JOINT STIFFNESS AND ‘ARTICULAR GELLING’: INHIBITION OF THE FUSION OF ARTICULAR SURFACES BY SURFACTANT

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SUMMARY

It was proposed some years ago that, in osteoarthritis, one source of joint stiffness arises from ‘articular gelling’, but, if so, why does this not occur in the normal joint? In a preliminary experiment using agar gels, it is shown how such fusion of gel surfaces can be inhibited by surface-active phospholipid (SAPL)—both synthetic and human—as quantified by the shear stress needed to cause cleavage between samples after prolonged contact. On the other hand, normal bovine articular cartilage (BAC) does not fuse to itself, but can be made to do so if rinsed with a powerful lipid solvent known to remove the outermost layer of adsorbed SAPL along with the hydrophobicity so characteristic of the normal ‘waxy’ surface it imparts. It is then shown how the inhibition of gel fusion can be restored by treating both bovine and human articular surfaces with exogenous SAPL derived from human AC and with synthetic SAPL. Samples of human articular cartilage excised from osteoarthritic hips and knees during total joint replacement showed a 55% greater tendency to fuse together than normal BAC. This was exacerbated by solvent rinsing and can be attributed to a deficiency in the outermost lining of SAPL previously studied as a load-bearing boundary lubricant capable of reducing friction and wear to the remarkably low levels observed physiologically. Hence, joint stiffness can be attributed, in part, to a deficiency in the lubricating layer of SAPL lining the normal articular surface where it can inhibit articular gelling/gel fusion, possibly imparting other desirable physiological functions. The possibility of clinical replenishment of SAPL in the osteoarthritic joint is discussed.

KEY WORDS: Articular gelling, Joint stiffness, Osteoarthritis, Synovial surfactant.

Joint stiffness can arise from several sources, and is often associated as much with structures around the joint as within the joint itself. In rheumatoid arthritis, a neurogenic component has also been identified [1], while in osteoarthritis, which is more a disease of the cartilage itself [2], the stiffness tends to be more localized [3] and is essentially mechanical in nature [4]. Focusing on cartilage, Wright [3, 4] made a most interesting observation when attributing stiffness to ‘articular gelling’, but, apparently, never pursued this phenomenon in detail.

Although the contribution of this component may not be as great as Wright originally envisaged, it emphasizes how the diarthrodial joint violates one of the basic principles of lubrication engineering. It is the golden rule, when designing moving systems, never to have two surfaces of the same material sliding against each other for fear of their ‘welding’ or binding to each other when they come into contact at start-up or shut-down as the fluid film is ‘squeezed out’. This aspect is very pertinent in the joint where the articular surface has been described in the light of more recent studies as a ‘proteoglycan-rich hydrated gel’ supported on a collagen matrix [5]. Gels such as these, whose rheology is determined largely by hydrogen bonding [5], are highly likely to fuse together, reviving the concept of ‘articular gelling’. At least, it poses the vital question of why this does not occur in the normal joint and what agent could be present to prevent gel fusion from occurring.

Problems of unwanted adhesion are usually resolved in the physical sciences by applying a very thin coating of a release (anti-stick) agent to the surface. These release agents are usually surfactants in which molecules bind to the surface by their polar ends, thus orientating their non-polar moieties outwards to impart a hydrophobic surface much less conducive to adhesion [6]. The layer may be no more than a monolayer, while, often, the same surfactant lining is also a good boundary lubricant, as witnessed in everyday life in many paper coatings [7].

It could, therefore, be particularly significant that a lining of surface-active phospholipid (SAPL) has been identified on the normal articular surface and shown to possess the capability to reduce friction to the very low levels recorded in the normal joint, and to do so under high load [8]. Moreover, this lining has been shown to be deficient in osteoarthritis [9]. As the outermost layer of boundary lubricant deposited from synovial fluid (SF), this coating of SAPL could also explain the extreme hydrophobicity of the normal articular surface [10, 11] and many other features reviewed elsewhere [12].

