample for 2 h under 60°C; acid hydrol and lotion: the degreased tissue sample hydrolyzes in nitric acid under 24°C, obtain and further separate supernatant suspension and abandon the rest, wash the supernatant liquid by nitric acid and distilled water twice each. Then add into solvent (Xu *et al*., 2008) (nitric acid or water) and centrifuge the sample; remove the under layer solvent, neutralize the rest lotion solvent by KOH and wash sample by distilled water according to the method mentioned above, then further purify the final 4 mL solvent (Galvin *et al*., 2005); finally segregate the wear debris by filter membrance. Acid digestion has several outstanding advantages such as low cost price and is suitable for segregating artificial joint wear debris in great numbers. However, there are also disadvantages: firstly, strong acid will induce metal particles to react, nitric acid, sulphuric acid and hydrochloric acid can all cause the reaction, resulting in the change of particle's properties. Acid digestion can be used to separate ultrahigh molecular weight polyethylene. Because of its high acid effect, acid digestion may induce the reaction of metal particles and change their property, which is potential for the suspension separation of polythene and ceramic.

Alkali digestion: There are metal, ceramic and polythene wear debris in nano-wear debris of artificial joint. Because of this, the influence of strong base on its chemical properties should be taken into account. Normally ceramic, polythene and titanium-alloy won't be corroded by strong base. Strong base could be adopted to digest protein degradation solution and then separate and extract wear debris particles. Specific steps are as follows (Miroslav *et al*., 2008): firstly, cut the tissue around prosthesis into pieces and put them into a solution of chloroform and methanol with a ratio 2:1 for 12 h, then dip into dimethyl benzene, wash with distilled water. After cleaning, put the sample into centrifuge tube and add into NaOH and incubate; put the sample into centrifuge tube again and add into cane sugar (Niedzwiecki *et al*., 2001), centrifuge for 1 h; move the solution into clean tube with absorption pipette, suspend in distilled water and use ultrasound crushing, keep it heating for 30 min under 80°C; then add into dimethyl-carbinol (Kowandy *et al*., 2006) and centrifuge for 2 h; when there appears band form, particle's existing condition can be tested by E-TOXATE (Mabrey *et al*., 2002); filter particles with filter membrane. The advantage of alkali digestion is the low cost. However, its disadvantage is the selection of nano-wear debris materials, limiting its application in abstraction of ceramic, polythene and titanium-alloy wear debris. Besides, alkali digestion asks for long time and the drug adopted may have adverse effecting and may be influenced by alkali crystal when observing it's morphous after extraction of nano-wear debris, leading to more doubtful result.

Enzyme digestion: Proteinase K is used to degrade protein in biological specimen (Slouf *et al*., 2004). By virtue of this, once prosthesis necrotic tissue with artificial joint wear debris is obtained from human body, protein can be digested by proteinase K (Gonçalves *et al*., 2010). The typical enzyme digestion process is as follows (Chen and Xu, 2008): Firstly, take the sample into glass slide with nippers, then cut into pieces with slicing knife, later weigh the weight required, then add the proteinase K into original mixed

solution which is heated under 37°Cand stirred at the rate of 350rpm by magnetic stirre (Hao *et al*., 2007). Secondly, extracted solution is added into distilled water and filtered with filter membrance to acquire wear debris particles. The advantage of enzyme digestion is that it's difficult to cause particle's reaction, thus it can be applied to any compatibility of articular head and articular mortar. Besides it is easy to operate and takes shorter time, acquiring a more convinced result. While its disadvantage is its high cost to experiment and can't suffer from long-time use.

Obtain and separation of wear debris particles in vitro: Different dosages of ox blood serum are adopted in different testing machines of article wear and different experiments. Since it is impossible to take out all the testing mediums of ox blood serum, typical blood serums with the same amount should be selected from testing mediums which is mixed uniformly and freezed for conservation. Before sampling, freeze-drying can be used to reduce the loss of ox blood serum (Niedzwiecki *et al*., 2001), promising a decreased blood serum amount. Then according to different materials used, the above acid, alkali or enzyme method should be adopted to separate particles.

BIOLOGICAL RESPONSE CAUSED BY NANO-WEAR DEBRIS

Specific onset process of aseptic loosening of artificial joint(Niki *et al*., 2003) has not been explained clearly until now, but normally it is said to be caused by various factors such as wear debris, jog, stress block and high liquid pressure. In particular, wear debris of implanted materials will induce activating reaction of cells, formation of foreign body reaction membrance of prosthesis-bone interface and cell factor release, etc. These biological responses are considered to have a close relationship with osteolysis and aseptic loosening around prosthesis during THA (total hip arthroplasty). The osteolysis degree is relevant to the activation of macrophage in interface and release of osteolytic cell factor (Hofbauer *et al*., 2000). Most scholars hold the view that there are 2 kinds of biology mechanism in osteolysis induced by wear debris (Zhao *et al*., 2004):

- Wear debris induce cells in foreign body reaction limiting membrane to release various kinds of cell factors, reinforcing the activity of osteoclast, thus activated osteoclast will accomplish bone resorption.
- Wear debris partially strengthen the cell infiltration in tissue, thus infiltrative mononuclear cell can play a role of mother nuclide of osteoclast.

