COMPARISON OF ACIDIC DECELLULARIZATION METHODS FOR PORCINE CORNEAS

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Abstract- Corneal diseases are one of the main causes of vision loss worldwide. Although cornea transplantation is a very useful treatment, the demand of corneas evidently exceeds the available supplement. Decellular corneal xenografts are thought to be potential resources for human corneas substitutes. In this work, we compared different acidic decellular protocols on porcine corneas. The porcine corneas were soaking in different concentrations of formic acid, acetic acid and citric acid solutions at 25℃ for 24 h on an orbital shaker. These acidic decellular porcine corneal scaffolds (dPC) were examined by scanning electronic microscope and showed uniform parallel extra cellular matrix structure and the average microstructure pore size was 220 ± 20 μm. According to DNA content, these acidic methods had great decellular efficiency. Examined by Masson trichrome staining, the collagen fibers arrangement was found similarly between control group and dPCs groups; and by Alcian blue staining, also confirmed that dPCs had an intact glycosaminoglycan preservation. These results revealed that acidic decelluarization for porcine corneas provided great microstructure and biological properties for further in vitro and in vivo tests, and suggested that acidic decellular scaffolds could have the potential for corneal tissue engineering applications.

Keywords- Tissue Engineering, Scaffold, Decellularization, Pig, Corneal Xenograft.

I. INTRODUCTION

Corneal diseases are the main causes of vision loss worldwide. Although cornea transplantation is a very useful treatment, the demand of corneas evidently exceeds the available supplement. More and more researchers are in interested in using tissue engineering concept for setting up cornea substitute. There are different biomaterials that have been studied for cornea substitutes including collagen, silk and fibrin, but all of them need to rely adding some biologic factors. Decellularization is one of the most potential methods for human cornea substitutes. This method preserve nature corneal ECM structure and original biologic proteins which are important for kerotocyte recellularization. Previous studies showed many available detergents for decellularizion, such as SDS, triton and SD, but these detergents are considerable for expensive costs and having concern about of the safety. Acidic methods are seen to be great methods for decellularization for having ability to disrupt cells and being neutralized into harmless salts. In this study, we compared different acidic decellular protocols on porcine corneas and evaluated the efficient method for decellularization.

II. MATERIALS AND PRODUCES

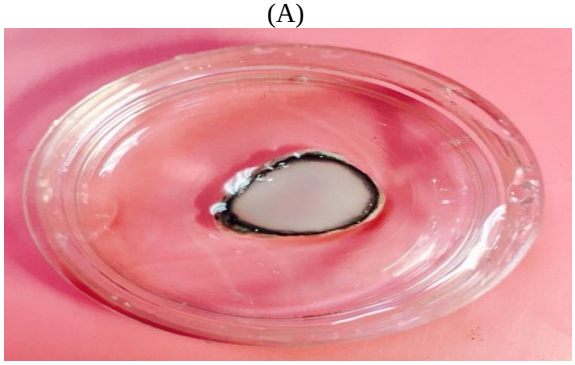
Fresh porcine corneas were obtained at a local slaughter house. After a complete washing, porcine corneas were soak in formic acid, acetic acid and citric acid solutions at 4℃ for 24 hours on an orbital shaker. After decellular process, samples were washed with PBS buffer and adjusted the pH-value to 7. The prepared formic acid, acetic acid and citric acid decellular porcine corneas (F-dPC, A-dPC and C-dPC) were sealed in plastic dishes, and stored at 4 ℃ before use.

III. RESULTS AND DISCUSSION

After the freeze-dry procedure, the acid decellular porcine corneal scaffolds showed an opaque appearance, but their transparency was recovered after treated with glycerol (Fig 1). These dPCs showed their original uniform parallel structure examined by scanning electronic microscope and the average microstructure pore size was 220 ± 20 μm (Fig 2).It is very important to maintain corneal parallel morphology, because this characteristic is relevant to the transparency. To determine the DNA content, the corneal scaffolds were verified by Quibt DNA assay. The F-dPC was showed the most efficient decellular procedure compared with the other two groups (Fig 3). Previous researches found that time is a crucial factor for decellularization. For A-dPC and C-dPC groups, it would be possible to obtain a better decellular efficiency by elongating the working time. Conserved collagen and GAGs in the

scaffolds is one of the most important factor which determines these scaffolds are useful or not in the further application. Examined by Masson trichrome staining, the collagen fibers arrangement was found similarly between native corneal scaffold group and dPCs groups (Fig 4). There was no significant difference between F-dPC, A-dPC and C-dPC in Masson trichrome staining. According to Alcian blue staining, it also confirmed that all the dPCs groups had an intact glycosaminoglycan preservation (Fig 5).