The question which this line of deduction poses is whether the lining of SAPL demonstrated on the normal articular surface can act as a release agent and, in particular, whether it can prevent gel fusion, including articular gels. SAPL has been shown to be a good release agent, maintaining patency in the Eustachian tube [13], but this need not apply to the aqueous environment of the articular surface. This study has been designed to address these questions by undertaking four basic experiments designed to test the concept of articular gelling as an example of gel fusion and its inhibition by SAPL by: (i) demonstrating that when
articular surfaces (human or bovine) are rinsed with a lipid solvent to remove all lipid in Zone I cartilage, those surfaces will fuse together if left in contact; (ii) demonstrating that when the lipid is left intact on normal bovine cartilage, fusion is inhibited; (iii) demonstrating that when articular surfaces from osteoarthritic hips and knees are placed in contact, there is some degree of fusion which can be eliminated by applying additional human SAPL; (iv) a very preliminary experiment is also included to demonstrate how SAPL can also inhibit the fusion of gels not specific to the joints, such as agar, which is a classical example of a structure held together by hydrogen bonding [14].

MATERIALS AND METHODS

In these studies, the degree of release is quantified as the shearing force needed to separate the surfaces after a standardized period of contact, shearing being considered more relevant to the joint than a perpendicular ‘pull’ as employed in standard adhesion/release tests. In the physical sciences, release is considered a different mechanism to lubrication, arising from overcoming fusion/binding as opposed to friction [7]; although the same agents may be used to reduce both.

Materials

Nutrient agar gel was prepared from Nutrient Agar Powder (Sigma N0394) as a solution of 23 g/l in water and allowed to set as a slab 3 mm in thickness. This slab was then cut into blocks 2 × 2 cm.

Bovine articular cartilage was obtained from the patellae of steers killed at the local abattoir and placed in saline at 0°C within 15 min of death. Strips of cartilage were excised from these bones, each measuring 2.5 cm × 1 cm × 2–3 mm. Similar strips of arthritic human cartilage were excised from discarded hips and knees replaced by the orthopaedic surgeons during routine total joint replacement. In the knees, tissue was excised from the lateral chondyles since those surfaces did not display the deep fibrillation found on the medial chondyles. In hips, care was taken to avoid any ‘worn’ surfaces and any marginal areas of hyaline cartilage were excised from the lateral chondyles since those surfaces did not display the deep fibrillation found on the medial chondyles. In hips, care was taken to avoid any ‘worn’ surfaces and any marginal areas of hyaline cartilage distinguishable as ‘opaque white tissue’ [15].

Thus, for human tissue, samples were standardized as 1 × 1 cm. Human SAPL was obtained by rinsing the articular surfaces of 12 hips and 32 knees replaced at surgery with Folch reagent (chloroform:methanol 2:1) and evaporating the eluant to dryness under N₂.

Preliminary experiment

A very simple experiment was performed in which two agar blocks, as used in culture dishes, were placed together face to face after removing any excess moisture by gently blotting those faces. The blocks were then laid horizontally on a watchglass and placed in a refrigerator at 4°C overnight after covering them with aluminium foil. The following day, they were found to be fused together and, if forced apart, the plane of cleavage did not follow the original boundary. Clearly, this was an example of gel fusion as depicted in Fig. 1.

The experiment was then repeated with the exception that 200 µl of a solution of DPPC in PG were spread across the surface (without flowing over the sides) before the agar gel surfaces were placed together. The following day, the two blocks separated very easily along their original plane of contact.

Quantification of gel fusion

In repeating the above experiment, the differences were not always as dramatic and it became clear that the degree of fusion needed to be quantified. The simplest index, and one considered most relevant clinically, was measurement of the shearing force needed to break the bonding caused by gel fusion, shearing also being the relevant mode of initiating cleavage in a stiff joint.

