博 士（医 学） 田 邉 芳 恵

学 位 論 文 題 名

Biological responses of novel high-toughness double network hydrogels in muscle and the subcutaneous tissues

（新しい高強度ダブルネットワーク・ハイドロゲルの筋および皮下組織内における生体反応）

学位論文内容の要旨

1. Introduction

Normal cartilage tissue is a kind of multi-functional hydrogel with high toughness. Recently, the authors developed 4 types of unique double network (DN) hydrogels as potential materials for artificial cartilage: The first gel is PAMPS/PDMAAm DN gel, which consists of poly (2-acrylamide-2-metyl-propane sulfonic acid) and poly (N,N′-dimetyl acrylamide). The second gel is PAMPS/PAAm DN gel, which consists of PAMPS and polyacrylamide. The third gel is Cellulose/PDMAAm DN gel, which is composed of bacterial cellulose and PDMAAm. The fourth gel is Cellulose/Gelatin DN gel, which consists of bacterial cellulose and gelatin. The purpose of this study is to evaluate any biological reaction of these DN gels implanted into the para-vertebral muscle and the subcutaneous tissue, using the biomaterial implantation test with the rabbit.

2. Materials and Methods

Pellet implantation test: A total of 90 pellets, rectangular parallelepiped specimens (1x1x10 mm), were created with each DN gel material. As for negative and positive controls for histological comparisons, high-density polyethylene and polyurethane containing 0.75% Zinc diethyldithiocarbamate were used, and the same shaped pellets were prepared. A total of 15 rabbits were used. Each pellet specimen was pushed into the tip of a needle. In each rabbit, 6 kinds of pellet specimens were randomly implanted in the 6 portions of the para-vertebral muscle. Five animals were sacrificed at 1, 4, and 6 weeks after implantation and the para-vertebral muscles were carefully harvested en block. Each specimen was fixed in 10% neutral buffered formalin. After fixation, the specimen was embedded in paraffin, and sliced with a microtome along the longitudinal axis of the implanted pellet. The consecutive 10 sections were stained with hematoxylin and eosin, and observed with light microscopy. To quantify the degree of inflammatory response of the muscle to the implanted materials, we took a photograph that showed a cross-section of the muscle. We measured the width of the reactive inflammatory zone against the pellet specimens. For statistical comparisons on the biodegradation in each parameter of each DN gel materials, we used the one-way analysis of variance. The significance limit was set at p=0.05.

Subcutaneous implantation test: A total of 20 rectangular parallelepiped specimens (10x10x5 mm) were prepared. Five mature rabbits were used. In each rabbit, 4 kinds of DN gel specimens were randomly implanted in the 4 subcutaneous spaces. At 6 weeks after surgery, each animal was sacrificed. Macroscopic situations of the implanted massive gel specimens in subcutaneous tissues were observed in careful dissection.

3. Results

Pellet implantation test: The implanted PAMPS/PDMAAm, PAMPS/PAAm, and Cellulose/PDMAAm DN gels induced various degrees of inflammation essentially similar to that around the positive control at 1 week. The gel was surrounded by numerous neutrophils with necrotic cells, and this zone was surrounded by fibrous tissues in which a number of macrophages including foreign body giant cells and fibroblasts with some eosinophils. At 1 week, the width of the inflammatory zone around the PAMPS/PDMAAm DN gel was significantly less than that around the positive control. On the other hand, the width around the PAMPS/PAAm and Cellulose/PDMAAm DN gels was significantly greater than that around the positive control. At 4 and 6 weeks, the width around the PAMPS/PDMAAm DN gel was the same as that of the negative control, while the width was the same around the Cellulose/PDMAAm DN gel as that of the negative control and significantly greater around the PAMPS/PAAm DN gel than that of the negative control. Specially around the PAMPS/PDMAAm gel, neutrophils disappeared and a thin fibrous capsule was formed. In contrast, around the PAMPS/PAAAm and Cellulose/PDMAAm DN gels, some neutrophils still remained with necrosis and the inflammatory zone was surrounded by thick fibrous tissue with infiltration of macrophages with some lymphocytes. The reactive inflammation around the implanted Cellulose/Gelatin DN gel was different from the area surrounding the other 3 gels. At 1 week, the inflammatory zone width around the gel was no significant difference from the negative control. Neutrophil infiltration or cell necrosis was rarely seen around the pellet, which was surrounded by a small amount of fibrous tissue with infiltration of many macrophages. At 4 and 6 weeks, the width became thicker than that observed at 1 week. In this area, foreignbody reaction that involved numerous lymphocytes, plasma cells, and macrophages including foreignbody macrophages, and some eosinophils were observed. The inflammatory zone width around the Cellulose/Gelatin DN gel was significantly less than the positive control at 1 week, while there was no significant difference from the negative control. At 4 and 6 weeks, the width became significantly greater than the negative control.