(A)



(B)

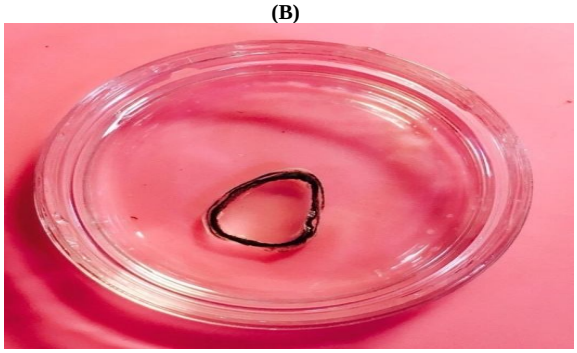
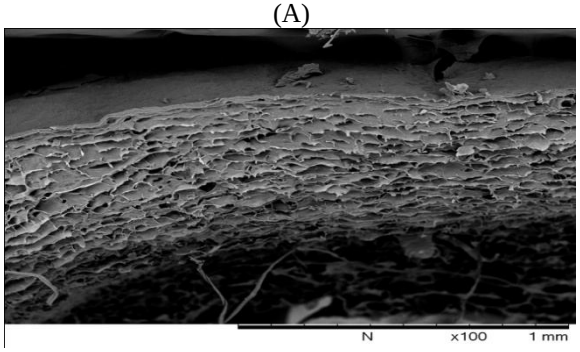
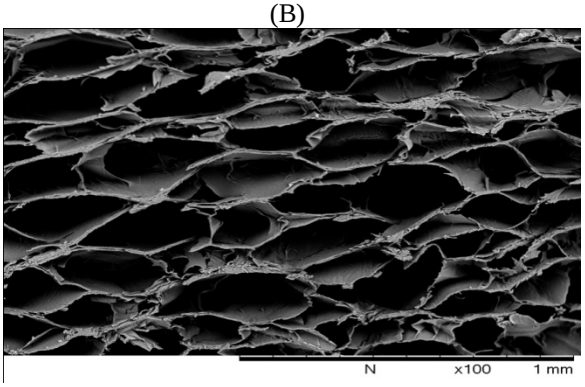


Fig.1. Decellular porcine corneal scaffolds showed (A)an opaque appearance, (B)but their transparency was recovered after treated with glycerol.

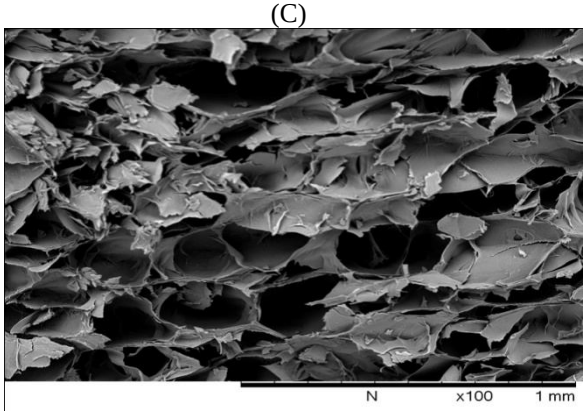
(A)



(B)



(C)



(D)

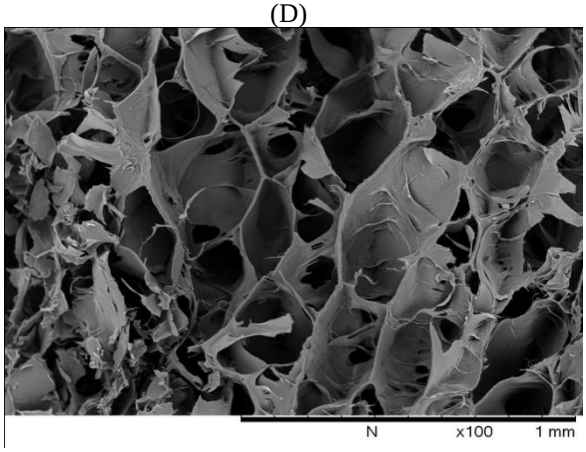


Fig.2. SEM revealed that the cross section of cornea had uniformed lamellar microstructure.
(A)Native cornea (B)F-dPC (C)A-dPC (D)C-dPC