This was achieved by attaching each agar block to a balsa wood strip using superglue and clamping these strips—one to the moveable carriage and the other to the base plate of the machine normally employed to measure the coefficient of friction. This device is essentially a modification of the apparatus devised by Radin and co-workers [16] for measuring kinetic friction, as described in detail elsewhere [8]. The basic configuration is shown in Fig. 2. Essentially, the carriage is moved at 3 mm/min, pulled by a cord attached to a

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**Fig. 1.**—Depicting how two blocks of an aqueous gel (a), if placed in contact (b), will tend to fuse together (c) and, depending upon contact time, will require an appreciable shearing force (F) to separate them; although cleavage (d) may not occur in the original plane of contact (b). This could be relevant to adhesive wear [22]. On the other hand, cleavage can occur spontaneously, or at a much lower shearing force (f), if the surfaces are coated with a monolayer of a release agent such as human SAPL prior to contact (e).
Following day. Since the cartilage was excised from a finding consistent with the basic theory of release (Folch reagent) prior to contact, partial fusion was There was no significant di.

The following day, the two samples fell apart upon In the preliminary experiment using the agar gels, clamped together with their articular surfaces in con- 70 and 74%, indicating the universal nature of the Bovine articular surfaces repeated at concentrations of 10, 50, 100, 150 and ent of DPPC concentration when deposited from

A preliminary experiment was performed in which On two further agar blocks, we used SAPL of human surfaces were now rinsed with a powerful lipid solvent of DPPC solution over one of the

A further control was performed with PG alone. Fig. 3. It can be seen that gel fusion was inhibited by

The procedure described above was repeated by depositing the 200 μl of SAPL solution over one of the surfaces prior to contact with its counterpart at concentrations of 10, 50, 100, 150 and 200 mg DPPC/ml PG. A further control was performed with PG alone.

In a second series of runs, 200 μl of DPPC solution in hexanecethanol were ‘touched off’ onto a horizontal gel surface over which it spread spontaneously as though on water. The solvent then evaporated, leaving the DPPC as a very thin coating. This procedure was repeated at concentrations of 10, 50, 100, 150 and 200 mg/ml, all runs being performed 10 times.

Bovine articular surfaces

A preliminary experiment was performed in which two samples of normal bovine articular cartilage were clamped together with their articular surfaces in contact and placed in the refrigerator as described above. The following day, the two samples fell apart upon removing the clamp.

When this simple experiment was repeated, but both surfaces were now rinsed with a powerful lipid solvent (Folch reagent) prior to contact, partial fusion was found to have occurred upon removing the clamp the following day. Since the cartilage was excised from curved surfaces, the clamp was also needed to keep them flat, a task made easier by reducing the sample size to 1 × 1 cm. The rinsing procedure followed that described in detail elsewhere [11] for the recovery of adsorbed SAPL from articular surfaces for analysis and evaluation of lubrication capability. The weight (W) needed to keep the surfaces flat was kept constant, gel fusion being conveniently quantified as a dimensionless ratio:

\[
\text{Cleavage index} = \frac{F}{W} 
\]

where \( F \) is the shearing force needed to initiate cleavage.

In an ancillary experiment, we attempted to restore the release/anti-fusion property of the original articular surfaces by applying SAPL in two forms: either synthetic DPPC or lipid harvested from rinsings of the articular surfaces of human hips and knees, as described in detail elsewhere [11].

**Human articular cartilage**

The above experiment was next performed upon human articular surfaces excised from arthritic knees replaced at surgery as described above. Unlike normal bovine articular cartilage, there was some degree of fusion and this was again quantified as the cleavage index defined in equation (2). This experiment was repeated upon the same pairs of surfaces after rinsing with Folch reagent to remove any adsorbed SAPL. It is important to remember that these values of the cleavage index, involving the rupture of gel SAPL, will be much higher than coefficients of friction (kinetic or static) which involves sliding only.

