The surface contour of articular cartilage in an intact, loaded joint

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ABSTRACT

The friction coefficients measured in diarthrodial joints are small. Theories of joint lubrication attribute this efficiency to entrapment or movement of synovial fluid, yet anatomical models of the surface are based on studies of isolated fragments of cartilage, not functional joints. To investigate the functional interrelationship of joint surfaces and synovial fluid, the ultrastructure of loaded joints was examined. Twenty-four New Zealand white rabbit knee joints were loaded either statically or moved ex vivo using simulated muscle forces and then plunge-frozen under load. After fixation in the frozen/loaded state by freeze-substitution fixation, the medial joint compartments were embedded in epoxy resin while still articulated. Bone was trimmed away from the articular surfaces, permitting the cartilage to be sectioned for light and electron microscopy. These joint surfaces were then compared with controls which were not loaded, not moved or had been disarticulated prior to embedding. Articular surfaces of loaded joints were smooth at magnifications from $\times 35$ to $\times 7500$, whereas the tibial surfaces of nonloaded joints were irregular. Small pools of joint fluid were observed at the meniscal edge and beneath the anterior horn of the meniscus. At magnifications of $\times 40000$, the joint surfaces were separated by a uniform 100 nm space containing fluid. An amorphous, electron dense articular surface lamina was present but, when loaded, was thicker and flatter than previously reported. No surface pits or bumps were visible in embedded, loaded joints. This is the first ultrastructural study of intact loaded joints. The findings suggest that fluid film lubrication is present in diarthrodial joints, but the fluid sequestration postulated in several models is not apparent.

Key words: Joints; articular cartilage.

INTRODUCTION

Normal human joints survive decades of heavy use, probably because they do not wear significantly. The coefficient of friction in joints has been reported to be as low as 0.002, a level 1 to 2 orders of magnitude better than artificial joint replacements (Linn, 1968; Unsworth et al. 1975). These remarkably low values have inspired diverse theories of joint lubrication, but the mechanism of lubrication is unknown at this time. In general, joint lubrication theories have been based on or supported by observations of specific anatomical features of the articular surface. For example, transmission electron microscopy (TEM) has revealed the consistent presence of a thin, negatively charged surface layer (Stanescu & Leibovitch, 1982; Laver–Rudich & Silbermann, 1985; Stanescu, 1985). This observation is evidence that joint lubrication may be augmented by specialised molecules which adhere to the articular surfaces (Davis et al. 1979; Schwarz & Hills, 1996, 1998; Williams et al. 1997).

Several types of articular surface irregularities, including pits and ridges, have been described in scanning electron microscopy (SEM) studies (Gardner & McGillivray, 1971). SEM also has been used to study the movement of joint fluid within or upon articular surfaces subjected to compressive loads (Walker et al. 1969; Kobayashi et al. 1996). These SEM observations lie at the core of theories which contend that joint lubrication involves displacement and/or entrapment of fluid between slightly incongruent articular surfaces (Walker et al. 1968). The
reliability of such microanatomical observations is questionable. Specimens prepared for SEM are prone to deformation. For example, the surface ridges were proven to be artefacts caused by curling of the cartilage as it was removed from bone (Clarke, 1972; Ghadially & Ghadially, 1976). Both fixation and drying cause specimen shrinkage (Boyle et al. 1977; Boyle & Boyle, 1980), and the surface depressions may be created or enlarged by shrinkage (Clark & Rudd, 1991; Bloebaum & Radley, 1995). TEM is less susceptible to the shrinkage artefacts inherent to SEM (Kääb et al. 1997), but TEM has other limitations. Due to concern about inadequate penetration of fixatives and the epoxy embedding resin, convention has dictated that TEM specimens must be small. Requisite dicing of the tissue into millimeter wide strips introduces mechanical trauma and usually includes removal of the cartilage from bone. Thus prepared, the specimens are too small and distorted to provide a perspective on surface contour.