Subcutaneous implantation test: The PAMPS/PDMAAm DN gel specimens were surrounded by a thin fibrous capsule, while the PAMPS/PAAm and Cellulose/PDMAAm DN gel specimens were surrounded by a fibrous capsule thicker than that around the PAMPS/PDMAAm DN gel specimens. The Cellulose/Gelatin DN gel specimens were surrounded by the thickest fibrous capsule. When the capsule was removed, a part of the specimens became absorbed.

4. Discussion

The histological reactions around the four DN gels examined in this study were essentially similar to the foreign body reaction, although the degree of the reaction was different among the implanted materials. The PAMPS/PDMAAm DN gel induced a mild inflammation the degree of which was between the negative and positive controls only at 1 week, but showed the same degree of inflammation as the negative control at 4 and 6 weeks. This DN gel is considered to be a potential material to be applied as a clinical implant, such as artificial cartilage. Further evaluations from various viewpoints should be performed in the near future. On the other hand, the implanted PAMPS/PAAm and Cellulose/PDMAAm DN gels induced a strong inflammation at each period. These materials are difficult to be applied as clinical implants in the current situation. Around the Cellulose/Gelatin DN gel, which was composed of the two natural polymers, inflammation was very mild at 1 week, but significantly increased at 4 and 6 weeks. In addition, the surface of the gel blocks implanted into the subcutaneous tissue was obviously absorbed. This material may be applicable to an absorbable implant, such as a material for drug-delivery system.

5. Conclusions

The implantation tests demonstrated that the PAMPS/PDMAAm DN gel has a possibility to be applied as artificial cartilage in the future. The Cellulose/Gelatin DN gel has a potential to be applied as an absorbable implant. The PAMPS/PAAm and Cellulose/PDMAAm DN gels are difficult to be applied as clinical implants in the current situation.

学位論文審査の要旨

主査　教授　三浪明男
副査　教授　山本有平
副査　教授　安田和則

学位論文題名

Biological responses of novel high-toughness double network hydrogels in muscle and the subcutaneous tissues

（新しい高強度ダブルネットワーク・ハイドロゲルの筋および皮下組織内における生体反応）

天然の高強度低摩擦ゲルである関節軟骨は自己再生能に乏しく、破壊された軟骨に対する有効な治療法は開発されていない。その治療のための選択肢の一つとしての人工関節軟骨の開発は関節外科における夢の一つであるが、その開発に向けての研究は有用な材料がないためにほとんど行われていない。申請者は共同研究者らが開発した正常軟骨と同等の超低摩擦特性と高い圧縮強度を有する４種類のダブルネットワーク・ハイドロゲル（DN ゲル）の異物特性と生体内劣化特性を ISO ガイドライン（10993-6）に則って、特に人工軟骨への応用の可能性を中心に評価した。