**RESULTS**

**Agar gel**

It was found that SAPL greatly reduced the shearing force needed to cause cleavage of agar gel blocks held together overnight in the refrigerator. The results are summarized in Table I and depicted graphically in Fig. 3. It can be seen that gel fusion was inhibited by SAPL whether this agent was dispensed in PG or hexanecethanol. It can be seen that the solvent PG promotes fusion, while hexanecethanol alone is not significantly different from the untreated control. It is also noted that the shearing force is independent of DPPC concentration when deposited from hexanecethanol, but somewhat dose dependent when the solvent is PG. Otherwise, the release properties are quite similar and range from 61 to 84%.

On two further agar blocks, we used SAPL of human origin in hexanecethanol when the release factors were 70 and 74%, indicating the universal nature of the natural material as a release agent.

In the preliminary experiment using the agar gels, application of Student’s \( t \)-test to the data in Table I showed a highly significant difference (\( P < 0.01 \)) between controls and surfaces treated with DPPC. There was no significant difference between shearing forces for different concentrations of adsorbed DPPC, a finding consistent with the basic theory of release.
TABLE I

<table>
<thead>
<tr>
<th>Surface treatment</th>
<th>Maximum shearing force ((f \text{ in mN}) \pm \text{s.e.m.})</th>
<th>(F = 196 \pm 8)</th>
<th>(n)</th>
<th>Release factor*</th>
</tr>
</thead>
<tbody>
<tr>
<td>None—control</td>
<td>(f = 366 \pm 18)</td>
<td>10</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>PG alone (control)</td>
<td>76 \pm 9</td>
<td>10</td>
<td>10</td>
<td>–7%</td>
</tr>
<tr>
<td>10 mg DPPC/ml PG</td>
<td>44 \pm 7</td>
<td>10</td>
<td>10</td>
<td>78%</td>
</tr>
<tr>
<td>50 mg DPPC/ml PG</td>
<td>57 \pm 8</td>
<td>10</td>
<td>10</td>
<td>71%</td>
</tr>
<tr>
<td>100 mg DPPC/ml PG</td>
<td>56 \pm 8</td>
<td>10</td>
<td>10</td>
<td>71%</td>
</tr>
<tr>
<td>200 mg DPPC/ml PG</td>
<td>31 \pm 3</td>
<td>10</td>
<td>10</td>
<td>84%</td>
</tr>
<tr>
<td>Hexane:ethanol control</td>
<td>207 \pm 9</td>
<td>10</td>
<td>–1%</td>
<td>–</td>
</tr>
<tr>
<td>10 mg DPPC/ml HexEth</td>
<td>77 \pm 6</td>
<td>10</td>
<td>10</td>
<td>61%</td>
</tr>
<tr>
<td>50 mg DPPC/ml HexEth</td>
<td>86 \pm 1</td>
<td>10</td>
<td>10</td>
<td>56%</td>
</tr>
<tr>
<td>100 mg DPPC/ml HexEth</td>
<td>61 \pm 6</td>
<td>10</td>
<td>10</td>
<td>69%</td>
</tr>
<tr>
<td>150 mg DPPC/ml HexEth</td>
<td>71 \pm 4</td>
<td>10</td>
<td>10</td>
<td>64%</td>
</tr>
<tr>
<td>200 mg DPPC/ml HexEth</td>
<td>65 \pm 4</td>
<td>10</td>
<td>10</td>
<td>67%</td>
</tr>
</tbody>
</table>

*As defined by equation (1).

Fig. 3.—Depicting the results quoted in Table I in which it can be seen that DPPC is an effective release agent inhibiting the fusion of agar blocks whether applied to the surfaces as a solution in propylene glycol (PG) or using hexane:ethanol (9:1) as a vehicle which is allowed to evaporate. Each result is the average of 10 runs \(\pm \text{s.e.m.} \). Agents in that a monolayer should suffice [6] and any additional surfactant is superfluous.