Perhaps the greatest weakness of previous anatomical studies is the setting in which the cartilage was prepared. Friction and wear are products of a dynamic interaction between the articulating surfaces and any interposed fluid. Therefore, the true functional anatomy of a joint is assumed when the surfaces are subjected to loads. To date, the articular cartilage studied by SEM and TEM has been removed from the joint prior to preparation. In this circumstance, these surface interactions cannot be observed. This laboratory has developed a technique whereby intact rabbit knee joints can be loaded by simulated muscle force, plunge-frozen while under load and then prepared for microscopy using freeze-substitution fixation. With SEM, we have described the deformation of cartilage collagen fibres in loaded joints (Nötzli & Clark, 1997). In the study presented here, we applied similar methods to examine the anatomy of articular surfaces under load. To address ultrastructural questions and to minimise shrinkage artefacts, the tissue was prepared for TEM. The fixation, embedding and sectioning techniques for TEM were modified so that the articular cartilage could be left intact throughout most stages of preparation.

MATERIALS AND METHODS

Whole knee joints including the entire femur and tibia, were removed from young adult (8 mo of age and 5 kg in weight) New Zealand white rabbits immediately after killing using pentobarbital, following the guidelines of our Institutional Animal Care and Use Committee.

Loading method (Fig. 1)

Within 1 h of death, the joints were loaded and plunge-frozen using techniques previously described (Nötzli & Clark, 1997). Twenty-four joints (right-left pairs from 12 animals) were loaded for 2 min with a simulated quadriceps force of 1.5 times body weight and then frozen under load by immersion in isopentane cooled to $-165\,^\circ C$. One knee in each pair was moved through an arc of $45^\circ$ (from $45^\circ$ to $90^\circ$) as the load was applied. Motion was achieved by moving the tibia while a constant quadriceps load, in the form of a hanging weight, was applied through a cable attached to the patella. The opposite knee was not moved and held at $90^\circ$ during the loading period. All knees were loaded and held at $90^\circ$ of flexion when plunge-frozen. To examine the effects of opening the joint capsule, the medial meniscus was removed from 2 pairs of these loaded joints; 1 of each pair was moved. In the remaining specimens, the joint capsule remained intact. As controls, 4 intact joints (2 pairs) and 2 missing the medial meniscus were placed into the device and frozen without load; 1 knee in each control pair was moved. During loading, the knees were immersed in Ringer’s solution at 20°C.

The plunge-frozen joints were fixed by freeze-substitution using successive solutions of 10% acrolein and 5% glutaraldehyde in acetone and methanol (Nötzli & Clark, 1997). All were then brought to room temperature over 24 h and transferred into absolute ethanol.
Fig. 2. Steps in preparation of specimens once loaded, frozen, fixed and substituted into ethanol. (a) The whole knee is divided into its medial and lateral compartments by a sagittal saw cut (cut i). (b) The size of the specimens is further reduced by cutting the femur and tibia without section of the collateral or cruciate ligaments (cut ii). (c) For TEM, the specimens are embedded in low-viscosity epoxy resin. (d) The embedded specimens are then sawn into halves for TEM, in the coronal plane (cut iii). (e) This sequence yields faces which include the apposed entire joint surface. To facilitate sectioning by a microtome, bone (b) is trimmed away from the embedded specimens, so that sections can be cut by an ultramicrotome without exposure to decalcification solutions. (f) Faced epoxy block showing relationship of the femur (f) and tibia (t). (g) Section cut from the medial compartment showing the articular cartilage of the femur (f) and tibia (t), subchondral bone (b) and the meniscus (m). These sections (and those shown in Fig. 3) wrinkle due to their size.

Preparation (Fig. 2)

Thirteen of the freeze-fixed joints (6 pairs, including 1 pair nonloaded control, 1 pair unloaded, medial meniscus removed and 1 loaded medial meniscus removed knee) were divided into medial and lateral compartments, using a scroll saw (Hegner Multimax 2, Hegner, Germany) with a thin kerf (1 mm) saw blade, leaving cruciate and collateral ligaments in place. These specimens, which were approximately 7 mm long on each side, remained articulated because the surrounding ligaments and capsule remained intact. Another 9 knees (4 pairs, including 1 nonloaded control pair and a loaded knee from which the meniscus was removed) were disarticulated following fixation. The femoral and tibial condyles were harvested, but not touched or exposed to air.