Biological response caused by wear debris with different particle sizes: Wear debris with different particle sizes cause different biological response. It is discovered that nano-wear debris are not only easier to be swallowed by larger macrophage, but also easier to be swallowed by smaller osteoblast, generating more osteolysis factors in human body, thus resulting in aseptic loosening of artificial joint and reducing service life of artificial joint. Horowitz's (Horwitz *et al*.,1993) research on bone cement-bone interface tissue around cinched prosthesis and tissue cultured in vitro revealed that macrophage can only swallow small bone cement particles with sizes of 1~12 μm, but can not swallow those of 20~30 μm. Macrophage and collage no blast adheres to the surface of these large particles, forming so-called kystis structure in the soft tissue surrounding prosthesis. Meanwhile, Howie *et al*. (1993) also revealed that wear debris less than 5 μm can be swallowed by macrophage, while particles of 15μm which is larger than macrophage tend to induce the circumvolution reaction of foreign body giant cells.

Roualdes *et al*. (2010) worked on the generation process of aluminium- zirconium alloy particle mixture and aluminium-zirconium nano-particles and revealed that particles have no harmful effect on biosystem both in vivo and in vitro. Besides they detected the influence of these particles on osteoblast and fibroblast, by testing human IN-form collagen protein and human fibrin, there was no evident change. However it requires a long time for us to experiment to confirm the effect of cell reaction induced by the long-time aggregation of nano-particles in cells. In particular, testing on inflammation of cells and aseptic loosening caused by different types of nano-particles in vivo are included.

Former researches have proved that nano-particles have less effect on osteoblast compared to microparticles, which makes it possible to observe how nano-particles lower the function of macrophage directly and completely. There are more macrophages in synovial fluid area and in addition, macrophage plays an important role in osteolysis. It is known that decreased reaction of macrophage contributes to decreased osteolysis and bone cinch, thus discussion (Dominique and Arlette, 2004) is needed to find out whether nano-particles of different shapes and sizes will have different effects on osteolysis and bone cinch in different inflammatory medium surroundings. Particles of different crystalline states have different surface roughness, resulting in different densities of macrophages, eventually resulting in different reactions of macrophages. Recent researches (Cai *et al*., 2005) revealed that changing surface roughness and crystalline state of aluminiumnano-particles can inhibit the activity of macrophages, furthermore inhibiting biological response of macrophages.

Tumor necrosis factor-a (TNF-a) is a major inflammatory medium released by inflammatory cells. It can stimulate inflammatory cells to excrete il-1 (interleukin-1) and il-6 (interleukin-6), both of which can induce the chemotaxis of macrophages and

osteoclasts, aggregating towards limiting membranes. Particles of various kinds of artificial joint materials may cause same histology reaction, promoting release of inflammatory medium TNF -α in tissue, further contributing to osteolysis, which is one important reason for artificial joint cinch. Observation of Zhao *et al*. (2002) revealed that TNF-a can encourage theexpress of Osteoprotegerin (OPG) of osteoblast, which further encourages mature differentiation of osteoclast (Yuan and Cheng, 2009) and enhances its function activity, resulting in prosthesis (Monika *et al*., 2009) cinch. Both osteoblast and marrow stroma cell in bone tissue can excrete OPG (Miroslav *et al*., 2008).

Co-Cr particles (Holmes *et al*., 2005) are of cytotoxicity to osteoblast and can stimulate osteoblast to release receptor or reactivator (RANKL) of NF-KB ligand, OPG and promote the increase of RANKL/OPG ratio, thus inhibiting the activity of osteoblast, encouraging differentiation and mature of osteoclast, decreasing formation of oseoblast and increasing absorption of osteoclast, leading to aseptic loosening of prosthesis.

Biological response caused by wear debris with different types: As three major implanted particles, Methyl Methacrylate (MMA), polythene and metal particles can cause aseptic loosening of prosthesis (John *et al*., 2004). However, there are many factors causing osteolysis. Darowish (Michael and Ra'Kerry, 2009) found the existence of Polymethacrylic Acid (PMAA) in osteolysis, which has no direct relationship with osteolysis. Human bone marrow cell mode and different immune reactions of polythene and titanium particles in vitro are observing (Heinrich *et al*., 2003). Polythene particles are mainly surrounded by cells but seldom are swallowed, while metal particles of small sizes are swallowed by cells. Thus it is easy to observe that metal particles are swallowed by cells, the result has been proved by many scholars (Ping and Wan-Chun, 2009). Experiments revealed (Naoya and Joscelyn, 2009) those metal particles, such as titanium alloy can limit the activity of osteoblast. Titanium particles can stimulate peripheral blood in vivo cells of human body to release TNF-a, which has a relationship with osteolysispathogenesy. Titanium particles can stimulate bone resorption and cause the differentiation of osteoclast and have an influence on activity and existence of osteoclast. Other researches also revealed that Ti-particles may stimulate PGE2, which constitutes the reason for osteolysis. After analyzing wear debris extracted from limiting membrane around prosthesis, the major wear debris that causes osteolysis is UHMWPE (Horikoshi *et al*., 1994). Ninety two percent of UHMWPE wear debris is less than 1μm and smaller wear debris would induce greater activity of macrophages. Most scholars believe that swallowed wear debris M<1μm in interface tissue is the key or essential factor of being activated (Blaine *et al*., 1997). But other researches show that large wear debris which cannot be swallowed can also be activated and release osteolysis factor such as IL- 1β and TNF. If pretreated with cytochalasin B, it would decrease swallowing by nearly 95%, but not decrease the release of TNF or IL-6. Thus swallowing is not essential condition of activation (Nakashima *et al*., 1999).