**Bovine articular cartilage**

When the shearing force needed to initiate motion was measured as a cleavage index as defined in equation (2), the result for 10 samples was a value of 0.096 \(\pm 0.006\) (mean \(\pm \text{s.e.m.}\)). When the surfaces were rinsed with either Folch reagent \((n = 4)\) or hexane:ethanol \((n = 5)\), the force needed to produce cleavage \((F')\) went off scale, even when \(W\) was reduced from 120 to 70 N (12 to 7 kg). However, when the SAPL was replenished on the surface by depositing a human extract \((100 \mu g)\) in hexane:ethanol as described above, the shearing force was greatly reduced, corresponding to a cleavage index of 0.140 \(\pm 0.009\) \((n = 10)\). When this experiment was repeated using DPPC \((100 \mu g)\) to replenish the SAPL elutriated by solvent rinsing, the shearing force was reduced further, corresponding to a cleavage index of 0.12 \(\pm 0.017\) \((n = 5)\). These results are depicted in Fig. 4.

The results for bovine articular cartilage, as depicted in Fig. 4, show a highly significant increase in shearing force needed to effect cleavage \((P < 0.001)\) when the surface is rinsed with either solvent for SAPL. However, any difference from normal becomes insignificant when SAPL is reapplied either as a human extract or as synthetic DPPC. This must add strong support to the concept that these treatments are replenishing SAPL extracted by solvent rinsing.

**Human articular cartilage**

Human articular cartilage from osteoarthritic hips and knees tended to fuse more readily than bovine articular cartilage, as witnessed by the results given in Table I. Depicting the relative shearing forces needed to cleave two normal bovine articular surfaces ‘clamped’ together and how they can be made to fuse together (cleavage index off scale) if these surfaces are first rinsed with two powerful lipid solvents (Folch reagent and hexane:ethanol 9:1). Results are plotted as the cleavage index defined by equation (2), the clamping load \((W)\) being the same in all runs. It is also shown how the application of human or synthetic SAPL, i.e. DPPC, almost restores the original release properties. The shearing force is quantified as the cleavage index as defined in equation (2).
Control treated with 500 mm
Control treated with 500 PF
Rinsed with Folch reagent (control)

in the cleavage index by comparison with any other indicated deficiencies of up to 60\% [9].

Shearing forces are compared as the outermost lipoidal layer is responsible for the very confirmed in other studies [20]. Note how synthetic DPPC reduces selves, whether bovine (Fig. 4) or human (Fig. 5). This implies a deficiency of SAPL on arthritic human cartilage as outermost lipid coating are prone to gelling to them-

<table>
<thead>
<tr>
<th>Surface treatment</th>
<th>Shear stress (N/cm²)</th>
<th>Cleavage index [equation (2)]</th>
<th>Release factor [equation (1)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>f = 4.36 ± 0.62</td>
<td>0.141 ± 0.020</td>
<td>11</td>
</tr>
<tr>
<td>Rinsed with Folch reagent (control)</td>
<td>F = 9.60 ± 1.09</td>
<td>0.310 ± 0.035</td>
<td>12</td>
</tr>
<tr>
<td>Control treated with 500 µg human SAPL</td>
<td>f = 3.36 ± 0.37</td>
<td>0.108 ± 0.012</td>
<td>12</td>
</tr>
<tr>
<td>Control treated with 500 µg DPPC</td>
<td>f = 1.16 ± 0.38</td>
<td>0.037 ± 0.012</td>
<td>12</td>
</tr>
</tbody>
</table>

\*n refers to samples from different joints.