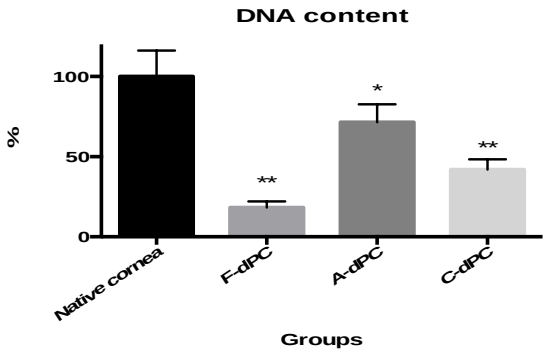
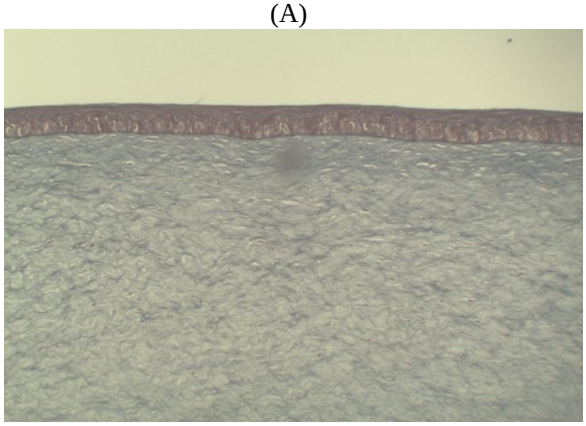
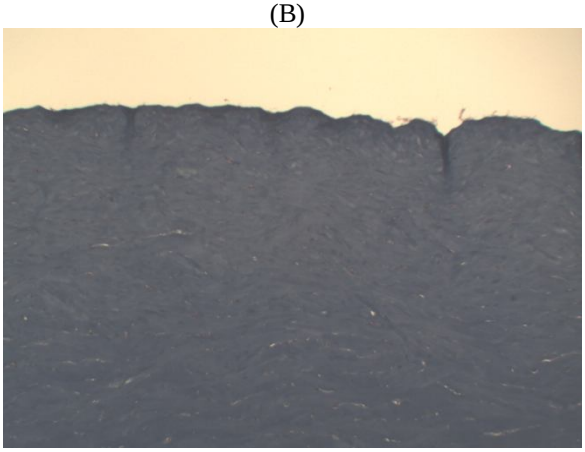


Fig.3. Qubit DNA assay evidenced the DNA was removed compared with native corneas.
p<0.01(*) p<0.001 (**)

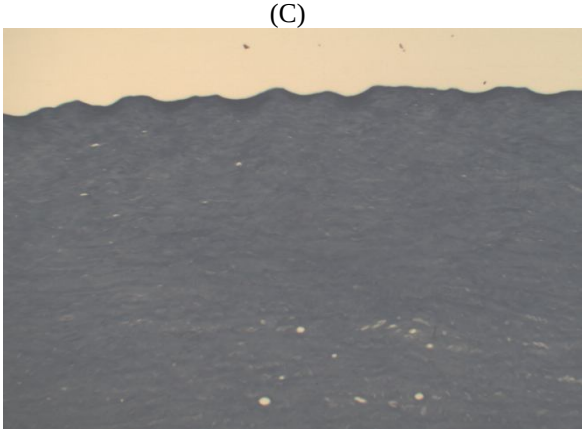
(A)



(B)



(C)



(D)