All freeze-fixed specimens were postfixed in a 1% solution of osmium tetroxide dissolved in ethanol at −80 °C. To facilitate penetration of the osmium into the tissue, the specimens were cooled and placed into the cold osmium solution for 48 h prior to elevation to room temperature.

Each specimen was then transferred into propylene oxide and embedded under vacuum in Spurr’s or ultra-low viscosity epoxy resin (Electron Microscopy Sciences, Fort Washington, PA). To allow penetration of the resin into these large specimens, the resin was not heated until it had begun to harden spontaneously, i.e. at 3 d for the ultra-low mixture and 11 d for the Spurr’s mixture. Once embedded, the specimens were bisected with the thin saw, creating coronal cuts through each joint surface (Fig. 2). The femoral and tibial bone was removed with a high-speed burr, leaving only the articular cartilage and a thin rim of subchondral bone. Each trimmed block was then sectioned using an ultramicrotome with glass knives to produce semithin sections which were stained with toluidine blue and studied by light microscopy (LM). Remaining bone was removed and thin sections were cut and contrasted with lead citrate/uranyl acetate for transmission electron microscopy (TEM). The thin
### Table. Number of joints prepared by each regimen

<table>
<thead>
<tr>
<th>Loading and fixation conditions</th>
<th>Joint integrity when fixed</th>
<th>Moved while loaded (one of each pair)</th>
<th>Preparation for microscopy</th>
<th>Condition when embedded for TEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frozen under load and fixed by freeze-substitution: 24</td>
<td>Intact when loaded/frozen: 20</td>
<td>10</td>
<td>Embedded for TEM: 14</td>
<td>Articulated, 8 knees</td>
</tr>
<tr>
<td></td>
<td>Meniscus removed before loading (capsule open): 4</td>
<td>2</td>
<td>Prepared for SEM: 6</td>
<td>Disarticulated, 6 knees</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Embedded for TEM: 2</td>
<td>Articulated, 1 knee</td>
</tr>
<tr>
<td>Frozen with no load and fixed by freeze-substitution: 6</td>
<td>Intact when frozen: 4</td>
<td>2</td>
<td>Prepared for SEM: 2</td>
<td>Disarticulated, 2 knees</td>
</tr>
<tr>
<td></td>
<td>Meniscus removed before freezing (capsule open): 2</td>
<td>1</td>
<td>Embedded for TEM: 2</td>
<td>Articulated, 2 knees</td>
</tr>
<tr>
<td>Fixed conventionally with no load: 4</td>
<td>Intact when immersed in fixative: 4</td>
<td>0</td>
<td>Prepared for SEM: 1</td>
<td>All disarticulated</td>
</tr>
</tbody>
</table>

Fig. 3. Light micrographs showing relationship between tibia (t), femur (f), and meniscus (m) under different circumstances. (a) Medial compartment fixed without load. Surfaces are separated by a space containing precipitated fluid. The tibial surface is irregular and does not match that of the femur. A fissure (arrow) is visible. Bar, 1 mm. (b) Medial compartment fixed under load. The meniscus, tibia and femur are in contact and thus conform. Bar, 1mm. (c) Lateral compartment fixed under load. The surfaces have separated during preparation, but still conform to one another in shape. Together, tibia and meniscus form a socket which matches the curvature of the femoral condyle. Bar, 0.5 mm. (d) Detail of medial compartment fixed under load. The joint line appears as a thin, flat line. The superficial cells in the articular cartilage are flat, and the meniscus is relatively acellular. Bar, 50 µm.

sections were viewed in a JEOL JEM 1200EXII TEM (JEOL, Tokyo).

The other 4 pairs (3 intact, 1 medial meniscus removed) of loaded knees and 1 nonloaded medial meniscus removed knee were cryofractured (Clark, 1985) apart while in ethanol, and the femoral condyles, lateral tibial plateaus and menisci were coated and examined by SEM after drying with t-
butyl alcohol (Inoue & Osatake, 1988). Each specimen was mounted, fractured surface up, and viewed in a JEOL JSM35C SEM.

