INTRODUCTION:
After analyzing SEM photomicrographs from a study of initial lesions of canine OA and being unable to interpret their morphology, this research group initiated an extensive literature search on the superficial zone (SZ) of articular cartilage (AC), began a histochemical and immunohistochemical dissection of the SZ matrix, chemically isolated the SZ, and initiated an ultrastructure study.

Great interest in the articular surface was shown by one person in the late sixties to the mid-seventies. In the mid-seventies, several scanning electron microscopists studied the structure. In the mid-nineties, investigators in Japan and U.S. and now in Wales have been investigating various aspects of the SZ. It appears that this global interest is warranted since the SZ may possess the key to the health, durability and possibly the regeneration of adult articular cartilage.

METHODS:
Only articular cartilage specimens collected after euthanasia were used, consequently IACUC approval was not needed. The SZ, principally from the articular cartilage of sheep, pigs and dogs was studied. Techniques used were histochemistry (Verhoff’s), with and without enzyme digestion (pancreatic elastase), immunohistochemistry (see below), polarized, light and fluorescence microscopy, SEM and TEM. Care was taken to divide the articular surface of the humeral head (shoulders in tetrapods support 60% of body weight) into weight and non-weight bearing area according to an in-vivo kinematics study correlated with histochromy. The SZ was isolated as a bipartite unit using maceration in 5% NaOH solution at 37°C. The thin superficial amorphous layer was studied microscopically and crystallized. The thicker multilayered unit was separated from the remainder of the articular cartilage and studied by polarized and fluorescence light microscopy as well as confocal microscopy. The antibodies employed in matrical immunohistochemistry were for Type II and Type III collagen, elastin, tropoelastin, microfibrillin and fibronectin counterstained with DAB or Alexa Fluor using appropriate positive and negative controls. At the SEM level, the SZ was processed through graded alcohols to absolute, critical point dried, gold sputtered, viewed in a JEOL JSM-840 and photomicrographs taken. At the TEM level, the articular cartilage was fixed in gluteraldehyde containing ruthenium red and negative controls. At the SEM level, the SZ was processed through graded alcohols to absolute, critical point dried, gold sputtered, viewed in a Philips EM400 Transmission electron microscope, and photomicrographs taken.

The results were tabulated, documented by photomicrography and correlated with data of the various techniques used as well as those in literature with a conceptual morphological model resulting.

RESULTS:
As the SZ was macerated, there was a staggered release of its components. After 2-4 hrs, a white, fluffy material was released from the surface; after 3-5 days, the underlying layer could be peeled from the rest of the articular cartilage. The surface layer was found to be amorphous, acellular and could be dried to a white powder. At the TEM level, it was ruthenium red positive and appeared attached to an underlying laminar layer through an electron dense line which was positive for fibronectin associated with a very thin line of Type II collagen. Between this line and the top of Zone II, an area negative for underlying laminar layer through an electron dense line which was positive for fibronectin associated with a very thin line of Type II collagen. Between this line and the top of Zone II, an area negative for underlying laminar layer through an electron dense line which was positive for fibronectin associated with a very thin line of Type II collagen. Between this line and the top of Zone II, an area negative for collagen, elastin, tropoelastin, microfibrillin and fibronectin counterstained with DAB or Alexa Fluor using appropriate positive and negative controls. At the SEM level, the SZ was processed through graded alcohols to absolute, critical point dried, gold sputtered, viewed in a JEOL JSM-840 and photomicrographs taken. At the TEM level, the articular cartilage was fixed in gluteraldehyde containing ruthenium red and negative controls. At the SEM level, the SZ was processed through graded alcohols to absolute, critical point dried, gold sputtered, viewed in a Philips EM400 Transmission electron microscope, and photomicrographs taken.

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DISCUSSION:
This is the first known demonstration of elastin, Type III collagen and material-filled reservoirs in the SZ known to the authors. The amorphous layer has been demonstrated by he Japanese and may have been chemically analyzed by Balaz. The laminae were defined by Wolf and beautifully demonstrated by Kobayashi, et al using CryoSEM. However, the amorphous layer may have been missed due to tissue processing and the laminae by type of technique used.

The presence of elastin in the SZ adds much to the physiology of the articular cartilage (e.g. protection of the chondrocytes, durable but elastic protective layer, possible aid in nutrition). The material-filled reservoirs may provide the morphological basis to explain boundary lubrication as well as a nutritional system for the large population of chondrocytes. It is possible that the one type of chondrocyte may be the stem cell described recently by Archer and postulated earlier, making the SZ an essential element in the maintenance and repair of articular cartilage.

All of these intriguing possibilities should offer investigators future research opportunities with the desired endpoint being delay of OA resulting in a better quality of life for the elderly.

REFERENCES: