The Physical and Mechanical Properties of Basement Membrane Hydrogels

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Introduction

Biomaterials play an important role in the ongoing demand for new tissue regeneration strategies. However, synthetic biomaterials currently available are limited in their ability to recapitulate the complexity and diversity of *in vivo* extracellular matrices (ECM). This limits their ability to serve as accurate mimetic of *in vivo* cell-ECM interactions. In order to accurately mimic the properties of natural materials we must first understand the physical and mechanical properties of natural self assembling scaffolds.

We have previously developed a technique that allows the isolation and gelation of basement membrane (BM) proteins hydrogels isolated from any tissue source[1]. The biological activity and mechanical properties of these matrices suggest their potential for application in tissue regeneration therapies. In addition, these gels can be used for investigation of tissue-specific aspects of cell-matrix interactions, scaffolds for tissue engineering therapies, or environments for guided stem cell differentiation. The goal of this work is to characterize the physical and mechanical properties of these biological matrices. These data can be used to better understand cell-ECM interactions and to serve as design criteria for the development of synthetic bio-mimetic materials.

Materials and Methods

Dermal BM hydrogels were isolated from rat dermis. Tissues were minced, suspended in dispase solution, and incubated for 30 mins at 4ºC. Solutions were homogenized in a high salt buffer and BM proteins were extracted with 2 M urea buffer. The mixture was centrifuged and the supernatant collected and analyzed using western blots, BCA protein assays, histology and ELISA. Dermis BM gels were formulated using two different methods: acid and temperature induced gelation. The BM gel samples were placed in glutaraldehyde solution and prepared for scanning electron microscopy (SEM) and transmission electron microscopy (TEM) imaging. Series of images were obtained from each BM gel formulation. The fiber and pore diameters were measured. Additionally mechanical properties of the dermis BM matrices surface using different BM concentrations (1, 5 and 10mg/ml) were calculated using multimode Nanoscope IIIa atomic force microscope (AFM). Images were captured using AFM tapping mode in air with silicon (Si_3N_4) probes at a frequency of 8-10 kHz.

Results and Discussion

The BM extracts assemble into 3D gels rapidly with a reduction in pH or more slowly with a change in temperature. The *in vivo* mechanism of gelation remains under debate, but greatly affects the physical properties of the resultant gels. Western blots and ELISAs have shown that the extracted BMs have a distinct composition and concentration of proteins (laminins, collagen IV, and nidogen) specific to the tissue source.

Nanostructure - The 3D gels were imaged with SEM and TEM. SEM revealing an interconnected network of fibers with structure nearly identical to BM *in vivo* regardless of the gelation mechanism (Fig. 1).

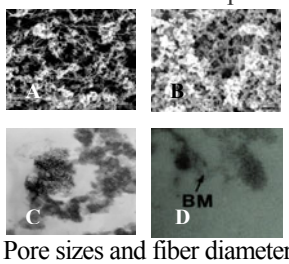


Figure 1: (A, B) SEM of 3D Dermis and U-87 BM gel respectively. **(C)** TEM image of gel showing diffusively flocculent structure similar to that seen in BM structure *in vivo*. **(D)** Image from literature showing structure of BM *in vivo*

Pore sizes and fiber diameters were compared to literature BM values of Matrigel, a commercially available and commonly used model of BM structure and function (Table 1). The fiber diameters of the dermis BM matrices were similar to the literature values of Matrigel however, the pore diameter of the dermis BM matrices were significantly larger. **Table 1:** The mean fiber and pore diameters of dermis BM and Matrigel.

Formulations	Dermis BM Temperature	Dermis BM Acid	Matrigel[2]
Fiber Diameter (nm)	72±12	166±25	69±35
Pore Diameter (nm)	602±407	475±253	105±70

Volume fractions and swelling ratios of the dermis BM were compared to the values of Matrigel.

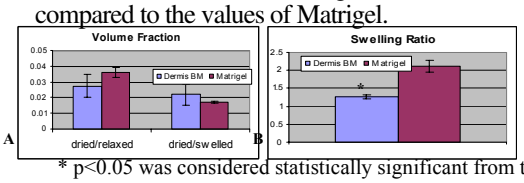
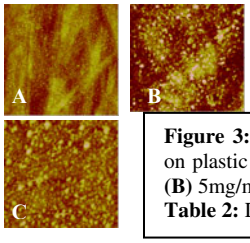


Figure 2: Volume fractions (A) and swelling rations (B) measurements for dermis BM and Matrigel.

* $p<0.05$ was considered statistically significant from the control

The 3D matrices were imaged using AFM. Contact of the AFM tip with the surface, measured the surface features digitally and provided a topological map of different concentrations of BM matrices on a plastic surface. Furthermore, the Young's modulus, a measurement of material stiffness, was calculated for the different BM matrices (Fig. 3).



Concentration (mg/ml)	RMS Roughness (nm)	E Stiffness (kPa)
Plastic	1.06	3,800
1.0 (A)	1.87	282
5.0 (B)	4.69	466
10.0 (C)	6.29	859

Figure 3: AFM height images of dermis BM matrices on plastic substrates. **(A)** BM concentration of 1 mg/ml **(B)** 5mg/ml, **(C)** 10 mg/ml (Scan size 2X 2 µm)
Table 2: Dermis BM mechanical properties

Conclusion

In this study we have characterized the physical and mechanical properties of natural BM hydrogels. The BM matrices can be formed by increasing temperature or reducing pH similarly to their self assembly *in vivo*. These results could be used to guide the design of synthetic materials for biomedical applications. Current work in our lab focuses on investigating the use of these gels for tissue regeneration.

References

1. Uriel S. et al. Regenerative Medicine World Congress. April 25-27, 2006 Oral Presentation.
2. Brody S. et al. Tissue Eng 2006;12(2):413-421.

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