Four intact nonloaded knees were fixed at room temperature with aqueous cacodylate-buffered 2% glutaraldehyde following a standard technique (Clark, 1985) and then disarticulated. The tibial and femoral condyles were harvested whole, postfixed with 1% buffered osmium tetroxide, embedded in resin and then sectioned for TEM after bone was trimmed away.

The preparation sequences used here permitted the following comparisons (Table): (1) loaded, intact and nonloaded intact joints; (2) intact joints and opened (meniscectomy) joints; (3) freeze-fixation and conventional fixation; (4) SEM and TEM; (5) articular surfaces of articulated joints and articular surfaces of disarticulated joints.

RESULTS

General

The medial compartments of the intact frozen joints remained tightly articulated during the embedding process for TEM and LM (Fig. 3). The entire posterior cruciate ligament remained attached to the tibial and femoral condyles and, along with the medial collateral ligament, stabilised the medial compartment. Because the anterior cruciate ligament was severed by the saw cut, the lateral compartments were not stable and motion or separation between femur and tibia were evident when the compartments were trimmed prior

Fig. 4. The appearance of joint fluid in loaded specimens. (a) By LM of sections stained with toluidine blue, collections of fluid are dark blue are visible here in proximity to the meniscal edge (arrows). Bar, 30 µm. (b) By TEM, the fluid deposits (arrow) are relatively electron dense and, as seen here at the meniscal edge (M), do not entirely fill the space between articular surfaces. Bar, 1 µm. (c) By SEM, the precipitated fluid (fl) is granular and identical to that described in other SEM studies. The collections of fluid seen here are located on the tibial plateau adjacent to the meniscus. Bar, 10 µm.
Fig. 5. TEM appearance of the loaded tibiofemoral articulation. (a) At lower magnification, the interface between tibia and femur appears as a thin, electron-dense line. The surface contour is not influenced by the underlying cell. Bar, 1 μm. (b) At magnifications greater than ×7500, the darkly-stained layer covering all articular surfaces is visible. The surfaces are slightly irregular, and typically are separated by a space 100 nm wide. Bar, 1 μm. (c) As this isolated tibial surface shows, a dark-staining layer covers each joint surface regardless of fixation technique or loading circumstance. Bar, 500 nm.
to epoxy embedding (Fig. 3c). Therefore, the LM and TEM observations on articulated, loaded and non-loaded joints were made from the medial compartments of frozen/freeze-fixed knees.

Due to the trauma of the fracturing process, the articular surfaces always separated in specimens prepared for SEM. When the SEM specimens were still held together by ligaments, matching areas of contact between tibia, femur and meniscus could be identified and compared with precision.

**Joint fluid**

Joints which were intact when fixed contained material identified as precipitated joint fluid solutes. The material occupied naturally occurring interstices in the intact joints, for example, along the meniscal edge, in fissures and under the posterior horn of the meniscus (Fig. 4). On LM preparations it stained with toluidine blue. The appearance of joint fluid in SEM preparations has been described by Walker et al. (1969) and material in these specimens displayed a similar granular texture. The TEM appearance of synovial fluid has not been definitively described, and was determined here by side-to-side comparison of TEM and SEM micrographs. In our TEM preparations, the precipitate was electron dense in comparison with other structures. By TEM and SEM, the pattern of precipitation was granular (Fig. 4c, d). Fluid was sparse in the joints opened for meniscectomy prior to freeze-fixation and stained less intensely in the joints fixed by conventional methods with aqueous solutions of glutaraldehyde and osmium. The distribution of joint fluid was affected by loading.