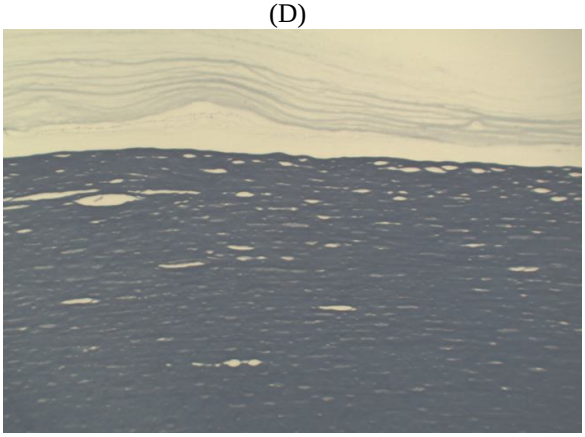
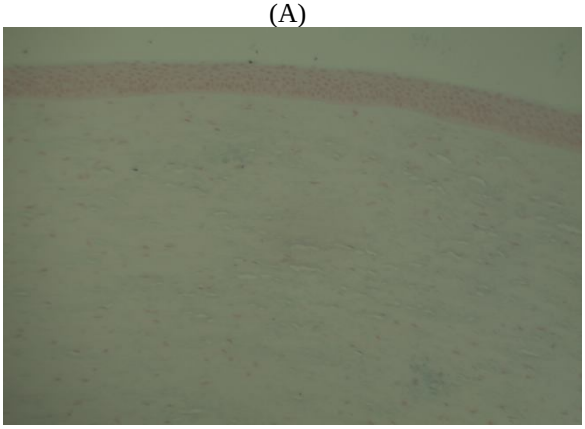
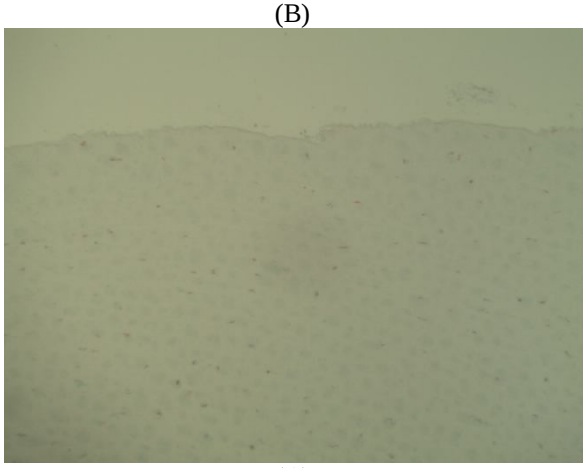


Fig.4. Masson's trichrome staining showed collagen fibres arranged similar to the native corneas.
(A)Native cornea (B)F-dPC (C)A-dPC (D)C-dPC

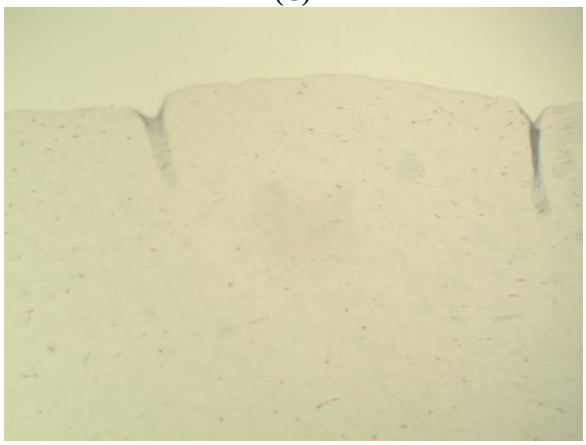
(A)



(B)



(C)



(D)

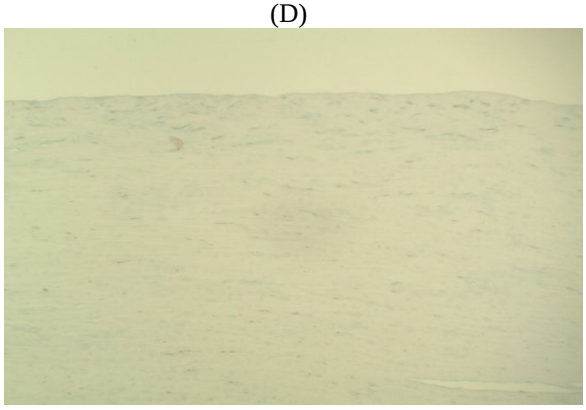


Fig.5. Alcian blue staining confirmed an intact GAGs preservation of the corneal stroma.
(A)Native cornea (B)F-dPC (C)A-dPC (D)C-dPC

CONCLUSIONS

In this study, we have explored a new decellularized procedure for corneal scaffolds. The results demonstrated that this method for porcine corneas had favorable biological properties and suggested it could be a potential scaffold substitutes for clinical corneal replacement.

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