After activated, macrophages firstly release tumor necrosis factor α, then stimulate osteoblast to release macrophages colony, stimulating factor, interleukin-6, prostaglandin E2. The tendency and differentiation of these factors can further induce the propagation and differentiation of macrophage, osteoclast and fibroblastic, synthesize and excrete more cell factors and kinds of protease. Tumor necrosis factor α is the major factor causing osteolysis around prosthesis (Boyce *et al*., 2005). Even though wear particles mainly cause aseptic loosening which is characterized by osteolysis around prosthesis, but meantime, there might also be protection reaction in organism during this process. Vermes *et al*. (2001) discovered that cells can swallow titanimun-particles and obviously inhibited precollagen α1 (I), at the meantime, they stimulate osteoblast to excrete a small amount of transforming growth factor-β1 and the latter can evidently increase precollagen α1 (I). It means that the changed function of osteoblast can be compensated by growth factor-β_1. Murakami *et al*. (1998) found that transforming growth factor-β1 can increase OPG and decrease OPG-L.

Contrast of influences on cells between nano-particles and microparticles: Compared with microparticles, nano-particles will generate mor free radicals around non-cellular tissue, damaging more DNAs while generating more cytotoxicity at the same condition. Compared with microparticles, nano-particles will split more cells under electron-dense sedimentum of cells. Researches on microparticles and nano-particles revealed that their damaging function to cells are different, thus it is an attracting part to research nano-wear debris in the field of implanted materials.

Papageorgiou *et al*. (2007) compared the influences of nano-particles and microparticles on genetic toxin with two factors and drew on conclusions. The first factor is testing changes of single strand and double strand of DNA by controlling alkalinity. It turned out that the testing result of damaging to DNA under high dose of particle concentration and after 24 h is 4 times as much as that of micro-particles (5000 μm^3/cell). The second factor is particles' influence on potential cytotoxicity. Taking cytochalasin B as recording factor after 12 h' tracing of particles' influence on cells, it turned out that nano-particles increase more cytochalasin B than microparticles under high concentrations.

Two methods can be used to test intact cyton: One is to use MTT (Mono-nuclear cell direc cytotoxicity assay (Eileen and John, 2005) to test cell's function of chondriosome, the other is to use LDH (Lactate dehydrogenase) to test ecto-synovial fluid. After 1~5 days' cultivation under the same dose (5 μm^3/cell), it can be directly seen that the decrease of MTT of nano-particles is more notable than that of microparticles. The contrast on MTT revealed that particles did not release directly in the first three days, however the ultimate release of LDH caused by nano-particles is larger than microparticles and showing evident statistical effect ($p<0.05$) . By using ELISA (Enzyme Linked-Immunosorbent Assay) to test cytokines (Thomas *et al*., 2003) IL-6, IL-10, TNF-α, TGF-β1, TGF-β2 and cell increase factor FGF-23, it is found that there was evident increase of IL-6 and TNF-a compared with control groups while IL-10 and FGF-23 showed no increase. In other cytokines, nano-particles and microparticles showed a decrease of TGF-β1while TGF-β2 increasing. After 3 h' dyeing there was no increase of 8-OH-dG caused by nano-particles. Besides, after 24 h' dyeing (Liang and Godley, 2003), nano-particles usually decreased the amount of 8-OH-dG compared with microparticles while microparticles increase 8-OH-dG after 3 h and decreased after 24 h. It is concluded that the influence of particles on the amount of 8-OH-dG is relevant to the concentration of cells and particles.

CONCLUSION

Biological response caused by wear debris may lead to osteolysis and cinch, which account for two important reasons for the implantation failure of artificial joint. Even though it has witnessed limited success in researches, there are still many problems to be studied and solved. Nano-wear debris are mainly obtained from joint emulator for separation, while it is harder to extract wear debris from human body repaired tissue directly. Because of its light weight and hard to centrifuge and separate, it is particularly difficult to extract High Molecular Weight Polyethylene (HMWPE) nano-wear debris. Researches on extraction of nano-wear debris in vivo and in vitro and its biological response will be continued to find an easier way of operation and to acquire overall and precise information of wear debris, thus providing info to research its formation mechanism and develop new artificial joint material.

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