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**Fig. 6.** High resolution TEM micrographs of the articular surface. Bars, 200 nm. (a) Loaded joint. The joint surfaces are separated by a space 100 nm wide and do not interdigitate. A thin line (arrows), presumably precipitated joint fluid, runs within this space. The articular surfaces of the tibia and femur are both darkly stained. The thickness of the darkly stained region varies. (b) Loaded joint. Collagen fibrils can be identified by their prominent banding pattern. In loaded specimens, collagen fibrils are found immediately adjacent to the articular surface (arrow) where they are partially obscured by the presence of dark-stained material. (c) Nonloaded tibia fixed by freeze-substitution. In this specimen, the articular surface (1) is darkly stained but, in contrast to loaded specimens, the darkly stained layer is separated from the collagen fibrils of the tangential zone by an amorphous layer (2). (d) Nonloaded tibial plateau, fixed by aqueous glutaraldehyde. The surface layer stains darkly. As with all nonloaded knees, the tibial plateau surface is convoluted and the collagen fibrils are not as closely packed together in comparison to loaded specimens.
Fig. 7. Pattern of joint fluid deposition under the meniscus. (a) SEM micrograph showing concentric lines of fluid (arrows) on the tibial surface. These lines run parallel to the meniscal edge. Bar, 100 µm. (b) TEM micrograph of fluid collection (arrow) between tibia (at bottom) and meniscus. These lines are associated with indentations or grooves in the articular surfaces. Bar, 1 µm.

Surface contour of loaded and nonloaded knees prepared for light microscopy and TEM

In the loaded joints, the articular surfaces of femur, tibia and meniscus were in contact over broad areas when observed by LM (Fig. 3). Small deposits of joint fluid could be detected between the articular surfaces at the meniscal edge, otherwise the joint surfaces of the tibia and femur appeared to be perfectly congruent. In these areas, the interface between the femur and tibia was smooth when viewed by LM and TEM at magnifications up to ×5000 (Fig. 5). Specifically, no surface projections or depressions were observed. In comparison with nonloaded joints, the tibial surface was indented by the meniscus.

In all control knees which had been fixed without
load, the tibia and femur were separated by a uniform gap of about 0.1 mm which contained precipitated joint fluid (Fig. 3a). Peripherally, areas of contact between meniscus and femur or tibia were present. In these nonloaded joints, the articular surface of the femoral condyle appeared smooth at low magnification, but the tibial plateau was irregular. Examination at higher magnification showed small irregularities of the tibial, meniscal and femoral surfaces, and fissures on the tibial surface. The fissures located in the tibial plateau contained precipitated joint fluid.

By TEM, the most superficial layer of the articular cartilage appeared as a thin electron-dense (black) line, 20–200 nm in thickness (Figs 5, 6). This surface layer was present on all specimens including those which were disarticulated before embedding, those which were opened before loading and those fixed by conventional methods. The structure of the surface of the articular surfaces was similar in all loaded joints. The electron dense layer appeared to lie directly on, or even intermingle with the large collagen fibrils of the underlying tangential zone (Fig. 6). These collagen fibrils were closely spaced and straight. At magnifications between ×7500 and ×40000, the loaded articular surfaces displayed minor roughness; small projections or depressions extended 10–30 nm above or below the general plane of the surface. In the nonloaded joints, the electron-dense superficial layer generally was more irregular than in loaded joints (Fig. 6c, d). The collagen fibres in the subjacent tangential zone were not straight, and appeared to be less tightly packed together. In some areas of nonloaded joints, the electron-dense surface layer appeared to be separated from the tangential zone by a space. An amorphous material, staining less densely than the immediate surface, was often visible in this space.

At high magnifications, the articular surfaces of loaded intact joints were never observed in direct contact but were instead separated by a narrow space approximately 100 nm wide (Fig. 6). This space contained what appeared to be precipitated joint fluid. The precipitated fluid formed a densely stained zone approximately 10 nm wide separated from the articular surfaces by a narrow gap. This line was continuous with larger collections of fluid such as those at the meniscal edge. An identical fluid plane was observed in areas of femur to tibia contact when the meniscus was removed, but not on the open surfaces. Instead, irregular clumps of precipitate or other contaminates were occasionally seen in these noncontacting regions.