DISCUSSION

The simple experiment with the agar gel demonstrates the principle of gel fusion and how effectively it can be inhibited by SAPL. At the molecular level, gelling has always been associated with hydrogen bonding [14] and so its inhibition by SAPL could be related to the finding that even an adsorbed monolayer of DPPC can impose a barrier to hydrogen ions, decreasing permeability by an order of magnitude [17]. Hydrogen bonding has also been emphasized as a major factor in the structure of the very hydrophilic proteoglycan gel [5] comprising the thick surface layer of articular cartilage supported on the tough collagen matrix providing structural integrity. Hence, it is not surprising that articular surfaces rinsed free of the outermost lipid coating are prone to gelling to themselves, whether bovine (Fig. 4) or human (Fig. 5). This outermost lipid layer is responsible for the very hydrophobic nature of the normal articular surface [10, 11], and is also an excellent boundary lubricant capable of reducing wear [18] and friction to the physiological range, which has been described as ‘miraculously’ low by engineering criteria [19]. Moreover, it imparts such remarkable lubrication under high load of >130 N/cm² (>13 kg/cm²). This same release mechanism might still apply even if other mechanisms, e.g., related to synovial cells, were contributing to the mechanical component of joint stiffness.

The observation that articular surfaces from arthritic joints demonstrated a greater tendency to fuse together than normal bovine articular cartilage is compatible with Wright’s original concept [4] of ‘articular gelling’ as the mechanism responsible for stiffness in osteoarthritis. This is demonstrated by the higher shearing force, as quantified by the higher cleavage index in Fig. 5 than recorded for normal bovine articular cartilage in Fig. 4. The difference could be attributed to a deficiency of SAPL. The origin of such a deficiency is probably related to the change in the lipid profile of synovial fluid recorded soon after a non-fracture traumatic injury of the type expected to induce osteoarthritis in the abused joint [20]. Our recent studies analysing solvent rinsings of the articular surfaces of hips and knees replaced at surgery have indicated deficiencies of up to 60\% [9].
In the preliminary experiment with agar, it was found that when untreated gel surfaces fused together, the plane of subsequent cleavage did not coincide with the original plane of contact—an observation consistent with the concept of ‘adhesive wear’ in osteoarthritis [21].

It was interesting that the potentiation of gel fusion introduced by rinsing normal (bovine) articular cartilage with a lipid solvent could be largely reversed by depositing more SAPL (see Fig. 4), either as the lipid elutriated from human articular cartilage or as synthetic DPPC. This indicates that these treatments are actually replenishing the SAPL known to be elutriated by analysis of solvent rinsings [11]. The fact that replenishment reduces the shearing force for cleavage to values appreciably lower than the original value (see Fig. 5) indicates that the latter was already deficient in SAPL. This indication is also borne out by the moderately significant difference between normal bovine and normal bovine values. The observation that human SAPL also inhibited the fusion of agar gel tends to confirm that it is acting as a general release agent.

The significantly better inhibition of articular gelling/gel fusion obtained with synthetic DPPC indicates a possible clinical application of this agent in relieving joint stiffness in osteoarthritis. Many other agents have been administered to the joints as lubricants by intra-articular injection, as reviewed elsewhere [22], but most proved biologically incompatible with the human joint. This should not apply to DPPC, which is normally present in the joint, produced as lamellar bodies identified in Type B synoviocytes [23, 24] just as alveolar Type II cells produce DPPC as surfactant in the lung [25]. Current clinical trials administering exogenous SAPL (formulated in this laboratory) to osteoarthritic knees, ostensibly as a lubricant [26], have indicated a reduction in joint stiffness lasting for 2 months or more.

This study demonstrates that the adsorption of SAPL tends to prevent the fusion of adjacent gel surfaces, including articular surfaces, thus adding another highly desirable physiological function for the outermost lipoidal layer of SAPL, originally studied for its lubricating capabilities [27]. Other possible functions include chondroprotection and control of the water content of articular cartilage by means of the hydrophobicity imparted by adsorbed SAPL, hydration being a common finding in osteoarthritic cartilage [28]. In recent pulmonary studies [29], it has been shown that SAPL is an effective scavenger for oxygen free radicals which have been implicated in the death of synoviocytes [30]. Whether such speculative properties are ultimately confirmed or not, the lubricating and release properties alone would make it highly desirable to maintain the integrity of that hydrophobic outermost lining of SAPL in combating osteoarthritis.

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**References**