使用した DN ゲルは PAMPS/PDMAAm ゲル、PAMPS/PAAm ゲル、Cellulose/PDMAAm ゲル、Cellulose/Gelatine ゲルである。評価として計 21 羽の家兎を用い、ペレット筋内埋植試験と皮下埋植試験を行った。ペレット筋内埋植試験では、４種類の DN ゲルと比較検討用の陰性対照および陽性対照を左右の傍脊柱筋内へ１種類ずつランダムに刺入した。埋植期間は、１、４、６週間とした。埋植物周囲の評価として、組織を HE 染色した後に、細胞浸潤の観察と炎症範囲の計測を行なった。その結果、PAMPS/PDMAAm DN ゲルは１週で周囲の狭い範囲に単球/マクロファージ系細胞の浸潤を認め、４週以降は薄い線維性被包化が見られた。炎症程度は１週では陰性対照と陽性対照の中間であり、４週以降では有意に減少し陰性対照とほぼ同等であった。PAMPS/PAAm DN ゲルは埋植後１週で周囲に非常に強い炎症細胞浸潤と横紋筋の壊死を認め、４週以降で炎症範囲が減少しこの周囲に線維性の被包化がみられた。炎症の程度は全ての時期で陽性対照に比べて有意に高値を示した。Cellulose/PDMAAm DN ゲルは１週で強い顆粒球浸潤と壊死した横紋筋と線維性の被包化が認められ、多数の多核巨細胞を含む単球/マクロファージ系細胞の浸潤が見られた。４週以降では筋の壊死と顆粒球浸潤は減少していた。炎症程度は１週で陽性対照よりも有意に高値を示し、４週以降は差を認めなかった。Cellulose/Gelatin DN ゲルは１週での細胞浸潤は軽微であったが、４週では広範囲な細胞浸潤を認めリンパ球や形質細胞の浸潤が目立った。炎症程度は１週では陰性対照との差を認めなかったが、４週以降では有意に高値を示

した。皮下埋植試験では、側背部の皮下へ４種類のゲル材料を別々に埋植した後、６週で各材料を回収した。異物反応性被膜の観察と埋植材料の圧縮破壊試験を行ない、埋植前の物性と比較した。その結果、PAMPS/PDMAAm DN ゲルは被膜形成が少なくゲル材料の変形は認めず、含水率の低下と力学的特性の有意の増加を認めた。PAMPS/PAAm DN ゲルでは、やや厚い皮膜形成とゲル全体の白濁を認めた。含水率と破断応力に変化は無く、初期弾性率が有意に低下した。Cellulose/PDMAAm DN ゲルでは厚い被膜形成と材料表面の塑造化を認めたが、含水率および力学的特性に変化は認めなかった。Cellulose/Gelatin DN ゲルでは厚い被膜形成と材料の変形を認め、含水率の増加と破断応力の有意の低下を認めた。これらの試験の結果、それぞれのゲルは含まれている高分子によって特徴的な異物特性と生体内劣化特性を有していることが示された。PAMPS/PDMAAm DN ゲルは異物反応が軽度で力学的特性の劣化を認めず、新たな人工軟骨開発の基礎的材料として期待が持てた。Cellulose/Gelatin DN ゲルは１週での異物反応が陰性対照と同等であり、４週以降で生体吸収性を示し吸収性医用材料への応用が期待された。他の２種の DN ゲルは力学的特性の劣化は認めないものの強い異物反応を示し、人工軟骨用生体材料としては不利であった。

口頭発表の後、副査の山本教授より、PAMPS/PDMAAm DN ゲルの破断強度増加の原因について、評価した４つの DN ゲル材料の含水率について、人工軟骨に最適な含水率について、および人工軟骨開発のために今後行うべき評価方法について質問があった。また主査の三浪より、この４種類の DN ゲルを人工軟骨開発の対象に選んだ理由について、他の治療法と比較した人工軟骨の利点について、および臨床応用時の DN ゲルと骨の接着方法について質問があった。最後に副査の安田教授より、筋内埋植試験における PAMPS/PDMAAm DN ゲル周囲の炎症細胞浸潤の特徴と意義について、この人工軟骨が目指す臨床応用のゴールについて質問があった。いずれに対しても申請者は、自己の研究結果と文献的考察に基づいて概ね妥当な回答を行った。

本研究は独創的な４種類の高機能 DN ゲルの異物特性と生体内劣化特性を初めて明らかにし、今後の人工軟骨の開発に重要な情報を与えた。審査員一同は、これらの成果を高く評価し、申請者が博士(医学)の学位を受けるのに十分な資格を有するものと判定した。