In peripheral areas beneath the meniscus, the tibial surfaces of loaded joints were covered with small concentric ridges 50 µm apart (Fig. 7). By SEM and TEM, these ridges appeared to consist of precipitated joint fluid. They corresponded to grooves in the overlying meniscus. Shallow round depressions and bumps were present on the articular surface of the tibial plateau under the tibial side of the meniscus. These corresponded to subsurface cells. Such irregularities were not present in regions of tibia to femur contact or femur to meniscus contact, nor in specimens prepared without a meniscus.

In side-by-side comparison at magnifications less than ×2000, the surfaces of material prepared for SEM was more irregular than in that examined by TEM. This was particularly evident on the femoral aspect of the meniscus. Fissures were present in the medial tibial plateau of loaded joints; these were narrower and less deep in comparison with those in unloaded specimens. No differences in surface contour or fluid distribution were observed between loaded joints which were moved and those which were held motionless.

**Discussion**

This study presents the first ultrastructural images of intact joint surfaces under load. The findings show that the tibial and femoral surfaces in loaded rabbit knee joints are smooth, concentric and congruent in the centre of the tibial plateau. Synovial fluid entrapment, a central feature in theories of joint lubrication, was observed but not in patterns predicted by these theories. The fluid filled a gap at the meniscal edge and formed ridges beneath the meniscus. Elsewhere, the fluid formed a thin, uniform layer between articular surfaces.

The contour of the normal articular surface has been described or depicted in numerous publications. Generally, surface roughness of 2 types is illustrated in these papers. One type of roughness is relatively gross and includes the pits, humps and wrinkles detected by SEM, optical microscopy and profilometry of surface casts. Current knowledge of these larger irregularities has been thoroughly reviewed by Gardner & McGillivray (1971) and, more recently, by Bloebaum & Radley (1995). A second type of roughness includes much smaller irregularities visible by TEM at magnifications between 5000 and 60000 times (Silberberg et al. 1961; Barnett et al. 1963; Weiss et al. 1968; Bullough & Goodfellow, 1971; Stanescu & Leibovitch, 1982; Jurvelin et al. 1985; Laver-Rudich & Silbermann, 1985; Orford & Gardner, 1985). At these magnifications, the surface
usually appears rough and this roughness increases with age and/or degeneration.

Roughness can be quantified in several ways. For example, Wright & Dowson (1976) reported that the centre line average of intact cartilage varied between 1 μm and 2.75 μm, and was 5.25 μm in osteoarthritic cartilage. All such indices are difficult to apply to micrographs, because one set of irregularities is visible only at low magnification, and the other is visible only at high magnification. Except for the normal joint curvature, the surfaces of loaded joints studied here were smooth by LM and low magnification electron microscopy. The projections observed at high magnification never extended more than 0.5 μm above or below the general plane of the surface. We did not further quantify the roughness created by these irregularities (nor is this done in other TEM studies). Clearly, the tibial surface roughness in our loaded joints was less than that observed in unloaded joints or pictured in most other studies. Perhaps of greater significance, irregularities of one surface had little or no effect on the contour of the opposing surface, due to the interposed space containing fluid. Thus, even in the joints which were loaded without motion, the tibial and femoral surfaces did not interdigitate.

Fissures are a normal feature of the medial tibial plateau. They were prominent on our earlier SEM studies of loaded cartilage, in part because they probably are enlarged by the drying necessary for scanning. The appearance of fissures in the light and transmission material here is probably more representative of their true morphology. In these preparations, they contain precipitated joint fluid and are lined by a thin osmiophilic border similar to that seen on the intact surfaces. As in our previous SEM studies, the fissures are smaller in the region impressed by the femoral condyle in loaded specimens, suggesting that loading closes the fissures to an extent.

Virtually all TEM studies of articular surfaces have reported that the surface is covered by a thin electron-dense line or membrane. Some of these observed a second, deeper layer separating the surface membrane from the collagen fibrils of the tangential zone. Individual characteristics of this layer have been identified using selective enzymatic degradation and ruthenium staining (Laver-Rudich & Silbermann, 1985; Stanescu, 1985; Jurvelin et al. 1996). The composition of the deeper layer remains unclear. Both TEM and SEM papers have described the presence of fine fibrils within a subsurface layer (Weiss et al. 1968; Teshima et al. 1995). This finding conflicts with observations of an amorphous substance removed with enzymes such as chondroitinase. The electron-dense membrane present on our freeze-fixed and conventionally prepared TEM specimens seems to be analogous to the surface membrane described in other work. In our loaded freeze-fixed material it often was thicker than the surface layer depicted in other papers, but we observed no separate subsurface layer (Fig. 6).

The absence of a distinct layer between the surface membrane and the tangential zone in our loaded specimens suggests that this layer may be compressed by surface pressure, or is an artefact caused by unopposed swelling in nonloaded tissue. Morphometric studies of loaded cartilage show that the superficial layer displays relatively more volume loss than the deep layers when compressed with a probe (O'Connor et al. 1988). Load-free explants swell (Bonassar et al. 1997). Swelling in the subsurface layer probably cannot be proved or disproved by current techniques, but would explain many of the contested observations concerning surface contour. For example, Bloebaum & Radley (1980) showed that the surface pitting could be eliminated by coating the surface with an osmium ligand, in effect stiffening the osmiophilic layer there. Also, pitting and elevations are minimised by use of isosmolal fixative solutions (Clark & Rudd, 1991).

The plunge-freezing/freeze-substitution technique enables the general distribution of joint fluid to be examined. Hyaluronate and proteoglycans in the fluid are insoluble in acetone/methanol and ethanol mixtures, and the joints are left intact while prepared. Thus these solutes precipitate as the tissue water is extracted and remain in place because they are never again exposed to water during preparation. The absence of the precipitate on disarticulated and opened joint surfaces confirms that the material identified as joint fluid is not a byproduct of the fixation process alone. Because only the precipitated solute is visible by microscopy, the exact distribution of the joint fluid water is not apparent. This may explain the observation that the electron-dense joint surfaces are separated from the interposed joint fluid by a clear space on high-resolution TEM micrographs (Fig. 6 a, b).

This study supports the theory that diarthodial joints utilise fluid film lubrication, because the surfaces were always separated by a space containing fluid. No pools of fluid suggesting ‘boosted’ or ‘hydrodynamic’ mechanisms were seen. The thin lines of fluid found beneath the meniscus were the only evidence of joint fluid sequestration in the loaded joints. There is no obvious explanation for the linear arrays of joint fluid beneath the meniscus (Fig. 6). This distribution could be influenced by the longitudinal collagen fibre...
arrangement in the body of the meniscus, although we found no direct evidence of such a correlation. On the basis of computer simulation, Tepic et al. (1985) have proposed that synovial fluid in the human hip joint will flow in linear patterns. In that model, however, the fluid lines ran toward the periphery of the joint whereas the lines in our study were parallel to the tibial margin.

SEM is a powerful tool, but because of the dimensional changes inherent with tissue drying, any irregularities on the surface of cartilage must be suspect when seen by SEM in that circumstance. In this study, SEM showed pitting on both tibial and femoral joint surfaces, yet such irregularities were never seen on the embedded specimens. We therefore interpret these to be artefacts of shrinkage inherent to the drying process necessary for conventional SEM. The fluid layer in loaded joints is almost as thin as the coating used in high-resolution SEM. TEM preparation is difficult in this application, but can be done reproducibly. The freeze substitution process provided good dimensional and histological preservation. Collagen and cell membrane staining, which were problematic in an earlier study (Nötzli & Clark, 1997), were definitely improved by the use of cooled osmium postfixation. The abiding problem with the embedded tissue was wrinkling of the sections, particularly the semithin sections used for LM.

This study was limited to 2 basic loading situations. The ‘nonloaded’ situation with no muscle force is not physiological, since living joints are probably never load-free. The intact, nonloaded joints may actually be exposed to negative pressure, and it will be important to identify the load at which fluid is displaced from between the surfaces of the tibia and femur. The loading conditions were relatively mild. We estimate that the quadriceps may generate forces of 10 times body weight (Nötzli & Clark, 1997). Although the surface contour did not appear different in the joints that were moved here, differences may occur with higher loads.

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